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intelligent measurement and control systems

Time-Correlated Single Photon Counting

Time-Correlated Single Photon Counting (TCSPC) is a technique to record low level light signals with ps time resolution. Typical applications are

Ultra-Fast Recording of Optical Waveforms
Fluorescence Lifetime Measurements
Detection and Identification of Single Molecules
Fluorescence Correlation Spectroscopy (FCS)
DNA Sequencing
Optical Tomography
Photon Correlation Experiments
Fluorescence Lifetime Imaging (FLIM)
Fluorescence Resonance Energy Transfer (FRET)

The method has some striking benefits:

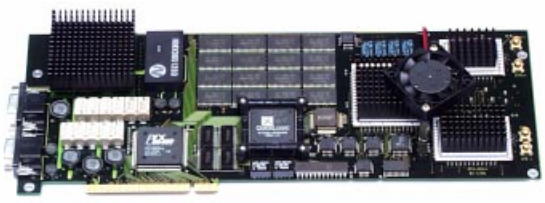
Ultra-High Time Resolution - 25 ps fwhm with the best detectors
Ultra-High Sensitivity - down to the Single Photon Level
Short Measurement Times
High Dynamic Range - Limited by Photon Statistics only
High Linearity
Excellent Signal-to-Noise Ratio
High Gain Stability

TCSPC works best for

High Repetition Rate Signals
Wavelength from 160 nm to 1000 nm

Principle of TCSPC Technique

Time-Correlated Single Photon Counting (TCSPC) is based on the detection of single photons of a periodical light signal, the measurement of the detection times of the individual photons and the reconstruction of the waveform from the individual time measurements.



Complete electronics on board - a TCSPC Module of Becker & Hickl

The method makes use of the fact that for low level, high repetition rate signals the light intensity is usually so low that the probability to detect one photon in one signal period is much less than one. Therefore, the detection of several photons can be neglected and the principle shown in the figure below be used:

The detector signal consists of a train of randomly distributed pulses due to the detection of the individual photons. There are many signal periods without photons, other signal periods contain one photon pulse. Periods with more than one photons are very rare.

When a photon is detected, the time of the corresponding detector pulse is measured. The events are collected in a memory by adding a '1' in a memory location with an address proportional to the detection time. After many photons, in the memory the histogram of the detection times, i.e. the waveform of the optical pulse builds up.

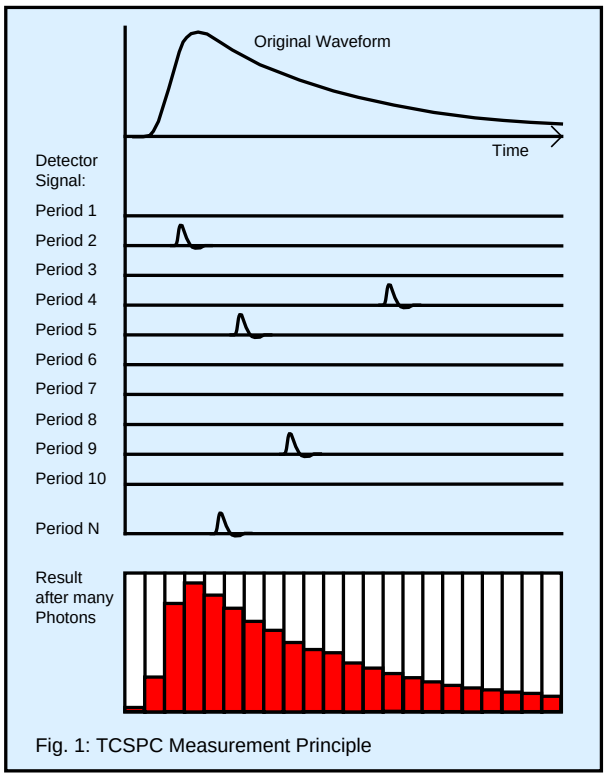


Fig. 1: TCSPC Measurement Principle

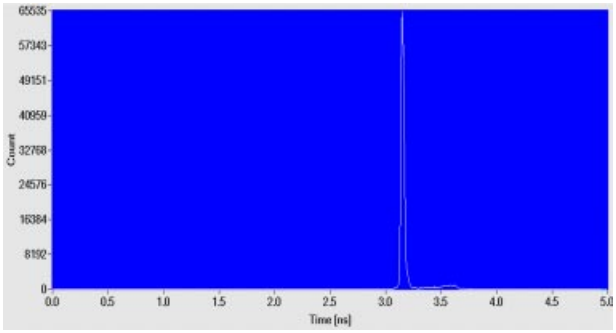
Although this principle looks complicated at first glance, it has a number of striking benefits:

