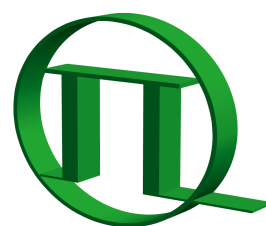


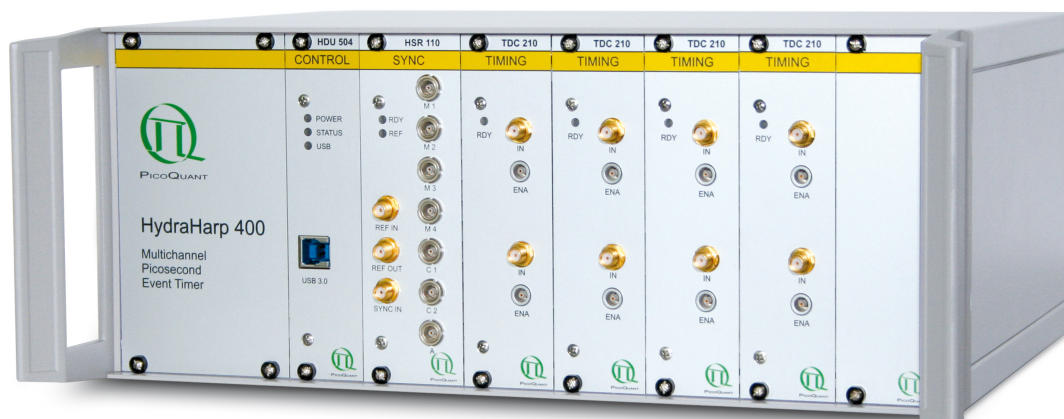
# HydraHarp 400

Picosecond Histogram  
Accumulating Real-time Processor



PICOQUANT

Time-Correlated  
Single Photon Counting System  
with USB Interface



User's Manual and Technical Data

Version 3.0.0.4

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**Acknowledgements**

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# 1. Introduction

While fluorescence spectroscopic investigations are fairly common today, extracting additional temporal information from molecules via laser induced fluorescence is a relatively new and much more powerful technique. The temporal analysis can reveal information about the molecule not available from spectral data alone. This is why lifetime analysis of laser induced fluorescence by means of Time–Correlated Single Photon Counting (TCSPC) has gained importance over the recent years. The difference in the fluorescence decay times of appropriate fluorescent dyes provides a powerful discrimination feature to distinguish molecules of interest from background or other species. This has made the technique very interesting for sensitive analysis, even down to the single molecule level.

The acquisition of fluorescence decay curves by means of TCSPC provides resolution and sensitivity that cannot be achieved with other methods. In practice it is done by histogramming arrival times of individual photons over many excitation and fluorescence cycles. The arrival times recorded in the histogram are relative times between laser excitation and corresponding fluorescence photon arrival (start / stop times) ideally resolved down to a few picoseconds. The resulting histogram represents the fluorescence decay. Although fluorescence lifetime analysis is a main field of application for the HydraHarp, it is in no way restricted to this task. Other important applications are e.g. Quantum Optics, Quantum Cryptography (QC) Time–Of–Flight (TOF) and Optical Time Domain Reflectometry (OTDR) as well as any kind of coincidence correlation.

The HydraHarp 400 is a cutting edge TCSPC system with Universal Serial Bus (USB) interface. Its modular design provides a scalable number of input channels and allows innovative measurement approaches. The timing circuits allow high measurement rates up to 12.5 Mcounts/s per channel and provide a time resolution of 1 ps. The input channels are programmable for a wide range of input signals. They all have programmable Constant Fraction Discriminators (CFD) and a programmable input offset replacing cumbersome cable delays. These specifications qualify the HydraHarp 400 for use with all common single photon detectors such as Single Photon Avalanche Photodiodes (SPAD), Photo Multiplier Tubes (PMT), Micro-Channel Photo Multiplier Tubes (MCP–PMT), Hybrid Photodetectors (HPD) and Superconducting Nanowire Single Photon Detectors (SNSPD). The time resolution is well matched to these detectors and the overall Instrument Response Function (IRF) will typically not be limited by the HydraHarp electronics. Similarly, a wide range of excitation sources can be used, e.g. the interchangeable, easy–to–use LDH diode laser series, as well as mode locked lasers such as a fs Ti:Sapphire laser. This permits lifetime measurements down to under ten picoseconds with deconvolution, e.g. using the FluoFit Fluorescence Decay Fit Software.

The HydraHarp 400 can operate in various modes to adapt to different measurement needs. The standard histogram mode performs real–time histogramming in on–board memory. Other modes are available via software re–configuration. For instance, two different Time–Tagged–Time–Resolved (TTTR) modes allow recording of each photon event on separate, independent channels, thereby providing unlimited flexibility in off–line data analysis such as burst detection and time–gated or lifetime weighted Fluorescence Correlation Spectroscopy (FCS) as well as picosecond coincidence correlation, using the individual photon arrival times. The HydraHarp 400 is furthermore supported by a variety of accessories such as pre–amplifiers and an optional hardware and software add–on allowing control of a monochromator from within the HydraHarp software. The latter supports automated measurement of Time–Resolved Excitation/Emission Spectra (TRES).

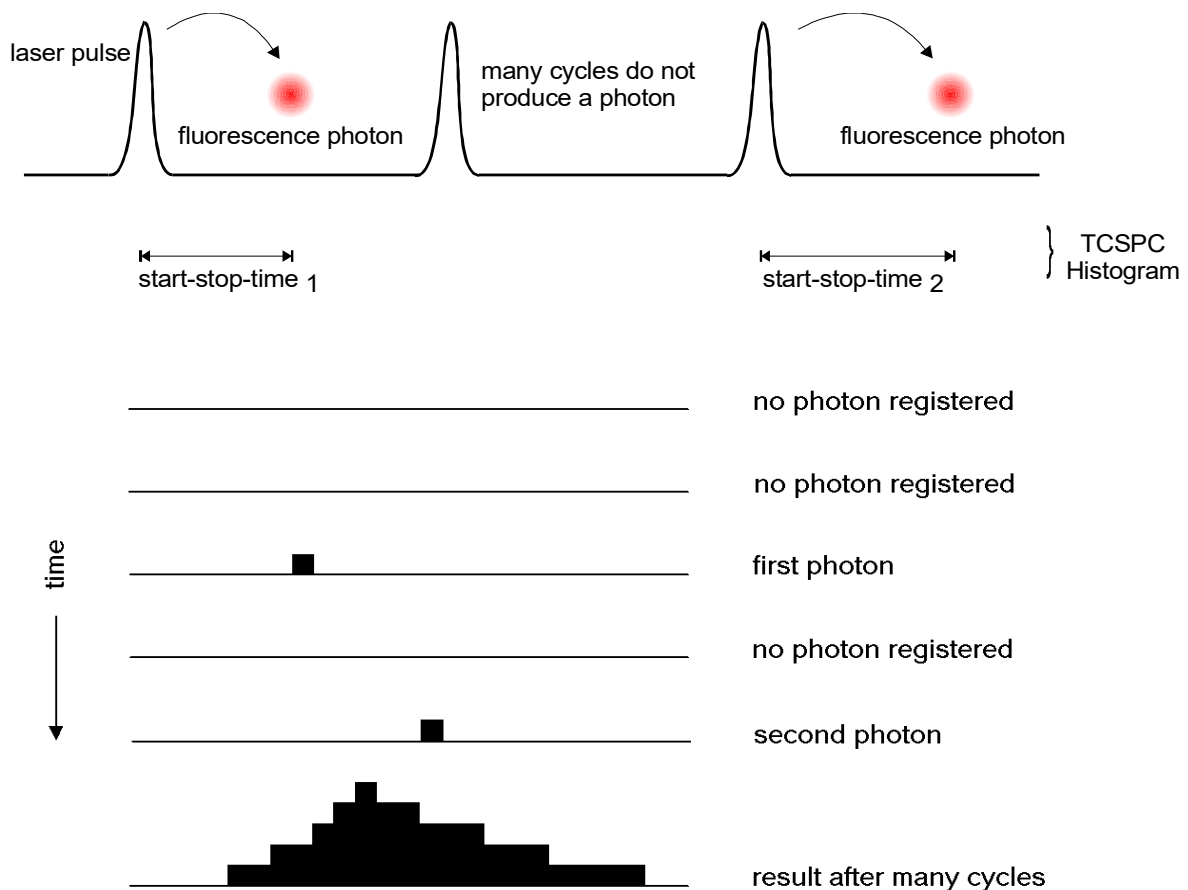
The HydraHarp software runs on all recent Windows PC platforms with Windows versions 8.1 and 10, including the x64 editions. It provides functions such as the setting of measurement parameters, display of measurement results, loading and saving of measurement parameters and decay curves. Important measurement characteristics such as count rate, count maximum and position, histogram width (FWHM) are displayed continuously. Data can conveniently be exported via the clipboard, e.g. for immediate processing by the FluoFit Fluorescence Decay Fit Software. An optional programming library (DLL) enables users to write custom data acquisition programs for the HydraHarp in all modern programming environments.

For details on the Time–Correlated Single Photon Counting method, please read the next section as well as our TechNote on TCSPC and consult the literature referenced at the end of section 2.4. Experienced users of the method should be able to work with the HydraHarp straight away. Nevertheless, we recommend carefully reading of the sections 3.2 and 3.3 on software and hardware installation to avoid damage. Later, the comprehensive online–help function of the HydraHarp software will probably let the manual gather dust on the shelf.

## 2. Primer on Time–Correlated Single Photon Counting

In order to make use of a powerful analysis tool such as time–resolved fluorescence spectroscopy, one must 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 a few hundred picoseconds to some hundred nanoseconds. In order to recover fluorescence lifetimes as short as e.g. 200 ps, 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 sample points in time. This means the transient recorder required would have to sample at e.g. 10 ps time steps. This is hard to achieve with ordinary electronic transient recorders of reasonable dynamic range. Secondly, the light available may be simply too weak to sample an analog time decay. Indeed the signal may consist of just single photons per excitation / emission. This is typically the case for single molecule experiments or work with minute sample volumes / concentrations. Then the discrete nature of the signal itself prohibits analog sampling. Even if one has more than just a single molecule and 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 excitation/emission cycles, one can reconstruct the single cycle decay profile from single photon events collected over many cycles.

The TCSPC 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 detector such as a Photo Multiplier Tube (PMT) or a Single Photon Avalanche Photodiode (SPAD) is used to capture the fluorescence photons. 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 attenuating the light level at the sample if necessary. If the single photon probability condition is met, there will actually be no photons registered in many of the excitation 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 (historically) 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 electrical 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 we will expand on the various steps involved in the method and associated issues of importance.

## 2.1. Count Rates and Single Photon Statistics

It was already 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 tens of 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 one or more. This would lead to an over-representation of early photons in the histogram, an effect called 'pile-up'. This leads to distortions of the fluorescence decay, typically the fluorescence lifetime appearing shorter. 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 mathematical 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 to 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 % to 5 % of the excitation rate. E.g. with the diode laser PDL 800-B, pulsed at 80 MHz repetition rate, the average detector count rate should not exceed 4 Mcps. This leads to another issue: the count rate the system (of both detector and electronics) can handle. Indeed 4 Mcps may already be stretching the limits of some detectors and usually are beyond the capabilities of older TCSPC systems. Nevertheless, 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). This is why high laser rates (such as 40 or 80 MHz from the PDL 800-B) are important. PMTs can safely handle TCSPC count rates of up to 10 Mcps, standard (passively quenched) SPADs saturate at a few hundred kcps, actively quenched SPADs may operate up to 5 Mcps but some types suffer resolution degradation when operated too fast. Old NIM based TCSPC electronics can handle a maximum of 50 to 500 kcps, newer integrated TCSPC boards may reach peak rates of 5 to 10 Mcps. With the HydraHarp 400, in each channel average count rates of 6 Mcps and peak rates up to 12.5 Mcps can be collected. It is worth noting that the photon arrival times are typically 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 count rates considered here and elsewhere. The specifications for TCSPC systems may interpret their maximum count rates differently in this respect. This is why another parameter, the so called dead-time is also of interest. This quantity describes the time the system cannot register photons while it is processing a previous photon event. The term is applicable both to detectors and electronics. Dead-time or insufficient throughput of the electronics do not usually have a detrimental effect on the decay histogram or, more precisely, the lifetime to be extracted from the latter, as long as single photon statistics are maintained. However, the photon losses prolong the acquisition time or deteriorate the signal to noise ratio (SNR) if the acquisition time remains fixed. In applications where the photon burst density must be evaluated (e.g. for molecule transition detection) dead-times can be a problem. The HydraHarp 400 has an extremely short dead time of typically less than 80 ns, imposing the smallest losses possible with instruments of comparable resolution today.

## 2.2. 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 that the detector introduces in the conversion from a photon to an electrical pulse. This timing error or uncertainty can be as much as ten times smaller than the detector's pulse response. The timing uncertainties are usually quantified by specifying the rms error (standard deviation) or the Full Width Half Maximum (FWHM) of the timing distribution or instrument response function (IRF<sup>1</sup>). Note that these two notations are related but not identical<sup>2</sup>. Good, but also very expensive detectors, notably Micro Channel Plate PMTs, can achieve timing uncertainties as small as 25 ps FWHM. Lower cost PMTs or SPADs may introduce uncertainties of 50 to 500 ps FWHM.

The second most critical source of IRF broadening in fluorescence lifetime measurements with TCSPC is usually the excitation source. While many laser sources can provide sufficiently short pulses, it is also necessary to obtain an electrical timing reference signal (sync) for comparison with the fluorescence photon signal. The type of sync signal available depends on the excitation source. With gain switched diode lasers (e.g. PDL 800-B) a low jitter electrical sync signal is readily available. The sync signal used here is typically a narrow negative pulse of -800 mV into 50 Ω (NIM standard). The sharp falling edge is synchronous with the laser pulse (<3 ps rms jitter for the PDL 800-B). With other lasers (e.g. Ti:Sa) 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). The light for this reference detector must be derived from the excitation laser beam e.g. by means of a 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 electrical signals used for the time measurement. At the trigger point of comparators, logic gates etc., the amplitude noise (thermal noise, interference etc.) always present in these signals is transformed to a corresponding timing error (phase noise). However, the contribution of the electronics to the total timing error is usually small. For the HydraHarp it is typically 10 ps. Finally, it is always a good idea to keep electrical noise pick-up low. This is why signal leads should be properly shielded coax cables, and strong sources of electromagnetic 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 overall IRF is the convolution of all component IRFs. An estimate of the overall IRF width, assuming independent noise sources, can be obtained from the geometric sum of the individual components as an rms figure according to statistical error propagation laws:

$$e_{system} = \sqrt{\sum e_{component}^2}$$

Due to the squares in the sum, the total will be 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 width of 200 ps FWHM, shortening the laser pulse from 50 ps to 40 ps is practically of no effect. However, it is difficult to specify a general lower limit on the fluorescence lifetime that can be measured by a given TCSPC instrument. In addition to the instrument response function and noise, other factors such as time bin width, quantum yield, fluorophore concentration, and decay kinetics will affect the measurement. However, as a limit, one can assume that under favourable conditions lifetimes down to 1/10 of the IRF width (FWHM) can still be recovered via deconvolution.

A final time-resolution related issue worth noting is the channel width of the TCSPC histogram. As outlined above, the analog electronic processing of the timing signals (detector, amplifiers, CFD etc.) creates a continuous distribution around any true time value. In order to form a histogram, at some point the timing results must be quantized. This is done by an Analog to Digital Converter (ADC) or an integrated Time-to-Digital Converter (TDC). This quantization introduces another random error, if chosen too coarse. The quantization step width (i.e. the resolution) must therefore be small compared with the continuous IRF. As a minimum sampling frequency, from the information theoretical point of view, one would assume the Nyquist frequency. That is, the signal should be sampled at least at twice the highest frequency contained in it. For practical purposes one may wish to exceed this limit where possible, but there is usually little benefit in sampling the histogram at resolutions much higher than 1/10 of the overall IRF width of the analog part of the system.

Some more insights on the complicated topic can be found in the literature [1, 7..9], listed in section 2.5.

1 This is not the same as an (electrical) impulse response function which is sometimes also abbreviated as IRF.

2 In case of a Gaussian distribution the r.m.s deviation corresponds to the standard deviation  $\sigma$  and FWHM  $\approx 2,35 \sigma$ .



## 2.3. Photon Counting Detectors

### 2.3.1. Photomultiplier Tube (PMT)

A PMT consists of a light-sensitive photo cathode that generates electrons when exposed to light. These electrons are directed onto a charged electrode called 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 photo cathode. Because of the multiplicative effect of the dynode chain, the PMT is a photo electron amplifier of high sensitivity and remarkably low noise. The high voltage driving the tube may be varied to change the sensitivity of the PMT. Current PMTs have a wide dynamic range, i.e. they can also measure relatively high levels of light. They are furthermore very fast, so that rapid successive events can be reliably monitored. 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 photo cathode 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 Mcps, dependent on the model. Similarly, in TCSPC applications, individual photon pulses may merge into one as the count rate increases. This leads to pulse pile-up and distortions of the collected histograms.

The timing uncertainty between photon arrival and electrical output (transit time spread) is usually 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 greatest quantum efficiency in the blue-green region, depending upon photo-cathode materials. Typical quantum efficiencies are about 25 %. For spectroscopy experiments in the ultraviolet / visible / near infrared region of the spectrum, a PMT 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 true photon event. Thermal emission from the cathode that undergoes the full amplification process can usually not be suppressed this way. In this case cooling of the detector is more helpful.

### 2.3.2. Micro Channel Plate PMT (MCP)

A Micro Channel Plate PMT consists of an array of glass capillaries (5–25  $\mu\text{m}$  inner diameter) that are coated on the inside with an 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. Due to the narrow and well defined electron path inside the capillaries, the transit time spread of the output pulses is much reduced compared to a normal PMT. The timing jitter of MCPs is therefore sufficiently small to perform time-resolved photon counting on a picosecond scale, usually outperforming PMTs. Good but also expensive MCPs can achieve timing uncertainties as low as 25 ps. Microchannel plates are in this respect the best match for the HydraHarp 400 but they are limited in permitted count rate and provide lower sensitivity towards the red end of the spectrum compared to suitably optimized SPADs.

### 2.3.3. Single Photon Avalanche Photo Diode (SPAD)

Avalanche Photo Diodes (APDs) are semiconductor devices, usually restricted to operation in the visible to infrared part of the spectrum. Generally, APDs may be used for ultra-low light detection (optical powers  $< 1$  pW), and can also be used 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 and small devices may achieve timing accuracies down to 50 ps, but small devices are usually hard to align and difficult to focus into. Single-photon detection probabilities of up to approximately 50 % are possible. APDs are often noisier than

PMTs, but can have a greater quantum efficiency. Maximum quantum efficiencies reported are about 70 %. The popular commercial devices from Excelitas, formerly Perkin Elmer (SPCM–AQR) provide a timing accuracy of ~400 ps and are specified to have a quantum efficiency of 60 %. These modules are thermoelectrically cooled for low dark count rate and deliver pre-shaped TTL pulses. They are the most common detectors for applications where NIR sensitivity is important, e.g. single molecule detection. To achieve the specified timing accuracy, exact focusing into the center of the active area is necessary. More recent SPAD designs such as the PDM family from Micro Photon Devices have the benefit of much better timing resolution and robustness, however, at the expense of a lower sensitivity at the red end of the spectrum.

### 2.3.4. Other and Novel Photon Detectors

The field of photon detectors is still evolving. Recent developments that are beginning to emerge as usable products include so called silicon PMTs, Hybrid PMTs, superconducting nanowire detectors and APDs with sufficient gain for single photon detection in analog mode. Each of these detectors have their specific benefits and shortcomings. Only a very brief overview can be given here.

Silicon PMTs are essentially arrays of SPADs, all coupled to a common output. This has the benefit of creating a large area detector that can even resolve photon numbers. The drawback is increased dark count rate and relatively high afterpulsing.

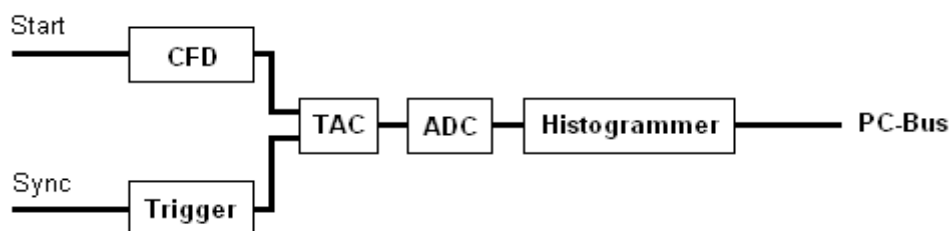
Hybrid Photodetectors (HPDs) make use of a combination of a PMT front end followed by an APD structure. The benefits are good timing and virtually zero afterpulsing while the need for very high voltage is a disadvantage. The HPD modules of PicoQuant's PMA Hybrid series alleviate this problem by encapsulating the high voltage supply, the detector, a Peltier cooler and even a protective shutter in a compact housing.

Superconducting nanowire single photon detectors (SNSPDs or SSPDs) routinely achieve excellent timing performance (<30 ps jitter) and high sensitivity from the visible to the near infrared with overall system detection efficiencies in excess of 90%. They operate at cryogenic temperatures, typically between 0.8 K and 4 K, which increases cost, footprint and power consumption and makes them less practical for applications where these parameters are of concern.

Another class of potentially interesting detectors are recently emerging APDs with very high gain. In combination with an electronic amplifier they have been shown to detect single photons. As opposed to Geiger mode, this avoids afterpulsing and allows very fast counting rates. The disadvantage is a high dark count rate, currently way too high for any practical TCSPC application.

## 2.4. Principles Behind the TCSPC Electronics

For introductory purposes it is worth looking at the design of conventional TCSPC systems first. They 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 of the random background signal can be suppressed. The same could not be achieved with a simple threshold detector (comparator). Especially with PMTs, constant fraction discrimination is very important, because their pulse amplitudes vary significantly. Particularly pulses originating from random electrons generated at the dynodes of the PMT can be suppressed because their avalanches had less time to amplify, and their corresponding output pulses are small.

The principle of a classic CFD is the comparison of the original detector signal with an amplified, inverted 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. In practice the comparison is done by a summation. The timing is done by a subsequent threshold trigger of the sum signal using a settable level, the so

called zero cross trigger. Newer CFD designs achieve the same objective by differentiating the input signal and triggering on the zero crossing of the differentiated signal. This is the case for the HydraHarp 400. This has the benefit of adaption to different detector types without a need for changing physical delay lines.

