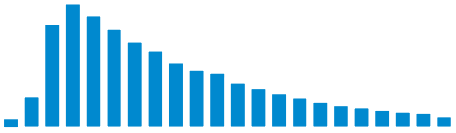

High Performance
Photon Counting

User Handbook

DPC-230

16 Channel

Photon Correlator

Becker & Hickl GmbH

(c) Becker & Hickl GmbH

Becker & Hickl GmbH
High Performance
Photon Counting

April 2008

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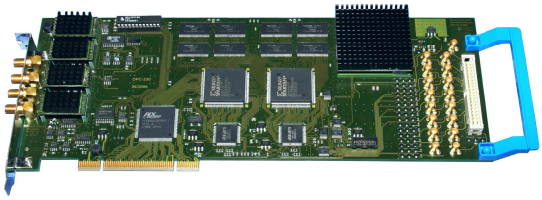
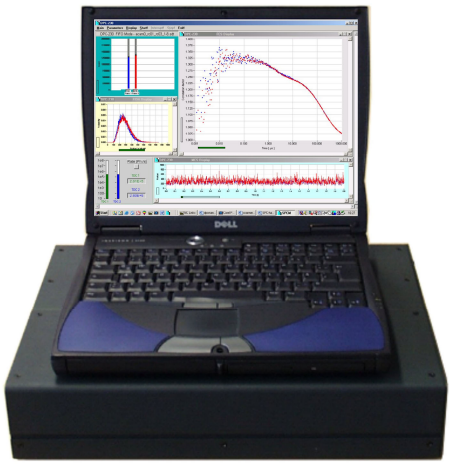
DPC-230
16 Channel Photon Correlator

Photon correlation down to the ps range

16 fully parallel time-resolved photon-counting channels
16 LVTTL inputs for SPADs or 4 CFD inputs for PMTs
Recording of absolute photon times
Autocorrelation within 16 LVTTL or 4 CFD channels
Cross-correlation between any pairs of LVTTL or CFD channels
Full correlation down to the picosecond range
FCS and anti-bunching from one experiment
3-channel TCSPC mode with 165 ps time channel width
Multiscaler operation of 15 LVTTL or 3 CFD channels
Imaging capability in TCSPC and multiscaler modes
Time-tag data available in all modes
Available as single PCI cards or laptop-based stand-alone systems





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Introduction

The DPC-230 photon correlator card records absolute photon times in up to 16 parallel detection channels. Depending on how the photons are correlated, fluorescence correlation (FCS), fluorescence cross correlation (FCCS), photon counting histograms, or waveforms of the light signals are obtained. In combination with optical scanning time-resolved images can be recorded. The maximum time resolution of the recording is 165 ps per time channel.

The DPC-230 is available as a single PCI card for installation in a standard PC, or as a compact 'Simple-Tau DPC' system based on a laptop computer with an extension box, see Fig. 1.

Fig. 1: DPC-230 card (left) and Simple-Tau DPC system (right)

The DPC-230 especially targets on a new type of fluorescence correlation spectroscopy that records correlation over a time interval of 10 orders of magnitude [12]. Thus, singlet decay rates, rotational relaxation rates, rate constants of conformational changes, triplet transition and decay rates, and diffusion times of free fluorophores and fluorophores bound to proteins, lipids or nanoparticles can be obtained within a single measurement.

Moreover, the DPC-230 can be used for recording luminescence decay curves or luminescence lifetime images from the sub-nanosecond to the millisecond range, for LIDAR experiments, FCS, time-of flight mass spectroscopy, or any other experiments based on acquiring the temporal distribution of detection events in a large number of detector channels.

The DPC-230 is operated by the same software as the bh SPC modules [2]. Although the system parameters and operation modes of the DPC differ from those of the SPC modules the general software philosophy is the same. Moreover, the DPC can be used with the same detectors, detector assemblies, detector controllers, excitation sources, and optical systems as the SPC modules. This handbook should therefore considered an addendum to the 'bh TCSPC Handbook' available on www.becker-hickl.com [2].

Principle of Data Acquisition

Time-to-Digital Conversion

The recording process in the DPC-230 is based on a digital TDC ('Time to Digital Converter') principle. A TDC uses the delay of fast CMOS gates as a timing reference. A reference pulse is cycling within a ring structure consisting of a number of similar CMOS gates, see Fig. 2. When a photon (or any other input event) is detected the position of the reference pulse in the

ring is read, and used to determine the detection time. Times longer than the reference cycle time are determined by counting the reference cycles.

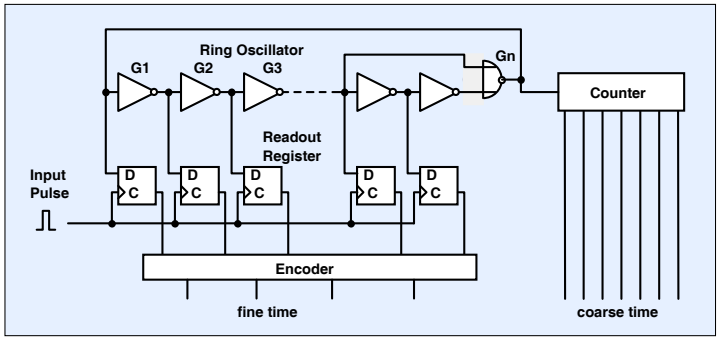


Fig. 2: Principle of a digital TDC. The time of the input pulse is derived from the location of a reference pulse cycling within a ring oscillator

Compared with the TAC-ADC principle used in fast TCSPC devices [1, 2] a TDC delivers coarser time-channels. A TDC can, however, be built at a lower price and with considerably lower power consumption. More important, a large number of TDC channels can be synchronised to obtain comparable photon times in a large number of recording channels.

DPC-230 Architecture

Absolute Timing

A general block diagram of the DPC-230 is shown in Fig. 3. The device has two TDC chips, each of which contains eight TDC channels. Both TDC chips are synchronised via a common clock oscillator. Both TDC chips can be operated either with 8 LVTTL (Low-Voltage TTL) inputs or with two ECL (Emitter-Coupled Logic) inputs. The LVTTL inputs are compatible with all commonly used single-photon avalanche photodiode (SPAD) detectors. The ECL inputs are driven by constant-fraction discriminators (CFDs). The CFD inputs are compatible with the output pulses of photomultiplier tubes (PMTs).

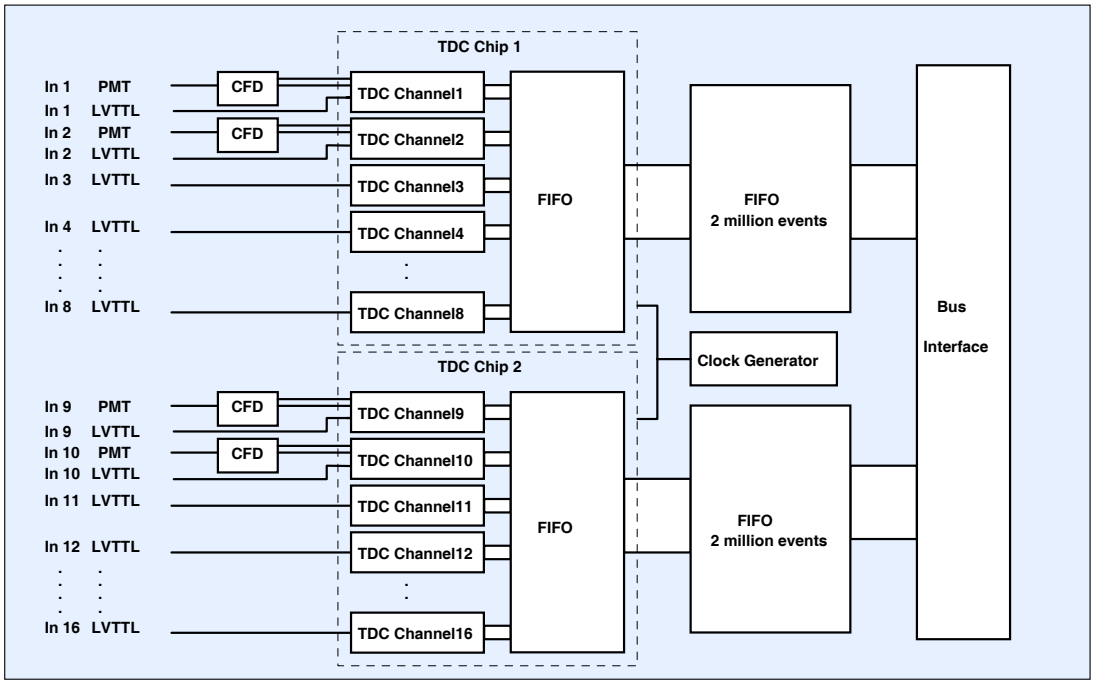


Fig. 3: General architecture of the DPC-230

When a measurement is started the TDCs in all active channels simultaneously start running. Any event detected at one of the 16 LVTTL inputs or at one of the four CFD inputs triggers a time readout from the TDC of the corresponding channel. The time is first buffered in an internal first-in-first-out (FIFO) memory of the TDC chip. The data from the internal FIFO are then written into an external FIFO that buffers up to 4 million events. The output of the external FIFO is read by the computer, and the data are transferred into the main memory or to the hard disc. The double-buffered structure avoids loss of photons by read-write collisions. Moreover, the external FIFO is large enough to buffer the photon data during possible background operations of the computer.

In the configuration shown in Fig. 3 the DPC delivers the times of the photons from the start of the measurement. Such data are often called ‘time -tagged photon data’. The interpretation of the data is merely a matter of the software. Thus, intensity traces, photon counting histograms, and fluorescence correlation and cross-correlation curves can be obtained (see ‘Operation Modes’ page 5).

Relative Timing

Relative timing is required if the waveform of an optical signal is to be recorded. The times of the photons have then to be measured with reference to the pulses of the excitation source. Relative timing can be achieved by recording reference pulses from the excitation source in one of the TDC channels. The times of the photons in the excitation period are then obtained by determining the differences of the photon times to the previous excitation pulse. This kind of relative timing is used in the multichannel-scaler mode of the DPC (see ‘Multichannel Scaler Mode’, page 8).

Excitation pulse rates in the MHz range, as they are typical of TCSPC measurements, do, however, cause a problem if direct relative timing is used: The high repetition rate of the reference pulses would saturate the TDC channel that is used for the reference. Therefore, the DPC has a TCSPC configuration implemented that records only reference pulses of excitation periods that contain valid photons. The DPC-230 architecture in the TCSPC mode is shown in Fig. 4.

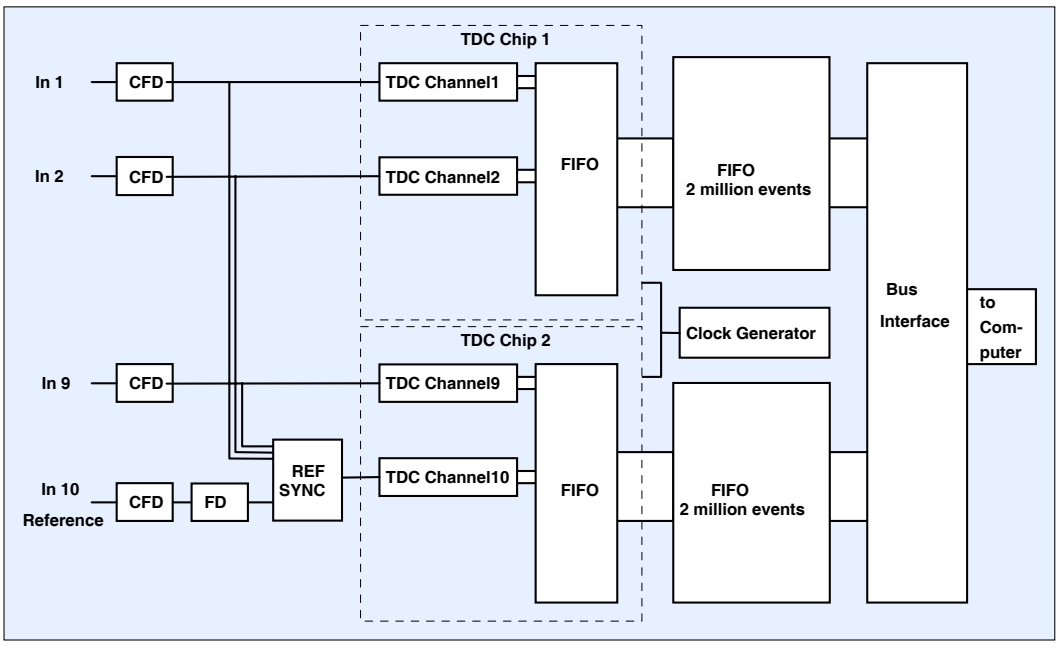


Fig. 4: DPC-230 architecture in the TCSPC mode

Three of the CFD channels are used for the detector signals. The CFD output pulses of these channels are fed directly into the ECL inputs of the TDC chip. The fourth CFD input is used for the reference from the laser. Before the reference pulses enter the TDC chip they pass a frequency divider, FD, and a synchronisation circuit, SYNC. The divider ratio of FD is selectable and can be set to 1:1, 1:2, or 1:4. The subsequent synchronisation circuit transmits one - and only one - synchronisation pulse if one of the first three CFDs has received a photon pulse within the previous pulse period of the divided reference signal. Thus, TDC2 receives one reference pulse when at least one photon was recorded in the previous 1, 2, or 4 excitation periods. As a result, the reference pulse rate is reduced to less than the photon rate. The reduction is achieved without sacrificing any excitation periods or photons.

Pulse diagrams for FD = 1:1 and FD = 2:1 are shown in Fig. 5 and Fig. 6, respectively. With FD = 1:1 the time of a photon is measured with respect to the next reference pulse. Thus, the measurement time interval extends over one period of the light source.

With FD = 2:1 (Fig. 6) the photon time is measured with respect to the next or second next excitation pulse, depending on whether the photon was detected in an even or uneven excitation period. Thus, the measurement time interval extends over 2 laser periods. A frequency divider ratio of four results in a recording over four signal periods.

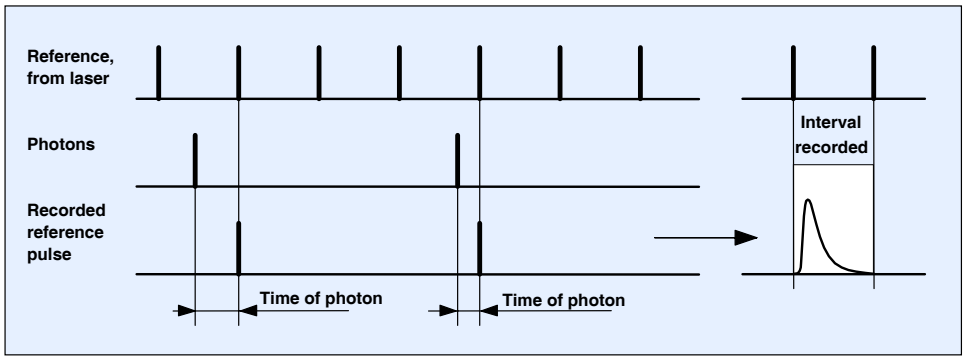


Fig. 5: Reference synchronisation for FD=1:1. After the detection of a photon the next reference pulse is recorded. The time of the photon is measured with respect to the selected pulse.

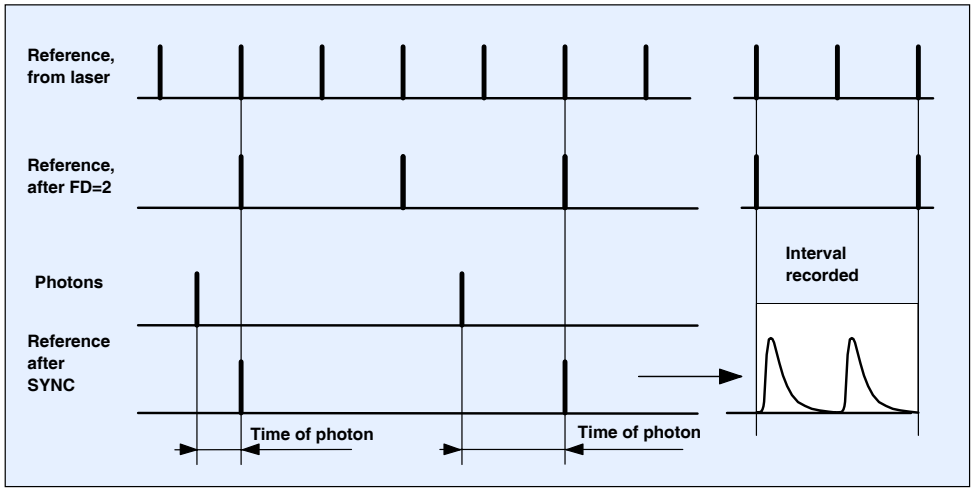


Fig. 6: Reference synchronisation for FD=2:1. The reference pulses are divided by a ratio of 2:1. After the detection of a photon the next pulse from the frequency divider is recorded. The time of the photon is measured with respect to this pulse. The measurement time interval extends over 2 reference periods.

Operation Modes

Absolute-Timing Modes

In the ‘Absolute Time’ mode every photon is characterised by its time from the start of the measurement and its input channel number. The interpretation of the data is merely a matter of the software. The different ways of analysing the data and the presenting the results are described below. The results can be built up online from the incoming data stream, or off-line from the time-tag data files. Please ‘Configuring the Runtime Display’, page 45.

Intensity Traces

The simplest way of interpreting the TDC data is to calculate intensity traces from the photon times. The photon numbers within consecutive time intervals of the photon data stream are determined, and displayed as functions of time, see Fig. 7. Although the procedure is trivial, intensity traces can be used to determine on-off states of molecules, and to check whether an experiment is running as expected.

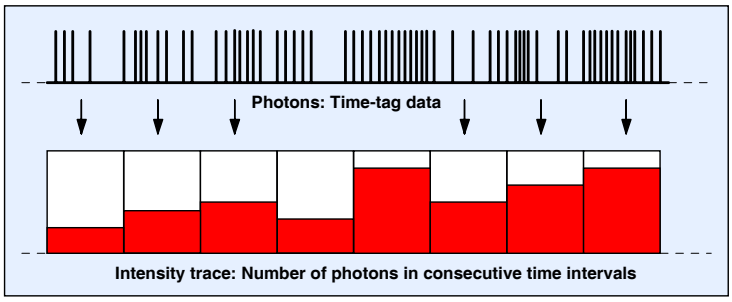


Fig. 7: Buildup of intensity traces from the time-tag data delivered by the TDCs

Photon Counting Histograms

The photon counting histogram (PCH) of an optical signal is obtained by recording the photons within consecutive sampling-time intervals and building up the distribution of the frequency of the measured counts versus the count numbers, see Fig. 8. For a classic light signal of constant intensity the PCH is a Poisson distribution. If the light fluctuates, e.g. by fluctuations of the number of fluorescent molecules in the focus of a laser, the PCH is broader than the Poisson distribution.

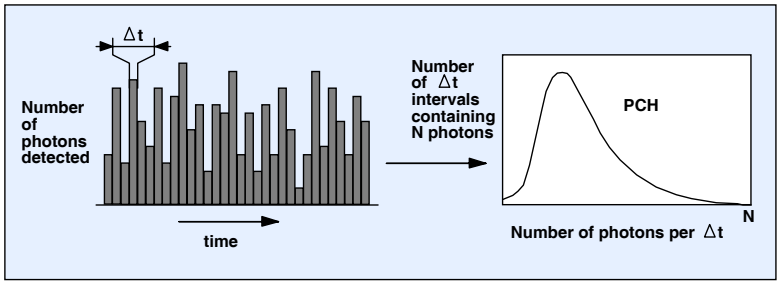


Fig. 8: Photon counting histogram. Left: Photons counted in successive sampling time intervals. Right: Histogram of the number of time intervals containing N photons

A first characterisation of the PHC was given in 1990 by Qian and Elson [26]. The PCH delivers the average number of molecules in the focus and their molecular brightness. Several molecules of different brightness can be distinguished by fitting a model containing the rela-

tive brightness and the concentration ratio of the molecules to the measured PCH. The technique is also called 'fluorescence intensity distribution analysis', or FIDA. The theoretical background is described in [9, 10, 15, 17, 21, 23, 24].

The PCH/FIDA technique can be extended for multi-dimensional histograms of the intensity recorded by several detectors in different wavelength intervals or under different polarisation. Multi-dimensional photon counting histograms have been shown to deliver a substantially improved resolution of different fluorophores [16, 17]. With the large number of input channels available in the DPC-230 recording multi-dimensional PCHs is merely a matter of the optical system.

Fluorescence Correlation

Fluorescence correlation spectroscopy (FCS) is based on exciting a small number of molecules in a femtoliter volume and correlating the fluctuations of the fluorescence intensity. The fluctuations are caused by diffusion, rotation, intersystem crossing, conformational changes, or other random effects. The technique dates back to a work of Magde, Elson and Webb published in 1972 [20]. Theory and applications of FCS are described in [8, 27, 28, 29, 30].

The (un-normalised) autocorrelation function $G(\tau)$ of an analog signal $I(t)$ and the cross-correlation function, $G_{12}(\tau)$, of two signals $I_1(t)$ and $I_2(t)$ are

$$G(\tau) = \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{+T} I(t)\, I(t+\tau)\, dt \qquad G_{12}(\tau) = \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{+T} I_1(t)\, I_2(t+\tau)\, dt$$

For photon counts, N, in consecutive, discrete time channels $G(\tau)$ and $G_{12}(\tau)$ can be obtained by calculating

$$G(\tau) = \sum N(t) \cdot N(t+\tau) \qquad G_{12}(\tau) = \sum N_1(t) \cdot N_2(t+\tau)$$

For a randomly fluctuating signal, $I(t)$, the autocorrelation function $G(\tau)$ assumes high values only if the values if intensity values, I, at a given time, t, and at a later time, $t+\tau$ are correlated. Uncorrelated fluctuations of I cancel over the integration time interval. Similarly, $G12(\tau)$ assumes high values if the fluctuations in both signals, $I_1(t)$ and $I_2(t+\tau)$, correlate with each other. The drop of $G(\tau)$ and $G_{12}(\tau)$ over the shift time, τ, shows how far the fluctuations are correlated in time. The general behaviour of the auto- and cross-correlation functions is illustrated in Fig. 9.

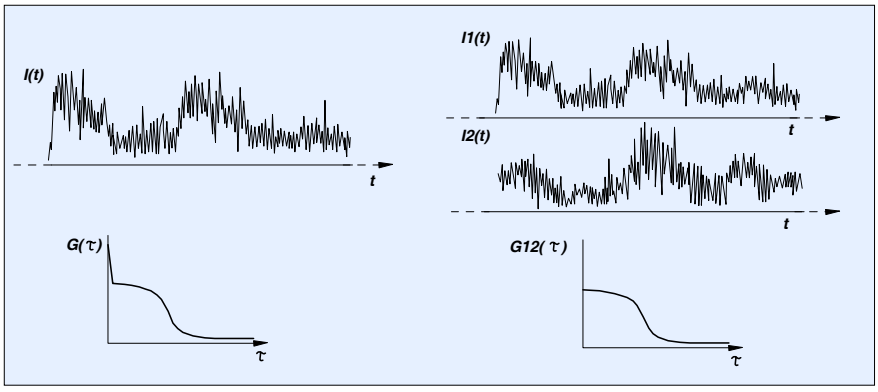


Fig. 9: General behaviour of the autocorrelation function, $G(\tau)$,of a signal I and the cross-correlation function, $G_{12}(\tau)$, of the signals I_1 and I_2

The autocorrelation (shown left) has a sharp peak at $\tau = 0$. (The function correlates perfectly with itself). The slow fluctuations in I(t) represent themselves in a high correlation at medium τ. For τ longer than the typical time of the slow fluctuations $G(\tau)$ drops to very low values.

The cross-correlation function of the two signals, *I1* and *I2*, is shown right. The slow fluctuations in I_1 and I_2 have the same general appearance. However, the noise in I_1 and I_2 is different. Therefore only the fluctuations correlate and give a noticeable contribution to $G_{12}(\tau)$.

