

Time-Correlated Photon Counting

Tech Note TCSPC 1.2

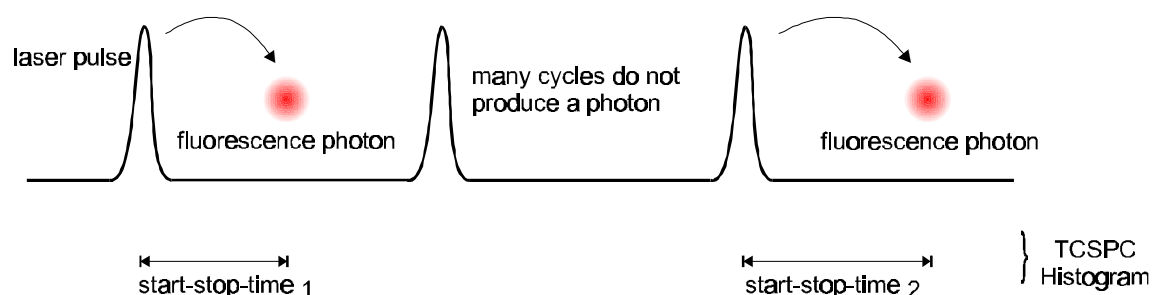
Michael Wahl, PicoQuant GmbH, January 2000

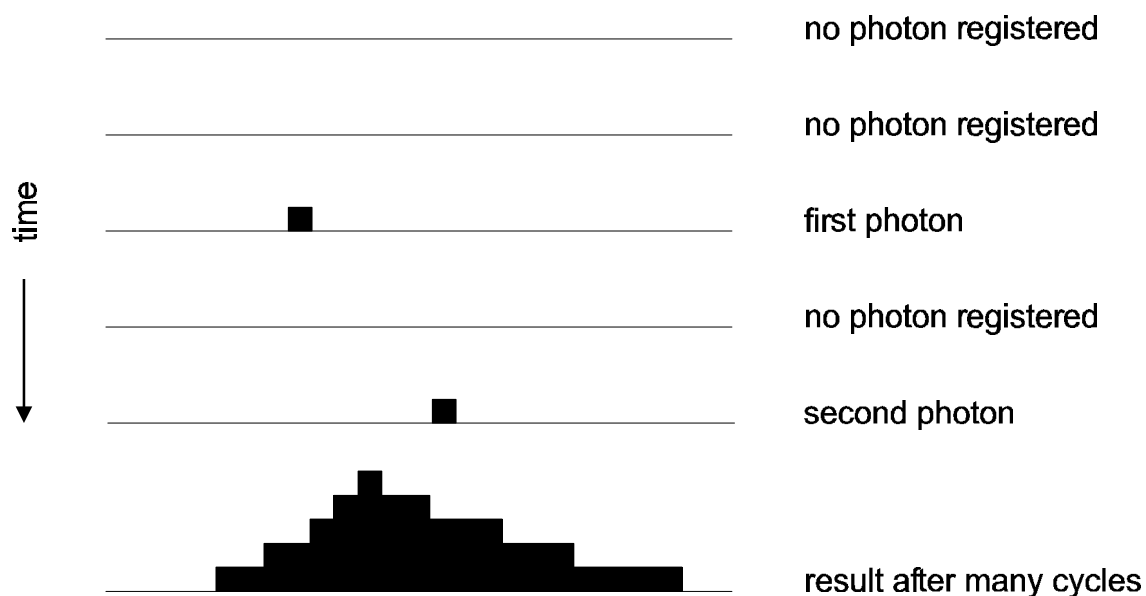


The Principle of Time-Correlated Single Photon Counting

In order to exploit the merits of a powerful analysis tool such as time-correlated fluorescence spectroscopy, one must in some way or other record the time dependent intensity profile of the emitted light. While in principle, one could attempt to record the time decay profile of the signal from a single excitation-emission cycle, there are practical problems to prevent such a simple solution in most cases. First of all the decay to be recorded is very fast. Typical fluorescence from organic fluorophores lasts only some hundred picoseconds to some hundred nanoseconds. In order to recover fluorescence lifetimes as short as e.g. 500ps, one must be able to resolve the recorded signal at least to such an extent, that the exponential decay is represented by some tens of samples. This means the transient recorder required would have to sample at e.g. 50ps time steps. Clearly this is hard to achieve with ordinary electronic transient recorders. Secondly the light available may be simply too weak to sample an analog time decay. Indeed the signal may consist of just a few photons per excitation/emission. Then the discrete nature of the signal itself prohibits analog sampling. Even if one has some reserve to increase the excitation power to obtain more fluorescence light, there will be limits, e.g. due to collection optic losses, spectral limits of detector sensitivity or photo-bleaching at higher excitation power. The solution is Time-Correlated Single Photon Counting (TCSPC). Since with periodic excitation (e.g. from a laser) it is possible to extend the data collection over multiple cycles, one can reconstruct the single cycle decay profile from single photon events collected over many cycles.

The method is based on the repetitive precisely timed registration of single photons of e.g. a fluorescence signal. The reference for the timing is the corresponding excitation pulse. As a single photon sensitive detector a Photomultiplier Tube (PMT), Multi Channel Plate (MCP) or a Single Photon Avalanche Photodiode (SPAD) can be used. Provided that the probability of registering more than one photon per cycle is low, the histogram of photon arrivals per time bin represents the time decay one would have obtained from a single shot time-resolved analog recording. The precondition of single photon probability can (and must!) be met by simply attenuating the light level at the sample if necessary. If the single photon probability condition is met, there will actually be no photons at all in many cycles. The diagrams below illustrate how the histogram is formed over multiple cycles.





The histogram is collected in a block of memory, where one memory cell holds the photon counts for one corresponding time bin. These time bins are often referred to as time channels. In practice the registration of one photon involves the following steps: first the time difference between the photon event and the corresponding excitation pulse must be measured. For this purpose both signals are converted to electric signals. For the fluorescence photon this is done via the single photon detector mentioned before. For the excitation pulse it may be done via another detector if there is no electrical sync signal supplied by the laser. Obviously all conversion to electrical pulses must preserve the precise timing of the signals as accurately as possible. The actual time difference measurement is done by means of fast electronics which provide a digital timing result. This digital timing result is then used to address the histogram memory so that each possible timing value corresponds to one memory cell or histogram channel. Finally the addressed histogram cell is incremented. All steps are carried out by fast electronics so that the processing time required for each photon event is as short as possible. When sufficient counts have been collected the histogram memory can be read out. The histogram data can then be used for display and e.g. fluorescence lifetime calculation. In the following sections we will expand on the various steps involved in the method and associated issues of importance.

Count rates and single photon statistics

We mentioned that it is necessary to maintain a low probability of registering more than one photon per cycle. This was to guarantee that the histogram of photon arrivals represents the time decay one would have obtained from a single shot time-resolved analog recording (The latter contains the information we are looking for). The reason for this is briefly the following: Due to dead times of detector and electronics for at least some nanoseconds after a photon event, TCSPC systems are usually designed to register only one photon per excitation/emission cycle. If now the number of photons occurring in one excitation cycle were typically >1 , the system would very often register the first photon but miss the following ones. This would lead to an over-representation of early photons in the histogram, an effect called ‘pileup’. It is therefore crucial to keep the probability of cycles with more than one photon low.