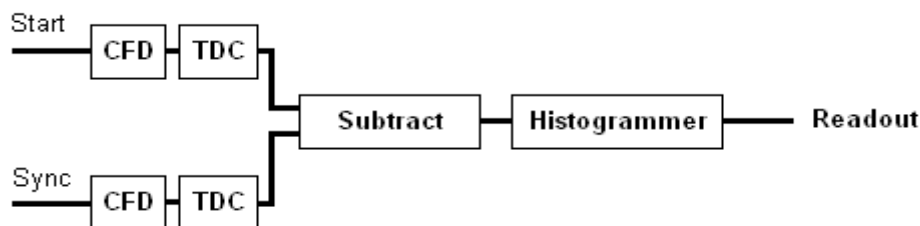
Making the zero cross level adjustable (slightly above zero) allows to adapt to the noise levels in the given signal, since otherwise an infinitely small signal could trigger the zero cross comparator. Typical CFDs furthermore permit setting of a discriminator threshold that determines the lower limit the detector pulse amplitude must pass. This is primarily used to suppress dynode noise from PMTs.

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 often sufficient to adapt to different sync sources. Nevertheless, it can be valuable to have a CFD also on the sync channel. A suitably designed CFD is also usable with pre-shaped signals and simply becomes a level trigger in this case. This is the case also for the HydraHarp 400. Note that the input signals must have sufficiently fast rise / fall times in the sub-nanosecond to 10 nanosecond range.

The signals from the two input discriminators / triggers are in conventional systems fed to a 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. In conventional systems 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. 12 bits) 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 must also be available for data readout, the histogrammer must occasionally 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 worth noting that the design of the HydraHarp 400 is somewhat different. Today, it is state-of-the-art that the tasks conventionally performed by TAC and ADC are carried out by a so called Time to Digital Converter (TDC). These circuits allow not only picosecond timing but can also extend the measurable time span to virtually any length by means of digital counters. The HydraHarp 400 does not use one such circuit but one for each input channel and one for the SYNC input. They independently work on each input signal and provide picosecond arrival times that then can be processed further, with a lot more options than in conventional TCSPC systems. In the case of classical TCSPC, this processing consists of a subtraction of the two time figures and histogramming of the differences. This is identical to the classical start-stop measurements of the conventional TAC approach. The following figure exemplifies this for one detector channel (Start).



The full strength of the HydraHarp design is exploited by collecting the unprocessed independent arrival times as a continuous data stream for more advanced analysis. Details on such advanced analysis can be found in the literature. In this case the on-board memory is reconfigured as a large data buffer (FIFO) so that count rate bursts and irregular data transfer are decoupled. This permits uninterrupted continuous data collection with high throughput. This mode of operation is called Time-Tagged Time-Resolved (TTTR) mode. Details are described in section 5.3.

## Forward and Reverse Start–Stop Mode

For simplicity it is most convenient to assume that the time delay measurement is directly causal, i.e. the laser pulse causes a photon event and one measures the time delay between laser pulse and the subsequent photon event. However, most conventional TCSPC systems need to give up this logical concept because of the high repetition rates of the typical excitation lasers: Since the time measurement circuit 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 conventionally conversion times are in the region of 0.3 to 2  $\mu\text{s}$ , any excitation rate in excess of 0.5 to 3 MHz 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. By 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 to 5 % of the excitation rate and can therefore be handled easily by the TAC / ADC. The consequence of this approach, however, is that the times measured are not those between laser pulse and corresponding photon event, but those between photon event and the next laser pulse. This still works (by software data reversing) but is inconvenient in two ways:

- 1) Having to reverse the data leads to unpleasant relocation of the data displayed on screen when the time resolution is changed.
- 2) Changing between slow and fast excitation sources requires reconnecting to different inputs, possibly causing trouble when there is no CFD at one of the inputs.

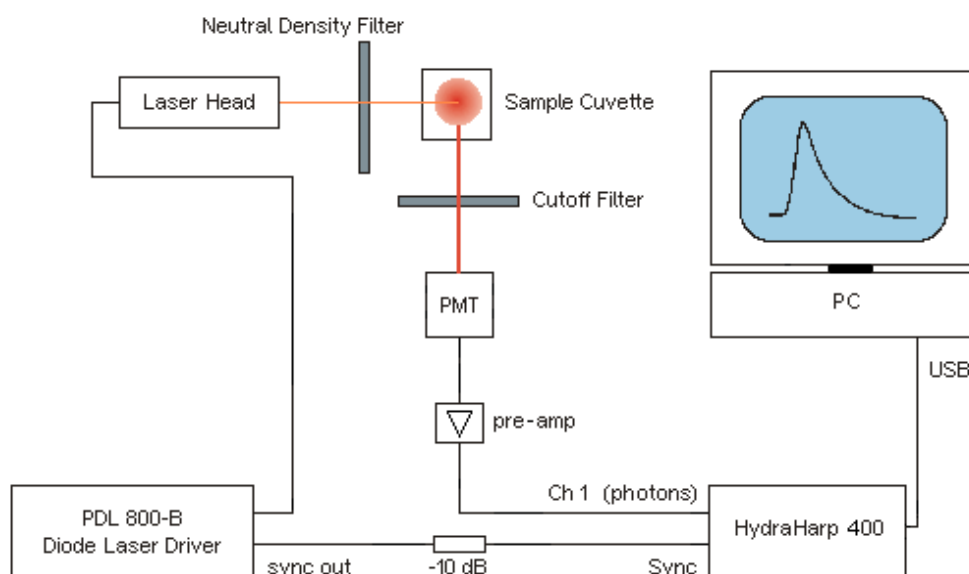
The HydraHarp 400 is revolutionary in this respect, as it allows to work in forward start stop mode, even with fast lasers. This is facilitated by two design features:

- 1) The independent operation of the time digitizers, and
- 2) a programmable divider in front of the sync input.

The latter allows to reduce the input rate so that the period is at least as long as the dead time. Internal logic determines the sync period and re-calculates the sync signals that were divided out. It must be noted that this only works with stable sync sources that provide a constant pulse-to-pulse period, but all fast laser sources known today meet this requirement within an error band of a few picoseconds. Note: for slow sync sources (< 1 MHz) the sync divider must not be used (set to None). Similarly, the divider must not be used for coincidence correlation measurements etc. since the sync rate has no influence on this kind of measurements. In summary: The HydraHarp 400 is designed to always work in forward start–stop mode.

## Experimental Setup for Fluorescence Decay Measurements with TCSPC

The figure below shows a typical setup for fluorescence lifetime measurements using one input channel of the HydraHarp 400.

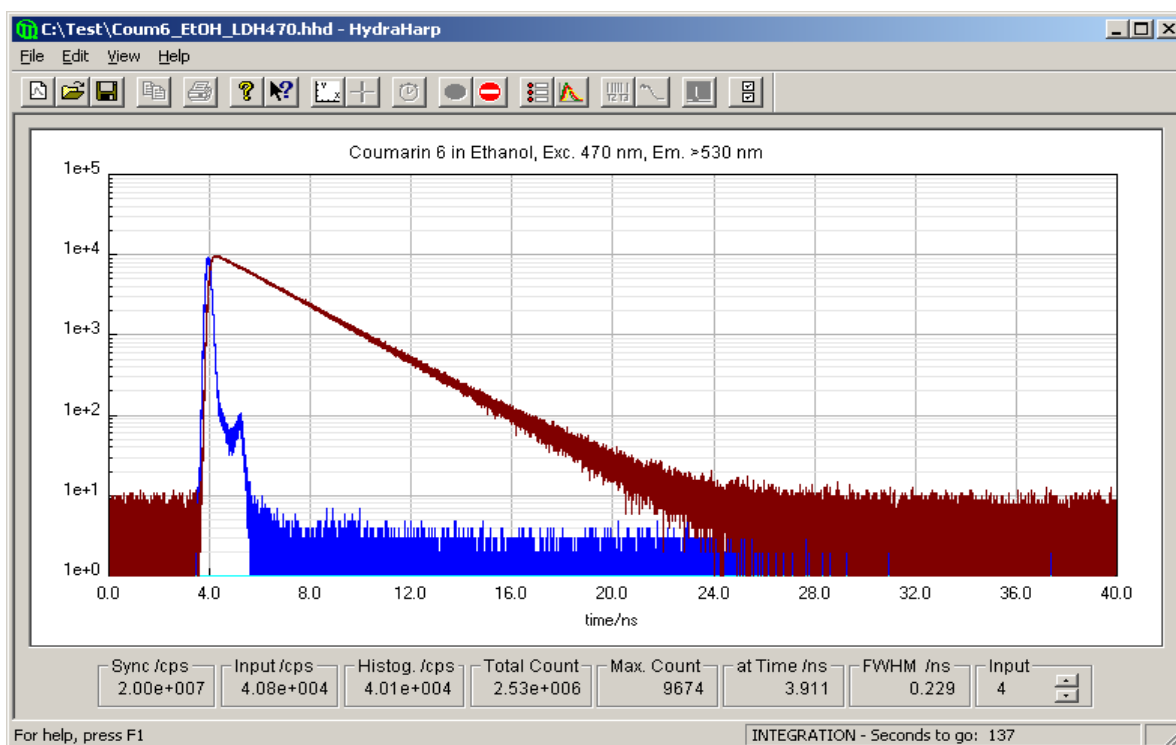


The picosecond diode laser (PDL 800–B) is triggered by its internal oscillator (settable at 2.5, 5, 10, 20 and 40 MHz). The driver unit is physically separate from the actual laser head, which is attached via a flexible cable. This permits to place the small laser head conveniently anywhere in the optical setup. The light pulses of typically  $<70$  ps FWHM, are directed toward the sample cuvette via 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 from scattered excitation light by means of an optical cut-off filter (other configurations may utilize a monochromator here). Then it is directed to the photon detector, again possibly via some appropriate collection optics, e.g. a microscope objective or a lens.

As a photon detector the H5783 PMT from Hamamatsu is very convenient. It only needs a 12 V supply and permits an instrument response width of  $<250$  ps, allowing lifetime measurements even much shorter than this via deconvolution. If a higher time resolution is required, the detector of choice is an MCP–PMT. The electrical signal obtained from the detector (small negative pulses of typically  $-10$  to  $-50$  mV) is fed to the TCSPC electronics via a preamplifier (e.g. PAM 105 from PicoQuant). This gives pulses of  $-100$  to  $-500$  mV. Cabling is double shielded  $50 \Omega$  coax cable. If the detector is a SPAD module with TTL output (SPCM–AQR from Excelitas, formerly Perkin Elmer) then an inverter / attenuator (SIA 400) must be inserted. Modern SPAD devices like the PDM series from MPD directly provide negative timing signals.

The PDL 800–B laser driver readily provides the electric sync signal needed for the photon arrival time measurement. This signal (a narrow negative pulse) is also fed to the TCSPC electronics via a high quality  $50 \Omega$  coax cable. When using the HydraHarp 400 in combination with the PDL 800–B, the sync pulse adapter LTT 100 (brass cube with SMA connectors) or a 10 dB attenuator should be inserted directly at the sync output of the laser driver. This reduces crosstalk into the relatively weak detector signals. If the laser does not provide an electrical sync signal (e.g. Ti:Sa lasers), a sync detector (photo diode) such as the TDA 200 must be used.

The following figure shows TCSPC histograms obtained with this setup. Excitation source was a PDL 800–B with a 470 nm laser head running at 20 MHz repetition rate. The narrower (blue) curve represents the system IRF, here dominated by the detector (PMT). The other curve (dark red) is the fluorescence decay from a solution of Coumarin 6 in ethanol, a fluorescent dye with fairly short fluorescence lifetime ( $\sim 2.5$  ns). The count rate was adjusted to  $<1\%$  of the laser rate to prevent pile-up. The plot in logarithmic scale shows the perfect exponential nature of the decay curve, as one would expect it. Note that this is obtained even without a deconvolution of the IRF.



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  $1 : 1/e$  (e.g. 100 000 : 36 788). In this particular experiment this resulted in a lifetime estimate of 2.55 ns in good agreement with published results for this dye. For a precise measurement one would perform a numerical exponential fit with IRF deconvolution (typically implemented as an iterative reconvolution). This would result in slightly shorter lifetimes since the IRF broadens the decay. Indeed one can measure lifetimes significantly smaller than the IRF with this method. Additionally, the rms 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.

## 2.5. Further Reading

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Bibliography listing all publications with work based on the HydraHarp 400

## 3. Hardware and Software Installation

### 3.1. General Installation Notes – Read This First

When handling the HydraHarp 400 system, be sure to avoid electrostatic discharges, especially when connecting cables. Before connecting any signals, carefully study the maximum ratings given in section 8.3.1.

HydraHarp software version 3.0.0.4 is supported under Windows 8.1, 10 and 11<sup>1</sup>. Windows 7 probably still works but is no longer actively tested and supported. In order to use the HydraHarp software with the HydraHarp 400 hardware, a device driver must be installed. The HydraHarp 400 is a USB ‘plug and play’ device, this means the necessary resources and drivers are allocated automatically by the operating system. Windows will automatically recognize when such a device is connected and load the appropriate driver. The software setup for the HydraHarp 400 installs the driver so that upon connecting the device it is readily available for the Windows plug&play device management.

On some Windows versions you may need administrator status to perform the software setup and de-installation. For the driver installation, it is needed in any case. Installing as Administrator has the benefit that you can install the software for use by all users on that computer even if they have limited access rights. The HydraHarp software will maintain individual settings for each user in the Windows registry. When switching between users with sessions still running in the background you cannot run the HydraHarp software in multiple sessions at the same time.

The HydraHarp software 3.0.0.4 is also suitable for 64-bit versions of Windows, where a suitable 64-bit device driver will be installed. The HydraHarp software as such runs as a 32-bit application on WOW64.

#### Important Note for Software Version 2.0 and higher

Software versions 2.0 and higher require a firmware update of the HydraHarp device if it was purchased before August 2012 and was not upgraded to new firmware since then. The HydraHarp software can perform this update when it is first started.

The firmware update requirement has consequences that you must observe:

1. Once the update is performed you will no longer be able to use any HydraHarp software prior to version 2.0.
2. Custom software you may have written for file import of 1.x versions will require minor adaptations.
3. You will no longer be able to use custom software based on HHLib.dll prior to version 2.0.
4. Custom HHLib-based software you may have written for 1.x versions will require minor adaptations.
5. After the update you may need small CFD adjustments for any known good setup.
6. In case of a power failure or computer crash during the update the device may become inoperational.
7. Reverting to old firmware or repairing a disrupted update requires a return to factory and may incur costs.

Also note: When the firmware update has been performed the device must be switched off and on again in order to become operational with the new software.

#### Important Note for Version 3.0 and higher

Software version 3.0 and higher uses a new file format with the file name extensions \*.ptu for TTTR mode files or \*.phu for histogram data files. The idea of this new format is to place individual header data items not in a strict file position and order but to “tag” the items by name, so that future additions do not harm existing software. The new file format will therefore be “future proof” in terms of maintenance and compatibility across new versions. The downside is that users who wrote their own data import routines for older formats will need to adapt them to the new format one more time. This should be quite easy by means of demo code in various programming languages provided as part of the software installation. For backward compatibility version 3.0 can still read (but not write) the previous format version 2.0.

## 3.2. Software Installation

Before installing the new software you should (if applicable) back-up all HydraHarp data files you created in any older HydraHarp directory (previous versions etc.) as well as any critical data on your PC. For a clean start we also recommend to uninstall any older HydraHarp software.

The HydraHarp 400 setup files are normally provided on a CD supplied with your HydraHarp device. The installer program file containing the complete distribution is `setup.exe`. If you received the software package by download or any other means of electronic distribution, it will probably be packed in a ZIP-File. In this case you can unzip this file to a temporary hard disk location of your choice and run the setup from there.

In order to use the HydraHarp software with the HydraHarp 400 hardware, a device driver must be installed. The HydraHarp 400 is a USB 'plug and play' device, this means the necessary resources and drivers are allocated automatically by the operating system. Windows will automatically recognize when such a device is connected and load the appropriate driver. Note that the software setup for the HydraHarp 400 installs the driver so that upon connecting the device it is readily available for the Windows plug&play device management. It is recommended to perform the software setup before connecting the device. Dependent on your version of Windows you may be prompted to confirm the final driver installation upon connecting the device.

On some Windows versions you will need administrator status to perform the software setup. For the driver installation you need it in any case. You can run the setup program directly from the CD or from the hard disk location where you unpacked the electronic distribution.

To perform the installation, insert the installation disk in your CD drive. Open the CD location either from the Windows desktop or via the Windows Explorer. If you unpacked the setup file to a hard disk location, open that location. Run `setup.exe`. The setup program will guide you through the installation process step by step.



When asked for a destination folder for the new software, please accept the default path or select another according to your program storage policies. This is where the HydraHarp files will be installed. To avoid confusion, make sure not to specify the path of an older HydraHarp version that you have not uninstalled or that of any other program on your PC.

The default location is: `\Program Files\PicoQuant\HydraHarp400v30`.

Setup will also create a dedicated "program folder" for the new HydraHarp 400 software that will later appear in the Start Menu. You can accept the default folder name or select another according to your own naming policies. However, you should make sure not to specify the folder name of an older HydraHarp version that you have not uninstalled nor the dedicated folder of any other program.

The setup program will install the following files in the chosen destination folder:

<code>HydraHarp.exe</code>	the main executable program
<code>HydraHarp.chm</code>	the HydraHarp compiled HTML-Help file
<code>Manual.pdf</code>	the manual in Adobe PDF format
<code>License.txt</code>	a text file with license information
<code>Readme.txt</code>	a text file with program information
<code>pquwstub.dll</code>	a helper DLL for operation under Linux with Wine
<code>unins000.exe</code>	the uninstaller program file
<code>unins000.dat</code>	the uninstaller information file

Setup will also create a subdirectory `\filedemo` which contains demo source code for access to HydraHarp data files in various programming languages. Another folder `\sampledata` will be created with samples of HydraHarp data files. Other necessary files such as setup information and the device driver will be installed in the standard places in your Windows directory tree.

Setup will also install a *File Info* shell extension that you can use to inspect individual header items of a `*.ptu` or `*.phu` file. This includes the measurement mode. Just right-click on the file in Windows explorer and select *Properties*. Then look at the tab *PQ File Info* and the tab *PQ File Comment*.

After the installation the HydraHarp software should be available in the Windows Start Menu under *Programs | PicoQuant – HydraHarp 400 v3.0* (or the folder name you chose during setup). However, before running the software, please proceed to the hardware installation step.



### 3.3. Device Installation

Make sure to avoid electrostatic discharge when handling the HydraHarp system, especially when connecting cables. Note that PMT detectors operate with high voltage that may discharge through the signal cable. Make sure such detectors are switched off and fully discharged before connecting them.

Dependent on the model of the HydraHarp device you purchased it has a USB 2.0 or USB 3.0 interface. Consult your PC manual as to whether and where it provides high speed USB 2.0 or super speed USB 3.0 connectors. The latter are typically blue. The minimum requirement is a USB 2.0 connection. The HydraHarp system will NOT work through USB 1.x. All current PCs should provide at least USB 2.0 connectivity. Most recent PCs will provide USB 3.x out of the box. If you purchased a HydraHarp with USB 3.0 interface but the PC has no USB 3.x support you can install a USB 3.x adaptor card. It is also possible to run a USB 3.0 HydraHarp device via USB 2.0 but performance will be poor. Use a proper USB 3.0 connection whenever possible.

Always use a quality USB cable rated for the chosen USB speed. The cable length must not exceed 5 metres (~16 ft). For best reliability we recommended to use the provided cable of 3 metres length. Note that the USB specification does not allow cable extensions other than dedicated active extension cables or hubs. The HydraHarp device should work flawlessly through suitable hubs. This is also a valid way of extending the maximum cable length. After a hub, another cable of up to 5 metres is allowed. Note, however, that hubs may lower the data throughput. For the same reasons it is recommended not to connect other bandwidth demanding devices to the same hub.

The inputs for the detector / sync signals are SMA connectors located on the front panel of the HydraHarp 400, labelled IN and SYNC IN. In case of time resolved fluorescence experiments with a pulsed excitation source, the sync signal must be connected to the SYNC IN input and the detector signal must be connected to an IN port. If coincidence correlation experiments between two (or more) detector signals are to be carried out, you need to decide whether you will be using on-board histogramming or TTTR mode. In the case of on-board histogramming connect one detector to the SYNC input and one or more to the regular IN ports. Coincidence timing and histogramming will always be with respect to the SYNC input. In TTTR mode it is possible to determine the relative timing between all inputs but this requires off-line data analysis (see section 5.3).

All inputs are terminated with 50  $\Omega$  internally. Note that they need negative going input signals. Use quality 50  $\Omega$  coax leads with appropriate connectors. For interfacing to BNC connectors use standard adaptors. Carefully screw on the SMA connectors for sync and detector until they are moderately tight. Do not use wrenches. Connect the cable ends to the appropriate signal sources (50  $\Omega$ ) in your experimental setup. The HydraHarp 400 inputs accept negative pulses going from 0 V to max. -1 V. Both inputs should be operated with similar pulse amplitudes to minimize crosstalk. The optimum range is -100 mV to -500 mV. Below this range you may pick up noise, above there may be crosstalk. Most PMT and MCP detectors will require a pre-amplifier to reach enough signal level. TTL-SPAD-detectors (e.g. Excelitas SPCM-AQR) must be connected through a pulse inverter (PicoQuant SIA 400). Weak PMT detectors should be connected through a 20 dB high speed pre-amplifier. MCP-PMT detectors should be connected through an amplifier with slightly higher gain. Suitable devices are available from PicoQuant. Be sure to switch the high voltage supply of PMTs off and allow their electrodes to discharge before connecting them. Their high voltage charge may damage the pre-amplifier. Observe the allowed maximum ratings for the input signal levels. If you are not sure what signals your setup delivers, use a fast oscilloscope to check the signal level and shape before connecting them to the HydraHarp. All signals should have rise/fall times of no more than 2 ns. Slower signals may not be seen by the HydraHarp and will certainly degrade timing accuracy.

When using the HydraHarp 400 in combination with the PDL 800-B, the sync pulses from the laser driver should be attenuated by 10 dB to 15 dB to fall in the optimum range for smallest crosstalk.

Do not connect anything other than dedicated hardware to the other HydraHarp 400 connectors. They are provided for hardware expansion (notably experiment control) and must not be used otherwise. See section 8.3.2 for pin assignments. It is recommended to start instrument setup without anything connected to the control ports.

After connecting the HydraHarp to the PC via USB for the first time, Windows should detect the device and perform the final driver installation. Note that the software setup for the HydraHarp 400 pre-installs the driver. Dependent on your version of Windows you may be prompted to confirm the final driver installation. Some older windows versions verbosely warn about drivers not being validated by Microsoft. You can safely ignore these warnings. After driver installation Windows should report the device as ready to use. If you wish to verify correct installation you can check the presence of 'HydraHarp 400' as a USB device in the Windows Device Manager. For troubleshooting see the next section.

### 3.4. Installation Troubleshooting

After completion of the software setup and device installation the HydraHarp device should be listed in the Windows Device Manager under the USB tree. Right click *My Computer* > left click *Properties* > *Device Manager* to check if the device is free of conflicts and / or if the device driver is installed correctly. Under *USB* look for a device named *HydraHarp 400* and inspect its *Properties*.