The equations shown above are appropriate to calculate correlation functions from analog signals. The do, however, not well apply to time-tagged photon counting data recorded at high time resolution. Such data do not display a continuous waveform as shown in Fig. 9 but are rather a random sequence of individual detection events.

The general correlation procedure for time-tagged photon data is demonstrated in Fig. 10. In typical TDC data, the time-channel width, T, is shorter than the dead time of the detector/photon counter combination. Therefore only one photon can be recorded in a particular time channel. Consequently, $N(t)$ and $N(t + \tau)$ can only be 0 or 1. The calculation of the autocorrelation function therefore becomes a simple shift, compare, and histogramming procedure. The times of the individual photons are subsequently shifted by one time-channel interval, *T*, and compared with the original detection times. The coincidences found between the shifted and the unshifted data are transferred into a histogram of the number of coincidences, *G*, versus the shift time, τ. The obtained $G(\tau)$ is the (un-normalised) autocorrelation function.

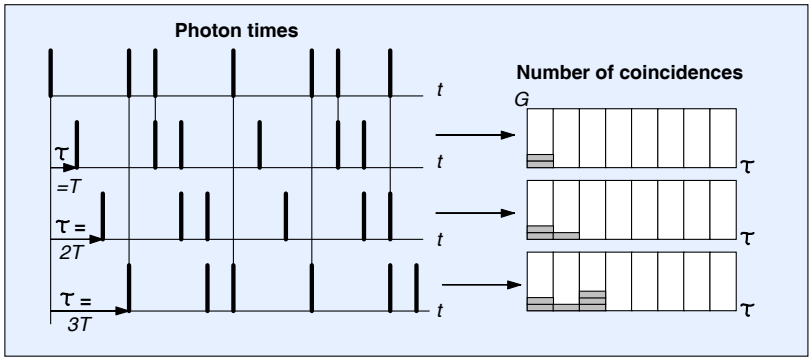


Fig. 10: Calculation of the autocorrelation function from TCSPC time-tag data

The cross-correlation function between two signals is obtained by a similar procedure. However, the photon times of one detector channel are shifted versus the photon times of another channel.

The result of the shift-and-compare procedure is not normalised. Normalisation can be interpreted as the ratio of the number of coincidences found in the recorded signal to the number of coincidences expected for an uncorrelated signal of the same count rate. The normalised autocorrelation and cross-correlation functions are

$$G_n(\tau) = G(\tau)\frac{n_T}{N_P^2}$$

with n_T = total number of time intervals, *Np* = total number of photons, and

$$G_{12n}(\tau) = G_{12}(\tau)\frac{n_t}{N_{P1}N_{P2}}$$

with n_T = total number of time intervals, N_{p1} = total number of photons in signal 1, N_{p2} = total number of photons in signal 2.

The procedure illustrated in Fig. 10 yields $G(\tau)$ in equidistant τ channels. The width of the τ channels is equal to the time-channel width of the DPC-230, T. The algorithm is known as the 'Linear Tau' algorithm. The equidistant τ channels result in an easily predictable statistics. This is a benefit if a model function is to be fitted to the correlation function. Unfortunately, for the small time-channel width of the DPC the algorithm would result in an extremely large number of τ channels in $G(\tau)$, and in intolerably long calculation times.

The operating software of the DPC therefore applies binning steps to the photon data during the correlation procedure. The general procedure is illustrated in Fig. 11.

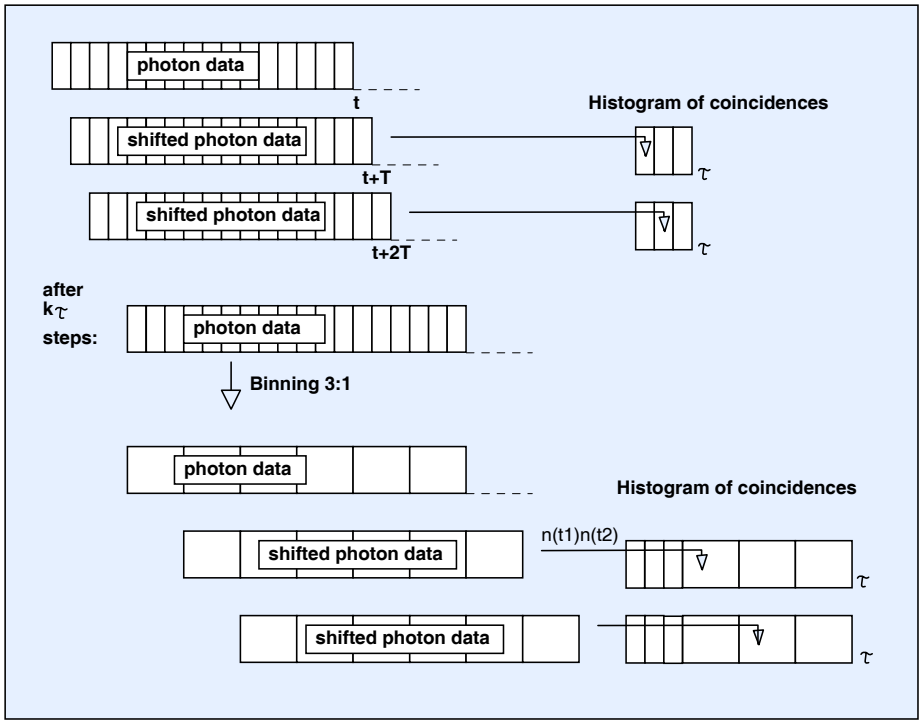


Fig. 11: Correlation procedure with progressive binning

The procedure starts with a number of shift-and-compare operations as shown in Fig. 10. However, after a number, k_τ, of shift-and-compare operations the remaining photon data are binned by a factor of three. (The factor of three is used to keep the new time intervals centred at multiples of the original time channel width, T.) Then the shift-and-compare operation is continued on the binned data. After every k_τ operations a new binning step is performed. Thus, the width of the τ channels increases progressively. The progression is the faster the smaller the progression parameter, k_τ is chosen. The procedure shown in Fig. 11 delivers similar results as the 'multi-τ algorithm' commonly used in conventional correlators [18].

Relative Timing Modes

In the relative timing modes reference pulses are recorded in one channel of the DPC-230. The times of the reference pulses are recorded together with the photon times, and relative times between the photons and the reference pulses are calculated.

Multichannel Scaler Mode

The multichannel-scaler mode records the waveform of a light signal, or, in the general case, the density of the detected events as a function of the time within the period of a repetitive excitation signal. In other words, the photon times are measured with reference to the pulses

of the excitation source, and the distribution of the events within the excitation period is built up. Thus, the multichannel scaler mode is a relative-timing mode.

Multichannel scaler operation is illustrated in Fig. 12. The hardware structure is the same as in the 'absolute time' mode. However, one channel of the DPC is used as a reference channel. The reference channel receives synchronisation pulses from the light source. The other channels receive the single-photon pulses from the detectors. The data stream of the DPC contains the times of the excitation pulses, and the times of the detected photons. From these data, the operating software extracts the relative times of the photons since the last excitation pulse. These times are used to build up the distribution of the photon density over this time. Because several detectors can be active, several waveforms for the individual detectors can be obtained within the same measurement.

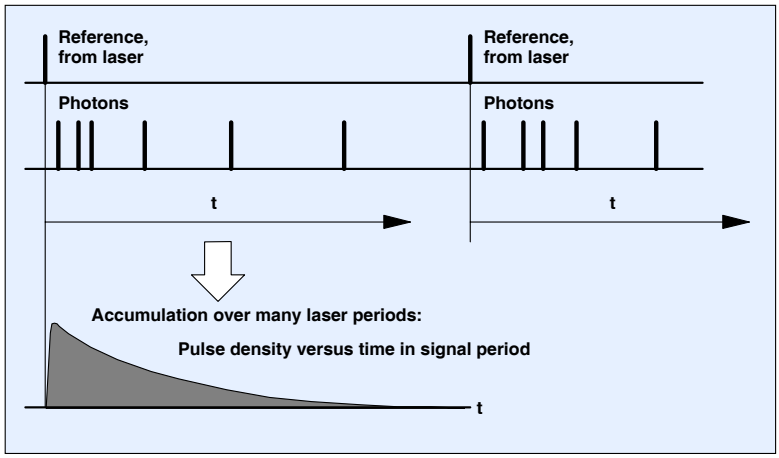


Fig. 12: Multiscaler mode of the DPC. One channel records reference pulses from the excitation source, the other channel records the photons. Relative times of the photons are determined, and curves of the photon density over the time within the excitation period is built up.

The multichannel scaler mode is recommended for excitation rates up to about 1 MHz. For higher excitation rates the high reference pulse rate can cause data transfer problems, and the TCSPC mode should be used.

TCSPC Mode

The TCSPC mode was implemented for waveform measurements at high repetition rate. The TCSPC mode measures the photon times with reference to the *next* excitation pulse. In classic TCSPC the principle is known as 'reversed start-stop' technique [1, 2, 22]. The benefit of the reversed start-stop principle is that reference pulses need to be recorded only for excitation periods that contain valid photons. In classic TCSPC reversed start-stop avoids the problem of excessive TAC start rates; in TDC-based instruments it avoids saturation of the TDC channel of the reference. The configuration of the DPC-230 in the TCSPC mode is shown in Fig. 4, page 3.

The build-up of the signal waveform in the TCSPC mode is illustrated in Fig. 13. In the typical TCSPC applications the count rate is considerably lower than the signal repetition rate. Thus, there are a large number of signal periods in which no photons are detected. In these periods the reference synchronisation circuit (see Fig. 4 and Fig. 5) suppresses the reference pulses, and nothing is recorded. In signal periods that contain a photon both the photon and the subsequent reference pulse are recorded. The software analyses the data stream, determines the relative times from the photons to the next reference pulse, and builds up the distribution of the photon density over the relative time. Except for a reversal of the time scale, the

result is the waveform of the light signal. Because three input channels are available in the TCSPC mode three signals from three detectors can be recorded simultaneously.

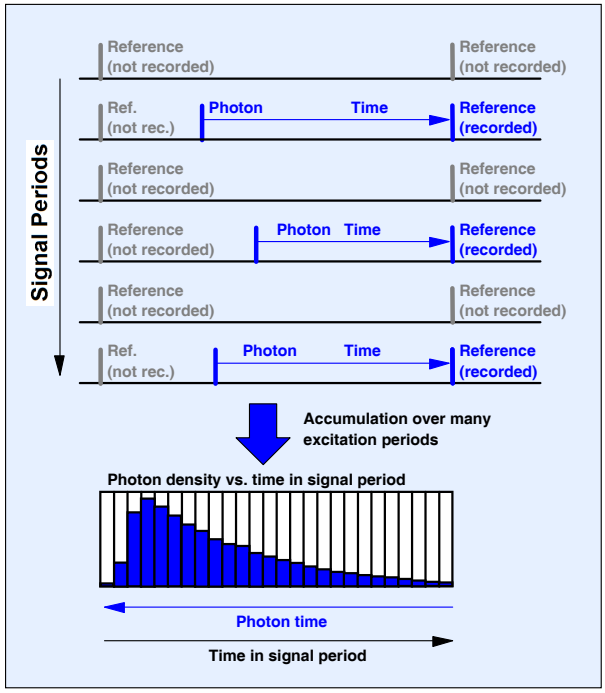


Fig. 13: Build-up of the signal waveform in the TCSPC mode

In contrast to TCSPC devices using the TAC/ADC principle the DPC is able to record several photons per signal period. This 'multi-hit' capability of a TDC is often praised as a panacea against pile-up distortions. However, in the typical fluorescence lifetime applications the lifetime is on the order of a few nanoseconds. The dead time of the detector/CFD/TDC combination is at least at the same order, if not longer (about 5 ns for PMTs and 50 ns for SPADs). Thus, there is little chance for a single detector to record several photons within the fluorescence decay. In standard fluorescence applications with lifetimes on the order of a few ns the multi-hit capability is therefore of little benefit. It is, however, important when fluorescence decay functions in the 100 ns range or above are recorded, i.e. from quantum dots, semiconductors, or rare-earth chelates.

As all bh TCSPC devices, the DPC has a selectable frequency divider in the reference channel. The divider ratio determines the number of signal periods over which the signal is recorded (see Fig. 4 through Fig. 6). It should be noted here that a result containing several signal periods normally cannot be fully exploited by standard data analysis programs. Nevertheless, recording several signal periods can be useful when the time scale is to be checked by a signal of known period, or when a TCSPC device is used as an optical oscilloscope [1, 2].

TCSPC with Absolute Timing

The time-tag data of the TCSPC mode still contain the absolute times of the detected photons. Thus, a TCSPC measurement can be used to simultaneously obtain the waveform of the optical signal and the autocorrelation or cross-correlation within one or between different detector channels [1, 2]. The waveforms of the detector signals are obtained as shown in Fig. 13, the correlation curves as shown in Fig. 10. The result is the same as for the FIFO (or time-tag) mode of the bh TCSPC mode where the fluorescence decay curves are obtained from the relative ('micro') time and the FCS curves from the absolute ('macro') times [2]. The results can be built up online from the incoming data stream, or off-line from the time-tag data files. Please see [2] and 'Configuring the Runtime Display' page 45.

Other applications of the absolute times in TCSPC data are multi-parameter single-molecule spectroscopy [19, 25, 31], burst-integrated lifetime (BIFL) experiments, and fluorescence intensity and lifetime distribution analysis (FILDA) [23].

Imaging

The DPC-230 can be used for imaging applications. The sample is scanned by a focused laser beam, the photons are detected with their absolute or relative times, and the image is built up from the recorded data.

The imaging modes of the DPC-230 use the parallel architecture of the DPC module to record both the photons and the synchronisation pulses from the scanner. Typically three scan synchronisation signals are used. A 'Frame Clock' edge indicates the start of a new frame, a 'Line Clock' edge the start of a new line, and a 'Pixel Clock' the transition to a new pixel. The clock edges are recorded in parallel with the photons, and with their absolute times. By identifying the clock edges in the data stream every photon is assigned to the pixel the scanner was on in the moment when the photon was detected. The method is similar to the FIFO Imaging mode of multi-dimensional TCSPC, a technique routinely used for fluorescence lifetime imaging (FLIM) with laser scanning microscopes [1, 2]. The principle is illustrated in Fig. 14.

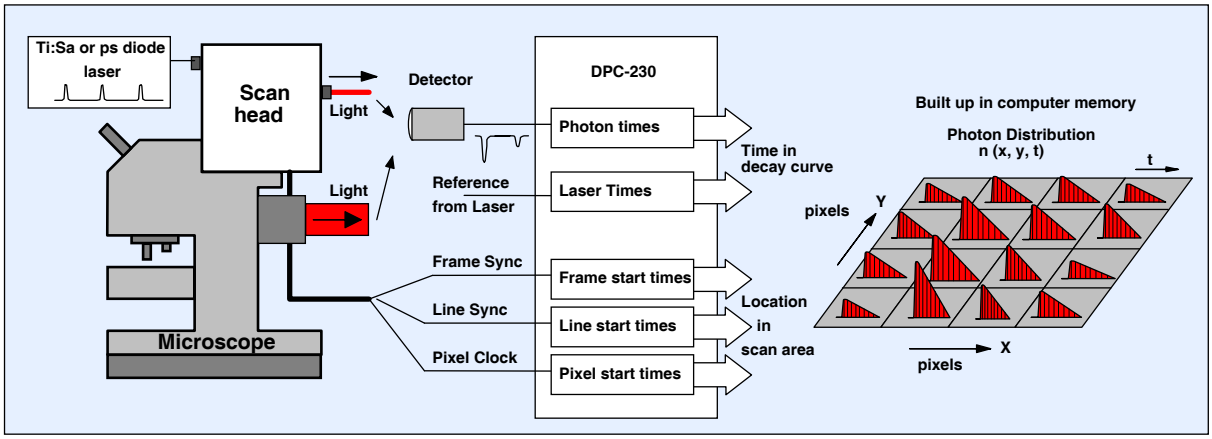


Fig. 14: DPC-230 Imaging: FLIM with laser scanning microscope

The laser scanning microscope scans the sample with a pulsed laser beam. The fluorescence light is fed to a photon counting detector via a suitable optical port of the microscope. The single-photon pulses of the detector are connected to one channel of the DPC-230. A second channel records reference pulses from the laser. The relative photon times, i.e. the differences of the photon times recorded in the photon and laser channel, are the times of the photons within the fluorescence decay.

The frame clock, line clock, and pixel clock pulses from the scanner are connected to three other channels of the DPC-230. By analysing the events recorded in these channels, the location of the laser beam in the scan area is obtained for each individual photon.

The fluorescence lifetime image is built up in the memory of the computer. A data array contains memory space for all pixels of the scan, and, within each pixel, memory space for a large number of time channels within the fluorescence decay. The software extracts the relative photon times and the locations from the data stream, and builds up a photon distribution over these parameters.

Please note that the principle not only works for slow scanning with piezo stages but also for fast scanning by galvanometer mirrors. In the typical fast-scan applications the pixel dwell time is on the order of a few microseconds or less. The pixel rate is then higher than the pho-

ton count rate. This makes the recording process more or less random. The recording is continued over as many frames as necessary to obtain the desired number of photons per pixel.

There are many modifications of the principle shown in Fig. 14. Imaging is possible both in the multiscaler mode and the TCSPC mode. Other operations than FLIM calculation can be applied to the photon and pixel data, and several detectors can be used simultaneously. By storing the raw data, i.e. the photon times, the laser pulse times and the scan clock times, any kind of processing can be applied to the data. The technique can therefore by used also for multi-parameter fluorescence detection and correlation imaging [11, 19].

Installation

Computer

In principle, the DPC-230 module can be installed in a PCI slot of any Pentium PC. However, the SPCM software runs the data transfer and the online-data processing in parallel 'threads'. To achieve a reasonable data transfer and processing rate the computer should be one with a dual-core architecture.

The graphics card should have 1024 by 628 resolution or more. Both table-top and tower versions can be used. However, the computer must accept a full-size PCI card. It is recommended to have two or three free PCI slots available. The DPC-230 can then be used in conjunction with a bh DCC-100 detector controller card or a bh GVD-100 scan controller card.

The operating system can be Windows 2000, NT, XP, or Vista. The computer should have 1 Gigabyte of main memory. If the DPC is used for long measurements at high count rates a main memory of 2 Gigabytes or more is recommended. The large memory helps to avoid hard disc actions during the measurement and thus increases the maximum sustained count rate.

The DPC is operated via the SPCM software that is also used for the bh SPC modules. Although the SPCM operating software requires only a few Mb of hard disk space, much more space should be available to save the measurement results. The time-tag data files can be as large as several Gigabytes. It is therefore recommended to use a hard disc of several 100 Gb. A DVD drive or another backup medium is also recommended.

Software Installation

The software installation starts automatically after the insertion of the installation CD. You may also install or update the software form the web. In this case, open www.bekcer-hickl.com, and click on the 'Software' button. On the 'Software' page (Fig. 15, left), click on 'TCSPC Modules', 'Operating Software'. Then click on 'Setup' for SPC-830, SPC-730, SPC-630, SPC-134, SPC-144, SPC-154 ((Fig. 15, right)). Download TCSPC_setup_web.exe and start it.

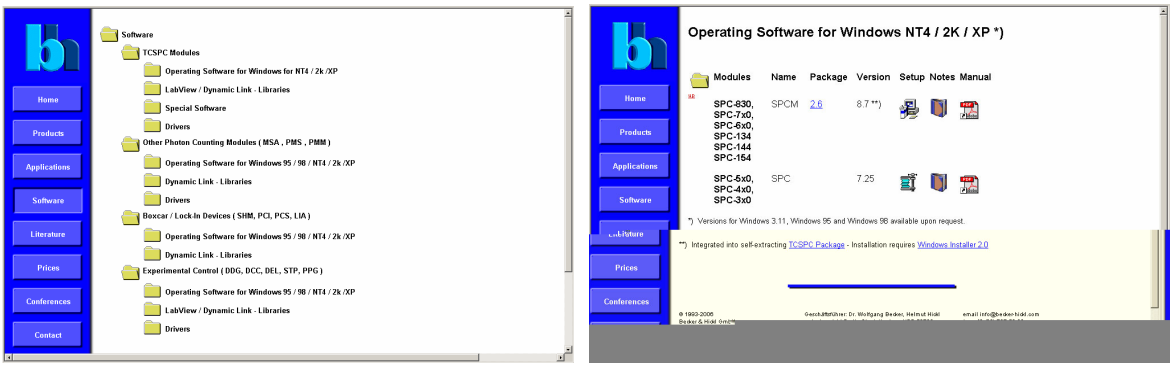


Fig. 15: Software download from the web. Left: 'Software' page. Right: Page for downloading the SPCM software

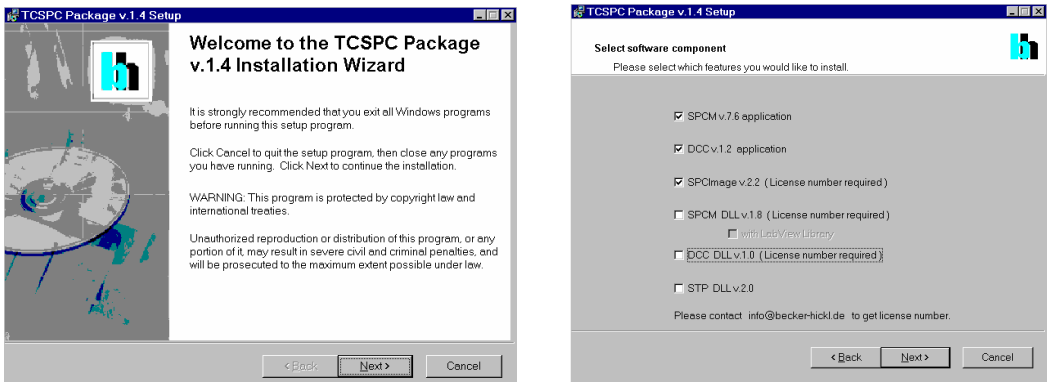


Fig. 16: Installation panels

The installation works the same way as the installation from the CD. Check the boxes of the software components you want to install (Fig. 16, right). The operating software of the DPC is 'SPCM'. If you operate the DPC together with the DCC-100 detector controller you need also 'DCC'. The SPCM and DCC software components are all you need for DPC standard applications. The components are free and can be installed without any password.
The installation package also contains a FLIM data analysis software, SPCImage, and DLL libraries for the bh DPC, SPC and DCC modules. These components are not needed for DPC standard applications. Their installation requires a licence number. You get this number if you purchase these software components from bh. *Please note that the DLL and Lab View libraries are only required if you plan to design your own experiment software. They are not required to run the SPCM software.*

For details of the installation please see The bh TCSPC Handbook [2].

Hardware Installation

To install the DPC-230 module, switch off the computer and insert the board (and the DCC-100 board, if you have one) into a free slot PCI slot. To avoid damage due to electrostatic discharge we recommend to touch a metallic part of the computer with one hand and then to grasp the module at the metallic back shield with the other hand. This will drain any potentially dangerous charge from you and the module. Then insert the module into a free slot of the computer. Keep the DPC module as far as possible apart from loose cables or other computer modules to avoid noise pick-up.

The DPC-230 modules has a PCI interface. Windows has a list of PCI hardware components. On the start of the operating system, it automatically assigns the required hardware resources to the components of this list. If you have several DPC or DCC modules in the computer each module automatically gets its own address range.

Driver Installation

When the computer is started the first time with a DPC module Windows detects the new module and attempts to update its list of hardware components. Therefore it may ask for driver information from a disk. When this happens, put the installation CD into the drive and select the drivers for Windows 2000, NT, XP an Vista. If you don't have the installation disk at hand you can also download the driver file from the bh web site, see Fig. 17.