To quantify this demand one has to set acceptable error limits and apply some statistics. For practical purposes one may use the following rule of thumb: In order to maintain single photon statistics, on average only one in 20..100 excitation pulses should generate a count at the detector. In other words: the average count rate at the detector should be at most 1..5% of the excitation rate. E.g. with the diode laser PDL 800, pulsed at 80MHz repetition rate, the average detector count rate should not exceed 4MHz. This leads to another issue: the count rate the system (of both detector and electronics) can handle. Indeed 4MHz are stretching the limits of many detectors and certainly are way beyond the capabilities of most conventional NIM based systems. On the other hand, one wants high count rates, in order to acquire fluorescence decay histograms quickly. This may be of particular importance where dynamic lifetime changes or fast molecule transitions are to be studied or where large numbers of lifetime samples must be collected (e.g. in 2D scanning configurations). PMTs can handle count rates of up to 1..10 Millions of counts per second (cps), standard (passively quenched) SPADs saturate at a few hundred kcps. Typical NIM based TCSPC electronics handle a maximum of 50,000 to 500,000 cps. With modern integrated TCSPC-designs on a single PC-board count rates up to 2..8 Mcps can be achieved. It is also worth noting that the actual count arrival times of course are random so that there can be bursts of high count rate and periods of low count rates. Bursts of photons may still exceed the average rate. This should be kept in mind when comparing average count rates considered here and elsewhere. The specifications of TCSPC systems may interpret their maximum count rates differently in this respect. This is why another parameter, the so called dead-time is of interest too. This quantity describes the time the system cannot register photons while it is processing a previous photon event. The term is applicable to both detectors and electronics. Dead-time or insufficient throughput of the electronics are usually not of detrimental effect on the decay histogram or more precisely the lifetime to be extracted from the latter. However, the photon losses prolong the acquisition time or deteriorate the SNR if the acquisition time remains fixed. In applications where the photon burst density must be evaluated (e.g. for molecule transition detection) excess dead-times can be a problem. The matter is no issue if the excitation period is longer than the dead-time.

Timing Resolution

The most critical component in terms of timing resolution in TCSPC measurements will usually be the detector. However, as opposed to analog transient recording the time resolution of TCSPC is not limited by the pulse response of the detector. Only the timing accuracy of registering a photon determines the resolution. The timing accuracy is limited by the timing uncertainty the detector introduces in the conversion from a photon to an electrical pulse. This timing error or uncertainty can be as much as 10 times smaller than the detectors pulse response. The timing uncertainties are usually quantified by specifying the r.m.s. error or the Full Width Half Maximum (FWHM) of the timing distribution. Note that these two notations are related but not identical. Good but also expensive detectors, notably MCPs, can achieve timing uncertainties as small as 20ps FWHM. Cheaper PMTs or SPADs may introduce uncertainties of 200 to 400ps FWHM.

The second most critical source of IRF broadening usually is the excitation source. While most laser sources can provide sufficiently short pulses, it is also necessary to obtain an electrical timing reference signal (sync) to compare the fluorescence photon signal with. Where this signal is derived from depends on the excitation source. With gain switched diode lasers (e.g.

PDL 800) a low jitter electrical sync signal is readily available. The signal type used here is commonly a narrow negative pulse of -800mV into 50 Ohms (NIM standard). The very sharp falling edge is synchronous with the laser pulse (<10ps r.m.s. jitter for the PDL 800). With other lasers (e.g. the Ti:Sa laser TIF-100) a second detector must be used to derive a sync signal from the optical pulse train. This is commonly done with a fast photo diode (APD or PIN diode, e.g. the PHD-400). The light for this reference detector must be coupled out from the excitation laser beam e.g. by means of some semi-transparent mirror. The reference detector must be chosen and set up carefully as it also contributes to the overall timing error.

Another source of timing error is the timing jitter of the electronic components used for TCSPC. This is caused by the finite rise/fall-time of the electric signals used for the time measurement. At the trigger point of e.g. compactors, logic gates etc. the amplitude noise (thermal noise, interference etc.) always present on these signals is transformed to a corresponding timing error (phase noise). However the contribution of the electronics to the total timing error usually is relatively small. Modern TCSPC electronics cause an r.m.s. jitter of < 10ps. Nevertheless it is always a good idea to keep the RF noise low. This is why signal leads should be properly shielded coax cables and strong sources of RF interference should be kept away from the TCSPC detector and electronics.

The contribution of the time spread introduced by the individual components of a TCSPC system to the total IRF strongly depends on their relative magnitude. Strictly the total IRF is the convolution of all component IRFs. An estimate of the overall IRF can be obtained from the geometric sum of the individual components e.g. as r.m.s. error or FWHM (Full Width Half Maximum) values according to statistical error propagation laws:

$$e_{\text{IRF}_{\text{system}}} \approx \sqrt{\sum e_{\text{component}}^2} \quad (1)$$

Obviously due to the squares in the sum the total will be more than proportionally dominated by the largest component. It is therefore of little value to improve a component that is already relatively good. If e.g. the detector has an IRF of 200ps FWHM, shortening of the laser pulse from 50ps to 40ps is practically of no effect.

It is difficult to specify a general lower limit on the fluorescence lifetime that can be measured by a given TCSPC instrument. Apart from the instrument response function and noise, factors such as quantum yield, fluorophore concentration, and decay kinetics will affect the measurement. However, as a rule of thumb one can assume that under favourable conditions lifetimes down to 1/10 of the IRF (FWHM) can still be recovered via deconvolution.

A final time-resolution related issue worth noting here is the channel width of the TCSPC histogram. As outlined above, the analog electronic processing of the timing signals (detector, amplifiers, TAC etc.) creates a continuous (e.g. gaussian) distribution around the true value. In order to form a histogram, at some point the timing signal must be quantised. This is done by the ADC or a direct Time-to-Digital Converter (TDC). This quantisation introduces another random error if chosen too coarse. The quantisation step width (i.e. the resolution) must therefore be small compared with the IRF given by the analog noise. As a minimum from the information theoretical point of view one would assume the Nyquist frequency. I.e. the signal should be sampled at least at twice the highest frequency contained in it. For practical purposes there is no point in sampling the histogram at resolutions much higher than 1/10 of the overall IRF of the analog part of the system.

Photon Counting Detectors

Photomultiplier Tube (PMT)

A PMT consists of a light-sensitive photocathode that generates electrons when exposed to light. These electrons are directed onto a charged electrode called a dynode. The collision of the electrons with the dynode produces additional electrons. Since each electron that strikes the dynode causes several electrons to be emitted, there is a multiplication effect. After further amplification by multiple dynodes, the electrons are collected at the anode of the PMT and output as a current. The current is directly proportional to the intensity of light striking the photocathode. Because of the multiplicative effect of the dynode chain, the PMT is a photoelectron amplifier of high sensitivity and remarkably low noise. PMTs have a wide dynamic range, i.e. they can also measure relatively high levels of light. They furthermore are very fast, so rapid successive events can be reliably monitored. PMTs are also quite robust. The high voltage driving the tube may be varied to change the sensitivity of the PMT.