- The time resolution of TCSPC is limited by the transit time spread, not by the width of the output pulse of the detector
- TCSPC has a near-perfect counting efficiency and therefore achieves optimum signal-to-noise ratio for a given number of detected photons
- TCSPC is able to record the signals from several detectors simultaneously
- TCSPC can be combined with a fast scanning technique and therefore be used as a high resolution high efficiency lifetime imaging (FLIM) technique in confocal and two-photon laser scanning microscopes
- TCSPC is able to acquire fluorescence lifetime and fluorescence correlation data simultaneously
- State-of-the-art TCSPC devices achieve count rates in the MHz range and acquisition times down to a few milliseconds

Time resolution

The TCSPC technique differs from methods with analog signal processing in that the time resolution is not limited by the width of the detector impulse response. Instead, for TSPC the timing jitter in the detection channel is essential. This accuracy is determined by the transit time spread of the single photon pulses in the detector and the timing jitter in the electronic system. When photomultipliers are used as detectors the half-width of the instrument response function (IRF) is usually 10 times shorter than the half width of the detector impulse response. Some typical values for different detector types are given below.

conventional photomultipliers	
standard types	0.6 ... 1 ns
high speed (XP2020)	0.35 ns
Hamamatsu TO8 photomultipliers	
R5600, H5783	140 ... 220 ps
micro channel plate photomultipliers	
Hamamatsu R3809	25 ... 30 ps
single photon avalanche photodiodes	60 ... 500 ps



A laser pulse recorded with 30 ps fwhm

Efficiency

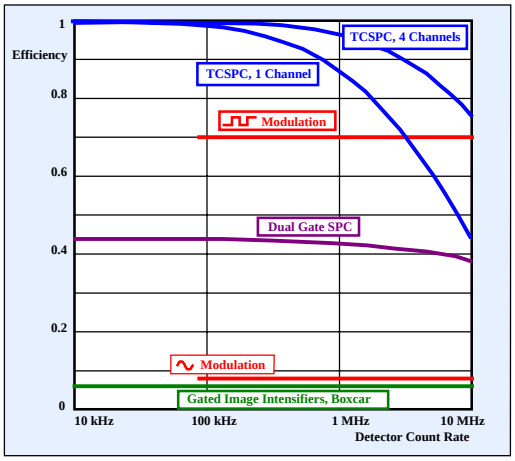
Different time-resolved optical signal recording techniques differ considerably in terms of recording efficiency, i.e. in the exploitation of the detected photons. Taking into regard that the available number of photons is limited by the photostability of the sample or by the acceptable acquisition time, recording efficiency is the most important parameter next to time resolution. The efficiency is defined by the ratio of the number of photons actually recorded, $N_{recorded}$, and the number of photons seen by the detector, $N_{detected}$:

$$E = N_{detected} / N_{recorded}$$

Since the SNR is proportional to the square root of the number of detected photons the efficiency is also

$$E = (SNR_{real} / SNR_{ideal})^2$$

A comparison of the efficiency for TCSPC with one channel and four parallel channels, for single channel modulation techniques with sine wave and square wave modulation, modulated and gated image intensifiers, boxcar, and dual-gate photon counting is given in the figure right. TCSPC features a near-perfect counting efficiency up to a detector count rate of 1 MHz. The reason is that TCSPC does not involve any gating process or gain modulation. Surprisingly, TCSPC beats the other methods in efficiency even for detector count rates of the order of 5 to 10 MHz.



Efficiency of different time-resolved signal recording techniques

Sensitivity

The sensitivity of the SPC method is limited mainly by the dark count rate of the detector. Defining the sensitivity as the intensity at which the signal is equal to the noise of the dark signal the following equation applies:

$$S = \frac{(Rd * N/T)^{1/2}}{Q}$$

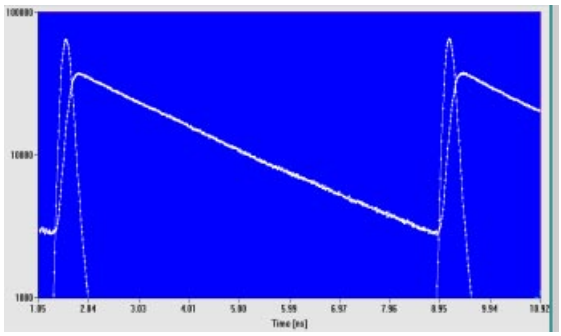
(Rd = dark count rate, N = number of time channels, Q = quantum efficiency of the detector, T = overall measurement time)

Typical values (PMT with multialkali cathode without cooling) are $Rd=300s^{-1}$, N=256, Q=0.1 and T=100s. This yields a sensitivity of S=280 photons/second. This value is by a factor of 10^{15} smaller than the intensity of a typical laser (10^{18} photons/second). Thus, when a sample is excited by the

laser and the emitted light is measured, the emission is still detectable for a conversion efficiency of 10^{-15}.