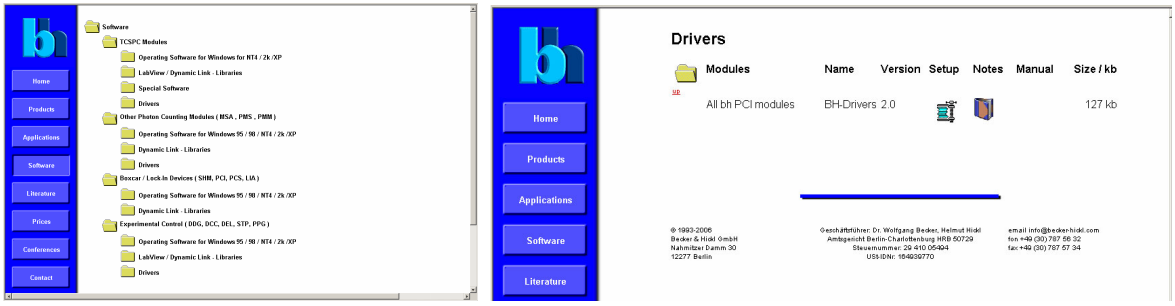


Fig. 17: Downloading the drivers from the bh web site

Open www.becker-hickl.com and click on ‘Software’. On the ‘Software’ page, click on ‘TCSPC Modules’, ‘Drivers’. Then click on ‘Setup’ and download the drivers.

Software Start

When the module is inserted and the driver is installed, start the SPCM Software. The initialisation panel shown in Fig. 18, left should appear. The installed modules (there can be up to four) are marked as ‘In use’. The modules are shown with their serial number, PCI address and slot number.

Important: If the DPC module is not found at this stage, e.g. because the driver was not installed, the software starts in an emulation mode (see below, ‘Starting the SPC software without an SPC module). What you see in this mode is generated by the software or loaded from a file - it is not the data recorded by your DPC-230 module!

The software runs a simple hardware test when it initialises the module. If an error is found, a message ‘Hardware Errors Found’ is given and the corresponding module is marked red. In case of non-fatal hardware errors you can start the SPCM main window by selecting ‘Hardware Mode’ in the ‘Change Mode’ panel. Please note that this feature is intended for trouble shooting and repair rather than for normal use.

When the initialisation window appears, click on ‘OK’ to open the main window of the SPCM Software. At the first start the software comes up with default parameter settings which may be not appropriate for your measurement problem. Therefore, changes may be required for your particular application, please see ‘Configuring the SPCM Main Panel’, page 37.

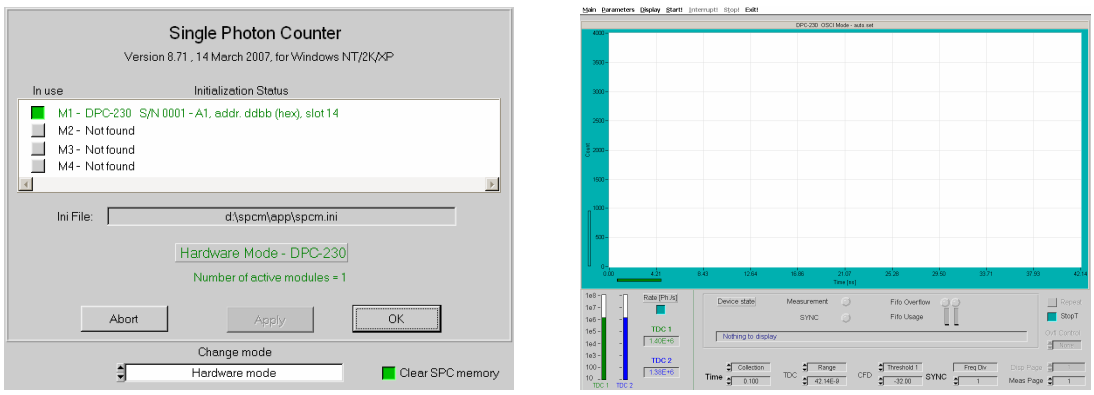


Fig. 18: Starting the SPCM software. Initialisation panel (left) and main panel of SPCM (right)

When you exit the SPCM software after changing parameters, the system settings are saved in a file ‘auto.set’. This file is automatically loaded at the next program start. So the system will come up in the same state as it was left before.

Starting the SPCM Software without a DPC-230 Module

You can use the Multi SPC Software without a DPC module. In its start window the software will display a warning that the module is not present, see Fig. 19, left.

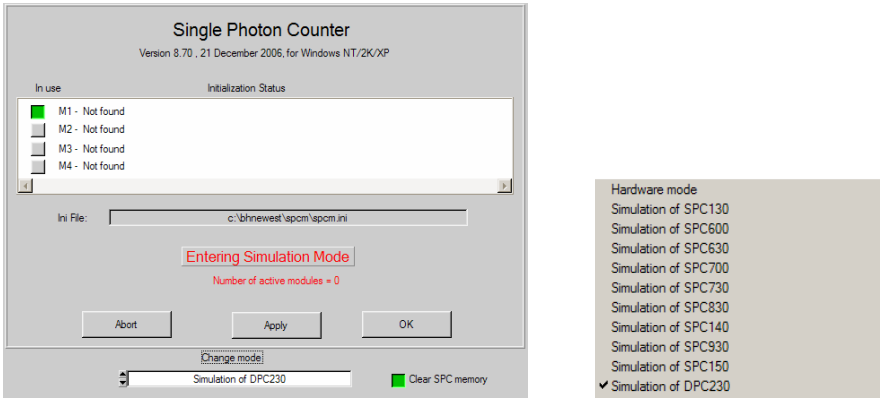


Fig. 19: Startup panel in the simulation mode (without a TCSPC module)

To configure the software for the desired module type, click into the ‘Change Mode’ field and select the module type (DPC-230) from the list which is opened (Fig. 19, right). Click on the ‘in use’ buttons for the modules you want to have active. Then click on ‘Apply’, ‘OK’. The software will start in a special mode and emulate the DPC device memory in the computer memory. You can set the system parameters, load, save, process, convert and display data, i.e. do everything except for a real measurement.

Operating the DPC-230

LVTTL Inputs

The LVTTL inputs of the DPC-230 are designed to receive single-photon pulses from SPAD (single-photon avalanche photodiode) modules. LVTTL means 'low voltage TTL', i.e. the inputs are triggered by signals as low as +1.5 V. The pulse duration can be as short as 2 ns.
The location of the LVTTL inputs at the DPC board are shown in Fig. 20. The signals are fed trough the back panel of the computer via cable adapters (Fig. 20, middle and right).

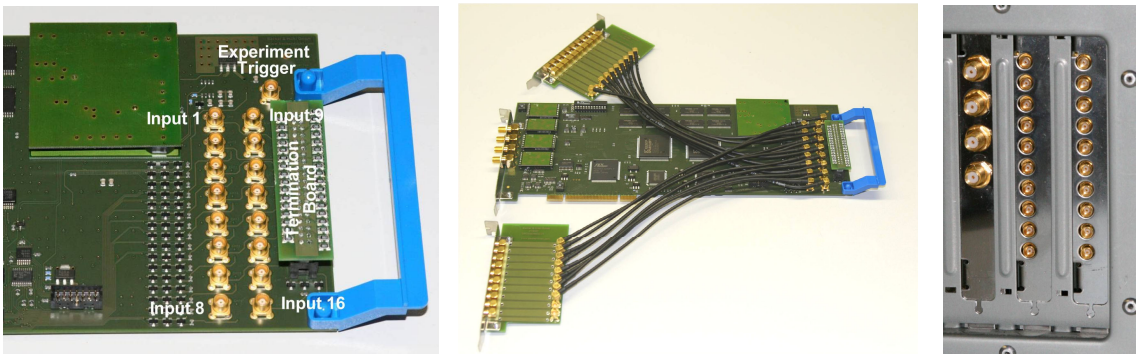


Fig. 20: Location of the LVTTL inputs at the DPC board (left), cable adapter (middle), DPC and adapters inserted in computer back panel (right)

Due to the limited size of the computer back panel MCX connectors had to be used for the LVTTL inputs. Please contact bh if you need additional cables with MCX connectors.
The LVTTL inputs are overload-protected and accept input voltages up to +5V. The input equivalent circuit is shown in Fig. 21.

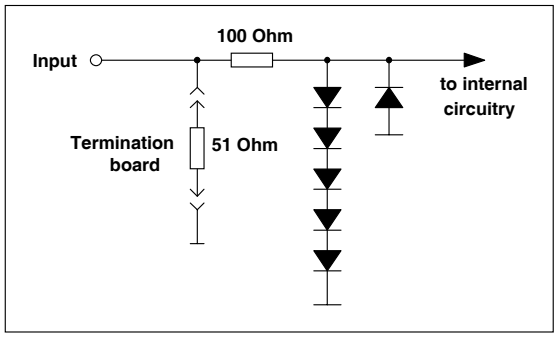


Fig. 21: Equivalent circuit of the LVTTL inputs

The LVTTL inputs of the DPC-230 board are terminated with 50 Ω via a termination board. The board is plugged into a connector close to the LVTTL inputs at the DPC board, see Fig. 20, left. We recommend to use the termination whenever the signal source is able to drive a 50 Ω load. For signals from standard TTL and LVTTL logics it may be necessary to remove the termination. Operation without termination can cause reflections on the signal lines and thus lead to double triggering. However, for standard TTL or LVTTL sources reflections are damped by the source impedance and are thus rarely troublesome.

Connecting SPAD and PMT Modules to the LVTTL Inputs

The LVTTL inputs can be used for all commonly used SPAD modules and a number of PMT modules with internal discriminators and TTL outputs. Some frequently used detectors are listed in the table below.

Manufacturer	Type	Pulse Amplitude	Pulse Width	Remark
id Quantique	id 100-xx	+2 V	20 ns	Versions of different area available
MPD	PDM-xxCT	+2 V	20 ns	Use TTL output. Versions of different area available
Perkin Elmer	SPCM-AQR	+3.5 V	35 ns	
Hamamatsu	H7421-40	+3V	30 ns	GaAsP PMT module, QE = 40% at 500nm, large area

When using LVTTL- or TTL-compatible detectors, please make sure that the corresponding DPC-230 input is configured for ‘rising edge’, see ‘Configuring the Inputs’ page 46.

Synchronisation to Light Sources via the LVTTL Inputs

When operated in the multichannel-scaler mode the DPC-230 needs a timing reference signal from the light source. Pulses from commonly used PIN or avalanche photodiode modules are usually not LVTTL-compatible. If the DPC has to be synchronised via a photodiode the circuit shown in Fig. 22 can be used.

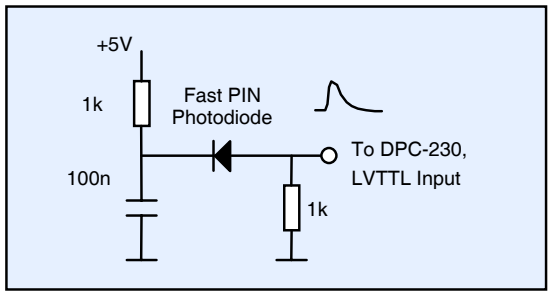


Fig. 22: Generating an LVTTL-compatible trigger signal via a PIN photodiode

Marker Pulses

Marker pulses can be used to identify external actions within the data stream delivered by the DPC. Typical examples are pixel and line synchronisation pulses of a scanner. Marker pulses can be connected to any LVTTL input that is not used for a detector. The pulses should be LVTTL or TTL. Please note that only the low-high or the high-low transition of the signal appears in the data. Thus, make sure that the DPC inputs are correctly configured, see ‘Configuring the Inputs’ page 46.

CFD Inputs

The DPC-230 has four CFD (constant fraction discriminator) inputs. These inputs are designed to trigger on single-photon pulses of PMTs. For best high-frequency behaviour, SMA connectors are used. CFD The CFD inputs are shown in Fig. 23.

Fig. 23: CFD inputs

The pulse amplitudes at the CFD inputs should be negative and within a range of -50 mV to -500 mV.

Connecting PMTs to the CFD Inputs

PMTs use secondary-electron emission to multiply a single photoelectron by a factor of 10^6 to 10^8. The output pulses of a PMT are negative, with a average amplitude between 10 mV and 100 mV and a duration between 300 ps and a few ns. Due to the random gain mechanism the pulse amplitude varies randomly. The variation can be on the order of 1:10. Moreover, there are a large number of small-amplitude background pulses from the dynode system of the PMT.

A conventional discriminator would suppress the background pulses, but introduce a timing jitter on the order of the duration of the leading edge of the pulses. Accurate triggering on PMT pulses therefore requires a trigger circuit that not only suppresses pulses below a given amplitude but also triggers at a time independent of the pulse amplitude. A CFD solves the problem by a double-discriminator principle, see Fig. 24. One discriminator, D1, triggers when the differentiated pulse crosses the signal baseline. The moment when the differentiated pulse crosses the baseline is independent of the pulse amplitude. However, because the threshold of the zero-cross discriminator must be close to the baseline, it also triggers to small pulses and noise. Therefore a second discriminator, D2, is used to select input pulses above a given amplitude. Only if the pulse amplitude is larger than a selectable threshold the pulse edge of the zero cross discriminator is passed to the subsequent timing circuitry. Details of the CFD design are described in [1, 2].

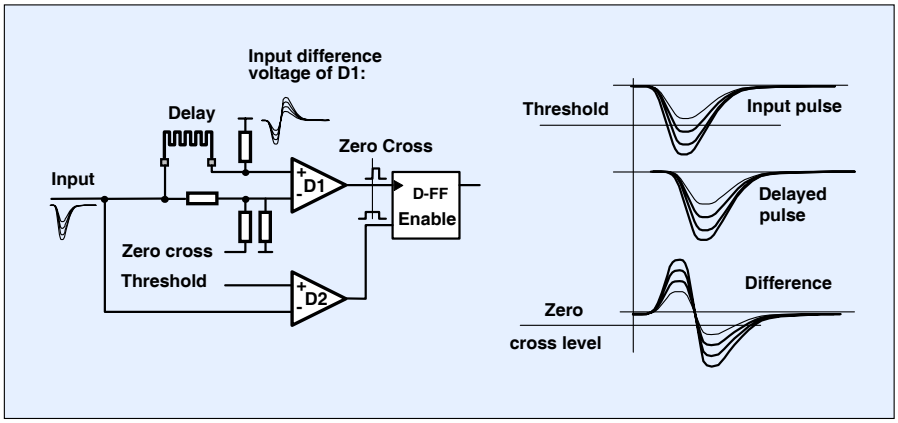


Fig. 24: Constant-fraction discriminator (CFD)

The threshold of the DPC CFDs can be set from 0 to -500 mV. In principle, all commonly used PMTs could be connected directly to the CFD inputs. There are, however, reasons why this is not recommended:

PMTs can deliver pulses of extremely high amplitude. Such pulses can be caused by radioactive particles generating light pulses in the glass of the tube, by instability in the PMT, or by illumination by strong laser pulses. Although the CFD inputs are overload-protected extremely strong pulses can damage the sensitive discriminator chips of the CFDs. Moreover, when the PMT output is connected directly to the CFD there is no control about the output current of the PMT. However, a PMT can easily be damaged by operation at excessive light intensities. PMTs should therefore always operated via a preamplifier. The amplifier not only protects the CFD against damage, it also delivers an overload warning if the maximum safe output current of the PMT is exceeded. Please see [2, 3] for details.

The recommended operating conditions for a number of PMTs and PMT modules are shown in the table below.

Manufacturer	Type	Preamplifier	HV / Gain	CFD Thresh.	Remark
bh	PMC-100-20[1)]	internal	100 %[2)]	-40 mV	cooled PMT module
bh	PMC-100-01[1)]	internal	95%[2)]	-80 mV	cooled PMT module
Hamamatsu	H7422P-40[1)]	HFAC-26-2	90%[2)]	-100 mV	cooled GaAsP PMT module
Hamamatsu	H5773P-00, -01	HFAC-26-10	0.9V[3)]	-100 mV	PMT module
Hamamatsu	R3809U	HFAC-26-01	-3000V	-50 mV	MCP PMT

1) Operated via bh DCC-100
2) DCC-100 gain setting
3) Gain control voltage

Please note that the gain of a PMTs strongly depends on its operating voltage. Moreover, the gain of different PMTs of the same type may be different by almost an order of magnitude. The values given in the table above are typical values. To obtain best counting efficiency and best IRF shape, please follow the instructions given in [2].

SPADs

Although the CFD inputs are primarily designed to accept single-photon pulses from PMTs they can also be used for recording pulses from SPAD modules. Because the SPAD modules have internal discriminators the threshold and zero-cross setting of the CFD have negligible influence on the counting efficiency and the instrument response width. SPAD modules having an output for negative pulses can be connected directly to the CFD inputs; SPAD modules with positive outputs need an A-PPI pulse inverter.

Manufacturer	Type	CFD Thresh.	CFD Zero cross	Remark
id Quantique	id-100	-100 mV	-20 mV	use A-PPI-D pulse inverter
MPD	PDM-xx-CD	-100 mV	-20 mV	use ‘Timing’ output
Perkin Elmer	SPCM-AQR	-100 mV	-20 mV	use 10 to 20dB attenuator plus A-PPI-D

Synchronisation Pulses from Light Sources

For TCSPC operation the timing reference signal has to be connected to CFD input number four. The input accepts pulses of negative polarity. The amplitude should be 100 mV to 500 mV; the duration can be anywhere from 300 ps to 5 ns. Thus, synchronisation signals from the bh BHL and BHLP diode lasers can be connected directly. For the bh BDL lasers an A-PPI-D pulse inverter is required (see Fig. 25, left). Also synchronisation signals from Titanium-Sapphire lasers can usually be used. Depending on the polarity, an A-PPI-D pulse inverter may or not be required.

If synchronisation signals are not available from the light source, a suitable signal can usually be generated by a bh PHD-400 photodiode module (see Fig. 25, right).

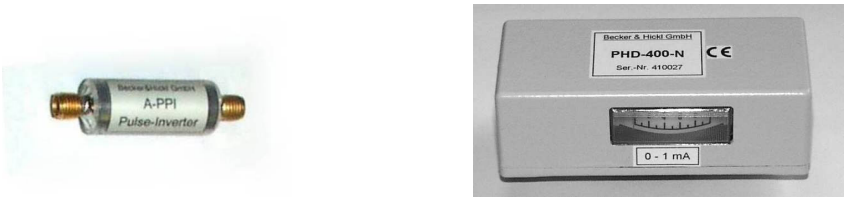


Fig. 25: Left: A-PPI-D pulse inverter. Right: PHD-400-N photodiode module

Typical Applications

Fluorescence Decay Measurements

A typical experiment setup for fluorescence decay measurement is shown in Fig. 26. The sample is excited by a bh BDL-SMC picosecond diode laser [5]. The pulse repetition rate is 20, 50, or 80 MHz, the pulse width between 50 and 90 ps. The laser is operated from a simple +12V wall-mounted power supply. The laser power is controlled by a signal from the bh DCC-100 detector controller [3].

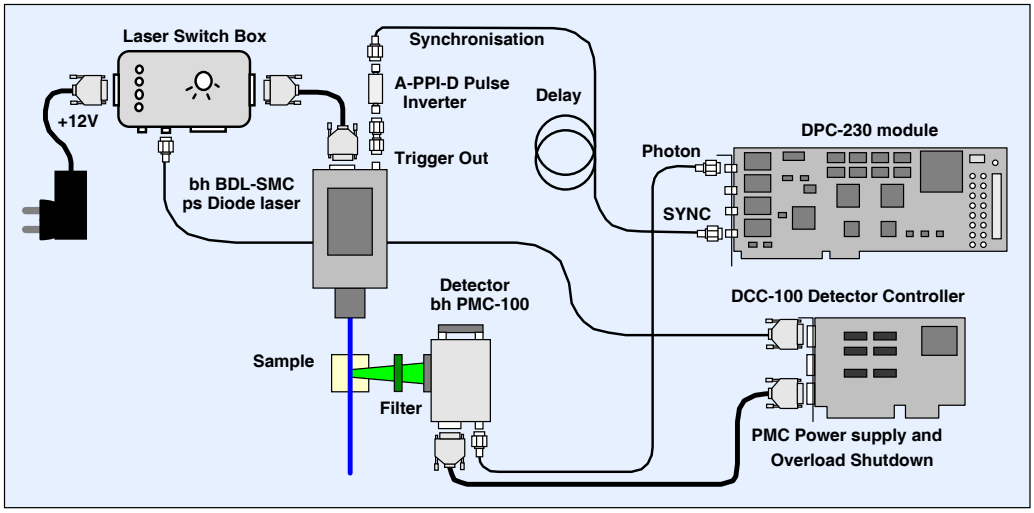


Fig. 26: Fluorescence decay measurement

The fluorescence is separated from scattered excitation light by a bandpass filter. Please note that the optical setup is shown simplified. In practice often a monochromator is used, and the fluorescence light is transferred to the detection system by a lens. Moreover, a polariser may be inserted in the beam path to remove the effect of rotational polarisation from the fluorescence decay. Please see [1, 2] for details.

The fluorescence is detected by a bh PMC-100 PMT module. The PMT module is controlled by a bh DCC-100 detector controller. The DCC-100 delivers the power for the PMT and the thermoelectric cooler of the PMC-100. It also provides overload shutdown of the detector if the light intensity becomes too high [2, 3].

The photon pulses of the PMC-100 are fed into a CFD input of the DPC-230 module. More PMC-100 modules can be added to the system and connected to the CFD inputs number 2 and 3. A synchronisation signal from the laser is connected to CFD input number 4. It is recommended that the synchronisation signal be delayed by a few meters of cable. With the right delay, the synchronisation pulse arrives later than the pulse of a photon detected in the same laser period. The location of the curves in the recorded time interval then becomes independent of the laser repetition rate [2].

For fluorescence decay measurement the DPC-230 is used in the TCSPC mode. It builds up a photon distribution over the time in the laser pulse period. The typical DPC system parameters are shown in Fig. 27. The measurement control parameters are located in the left part of the system parameter panel. The operation mode is ‘TCSPC FIFO’, ‘Single’. The measurements starts and stops by operator commands. If you want the measurement to stop after a specified time, switch in ‘Stop T’, and specify the desired ‘Collection Time’.

The right part of the panel shows the discriminator parameters of the CFD inputs and the TDC parameters. The optimal discriminator settings depend on the detectors; the parameters shown are typical of the bh PMC-100 module. The TDC parameters contain the time-channel width, the number of time channels, and the time range of the recording. Moreover, the curves can be shifted in time by applying an offset to the TDC times.

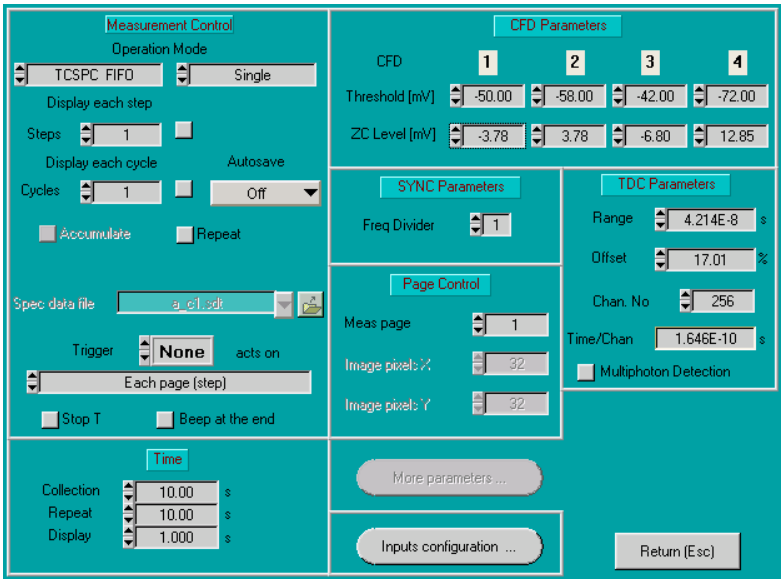


Fig. 27: System parameters for fluorescence decay measurement

The input configuration button opens the panel shown in Fig. 28. Both CFD channels of both TDCs are activated. CFD 1, 2, and 3 are used as detector inputs. CFD number four is used as a reference input for the laser pulses.