One photon on the photo-cathode can produce a short output pulse containing millions of photoelectrons. PMTs can therefore be used as single photon detectors. In photon counting mode, individual photons that strike the photocathode of the PMT are registered. Each photon event gives rise to an electrical pulse at the output. The number of pulses, or counts per second, is proportional to the light impinging upon the PMT. As the number of photon events increase at higher light levels, it will become difficult to differentiate between individual pulses and the photon counting detector will become non-linear. This usually occurs at 1-10 millions of counts per second.

The timing uncertainty between photon arrival and electrical output is small enough to permit time-resolved photon counting at a sub-nanosecond scale. In single photon counting mode the tube is typically operated at a constant high voltage where the PMT is most sensitive.

PMTs usually operate between the blue and red regions of the visible spectrum, with greater quantum efficiency in the blue-green region, depending upon photo-cathode materials. Maximum quantum efficiencies are about 25%. For spectroscopy experiments in the ultraviolet-visible-near infrared region of the spectrum, a photomultiplier tube is very well suited.

Because of noise from various sources in the tube, the output of the PMT may contain pulses that are not related to the light input. These are referred to as dark counts. The detection system can to some extent reject these spurious pulses by means of electronic discriminator circuitry. This discrimination is based on the probability that some of the noise generated pulses (those from the dynodes) exhibit lower signal levels than pulses from a photon event.

Microchannel Plate PMT (MCP)

A microchannel plate PMT is also a sensitive photon detector. It consists of an array of glass capillaries (10-25 μm inner diameter) that are coated on the inside with a electron-emissive material. The capillaries are biased at a high voltage. Like in the PMT, an electron that strikes the inside wall of one of the capillaries creates an avalanche of secondary electrons. This cascading effect creates a gain of 10^3 to 10^6 and produces a current pulse at the output. The timing jitter of MCPs is sufficiently small to perform time-resolved photon counting on a sub-nanosecond-scale, usually outperforming PMTs. Good but also expensive MCPs can achieve

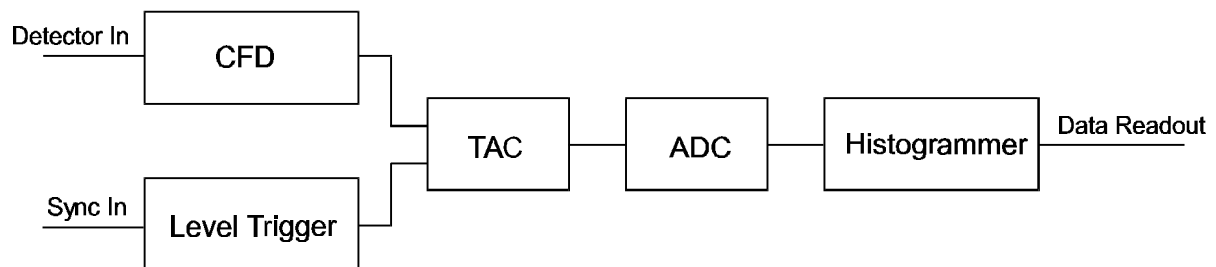
timing uncertainties as low as 20ps. Microchannel plates are also used as an intensifier for low-intensity light detection with array detectors.

Avalanche Photo Diode (APD)

APDs are the solid-state equivalent of PMTs, usually restricted to operation in the red to infrared part of the spectrum. Generally, APDs may be used for ultra-low light detection (optical powers < 1 pW), and can be used in either "linear" mode (bias voltage slightly less than the breakdown voltage) at gains up to about 500, or as photon-counters in the so-called "Geiger" mode (biased slightly above the breakdown voltage). In the case of the latter, a single photon may trigger an avalanche of about 10^8 carriers. In this mode the device can be used as a detector for photon counting with very accurate timing of the photon arrival. In this context they are also referred to as Single Photon Avalanche Photo Diodes (SPAD). Selected devices may achieve timing accuracies of < 200 ps. Single-photon detection probabilities of up to approximately 50% are possible. APDs are often noisier than PMTs but have greater quantum efficiency. Maximum quantum efficiencies reported are about 80%.

Principles behind the TCSPC electronics

Conventional TCSPC systems consist of the following building blocks:



The CFD is used to extract precise timing information from the electrical detector pulses that may vary in amplitude. This way the overall system IRF may be tuned to become narrower and some random background signal can be suppressed. The same could not be achieved with a simple threshold detector (comparator). Particularly with PMTs, constant fraction discrimination is very important as their pulse amplitudes vary significantly. Particularly pulses originating from random electrons generated at the dynodes of the PMT can be suppressed as their avalanches had less time to amplify, so that their output pulses are small.

The principle behind a CFD is the comparison of the original detector signal with an amplified and delayed version of itself. The signal derived from this comparison changes its polarity exactly when a constant fraction of the detector pulse height is reached. The zero crossing point of this signal is therefore suitable to derive a timing signal independent from the amplitude of the input pulse. This is done by a subsequent comparison of this signal with a settable zero level, the so called zero cross trigger. Making this level settable allows to adapt

to the noise levels in the given signal, since in principle an infinitely small signal could trigger the zero cross comparator. Typical CFDs furthermore permit the setting of one or two so called discriminator thresholds, determining the lower and upper limits of a window discriminator the detector pulse amplitude must pass.

Similar as for the detector signal, the sync signal must be made available to the timing circuitry. Since the sync pulses are usually of well-defined amplitude and shape, a simple settable comparator (level trigger) is sufficient to adapt to different sync sources.

The signals from the CFD and SYNC trigger are fed to the Time to Amplitude Converter (TAC). This circuit is essentially a highly linear ramp generator that is started by one signal and stopped by the other. The result is a voltage proportional to the time difference between the two signals.

The voltage obtained from the TAC is then fed to an Analog to Digital Converter (ADC) which provides the digital timing value used to address the histogrammer. The ADC must be very fast in order to keep the dead time of the system short. Furthermore it must guarantee a very good linearity, over the full range as well as differentially. These are criteria difficult to meet simultaneously, particularly with ADCs of high resolution (e.g. 12bits) as is desirable for TCSPC over many histogram channels.

The histogrammer has to increment each histogram memory cell whose digital address in the histogram memory it receives from the ADC. This is commonly done by fast digital logic e.g. in the form of Field Programmable Gate Arrays (FPGA) or a microprocessor. Since the histogram memory at some point also must be available for data readout, the histogrammer must stop processing incoming data. This prevents continuous data collection. Sophisticated TCSPC systems solve this problem by switching between two or more memory blocks, so that one is always available for incoming data.

While this section so far outlined the typical structure of conventional TCSPC systems, it is also worth noting that the tasks performed by TAC and ADC can be carried out by a single fully digital circuit, a so called Time to Digital Converter (TDC). These circuits can measure time differences based on the delay times of signals in semiconductor logic gates or the conductor strips between them. The relative delay times in different gate chains can be used to determine time differences well below the actual gate delay. This permits exceptionally small, compact and affordable TCSPC solutions, as the circuits can be implemented as Application Specific Integrated Circuits (ASICs) at low cost and high reliability. The TCSPC system TimeHarp 100 makes use of such a design, permitting a resolution of 30-40ps. This resolution is well matched to the excitation pulse widths possible with diode lasers and the resolution permitted by affordable compact PMTs (e.g. IRF 200ps).