You can also use the Windows system information facilities (*Start* > *Run* > *msinfo32*). In the System Information utility inspect *Software environment* > *System Drivers* to check if the HydraHarp device driver PQUSB.SYS (PQUSB64.SYS on x64) is correctly installed and running.

You can also repeat the software installation if necessary. Disconnect the device, uninstall the software and repeat the setup procedure. Make sure the software is not installed in multiple places. If this does not resolve the problem, try a different computer. If problems persist, see section 7 for support.

### 3.5. Uninstalling the Software

Before uninstalling the HydraHarp software you should back-up all valuable data files you might have created in the HydraHarp installation directory.

Do not manually delete any program files from the installation folder as it will render a clean uninstall impossible. Also do not delete any driver files manually.

To uninstall the HydraHarp software from your PC you may need administrator rights (dep. on Windows version and security settings). Under Windows 10/11 you navigate to *Apps and Features*, under Windows 8 you go to *Control Panel* > *Programs*. Then select *PicoQuant – HydraHarp 400 vx.x* for un-installation. This will remove all files that were installed by the HydraHarp setup program but not the user data that may have been stored. If there was user data in the program folders or any subfolders, these will not be deleted by the uninstall program. If intended, you need to delete these files or folders manually. Nevertheless, it is recommended to back-up valuable measurement data before uninstalling the software.

Note that un-installation of the data acquisition software does not uninstall the device driver. In HydraHarp software version 2.1 the driver package appeared as a separate software installation that can be uninstalled like any other software through the standard Windows Control Panel mechanisms (*Add/Remove Software* or *Apps and Features*). Look for items called *Windows Driver Package - PicoQuant (PQUSB)*. If the driver package does not appear there you can delete the driver software from within Device Manager. Note, however, that the driver may still be needed by other PicoQuant devices. Uninstall it only if you are sure it is no longer required.

Note also that un-installation of the data acquisition software does not automatically uninstall the PQ File Info shell extension. It can be un-installed as a separate item through the standard Windows Control Panel mechanisms.

## 4. Software Overview

### 4.1. Starting the Program

After correct installation the Start Menu *Programs* contains a shortcut to the HydraHarp software. To start the HydraHarp software select *PicoQuant – HydraHarp 400 v3.0.0.4*. Note that after switching the HydraHarp on, you need to allow a warm-up period of at least 20 minutes before using the instrument for serious measurements. You can use this time for set-up and preliminary measurements.

If the HydraHarp software cannot find the device (or if there are driver problems) it will display a notification message, but it can still start. Device dependent toolbar buttons and functions of the program will then remain disabled. This allows you to use the software without the HydraHarp hardware, e.g. to view or print files on another computer.

If the HydraHarp 400 is correctly installed and there are still device related errors, you can use the Windows Device Manager for troubleshooting (see the corresponding section above). If problems cannot be resolved, see section 7 for support. If possible, try the device on another computer.

For regular use of the HydraHarp you may want to create an icon for it on your Windows desktop. If you missed this during software setup you can do it any time later via a right mouse click on the start menu entry (*send to > Desktop – create shortcut*). Alternatively use the Windows Explorer to locate the file `HydraHarp.exe` in the directory you selected for installation and drag the icon onto the desktop. This will create a link to the HydraHarp executable file.

You can also start up the HydraHarp software directly from a HydraHarp histogram file by double-clicking on the file or dragging it onto the HydraHarp icon.

### 4.2. The Main Window

The HydraHarp software provides a measurement control interface to the HydraHarp hardware and an online histogram display. The HydraHarp main window accommodates the histogram display area.

Above the display area is the toolbar. Here you can access frequently used commands by simple mouse click. Above the toolbar is the menu bar for access to additional commands. At the bottom of the histogram display area is a set of 'panel meters' showing count rates, count sums, and histogram peak characteristics. These will be updated continuously, some only when a measurement is in progress. Note the selector at the right of the panel meters. This selects the channel the meters are displaying. Instead of a single channel you can also select Sum, which then displays the summed rates from all channels. The panel meters can be enlarged by double-clicking, which is useful for optical alignment etc. when the PC is at some distance.

In the top center of the display area a title line is shown. This can be double-clicked to edit the title. When editing the title, note that only the first line will appear in the display. The remaining lines are meant to be used as a file comment. All lines will be stored with the file data, but the maximum total is 256 characters.

The main window is resizeable and the actual histogram display will adapt its size accordingly. If you make the window smaller than the minimum histogram display, two scrollbars will permit access to hidden window areas. Note that the actual size of the main window depends on the system font selected. It will scale to the size of the current system font. Screen resolutions under 800x600 are not suitable for serious work with the software.

Note that the position and size of the main window on the screen will be stored in the Windows registry and retrieved during the next program start. The registry settings are kept separately for each user, provided he / she is logged on with a unique user name. Consult the section 6.1 for further main window command descriptions. Toolbar, Menu, panel meters etc. will be explained in the next sections.

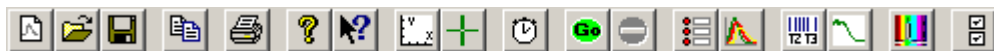
At the very bottom of the main window there is a status bar. The leftmost area of the status bar describes actions of menu items as you navigate through menus. Similarly it shows messages that describe the actions of toolbar buttons. The second status bar area from the left shows the current measurement status of the HydraHarp. The rightmost area of the status bar indicates if the `<Caps>` and `<NumLock>` keys are latched down.

When the HydraHarp software is running with functional hardware it continuously collects information about the input signals and the current acquisition settings. If these settings together with the input rates indicate possible errors, the software will activate a warning icon in the status bar. The warning icon can be clicked to display a list of current warnings together with a brief explanation of each warning (see also section 8.1).





















## 4.3. The Toolbar

The toolbar is displayed across the top of the HydraHarp main window, below the menu bar. The toolbar provides quick mouse access to frequently used commands and tools. Note that some buttons may be grayed out (disabled) dependent on the installed software components and / or the state of the hardware.



To hide or display the Toolbar, choose Toolbar from the View menu (<Alt>+V T). The toolbar is capable of 'docking', i.e. you can move it to another edge of your window or even have it displayed as a separate window. The following table explains the individual buttons.

Click...	to...
	open a blank histogram with default control panel settings
	open an existing histogram file. Displays the <i>Open</i> dialog box, in which you can locate and open the desired file.
	save the current histogram data with its current name. If you have not named the file, the <i>Save As...</i> dialog box is displayed.
	copy the currently displayed curves to the clipboard (ASCII export).
	print the currently displayed histogram curves.
	display the <i>About...</i> window. This is where you can determine the version of your HydraHarp software and hardware. Also provides links for updates etc.
	activate context help.
	launch the axis panel.
	launch the data cursor dialog.
	calibrate the time measurement hardware
	start measurement based on current HydraHarp control panel settings
	stop measurement and histogram accumulation.
	launch the HydraHarp control panel.
	launch the trace mapping dialog.
	launch the TTTR mode dialog.
	Launch the TTTR mode real-time correlator dialog.
	launch the dialog for monochromator control and TRES setup.
	launch the general settings dialog.

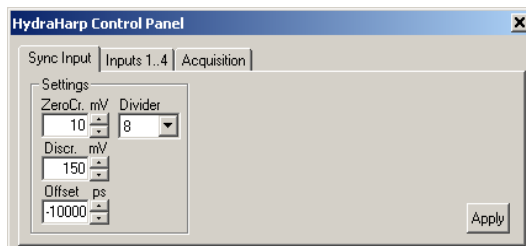
## 4.4. The Control Panel

The HydraHarp control panel is a dialog box for setting the parameters for hardware adjustment and data acquisition. It is implemented as a 'non-modal' dialog box, i.e. it does not have to be closed before the main window can continue. This way you can make changes to your settings in the control panel and watch their effect on a running measurement in the main window immediately. Nevertheless, you may close the control panel and restore it at any time by clicking the control panel button on the toolbar or pressing <Alt>+C.

The control panel consists of several pages (tabbed sheets) containing groups of edit boxes and other controls for related parameters. These pages and their respective controls are:

### Sync-Input

Zero Cross	edit box and spin control
Discriminator Level	edit box and spin control
Offset	edit box and spin control
Sync Divider	edit box and spin control

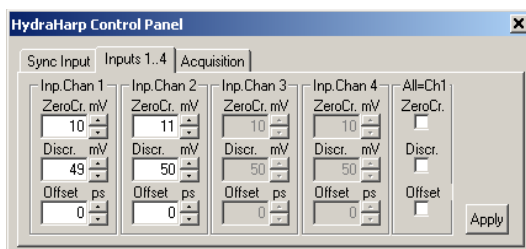


### Inputs 1...4

Zero Cross	edit box and spin control
Discriminator Level	edit box and spin control
Offset	edit box and spin control

All=Ch1, ZeroCr.	tick box
All=Ch1, Discr.	tick box
All=Ch1, Offset	tick box

Controls for channels that do not exist are gray.

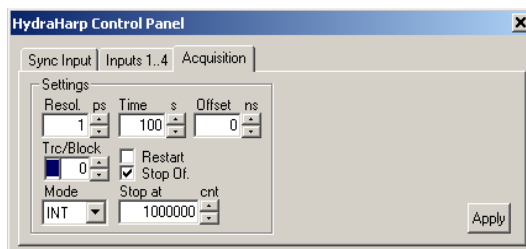


### Inputs 5...8

This page only exists when more than 4 input channels are installed. It has the same controls as the page for inputs 1..4 and follows the same logic of operation.

### Acquisition

Resolution	edit box and spin control
Time	edit box and spin control
Offset	edit box and spin control
Trc/Block	trace colour indicator, edit box and spin control
Restart	check box
Stop on Overflow	check box
Mode	drop down selection box
Stop At	edit box and spin control



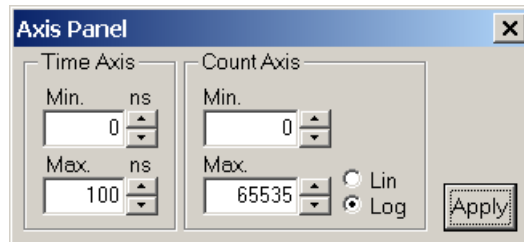
Edit boxes are for keyboard entry. The values must be confirmed with either the <Enter> key or the *Apply* button. The spin controls can be used to increment or decrement the edit box values. In this case the changes take effect immediately without need for <Enter> or *Apply*. Check boxes have their denoted effect when the tick is shown. They can be toggled with a mouse click. Groups of radio buttons are like check boxes but mutually exclusive.

Note that the settings of the control panel as well as the positions of control panel and main window on the screen will be stored in the Windows registry and retrieved during the next program start. The registry settings are kept separately per user. When a HydraHarp data file is loaded, the control panel settings will change according to the settings in that file.

The individual control panel items are discussed in the section 6.4 Control Panel in the Controls and Commands Reference.

## 4.5. The Axis Panel

The axis settings panel is a dialog box for setting the axis range for the histogram display in the main window. It is implemented as a 'non-modal' dialog box, i.e. it does not have to be closed before the main window can continue. This way you can make changes in the axis panel and watch their effect in the main window immediately. Nevertheless, you may close the axis panel and restore it at any time by clicking the axis panel button on the toolbar or pressing <Alt>+A. The panel will also open if you double-click the axes in the main window.



The axis panel consists of two groups containing edit boxes and other controls for related parameters. These groups and their respective controls are:

### Time Axis

<i>Minimum</i>	edit box and spin control
<i>Maximum</i>	edit box and spin control

### Count Axis

<i>Minimum</i>	edit box and spin control
<i>Maximum</i>	edit box and spin control
<i>Linear</i>	radio button
<i>Logarithmic</i>	radio button

The edit boxes are for keyboard entry. The values must be confirmed with either the <Enter>-key or the *Apply*-button. The spin controls can be used to increment or decrement the edit box values. In this case the changes take effect immediately without need for <Enter> or *Apply*. Check boxes have their denoted effect when the tick is shown. They can be toggled with a mouse click. Groups of radio buttons are like check boxes but mutually exclusive. They work like on an old radio: one that was pressed pops out when another one is pressed.

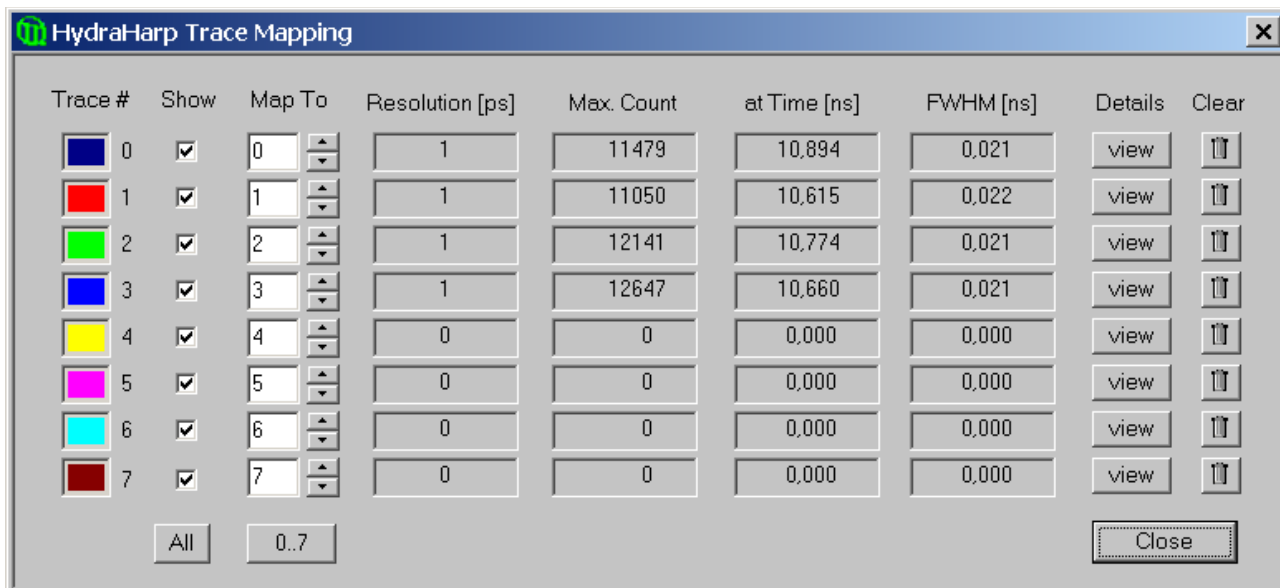
Note that the settings of the axis panel as well as the positions of the panel on the screen will be stored in the Windows registry and retrieved during the next program start. The registry settings are kept separately per user.

The individual axis panel items are discussed in the section 6.5. "Axis Panel" in the Controls and Commands Reference.

To begin, use logarithmic display to make sure even weak signals can be seen.

## 4.6. The Trace Mapping Dialog

The HydraHarp software can measure histograms in up to 512 memory blocks. Out of these up to 8 curves can be displayed. The *Trace Mapping* dialog is used to select the curves to display. It also allows to view curve details and cleanup. You can use the *Trace Map* button on the Toolbar or click the curve colour indicator in the control panel to launch the dialog.



In the *Trace Mapping* dialog you can tick the individual boxes 'Show' to display a curve. Select the number of the memory block you wish to map the individual display curve to. Choose the active memory block you wish to use for the next measurement in the HydraHarp control panel with the *Map To* edit box and spin control.

The dialog also provides some statistics on each curve (central matrix of figures). *Resolution* is the channel width (bin width) of the histogram in picoseconds. Next is the *Max. Count*, the count in the highest point of the curve. The column *At Time* shows the time corresponding to the *Max. Count* channel. Leftmost there is the Full Width Half Maximum (*FWHM*) of the curve peak (usually for IRF traces).

There are also some buttons for frequently required actions: The button 'view' can be clicked to see more detailed curve information such as time of recording, acquisition settings and count rates. The button *All* can be clicked to tick all traces as shown. The button *0..7* can be clicked to set the default mapping of trace 0..7 to block 0..7. The *Clear* buttons (trash cans) can be clicked to delete the contents of individual blocks.

Note that the Trace Mapping Dialog is non-modal. This means the dialog can remain open while a measurement is in progress, so that adjustments can be made under immediate visual control, similar to the operation of the control panel. Note also that a measurement can be running in a block that is not mapped or shown.

The individual axis panel items are discussed in the section 6.6. "Trace Mapping" in the Controls and Commands Reference.

## 4.7. Other Dialogs

In order to keep this manual readable, the dialogs described here are limited to the most important ones the reader should know about before starting practical work with the software. Some more dialogs will be described implicitly in the following sections in the context of specific measurement tasks. For information on all other dialogs the user is kindly requested to refer to the controls and commands reference (section 6) or consult the on-line help facility of the software. Pressing F1 in any given dialog will provide help on that dialog.

## 5. Specific Measurement Tasks

### 5.1. Setting Up the Input Channels

This section provides help and instructions for the first basic steps of setting up the instrument. However, if you are running TCSPC measurements for the first time we strongly recommend you read our TechNote on TCSPC first. The document is on your distribution CD. This will help you getting started more quickly.

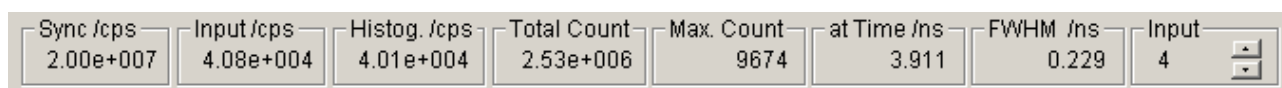
In order to acquire any data, the input channels and the SYNC input of the HydraHarp 400 must be set to match their electrical input signals. The HydraHarp input channels are designed identically, all with a Constant Fraction Discriminator (CFD). They can handle an input pulse peak range from 0 to  $-1$  V for regular operation. **The absolute maximum rating (beyond which damage may occur) is  $\pm 1.5$  V.**

In case of coincidence correlation experiments between two or more detectors, the channels are typically used for one detector each and data is collected in T2 mode. The SYNC is then usually not required. If a quick visualization of coincidence is required, it is also possible to use histogramming mode. In that case, one detector is connected to the SYNC input. Coincidence histograms will be collected for each input channel with respect to the SYNC input. In time-resolved fluorescence measurements with a pulsed excitation source (typically a laser) the SYNC input receives a sync signal from the laser. Here we focus on the latter, more common case. Perform the following steps to set up the input discriminators.

#### Using the Panel Meters

At the bottom of the main window you find a set of panel meters. These are very important during set-up. The meters showing units of cps (counts per second) in their title are rate meters. The leftmost rate meter shows the Sync input rate. The next meter shows the channel input rate. Note the selector at the right of the panel meters. This selects the channel the rate meters (except Sync) are referring to. Select the channel you are currently setting up. The HydraHarp software enumerates the physical input channels as logical input channels 1 to N, where N depends on the number of installed TDC modules. The mapping of physical to logical channels is obtained by counting as follows: start from the leftmost TDC module and count top to bottom within each TDC module. At the time of factory assembly this mapping will be marked by plastic labels at each IN connector.

The other meters show the histogramming rate, the total count in the histogram, the maximum (peak) count and the position of the maximum. They all depend on the input selector.



Note that the Input selector also has a selection option *Sum*. In this case the meters are fed from the sum of all input channels. Also note that the internal rate counters have a gate time of 100 ms. Very low rates can therefore not be measured very accurately and the meter reading may fluctuate between two values.

#### Setting up the SYNC Input

For typical fluorescence decay measurements, the HydraHarp needs an electrical SYNC signal from the light source. The PDL 800 family of diode lasers from PicoQuant provides this signal directly. If the laser does not provide an electrical sync signal (e.g. Ti:Sa lasers), a SYNC detector (photo diode) such as the TDA 200 must be used. The SYNC signal must consist of pulses with falling active edge. The pulse edges must be steep (2 ns rise/fall time or faster). For example, a NIM type signal is appropriate. This is a steep negative pulse (0.5 ns ... 1 ns wide) of typically  $-800$  mV into  $50 \Omega$ . The HydraHarp can actually handle  $-1$  V but amplitudes in excess of  $-800$  mV may cause excess interference and crosstalk between the input channels. Amplitudes around  $-200$  mV ...  $-300$  mV are best in terms of timing accuracy and lowest histogram ripple. Another rule of thumb for lowest signal distortion is to use signals of similar amplitude on all channels. SMA in-line attenuators of suitable bandwidth can be used to adjust this. The adjustable CFD level is restricted to  $-1000$  mV to  $0$  mV. Initially you should set it to half of the SYNC pulse amplitude. The zero cross setting should be set to  $10$  mV initially.



Unless you are sure what kind of signal your SYNC source delivers, use a fast oscilloscope (50  $\Omega$  input) to check the pulse shape, polarity and amplitude. The leading edge should be steep (2 ns fall time or faster), there should also be no excessive overshoot or ringing. The pulse width should be 0.5 ns ... 30 ns. The upper limit is not critical, however, the pulse width should be as small as necessary to fit in half the SYNC period.

If the signal is satisfactory, connect the source to the SYNC input and start the HydraHarp software if it is not yet running. A detector signal (Channels 1..8) is not required at this point but it does no harm if it is also connected. Open the HydraHarp control panel. Leftmost, there is a tab with a group of controls for the SYNC input. Select the corresponding tab and find the edit box and spin control for the CFD level. The level should be set to a value around half of the amplitude of the SYNC pulses. Note that the control panel shows absolute numbers from 0 to 1000 mV despite the real signals are negative going. Next, set the Zero Cross level to 10 mV. Then look at the Sync Divider. It must be set so that the sync rate divided by the shown divider value does not exceed 12.5 MHz, e.g., for a diode laser with 80 MHz repetition rate it must be set to 8. For a slow source such as a flash lamp it must be set to None.

If the SYNC source is active, the Sync rate will now be displayed at the bottom left rate meter in the HydraHarp main window. Note that the rate meter internally corrects for the chosen divider setting, so that the meter always shows the undivided input rate. The meter display should therefore match the rate delivered from the source. The rate meter will be refreshed every 0.1 to 1 seconds, determined by the value in the General Settings Dialog. The Sync rate should be displayed very accurately unless it is very low. Large fluctuations or occasional zeros indicate an incorrect discriminator level setting or an unstable Sync signal. Try to vary the discriminator level to obtain a stable Sync rate display. If the rate is stable and as expected, you can proceed to the set-up of the other input. A last fine tuning of the discriminator and zero cross level can be done when the detector inputs are up and running.