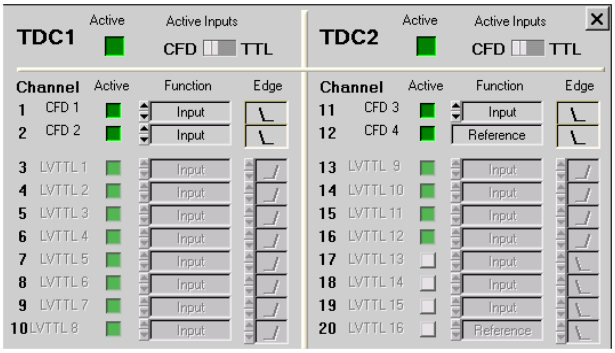


Fig. 28: Input configuration for TCSPC with up to three PMT detectors

The display of the curves is controlled by the trace parameters and display parameters, see Fig. 29. The trace parameters define which curves are to be displayed, and assign different colours to the individual curves. The trace parameters shown in Fig. 29, left, display the data recorded by a detector connected to channel 1, i.e. CFD 1. Curves of several measurements can be recorded into subsequent ‘pages’ of the data memory. The trace parameters shown display the curves recorded into page 1 and page 2.

The scale of the display and other details of the display are defined in the display parameters, see Fig. 29, right. A linear scale was selected, and the autoscale function was turned on. Two decay curves recorded and displayed with the settings of Fig. 27 through Fig. 29 are shown in Fig. 30.

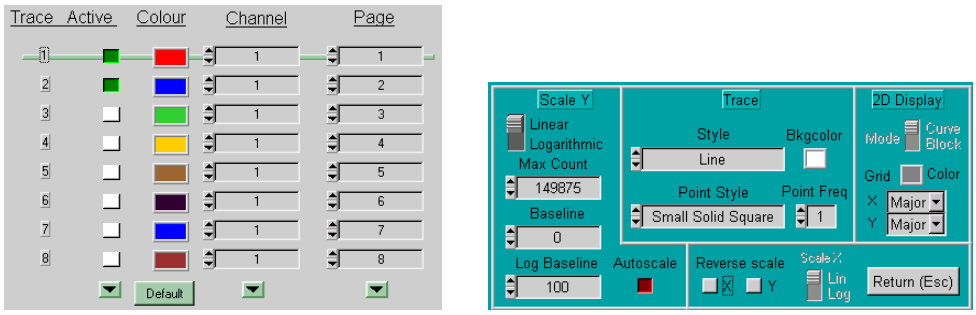


Fig. 29: Trace parameters and display parameters recommended for fluorescence decay measurement

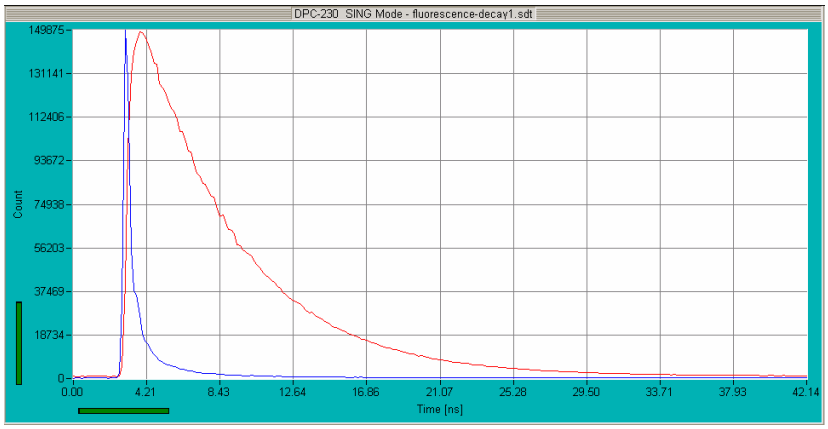


Fig. 30: Decay curves recorded with the setup parameters shown in Fig. 27 through Fig. 29

Luminescence Decay Measurement in the Microsecond Range

A suitable setup for luminescence decay measurements in the µs range is shown in Fig. 31. For extremely long decay times the laser pulse repetition rate must be reduced to a few 10 kHz or less. Using ps pulses at a repetition rate this low would result in an extremely low average excitation power. It is therefore better to use excitation pulses with a duration of a few 100 ns to several µs. The bh BDL-SMC diode laser is therefore operated in the CW mode [5]. The laser is switched on and off periodically via its on/off input. To generate pulses of appropriate duration suitable on/off signal is generated by a bh DDG-200 pulse generator module. The pulse width and repetition rate of the excitation pulses is defined by the DDG-200 setup parameters.

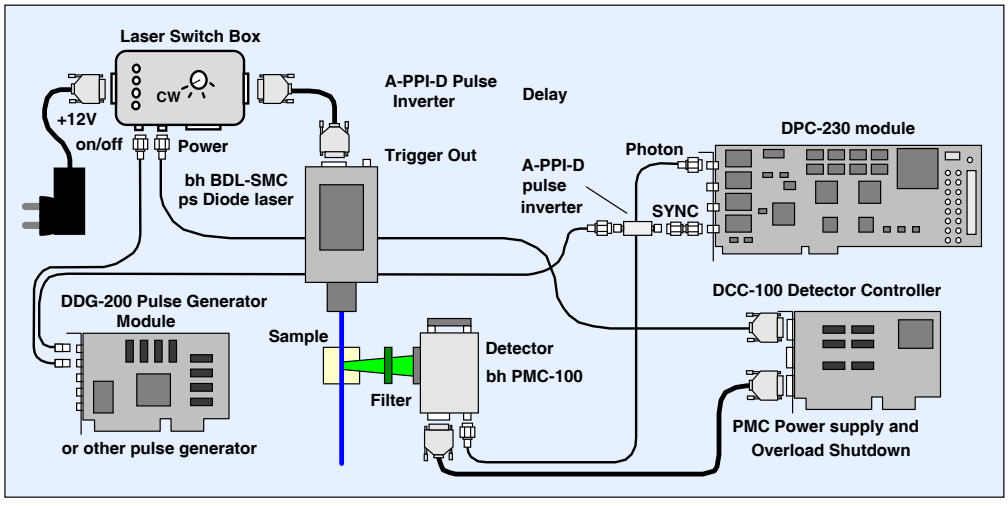


Fig. 31: Luminescence decay measurement in the microsecond range

The luminescence light is detected the same way as for fluorescence decay measurement. The reference pulses for the DPC-230 are obtained from a second output of the DDG-200 pulse generator.

For microsecond lifetime measurement the DPC-230 is operated in the multiscaler mode. The system parameters are shown in Fig. 32. The other parameters are the same as for fluorescence decay measurement. A typical result is shown in Fig. 33.

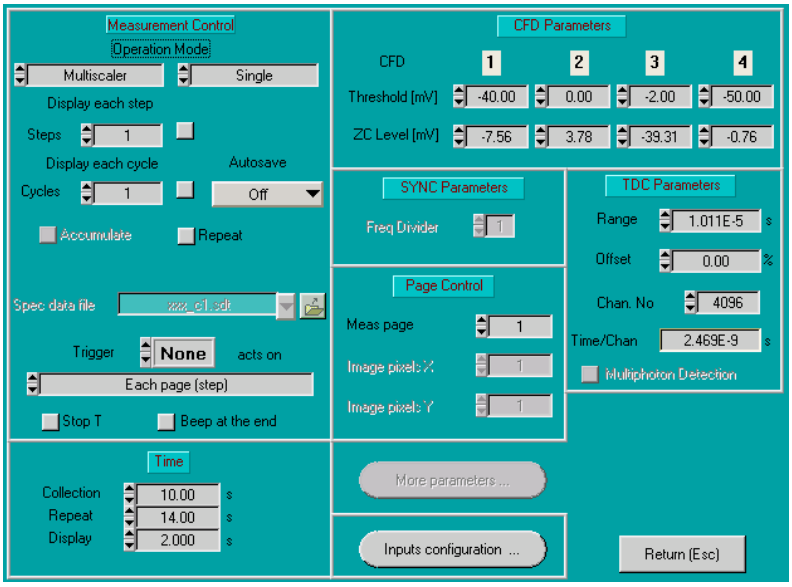


Fig. 32: System parameters for microsecond-luminescence decay measurement

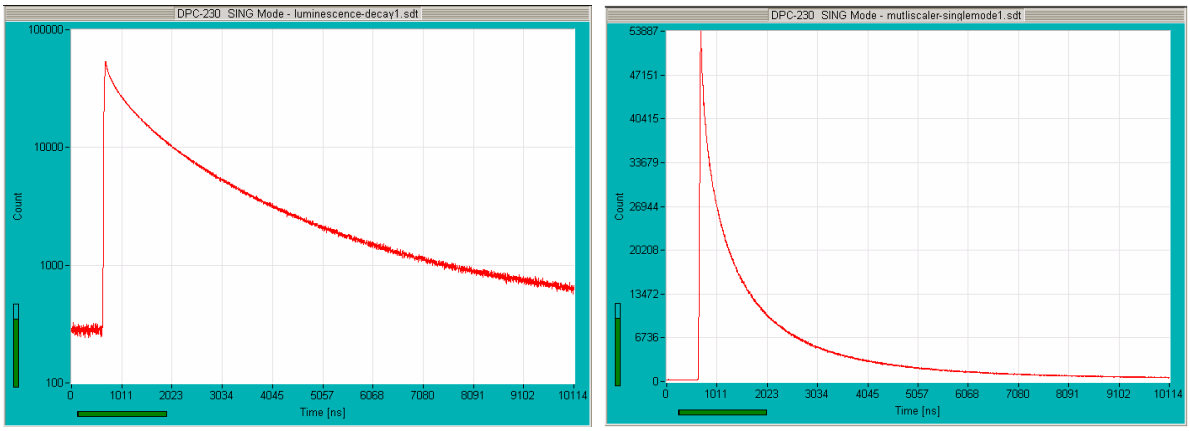


Fig. 33: Luminescence decay recorded with the system parameters shown in Fig. 32. Left: Logarithmic scale. Right: Linear scale

Fluorescence Correlation

Fluorescence correlation spectroscopy (FCS) is based on exciting a small number of molecules in a femtoliter volume and correlating the fluctuations of the fluorescence intensity. The fluctuations are caused by diffusion, rotation, intersystem crossing, conformational changes, or other random effects. The required femtoliter volume can be obtained one-photon excitation and confocal detection or by two-photon excitation. The technique dates back to a work of Magde, Elson and Webb published in 1972 [20]. Theory and applications of FCS are described in [8, 27, 28, 29, 30].

A typical optical setup is shown in Fig. 34. A CW laser beam is focused into the sample through a microscope objective lens. The fluorescence light from the sample is collected by the same lens, separated from the laser by a dichroic mirror, and fed through a pinhole in the upper image plane of the microscope lens. Light from above or below the focal plane is not focused into the pinhole and therefore gets substantially suppressed. With a high-aperture objective lens the effective sample volume is of the order of a femtoliter, with a depth of about 1.5 µm and a width of about 400 nm. A similarly small sample volume can be obtained by two-photon excitation. Typical FCS instruments use several detectors in different wavelength intervals or under different polarisation. Due to the similarities in the optical system, FCS is possible with most confocal laser scanning microscopes [2].

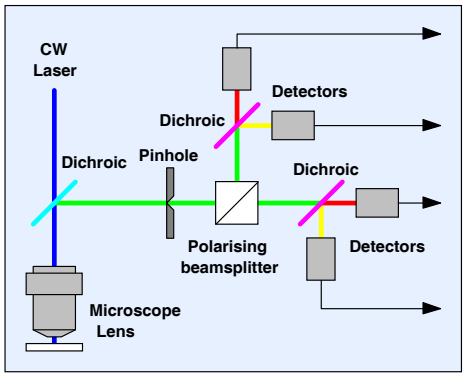


Fig. 34: Optical setup for FCS measurements

For good FCS results high detection efficiency is essential. The reason is the unusual signal-to-noise behaviour of correlation experiments. In a fluorescence lifetime or FLIM measurement a 50% loss in efficiency can be compensated by increasing the acquisition time by a factor of two. In an FCS measurement a 50% loss in detection efficiency requires the acquisition time to be increased by a factor of four to obtain the same signal-to-noise ratio. The detectors of FCS instruments are therefore single-photon avalanche photodiodes (SPADs) or high-efficiency PMTs with GaAsP cathodes [2].

The electronic setup of an FCS system is shown in Fig. 35. SPADs are connected via a cable adapter (see Fig. 20) to the LVTTL Inputs of the DPC. Any SPAD module with TTL or LVTTL output can be used. PMTs are connected to the CFD inputs, see Fig. 35, right.

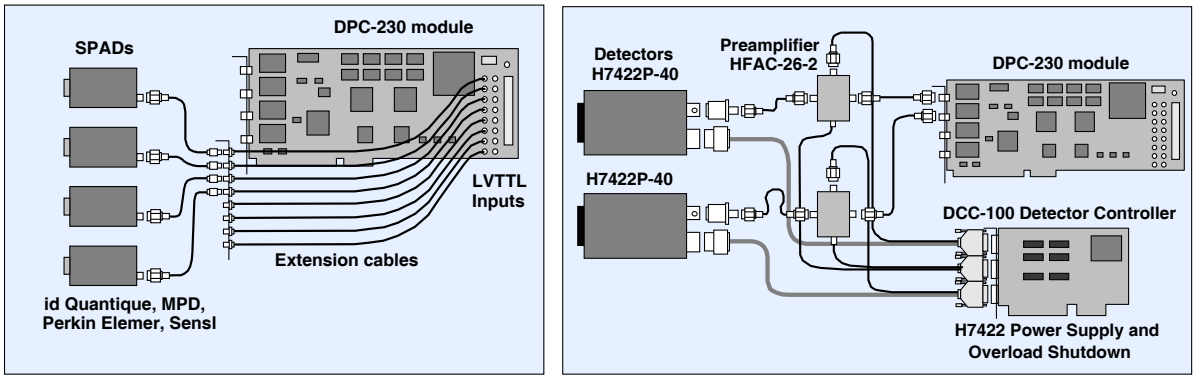


Fig. 35: FCS setup with DPC-230. Left: SPAD detectors. Right: GaAsP PMT modules

The measurement control part of the System parameters is shown in Fig. 36. ‘Operation Mode’ is set to ‘Absolute Time’, ‘Correlation’. The settings shown on the left calculate and display FCS curves, the accumulated number of counts, and an MCS (intensity) trace online. The time-tag data are not saved. The settings shown on the right calculate the same curves, but simultaneously save the time-tag data into a file ‘fluorescein-a.spc’. The maximum file size is 1000 Mb. The measurement stops when this file size is reached. The measurement can also be

stopped after a defined acquisition time. Activate the ‘Stop T’ button if you want to stop after a specified time.

‘Max Buffer Size’ defines a buffer in the RAM of the computer. If enough RAM is available the buffer size should be as large as or larger than the specified file size. The SPCM software then stores the time-tag data into the RAM before it writes them to the hard disc. The data readout is then not slowed down by hard disc operations.

The experiment trigger is not used; the setting is ‘none’. You may use the experiment trigger if you want to start the recording synchronously with an external event.

‘Collection time defines the acquisition time for the ‘Stop T option. ‘Display Time’ is the update interval for the online display.

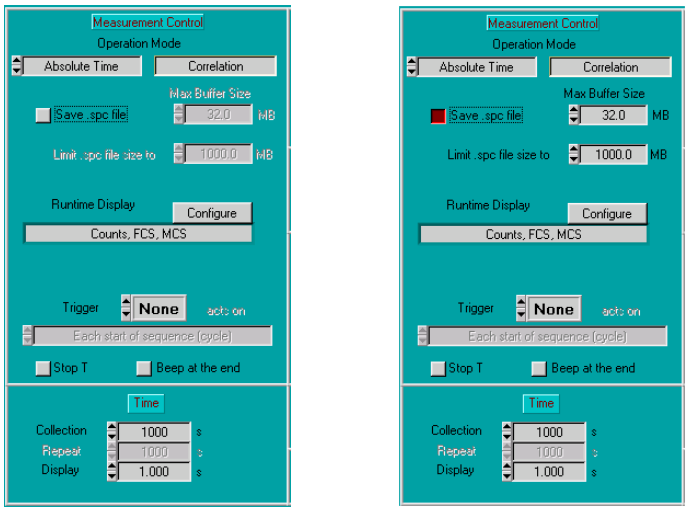


Fig. 36: FCS: Measurement control part of the ‘System Parameters’ panel. Left without, right with saving of the time-tag data.

The online display is configured via the configure panel shown in Fig. 37. With the settings shown decay curves, FCS curves, and MCS (intensity) traces are calculated. FCS curves are calculated by a multi-tau algorithm, and within a correlation time window of 1 ms. The MCS traces are displayed at a resolution of 1 ms per point.

The SPCM software is able to run a fit on the calculated FCS data. The setting shown left are without, the settings shown right with an FCS fit. The model contains two diffusion times, tau 1 and tau 2, and a triplet time, Tau trip. F is the relative triplet population, N the number of molecules in the detection volume, and Y the relative amplitude of the fast diffusion component. For the online fit we recommend to use a simple model, preferably one diffusion time and a triplet time. The start values of the fit procedure are defined on the right of the ‘Configure’ panel.

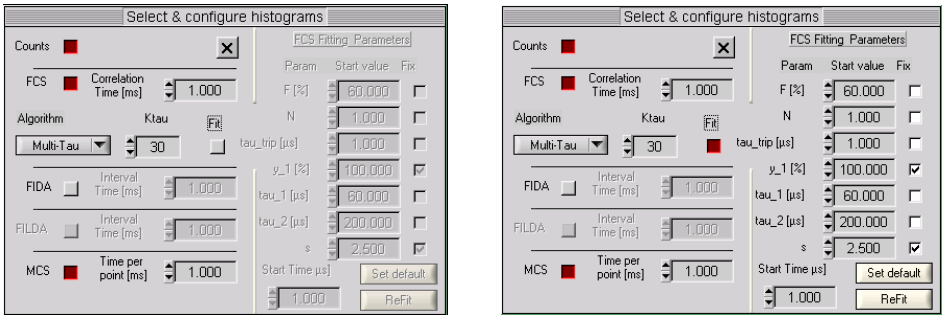


Fig. 37: Configuration of the online display. Decay curves, FCS curves, and an MCS trace are displayed. The left setup is without, the right setup with an FCS fit.

The curves to be displayed in the display windows for the accumulated counts, FCS curves, and MCS traces are defined under ‘Trace Parameters’. Each display window has its own trace parameters. To open the trace parameters, click on ‘Display’, ‘Trace Parameters’. A click into one of the curve windows selects the right trace parameter set. Alternatively, you can click in the desired curve panel with the right mouse key, and select ‘Trace Parameters’. Examples of the trace parameters are shown in Fig. 38. The trace parameters for the ‘Counts’ window activate the display of the accumulated counts for two detectors, connected to channel 4 and channel 6. The trace parameters of the FCS window are set to calculate and display an FCS curve for channel 4, and a fit to the data in channel 4. The trace parameters for the MCS window define traces for channel 4 and channel 6.

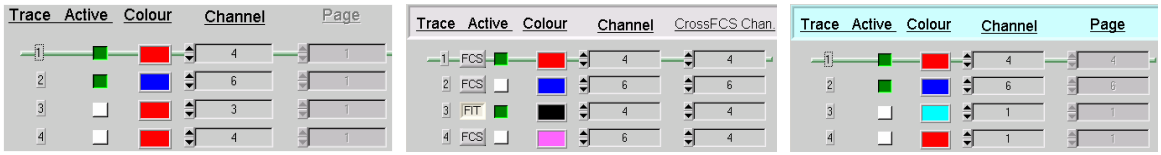


Fig. 38: Left to right: Trace parameters for Counts window, FCS window, and MCS window

A result of an FCS measurement with these parameters is shown in Fig. 39. The photons were acquired by two detectors, at an average total count rate of 260,000 photons per seconds. The total acquisition time was 46 seconds. The figure shows the accumulated counts of both detectors, the FCS (autocorrelation) of the detector connected to channel 4, and MCS traces for both detectors.

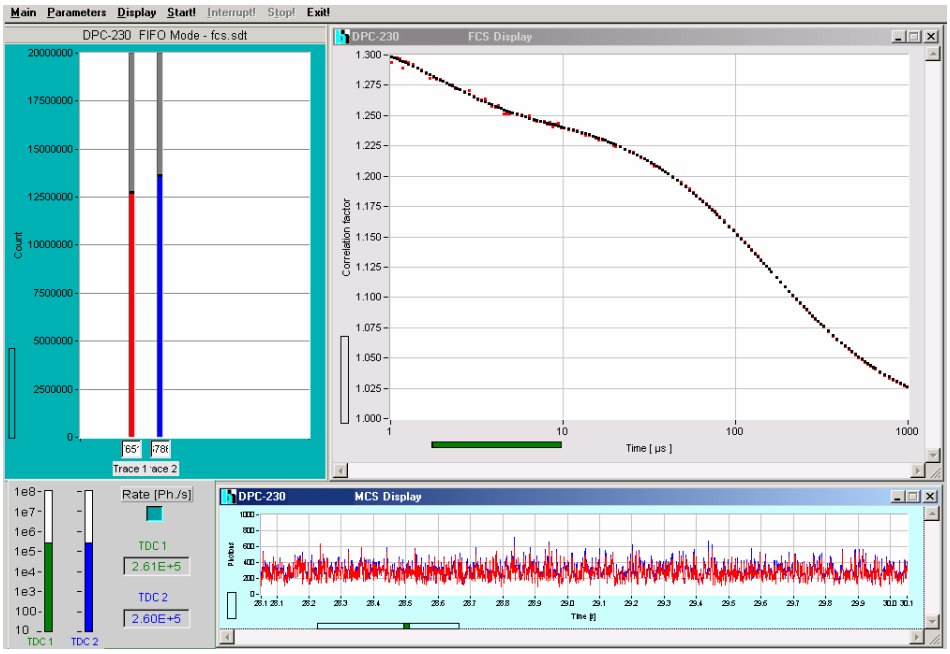


Fig. 39: Result of an FCS measurement

Cross-Correlation between different detector channels is obtained by simply defining the channels to be correlated in the trace parameters, see Fig. 40, left. The parameters shown correlate DPC channel 4 against channel 6, and run a fit on the calculated cross-FCS curve. The result is shown in Fig. 40, right. The data points are shown pink, the fit with a single diffusion time and a triplet time is shown black. The result of the fit is displayed by clicking on the ‘Trace Statistics’ button. They are shown as an insert in Fig. 40, right.

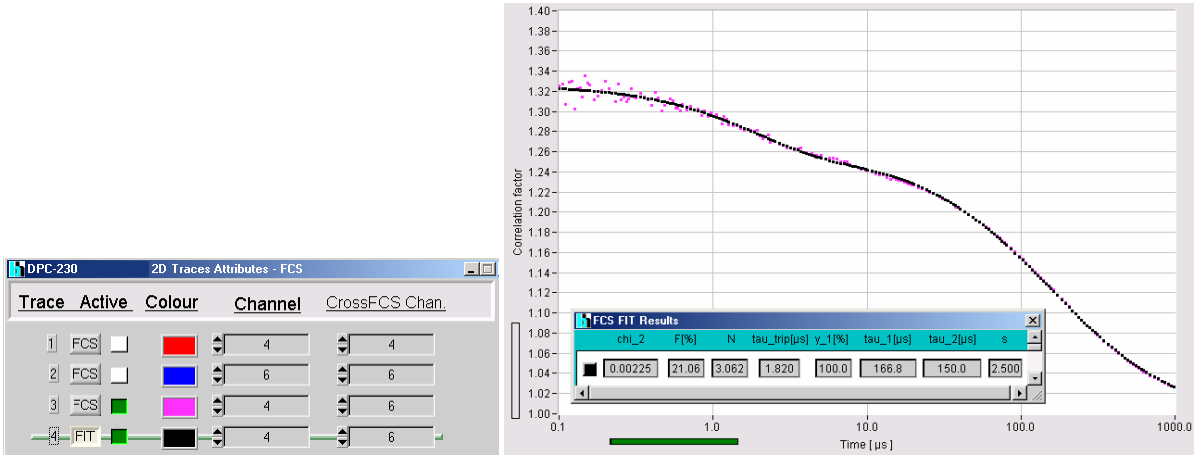


Fig. 40: Cross-Correlation between two detector channels

Picosecond Fluorescence Correlation

Fluorescence correlation down to the picosecond range has first been described in [12]. The authors used two bh SPC-130 modules with synchronised macro times, and included the TAC times in the calculation. The time resolution of this system is extremely high. The time-channel width can be as small as 820 fs; the electronic IRF width is about 5 ps FWHM. The resolution is therefore only limited by the transit-time spread of the detector. However, because the TAC times are not synchronous with the macro time clock the approach requires relatively complicated data analysis [13]. Of course, detector dead time and afterpulsing preclude ps correlation to be obtained from a single detector. Therefore, at least two detectors have to be used and the photon be data cross-correlated.