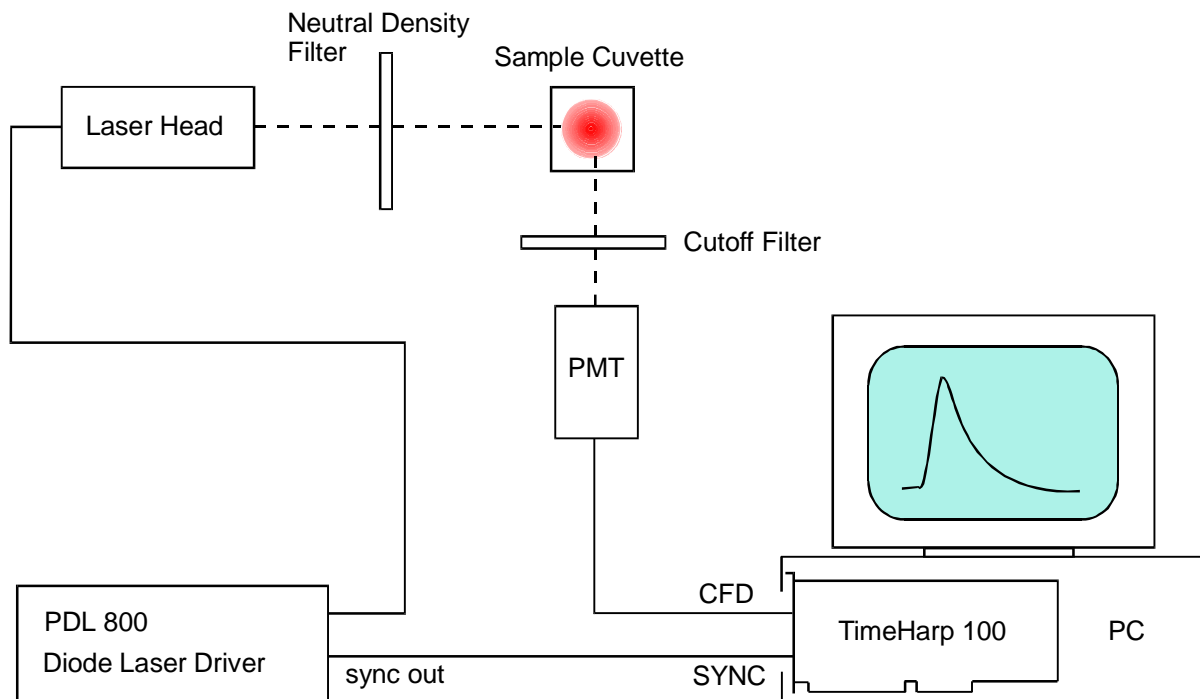
Reverse Start-Stop Mode

So far we have always assumed that the time delay measurement should be nicely causal, i.e. the laser pulse causes a photon event and therefore we measure the time delay between laser pulse and subsequent photon event. However, there are practical reasons to give up this convenient concept. The reasons are in the high repetition rates of the typical excitation lasers: Since the time measurement circuit (TAC, ADC) cannot know in advance, whether there will be a fluorescence photon, it would have to start a time measurement upon each laser pulse. Considering that typical conversion times are in the region of 0.5 to 2 μ s, any excitation rate in excess of 0.5 .. 2MHz would overrun the time measurement circuits. In fact they would most

of the time be busy with conversions that never complete, because there is no photon event at all in most cycles. The solution lies in the precondition of the single photon counting statistics we must maintain anyhow. By simply reversing the start and stop signals in the time measurement, the conversion rates are only as high as the actual photon rates generated by the fluorescent sample. These are (and must be) only about 1..5% of the excitation rates and can therefore be handled easily by the TAC/ADC or TDC. The consequence of this approach, however, is that the times we measure are not those between laser pulse and corresponding photon event but those between photon event and the next laser pulse. This is not too much of a problem, since the laser excitation is periodical and the times we measure are directly related to the ones we wanted to measure via the excitation period. As simple as this may sound, there may be yet more problems if the excitation period is very long. This may indeed be of practical relevance, e.g. with flash lamps (<100kHz). The reverse Start-Stop Principle as explained so far would lead to time delays as long as e.g. 10 μ s for 100kHz excitation rate. These delays are much too long to be measured by the TAC/ADC while the region of interest in this time span (i.e. the actual fluorescence decay) is as short as a few hundred nanoseconds. Again, there is a solution: One just has to delay the sync signal corresponding to the true excitation pulse relative to the photon detector signal. This can be done just by a few metres of cable or some sufficient optical path. The detector pulse can thereby ‘overtake’ the sync pulse and reach the timing electronics first. There it can start the time measurement and the sync pulse arriving later will stop the measurement. This works fine because the cable delays etc. remain constant.

Experimental Setup for Fluorescence Decay Measurements with TCSPC

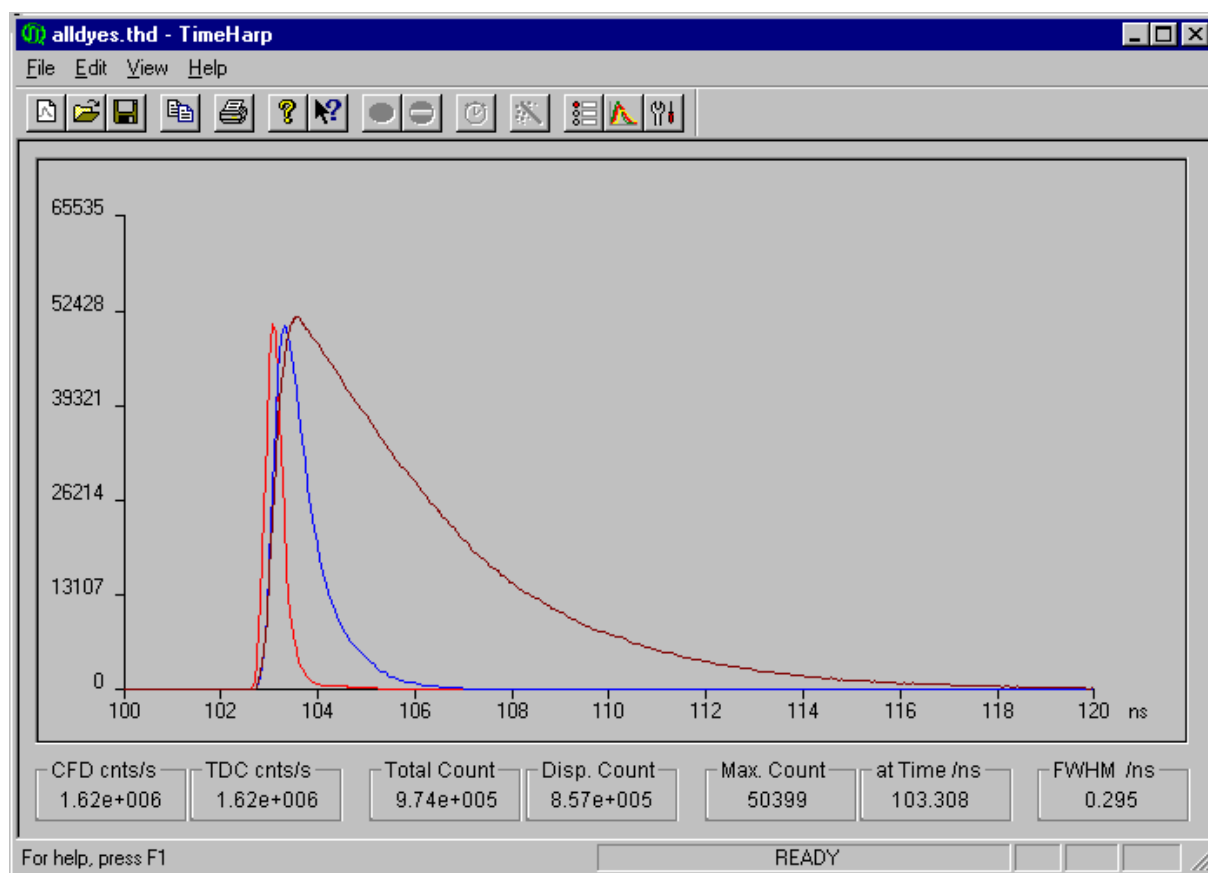
The figure below shows a typical setup for fluorescence lifetime measurements with TCSPC.