### Setting up the Photon Detector Inputs (Channel 1...8)

Dependent on the number of installed TDC modules your HydraHarp may have 2, 4, 6 or 8 input channels. As noted before, the HydraHarp 400 input channels are designed electrically identical, each with a CFD. The signals must consist of pulses with falling active edge. The pulse edges must be steep (2 ns rise / fall time or faster). The inputs can handle a voltage range of 0 to -1 V. **Note that some SPAD-detectors (e.g. SPCM-AQR) deliver positive pulses of ~3 V (TTL) and must therefore be connected through a pulse inverter with attenuation (PicoQuant SIA 400). Connecting them directly will cause damage.** PMTs should be connected through a preamplifier (10 dB ... 20 dB). MCP-PMT detectors should be connected through an amplifier with slightly higher gain. All accessories are available from PicoQuant. Be sure to **switch the high voltage supply of PMTs off and allow their electrodes to discharge before connecting/disconnecting them. Their high voltage charge may damage the preamplifier.** Observe the allowed input signal levels including those of the pre-amplifier. Again, in a new experimental setup, to be absolutely sure, please check your detector pulses as well as the preamp output with a fast oscilloscope. Start timing is on the leading edge, so it should be steep. Ringing and overshoot should be as small as possible. Do not over-illuminate the detector to avoid damage.

If the signals are appropriate, connect a detector at channel 1. Starting with only one detector makes the first steps easier. Preliminary adjustments can actually be done with uncorrelated light, e.g. daylight. For safety of your detector, use strongly attenuated light, even when the detector is off. Start the HydraHarp software and open the Control Panel. In the Control Panel section for the chosen Channel, initially set the CFD Discriminator Level to 30 mV and the CFD Zero Cross level to 10 mV.

Using the input selector at the right of the rate meters, select the channel you are currently using. The rate meter next to the sync rate meter will now show the input rate at that channel. Make sure that there is actually a signal coming in (some strongly attenuated light on the detector) and try to adjust the CFD discriminator level. To monitor this, watch the count rate meter. The adjustment range is 0 mV ... 1000 mV for the CFD discriminator level and 0 mV ... 40 mV for the CFD zero cross level. Note that a precise tuning of the CFD settings is only critical with PMT or MCP-PMT detectors. For such detectors the initial setting of the discriminator level of 30 mV should be approximately right to suppress any electrical noise. If you lower it, you should see an increase in count rate (probably mostly noise). If you raise it you will see a reduced rate. Thus, you should stay just slightly above the noise level. If you still see millions of counts per second at levels over 50 mV, reduce the detector illumination. Your detector may be at risk at such high count rates. You can then also try out responsivity to illumination changes. The Zero Cross level is not yet meaningful. It can be adjusted for optimum timing later. For SPAD detectors that typically deliver pre-shaped pulses of constant amplitude the CFD set-up is very simple. Just set the level to approximately half of the pulse amplitude. Again, the Zero Cross level is not yet critical. It can be adjusted for optimum timing later.

To actually collect histograms, select a measurement range large enough (determined by the chosen resolution) and a display range to cover your SYNC interval (i.e. 1 / sync) if possible. Set Offset = 0 and StopAt = 4,294,967,295. Start a measurement in oscilloscope mode with e.g. 1 second acquisition time (see the next sec-

tion for instructions). If there are counts coming in, you can try to set an upper limit of the count axis so that the histogram is scaled for best viewing. Start with logarithmic display so that weak signals can be seen.

If so far you used uncorrelated light, you may now want to move to time-correlated measurements. With a detector signal e.g. laser induced, or a 'fake' electrically derived from the laser SYNC, you can try to obtain a histogram that should be a narrow peak, as opposed to the flat distribution of uncorrelated light. This requires an experimental setup that delivers the signals at the two input channels with a more or less constant relative delay. For the HydraHarp in histogramming mode, this delay must be such that the Channel 1..8 (detector) signal comes later than the SYNC signal (forward start-stop mode). In addition, the delay must be chosen so that it fits in the measurement range of the histogrammer (max. 67  $\mu$ s). To obtain such a timing it may be necessary to adjust the input offsets.

If you have an appropriate setup installed and a measurement running, you can slightly vary all levels (including SYNC) for best timing response. If there is no clear optimum for any level, return to the center of its stable working range. For optimization purposes you also may want to switch to a higher resolution. If the rough setup is complete and a time-correlated signal peak is present in the histogram, the Full Width Half Maximum (FWHM) of the signal peak is displayed on-line as a figure of merit. You can adjust the input offsets to place the signal properly within the boundaries of the SYNC frame. You can also adjust your axis limits to optimize the display. Note that you can double-click all the rate meters to enlarge them as separate windows. This is useful for optical alignment work, if the PC screen is in some distance from your optical table.

### Input Troubleshooting

Whenever there is a problem, first check your cabling, detector power supply and SYNC source. To be absolutely sure, check the signals with a fast oscilloscope (50  $\Omega$  input). Disconnect the scope when you are done. Never try to deliver the signals to multiple 50  $\Omega$  loads in parallel, using simple T-pads. If you have multiple detectors, try to test your setup with one detector first to keep things simple.

If you cannot get a stable SYNC rate reading of expected figures, there may be several reasons:

- there is no proper SYNC signal (voltage, polarity, pulse width, frequency)
- the SYNC CFD level setting is inappropriate
- the SYNC divider setting is inappropriate
- Note that at small rates the meter display may fluctuate between two discrete values.

Reasons for zero input channel counts are:

- wrong input selection for the rate meters
- no or inappropriate signal (voltage, polarity, pulse width, frequency)
- inappropriate CFD levels
- preamplifier or detector failure

Note that all rate meters are updated at the display refresh rate you have selected in the general settings dialog. In oscilloscope mode the update frequency is equal to the acquisition time. If nothing seems to happen at all, you may be in oscilloscope mode with a very long acquisition time set.

Once count rates  $> 0$  are being displayed you should then also see counts appearing in the histogramming rate meter, if your measurement range is large enough. Make sure the offset is set to 0. If there are histogramming counts but no histogram is building up on the screen, check the time and count axis bounds. It is best to start with a wide display range and then narrow it down. Similarly, a logarithmic count axis setting is the safest way to see even small histograms. If your measurements stop earlier than expected, make sure the Stop at level is not set to less than 4,294,967,295 unless you have experimental reason to limit the count height.

In the process of set-up you should observe the warning icon that may appear at the bottom right of the main window. When the HydraHarp software is running with functional hardware it continuously collects information about the input signals and the current acquisition settings. If these settings together with the input rates indicate possible errors, the software will activate the warning icon.



The warning icon can be clicked to display a list of current warnings together with a brief explanation of each warning (see also section 8.1). Note that the software can detect only a small subset of possible error conditions. It is therefore not safe to assume “all is right” just by seeing no warning. On the opposite, if any of the warnings turns out to be an unnecessary nuisance, e.g. because your specific measurement conditions will expectedly and inevitably cause it, you can disable that warning via the general settings dialog (see section 6.7).

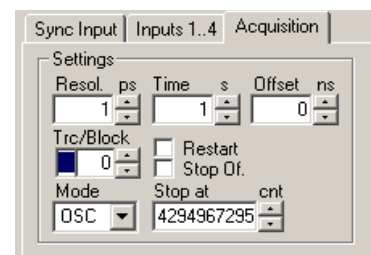
## 5.2. Setting Up and Running Interactive Measurements

The primary mode of operation of the HydraHarp software is interactive histogramming mode. This is what the main window of the software is dedicated to. The user can set up measurement parameters, start measurements and immediately see histogram data on the screen. In further sections, e.g. on TTTR mode, you will learn about other modes of operation with less user interaction that will collect data straight to disk without immediate visualization. Here, we focus on the interactive histogramming mode of operation.

To set up measurement parameters use the HydraHarp control panel. The control panel can be opened by clicking the control panel button on the toolbar or by pressing <Alt>+C.



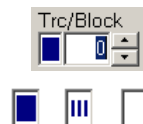
In the control panel section 'Acquisition' you can set the resolution (time per bin), the offset, the measurement time, and the block of memory to use for this measurement. To begin, use a measurement time of 1 second and an offset of 0. There are always 65,536 time channels (bins) per HydraHarp histogram. Histograms can be measured in 512 memory blocks. Out of these up to 8 curves can be displayed and one 'active curve' can be used for a measurement. You can designate the active memory block you wish to use for the next measurement by selecting the block number in the control panel. Never forget to select a new block when collecting new data and old data is intended to be preserved.



Use the trace mapping dialog to select up to 8 curves for display. Make sure the memory block you measure into is mapped to a display trace that is switched on, so that you can see the trace. You can reach the trace mapping dialog from the toolbar or by clicking on the trace colour indicator next to the block selector.



The colour indicator shows the trace colour the chosen block is currently mapped to. If it is a solid square, the curve is mapped and shown. If it is mapped but not shown, the indicator shows a small striped square. If the curve is not even mapped for display the indicator remains white.



There are two basic histogramming modes for interactive measurements: Oscilloscope and Integration mode. Oscilloscope mode repeatedly collects histograms with a fixed measurement time and displays them on the screen. This lets you see fast changes in the histogram, e.g. for optical setup and adjustments. Usually this only makes sense with relatively strong signals and short acquisition times. Integration mode is usually operated with longer acquisition times. In this case the histogram continues to grow over a longer time and the display is updated at regular intervals, so that the accumulation process can be observed.



To start a measurement with the current control panel settings, use the start button (GO) on the toolbar or press <Alt>+G.



To stop a measurement use the stop button on the toolbar or press <Alt>+S.



Note that a measurement may automatically stop and / or restart, dependent on the current settings of 'stop on overflow' and 'restart' in the control panel.

Note: to actually run a meaningful measurement you will need to set up the input channels, most importantly with appropriate CFD levels as outlined in the previous section. Also allow a warming-up period of 20 to 30 minutes dependent on lab temperature before using the HydraHarp for final measurements. You can use this time for set-up and preliminary measurements. Click the calibrate button after warming up.



The rate meters (bottom of main window) permit visual control of the data acquisition. Note the selector at the right of the rate meters. This selects the channel the rate meters are referring to. Select the channel you are currently using. The meters are often too small to view from further away, e.g. when adjusting the optical setup. You can in this case simply double-click the rate meter of interest. This opens a large meter display that you can then size and move on the screen as you like.

Once you have established standard settings for your experimental setup you may want to save them to a file. The control panel settings can then be recalled at any time. The program stores all settings together with the histogram data of those curves in memory (max. 512) that have been filled by a measurement. In addition to this, all settings are memorized in the Windows registry, so that at program startup you find the control panel as it was when you last closed the HydraHarp software.

## 5.3. Time Tagged Mode Measurements

Time-Tagged Time-Resolved (TTTR) mode allows the recording of individual count events directly to hard disk without on-board forming of histograms. It originates from the TimeHarp TCSPC systems and has been further improved for the HydraHarp 400.

In TTTR mode, the timing of individual events is faithfully recorded to disk rather than just histogrammed. This time-tagging is particularly interesting where the dynamics in a fluorescence process are to be investigated. The availability of the time-tags permits photon burst identification, which is of great value e.g. for Single Molecule Detection in a liquid flow. Other typical applications are Fluorescence Correlation Spectroscopy (FCS) and Burst Integrated Fluorescence Lifetime (BIFL) measurements. Together with an appropriate scan controller, TTTR mode is also suitable for ultra fast Fluorescence Lifetime Imaging (FLIM).

The HydraHarp 400 actually supports two different Time-Tagging modes, T2 and T3 mode, which will be explained further below. When referring to both modes together we use the general term TTTR.

### 5.3.1. System Requirements

In cases where the time-tagging modes are to be used with high continuous count rates ( $> 1$  Mcps) the PC system must meet some performance criteria, that usually are insignificant in standard interactive use of the HydraHarp. The reason for this is the large amount of data being generated in TTTR mode. In order to prevent an overflow in the recording, the data must be transferred to the computer in real-time. This requires a modern PC with a fast CPU and I/O subsystem. For the best possible performance in TTTR mode a modern hard disk with high throughput is recommended. If it is intended to make use of the full bandwidth of a HydraHarp with USB 3.0 interface then the hard disk must be able to handle sustained write rates of 160 MBytes/s. This can be achieved with RAID arrays or modern solid state disks.

At the same time, the USB subsystem must be configured correctly. In order to deliver maximum throughput, the HydraHarp 400 uses state-of-the-art USB 2.0/3.0 bulk transfers. This is why the HydraHarp must rely on having a suitable host interface of corresponding speed rating. USB 2.0 and 3.x host controllers of modern PCs are usually integrated on the mainboard. Otherwise USB 3.0 interfaces can be upgraded as PCIe cards. Make sure that the host controller drivers are correctly installed. This may require using the mainboard manufacturer's driver disk rather than relying on Windows drivers. Do not run other bandwidth demanding devices on the same USB interface when working with the HydraHarp.

### 5.3.2. T2 Mode

In T2 mode all timing inputs of the HydraHarp 400 and the SYNC input are functionally identical. There is no dedication of the SYNC input channel to a sync signal from a laser. It may be left unconnected or can be used for an additional detector signal. The sync divider should then be set to None. If a sync signal is to be used for some special experiment then it must be slow enough to avoid swamping the data stream with too many events. The sync divider can be used to achieve this but then the sync signal must be strictly periodic. SYNC events removed by the divider are not reconstructed. Custom software can of course do this.

Usually in T2 mode all regular inputs are used to connect photon detectors. The events from all channels including SYNC are recorded independently and treated equally. In each case an event record is generated that contains information about the channel it came from and the arrival time of the event with respect to the overall measurement start. The timing is recorded with the highest resolution the hardware supports (currently 1 ps). Each T2 mode event record consists of 32 bits. There are 6 bits for the channel number and 25 bits for the time-tag. If the time tag overflows, a special overflow record is inserted in the data stream, so that upon processing of the data stream a theoretically infinite time span can be recovered at full resolution. Dead times exist only within each channel (80 ns typ.) but not across the channels. Therefore, cross correlations can be calculated down to zero lag time. This allows powerful new applications such as FCS with lag times from picoseconds to hours. Autocorrelations can also be calculated at the full resolution but of course only starting from lag times larger than the dead time.

The 32 bit event records are queued in a FIFO (First In First Out) buffer capable of holding up to 2 M event records. The FIFO input is fast enough to accept records at the full speed of the time-to-digital converters (up to 12.5 Mcps each). This means, even during a fast burst no events will be dropped except those lost in the dead time anyhow. The FIFO output is continuously read by the host PC, thereby making room for fresh incoming events. Even if the average read rate of the host PC is limited, bursts with much higher rate can be recorded for some time. Only if the average count rate over a long period of time exceeds the readout speed of the PC, a FIFO overrun could occur. In case of a FIFO overrun the measurement must be aborted because data integrity cannot be maintained. However, on a modern and well configured PC a sustained average count rate of 9 Mcps is possible with USB 2.0. If your device supports USB 3.0 and is connected appropriately the throughput can be as high as 40 Mcps. This total transfer rate must be shared by the input channels. For all practically relevant photon detection applications the effective rate per channel is more than sufficient.

For maximum throughput, T2 mode data streams are normally written directly to disk, without preview other than count rate and progress display. However, it is also possible to analyze incoming data "on the fly". The HydraHarp software provides a real-time correlator for preview during a T2 mode measurement (see section 5.3.7). Other types of real-time processing must be implemented by custom software. The HydraHarp software installation CD contains demo programs to show how T2 mode files can be read by custom software. The implementation of custom measurement programs requires the HydraHarp programming library, which is available as a separate option.

### 5.3.3. T3 Mode

In T3 mode the SYNC input is dedicated to a periodic sync signal, typically from a laser. As far as the experimental setup is concerned, this is similar to histogramming mode. The main objective is to allow high sync rates (up to 150 MHz) which could not be handled in T2 mode. Accommodating the high sync rates in T3 mode is achieved as follows: First, the sync divider is employed as in histogramming mode. This reduces the sync rate so that the channel dead time is no longer a problem. The remaining problem is now that even with a divider of 16, the sync rate is still too high for collecting all individual sync events like ordinary T2 mode events. Considering that sync events are not of primary interest, the solution is to record them only if they arrive in the context of a photon event on any of the input channels. This is the same as the original TTTR mode concept as it was first conceived for the TimeHarp system. The event record is then composed of two timing figures: 1) the start-stop timing difference between the photon event and the last sync event, and 2) the arrival time of the event pair on the overall experiment time scale (the time tag). The latter is obtained by simply counting sync pulses. From the T3 mode event records it is therefore possible to precisely determine which sync period a photon event belongs to. Since the sync period is also known precisely, this furthermore allows to reconstruct the arrival time of the photon with respect to the overall experiment time.

Each T3 mode event record consists of 32 bits. There are 6 bits for the channel number, 15 bits for the start-stop time and 10 bits for the sync counter. If the counter overflows, a special overflow record is inserted in the data stream, so that upon processing of the data stream a theoretically infinite time span can be recovered. The 15 bits for the start-stop time difference cover a time span of  $32,768 \times R$  where  $R$  is the chosen resolution. At the highest possible resolution of 1 ps this results in a span of 32 ns. If the time difference between photon and the last sync event is larger, the photon event cannot be recorded. This is the same as in histogramming mode, where the number of bins is larger but also finite. However, by choosing a suitable sync rate and a compatible resolution  $R$ , it should be possible to reasonably accommodate all relevant experiment scenarios.  $R$  can be chosen in doubling steps between 1 ps and 33.5  $\mu$ s.

Dead time in T3 mode is the same as in the other modes (80 ns typ.) Within each photon channel, autocorrelations can be calculated meaningfully only starting from lag times larger than the dead time. Across channels dead time does not affect the correlation so that meaningful results can be obtained at the chosen resolution, all the way down down to zero lag time. This requires dedicated software.


The 32 bit event records are queued in a FIFO (First In First Out) buffer capable of holding up to 2 M event records. The FIFO input is fast enough to accept records at the full speed of the time-to-digital converters (up to 12.5 Mcps each). This means, even during a fast burst no events will be dropped except those lost in the dead time anyhow. The FIFO output is continuously read by the host PC, thereby making room for new incoming events. Even if the average read rate of the host PC is limited, bursts with much higher rate can be recorded for some time. Only if the average count rate over a long period of time exceeds the readout speed of the PC, a FIFO overrun could occur. In case of a FIFO overrun the measurement must be aborted because data integrity cannot be maintained. However, on a modern and well configured PC a sustained average count rate of 9 Mcps is possible. If your device supports USB 3.0 and is connected appropriately, the throughput can be as high as 40 Mcps. This total transfer rate must be shared by the input channels. For all practically relevant photon detection applications it is more than sufficient.

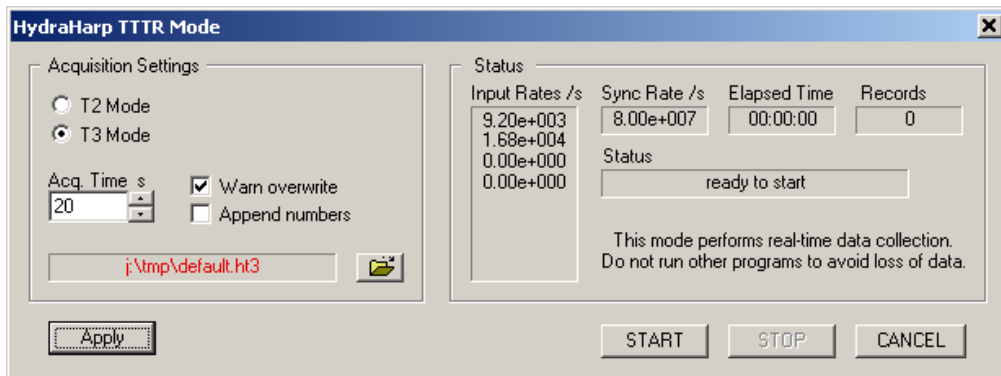
For maximum throughput, T3 mode data streams are normally written directly to disk. However, it is also possible to analyze incoming data "on the fly". One such analysis method is the on-line correlation implemented in the HydraHarp software. Other specialized analysis methods must be implemented by custom software. The HydraHarp software installation CD contains demo programs showing how T3 mode files can be read. The implementation of custom measurement programs requires the HydraHarp programming library, which is available as a separate option. Another alternative for advanced TTTR data collection and analysis is the SymPhoTime software offered by PicoQuant.

### 5.3.4. Running a basic TTTR Mode Measurement

A TTTR mode measurement (T2 or T3 mode) will typically be started after all control panel settings have been tested in normal interactive histogramming mode (oscilloscope or integration). The acquisition time (measurement time) and the file for saving the data are the only parameters that can be set separately.

A typical approach to set up a TTTR mode measurement would be by first starting oscilloscope mode with an acquisition time of e.g. 1 second. Then all control panel settings should be optimized to reliably obtain the expected data.

Once all settings are satisfactory, click the "TTTR Mode" button  on the toolbar. This will bring up the "TTTR Mode" dialog.



The dialog section *Acquisition Settings* is for the selection of the measurement mode (T2 / T3), an overall acquisition time and a file name. Note that switching between T2 and T3 mode takes some time because the hardware must be reconfigured. Normally such a switching should not occur often because the two modes usually require a very different experiment setup.

The section *Acquisition Settings* also has two tick boxes for the handling of existing files. You can turn on a warning and / or automatically have numbers appended to the file name, so that you can conveniently perform series of measurements. The file name is shown in red if the file already exists. The button with the file icon will open a standard Windows file dialog. You can select an existing file or choose a new name. The HydraHarp TTTR mode files (starting from software version 3.0) have the file name extension `.ptu`. For maximum count rate throughput you should choose a file destination on a fast local hard disk as outlined above. Network drives are often too slow.

The dialog section *Status* shows elapsed time, the count rates and the number of collected records. Below these figures there is a status line showing what is currently happening. Further below there are buttons for Start, Stop and Cancel. Start and Stop control the actual TTTR measurement run. Cancel is for leaving the TTTR mode dialog.

The TTTR mode measurement will start as soon as you click the Start button. You will then be able to watch the progress of your measurement in the status boxes. The leftmost status box shows the elapsed measurement time. The two boxes in the center show the count rates. The rightmost box will show the number of events that have been recorded. Note that this includes overflow and marker records. Therefore, the number of records will be somewhat larger than the true photon counts. The overflow and marker records will be removed by the data analysis / processing software.

Next to the status line you may see a warning icon. When the TTTR mode dialog is running it continuously collects information about the input signals and the current acquisition settings. If these settings together with the input rates indicate possible errors, the software will activate the warning icon. The warning icon can be clicked to display a list of current warnings together with a brief explanation of each warning (see also section 8.1). In order to fix wrong settings you may have to close the TTTR mode dialog and return to the control panel.



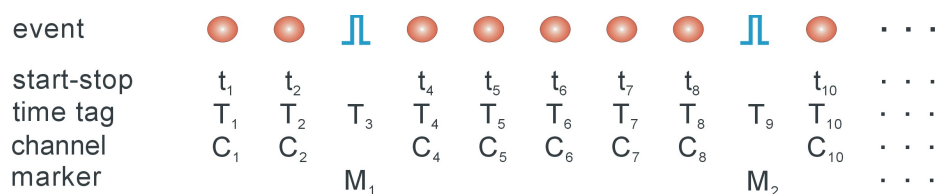
A measurement can be stopped at any time by clicking the Stop button. The data recorded up to this point will be in the file. When the measurement has completed, the Stop button will change to grey (disabled). Use the Cancel button to return to the normal interactive mode. Again, this will take some time for hardware reconfiguration.