With the DPC-230, picosecond fluorescence correlation is merely a matter of the display scale defined in the FCS display. A result is shown in Fig. 41. The trace parameters define two traces, one for DPC channel 4 correlated versus channel 6, the other for channel 6 correlated versus channel 4. The data points are shown red and black, respectively.

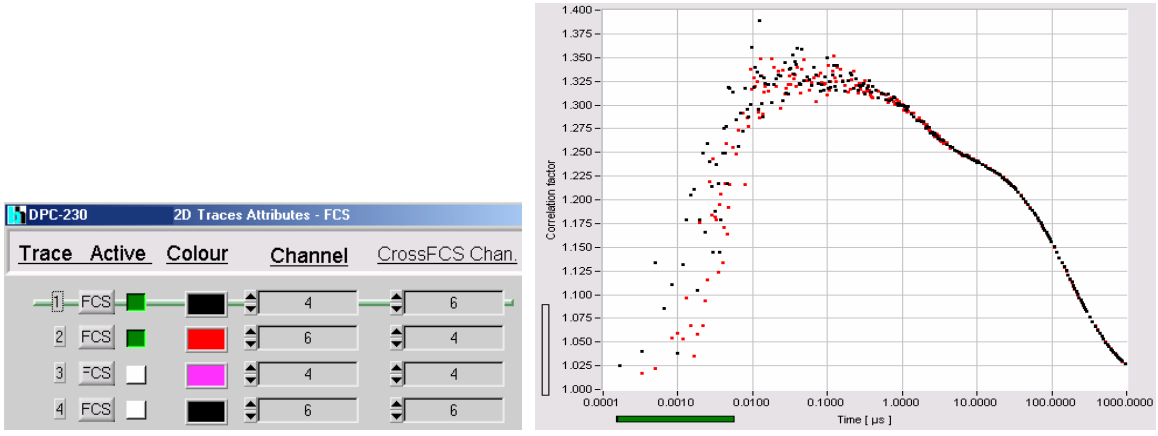


Fig. 41: Picosecond FCS. Left: Trace parameters. Right: FCS data. Time axis from 100 ps to 1 ms.

In contrast to classic FCS, in picosecond FCS transit time differences of the signals are noticeable. The result of such differences is a shift in the curves depending on whether detector one is correlated against detector 2 or vice versa. Therefore, make sure that the optical path lengths and the cable lengths are the same in all detector channels.

Anti-Bunching

Anti-bunching information is contained in picosecond fluorescence correlation data, see Fig. 41. However, anti-bunching curves can also be recorded via the classic Hanbury-Brown-Twiss start-stop experiment [14]. The light is split in two or more components and fed into separate detectors. The photons of one component are used as start pulses, the photons of another detector as stop pulses. The photon pulses in the stop channels must be delayed in order to obtain positive start-stop times. A delay can be introduced by increasing the optical path length, by increasing the cable length, or, in the DPC-230, by defining a TDC offset in the system parameters. Except for the delay, the optical and the electrical setup are the same as for fluorescence correlation. The difference is only in the interpretation of the data. Picosecond FCS calculates cross-correlation between different detectors, the classic start-stop experiment builds up histograms of the times between the photons recorded in different detectors.

DPC system parameters for classic anti-bunching are shown in Fig. 42. In the simplest case, the DPC builds up the start-stop histograms online, without saving any data of the individual photons Fig. 42, left. However, the DPC can also build up FCS curves simultaneously with the start-stop histograms (Fig. 42 middle and right). The single-photon data can be discarded (Fig. 42, middle), or saved for later analysis (Fig. 42, middle).

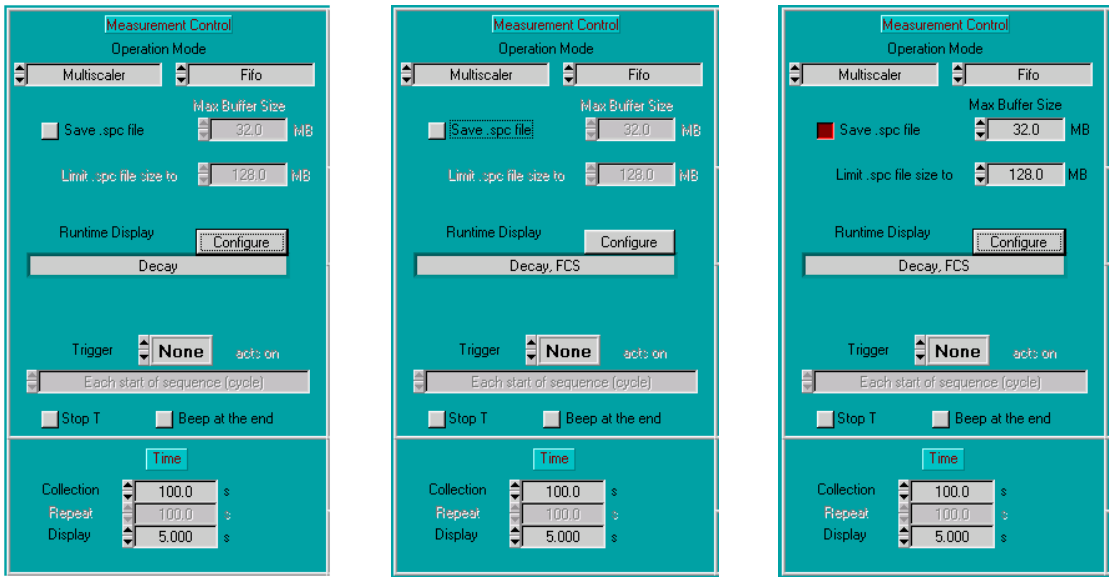


Fig. 42: System parameters for classic start-stop experiments. Left: Histograms built up online, single-photon data discarded. Middle: Histograms and FCS curves built up online, single photon data discarded. Right: Histograms and FCS curves built up online, single-photon data saved.

The input configuration panel is shown in Fig. 43. In the example shown two detectors are used. The start detector is connected to channel 4 (LVTTL Input 2), and declared as ‘Reference’. The second detector is connected to channel 6 (LVTTL channel 4) and used as a stop detector. More stop detectors can be used, and declared as ‘Input’ channels.

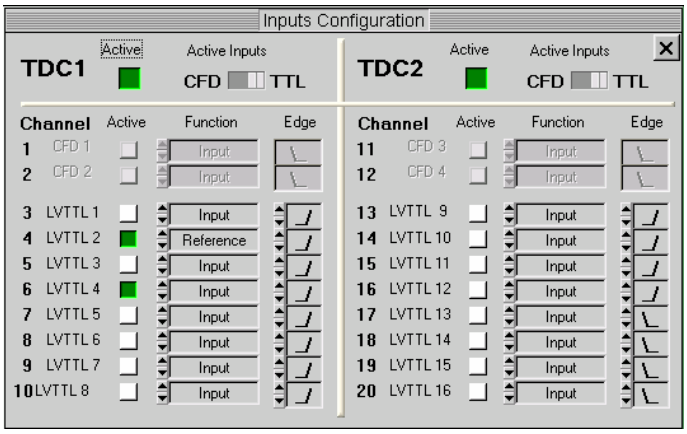


Fig. 43: Input configuration for a classic anti-bunching start-stop experiment

To see the desired start-stop histograms, activate the traces for the input channels (i.e. the stop channels) used in the trace parameters. The parameters for the two-detector setup defined in the input configuration are shown in Fig. 44. A result obtained from a Rhodamine 110 solution is shown in Fig. 45.

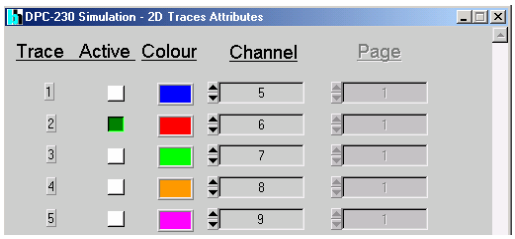


Fig. 44: Trace parameters for a start-stop experiment, detector channel 6 against the detector declared as 'reference' in the input configuration.

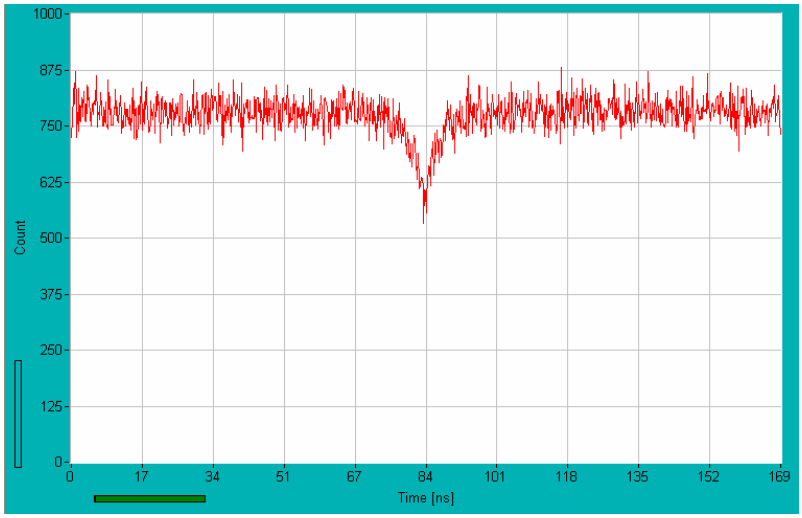


Fig. 45: Anti-bunching curve obtained with the settings shown above

With the setups shown in Fig. 42, middle or right, classic anti-bunching histograms together with correlation curves are obtained. A main panel with a start-stop histogram and an FCS curve is shown in Fig. 46.

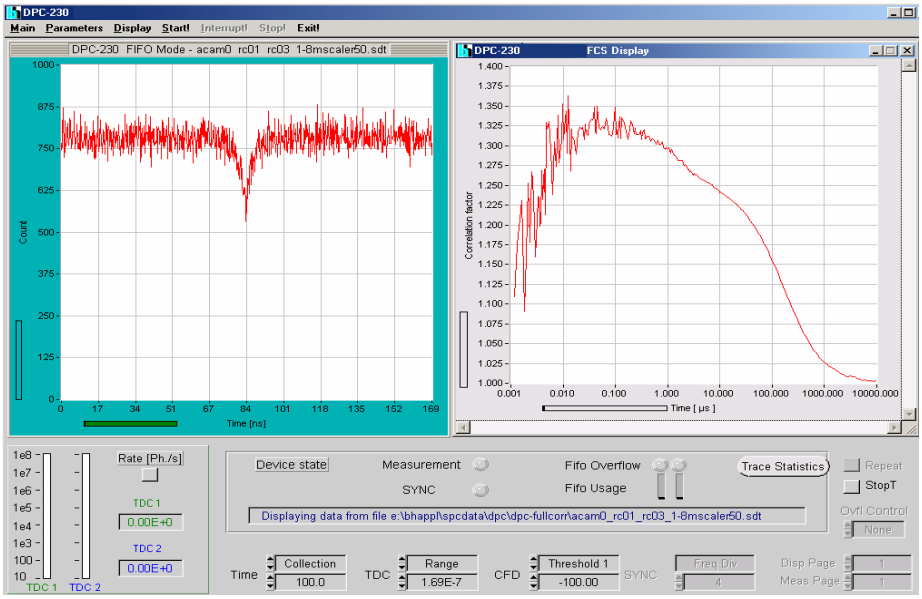


Fig. 46: Start-stop histogram and ps correlation obtained in the same measurement

Fluorescence Lifetime Imaging

The general principle of the TCSPC FLIM mode of the DPC-230 is the same as for the FIFO Imaging mode of the bh SPC modules [2]. The DPC-230 records the photons detected in one detector together with the synchronisation pulses from the scanner into a common time-tag data stream. By analysing these data, the SPCM software assigns each individual photon to a particular pixel of the scan area and to a particular time channel within this pixel, see Fig. 14, page 11.

The basic system setup is illustrated in Fig. 47. The laser scanning microscope must either be a multiphoton microscope (with a Ti:Sapphire laser) or a ps diode laser must be connected to the scan head. The fluorescence light is coupled to the detector either from a non-descanned port or a confocal output of the scan head. The single-photon pulses of the detector are fed into CFD input 3 of the DPC module. A synchronisation signal from the laser is connected to CFD input 4. For lasers with variable pulse period the SYNC pulses are delayed to make the recording independent of the pulse period, see [2].

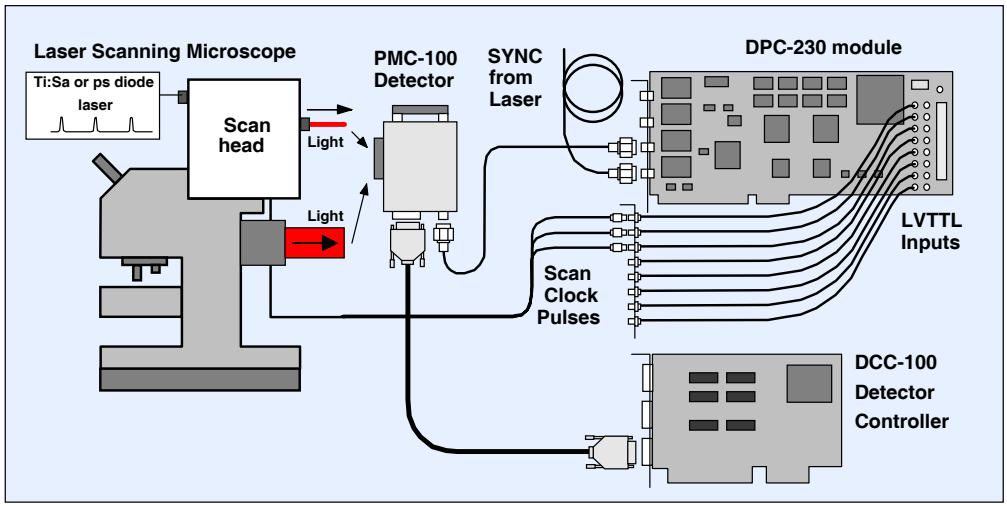


Fig. 47: Basic system setup of a FLIM system

The scan clock pulses (pixel clock, line clock, frame clock) of the microscope are connected into three LVTTL channel of TDC 1. Thus, TDC 1 records the times of the scan clocks, TDC 2 the times of the photons and the times of the laser pulses. The FLIM image is reconstructed from these data.

The system parameters for FLIM operation are shown in Fig. 48. The DPC works in the 'TCSPC FIFO' 'FIFO Imaging' mode. With the settings shown, a FLIM image with 256x256 pixels and 256 time channel per pixel is produced. Intermediate results are displayed in intervals of 'Display time'. The image data are calculated online, the time-tag data are not saved. If you need the time-tag data, e.g. for image correlation within a recording over a large number of frames, activate the 'Save .spc file' button. No stop condition is set; the measurement runs until it is stopped by the operator. If you want the measurement to stop after a specified 'Collection Time', activate the 'Stop T' button.

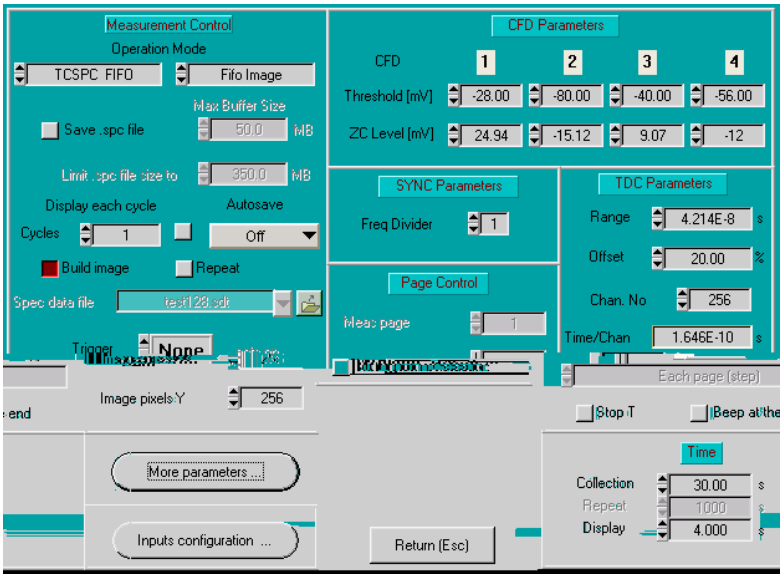


Fig. 48: System parameters for FLIM operation

The input configuration is shown in Fig. 49. TDC 1 works in the LVTTL mode. The inputs 3, 4, and 5 are defined as frame clock, line clock, and pixel clock inputs. TDC2 works in the CFD input mode. CFD 3 receives the photon pulses, CFD 4 the laser reference pulses.

Fig. 49: Input configuration for FLIM operation

The details of the scan synchronisation are defined under 'More Parameters'. In the simplest case (i.e. for the bh DCS-120 scanner), the FLIM image is recorded at the same resolution the microscope uses to scan the sample. The 'More Parameters' panel for this situation is shown in Fig. 50, left. However, the DPC-230 can also apply line and pixel binning to the FLIM data. Binning can be useful if a microscope scans at a pixel resolution higher than appropriate for

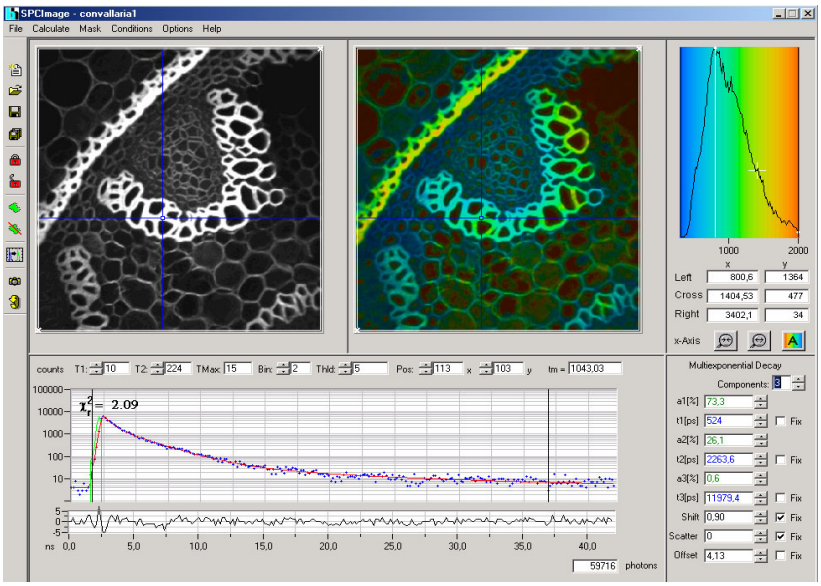


Fig. 52: Fluorescence lifetime image recorded with DPC-230 and bh DCS-120 confocal scanning FLIM system [4]. Data analysed by SPCImage data analysis routines of SPCM software [4, 6]. Intensity image, lifetime image, decay curve and fit at cursor position.

Luminescence Lifetime Imaging in the Microsecond Range

Fluorescence lifetime imaging in the microsecond range requires low repetition rate of the excitation pulses. Low repetition rate causes two problems. The first one is that the laser repetition rate interferes with the pixel rate. Unless extremely slow pixel rates are used the result is stripes in the image. Second, the ratio of peak power to average power becomes large. Especially if femtosecond or picosecond lasers are used the high peak power may cause multiphoton excitation and excited-state absorption. If the peak power is reduced the average power becomes low, resulting in low emission intensity and long acquisition time.

The solution is to use excitation pulses of nanosecond or microsecond duration, and to synchronise these pulses with the pixel clock of the scanner. The principle is shown in Fig. 53.

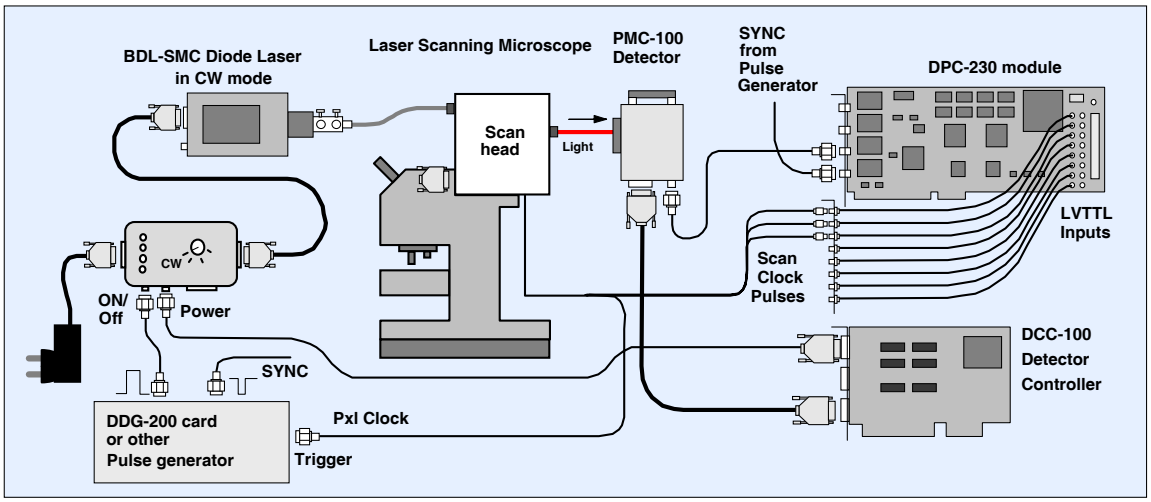


Fig. 53: Microsecond FLIM. The laser pulses are synchronised with the pixel clock.

The general setup is the same as for FLIM in the nanosecond range, compare Fig. 47. However, the BDL-SMC laser is operated in the CW mode [5] and controlled by a bh DDG-200 card or another pulse generator. The laser is switched on for a specified time interval at the

beginning of each pixel. The pixel time is made long enough to observe the full luminescence decay before the scanner goes to the next pixel.

Because of the low laser repetition rate the DPC-230 is best operated in the 'Multiscaler Imaging' mode. This makes a delay in the SNC path unnecessary. Moreover, multiscaler operation does not require any gating of the stop pulse with the previously detected photons (see Fig. 4). Therefore, 12 LVTTL input channels (16 minus 4 for the scan clocks and the SYNC) are available to connect detectors. An example of a system with several SPADs is shown in Fig. 54.