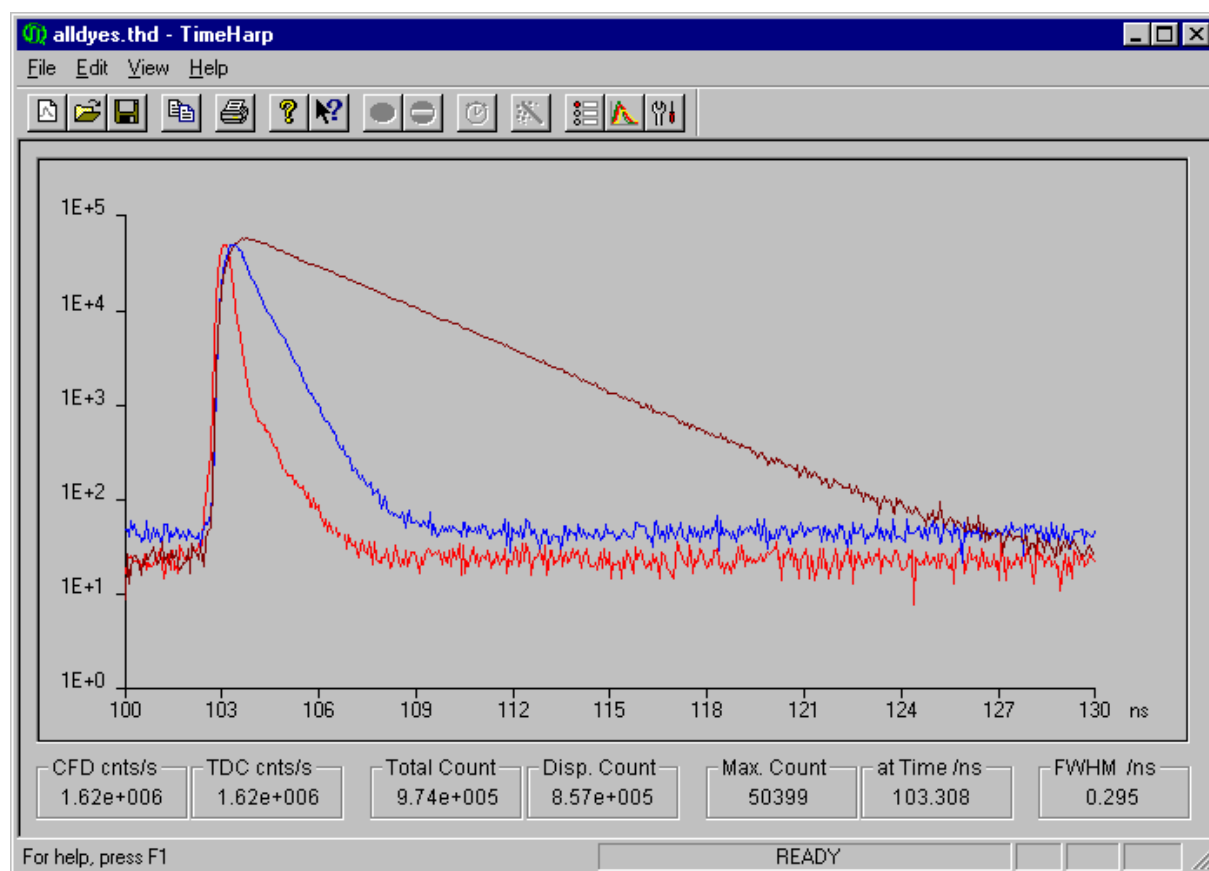
The picosecond diode laser (PDL 800) is running on its internal clock (settable at 5, 10, 20, 40 or 80 MHz). The driver box is physically separate from the actual laser head, which is attached via a flexible lead. This permits to conveniently place the small laser head anywhere in the optical setup. The light pulses of e.g. 50ps FWHM, are directed at the sample cuvette, possibly via some appropriate optics. A neutral density filter can be used to attenuate the light levels if necessary. Upon excitation, the fluorescent sample will emit light at a longer wavelength than that of the excitation light. The fluorescence light is filtered out against scattered excitation light by means of some optical cutoff filter. Then it is directed to the photon detector, again possibly via some appropriate collection optics, e.g. a microscope objective or just a lens. For timing accuracies ≥ 200 ps FWHM (permitting lifetime measurements even shorter than this via deconvolution) a cheap PMT is sufficient. The electrical signal obtained from the detector (e.g. a small negative pulse of -20mV) is fed to the TCSPC electronics via a standard 50Ohms coax cable. The complete TCSPC electronics (e.g. TimeHarp 100) are contained on a single PC-board, placed in a 486 or Pentium™ PC. In most cases there is not even a need for a preamp.

The laser driver also provides the electric sync signal needed for the photon arrival time measurement. This signal (NIM standard, a narrow pulse of -800mV) is also fed to the TCSPC electronics via a standard 50Ohms coax cable.

The following figure shows two fluorescence decay curves obtained with such a simple setup. The narrowest curve represents the system IRF, dominated by the detector. The second widest curve is a fluorescence decay from a solution of Tolnidin, a fluorescent dye with relatively short fluorescence lifetime. The widest curve is from Oxazin 4, another typical fluorescent dye. The excitation source was a PDL 800 at 80MHz repetition rate, while the TCSPC data was collected by the TimeHarp 100.



A second plot in the logarithmic scale reveals the nearly perfect exponential nature of the decay curves, as one would expect them. Note that this is even without a deconvolution of the relatively broad IRF (300ps).