As outlined above, TTTR data collection at high rates is a demanding real-time streaming process. The hardware and software must ensure not to lose any data. In order to implement this efficiently the HydraHarp software employs multiple threads (concurrent CPU processes). A first thread continuously reads the HydraHarp's hardware FIFO and puts the retrieved data in a software queue. A second thread concurrently reads this software queue and writes the data to disk. If real-time correlation is being performed then this is done in further separate threads. User interface and interaction are handled in yet another thread. Multi-core CPUs are particularly useful here as they can run the threads in parallel rather than switching between them. There are two typical error scenarios that you may encounter in this process. The first is a situation where the first thread does not empty the hardware FIFO quickly enough and the FIFO runs full. The software then reports the error FIFO\_OVERRUN. In order to avoid this you may have to reduce the input data rate. Another error situation may result when the second thread cannot write to disk quickly enough and the software queue runs full. The software then reports the error STORAGE\_QUEUE\_OVERRUN. In order to avoid this you may want to check the write speed of your hard disk and see how it can be improved.

### 5.3.5. External Markers

Often it is desirable to synchronize TCSPC measurements with other information or processes of complex measurement tasks. In order to perform e.g. Fluorescence Lifetime Imaging, the spatial origin of the photons must be recorded as well. For this purpose one needs a mechanism to assign external synchronization information to the TCSPC data. For the special case of Fluorescence Lifetime Imaging, conventional systems use large arrays of on-board memory and switch to new blocks of memory upon arrival of e.g. a pixel clock pulse. To accommodate the large amount of data generated due to the 3-dimensional matrix of pixel co-ordinates and lifetime histogram channels causes serious problems. Even with modern memory chips, this approach is limited in image size and / or number. In addition, it is expensive, and implies loss of information about the individual photon arrival times. To solve the problem much more elegantly, the TTTR data stream generated by the HydraHarp can contain markers for synchronization information derived from an imaging device, e.g. a scan controller. For this purpose the front panel of the HydraHarp SYNC module HSR 110 provides four TTL inputs for synchronization signals (M1..M4).

The figure below illustrates how the external marker signals are recorded in the data stream.



Bullets denote a photon, blue pulses denote a marker signal. The external markers are treated almost as if they were regular photon event records. A special channel code allows to distinguish true photon records from the marker records. Software reading the TTTR file can thereby filter out the markers, e.g. for line and frame clock in imaging applications. This makes it possible to reconstruct the 2D or 3D image from the stream of TTTR records, since the relevant XYZ position of e.g. a scanner can be determined during the data analysis. The data generated is nearly free of redundancy and can therefore be transferred in real-time. The image size is unlimited both in XYZ and in count depth. Since there are up to four such synchronisation signals, all imaging applications can be implemented and even other experiment control signals can be recorded. This marker scheme is a very special innovative feature of the PicoQuant TCSPC electronics. It is worth noting that this technology enabled PicoQuant GmbH to develop the leading edge MicroTime 200 Fluorescence Lifetime Microscope.

The TTL compatible inputs accept the synchronization signals that will be recorded as markers. The active edges of these signals can be chosen in the general settings dialog (available through the Toolbar). Both high and low state must be at least 50 ns long. The period may therefore (in principle) be as short as 100 ns but data bus throughput constraints will apply. Each marker creates an additional TTTR record, so that one must ensure not to swamp the data stream with too many marker records. Markers also take precedence over photon records, so that excess marker traffic can suppress photon records. In fast imaging applications it is therefore recommended not to use a pixel clock but a line clock only. Since each photon has a time tag, it is usually not necessary to use an additional pixel clock. The accuracy of marker timing is on the order of 50 ns.

The HydraHarp provides a programmable marker holdoff time. This can be used to suppress glitches on the marker signals that some poorly designed scan hardware or cable reflections might create. The idea is that when a marker signal was detected the next (spurious) marker will be suppressed if it occurs within the holdoff time after the first detection. The holdoff time can be chosen in the software settings dialog available through the Toolbar.

For each channel there is another TTL input that can be used to externally enable / disable the recording of incoming counts (see section 8.3.2). Counting is enabled when this input is held high (or left open). Pulling it low disables counting. This mechanism can be used to blank out periods where e.g. an external scanner is moving to the next position. The count enable signal is also active in interactive mode. It may be used to synchronize the start of acquisition with external events.

### 5.3.6. Using TTTR Mode Data Files

For diagnostic purposes you may re-load a T3 mode file into the HydraHarp software. The limitation is that you will only be able to form a histogram over the start-stop times in your T3 mode data. The time-tag information will not be used here. The HydraHarp software will recognize that you are loading a T3 mode file and how many records there are contained in it. It will then prompt you for a range to use for histogramming. The histogram will go to blocks 0..N-1 of the histogram memory where N is the number of channels that were used in the T3 mode measurement. A TTTR mode file also contains all control panel settings that were active at the time of the measurement run. After loading a TTTR mode file, you will find the document title reading "Histogram from...". If you choose to save such data you will have to give it a new file name (\*.phu). This is because now a histogram has been formed, and saving it to the same file name would destroy the original TTTR mode file. However, you may save the previously formed histogram as if it were obtained in normal interactive mode, to a standard HydraHarp histogram data file (\*.phu).

Reloading T3 mode files serves as a quick diagnostic tool only. For T2 mode files such a feature is currently not available. Further processing or analysis of TTTR mode data must therefore be performed through external data analysis software. Such software is available from PicoQuant for a wide range of analysis tasks (SymPhoTime). Further specialized analysis can be performed by dedicated custom software. If you wish to save the cost for the commercial TTTR analysis software or if you require special analysis algorithms you may want to program your own analysis software. For development of your own custom programs, please refer to the demo code provided. Demo source code is contained on your HydraHarp installation disks and will be installed by the software setup. Also see section 8.2 for the file format specifications. The paragraph below can only give an outline.


The first part of a TTTR mode file is a header with the basic setup information, similar to that of the other modes. What follows after the header is a sequence of 32 bit TTTR records. The TTTR records in the file consist of different pieces of information in groups of bits. These pieces of information must be extracted by a bit masking / shifting operation. Their specific layout is different for T2 and T3 mode. In both cases, in addition to extracting the bit fields, the software must step through the whole file and interpret the overflow records and correct the overall time axis accordingly. Details on how this can be done should be looked up in the demo programs that are installed as part of the software distribution.

Note that T2 and T3 mode files cannot be distinguished by their file extension. They all get stored as \*.ptu. However, you can use the PicoQuant File Info shell extension that will be installed by the HydraHarp software setup to inspect individual header items of a \*.ptu or \*.phu file. This includes the measurement mode. Just right-click on the file in Windows explorer and select *Properties*. Then look at the tab *PQ File Info*.

### 5.3.7. TTTR Mode Measurements with Real-Time Correlation

An important application of the HydraHarp and its TTTR mode is Fluorescence Correlation Spectroscopy. While TTTR data collection and off-line software correlation has been used for quite some time, PCs have only recently become fast enough to calculate the correlation function "on the fly" while the data is being collected. This capability can be very useful in setting up and monitoring of FCS experiments. The HydraHarp software provides such a real-time software correlator for T2 and T3 mode.



Although it collects the same TTTR mode files, the real-time correlator has its own button on the Toolbar. 

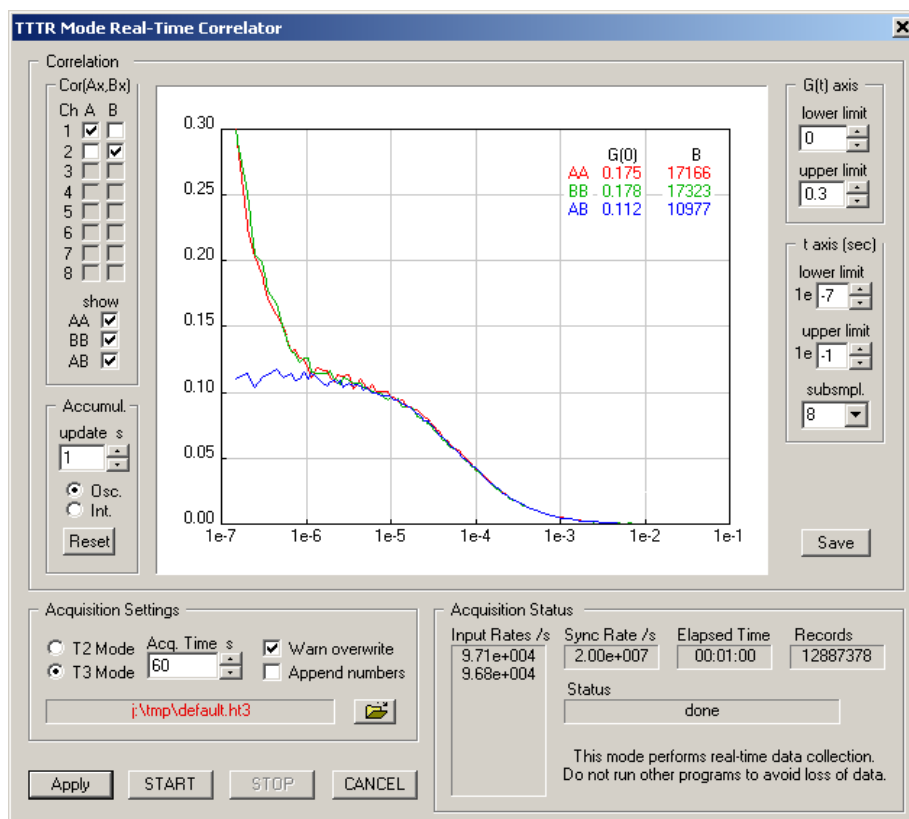
The next figure shows the TTTR mode correlator dialog. Its lower section provides the same file handling and progress indicators elements as in plain TTTR mode. Please refer to section 5.3.4 for details. The upper section of the dialog contains the correlator display and various control elements.

The dialog section  $Cor(Ax, Bx)$  allows to select individual input channels for the correlation. A and B are the virtual input channels to be correlated. All input channels that are ticked in columns A resp. B will be used in the corresponding correlation channel. If your selection is empty, the heading of the section  $Cor(Ax, Bx)$  will turn red to indicate the problem. If you proceed anyway you will get error messages upon start.

The correlator always calculates the autocorrelations of the two virtual channels (AA and BB) as well as the cross-correlation (AB). There are three tick boxes that select which of these are shown.

The dialog section *Accumul.* allows to select an update time for the correlation display. Furthermore it allows to select between repetitive updates (*Osc.*) and cumulative collection (*Int.*). If the latter is chosen one can manually reset the correlator by clicking the *Reset* button. Note that all these settings affect only the real-time correlator. The raw TTTR mode data will be collected continuously and completely independent from the correlator settings.

The *Save* button under the *Accumul.* section allows to save the correlator results as they were last shown. The saved result is an ASCII file with some header information. The format is self-explanatory. Note that the real-time correlator is primarily a tool for preview during measurement setup and optimization. It does not allow to correlate data from TTTR files. If you need the correlation over the entire stream of collected data you need to make sure you run in Integrating mode and do not press reset during the measurement. If you need the correlation results you should save them here. If you need to perform further or more thorough FCS analysis on the collected data you can use the SymPhoTime software from PicoQuant.



On the right hand side there are controls for the axis ranges of the correlator display. Note that they affect only the display. Collected data is always complete, independent from the axis settings. In contrast, the selection box *subsmpl* has an effect on the correlator results. It determines how many tau points are calculated. The correlator works in a logarithmic multiple tau scheme and *subsmpl* specifies the number of linear subsamples in each log stage. A higher number of subsamples increases the resolution of the correlation curve. Calculating more points is more time consuming and therefore it may lead to lower count rate limits that can be handled. In this

case you may get FIFO overruns. The default of `subsmpl=8` is a reasonable trade-off between speed and resolution. On faster computers the setting of `subsmpl=16` is a good choice.

Note that the starting point and spacing of the Tau sampling points also depends on the time tag resolution. In T3 mode this corresponds to the sync period. In T2 mode the native resolution of 1 ps is binned down to a time tag resolution of 25 ns in order to make the data manageable for the real-time correlation algorithm.

The correlation curve display can be shown with a grid (see general settings dialog, accessible from the toolbar of the main window). It also shows two useful figures obtained from the collected data (top right of curve window). The first figure is an approximation of  $G(0)$ . In classic FCS experiments this corresponds to the inverse of the number of particles in the focal volume. It is continuously updated together with the curve display, which can be useful for system adjustment, notably when using the repetitive accumulation mode (Osc.). Note that the approximation is a simple averaging over the first ten Tau points. The figure B is an indicator for molecular brightness. It is also updated continuously. Note that it is also only an approximation obtained by multiplying the  $G(0)$  approximation with the average count rate on both virtual correlation channels A and B. Dependent on the chosen channels in A and B this may lead to figures that are not truly molecular brightnesses, however, as an adjustment aid they should be useful in any case.


All other aspects of TTR data collection with correlator preview are the same as in plain TTR mode as described in the previous subsections. Topics such as using external markers or how to use the data files should be looked up there.

## 5.4. Time-Resolved Excitation and Emission Spectra

In time-resolved fluorescence research it is often of great interest to observe time-dependent spectral shifts and decay changes, e.g. in the context of solvent relaxation dynamics and general spectral evolution. This requires recording Time-Resolved Excitation/Emission Spectra (TRES), ideally with automated wavelength scan and TCSPC data collection under full software control.

In order to measure excitation or emission spectra in combination with fluorescence lifetime, the HydraHarp software provides an automated TRES measurement mode. This mode allows to control a monochromator via a stepper motor and automated collection of spectrally resolved lifetime histograms. Note, however, that this feature is only maintained for legacy systems and does not support modern monochromators.

For TRES data is collected as in standard "Integration Mode" and saved in different blocks of memory for each wavelength. Note, however, that TRES data is always collected through input channel 1 only.

If a monochromator with appropriate stepper motor and associated drivers is installed, a specialized dynamic link library (Mono.dll) can be installed together with the HydraHarp software. The presence of this DLL will be detected by the software. In this case the button for Monochromator and TRES control on the toolbar will be enabled. 

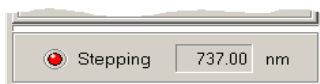
Note that most of the monochromators can be controlled only through dedicated stepper motor hardware from PicoQuant or selected vendors, usually installed as part of a complete PicoQuant spectrometer. Custom configurations can only be supported upon special request.

Clicking the monochromator button will launch a dialog for manual monochromator control and TRES setup. There you can set up parameters such as the start, step and end of the wavelength scan. There will be error messages if the monochromator / stepper and associated drivers are not configured properly.

The monochromator used for TRES measurements is controlled by a special dialog. It shows a tab control and a status panel at the bottom of the dialog. The three tabs give access to the *Initialization*, *TRES* and *Manual* page of the dialog.



The status panel shows the current monochromator position in *nm* and whether the monochromator is currently moving (the red "LED" labelled *Stepping* is flashing, when it moves).



The dialog is "non modal", i.e. it does not need to be closed before other windows can be accessed. It has its own icon in the Windows Taskbar, from where it can be brought back to the top, should it have been covered by other windows.

### Monochromator Models

Please note that the TRES feature implemented in the HydraHarp software is only maintained for continued support of legacy systems and does not support modern monochromators.

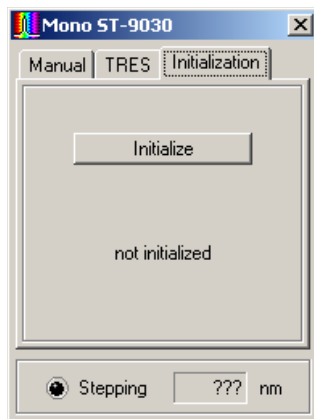
The type of the current monochromator is displayed in the title bar of the dialog. Apart from a simulation mode, as of this release the following monochromator models are supported: Scientech ST-9030, ST-9030DS and ST-9055, Acton Research SP-2150, SP-2155, SP-150 and SP-275. Some other Acton Research models are partially supported. Note that some monochromators can only be controlled through dedicated stepper motor hardware from PicoQuant or the monochromator supplier. Usually these were installed as part of a complete PicoQuant spectrometer.

If your system is not configured for use of a real monochromator, the Monochromator / TRES dialog shows the caption *Simulation*. This is for demonstration purposes only. It will work the same way as the "real" ST-9030 monochromator described below, except that it controls no real hardware and just simulates the monochromator adjustments. Of course the simulated monochromator adjustments will not have any effect on the measured TCSPC histograms.

### Monochromator Initialization

Before use, the monochromator must be initialized. This means, it has to seek its mechanical reference position (determined by a micro switch). Physical calibration values are kept in a monochromator configuration file (monochromator.cfg) which is generated by the system supplier at the time of delivery. Do not edit this file unless you have exact instructions for doing so.

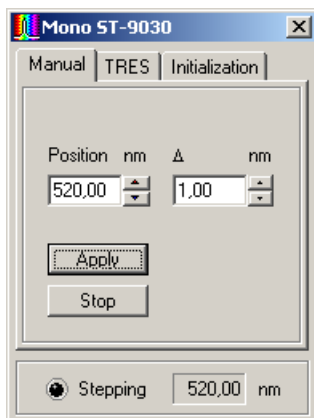
Position initialization is required each time the software is started. It can be controlled on the *Initialization* page of the dialog. At first startup the dialog shows the state "not initialized". This means the software has no information about the current monochromator / stepper position.



The position initialization is started by the *Initialize* button. If the initialization is running, the caption of the button will change to *Stop* and the LED will start flashing. You should also hear the stepper motor. If the initialization is successful, the string "not initialized" will change to "Initialization OK", otherwise it will change to "Initialization failed!". In this case, check cabling and power supply of the system. This may happen if either the *Stop* button was pressed or an error occurred during the initialization process. The *Manual* and *TRES* pages will not be available unless the initialization was successfully performed. If for any reason the initialization becomes invalid (e.g. if the monochromator was turned manually and subsequently accidentally reached the end point switch) the *Initialization* page will be activated and access to the *Manual* and *TRES* pages will be blocked.

## Manual Monochromator Control

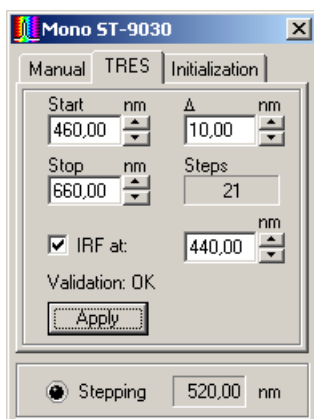
The *Manual* page of the dialog can be used to position the monochromator manually at a given wavelength. The desired position can be entered in the *Position* edit box. Clicking *Apply* or Pressing <Enter> will cause the monochromator to move to the specified position. Alternatively, the spin buttons right to the edit box can be used to modify the position. The step width of this action is defined in the  $\Delta$  (delta) edit box. Pressing arrow buttons will make the change take effect immediately; entering values by keyboard require pressing *Apply* or <Enter> to show an effect.



If there is a large difference between the current monochromator position and the position entered, the movement will take some time. If you wish to interrupt this process, press the *Stop* button. The monochromator immediately will slow down and stop. The deceleration is necessary to preserve the calibration.

## Running TRES Measurements

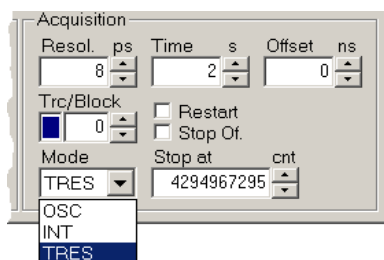
The *TRES* page of the dialog contains the controls used to set up an automated scanning of the monochromator. The start and stop wavelengths of the scan can be entered in the corresponding edit boxes, the  $\Delta$  (delta) box defines the step width of the scan. The scan starts always exactly at the *Start* value. If the *Stop* position cannot be reached by an integer multiple of the step width, the scan stops at the last position within the start / stop range. If the start position is larger than the stop position, the scanning direction will be reversed. The maximum range and number of wavelength steps is determined by the physical characteristics of the monochromator as well as the maximum number of memory blocks to record histograms in (512). Again, pressing the spin buttons will apply the change in the corresponding edit boxes immediately; entering values manually require pressing <Enter> or the *Apply* button.



The IRF can be measured automatically as part of the scan, at a separately chosen wavelength. To do so, check the box entitled IRF at and enter the wavelength at which to measure the IRF in the corresponding edit box. The monochromator will go to the IRF position at the beginning of the TRES measurement. After the IRF was measured, the actual TRES scan will be performed. The IRF will always be recorded in memory block 0, while the wavelength scan always starts in block 1. Note that starting a TRES measurement will clear and sub-

sequently overwrite all curves in memory that may have been recorded previously. If you wish to measure the IRF yourself you must do this AFTER the TRES measurement. If the curves in memory were loaded from a file, the actual file on disk will not be overwritten until you save to the file (Save menu or corresponding toolbar button). Use Save As and select a new file name if you want to preserve the old file. Note that a TRES file will remain a TRES file for its lifetime. This means that after creating or re-loading such a file you can do only two things: perform a new TRES measurement (effectively overwriting the old file contents) or measure an IRF into curve 0 (overwriting only curve 0). If you want to perform any other measurement you will need to create a new file or load a non-TRES file.

The TRES measurement is selected in the HydraHarp control panel. You need to use the selection box for the measurement mode to select TRES mode (instead of OSC or INT).