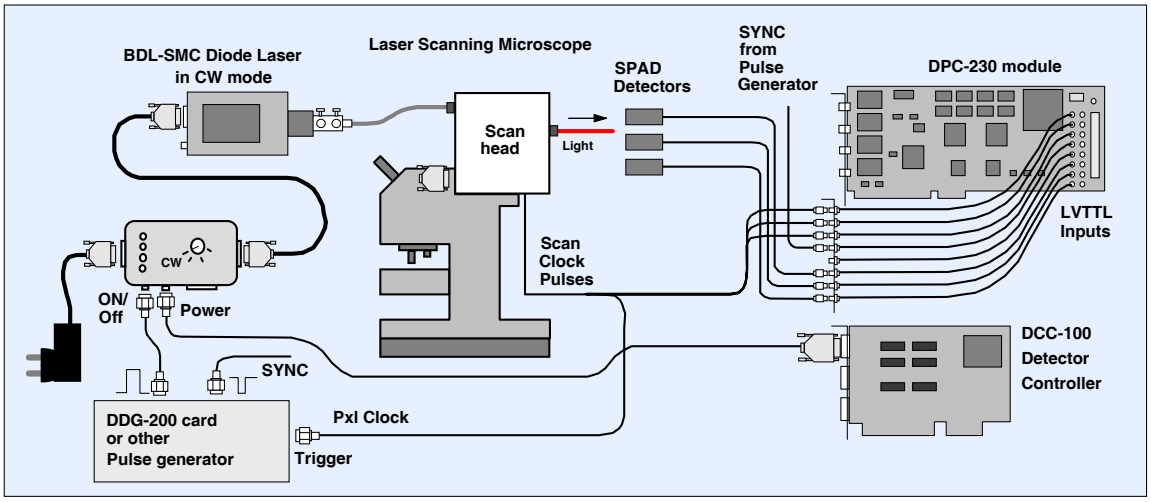


Fig. 54: Microsecond FLIM system with SPAD detectors

System parameters for a microsecond FLIM system are shown in Fig. 55. The operation mode is 'Multiscaler, 'FIFO Image'. An image of 256 x 256 pixels is acquired; the acquisition runs until the measurement is stopped by the operator. Each pixel contains 256 time channels, with a width of 39 ns. The total time range covered is about 10 µs. The image is built up online; the time-tag data are not saved. If you want to save the data, e.g. for running image correlation on data acquired over many frames, activate the 'Save .spc file' button.

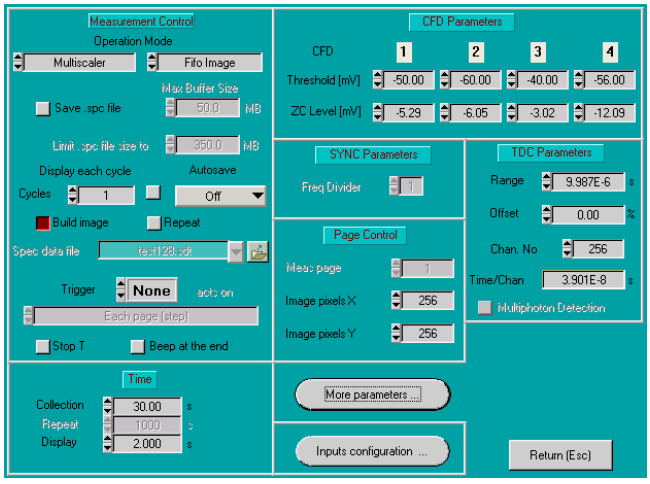


Fig. 55: DPC System parameters for microsecond FLIM

The input configuration panel is shown in Fig. 56. The configuration for a setup as shown in Fig. 53 is shown left. The PMT is on CFD 3, the reference on CFD 4. The scan clock pulses are connected to LVTTL 1, 2, and 3.

The input configuration panel for the setup shown in Fig. 54 is shown right. The reference is on LVTTL 4, LVTTL 5 through LVTTL 20 record signals from SPADs.

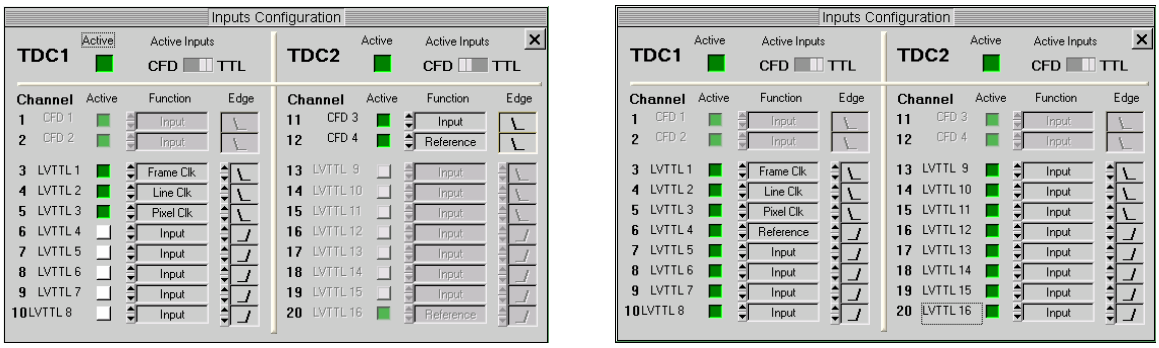


Fig. 56: Input configuration panel. Left: PMT on CFD 3, reference on CFD 4. Right: SPADs on LVTTL 5 through 20, reference on LVTTL 4.

Fig. 57 shows a luminescence lifetime image of a luminescent glass embedded in a Rhodamin 6G solution. The image was recorded by a bh DCS-120 confocal scanning FLIM system [6] and analysed by the bh SPCImage routines embedded in the SPCM software. The glass fluorescence has a triple-exponential decay, with components of 140 ns, 689 ns, and 1,79 µs.

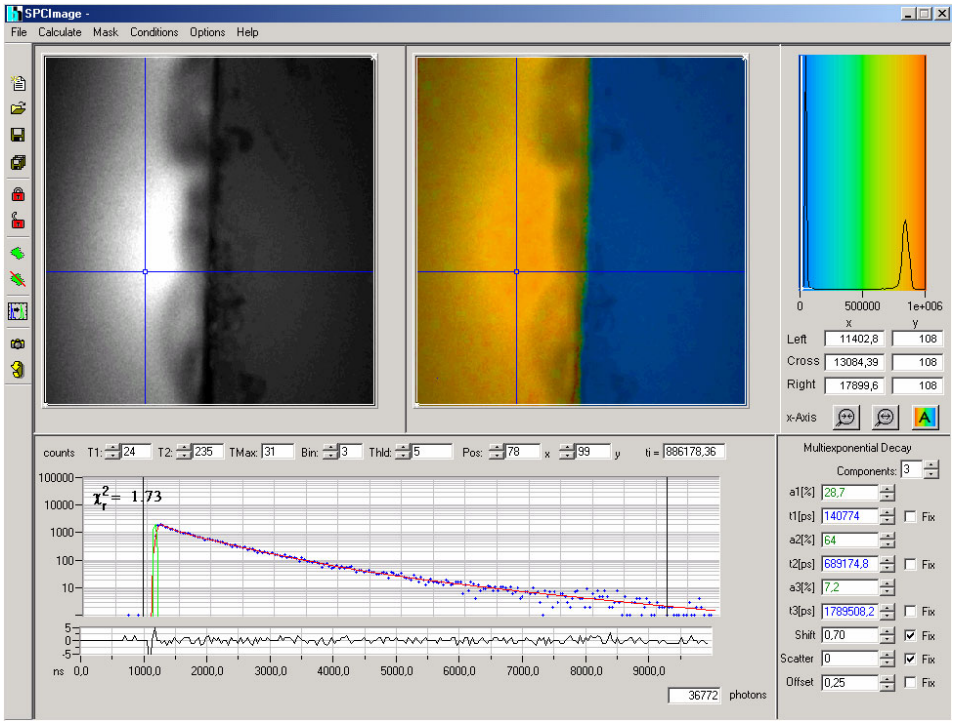


Fig. 57: Microsecond FLIM, analysed with SPCImage. Fluorescent glass (left) embedded in Rhodamin 6G solution (right). Colour represents lifetime. Triple-exponential decay analysis, decay components of glass fluorescence 140 ns, 689 ns, and 1,79 µs. bh DCS-120 confocal scanning FLIM system with bh BDL-405SMC laser.

SPCM Software

The DPC-230 comes with the 'Multi SPC Software', or 'SPCM' operating software. The SPCM software is not only used for the DPC-230 but also for all bh SPC (TCSPC) modules. It allows the user to operate up to four DPC-230 modules, or up to four SPC-630, -730, -830, 130, 140, or 150 modules. The SPCM software includes measurement parameter setting, measurement control, loading and saving of measurement and setup data, and data display and evaluation in 2-dimensional and 3-dimensional modes. The SPCM software runs under Windows 2000, NT, XP, and Vista. For installation of the SPCM software, please see 'Installation' page 13. The SPCM software is described in detail in the 'bh TCSPC Handbook' [2]. The following chapter focuses mainly on the features which are specific of the DPC-230.

Configuring the SPCM Main Panel

The main panel of the SPCM software can be adjusted to different instrument configurations. For details please see [2], 'SPCM Software'. There may be several windows for the measurement results, or different results may be displayed within one window. Frequently used control panels may be kept open at any time. Some typical configurations are shown in Fig. 58.

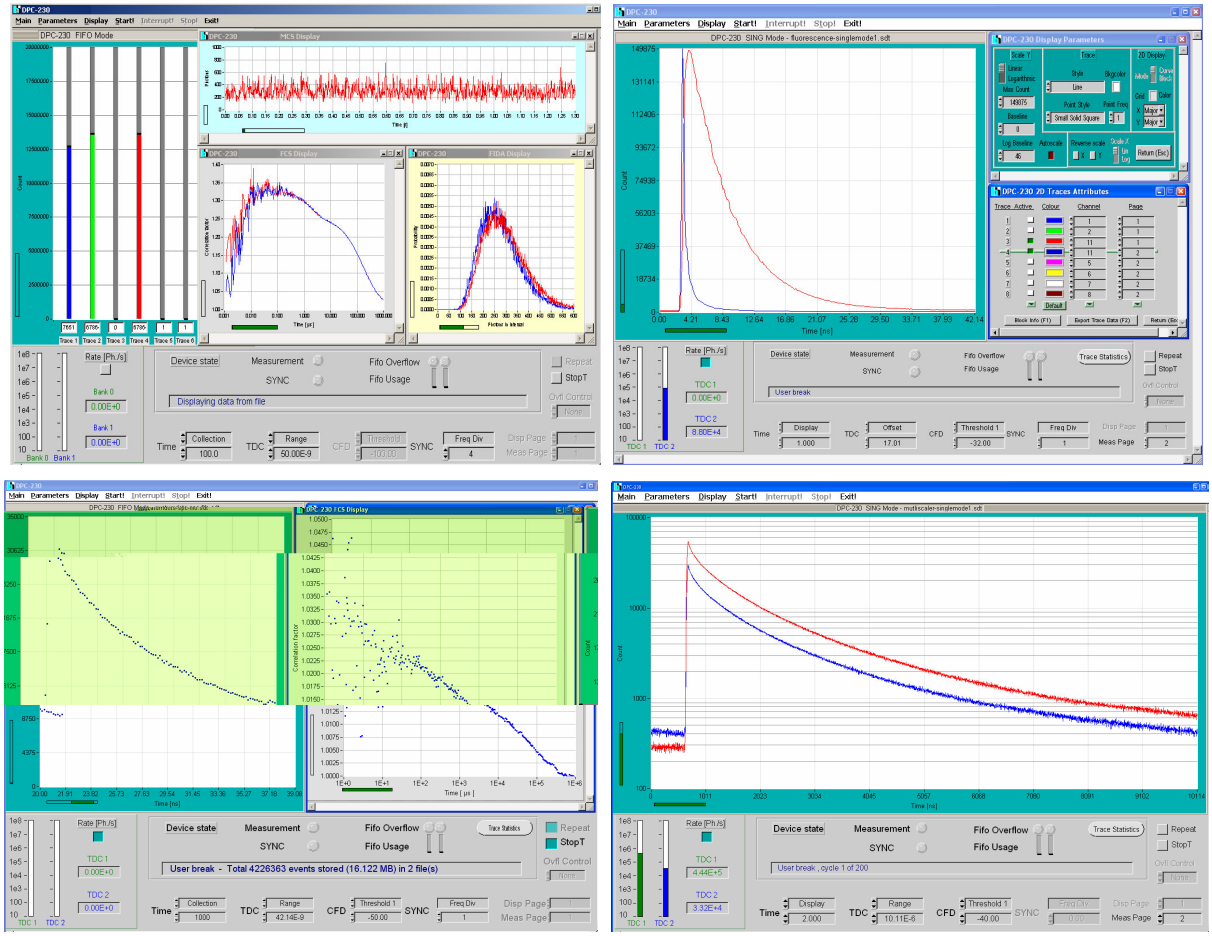


Fig. 58: Different configurations of the main panel. Upper row, left: Absolute time mode, with numbers of photons accumulated, intensity trace, cross-correlation of two channels, and photon counting histograms. Upper row, right: TCSPC measurement. Lower row, left: TCSPC FIFO measurement. Fluorescence decay and FCS curve. Lower row, right: Measurement in the multichannel scaler mode. Waveforms recorded into different measurement pages.

A typical main panel of a correlation measurement in the ‘Absolute Time’ mode is shown in the upper row, left. It shows the photon numbers accumulated in four detection channels, the intensity trace of one channel, correlation between two channels, and photon counting histograms of two channels.

A TCSPC measurement is shown in the upper row, right. It shows a fluorescence decay curve and an IRF recording. The recordings were taken in different ‘Measurement Pages’. The panels of the ‘display parameters’ and the ‘trace parameters’ are kept open on the right.

The lower row, left, shows a combined decay/FCS measurement in the TCSPC FIFO mode. It shows the decay curve, the intensity trace, and the FCS curve for one detector.

A measurement in the Multichannel Scaler mode is shown in the lower row, right. Two subsequent measurements were recorded into subsequent ‘pages’ of the memory, and are displayed simultaneously.

Pleas note that the setup parameters for frequently configurations can be put in a list of ‘Predefined Setups’, see ‘Predefined Setups’, page 51. Changing between different instrument configurations is then a matter of a single mouse click, see below.

Changing Between Different Instrument Configurations

The complete instrument configuration is saved together with the data or setup files [2]. Thus, the main panel configuration used for a particular experiment is restored when the corresponding measurement or setup data are loaded. Moreover, you may put frequently used system configurations into a list of ‘predefined setups’, see page 51. By using the panel shown in Fig. 59 you can change between different instrument configurations by a single mouse click.

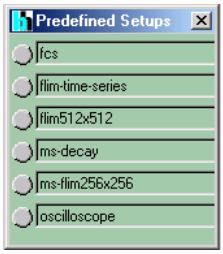


Fig. 59: Predefined-Setup panel. You can change between different instrument configurations by a single mouse click

Display and Trace Parameters

The display windows of the main panel are configured by the ‘Display Parameters’ the ‘2D Trace Parameters’, and the ‘3D Trace Parameters’. The corresponding panels are shown in Fig. 60.

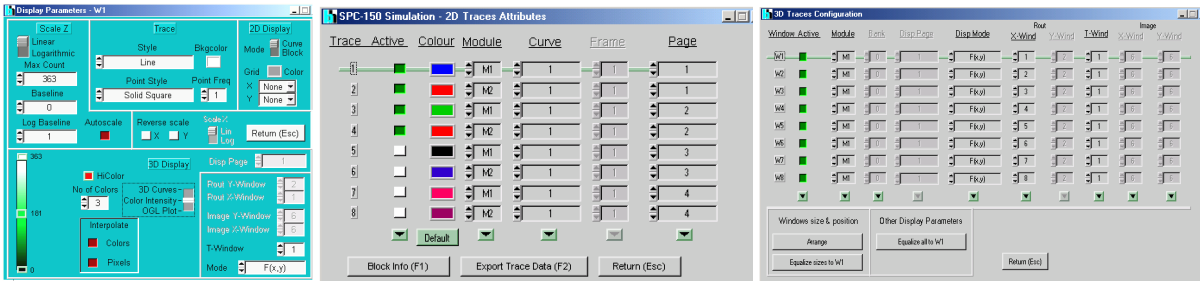


Fig. 60: Left to right: Display parameters, 2D Trace parameters, and 3D Trace Parameters

The *Display Parameters* (shown left) allow you to define how your results will be displayed. The upper part refers to the display of curves, the lower part to the display of images or other multi-dimensional data. The range of the count numbers to be displayed can be defined in the upper left. The style of the curves displayed can be defined under ‘Trace’ and ‘2D display’. The colours of images are defined in the lower left. A 3D curve mode, a colour-intensity mode, and an OGL plot are available. Sub-sets of multi-dimensional data sets, e.g. images in different time or wavelength windows, can be selected in the lower right part of the panel. Examples are given in section. For details, please see ‘The bh TCSPC Handbook’ [2]..

As shown in Fig. 58, the main panel may have several display windows open. The windows have separate Display and Trace Parameters. To select the right set of display parameters, click into the top bar of the particular display window, or click into the desired display window with the right mouse key.

The *2D Trace Parameters* define the curves displayed 2-dimensional display modes. Curves from different modules of a multi-module TCSPC system (‘Module’), individual curves from a multi-detector measurement (‘Curve’), and from different measurements or steps of a measurement sequence (‘Frame’ or ‘Page’) can be selected. A curve may also contain accumulated data of several detector channels or measurement steps.

The *3D Display Parameters* define up to eight display windows for multi-dimensional measurements. For each window, the display mode can be defined individually. Thus, images in defined time windows, sequences of curves in defined windows of an image, or a defined windows of the detector number can be defined. Each window has separate display parameters. Please see also [2].

Resizing and Positioning the Display Windows

To make the windows of the main panel resizable click into ‘Display’ and select ‘Scale Contents on Resize’. To resize a window, seize the edge of the window with the mouse cursor and pull the panel to the desired size. To shift a window, seize the top bar of the window and shift it into the desired position, see Fig. 61.

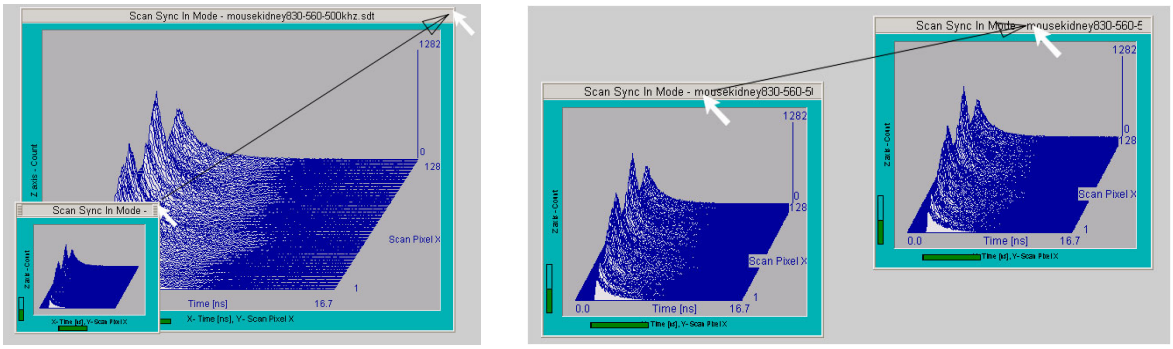


Fig. 61: Resizing and shifting windows of the main panel

Clicking into a display window area by the right mouse key opens the select panel shown in Fig. 62. ‘Proportional Graph’ sets the display proportions according to the ‘Scan Pixels X’ and ‘Scan Pixels Y’ of a Scan measurement. ‘Full Size Graph’ spreads the display window over the maximum available area. The panel also allows you to enable or disable the cursors, and to access the Display Parameters and Trace Parameters.

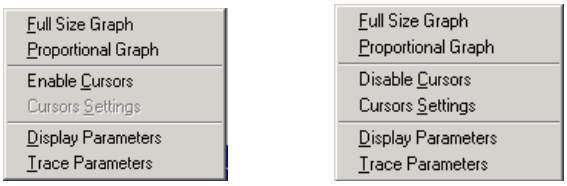


Fig. 62: Select panel for display size, cursor display, and display and trace parameters

It can happen that a display window has disappeared behind the edge of the screen or otherwise got out of control. (This can happen if a file from a dual-screen system is loaded in a computer with only one screen.) In that case, click into ‘Display’, and ‘Default Size and Position’. When the window is back in the screen area, set it to ‘Scale Contents on Resize’, see Fig. 63.

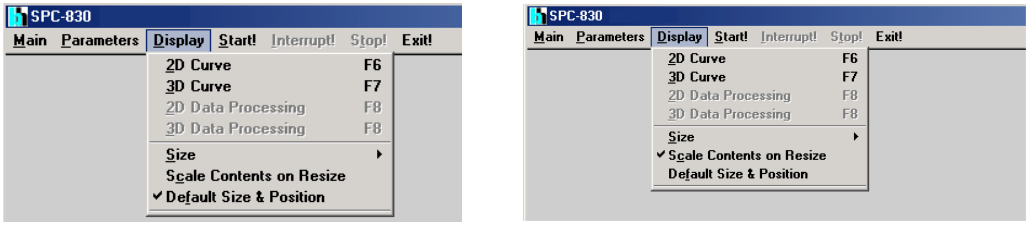


Fig. 63: Left: Setting window sizes and positions to default. Right: Window sizes user-definable

Cursors in the Display Windows of the Main Panel

Cursors in the display windows of the main panel are enabled by clicking into the window with the right mouse key. This opens a small panel in which the cursor functions can be enabled or disabled, see Fig. 64, left. A display window with cursors is shown in Fig. 64, middle. Two cursors and a ‘data point’ are available.

When the cursors are enabled a window with the cursor settings is displayed, see Fig. 64, right. The cursor settings window can be placed anywhere in the screen area. It can be closed by clicking on the ‘close’ symbol in the upper right corner, and re-opened by a right mouse click into the display window and selecting ‘Cursor Settings’.

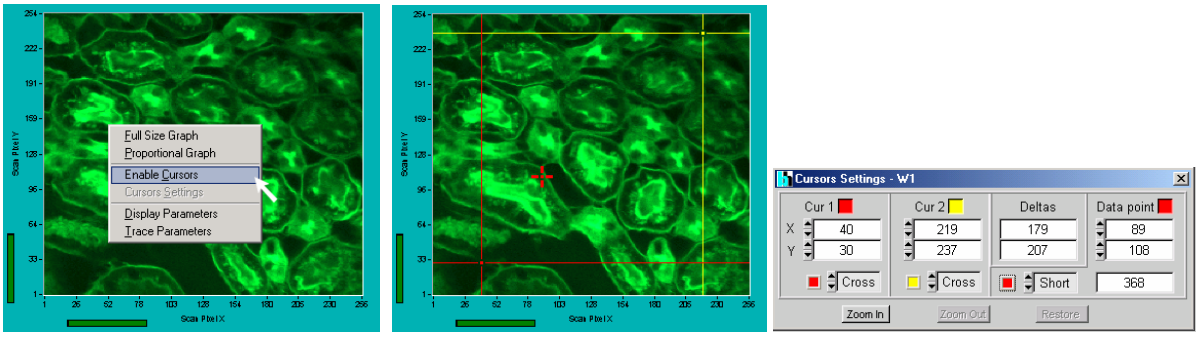


Fig. 64: Cursors in the display windows of the main panel

The cursors and the data point can be shifted by the mouse cursor, or by changing the cursor positions in the cursor settings window. Furthermore, the style and the colour of the cursors can be changed and a zoom function is available. The cursors in the main window interact with the parameters of the bh DCS-120 confocal scanning system, see [6].

Link to Data Analysis

The SPCM data acquisition software has a direct link to the SPCImage data analysis. Data are sent to the data analysis by clicking on ‘Main’, ‘Send Data to SPCImage’. For a number of

instrument configurations several data sets may have been recorded. In this case click on the display window that shows the data to be analysed, and then click on ‘Send Data to SPCImage’, see Fig. 65. For details of the SPCImage data analysis, please see [4] and [6].

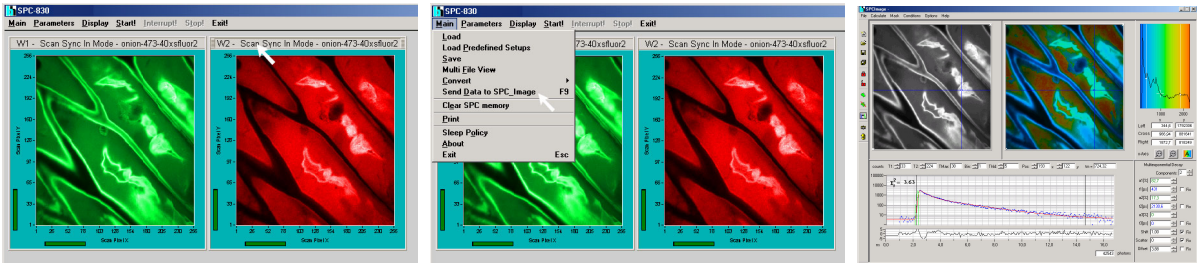


Fig. 65: Sending data to the data analysis. Click on data set to be sent (left), click on ‘send data to SPCImage’ (middle), SPCImage opens with the data selected (right)

Trace Statistics

Clicking on the 'Trace Statistics' button opens a window which displays information about the data shown in the curve windows. For decay curves or other waveforms the FWHM values, the peak counts, the total counts and the first moment of the photon distribution are displayed, see Fig. 66, left. For an FCS window the trace statistics window displays the results of the fit, see Fig. 66, right.