Accuracy

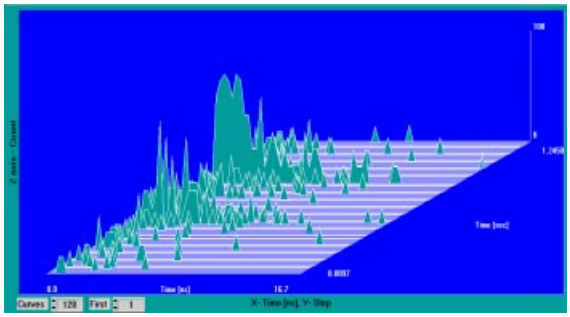
The accuracy of the measurement is given by the standard deviation of the number of collected photons in a particular time channel. For a given number of photons N the signal-to-noise ratio is $SNR = N^{-1/2}$. If the light intensity is not too high, all detected photons contribute to the result. Therefore, TCSPC yields an ideal signal-to-noise ratio for a given intensity and measurement time. Furthermore, in the TCSPC technique noise due leakage currents, gain instabilities, and the random gain mechanism of the detector does not appear in the result. This yields an additional SNR improvement compared to analog signal processing methods.



Fluorescence decay curves, excitation with Ar+ laser

Acquisition Time

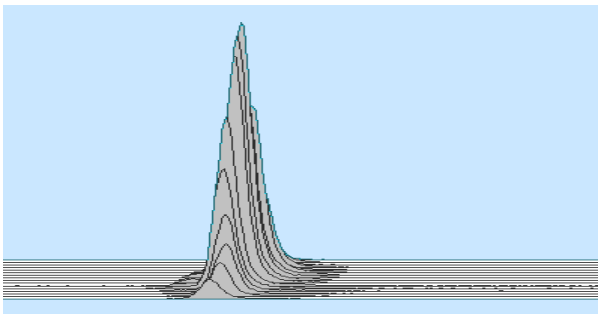
The TCSPC method is often thought to suffer from slow recording speed and long measurement times. This ill reputation comes from traditional TCSPC devices built up from nuclear instrumentation modules which had a maximum count rate of some 10^4 photons per second. Due to a proprietary AD conversion principle the TCSPC devices from Becker & Hickl achieve count rates of several 10^6 photons per seconds. Thus, 1000 photons can be collected in less than 1 ms, and the devices can be used for high speed applications as the detection of single molecules flowing through a capillary, fast image scanning, for the investigation of unstable samples or simply as optical oscilloscopes.



Fluorescence decay signals from single molecules running through a capillary. Collection time 1 ms per curve.

Multidetector Capability

Becker & Hickl have introduced a proprietary TCSPC multidetector technique. Multidetector operation makes use of the fact that at the low light intensities typical for TCSPC the detection of several photons in the same laser period is unlikely.



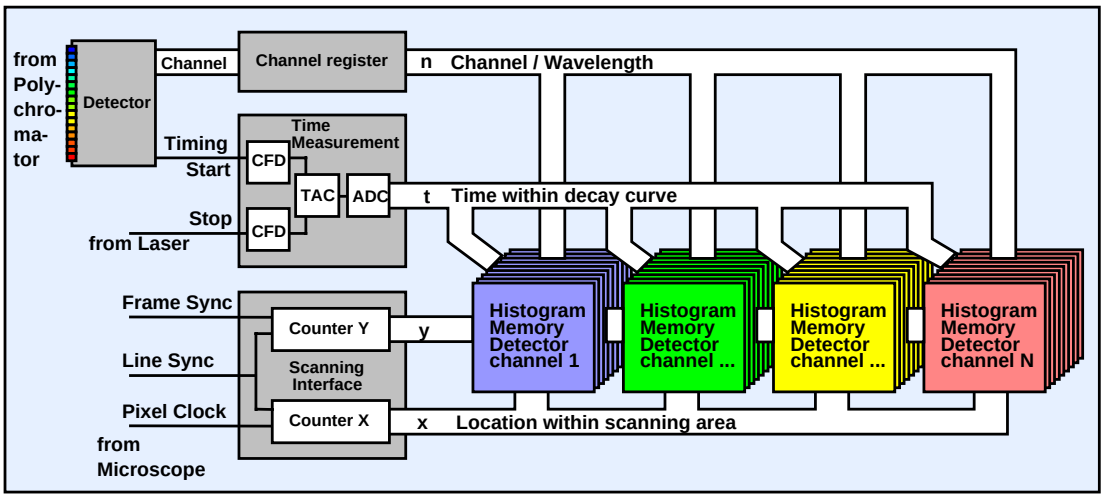
16 signals measured simultaneously with a 16 channel PMT