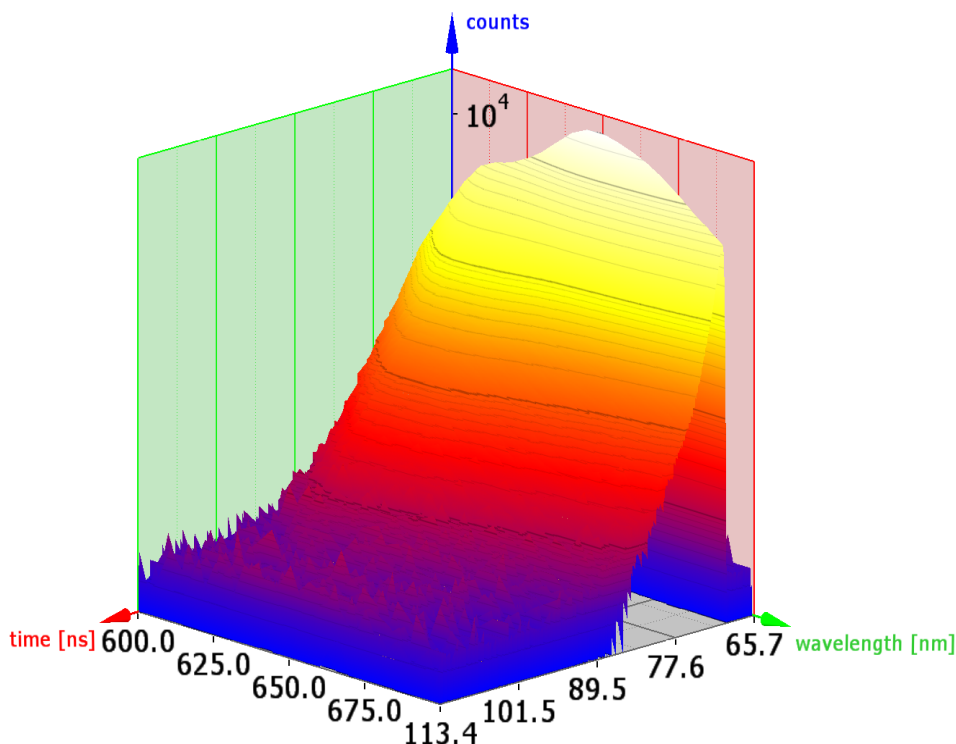
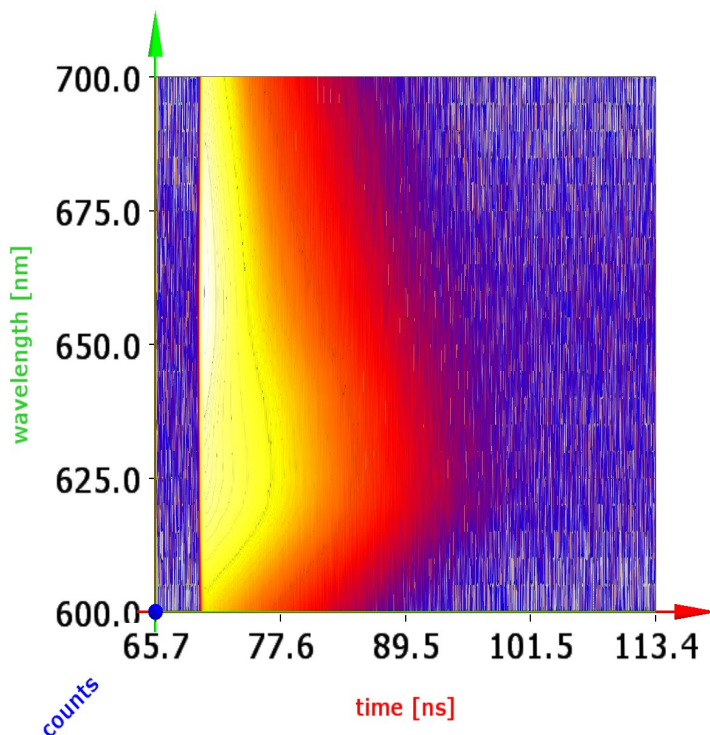
A TRES measurement is started or canceled as usual via the toolbar buttons for start and stop. The data acquisition will be performed like a standard "Integration Mode" measurement, while the block number is incremented for each new wavelength.



At the beginning of each TRES run, the active curve (block) will be set to 0, or to 1 if no IRF is collected. All curves that were previously collected and not saved to a file will be overwritten with new data. During a TRES run, the currently collected data will always be shown as Trace 0 (dark blue), even though the block (Trace) number is actually incremented at each wavelength step. This is to overcome the limitation of only 8 display curves being available. You can watch the block number in the control panel to see the curve currently being collected. Manual entry will be disabled during the run. Also, you can watch the status bar to see the current wavelength and stepping activity. Note that for a TRES measurement the control panel setting 'Stop Of' applies as in Integration mode, while 'Restart' is meaningless in TRES mode.

### TRES Data Analysis and Visualization

Having collected a complete TRES data set you can save it to a regular \*.phu file. You can also inspect individual curves via the Trace Mapping dialog. You can furthermore use the software tool FluoPlot from PicoQuant to visualize and analyze the data in various 2D and 3D representations with a multitude of options for colouring, scaling and changing view aspects in 3D. The figures following below show FluoPlot visualizations from a TRES measurement of mixed oxazine dyes in ethanol.






FluoPlot is provided on your software installation media. Just run FluoPlotSetup.exe from there. If you received your HydraHarp software by download or email you may need to request FluoPlot separately. Note that the program requires modern hardware acceleration with support for OpenGL version 1.5 or higher. Speedy handling of large data files requires sufficient memory. Further data analysis may need to be performed by dedicated software, either custom programs or specialized solutions available from PicoQuant.

## 6. Controls and Commands Reference

### 6.1. Main Window

#### The Title Bar

The title bar is located along the top of a window. To move the window, drag the title bar. Note: You can also move dialog boxes by dragging their title bars. The title bar may contain the following elements:

System Menu	
HydraHarp – [Name of the file or dialog]	
<i>Minimize</i> Button	
<i>Maximize</i> or <i>Restore</i> Button, resp.	
<i>Close</i> Button	

#### Scroll bars

Displayed at the right and bottom edges of the HydraHarp window if a certain minimum window size is reached. The scroll boxes inside the scroll bars indicate your vertical and horizontal location in the display area. You can use the mouse to scroll to other parts of the window.

#### Size command

Use this command to resize the active window.

Note: The command is unavailable for already maximized or minimized windows.

##### Shortcut

Mouse: Drag the corners or edges of the window.

Keys: <Alt>+<Space> S

#### Minimize command

Use this command to reduce the HydraHarp window to an icon. Running measurements will continue.

Note: The command is unavailable for already minimized windows.

##### Shortcut

Mouse: Click the minimize button  on the title bar.


Keys: <Alt>+<Space> N

#### Maximize command

Use this command to enlarge the active window to fill the available space.

Note: The command is unavailable for already maximized windows.

##### Shortcut

Mouse: Click the maximize button  on the title bar; or double-click the title bar of a not-enlarged window.


Keys: <Alt>+<Space> X

### Restore command

Use this command to return from an enlarged window to the previous size.

Note: The command is only available for already maximized or minimized windows.

#### Shortcut

Mouse: Click the maximize button  on the title bar;  
or double-click the title bar of the enlarged window.


Keys: <Alt>+<Space> R

### Close command

Use this command to close the active window or dialog box.

Double-clicking a system menu box is the same as choosing the Close command.

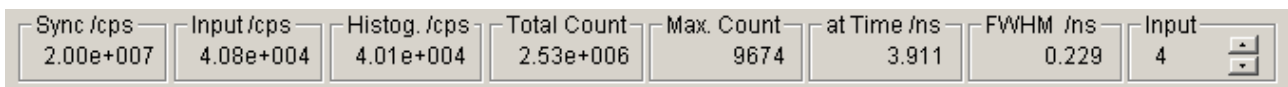
#### Shortcuts

Mouse: Click the close button  on the title bar.

Keys: <Alt>+<F4>  
<Alt>+<Space> C

### Panel Meters

At the bottom of the main window there is a set of panel meters. The meters showing units of cps (counts per second) in their title are rate meters. The leftmost rate meter shows the Sync input rate. The next meter shows the channel input rate. Note the selector at the right of the panel meters. This selects the channel the rate meters (except Sync) are referring to. The other meters show the histogramming rate, the total count in the histogram, the maximum (peak) count and the position of the maximum. They all depend on the input selector.



Note that the Input selector also has a selection option *Sum*. In this case the meters are fed from the sum of all input channels. Also note that the internal rate counters have a gate time of 100 ms. Very low rates can therefore not be measured very accurately and the meter reading may fluctuate between two values.



## 6.2. Menus

### 6.2.1. File Menu


The *File* menu offers the following commands:

<i>New</i>	Clears all histogram data and restores default settings.
<i>Open</i>	Opens an existing histogram file.
<i>Save</i>	Saves a histogram file.
<i>Save As...</i>	Saves an opened histogram file to a specified file name.
<i>Export ASCII</i>	Exports the currently shown histogram data as an ASCII file
<i>Print</i>	Prints the currently displayed histogram.
<i>Page Setup</i>	Allows modifying the page layout for printing.
<i>Print Preview</i>	Displays the layout as it would appear printed.
<i>Print Setup</i>	Selects a printer and printer connection.
<i>1...4 &lt;Recent Filename&gt;</i>	Opens one of the four last recently opened files
<i>Exit</i>	Exits HydraHarp software.

#### New command

Use this command to create a blank histogram with the last default settings. You can open an existing histogram file with the *Open* command.

##### Shortcuts

Toolbar: 

Keys: <Ctrl>+N  
<Alt>+F N

#### Open command

Use this command to open an existing histogram file. All control panel settings will be restored also. You can also open HydraHarp histogram files (\*.phu) by double clicking them or dragging them onto the HydraHarp icon. You can revert to a blank histogram and default control panel settings with the New command. You can also select other file types for loading as histogram data, notably T3 mode files. Loading such files as histogram data is for diagnostics only and cannot use their full information content.

##### Shortcuts

Toolbar: 

Keys: <Ctrl>+O  
<Alt>+F O

#### File Open dialog box

The following options allow you to specify which file to open:

*Look in:* Select (i.e. browse into) the directory in which the file that you want to open resides.

*Main box* In this box you see the content of the chosen directory, filtered by *Files of type*.

*File name:* Type or select the filename you want to open. This box lists files with the extension you select in the *Files of type* box.

*Files of type* Select the type of file you want to open. The default HydraHarp extension is `.phu`

### Save command

Use this command to save the current histogram data to its current name and directory. When you save a histogram for the first time, HydraHarp displays the *Save As...* dialog box so you can name your file. This command is unavailable if a measurement is running. If you want to change the name and directory of an existing file before you save it, choose the *Save As* command.

#### Shortcuts

Toolbar: 

Keys: `<Ctrl>+S`  
`<Alt>+F S`

### Save As... command

Use this command to (re-)name the current histogram data and save. The software displays the *Save As...* dialog box so you can name your file. To save a file with its existing name and directory, use the *Save* command.

#### File Save As... dialog box

The following fields allow you to specify the name and location of the file you are about to save:

- Save in:* Select (i.e. browse into) the directory where you want to store the file.
- Main box* In this box you see the content of the chosen directory, filtered by *Save as type*.
- File name:* Type a new filename to save a histogram with a different name. The software automatically adds the extension you specify in the *Save as type:* box.
- Save as type:* Selects a filter on the directory chosen in the *Save in:* box. Only files that pass the filter are shown in the dialog's main box. Additionally the software adds the extension associated with the chosen filter to a given file name if it was given without extension.

### Export ASCII command

Exports the currently displayed curves to a file in ASCII format. Note that only the currently selected curves within the current display limits are exported. If the curves in the display are of different resolution then all 65536 channels will be copied. The exported data is plain `<Tab>` separated ASCII and can be read by most commonly used spreadsheet programs or data analysis software.

#### Shortcuts

Keys: `<Alt>+F E`

### Print command

Use this command to print the currently displayed histogram curves. This command presents a *Print* dialog box, where you may specify the number of copies, the destination printer, the paper orientation and other printer setup options.

#### Shortcuts

Toolbar: 

Keys: `<Ctrl>+P`  
`<Alt>+F P`

### Page Setup command

Use this command to change the print layout. This command presents the *page setup* dialog box, where you may select or deselect various items to appear in the print. You can use *print preview* to check the resulting layout.

#### Shortcut

Keys: <Alt>+F U

### Print Preview command

Use this command to display the layout as it would appear when printed. The main window will be replaced with a print preview window in which one or two pages will be displayed in their printed format. The print preview toolbar offers you options to view either one or two pages at a time; move back and forth through the document; zoom in and out of pages; and initiate a print job.

#### Shortcut

Keys: <Alt>+F V

### Print Setup command

Use this command to select a printer and a printer connection. This command presents a Print Setup dialog box, where you specify the printer and its connection.

#### Shortcut

Keys: <Alt>+F R

### 1, 2, 3, 4 command (most recently used files)

Use the numbers and filenames listed at the bottom of the *File* menu to open the last four files you closed. Choose the number that corresponds with the file you want to open.


#### Shortcuts

Keys: <Alt>+F 1  
<Alt>+F 2  
<Alt>+F 3  
<Alt>+F 4

### Exit command

Use this command to end your HydraHarp session. Save your data before exiting.

#### Shortcuts

Mouse: Click the close button  on the title bar.  
Keys: <Alt>+<F4>  
<Alt>+F X

## 6.2.2. Edit Menu

The *Edit* menu offers these commands:

<i>Copy</i>	Copies the displayed curves.
<i>File Comment</i>	

### Copy command

Copies the currently displayed curves in ASCII format to the clipboard. This can be used to export histogram data to spreadsheet or data analysis software, e.g. the FluoFit Fluorescence Decay Fit Software. Note that only the currently selected curves within the current display limits are copied. If the curves in the display are of different resolution then all 65,536 channels will be copied. Copying data to the clipboard replaces the contents previously stored there.

The exported data is plain <Tab> separated ASCII and can be read by most of the common spreadsheet programs or data analysis software. This command is unavailable while a measurement is running.

### Shortcuts

Keys:           <Ctrl>+C  
                  <Alt>+E C

### File Comment command

This command opens a dialog to edit the file title / comment. The file title/comment editor can also be opened via double-click on the title or via the *Print Preview* Toolbar. The text you enter here will be stored in the data file(s) you save. The first line will be displayed as the file title over the histogram display area. The text you can enter here is limited to 4 lines and 255 characters. Upon loading of a data file, the title and comment will also be retrieved. It will also be included in prints, if the corresponding check mark is set in the page setup dialog box available through the *File* menu.

### 6.2.3. View Menu

The View menu offers the following commands:

<i>Toolbar</i>	Shows or hides the toolbar.
<i>Status Bar</i>	Shows or hides the status bar.
<i>Axis Panel</i>	Shows the axis settings panel.
<i>Control Panel</i>	Shows the control panel.
<i>Trace Mapping</i>	Shows the trace mapping dialog.

#### Toolbar command

Use this command to display and hide the Toolbar, which includes buttons for the most common commands, such as *File Open*. A check mark appears next to the menu item when the Toolbar is displayed.

##### Shortcut

Keys: <Alt>+V T

#### Status Bar command

Use this command to display and hide the status bar, which describes the action to be executed by the selected menu item or depressed toolbar button, the current measurement activity and keyboard latch state. A check mark appears next to the menu item when the status bar is displayed. The status bar is displayed at the bottom of the HydraHarp main window.

##### Shortcut

Keys: <Alt>+V S

#### Axis Panel command

Use this command to display the axis settings panel. The result is the same as the axis panel button in the toolbar or double clicking the on the histogram axes. The axis settings panel can be closed by clicking its close button.

##### Shortcut

Keys: <Alt>+V A

#### Control Panel command

Use this command to display the control panel. This is the same as the control panel button in the toolbar. The control panel can be closed by clicking its close button.

##### Shortcut

Keys: <Alt>+V C

#### Trace Mapping command

Use this command to display the trace mapping dialog. This is the same as the corresponding button in the toolbar. The trace mapping dialog can be closed by clicking its close button.

##### Shortcut

Keys: <Alt>+V M

## 6.2.4. Help Menu

The *Help* menu offers the following commands, which provide you assistance with this application:

<i>Help Topics</i>	Offers you the contents list of topics on which you can get help.
<i>Help Index</i>	Offers you an index to topics on which you can get help.
<i>Help Search</i>	Offers you a means of full text search on all help topics.
<i>Activate Context Help</i>	Switches the cursor mode to "point to item" for context help.
<i>About HydraHarp...</i>	Displays version information of the HydraHarp software.

Note: Online help (context help) on most functions, dialogs, control items etc. is available via the F1 key.

### Help Topics command

This command opens a browser offering a tree view like list of contents, and a help page. Browse through the contents with your mouse or the cursor keys. <Enter> opens / closes chapters, <Cur Up> / <Cur Down> turns the pages. The help page to the right of the browser will always be held up-to-date.

#### Shortcuts

Keys:           <F1>                   (if online help was last recently opened in topics mode)  
                  <Alt>+H T

### Help Index command

This command opens a browser offering a index list of keywords and a help page. Browse through the contents with your mouse or the cursor keys. <Enter> opens the current item, <Cur Up> / <Cur Down> navigates through the index.

#### Shortcuts

Keys:           <F1>                   (if online help was last recently opened in index mode)  
                  <Alt>+H I

### Help Search command

This command opens a browser offering full text search on words or word groups over the whole online help volume. You can enter more specific search pattern, using the operators "AND", "OR", "NEAR" and "NOT".

#### Shortcuts

Keys:           <F1>                   (if online help was last recently opened in search mode)  
                  <Alt>+H S

### Activate Context Help

Changes the mouse cursor mode to context help mode. You can choose context sensitive help for a visual element in the GUI by simply clicking on it.

#### Shortcuts

Keys:           <Shift>+<F1>  
                  <Alt>+H C

### About HydraHarp... command

Use this command to display the copyright notice and version number of your HydraHarp software and hardware, if installed. It also provides access to the PicoQuant Web site and software updates.

#### Shortcut

Keys:           <Alt>+H A

## 6.3. Toolbar

Toolbar buttons of special interest are explained in some more detail here:



### **Context Help**

When you choose the *Context Help* button, the mouse pointer will change to an arrow and question mark. Then click somewhere in the HydraHarp window, such as on another toolbar button. The help topic will be shown for the item you clicked.



### **Axis Panel**

Clicking this button opens the *Axis Panel*. This panel provides controls for the axis limits and lin / log display switching. Double-clicking the axes opens the same dialog. The dialog is non-modal, so it can remain permanently open. The panel will remember its last position when closed. When it is opened the next time it will be at that screen position again.



### **Data Cursor**

Opens the data cursor dialog box. You can use this dialog to mark and retrieve the data values of individual histogram channels. A pair of cross hairs will be provided for marking data points with the mouse or keyboard. When the data cursor dialog is activated, the cross hairs will appear in the histogram display area. By clicking on or near a data point, the current cross hair (black) will snap to that point. At the same time a second cross hair (grey) will jump to the previously marked point. The data cursor dialog then shows the current count and time values for these points as well as the corresponding differences (deltas). The data cursor always applies to one "active" curve only. You can select this active curve at the top of the dialog where it is shown with its corresponding curve colour. While normally the grey cross hair jumps to the previous data point, you can modify this behaviour by holding the SHIFT key down while clicking on curve points. The grey cross hair will then remain at its previous position. This allows finding a particular point while keeping the other one fixed.

Another advanced mode of operation of the data cursor is possible via the LEFT and RIGHT arrows of the keyboard. Pressing these keys will direct the black cursor to the next left or right point in the curve. Again, the behaviour of the marker for the previous point (grey cross) can be controlled via the SHIFT key.



### **Calibrate**

Calibrates the time measurement circuits. This is necessary whenever temperature changes  $> 5$  K occurred. Calibrate after warming up of the instrument before starting serious measurements. Warming up takes about 20 to 30 minutes dependent on the lab temperature. It is completed when the cooling fan starts to run for the first time. Calibration takes only a few seconds. During calibration the status bar shows the message *CALIBRATING*. Calibration is only important for serious measurements. You can use the instrument during the warming-up period for set-up and preliminary measurements. For very long measurements, allow some more time for thermal stabilization, calibrate immediately before the measurement commences and try to maintain a stable room temperature during the measurement. The permissible ambient temperature is 15°C to 35 °C. Do not obstruct the cooling fan at the back and the air inlets at the bottom of the housing.



### **Control Panel**

Launches the HydraHarp *Control Panel*. If the control panel is already open, subsequent clicking of this button will just make the control panel the active window. The dialog is non-modal, so it can remain permanently open. The panel will remember its last position when closed. When it is opened the next time it will be at that screen position again, even in the next HydraHarp session.



### **Trace Map**

Launches the *Trace Mapping* dialog. The HydraHarp can measure histograms in up to 512 memory blocks. Out of these, up to 8 curves can be displayed. The trace map dialog is used to select the curves to display. Tick the individual "Show" boxes to display a curve. Select the number of the memory block you wish to map the individual display curve to. Choose the active memory block you wish to use for the next measurement in the HydraHarp control panel.



### **TTTR Mode**

Opens the TTTR mode dialog box. Use this dialog to enter the acquisition time and the destination file for a TTTR mode run. Make sure all other measurement parameters have been set and tested in interactive mode before entering TTTR mode.



### **TTTR Real-Time Correlator**

Opens the TTTR mode real-time correlator dialog box. Use this dialog for FCS preview during a TTTR mode run. Make sure all other measurement parameters have been set and tested in interactive mode before entering TTTR correlator mode. Changing to this mode takes a few seconds for reconfiguration of the hardware. See also section 5.4.



### **Monochromator / TRES**

In order to measure Time-Resolved Emission Spectra (TRES), the HydraHarp software provides a TRES measurement mode, that allows to control a monochromator via stepper motors and automated collection of spectrally resolved lifetime histograms. Clicking this button will launch the dialog for manual monochromator control and TRES setup. There you can set up parameters such as the start, step and end of the wavelength scan. There will be appropriate error messages if the monochromator / stepper and associated drivers are not configured properly.



### **General Settings**

Opens the software settings dialog box. Use this dialog to change standard settings of the HydraHarp software. Notably these are: *Display rate* (0.1 to 1s), *Draw mode* (lines, stairs), *Grid*-checkbox, *Prompt overwrite* (warning before overwriting existing data) and *TTTR Marker Settings*. The control connector of the HydraHarp 400 provides TTL inputs for synchronization signals. The markers can be enabled resp. disabled and recorded at the rising or falling edge of the corresponding TTL signal. The active edges can be chosen here. The dialog also allows selective disabling of warnings that you do not wish to receive. All settings will be kept in the Windows registry and will be retrieved at the next program start. They will be individual to each user.



## 6.4. Control Panel

The control panel consists of several pages (tabbed sheets) containing edit boxes and other controls for related parameters. These pages are described in the following subsections. Note that the settings of the control panel as well as the position of the control panel on the screen will be stored in the registry and retrieved at the next program start. When you load a HydraHarp data file the settings of the control panel will change according to the settings in that file. This can be used as a means of reverting to a standard setting for the given experiment.

### 6.4.1. Sync-Input

The settings in this group configure the behaviour of the Sync input. See section 5.1 “Setting Up the Input Channels” to get started.

#### **Zero Cross** edit box and spin control

Here the zero cross level of the Constant Fraction Discriminator (CFD) for the Sync input can be set. Units are millivolts (mV), the permitted range is 0 to 40. Type the value as an integer in the edit box and press `<Enter>` or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case changes take effect immediately without pressing `<Enter>`.

#### **Discriminator Level** edit box and spin control

Here the discriminator level of the Constant Fraction Discriminator (CFD) for the Sync input can be set. Units are millivolts (mV), the permitted range is 0 to 1,000. This corresponds to real input voltage levels of 0 to -1,000 mV. Type the value as an integer in the edit box and press `<Enter>` or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case changes take effect immediately without pressing `<Enter>`.

#### **Offset** edit box and spin control

Shifts the relative timing of Sync and photon events and is designed to compensate optical and electrical delays (e.g. in cables and optical paths), e.g. in order to shift your fluorescence decay within the sync frame so that it is not truncated. This feature completely eliminates the need for adjustable delay boxes.