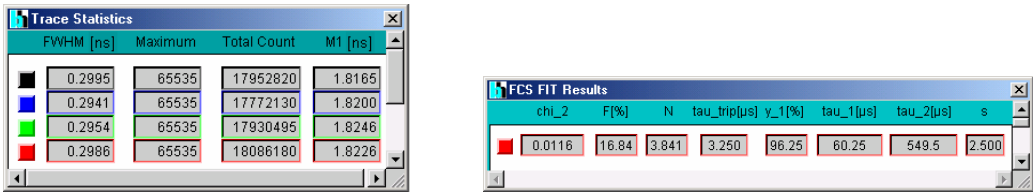


Fig. 66: Trace statistics panel. Left for a waveform window, right for FCS window

The window can be placed anywhere in the screen area. Please note that the trace statistics window works also in the oscilloscope mode. The window is thus an efficient tool to adjust the system for best time resolution, correct the signal transit time, and optimise the counting efficiency or IRF stability.

Status Information

Count Rate Display

The count rate display informs about the count rates of the two TDCs of the DPC-230. Both rates are the total rates for all active channels of the TDCs. Important: In contrast to the bh SPC modules the TDC chips have no counters to determine the count rates directly. The count rates have therefore to be determined by the SPCM software from the incoming photon data stream. Because the photon data of each TDC are buffered in FIFO there may be some latency in the count rate display.

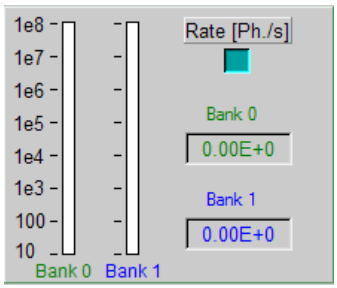


Fig. 67: Count Rate Display

FIFO Status

The status of the on-board FIFO buffers are displayed in the ‘Device State’ window. The filling of the buffers is shown by ‘FIFO Usage’. A FIFO overflow is shown by the indicator on top of the FIFO usage bars. When an overflow occurs there is a gap in the recorded photon data stream, and the absolute time scale may be

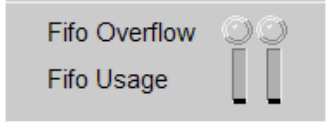


Fig. 68: FIFO status

lost. Strictly, such data cannot be correlated any more. However, as long as only a few overflows occurred correlation within one and the same TDC may still possible without noticeable errors. However, data with more than 10 overflows are useless for correlation.

Device State

'Device state' informs about the general status of the DPC-230 see Fig. 69.

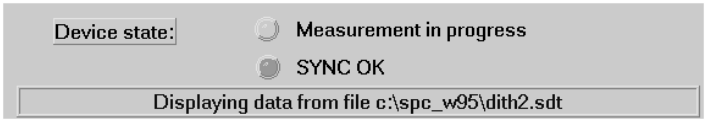


Fig. 69: Device status information

The most common status messages are

Collecting Data:	Measurement running
User break:	Measurement has been stopped by the operator
Displaying Data:	Measurement finished, final result is displayed
Displaying data from file:	Data loaded from an .sdt file are displayed
Total 275635 events collected:	Measurement has recorded the indicated number of events
Displaying data from file:	Displaying data loaded from an .sdt data file
Overflow(s) occurred:	The FIFO has run full, not all photons could be recorded
Waiting for Trigger:	Measurement started, waiting for experiment trigger

System Parameters of the DPC-230

The system parameters panel of the DPC-230 is shown in Fig. 70. The panel contains separate sections for the measurement control parameters and the hardware settings of the CFDs and the TDCs.

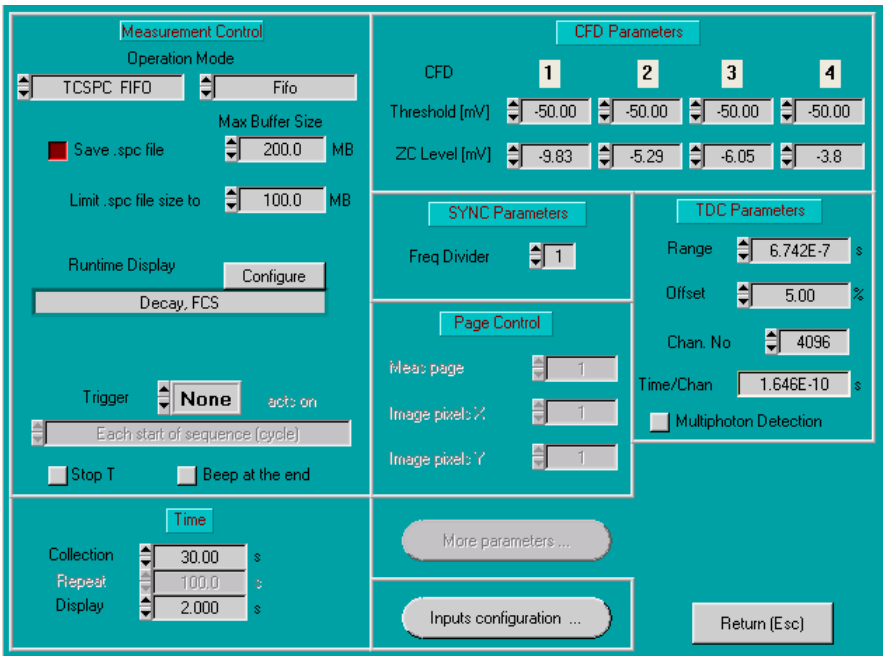


Fig. 70: System parameters of the DPC-230

Operation Mode

The operation mode of the DPC is selected by two parameters. The first parameter determines the hardware configuration, the second one the way the photon data are interpreted. The selection panel is shown in Fig. 71.

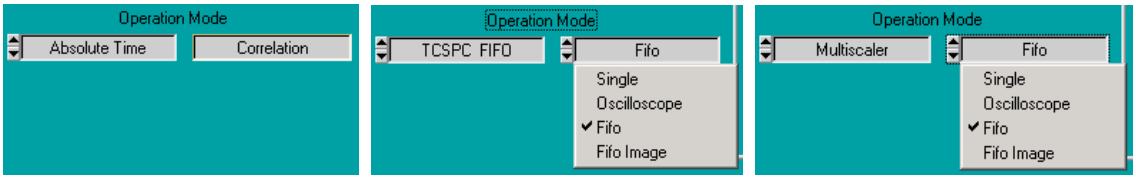


Fig. 71: Selection of the operation mode

Absolute Time

'Absolute Time' is an absolute timing mode. It records the photons of all active channels (see 'Absolute Timing', page 2) with their times from the start of the experiment. The way of interpretation is 'Correlation'. The Absolute Time mode is used to obtain intensity traces, photon counting histograms, and fluorescence correlation and cross-correlation functions for a large number of detector channels. Correlation can be obtained over a wide time range from picoseconds to seconds.

TCSPC FIFO

'TCSPC FIFO' records three detector signals and one reference signal via the CFD inputs. The mode delivers both relative and absolute photon times. The absolute times are referred to the start of the measurement, the relative times to reference pulses from the light source. The time measurement is 'reversed start-stop', i.e. the times are measured from the photons to the

next reference pulse (see ‘Relative Timing’, page 3). The data of the detector channels can be interpreted either as single waveforms (‘Single’), as oscilloscope traces (‘Oscilloscope’), or as fluorescence decay curves plus fluorescence correlation data (‘FIFO’). The ‘FIFO’ option is similar to the FIFO mode of the bh SPC modules [2]. Moreover, images can be acquired by recording synchronisation pulses from a scanner together with the photons (‘FIFO Image’). Please note that ‘TCSPC’ is available only for the CFD inputs.

Multichannel Scaler

The ‘Multiscaler’ mode records both relative and absolute photon times. The times are referred to a reference pulse from a light source (see ‘Multichannel Scaler Mode’, page 8). In contrast to ‘TCSPC FIFO’ every reference pulse is rescored, not only the next one after the detection of a photon. The times are measured from the reference pulses to the photons. The ‘Single’ option accumulates the photon density over the time after the reference pulses, i.e. builds up the waveform of the light signals. The ‘Oscilloscope’ mode runs a repetitive measurement and displays subsequent waveforms in short intervals. ‘FIFO’ is used when fluorescence correlation in combination with photon density histograms is required. Images can be acquired by recording synchronisation pulses from a scanner together with the photons (‘FIFO Image’). The ‘Multiscaler’ function is available both for the CFD inputs and for the LVTTL inputs.

Specification of the Time-Tag Date File

The DPC-230 records time-tagged data, i.e. the times and TDC channel numbers of the individual photons. From these data can be calculated waveforms, intensity traces, correlation curves, or images. Because these data operations are performed online on the incoming data stream the time-tag data need not necessarily be saved in a file. However, it may be useful to save the time-tag data, e.g. to be able to re-analyse the data later, or to import them into other analysis software. Therefore, the time-tag data can be stored in two data files, one for TDC 1, the other for TDC 2. For data format please see ‘Format of Time-Tag Data Files’, page 55. The corresponding control section of the system parameters is shown in Fig. 72.

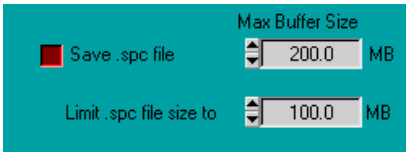


Fig. 72: Options for saving the raw data

The time-tag data recorded by the DPC-230 can easily reach sizes of tens or hundreds of megabytes. The maximum used disk space can therefore be limited by activating the ‘Limit Disk Space’ button and specifying the maximum file size. The measurement stops when the specified disk space has been filled.

‘Maximum Buffer Size’ defines a buffer in the computer memory. The buffer stores the data before they are saved to the hard disk. If possible, the buffer should be large enough to buffer the FIFO data of the complete measurement. Usually, the buffer size can be as large as 250 Mb for a computer with 1 Gb RAM. Under no circumstances a buffer size larger than the available memory space of the computer should be used. Windows may then attempt to provide virtual memory, i.e. to swap memory space with the hard disc. This makes the computer extremely slow and almost certainly causes problems with the data transfer from the DPC module.

If a sufficiently large buffer is not available use a buffer smaller than the value specified under ‘limit disk space’. Of course, the SPCM software writes to the hard disk when the buffer is

full. Although this may slow down the data transfer from the SPC module the loss is by far smaller than for memory swapping.

You may switch off the storing of the time-tag data altogether. However, in this case the software discards the raw data and delivers (and stores) only the results specified under 'Configure' and. *It is then impossible to run any later data processing on the single-photon data.* The option should therefore be used with care.

Name of Time-Tag Data File

During the measurement, the time-tag data are stored in a temporary file. The final data file is written when the measurement has been completed. The name of the time tag file time-tag data file is therefore specified at the end of the measurement. The definition panel is shown in Fig. 73. A list of previously used data file names is available by clicking on the ▼ symbol.

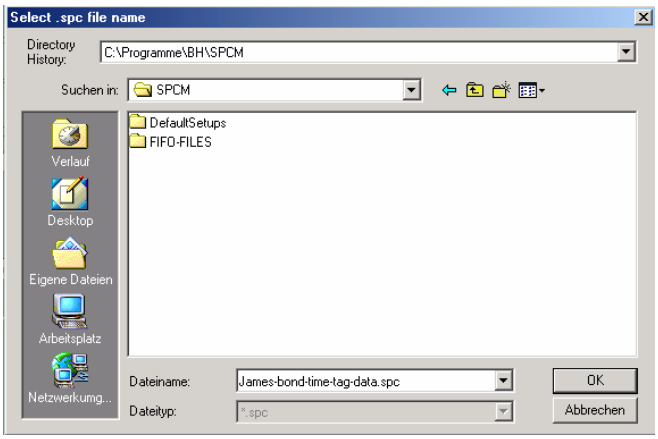


Fig. 73: Definition of the name of the time-tag data files

Configuring the Runtime Display

The SPCM software runs online processing on the incoming DPC data. The functions to be calculated on-line are shown in the field under 'Run Time Display'. The run time display can be configured by clicking on the 'Configure' button. This opens the panel shown in Fig. 74.

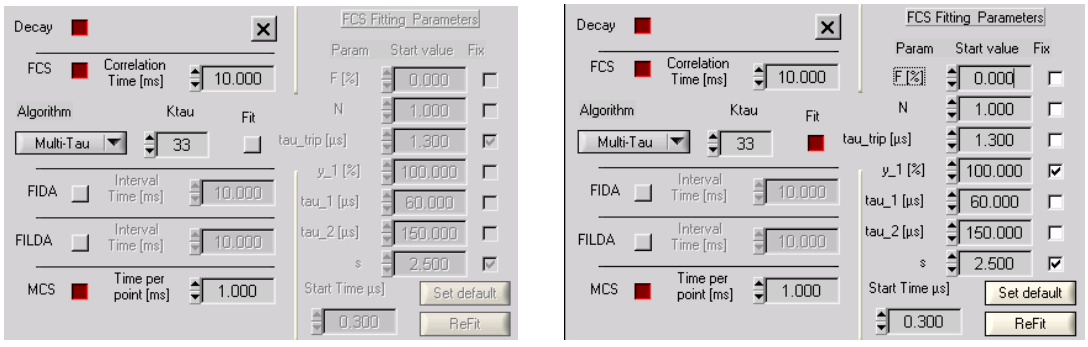


Fig. 74: Configuration panel for the runtime display. Left without, right with fitting of FCS data

The available on-line functions are

- Calculation of decay curves (photon density distributions) for the individual detectors
- FCS by a linear-τ algorithm with subsequent binning or FCS by a multi-τ algorithm. The maximum time up to which the correlation is calculated is defined by 'Correlation Time'.
- Fluorescence cross correlation between different detectors. (Use the 2D Trace parameters to define the detector channels)

- Calculation of photon counting histograms for the individual detectors (‘FIDA’). The sampling time interval is specified on the right.
- Calculation of lifetime histograms (FILDA) for all detectors. The sampling time interval is specified on the right.
- Intensity traces (MCS) for the individual detectors.

For all functions specified for runtime display individual display windows are provided in the main panel, see ‘Configuring the SPCM Main Panel’ page 37. The data displayed within these windows are defined by the ‘Trace Parameters’. Each display window has its own set of trace parameters. The trace parameter definitions for ‘Decay’, ‘FIDA’, ‘FILDA’ online display are shown in Fig. 75. Traces for DPC channels 1 to 4 are displayed.

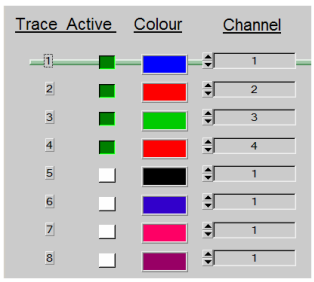


Fig. 75: Trace Parameters of the DPC-230, for decay curve, PCH, and MCS

The trace parameters for ‘FCS’ are shown in Fig. 76. The trance parameters for FCS without a fit are shown left. ‘Channel’ and ‘Cross FCS Channel’ define the DPC detector channel numbers for the data to be correlated and displayed. If ‘Curve’ and ‘Cross-FCS Curve’ are the same the autocorrelation function of the channel is calculated. Thus, the configuration shown calculates the autocorrelation functions of channel 4 and 5, and the cross-correlation of channel 4 versus channel 5.

If the fit function in the runtime configuration panel is enabled (see Fig. 74, right) the trace parameter panel is as shown in Fig. 76, right. For each trace can be defined whether an FSC curve or a fit curve is displayed.

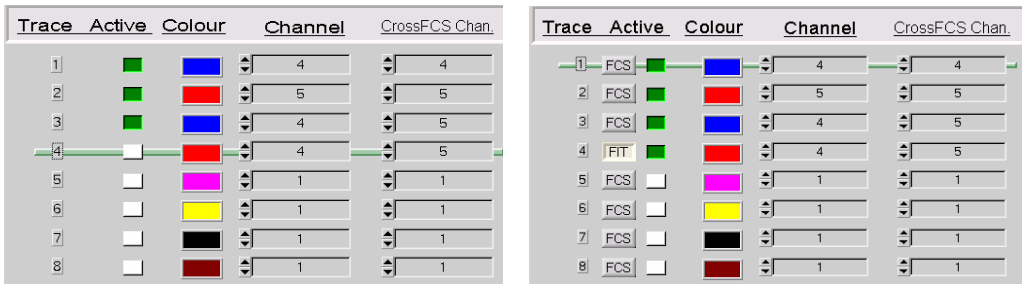


Fig. 76: Trace parameters for FCS display. Left without, right with fit of the FCS curves

For reasons of computation speed and memory space, the software calculates only one correlation function per detector channel. If the Trace Parameters define several correlation functions for one channel, e.g. an autocorrelation and a cross-correlation with another channel, only the first correlation is calculated during the measurement. The other ones are calculated and displayed after the measurement has been completed.

Configuring the Inputs

The configuration panel for the configuration of the DPC inputs is shown in Fig. 77. The panel is opened by clicking on ‘Input Configuration’ in the system parameter panel.

Both TDC chips can be switched on and off by the ‘active’ button. If one of the TDCs is not used it should be switched off to avoid unnecessary software actions.

For both TDCs the inputs can be switched to ‘LVTTL’ or ‘CFD’. Each TDC either has 8 LVTTL inputs or 2 CFD inputs. The LVTTL configuration is shown in Fig. 77, left, the CFD configuration right. It is possible to combine LVTTL operation in one TDC with CFD operation in the other.

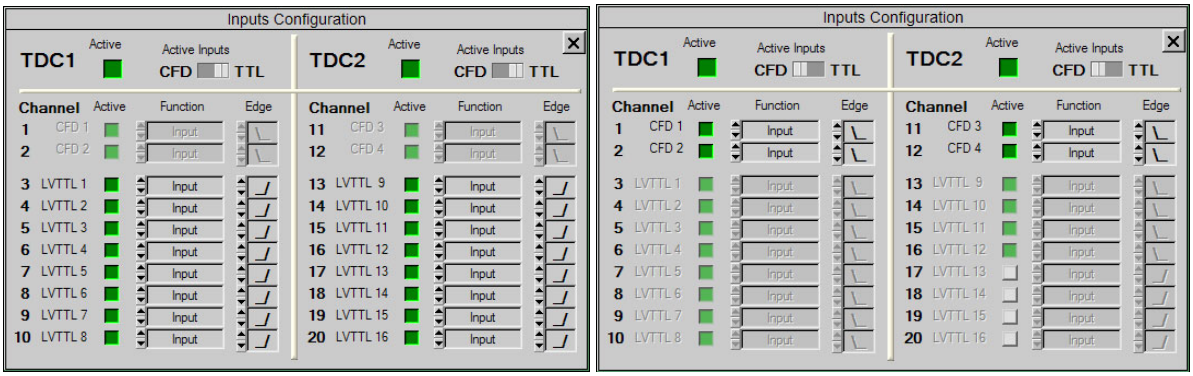


Fig. 77: Input configuration panel

All inputs can be individually switched on and off by the ‘active’ buttons. Moreover, the function of the inputs can be defined. The function can either be an input for a detector signal, a reference input from a light source, or a marker input. For TCSPC operation channel 12 (CFD 4) is always the reference.

‘Edge’ defines the active edge of the input signal. The active edge of the CFD inputs is always the falling edge; for the LVTTL inputs either the rising or the falling edge may be used.

Please note: For SPAD modules with TTL outputs ‘rising edge’ must be selected.

CFD and SYNC Parameters

The CFD parameters define the thresholds and the zero-cross levels of the CFD inputs. The thresholds can be set from 0 to -510 mV, the zero-cross levels from -96 to +96 mV. CFD thresholds smaller than -20 mV can result in noise pickup and should therefore be avoided. If a detector requires a threshold this low a preamplifier of sufficient gain should be used.

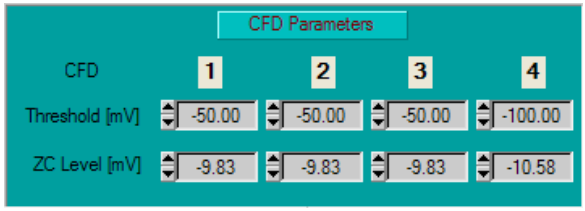


Fig. 78: CFD threshold and zero cross settings.

Please note that in the TCSPC mode CFD 4 is used for the reference input.

SYNC Frequency Divider

In the TCSPC mode the fourth CFD channel is used as a reference input. To display either 1, 2, or 4 signal periods of the recorded signal (see Fig. 6, page 4) the fourth CFD channel has a selectable frequency divider. The divider ratio is selected under ‘SYNC parameters’. The parameter is available only in the TCSPC mode.

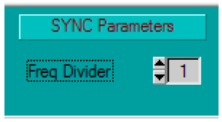


Fig. 79: SYNC frequency divider setting. The parameters determines the number of signal periods recorded in the TCSPC mode

TDC Parameters

The TDC parameters determine the recorded time interval and the number of time channels in the TCSPC mode and in the multichannel-scaler mode. The parameters are shown in Fig. 80.

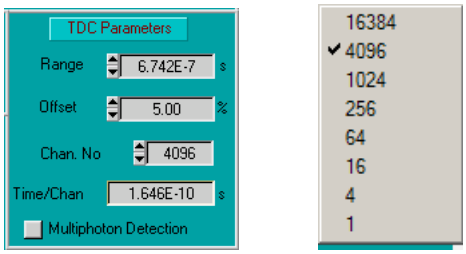


Fig. 80: Left: TDC parameters. Right: Selecting the number of time channels from a list

‘Range’ is the recorded time interval. ‘Offset’ provides a shift of the results in time. ‘Channel Number’ is the number of time channels. Values from 1 to 16384 can be selected from a list, see Fig. 80, right.

Time/Channel is either the time channel width of the TDC (164.6 ps) or a multiple of this value. Time/channel is set automatically depending on the selected ‘Range’ and ‘Channel Number’.

Because ‘Range’ must by a multiple of the time-channel width of the TDC the parameters ‘Range’ and ‘Channel Number’ are automatically corrected to the nearest possible combination. If the selected number of channels would result in a ‘Range’ larger than selected the value for ‘Range’ is automatically increased. If the desired ‘Range’ cannot be reached with the selected number of time channels the width of the time-channels is increased, i.e. several TDC channels are binned into one wider channel.

The ‘Multiphoton Detection’ button defines whether only one or several photons per signal period are recorded in the TCSPC mode. Please be cautious when using TCSPC with multiphoton detection. Even when multiphoton detection is enabled a second photon cannot be detected within the dead time of the detector. Multiphoton detection therefore does not necessarily remove curve distortions by pile-up effects. On the contrary, dead-time effects can make distortions even stronger, and less predictable than for single-photon detection.

Saving Setup and Measurement Data

The ‘Save’ panel is shown in Fig. 81. It contains fields to select different file types, to select or specify a file, to display information about existing file, and to select between different save options.