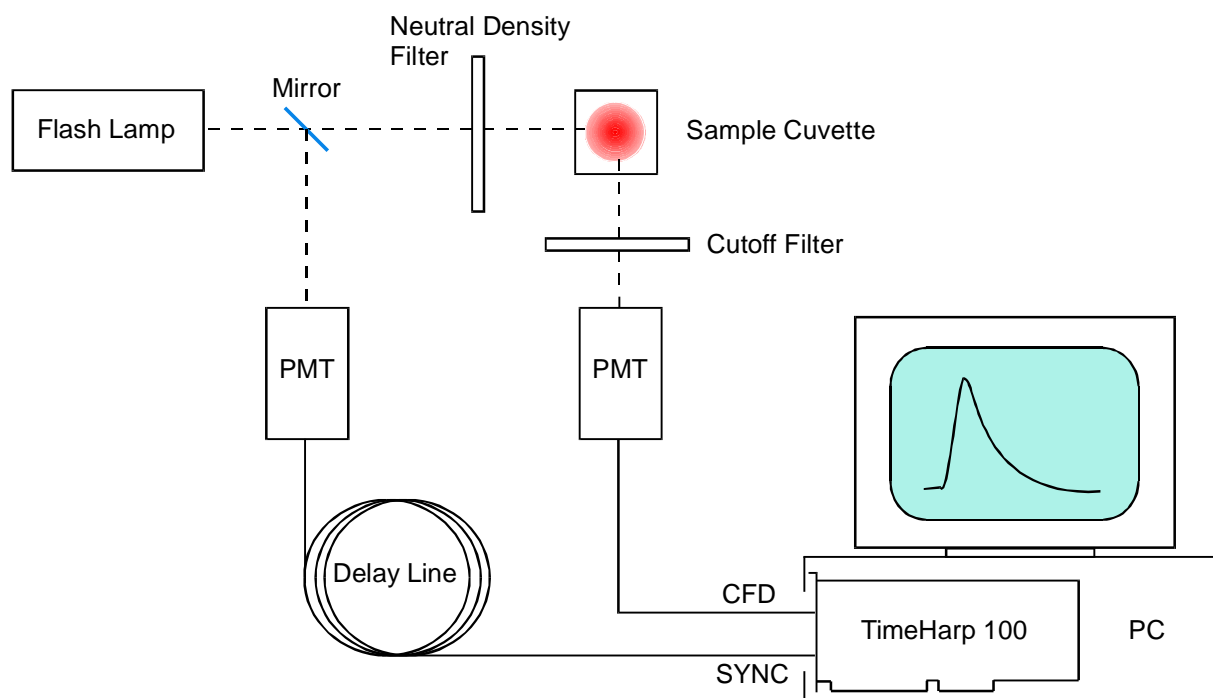


The approximate mono-exponential fluorescence lifetime can be obtained from a simple comparison of two points in the curve with count rates in the ratio of e:1 (e.g. 27,180:10,000). In this particular experiment this results in a lifetime estimate of 500ps for Tolnidin and 3.1ns for Oxazin. For a precise measurement one would perform a numerical exponential fit with IRF deconvolution, which would result in slightly smaller lifetimes since in this experiment the IRF (at least for Tolnidin) is nearly as broad as the lifetime to be measured. Nevertheless one can measure lifetimes significantly smaller than the IRF with this method. Additionally the r.m.s. residue from the fit can be used to assess the quality of the fit and thereby the reliability of the lifetime measurement. The FluoFit decay fit software package from PicoQuant provides this functionality. Of course it is easy to measure long lifetimes with or without deconvolution, since the IRF is of less influence.

Measurements at Low Repetition Rates

Since nowadays the most common excitation sources are lasers at high repetition rates (often >70MHz) the reverse start-stop concept has become standard for practically all modern TCSPC systems. This is why it is also standard for the TimeHarp 100 and the SPC family. The consequence is that for slow excitation sources such as flash lamps (some tens of kHz) the time

difference between start (photon) and stop (next sync pulse) is extremely long. Since the measurement range of TAC based systems is usually limited to about $1\mu\text{s}$ these time differences cannot be measured. Although TDC based systems like the TimeHarp 100 could be extended to measure some milliseconds, there is a more convenient way to overcome the problem. One just has to delay the stop pulse that corresponds to the photon event (i.e. the sync signal) relative to the photon event, so that the electronics will not take the next but the previous sync pulse for the stop of measurement. The delay has to be just long enough to span the time range of fluorescence decay one wants to record. The diagram below shows a setup for this mode of TCSPC (reversed start-stop at low repetition rates).



PicoQuant TCSPC Electronics and System Integration

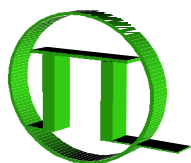
Besides pulsed diode lasers PicoQuant provides the fast timing electronics for acquisition of e.g. time resolved fluorescence decay profiles, bringing this technology to a degree of compactness and ease of use unseen before. Similar to the developments in fast solid state excitation sources this permits the transfer of revolutionary methods from the lab to real life industry applications e.g. in quality control or high throughput screening.

The PicoQuant TCSPC systems contain all components previously accommodated in bulky NIM racks on a single PC slot card. Nevertheless they outperform conventional systems in many parameters. Due to the versatile design the boards support many useful measurement modes such as oscilloscope mode for on-the fly optical alignment or the $F(t,\lambda)$ mode for time- and spectrally resolved measurements. The TCSPC board TimeHarp 100 is specially designed to reduce cost and size while maintaining the resolution and throughput required in combination with affordable excitation sources and photon detectors. It extends the range of powerful measurement modes to continuous and time tagged modes e.g. for single molecule detection. Hardware synchronisation pins permit real-time scanning setups with millisecond stepping. DLL libraries are available for custom programming or system integration.

Apart from supplying stand-alone components, PicoQuant develops complete instruments and supports system integration for specific research applications as well as for OEM needs. Of course PicoQuant does not leave the individual user alone with the sometimes tricky task of combining the components for a TCSPC system. We provide help, suggestions and professional consultancy to the individual researcher in the chemistry or biophysics lab as well as to the developer of an industry application.

Further Reading

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PICOQUANT
Unternehmen für optoelektronische
Forschung und Entwicklung

PicoQuant GmbH
Rudower Chaussee 29 (IGZ)
12489 Berlin

Tel.: (030) 6392 65 60 e-mail: photonics@pq.fta-berlin.de
Fax: (030) 6392 65 61 www: <http://www.picoquant.com>

Glossary

Absorption

When atoms or molecules absorb light, the incoming energy excites a quantised structure to a higher energy level. The type of excitation depends on the wavelength of the light. Electrons are promoted to higher orbitals by ultraviolet or visible light, vibrations are excited by infrared light, and rotations are excited by microwaves.

An absorption spectrum is the amount of absorption as a function of wavelength. The spectrum of an atom or molecule depends on its energy level structure, and absorption spectra are useful for the identification of compounds.