Units are picoseconds (ps), the permitted range is from -99,999 ps to +99,999 ps. Type the value as an integer in the edit box and press `<Enter>` or click on *Apply*. The offset value can be entered in steps of picoseconds. Alternatively, use the spin control next to the edit box to increment / decrement the current value. In this case changes take effect immediately without pressing `<Enter>`.

#### **Sync Divider** edit box and spin control

Here the programmable divider of the Sync input can be set. This allows to reduce the sync input rate so that the period is at least as long as the dead time. This is required for fast sync sources (>12.5 MHz) Internal logic determines the sync period and re-calculates the sync signals that were divided out. It must be noted that this only works with stable sync sources that provide a constant pulse-to-pulse period, but all fast laser sources known today meet this requirement within an error band of a few picoseconds. With random and low rate signals (< 1 MHz), the divider must be set to 'None'.

### 6.4.2. Inputs 1...4

The settings in this group configure the behaviour of the input channels 1..4. See section 5.1 “Setting Up the Input Channels” to get started. The HydraHarp device is modular and can have up to 8 input channels. In order to avoid excessive space requirements as well as unpleasant clutter in the controls for all those channels, they are organized in groups of 4. This page holds the controls for the input channels 1..4. If only two channels are installed, only the control elements for channels 1..2 will be accessible. If more than 4 channels are installed, the control elements of channels 5..8 will be located in a separate page of similar layout (see below). The specific controls for each channel on this page are described below.

**Zero Cross** edit box and spin control

Here the zero cross level of the Constant Fraction Discriminator (CFD) for the Input Channels 1...4 can be set. Units are millivolts (mV), the permitted range is 0 to 40. Type the value as an integer in the edit box and press <Enter> or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case changes take effect immediately without pressing <Enter>.

**Discriminator Level** edit box and spin control

Here the discriminator level of the Constant Fraction Discriminator (CFD) for the respective input channel can be set. Units are millivolts (mV), the permitted range is 0 to 1,000. This corresponds to real input voltage levels of 0 to -1,000 mV. Type the value as an integer in the edit box and press <Enter> or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case changes take effect immediately without pressing <Enter>.

**Offset** edit box and spin control

Shifts the relative timing of photon events and is designed to compensate optical and electrical delays (e.g. in cables and optical paths), e.g. in order to shift your fluorescence decay within the sync frame so that it is not truncated. This feature completely eliminates the need for adjustable delay boxes.

Units are picoseconds (ps), the permitted range is from -99,999 ps to +99,999 ps. Type the value as an integer in the edit box and press <Enter> or click on *Apply*. The offset value can be entered in steps of picoseconds. Alternatively, use the spin control next to the edit box to increment/decrement the current value. In this case changes take effect immediately without pressing <Enter>.

**All=Ch1** group of tickboxes

On the right hand side of the regular controls there is a special group "All=Ch1" with three tick boxes labelled "ZeroCr.", "Discr." and "Offset". When one of these is ticked, control of this parameter is coupled to the corresponding input setting for channel 1. All other channels will automatically take the same setting. This serves as a means of manipulating all channels at once, which can be helpful in the initial setup phase when there are a lot of channels that must be brought to identical standard settings. Later, when individual fine tuning is required, the tick can be removed and the individual channels can then be programmed individually again. Note that removing the tick does not bring the previous individual settings back. If you have lost some specially optimized settings you may load a data file with known good settings which will update the control panel accordingly. It is therefore a good idea to save a data file after optimizations have been completed and name it descriptively as something like "initial settings for experiment X".

### 6.4.3. Inputs 5...8

This page only exists when more than 4 input channels are installed. It has the same controls as the page for inputs 1...4 and follows the same logic of operation (as described above).

### 6.4.4. Acquisition

**Resolution** edit box and spin control

Use this set of input controls to specify the acquisition resolution. Units are Picoseconds (ps). Possible choices are the base resolution (1 ps) and binary multiples of this resolution (1, 2, 4, 8, 16, ... ps). Type the desired resolution value (ps) as an integer in the edit box and press <Enter> or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case, changes take effect immediately without pressing <Enter>. In case of entering values other than valid resolutions, the next suitable resolution step is chosen automatically.

There are always  $2^{16} = 65,536$  channels in one histogram. With the different resolutions, the approximate time ranges covered are between  $65,536 * 1 \text{ ps} = 65.5 \text{ ns}$  up to  $65,536 * 33,554,432 \text{ ps} = 2.199 \text{ s}$ . The choice of range must compromise between resolution and time span covered. The smallest range offers the best resolution and the shortest span (vice versa for the largest range). For sync rates > 20 MHz the highest resolution is usually most appropriate, since the smallest range still covers the full sync period. For lower sync rates the histogram range is too small to cover the full sync period. The decay curve region of interest may therefore lie outside the acquisition window of 65,536 time bins. Apart from switching to a lower resolution it is possible to shift

the acquisition window relative to the sync frame by means of the offset. Note that working with very long time spans (low sync rates) also requires long acquisition times to minimize noticeable "steps" in the acquired histograms.

#### **Acquisition Time** edit box and spin control

Set the desired measurement time here. Units are Seconds (s). The permitted range is 0.001 to 360,000 in steps of 0.001. Type the desired value in the edit box and press <Enter> or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case, changes take effect immediately without pressing <Enter>. The default increment / decrement per mouse click is logarithmic.

#### **Offset** edit box and spin control

For sync rates > 20 MHz the highest resolution is usually most appropriate, since the smallest range still covers the full sync period. The offset should in these cases always be set to 0.

For lower sync rates the histogram range may be too small to cover the full sync period. The decay curve region of interest may therefore lie outside the acquisition window of 65,536 channels. Apart from switching to a lower resolution it is possible to shift the acquisition window relative to the sync frame by means of setting the offset > 0.

Use this set of input controls to specify the desired offset. Units are Nanoseconds (ns). Possible offsets are in the range from 0 to 500000 ns. Type the value as an integer in the edit box and press <Enter> or click on *Apply*. The offset value can be entered in steps of nanoseconds. Alternatively, use the spin control next to the edit box to increment/decrement the current value. In this case changes take effect immediately without pressing <Enter>.

Internally the offset is subtracted from each start–stop measurement before it is used to address the histogram channel to be incremented or stored in a T3 mode record. Therefore, increasing the offset means shifting the signal on the screen to the left, towards earlier times.

Note that this offset has no effect in T2 mode and no effect on the relative timing of laser pulses and photon events. It merely shifts the region of interest where start–stop measurement data is to be collected. The relative timing of laser pulses and photon events can only be controlled by means of the input offsets or physical delays (optical or electrical) e.g. in order to shift your fluorescence decay within the sync frame so that it is not truncated.

#### **Trc./Block** trace colour indicator, edit box and spin control

The HydraHarp can measure histograms in up to 512 memory blocks. Out of these, up to 8 curves can be displayed and one "active block" can be used for measurements. Choose the active memory block you wish to use for the next measurement here. The trace mapping dialog is used to select the curves for display. Make sure the curve you are using is switched on (*shown*). You can open this dialog directly by clicking on the trace colour indicator. The colour indicator shows the trace colour the chosen block is currently mapped to. If it is a solid square, the curve is mapped and shown. If it is mapped but not shown, the indicator shows a small striped square. If the curve is not even mapped for display the indicator remains white. Make sure not to overwrite existing data in a block that was used before. In order to warn you if a trace is used, the heading of the Trc/Block selector will turn red.

#### **Restart** check box

If this box is ticked (checked) the measurement will automatically restart after the acquisition time has elapsed. Toggle the current setting with a mouse click. The setting is without effect in TRES mode.

#### **Stop on Overflow** check box

If this box is ticked (checked) the measurement will stop on overflow of any histogram bin. The overflow limit is set by the "*Stop At*" parameter. Toggle the current setting with a mouse click.

#### **Mode** selection box

You may here select between three different acquisition modes:

**Oscilloscope Mode:**

The selection *Oscilloscope* from the selection box "Mode" selects the current acquisition mode *Oscilloscope* for the HydraHarp. In oscilloscope mode the acquisition and display, once started, repeats at intervals given by the current acquisition time setting. The histogram accumulation always starts from scratch. This is useful for monitoring fast changes during optical alignment etc.

**Integration Mode:**

As opposed to oscilloscope mode, the histogram acquisition in integration mode is not reset to zero with each display refresh. The histogram continues to grow while the display is updated every 0.1 to 1 seconds determined by the refresh rate value selected in the *General Settings Dialog*. Acquisition can be manually started and stopped, additionally the option "*Stop on Overflow*" will stop the acquisition when the maximum count of any channel overflows. The option "*Restart*" will cause the acquisition to start again after the acquisition time has elapsed. This is similar to oscilloscope mode but allows watching the histogram grow if the acquisition time is fairly long.

**TRES Mode:**

Selects the measurement mode TRES (Time-Resolved Emission Spectra). This requires a monochromator and corresponding driver hardware / software to be installed. See section 5.3.7.

**Stop At** edit box and spin control

Here the stop count level for histogramming can be set. The setting is only meaningful if the check box "*Stop Off*" is active. Type the value as an integer in the edit box and press <Enter> or click on *Apply*. Alternatively, use the spin control (next to it) to increment / decrement the current value. In this case changes take effect immediately without pressing <Enter>. The minimum is 0. If the entered value exceeds the allowed maximum of 4,294,967,295, an error message appears. When using the spin control exceeding the maximum is simply not allowed. Instead of incrementing the value further, the last permitted value remains active.

## 6.5. Axis Panel

The controls of the *Axis Panel* are used to customise the main window's curve display.

### **Apply**-button

All mouse actions on the spin controls on this dialog result in instant modification of the display. If you choose to alter the values in the edit fields by keyboard, you have to finish your changes by pressing `<Enter>` or to click the *Apply*-button.

All other controls are grouped as follows:

### 6.5.1. Time Axis Group

Note that the time axis labelling is precise in terms of relative information only. At `offset=0` the absolute time difference between the inputs is close to the shown times but may be off by some tens of picoseconds. Offsets `>0` change the placement of the acquisition window and the true time differences are then off accordingly.

#### **Minimum** edit box and spin control

Here the start of the displayed time axis can be set. Units are nanoseconds (ns). The minimum is 0. If the entered value exceeds the current time axis maximum, an error message appears. When using the spin control exceeding the current time axis maximum is simply not allowed. Instead of incrementing the minimum value further, the last permitted value remains active. Observe the notes on time axis interpretation given in the paragraph *Time Axis Group* above.

#### **Maximum** edit box and spin control

Here the end of the displayed time axis can be set. Units are nanoseconds (ns). The maximum allowed here is 2.2 s. If the entered value becomes smaller than the current time axis minimum, an error message appears. When using the spin control, violating the current time axis minimum is automatically prevented. Instead of decrementing the minimum value further, the last permitted value remains active. Observe the notes on time axis interpretation given in the paragraph *Time Axis Group* above.

The time axis settings will determine the number of time bins exported via clipboard copy / paste. Only the bins within the displayed time range will be used.

### 6.5.2. Count Axis Group

#### **Minimum** edit box and spin control

Here the start of the displayed count axis can be set. Units are counts. In linear scale mode the default increment / decrement is 100. In log scale mode it is one decade. If the minimum is set to 0 in logarithmic mode, the resulting minimum as displayed is  $10^{-1}$ . If the entered value exceeds the current count axis maximum, an error message appears. When using the spin control, exceeding the current count axis maximum is automatically prevented. Instead of incrementing the minimum value further, the last permitted value remains active.

#### **Maximum** edit box and spin control

Here the upper end of the displayed count axis can be set. Units are counts. In linear scale mode the default increment / decrement is 100. In log scale mode it is one decade. If the entered value becomes smaller than the current time axis minimum, an error message appears. When using the spin control, violating the current count axis minimum is prevented automatically. Instead of decrementing the minimum value further, the last permitted value remains active.

#### **Lin / Log** radio buttons

These radio buttons change the inner scaling of the count axis from linear to logarithmic and vice versa. Despite the fact that in logarithmic scale the display will always show whole decades, the change of the scaling is applied without modifying the minimum / maximum range.

## 6.6. Trace Mapping Dialog

The HydraHarp software can measure histograms in up to 512 memory blocks. Out of these, up to 8 curves can be displayed. The trace mapping dialog is used to select the curves to display. Tick the individual boxes 'Show' to display a curve. Select the number of the memory block you wish to map the individual display curve to. The Trace Mapping Dialog also provides some statistics on each curve. These items are:

<i>FWHM</i>	The Full Width Half Maximum of the curve peak (usually for IRF traces)
<i>Max Count</i>	The count in the highest point of the curve
<i>At Time</i>	The time corresponding to the <i>Max Count</i> channel
<i>Resolution</i>	The channel width (time bin width) of the curve

Furthermore there are several buttons:

<i>Details</i>	Can be clicked to see more curve information
<i>All</i>	Can be clicked to tick all traces as shown
<i>0..7</i>	Can be clicked to set the default mapping of trace 0..7 to block 0..7
<i>Clear (trash can)</i>	Can be clicked to delete the contents of individual blocks

The trace mapping dialog can be launched from the corresponding button on the toolbar as well as through the trace colour indicator on the control panel.

## 6.7. General Settings Dialog

Use this dialog to change standard settings of the HydraHarp software. Notably these are: *Prompt overwrite* (warning before overwriting existing data) and *TTTR Marker Settings*. The control connector of the HydraHarp 400 provides TTL inputs for synchronization signals. The markers can be enabled resp. disabled and recorded at the rising or falling edge of the corresponding TTL signal. The active edges can be chosen here. All settings will be kept in the Windows registry and will be retrieved at the next program start. They will be individual to each user.

The dialog's controls are grouped as follows:

### Display Group

<i>Display Rate /s</i>	Sets the time interval between display updates from (0.1s to 1s).
<i>Draw mode</i>	Switches between different curve draw-modi (Lines / Stairs).
<i>Grid</i>	Checked: The curve display is grated with a light-grey grid Unchecked: The axes of the curve display are marked with ticks

### File Saving Group

<i>Prompt overwrite</i>	Activate this check box if you wish to be prompted before saving data over existing data in a file (recommended).
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### Warnings Group

Here you can selectively enable/disable individual warnings. Activate the check box for each warning you wish to receive. See section 8.1 for details.

### TTTR Marker Settings Group

Here you can set the signal specifications for external marker signals used in TTTR mode (T2 and T3 mode). The settings for the different markers are independent from each other. The following table applies to each of the four markers:

<i>enable</i>	Check this if you want the marker to be included into the TTTR data stream.
<i>rising / falling</i>	Radio buttons to identify the active edge of the signal.

### Reference Clock Source Group

The setting in this group takes effect after the next software start.

<i>internal / external</i>	Select whether the HydraHarp uses its internal clock or an external 10 MHz clock supplied at the REF IN connector.
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## 6.8. About HydraHarp... Dialog

This dialog provides version information on the HydraHarp software and hardware, the latter only if it is installed and operational. It can be opened via the Help menu or via the toolbar. The button *Request Support* opens a small text viewer window that provides a listing of HydraHarp hardware and software versions that you can copy and paste into your support enquiry. Note that this information is very important for adequate support. Support requests without this information cannot be processed and will be delayed by return questions for this information. If your system is not functional at all, the minimum information you must provide for support is the serial number of your HydraHarp. It can be found at the back of the housing. The dialog also provides buttons for links to relevant web pages and a button for download of software updates. Note that downloading a software update does not automatically install it. Typically the downloads contain zip files that must be unpacked and installed manually. Also note that it may not always be advisable to blindly install the latest software. This is the case especially when a user or his/her laboratory have developed custom software that relies on a certain file format. Updates may break such compatibility. Therefore, always study the release notes before installing.

## 6.9. Title and Comment Editor

You can use this dialog to edit the file title / comment. It can be opened via double-click on the title, via the *File* menu or via the *Print Preview* Toolbar. The text you enter here will be stored in the data file. The first line will be displayed as the file title over the histogram display area. The text you can enter here is limited to 4 lines and 255 characters. Upon loading of a data file, the title and comment will also be retrieved. It will also be included in prints, if the corresponding check mark is set in the page setup dialog box available through the *File* menu.

## 6.10. Print Preview Dialog

Use this Dialog to preview the layout as it would appear when printed. When you choose this command, the main window will be replaced with a print preview window in which one or two pages will be displayed in their printed format. The print preview dialog offers the following options:

### Print

Bring up the print dialog box, to start a print job.

### Next Page

Preview the next printed page.

### Prev Page

Preview the previous printed page.

### One Page / Two Page

Preview one or two printed pages at a time.

### Zoom In

Take a closer look at the printed page.

### Zoom Out

Take a larger look at the printed page.

### Page Setup

Change the layout of the printed page.

**Close**

Return from print preview to the editing window.



## 7. Problems, Tips & Tricks

### 7.1. PC Performance Issues

The HydraHarp device and its software interface are a complex real-time measurement system demanding appropriate performance both from the host PC and the operating system. This is why a fairly modern CPU and sufficient memory are required. The screen resolution should be at least 800x600. At least a 1.5 GHz dual core processor, 4 GB of memory and a fast hard disk are recommended.

In order to maintain correct interaction between the measurement hardware and the display of histogram curves and user input, the operating system's message passing mechanism is used. It is recommended not to overload the system by running other processes in the background while measuring with the HydraHarp. The PC's own occasional network activity should be no problem but running the machine e.g. as a server for other PCs is not recommended. In principle, of course, any kind of background activity is allowed. Should the system become overloaded the HydraHarp histograms will not be "wrong" or distorted, but measurement times and display rates may become irregular. You can even iconize the HydraHarp during measurements without doing damage. This may be of interest for lengthy measurements in integration mode, where one is only interested in accumulating a certain amount of counts without need for permanent monitoring. The "panel meters" showing the current count rates are not meant to be 100 % accurate. They merely serve as an aid for setting up the system. Some of them may suffer from system overload. Accordingly the values shown in the curve details (trace mapping dialog) are subject to such tolerances.

In TTTR mode, system overload will manifest itself in loss of data. Here no other background processing should be allowed. The faster the PC, the less these issues will matter. TTTR mode performance (i.e. max. throughput without loss) may also be improved by de-fragmenting the hard disk before long measurements.

### 7.2. USB interface

In order to deliver maximum throughput, the HydraHarp 400 uses (dependent on the model you purchased) USB 2.0 or USB 3.0 bulk transfers. Correspondingly, the HydraHarp must rely on having a suitable host interface of appropriate speed rating. USB 2.0 and 3.x host controllers of modern PCs are usually integrated on the mainboard. Otherwise USB 3.x interfaces can be upgraded as PCIe cards. Make sure that the host controller drivers are correctly installed and up to date. This may require using the mainboard manufacturer's driver disk rather than relying on Windows drivers. Note that some of the early USB 3.0 host controllers had immature drivers and possibly flawed firmware. Check the mainboard manufacturer's website for updates if you encounter problems. For best performance, do not run other bandwidth demanding devices on the same USB interface when working with the HydraHarp. USB cables must be rated for the intended USB speed. Older cables do not meet this requirement and can lead to errors and malfunction. Similarly, some PCs have poor internal USB cabling, so that USB sockets at the front of the PC are often unreliable.

### 7.3. Histogram Artefacts

Disturbing histogram ripple is strongly dependent on the quality of the input signals. Try to deliver the best possible signal quality with clean and steep active edges and without overshoot and ringing. It is recommended to use detector and sync signals of similar amplitude to avoid excess crosstalk. If the PDL 800-B picosecond diode laser is used, attenuating the sync pulses by 10 dB immediately at the laser driver may reduce histogram ripple (set CFD level accordingly). Always use good quality 50  $\Omega$  components and coax leads with proper shielding and correct termination. We recommend cables of type RG223 with double braided and silver plated screen. Check your setup for ground loops. Grounding different system components (PC, detector, detector power supply, diode laser driver, etc.) at different points can induce considerable noise in the ground lines. Because signal return paths may share the same ground lines, this noise is copied into the signal lines and causes increased timing jitter and / or histogram ripple. Network cables and mobile phones may also be sources of surprising noise. Use properly impedance matched power splitters (reflection-free T-pads) if signals must be fed to multiple 50  $\Omega$  inputs. Never use ordinary Tees. All accessories are available from PicoQuant. PMT detectors should be connected through a suitable high speed preamplifier (available from PicoQuant). MCP-PMT detectors should be connected through an amplifier with slightly higher gain (e.g. PAM 102-M from PicoQuant). TTL-SPAD-detectors (e.g. Excelitas SPCM-AQR) must be connected through a pulse inverter (PicoQuant SIA 400).

A problem often overlooked is pile-up and dead time related histogram distortion. It becomes noticeable if the detector count rate exceeds 2 % of the sync rate. This is why high excitation rates are so important. Pile-up is an inherent problem of TCSPC and not a fault of the hardware. The only way to handle it safely is to maintain count rates < 1% to 2 % of the sync rate. Pile-up may to some extent also be corrected for, but this can be extremely difficult in practice. For further details see our TechNote on TCSPC or the literature given in the end of section 2.4.

## 7.4. Warming Up and Calibration

Observe the warm-up period of at least 20 minutes (dep. on ambient temperature) before using the HydraHarp for serious measurements. Warming up takes about 20 to 30 minutes dependent on the lab temperature. It is completed when the cooling fan starts to run for the first time. You can use this time for set-up and preliminary measurements. Calibrate one more time before starting serious measurements. The calibration takes only a few seconds. For very long measurements allow some more time for thermal stabilization and avoid temperature changes > 5 K in the environment of the instrument. The permissible ambient temperature is 15°C to 35°C. Do not obstruct the cooling fan at the back and the air inlets at the bottom of the housing. Make sure that the cooling air can circulate freely and no other hot instrument is under the cooling inlets.

## 7.5. Custom Programming of the HydraHarp

A programmer's library (DLL) for custom Windows software development is available to build your own applications e.g. in LabVIEW, Matlab, C++, C#, Delphi, Lazarus, Python or Visual Basic. A rich set of demo code is provided for an easy start. A Library for Linux is also available. It provides the same interface so that applications can easily be ported between the platforms.

## 7.6. Software Updates

We constantly improve and update the software for our instruments. This includes updates of the configurable hardware (FPGA). Such updates are important as they may affect reliability and interoperability with other products. The software updates are free of charge, unless major new functionality is added. It is strongly recommended that you check for software updates regularly. You can go through the Web, alternatively you can click the button 'Check for Updates' in the Help-About dialog available through the main menu or the toolbar. Note that downloading a software update by means of this button does not automatically install it. Typically the downloads contain zip files that must be unpacked and installed manually. Also note that it may not always be advisable to blindly install the latest software. This is the case especially when a user or his/her laboratory have developed custom software that relies on a certain feature. Updates may break this compatibility. Therefore, always study the release notes before installing updates.