Thus, the output pulses of several detectors can be combined into one common timing pulse line and sent through the timing and histogramming circuitry of one TCSPC channel. An external 'Routing' device determines in which detector a particular photon was detected. This information is used to route the photons from different detectors into different memory blocks of the TCSPC module. As a result, separate histograms build up containing the waveforms for the individual detectors.

Multidetector operation can increase the efficiency of a TCSPC measurement considerably since photons from different wavelength intervals or from different spots of the sample are recorded simultaneously. Moreover, multidetector operation reduces classic pile-up-effects because multi-photon events are recognised and rejected by the routing electronics. Typical applications are optical tomography, multi-wavelength lifetime imaging and single molecule experiments.

Fluorescence Lifetime Imaging with Laser Scanning Microscopes

The SPC-730 and SPC-830 modules can be connected directly to a confocal or two-photon laser scanning microscope. The modules employ an advanced three-dimensional TCSPC technique and build up the photon density over the time, t, within the fluorescence decay, the image coordinates, x,y, and the detector number or wavelength, n or λ. The principle is shown in the figure below.



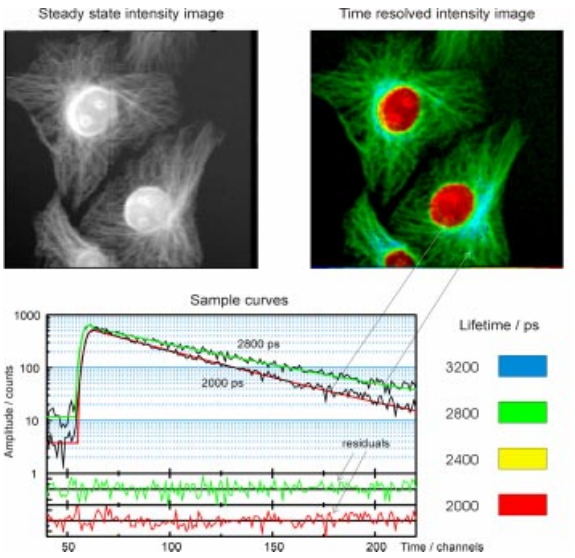
TCSPC imaging technique used in the SPC-730 and SPC-830

The TCSPC module receives the single photon pulses from the photomultiplier (PMT) of the microscope, the reference pulses from the laser and the Frame Sync, Line Sync and Pixel Clock signals from the scanning unit of the microscope. For each PMT pulse, i.e. for each photon, the TCSPC module determines the time of the photon within the laser pulse sequence and the location within the scanning area. These values are used to address the histogram memory in which the events are accumulated. Thus, in the memory the distribution of the photon density over the scan coordinates, x, y, and the time, t, within the fluorescence decay function builds up. The result can be interpreted as a two-dimensional (x, y) array of fluorescence decay curves or as a sequence of fluorescence images for different times (t) after the excitation pulse. Several such arrays exist depending on the number of detector or wavelength channels.

As for the basic TCSPC technique, there is virtually no loss of photons in the TCSPC imaging process. As long as the photon detection rate is not too high all detected photons are processed and accumulated in the histogram, thus providing near-ideal signal-to-noise ratio and maximum sensitivity. This is a key advantage of TCSPC imaging compared to gated photon counting, gated image intensifiers and modulation techniques.

The figure right shows a TCSPC image of a single cell layer (double staining with Hoechst for DNA and Alexa 488) obtained by two-photon excitation at 800 nm in a Zeiss LSM-510 microscope. The intensity image (containing the photons of all time channels) is shown left. Deconvolution analysis delivers the fluorescence lifetime τ in the individual pixels of the image. This allows to generate intensity-τ images that display the fluorescence intensity and the fluorescence time as brightness and colour (figure right). The quality of the fit is shown for two selected pixels (right, bottom).