Avalanche Photo Diode (APD)

APDs are the solid-state equivalent of PMTs, usually restricted to operation in the red to infrared part of the spectrum. Generally, APDs may be used for ultra-low light detection (optical powers < 1 pW), and can be used in either "linear" mode (bias voltage slightly less than the breakdown voltage) at gains up to about 500, or as photon-counters in the so-called "Geiger" mode (biased slightly above the breakdown voltage). In the case of the latter, a single photon may trigger an avalanche of about 10^8 carriers. In this mode the device can be used as a detector for photon counting with very accurate timing of the photon arrival. Selected devices may achieve timing accuracies of < 200 ps. Single-photon detection probabilities of up to approximately 50% are possible. APDs are often noisier than a PMTs but have greater quantum efficiency. Maximum quantum efficiencies reported are about 80%.

Calibration

The check or correction of the accuracy of a measuring instrument to assure defined operational characteristics.

Constant Fraction Discriminator (CFD)

The CFD is used to extract precise timing information from electro-optical detector pulses that may vary in amplitude. A CFD is based on the comparison of the original detector signal with an amplified and delayed version of itself. The signal derived from this comparison changes its polarity exactly when a constant fraction of the detector pulse height is reached. The zero crossing point of this signal is therefore suitable to derive a timing signal independent from the amplitude of the input pulse. This is done by a subsequent comparison of this signal with a settable zero level, the so called zero cross trigger. Typical CFDs furthermore permit the setting of one or two so called discriminator thresholds, determining the lower and upper limits of a window discriminator the detector pulse amplitude must pass.

Emission

Atoms or molecules that are excited to high energy levels can decay to lower levels by emitting radiation (emission, fluorescence or luminescence). For atoms excited by a high-temperature energy source this light emission is commonly called atomic or optical emission, and for atoms excited with light it is called atomic fluorescence. For molecules it is called fluorescence if the transition is between states of the same spin and phosphorescence if the transition occurs between states of different spin. The emission intensity of an emitting substance is proportional to analyte concentration at low concentrations, and is useful for quantitating emitting species.

Filters

Filters separate different parts of the electromagnetic spectrum by absorbing or reflecting certain wavelengths and transmitting other wavelengths. Colour filters are glass substrates containing absorbing species that attenuate certain wavelengths. A typical example is a cut-on colour filter, which blocks short wavelength light such as an excitation source, and transmits longer wavelength light such as fluorescence that reaches a detector.

Interference filters are made of multiple dielectric thin films on a substrate. They use interference to selectively transmit or reflect a certain range of wavelengths. A typical example is a bandpass interference filter that transmits a narrow range of wavelengths, and can isolate a single emission line from e.g. a discharge lamp.

Fluorescence

Fluorescence is the phenomenon in which absorption of light of a given wavelength by a fluorescent molecule is followed by the emission of light at longer wavelengths. The distribution of wavelength-dependent intensity that causes fluorescence is known as the fluorescence excitation spectrum, and the distribution of wavelength-dependent intensity of emitted energy is known as the fluorescence emission spectrum.

Fluorescent Probes

Specimens of interest that do not show suitable fluorescence themselves may be labelled with a fluorescent probe. Fluorescent probes are available for a wide range of biological preparations permitting the use of LIF to image or detect e.g. macromolecular structures (such as proteins, lipids, carbohydrates and nucleic acids). Several factors must be considered when selecting fluorescent probes. The major considerations are the excitation/emission wavelengths of the probe to be used, the laser and detector emission/absorption spectra available and the filter sets used. For time-resolved work the fluorescence lifetime must be matched by the time-resolution of the detector, electronics and duration of the laser pulse. Major factors that influence fluorophore selection are the emission spectrum and quantum efficiency of fluorescence Q_f and to a lesser extent the absorption spectrum and molar extinction coefficient (E). The product of these ($E_{\max} * Q_f$) can be used as a simplistic measure of relative brightness of the fluorophore. Both values (E and Q_f) can be greatly affected by environmental factors (e.g. pH). Note that fluorophores can change their properties after they are conjugated to other molecules, and after they are bound to their target.

Field Programmable Gate Array (FPGA)

Electrically programmable, erasable and reconfigurable logic device consisting of usually a large number of general purpose logic gates.

Full Width Half Maximum (FWHM)

Quantity for the description of pulse or distribution widths. The full width of the curve shape taken at a height of half of the maximum.

Laser

A laser is a coherent and highly directional light source. The acronym LASER stands for Light Amplification by Stimulated Emission of Radiation.

A laser consists of at least three components:

1. a gain medium that can amplify light that passes through it
2. an energy pump source to create a population inversion in the gain medium
3. two mirrors that form a resonator cavity

The gain medium can be solid, liquid, or gas and the pump source can be an electrical current or discharge or another light source. The specific design of a laser varies depending on the gain medium and whether the laser is operated continuously (cw) or pulsed. Some of the important considerations for selecting a laser for LIF investigations are cost, wavelengths of emission (laser lines), output power at the desired wavelength, efficiency, stability etc. Diode lasers are probably the most affordable and easy-to-use laser sources around today.

Laser-induced fluorescence (LIF)

Laser-induced fluorescence is the optical emission from molecules that have been excited to higher energy levels by absorption of electromagnetic radiation from a laser source. This emission is characteristic for the molecule. The main advantage of fluorescence detection compared to absorption measurements is the greater sensitivity achievable because the fluorescence signal has a very low background. For molecules that can be excited resonantly, LIF provides selective excitation of the analyte to avoid interferences. LIF is useful to study the electronic structure of molecules and to make quantitative measurements of analyte concentrations. Analytical applications include monitoring gas-phase concentrations in the atmosphere, flames, and plasmas.

The excitation source for molecular LIF was in the past typically a tunable dye laser in the visible spectral region. Studies in the near-ultraviolet and near-infrared became more common as near-infrared lasers and frequency-doubling methods improved. Cheap and easy-to-use semiconductor lasers (Diode Lasers) have greatly simplified LIF studies over the recent years but are currently available only in the visible red to infrared spectrum. Blue diode lasers are expected to be available soon.

Microchannel Plate (MCP)

A microchannel plate is a sensitive photon detector. It consists of an array of glass capillaries (6-25 μm inner diameter) that are coated on the inside with an electron-emissive material. The capillaries are biased at a high voltage and like the channeltron, an ion that strikes the inside wall of one of the capillaries creates an avalanche of secondary electrons. This cascading effect creates a gain of 10^3 to 10^5 and produces a current pulse at the output. The timing jitter of MCPs is sufficiently small to perform time-resolved photon counting on a subnanosecond-scale. Microchannel plates are also used as an intensifier for low-intensity light detection with array detectors.

Phase Fluorimetry

Phase-modulation fluorescence spectroscopy excites a fluorophore with light of a periodic, time-dependent intensity (e.g. sine modulated). As the fluorophore returns to its initial state after each excitation peak, the fluorescent light emitted is the same frequency as the excitation light. The emitted light, however, is phase shifted relative to the excitation light and the amplitude is modulated dependent on the fluorescence decay behaviour of the molecule. By measuring the phase shift and the modulation, it is possible to calculate the fluorescence lifetime. Because these two parameters, the phase shift and the amount of modulation of the emitted light, are independent measurements, they can be used for cross-validation. Limitations arise when the fluorescence decay is not a simple monoexponential. The complete description of the decay requires then the measurement at many modulation frequencies, making instrumentation expensive and data acquisition slow.