## 7.7. Support and Bug Reports

The HydraHarp 400 TCSPC system has gone through extensive testing. Nevertheless, it is a very cutting edge product and we would like to offer you our support in any case of problems with the system. Do not hesitate to contact PicoQuant in case of difficulties with your HydraHarp.

As a first step it is always advisable to study the manual or to click the help button. Should you observe persistent errors or bugs of the HydraHarp system please try to find a reproducible error situation. Then open *Help - Request Support*. This dialog provides version information on the HydraHarp software and hardware (the latter only if it is installed and operational). It can be opened via the Help menu or via the button *Request Support* in the *About HydraHarp* dialog. A small text viewer window then provides a listing of HydraHarp hardware and software version details that you can copy and paste into your support enquiry. Note that this information is very important for adequate troubleshooting. Support requests without this information cannot be processed and will be delayed by return questions for this information. If your system is not functional at all, the minimum information you must provide for support is the serial number of your HydraHarp. It can be found at the back of the housing. There may be other relevant circumstances, especially other new hardware installed in your PC, so please provide details. You can run *msinfo32* to obtain a listing of your PC configuration and attach the summary file to your error report to [www.picoquant.com/contact/support](http://www.picoquant.com/contact/support). If you cannot access the web form directly, please contact [support@picoquant.com](mailto:support@picoquant.com) and attach the same information to your email. Your feedback will help us to improve the product and documentation.

If you encounter serious problems that require the device to be sent in for inspection / repair, please request an RMA number before shipping the hardware. Unexpected shippings without this number may be rejected. Observe precautions against static discharge, moisture, and physical damage under all circumstances in handling, packaging and shipping. Use original or equally protective packaging material.

Of course we also appreciate good news: If you have obtained exciting results with one of our systems, please let us know, and where appropriate, please mention the instrument in your publications. The simplest way of doing this in case of the HydraHarp 400 is citing the original publication about the instrument<sup>1</sup>. At our web-site we also maintain a large bibliography of publications referring to our instruments. It may serve as a reference for you and other potential users. See <http://www.picoquant.com/scientific/references>. Please submit your publications for addition to this list.

1 Wahl M., Rahn H.-J., Röhlicke T., Kell G., Nettels D., Hillger F., Schuler B., Erdmann R.: Scalable time-correlated photon counting system with multiple independent input channels. *Review of Scientific Instruments*, Vol.79, 123113 (2008)

## 8. Appendix

### 8.1. Warnings

When the HydraHarp software is running with functional hardware it continuously collects information about the input signals and the current acquisition settings. If these settings together with the input rates indicate possible errors, the software will indicate this by means of a warning icon.



While the software is running in interactive histogramming mode the warning icon is displayed at the bottom of the main window in the status bar. In the case of TTR mode, it will appear directly in the TTR mode dialog. The icon can be clicked to display a list of current warnings together with a brief explanation of each warning.

Note that the software can detect only a subset of all possible error conditions. It is therefore not safe to assume “all is right” just by seeing no warning. On the opposite, if any of the warnings turns out to be an unnecessary nuisance, e.g. because your specific measurement conditions knowingly force it, you can disable that warning via the general settings dialog (see section 6.7).

The warnings are to some extent dependent on the current measurement mode. Not all warnings will occur in all measurement modes. Also, count rate limits for a specific warning may be different in different modes. The following table lists the possible warnings in the three measurement modes and gives some explanation as to their possible cause and consequences.

Warning	Histo Mode	T2 Mode	T3 Mode
<p><b>WARNING_SYNC_RATE_ZERO</b></p> <p>No counts are detected at the sync input. In histogramming and T3 mode this is crucial and the measurement will not work without this signal. Note that at very low sync rates this warning may occur spuriously due to the rate meter limitations. You may want to switch it off in this case.</p>	√		√
<p><b>WARNING_SYNC_RATE_TOO_LOW</b></p> <p>This warning should not occur with current software and firmware. It is kept in this list for backward compatibility only.</p> <p>Note, however, that there actually is a minimum sync rate of about 0.25 Hz in histogramming and T3 mode. Due to the lower limit of the rate meters this condition cannot be detected, hence no warning is issued.</p>			
<p><b>WARNING_SYNC_RATE_TOO_HIGH</b></p> <p>In histogramming or T3 mode:</p> <p>The divided pulse rate at the sync input is higher than 12.5 MHz (deadtime limit). Use a larger divider if possible.</p> <p>In T2 mode:</p> <p>The pulse rate at the sync input is higher than 9 MHz (bus limit if running at USB 2.0 speed) or higher than 12.5 MHz after the divider (deadtime limit if running at USB 3.0 speed). The most common reason for this error is that a laser sync signal is still connected. T2 mode is normally intended to be used without a sync signal. There are some rare measurement scenarios where this condition is expected and the warning can be disabled. Examples are measurements where the FIFO can absorb all data of interest before it overflows.</p>	√	√	√

Warning	Histo Mode	T2 Mode	T3 Mode
<p><b>WARNING_INPT_RATE_ZERO</b></p> <p>No counts are detected at any of the input channels. In histogramming and T3 mode these are the photon event channels and the measurement will yield nothing. You might sporadically see this warning if your detector has a very low dark count rate and is blocked by a shutter. In that case you may want to disable this warning.</p>	√		√
<p><b>WARNING_INPT_RATE_TOO_HIGH</b></p> <p>You have selected T2 or T3 mode and the overall pulse rate at the input channels is higher than the USB speed limit (9 MHz with USB 2.0 connection or 40 MHz with USB 3.0 connection). The measurement will inevitably lead to a FIFO overrun. There are some rare measurement scenarios where this condition is expected and the warning can be disabled. Examples are measurements where the FIFO can absorb all data of interest before it overflows.</p>		√	√
<p><b>WARNING_INPT_RATE_RATIO</b></p> <p>This warning is issued in histogramming and T3 mode when the rate at any input channel is higher than 5% of the sync rate. This is the classical pile-up criterion. It will lead to noticeable dead-time artefacts. There are rare measurement scenarios where this condition is expected and the warning can be disabled. Examples are antibunching measurements.</p>	√		√
<p><b>WARNING_DIVIDER_GREATER_ONE</b></p> <p>You have selected T2 mode and the sync divider is set larger than 1. This is probably not intended. The sync divider is designed primarily for high sync rates from lasers and requires a fixed pulse rate at the sync input. In that case you should use T3 mode. If the signal at the sync input is from a photon detector (coincidence correlation etc.) a divider &gt; 1 will lead to unexpected results. There might be some rare measurement scenarios where this condition is intentional and the warning can be disabled.</p>		√	
<p><b>WARNING_TIME_SPAN_TOO_SMALL</b></p> <p>This warning is issued in histogramming and T3 mode when the sync period (1/SyncRate) is longer than the start to stop time span that can be covered by the histogram or by the T3 mode records. You can calculate this time span as follows:                      Histogramming mode: Span = Resolution * 65536                      T3 mode: Span = Resolution * 32768                      Events outside this span will not be recorded. There are some measurement scenarios where this condition is intentional and the warning can be disabled.</p>	√		√
<p><b>WARNING_OFFSET_UNNECESSARY</b></p> <p>This warning is issued in histogramming and T3 mode when an offset &gt;0 is set even though the sync period (1/SyncRate) can be covered by the measurement time span without using an offset. The offset may lead to events getting discarded. There are some measurement scenarios where this condition is intentional and the warning can be disabled.</p>	√		√

If any of the warnings you receive indicate wrong pulse rates, the cause may be inappropriate input settings,

wrong pulse polarities, poor pulse shapes or bad connections. If in doubt, check all signals with an oscilloscope of sufficient bandwidth.

## 8.2. Data File Formats

While for many purposes the ASCII export of histograms to files or to the clipboard is sufficient and easy, you also may want to access the HydraHarp data files via custom programs. This section provides only a brief overview on the file format. For details please refer to the online help file available via the help menu.

To overcome certain limitations of the formats used in the past, the latest HydraHarp software uses a new file format previously proven in our SymPhoTime 64 software. It is designed to be future proof in the sense that files created by the current software version stay valid for future software revisions and, moreover, files created by future software versions will most likely still be readable by older software, although they might contain information, that software can't even "know" about. This is achieved by using a tagged format. Tags identify the data to follow, and give the type, length and even meta information. The exact location of an individual item in the file is then irrelevant. Version robustness is granted as long as version-breaking changes to the semantics of a given field are implemented by a tag with a new identifier rather than expanding the range or interpretation of the old one. The list of tags (identifiers) and their interpretation rules can be kept in a tag dictionary. With this as a precondition, the software only has to show tolerance on missing non-mandatory (i.e. optional) content.

The new format definition unifies PicoQuant's existing file formats which individually evolved over many years. The resulting new TTR file format with the extension `*.ptu` will be used for all current and future TCSPC products supporting TTR mode and enriches them by powerful new features. Similarly, a tagged file format with the extension `*.phu` will cover the histogram data formats of our current and future TCSPC products.

The new format may at first glance look more complicated than previous versions with hard coded structure. Nevertheless, we hope that the benefits of version tolerance and "durability" will outweigh the complications. To support understanding of the format and implementation of custom software accessing these files, a set of demos is provided in the subfolder `\Filedemo` in your chosen installation folder. If you need to evaluate more header items than the demos do, please refer to the HydraHarp online help file available via the help menu. A file format related html help file is also provided in the file demo folder. It contains a list of tag types and a tag dictionary that explains the individual items. Note that the dictionary contains more items than the HydraHarp software actually uses. It is recommended to go by a specific file, have one of the demos read it and then look at the list of header items you get. You can also use the PicoQuant File Info shell extension that will be installed by the HydraHarp software setup to inspect individual header items of a `*.ptu` or `*.phu` file. Just right-click on the file in Windows explorer and select *Properties*.

For backward compatibility version 3.0 of the HydraHarp software can still read old files of format version 2.0 (`*.hhd`, `*.ht3`). Note, however, that saving such files converts them to the new format. Despite the intended version tolerance of the tagged format, for consistency and safe version checking the new HydraHarp data files still carry a format version number, which is now called *content version* and currently has the string value "3.0". In order to identify a HydraHarp data file as a file created by and to be used by the native HydraHarp software there is a tag *assured content* which begins with the string "HydraHarp". There is also a pair of tags creator name and creator version that identify the creating software. Programmers of custom software writing such files **MUST USE THEIR OWN CREATOR NAME**.

Note that despite our best efforts towards version tolerance, file formats in future software releases are subject to change without notice.

### 8.2.1. Interactive Histogramming Mode File Format

The standard HydraHarp histogram data files created by the HydraHarp software in interactive histogramming mode (`*.phu`) are tagged binary files which contain both the setup parameters and the actual histogram data. The latter can be present multiple times, i.e. multiple measurements can be stored in one file. Relevant settings are stored for each measurement separately. In order to identify a HydraHarp data file as a file created by and to be used by the native HydraHarp software, a program reading in these files can read the tag *assured content* which begins with the string "HydraHarp". However, a piece of software aiming solely at retrieving the histogram data content can (and should) be tolerant about this tag and go for the pure histogram data. This tolerance will ensure compatibility for the future. Indeed, the demos in the subfolder `\Filedemo` in your chosen installation directory are following this tolerant approach. For more information on individual file tags and their content, please consult the online help file available via the help menu.

## 8.2.2. TTTR Mode File Format

HydraHarp data files from T2 and T3 Mode (\*.ptu) created by the HydraHarp software are tagged binary files which contain both the setup parameters and the actual event data. There can be only one measurement per file. The setup data in the file header which is similar to that in standard interactive mode files. In order to identify a HydraHarp data file as a file created by and to be used by the native HydraHarp software, a program reading in these files can read the tag *assured content* which begins with the string "HydraHarp". However, a piece of software aiming solely at retrieving the histogram data content can (and should be) be tolerant about this tag and go for the pure event record data. This tolerance will ensure compatibility for the future. Indeed, the demos in the subfolder `\Filedemo` in your chosen installation directory are following this tolerant approach. For more information on individual file tags and their content, please consult the online help file available via the help menu. It is worth noting that the actual TTTR record data following the file header has the same structure as that of the previous file format version 2.0 (\*.ht2, \*.ht3) and corresponds directly to the raw data obtained with custom programs using the HydraHarp programming library.



## 8.3. Hardware Technical Data

### 8.3.1. Specifications

All information given here is reliable to our best knowledge. However, no responsibility is assumed for possible inaccuracies or omissions. Specifications and external appearance are subject to change without notice.

#### Input Channels and Sync

Trigger principle	Vertex Finding CFD software adjustable
Trigger point	Falling edge (negative going)
Input voltage operating range (pulse peak, 50 $\Omega$ ) optimum:	0 mV ... -1 000 mV -100 mV ... -500 mV
Input voltage max. range (damage level)	$\pm 1\,500$ mV
CFD level adjust	0 mV ... -1 000 mV
CFD zero cross adjust	0 mV ... 40 mV
Trigger pulse width	0.5 ns ... 30 ns
Trigger pulse rise/fall time	$\leq 2$ ns
Max. count rate	$12.5 \cdot 10^6$ cps
Max. sync rate without divider	12.5 MHz
Max. sync rate with divider 1/16	150 MHz
Adjustable delay range for each input channel resolution:	$\pm 100$ ns 1 ps
Marker and Control inputs	standard TTL, 100 k $\Omega$ DC load

#### Time to Digital Converter

Base resolution (min. time bin width)	1 ps
Uncertainty of time difference measurements (rms)	$< 12 \text{ ps} + 10^{-8} \cdot \Delta t$
Differential non-linearity (full measurement range)	$< 2.0\%$ p.p. $< 0.2\%$ rms
Dead time	$< 80$ ns

#### External Reference Clock

Input	10 MHz 200 mV ... 1 000 mV p.p. 50 $\Omega$ ; AC coupled
Output	10 MHz 300 mV p.p. 50 $\Omega$ ; AC coupled

#### Histogrammer

Maximum number of time bins	65 536
Bin widths	1, 2, 4, 8, ..., 33 554 432 ps
Measurement ranges	65 536 x 1 ps ... 65 536 x 33.55 $\mu$ s
Count depth per time bin	4 294 967 296 (32 bit)
Acquisition time	1 ms ... 100 h

**TTTR Engine**

T2 mode resolution			1 ps
T3 mode resolution			1, 2, 4, 8, ..., 33 554 432 ps
FIFO buffer depth (records)			2 097 152
Sustained throughput limit (sum of all channels)*	USB 2.0	typ.	$9 \cdot 10^6$ events/s
* depends on host PC configuration and performance	USB 3.0	typ.	$40 \cdot 10^6$ events/s
Acquisition time			1 ms ... 100 h
External Marker input pulse duration			> 50 ns
External Marker time recording error in T2 mode			< 100 ns
External Marker time recording error in T3 mode			< Tsync + 100ns

**Operating Environments**

Ambient temperature			15°C ... 35°C 59°F ... 95°F
Relative humidity			< 80% non-condensing
Warm-up period			≥ 20 min
Bus interface			Model dependent: USB 2.0 or USB 3.0
Recommended PC specs			
CPU cores			≥ 2
CPU clock			≥ 1.5 GHz
RAM			≥ 4 GB
Operating system			Windows 8.1 / 10 / 11

**Power Consumption**

Line 100 V ... 240 V AC			small frame ≤ 50W, large frame ≤ 100W
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**Dimensions**

Small frame (up to 4 channels)			310 × 235 × 140 mm
Large frame (up to 8 channels)			310 × 340 × 140 mm

**Retraction of old Devices**

Waste electrical products must not be disposed of with household waste.

This equipment should be taken to your local recycling centre for safe treatment.



WEEE-Reg.-Nr. DE 96457402

## 8.3.2. Connectors

Apart from the SMA connectors for the input signals the HydraHarp 400 provides several connectors for other purposes such as experiment control. The following table lists their function and signal types, sorted by the module they belong to. For details on the signal specifications (voltages, pulse widths etc.) see section 8.3.1.

All SMA connectors are designed for 50 Ohms coaxial cable connections and are terminated accordingly. All Lemo connectors are for high impedance TTL signals and not terminated. In case of TTL outputs they must never be connected to loads with 50 Ohms termination.

HDU 404 (CONTROL)			
Connector Label	Connector Type	Signal Type	Function
USB 2.0	USB 2.0 Type B receptacle	USB 2.0	host communication
CAN	Sub-D 9 pin male	CAN Bus	control of external devices (proprietary)
HDU 504 (CONTROL)			
Connector Label	Connector Type	Signal Type	Function
USB 3.0	USB 3.0 Type B receptacle	USB 3.0	host communication
HSR 110 (SYNC)			
Connector Label	Connector Type	Signal Type	Function
REF IN	SMA female	10 MHz clock in	external reference clock, e.g. from a frequency normal or atomic clock, or from another HydraHarp 400
REF OUT	SMA female	10 MHz clock out	reference clock output e.g. for another HydraHarp 400
SYNC IN	SMA female	NIM in	timing signal from sync source (typically a laser)
M1 ... M4	NIM-CAMAC receptacle Lemo ERN.00.250**	TTL in	external markers for external device synchronization, e.g. line and frame in image scanning (select active edge and enabling via software)
C1 ... C2	NIM-CAMAC receptacle Lemo ERN.00.250**	TTL in	experiment control (requires dedicated software)
A	NIM-CAMAC receptacle Lemo ERN.00.250**	TTL out (active low)	'active' signal for use by external hardware (must not be loaded in excess of 10 mA)
TDC 210 (TIMING)			
Connector Label	Connector Type	Signal Type	Function
IN	SMA female	NIM in	timing signal input (typically from a photon detector)*
ENA	NIM-CAMAC receptacle Lemo ERN.00.250**	TTL in	count enabling of the corresponding input* (high or open = enabled, low = disabled)

\*Note: The HydraHarp software enumerates the physical input channels as logical input channels 1 to N, where N depends on the number of installed TDC modules. The mapping of physical to logical channels is obtained by counting as follows: start from the leftmost TDC module and count top to bottom within each TDC module. At the time of factory assembly this mapping will be marked by plastic labels at each IN connector.

\*\* Suitable plugs from the Lemo catalog are FFA.00.250.xxx. Alternatively the BNC adaptor ABF.00.250.CTA can be used.

### 8.3.3. Indicators

The HydraHarp 400 front panel provides several LED indicators signalling the instrument status. The following table lists their function, sorted by the module they belong to. Note that precise status information should be obtained from the software.

<b>HDU 404 (CONTROL)</b>		
<b>LED Label</b>	<b>LED Function</b>	<b>Colour Interpretation</b>
POWER	power status of entire instrument	green = OK dark = failure
STATUS	status of control unit	green = OK red = permanent: error (software will report details) brief flash: transient state, no error
<b>HDU 504 (CONTROL)</b>		
<b>LED Label</b>	<b>LED Function</b>	<b>Colour Interpretation</b>
POWER	power status of entire instrument	green = OK dark = failure
STATUS	status of control unit	green = OK red = permanent: error (software will report details) brief flash: transient state, no error
USB	status of USB connection	green = USB 3.0 super speed orange = USB 2.0 high speed red = no connection or error flicker = data transfer in progress (note that fast flicker may appear like orange light)
<b>HSR 110 (SYNC)</b>		
<b>LED Label</b>	<b>LED Function</b>	<b>Colour Interpretation</b>
RDY	status of sync unit	green = OK orange = thermal stabilization and subsequent calibration not yet completed red = error (software will report details)
REF	status of reference clock	green = OK red = error (probably external reference missing)
<b>TDC 210 (TIMING)</b>		
<b>LED Label</b>	<b>LED Function</b>	<b>Colour Interpretation</b>
RDY	status of timing unit	green = OK orange = thermal stabilization and subsequent calibration not yet completed red = error (software will report details)

## 8.4. Using the Software under Linux

The HydraHarp software can also be used under Linux (x86 platform only). This requires that Wine is installed (see <http://www.winehq.org>). You can run the regular software setup as explained in section 3.2. Instead of installing a device driver, running under Linux with Wine requires that you have Libusb 1.0 installed (see <https://libusb.info/>). Because HydraHarp.exe is a 32-bit application you will need the 32-bit version. We have successfully tested under Wine 5.0 and 7.0 with Libusb 1.0.23 on Ubuntu 20.04 LTS and Linux Mint 20.2. In all cases we used the package `winehq-stable` which is typically more mature than the version provided by the Linux distribution.

### Libusb Access Permissions

For device access through libusb, your kernel needs support for the USB filesystem (`usbfs`) and that filesystem must be mounted. This is done automatically, if `/etc/fstab` contains a line like this:

```
usbfs /proc/bus/usb usbfs defaults 0 0
```

This should routinely be the case if you installed any of the mainstream Linux distributions. The permissions for the device files used by libusb must be adjusted for user access. Otherwise only root can use the device(s). The device files are located in `/proc/bus/usb/`. Any manual change would not be permanent, however. The permissions will be reset after reboot or replugging the device. The proper way of setting the suitable permissions is by means of hotplugging scripts or `udev`. Most recent distributions use `udev`.

### Udev

For automated setting of the device file permissions with `udev` you have to add an entry to the set of rules files that are contained in `/etc/udev/rules.d`. `Udev` processes these files in alphabetical order. The default file is usually called `50-udev.rules`. Don't change this file as it could be overwritten when you upgrade `udev`. Instead, put your custom rule for the HydraHarp in a separate file. The contents of this file for the handling of the current HydraHarp 400 models should be:

```
ATTR{idVendor}=="0d0e", ATTR{idProduct}=="0004", MODE="666"  
ATTR{idVendor}=="0d0e", ATTR{idProduct}=="0009", MODE="666"
```

A suitable rules file *HydraHarp.rules* is provided in the folder `Linux/udev` on the distribution media. You can simply copy it to the `/etc/udev/rules.d` folder. The install script in the same distribution media folder does just this. Note that the name of the rules file is important. Each time a device is detected by the `udev` system, the files are read in alphabetical order, line by line, until a match is found. Note that different distributions may use different rule file names for various categories. For instance, Ubuntu organizes the rules into further files: `20-names.rules`, `40-permissions.rules`, and `60-symlinks.rules`. In Fedora they are not separated by those categories, as you can see by studying `50-udev.rules`. Instead of editing the existing files, it is therefore usually recommended to put all of your modifications in a separate file like `10-udev.rules` or `10-local.rules`. The low number at the beginning of the file name ensures it will be processed before the default file. However, later rules that are more general (applying to a whole class of devices) may later override the desired access rights. This is the case for USB devices handled through Libusb. It is therefore important that you use a rules file for the HydraHarp that gets evaluated after the general case. The default naming `HydraHarp400.rules` most likely ensures this but if you see problems you may want to check.

Note that there are different `udev` implementations with different command sets. On some distributions you must reboot to activate changes, on others you can reload rule changes and restart `udev` with these commands:

```
# udevcontrol reload_rules  
# udevstart
```

### Wine Limitations

Running the software under Wine is an experimental feature with limited support. A known issue is the current implementation of Wine's viewer for `chm` help files. You may observe failure or some glitches when using the

online help facility.

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