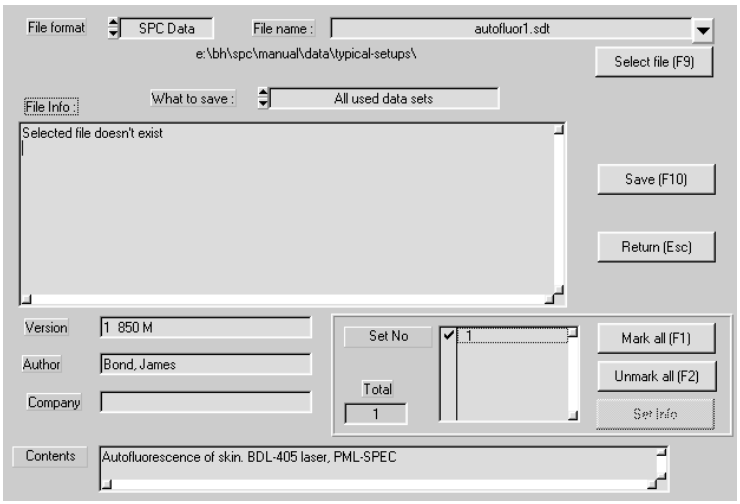


Fig. 81: Save panel

File Format

You can chose between ‘SPC Data’ and ‘SPC Setup’. The selection refers to different file types. With ‘SPC Data’ files are created which contain both measurement data and system parameters. When this file is loaded not only the measurement data are restored but also the complete system setup. With ‘SPC Setup’ files are created that contain the system parameters only. When such files are loaded the system setup is restored, but no data are loaded. Files created by ‘SPC Data’ have the extension ‘.sdt’, files created by ‘SPC Setup’ have the extension ‘.set’.

File Name / Select File

A file name can be written into the ‘File Name’ field. ‘Select File’ opens a dialog box that allows you to change or create directories. Moreover, it shows the names of existing files. These are ‘.sdt’ files or ‘.set’ files, depending on the selected file format. If you want to overwrite an existing file you can select it in the ‘File Name’ field. A history of previously saved files is available by clicking on the button.

File Info

After selecting the file text can be written into the ‘Author’, ‘Company’ and ‘Contents’ fields. Both for ‘SPC data’ and ‘SPC setup’ the file information is saved in the file. The file information helps considerably to later identify a particular measurement among a large number of data files. We therefore strongly recommend to spend a few seconds on typing in a reasonable file information.

If you have selected an existing file the file information contained in it is displayed in the ‘File info window’. If you want to overwrite this file you can edit the existing file information.

Selecting the data to be saved

Under ‘What to Save’ the options ‘All used data sets’, ‘Only measured data sets’ or ‘Selected data blocks’ are available, see Fig. 82. The default setting is ‘All used data sets’, which saves all valid data available in the memory of the SPC modules. These can be measured data, cal-

culated data or data loaded from another file. *Except for special cases (see[2]) we recommend to use the 'All used data sets' option.*

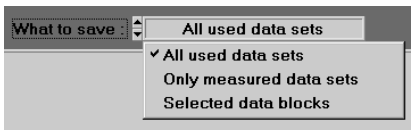


Fig. 82: Save options

Loading Setup and Measurement Data

The 'Load' menu is shown in Fig. 83. It contains fields to select different file types, to specify a file, to display information about the file selected, and to select different load options.

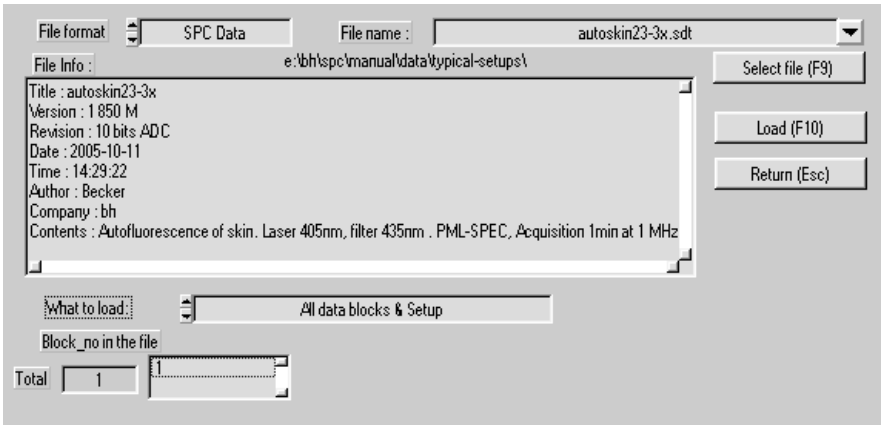


Fig. 83: Load panel

File Format

You can chose between 'SPC Data' and 'SPC Setup'. The selection refers to different file types. With 'SPC Data', .sdt files are loaded. These files contain both measurement data and system parameters. Thus the load operation restores the complete system state as it was in the moment when the file was saved.

If you chose 'SPC Setup', .set files are loaded. These files contain the system parameters only. The load operation sets the system parameters, but the actual measurement data are not influenced.

Note: Measurements in the 'FIFO' (time tag) mode deliver an .spc file that contains the micro time, the macro time, and the detector channel for each individual photon. These files are loaded by using the 'Convert' routines, see [2].

File Name / Select File

The file to be loaded can is selected in 'File Name' field. 'Select File' opens a dialog box that displays the available files. These are '.sdt' files or '.set' files depending on the selected file format. A history of previously loaded files is available by clicking on the button.

File Info

The file info window displays information about the file selected. The first three lines of the file info are inserted automatically when a file is saved. The last three items can be typed in by the operator, see 'Saving Setup and Measurement Data'.

Block Info

Activating a data block in the 'Block Number in File' field enables a 'Block Info Button'. Clicking on this button opens a list that contains the device number of the SPC modules by which the data were recorded, the time and data of the recording, and all system parameters, see Fig. 84. At the end of the block information the minimum and maximum count rates of the corresponding measurement are shown (see Fig. 84, right). The block info often helps to recover the exact recording conditions of an older measurement.

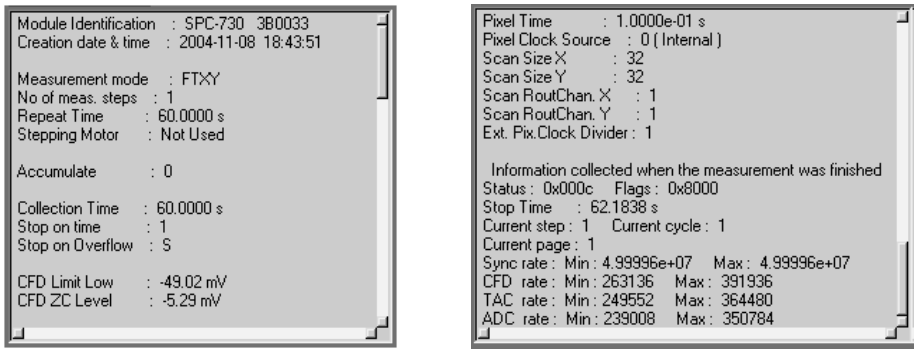


Fig. 84: Block info window of the load panel

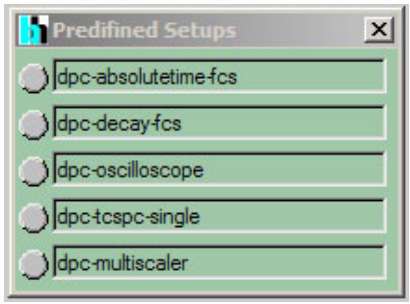
Load Options

Under 'What to Load' the options 'All data blocks & setup', 'Selected data blocks without setup' or 'Setup only' are available. The default setting is 'All data blocks & setup', which loads the complete information from a previously saved data file. *Except for special cases (see[2]) we recommend to use the 'All data blocks & setup' option.*

Predefined Setups

Setups of frequently used system configurations can be added to a list of 'predefined setups'. Changing between these configurations then requires only a mouse click.

To use the predefined setup option, click on 'Main', 'Load Predefined Setups'. This opens the panel shown right. A setup is loaded by clicking on the button left of the name of the setup.



To add or delete setups to or from the list, or to change the names of the setups, click into one of the name fields with the right mouse key. This opens the panel shown in Fig. 85.

To add a setup, click on the disc symbol right of the 'File Name' field and select a '.set' file. Default setups coming with the SPCM software are in the 'default setups' folder of the working directory defined during the software installation. Please note that there may by sub-directories for different classes of applications. Select the files you want to put into the list of predefined setups, and click on the 'Add' button. Every setup has a user-defined 'nickname'. The default nickname is the file name of the .set file. To change the nickname, click into the nickname filed and edit the name. Then click on 'Replace'.

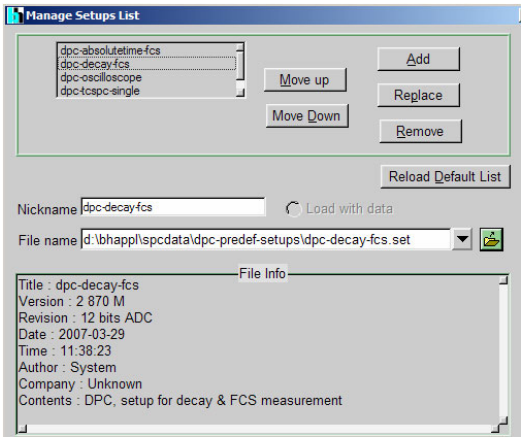


Fig. 85: Editing the list of predefined setups

To create your own predefined setups, first save a setup file of the system configuration you want to add the list. Use the ‘Save’ panel, option ‘setup’, as described under ‘Save’. Then add the file to the setup list as described above.

You can also add an ‘.sdt’ file to the setup list. The .sdt file contains not only the system settings but also measurement data. You can define whether the file is loaded with or without the data by clicking on the ‘load with data’ marker.

Importing FIFO Files

Measurements in the absolute time modes deliver an .spc file that contains time-tag data, i.e. the micro time, the macro time, and the detector channel for each individual photon [2]. During a FIFO measurement normally decay curves, FCS curves, or photon counting histograms are calculated on-line. These data are saved in normal .sdt files. However, it may happen that not all of the desired functions were calculated during the measurement, or that the calculation was not done with the optimal binning parameters or sampling time intervals. Moreover, on-line data analysis may have not been used altogether to increase the data throughput rate. In these cases, the 'Convert FIFO File' function allows you to read the data from the .spc file created during the measurement and calculate the desired correlation functions or histograms. The Convert FIFO panel is shown in Fig. 86.

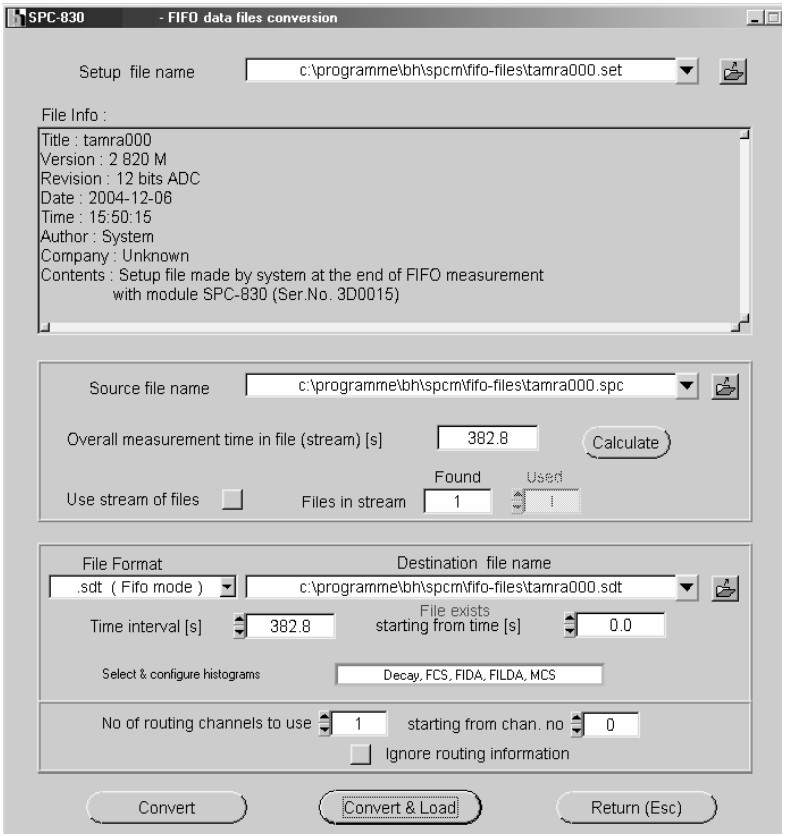


Fig. 86: Conversion of FIFO data into .sdt data

Each measurement creates a setup (.set) file which has the same name as the .spc file (or the last .spc file if several are recorded sequentially). The name of this setup file must be specified under 'Setup File name'. As in the load and save panels, the file is specified in file name field. Clicking on the disc symbol opens a dialog box that displays the available files. A history of previously used setup files is available by clicking on the button. The 'File Info' displays information about the corresponding measurement.

The FIFO (.spc) file is specified under 'Source File Name'. A FIFO measurement may produce several .spc files. The data of all these files are combined by activating the 'Use Stream of Files' button. 'Overall Measurement Time' informs about the total time over which the measurement has been run.

The lower part specifies the .sdt file to be created. The file name is specified under 'Destination file name'. The conversion routine suggests a file with the same name as the .spc file.

The ‘Convert FIFO’ routine allows you to convert .spc files into different destination file types. The destination file type is specified in the ‘File Format’ field in the lower part of the Convert panel. The most frequently used conversion is into .sdt files of the TCSPC FIFO mode, see Fig. 87.

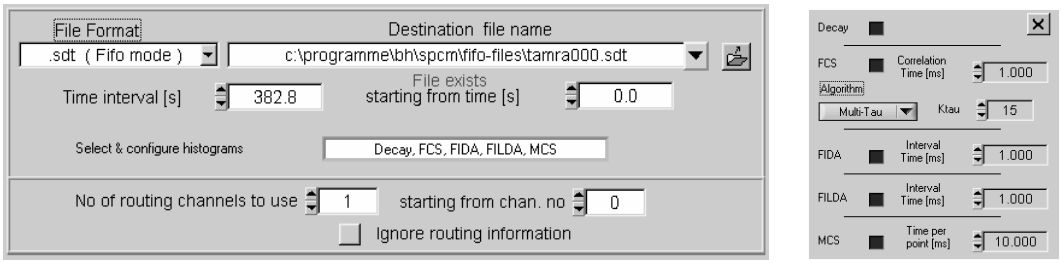


Fig. 87: Conversion of .spc files into .sdt files of the FIFO mode. Left: Configuration of destination data. Right: Selection of the functions to be calculated and calculation parameters.

The functions to be calculated are defined in the ‘Select & configure histograms’ field. A click into this field opens the panel shown in Fig. 87, right. It is the same panel used to configure the online display of the FIFO mode, see ‘Configuring the Runtime Display’, page 45. You can specify the calculation of decay curves, FCS functions, photon counting histograms (FIDA), photon counting lifetime histograms (FILDA), and FCS traces.

A FIFO measurement may have been run over a long acquisition time and contain data from several routing channels (detectors). Therefore, parameters are provided to control the structure of the calculated data. With ‘Time Interval’ and ‘Starting from Time’ a time interval within the ‘Overall measurement time’ of the FIFO measurement can be selected. Moreover, if the .spc data were recorded in several detector channels the full routing information may be used, only a specified range of channels may be converted, or the routing information may be ignored altogether, i.e. all detector channels merged.

Format of Time-Tag Data Files

In most of the operation modes the DPC-230 allows the user to record time-tag data of the individual photons. The format of the time-tag data files is described in this section.

Time-tag data files consists of a sequence of 32 bit records. The first record is a descriptor that identifies the file as a DPC-230 time-tag file and contains the time-channel width of the recording. The subsequent records contains photon data. Each record contains four bytes as shown in Table 1.

Byte 0	1st Record = Descriptor	Bits [7:0]
Byte 1		Bits [15:8]
Byte 2		Bits [23:16]
Byte 3		Bits [31:24]
Byte 0	2nd Record	Bits [7:0]
Byte 1		Bits [15:8]
Byte 2		Bits [23:16]
Byte 3		Bits [31:24]

Table 1: Byte order in the .spc files

Descriptor

The structure of the descriptor is shown in Table 2.

Bit 31	Bit 30	Bit 29	Bit 28	Bit 27	Bit 26	Bit 25	Bit 24
1	1	0	0	0	RAW	0	1
Bit 23	Bit 22	Bit 21	Bit 20	Bit 19	Bit 18	Bit 17	Bit 16
TPB[23]	TPB[22]	TPB[21]	TPB[20]	TPB[19]	TPB[18]	TPB[17]	TPB[16]
Bit 15	Bit 14	Bit 13	Bit 12	Bit 11	Bit 10	Bit 9	Bit 8
TPB[15]	TPB[14]	TPB[13]	TPB[12]	TPB[11]	TPB[10]	TPB[9]	TPB[8]
Bit 7	Bit 6	Bit 5	Bit 4	Bit 3	Bit 2	Bit 1	Bit 0
TPB[7]	TPB[6]	TPB[5]	TPB[4]	TPB[3]	TPB[2]	TPB[1]	TPB[0]

Table 2: Format of the descriptor

RAW Bit

The RAW bit identifies whether or not the data in the file are pre-processed. ‘Raw = 1’ identifies data as they are read from the incoming data stream. Such data are difficult to use for several reasons: The TDC channels of the DPC board have individual FIFOs. The software may therefore read the individual photons not in the order they were detected. Moreover, the internal clock of the TDC channels overflows in regular intervals. The clock overflows have to be marked in the data stream read from the DPC module and be taken into regard by the SPCM software. The raw data read from the DPC module are therefore difficult to interpret. Nevertheless, raw data can be stored by the SPCM software. Such data should, however, be used for test purpose only.

To facilitate users to analyse time-tag data the SPCM software generates files in which all these effects are already taken into regard. Please note that pre-processing does not cause any

loss of photons or any loss in photon information. Pre-processed data are identified by 'RAW = 0'. The description given below relates to pre-processed data.

Time per bin

TPB[23:0] defines the time unit in which all subsequent photon data are expressed. TPB is given in femtoseonds. All times are expressed in multiples of TPB.

Records of Photons

The records following the descriptor are photon data. All detection events are recorded with their channel number and a 54 bit integer time stamp. The time stamp reflects the absolute time from the start of the measurement. The time unit is the TPB entry in the descriptor, see Table 2.

To save disk space an event is normally stored with its detector channel number and the lower 24 bit of the time tag only. Only if the time in these 24 bits overflows the complete time is written. The bits 30 and 31 are used to identify whether the record is a photon with a 24 bit time or a record containing the higher bytes of the time of the previous photon. The meaning of bit 30 and bit 31 are shown in Table 3.

Bit 31	Bit 30	Type	Description
0	0	Event	Low Part of Time [TPB] incl. channel number
0	1	TIME[53:24]	Set Time to TIME[53:24]
1	0	Reserved	Reserved
1	1	Special	Descriptor, at start of file

Table 3: Bits identifying whether a record contains a 24 bit time or the higher bits for the complete 54 bit time

Event Record

The format of a photon record with 24-bit time is shown in Table 4.

Bit 31	**Bit 30**	**Bit 29**	**Bit 28**	**Bit 27**	**Bit 26**	**Bit 25**	**Bit 24**
0	0	GAP	Ch[4]	CH[3]	CH[2]	CH[1]	CH[0]
Bit 23	**Bit 22**	**Bit 21**	**Bit 20**	**Bit 19**	**Bit 18**	**Bit 17**	**Bit 16**
TIME[23]	TIME[22]	TIME[21]	TIME[20]	TIME[19]	TIME[18]	TIME[17]	TIME[16]
Bit 15	**Bit 14**	**Bit 13**	**Bit 12**	**Bit 11**	**Bit 10**	**Bit 9**	**Bit 8**
TIME[15]	TIME[14]	TIME[13]	TIME[12]	TIME[11]	TIME[10]	TIME[9]	TIME[8]
Bit 7	**Bit 6**	**Bit 5**	**Bit 4**	**Bit 3**	**Bit 2**	**Bit 1**	**Bit 0**
TIME[7]	TIME[6]	TIME[5]	TIME[4]	TIME[3]	TIME[2]	TIME[1]	TIME[0]

Table 4: Photon record (24 bit time)

CH[4:0] Channel number of the DPC in which the photon was recorded. The LVTTL have channel number from 1 to 16, the CFD channels have the channel numbers 1 and 2 for TDC 1 and 11 and 12 for TDC 2, see Fig. 77, page 47.

TIME[23:0] Event Time [TPB], low part. Only if bit 30 and bit 31 are 0, i.e. the higher part of the time has not changed.

GAP The FIFO has overflown. Data of a number of photons were lost before this entry.

High-Time Record

A record with bit 30 = 1 and bit 31 = 0 indicates that the higher part of the time has changed, and that a new high-part of the photon time has to be used starting from the previously recorded photon, see Table 5. In other words, both parts, TIME[53:24] of this and TIME[23:0] of the previous record, have to be used as a time tag.

Bit 31	**Bit 30**	**Bit 29**	**Bit 28**	**Bit 27**	**Bit 26**	**Bit 25**	**Bit 24**
0	1	TIME[53]	TIME[52]	TIME[51]	TIME[50]	TIME[49]	TIME[48]
Bit 23	**Bit 22**	**Bit 21**	**Bit 20**	**Bit 19**	**Bit 18**	**Bit 17**	**Bit 16**
TIME[47]	TIME[46]	TIME[45]	TIME[44]	TIME[43]	TIME[42]	TIME[41]	TIME[40]
Bit 15	**Bit 14**	**Bit 13**	**Bit 12**	**Bit 11**	**Bit 10**	**Bit 9**	**Bit 8**
TIME[39]	TIME[38]	TIME[37]	TIME[36]	TIME[35]	TIME[34]	TIME[33]	TIME[32]
Bit 7	**Bit 6**	**Bit 5**	**Bit 4**	**Bit 3**	**Bit 2**	**Bit 1**	**Bit 0**
TIME[31]	TIME[30]	TIME[29]	TIME[28]	TIME[27]	TIME[26]	TIME[25]	TIME[24]

Table 5: Record defining a new high-part of the photon time

Specification

LVTTL Inputs

No. of channels	16
Input Voltage	LVTTL
Threshold	1.4 V
Min. Input Pulse Width	2 ns
Min. Pulse Distance	5.5 ns
Connectors	MCX, on board

CFD Inputs

No of channels	4
Threshold	- 20 mV to - 500 mV
Zero Cross Adjust	- 100 mV to + 100 mV
Connectors	SMA, front panel

Experiment Trigger Input

Input Voltage	LVTTL
Threshold	1.4 V

Data Acquisition, Correlation Mode	
Method	Time-tag recording, absolute photon times
Correlation of photons	Multi tau or linear tau algorithm, online or offline
Autocorrelation	all channels
Cross-correlation	any pairs of channels
Time increment	164.61 ps
Dead Time	< 10 ns
No of parallel channels	16 LVTTL or 4 CFD channels
On-board FIFO Buffer size	$4 \cdot 10^6$ photons
Readout	continuous readout during measurement
Sustained readout rate (typ., depends on computer)	$7 \cdot 10^6$ photons

Data Acquisition, TCSPC Mode

Method	Time-tag recording, reversed start-stop
Correlation of photons	Start-stop histogram, online or offline
Start (photon) channels	3 CFD inputs
Dead Time	< 10 ns
Stop channel	1 CFD input
Stop input rate	max 150 MHz
Stop frequency divider	1 - 2 - 4
Time channel width	164.61 ps
On-board FIFO Buffer size	$4 \cdot 10^6$ photons
Readout	continuous readout during measurement
Sustained readout rate (typ., depends on computer)	$7 \cdot 10^6$ photons

Data Acquisition, Multiscaler Mode

Method	Time-tag recording, direct start-multistop
Correlation of photons	Start-stop histogram, online or offline
Start (reference) channel	1 CFD input or 1 LVTTL input
Stop (photon) channels	3 CFD inputs or 15 LVTTL inputs
Dead Time	< 10 ns
Time channel width	164.61 ps
On-board FIFO Buffer size	$4 \cdot 10^6$ photons
Readout	continuous readout during measurement
Sustained readout rate (typ., depends on computer)	$7 \cdot 10^6$ photons

Operation Environment

Computer System	Pentium PC
Recommended configuration	>1024 Mb RAM, >100 Gb HD
Bus Connector	PCI
Power Consumption	approx. 12 W from +5V
Dimensions	312 mm x 124 mm x 20 mm

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