Photon

A particle with energy equal to a single quantum. The energy of the particle is proportional to frequency. Light as a form of electromagnetic energy may be considered to be wave-like as well as particle-like. For fluorescence phenomena it is often more suitable to consider the energy to be carried by photons, rather than by waves. The energy in a photon is given by the product $h \cdot f$, where h is Planck's constant (6.63×10^{-34} Js) and f is the frequency. Usually, electromagnetic energy other than light is not described in terms of photons due to the fact that even for very low intensities, the number of e.g. radio-frequency photons arriving per second is very large and receiver system noise is dominated by the thermal kT component, where k is Boltzmann's constant (1.38×10^{-23} J/K) and T is the temperature in degrees K. Since optical frequencies are some five orders of magnitude higher than microwave frequencies, each optical photon is far more energetic than its microwave counterpart. Optical receiver noise is often dominated by the quantum hf component.

Photomultiplier Tube (PMT)

A PMT consists of a light-sensitive photocathode that generates electrons when exposed to light. These electrons are directed onto a charged electrode called a dynode. The collision of the electrons with the dynode produces additional electrons. Since each electron that strikes the dynode causes several electrons to be emitted, there is a multiplication effect. After further amplification by multiple dynodes, the electrons are collected at the anode of the PMT and output as a current. The current is directly proportional to the intensity of light striking the

photocathode. The PMT is a photoelectron amplifier of high sensitivity and remarkably low noise. PMTs have a wide dynamic range and they furthermore are very fast.

One photon on the photo-cathode can produce a short output pulse containing millions of photoelectrons. PMTs can therefore be used as single photon detectors. In photon counting mode, individual photons that strike the photocathode of the PMT are registered. Each photon event gives rise to an electrical pulse at the output. The number of pulses, or counts per second, is proportional to the light impinging upon the PMT. As the number of photon events increase at higher light levels, the photon counting PMT will become non-linear. This usually occurs at 10^6 - 10^7 counts per second.

The timing uncertainty between photon arrival and electrical output is small enough to permit time-resolved photon counting at a sub-nanosecond scale. In single photon counting mode the tube is typically operated at a constant high voltage where the PMT is most sensitive. PMTs usually operate between the blue and red regions of the visible spectrum, with greater quantum efficiency in the blue-green region, depending upon photo-cathode materials. Maximum quantum efficiencies are about 25%. Because of noise from various sources in the tube, the output of the PMT may contain pulses that are not related to the light input. These are referred to as dark counts.

Pile-up

TCSPC electronics are usually designed to register only one photon per excitation/emission cycle. If the number of photons occurring in one cycle is typically >1 , the system will very often register the first photon but miss the following ones. This leads to an over-representation of early photons in the histogram called 'pileup'. In TCSPC it is therefore necessary to maintain a low probability of registering more than one photon per cycle. This is to guarantee that the histogram of photon arrivals represents the time decay one would have obtained from a single shot time-resolved analog recording.

Quantum Efficiency

The ratio of hole-electron pairs or photoelectrons to the number of photons received by a photodetector. The quantum efficiency can be as high as 80% for an APD and 25% for a PMT.

Raman Scattering

Light scattering due to vibrations in molecules or optical phonons is called Raman scattering. Raman scattered light can be shifted by as much as 4000 cm^{-1} from the incident light.

Rayleigh Scattering

Light scattering at the same wavelength as the incoming light is called Rayleigh scattering. Random local variations of the molecular positions e.g. in glass create inhomogenities that act as tiny scattering centres. The amplitude of the scattered field is wavelength dependent, blue

light is scattered more than red. The attenuation caused by Rayleigh scattering decreases with wavelength $1/\lambda^4$.

Semiconductor Lasers

Semiconductor lasers basically are light-emitting diodes within a resonator cavity that is formed either on the surfaces of the diode or externally. An electric current passing through the diode produces light emission when electrons and holes recombine at the p-n junction. Because of the geometry of the active region, the laser output is divergent and the beam intensity profile is not ideal (mostly elliptical) It usually requires special optics to produce a good beam shape. Pulsed diode lasers can be used as excitation sources for time-resolved fluorescence investigations, however, they require sophisticated electrical driver circuitry and/or modification of the laser chip to generate pulses as short as e.g. 100ps and high repetition rates.

Signal-to-Noise Ratio (SNR)

SNR = signal level/rms noise level

Spectrometers

An optical spectrometer is an optical system that transmits specific bands of electromagnetic spectrum for selective measurement. Dispersion of different wavelengths is accomplished with the separating capability of refraction (prism) or diffraction (diffraction grating). Typical applications are isolation of a narrow band of radiation from a continuum light source for absorption measurements, or analysis of the emission from excited atoms or molecules.

Spectroscopy

Spectroscopy is the use of the absorption, emission, or scattering of electromagnetic radiation by atoms or molecules (or atomic or molecular ions) to qualitatively or quantitatively assess the atoms or molecules, or to study physical processes.

Stokes Shift

When a photon is absorbed by a fluorescent compound, electrons are excited to a higher energy level for a very short period of time. When the electrons return to their normal energy levels a photon is released. Not all of the energy is recovered in the emitted photon, therefore it has a lower energy than the excitation photon and is thus a longer wavelength. This difference in energy or wavelength is called the Stokes shift.

Transient Recorders

Transient recorders (or transient digitisers) are electronic instruments for recording fast transient signals such as a fluorescence decay over time. To record fast optical signals with

electronic transient recorders a fast detector is prerequisite. Transient averagers are transient recorders that store the data in memory where data can then be summed and averaged to improve the signal-to-noise ratio. Digital (sampling) oscilloscopes practically are transient recorders. There is a limit to which the time resolution (or respectively sampling frequency) of a transient recorder can be pushed. For sampling times $<1\text{ns}$ other methods such as gated (boxcar) integration or time-correlated single photon counting may be advantageous, particularly if, additionally, the signal levels are low. Streak tubes (or streak cameras) are an alternative but expensive tool to achieve high time resolution.

Time-Correlated Single Photon Counting (TCSPC)

With periodic light signals it is possible to reconstruct the signal shape of one cycle from single photon events collected over many cycles. The method is based on the repetitive precisely timed registration of single photons of e.g. a fluorescence signal. The reference for the timing is the corresponding excitation pulse. A single photon sensitive detector with accurate timing must be used. Provided that the probability of registering more than one photon per cycle is low, the histogram of photon arrivals per time bin represents the signal one would have obtained from a single shot time-resolved analog recording.

Time to Amplitude Converter (TAC)

Electronic circuit for precise time measurement via conversion to a proportional voltage. A typical TAC is essentially a highly linear ramp generator that is started by one signal and stopped by the other. The result is a voltage proportional to the time difference between the two signals.



PicoQuant GmbH
Rudower Chaussee 29
12489 Berlin

Tel.: (030) 6392 65 60 e-mail: photonics@pq.fta-berlin.de
Fax: (030) 6392 65 61 www: <http://www.picoquant.com>