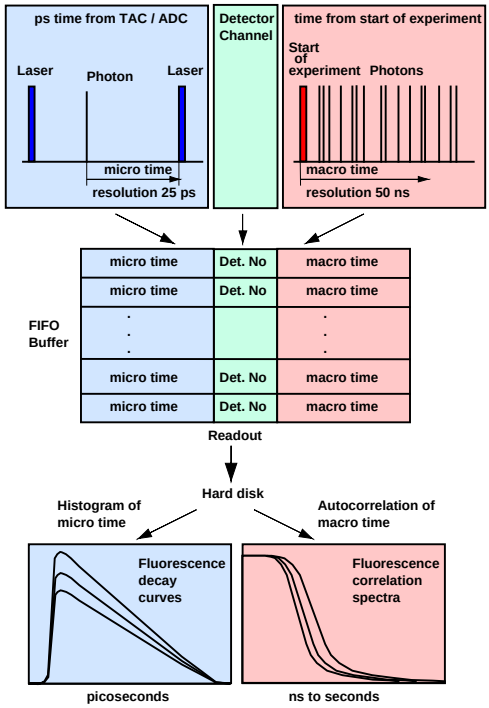
Main applications of TCSPC lifetime imaging are fluorescence quenching, fluorescence resonance transfer (FRET) and the separation of autofluorescence components in cells.



Lifetime imaging of cells. Intensity Image (top left), Intensity / τ Image (top right) and decay curves of selected pixels (bottom)

Simultaneous Lifetime and FCS data acquisition

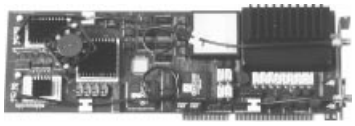
Fluorescence Correlation Spectroscopy (FCS) exploits intensity fluctuations in the emission of a small number of chromophore molecules in a femtoliter sample volume. The fluorescence correlation spectrum is the autocorrelation function of the intensity fluctuation. FCS yields information about diffusion processes, conformational changes of chromophore - protein complexes and intramolecular dynamics. These effects can be accompanied by lifetime fluctuations which, of course, should be recorded simultaneously from the same sample volume. The 'FIFO' mode of the SPC-630, SPC-134, and SPC-830 modules can be used for such measurements. This mode does not build up a histogram as the TCSPC imaging techniques do. Instead, it records the full information about each photon. Each entry contains the time of the photon in the laser pulse sequence, the time from the start of the experiment, and the detector channel. The data structure is shown in the figure right. For each detector an individual correlation spectrum and a fluorescence decay curve can be calculated. If several detectors are used to record the photons from different chromophores, the signals of these chromophores can be cross-correlated. The fluorescence cross-correlation spectrum shows whether the molecules of both chromophores and the associated protein structures are linked or diffuse independently.



Simultaneous FCS / lifetime data acquisition

BH TCSPC Modules

BH has developed and manufactures a wide variety of TCSPC modules for different applications. The most common modules are listed below.

Module	Count Rate MHz		Memory		Application	
	Saturated	Useful 50% loss	Histogram curves * channels	FIFO Buffer photons		
SPC-300 SPC-330	5 5	2.5 2.5	131,072	- -	traditional fluorescence lifetime measurement	
SPC-400 SPC-430	8 8	4 4	262,144 262,144	- -	fluorescence lifetime, single molecule detection	
SPC-500 SPC-530	3 3	1.5 1.5	4,194,304 4,194,304	- -	fluorescence lifetime, multi-parameter measurements FLIM	
SPC-630	8	4	262,144	131,072	fluorescence lifetime, single molecule detection, FCS, correlation experiments optical tomography stopped flow	
SPC-730	5.5	2.25	4,194,304	-	fluorescence lifetime, TCSPC imaging, laser scanning microscopy, FLIM, FRET, multi-parameter measurements, correlation experiments, stopped flow	
SPC-134	32	16	1,485,576	262,144	4 fully parallel TCSPC channels. optical tomography, photon migration single molecule detection, FCS correlation experiments, stopped flow	
SPC-830	8	4	16,777,216	8,388,608	fluorescence lifetime, TCSPC imaging, laser scanning microscopy, FLIM / FRET, single molecule detection, FCS correlation experiments, multi-parameter measurements, stopped flow	

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