FluoTime 200

Fluorescence Lifetime Spectrometer

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Unternehmen für optoelektronische Forschung und Entwicklung

User's Manual

Version 3.3

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1. Introduction

While steady-state fluorescence spectroscopic investigations are common for many years, extracting temporal information from molecules via laser induced fluorescence is a relatively new and powerful technique. The temporal analysis may reveal information about the molecule that is not available from spectral data alone. This is why lifetime analysis of laser induced fluorescence by means of time-correlated single-photon counting (TCSPC) has gained increasing importance over the recent years. The difference in the fluorescence decay times of appropriate fluorescent dyes provides a powerful feature to distinguish molecules of interest from background or other species. This has made the technique very interesting for analytical chemistry, even down to the single molecule level.

Acquisition of fluorescence decay curves by means of TCSPC provides resolution and sensitivity that cannot be achieved with other methods. In practice, it is done by histogramming the arrival times of individual photons over many excitation/emission cycles. The arrival times recorded in the histogram are relative times between laser excitation and corresponding fluorescence photon arrival (start-stop-times) are ideally resolved down to a few picoseconds. Due to the photon counting statistics it requires a large number of cycles to collect the histogram to such a degree of signal-to-noise, that a fluorescence lifetime can be calculated. Using high repetition rate lasers and fast photon counting electronics, this can be achieved within seconds.

The FluoTime 200 is a high performance lifetime spectrometer featuring a modular construction, single photon counting sensitivity and research flexibility. It can be equipped with various pulsed light sources, like pulsed picosecond diode laser, other (e.g. mode locked) laser excitation source with MHz repetition rate, sub-nanosecond LED or xenon flash lamp. The optical layout and photon counting detection ensures the ultimate sensitivity and time resolution.

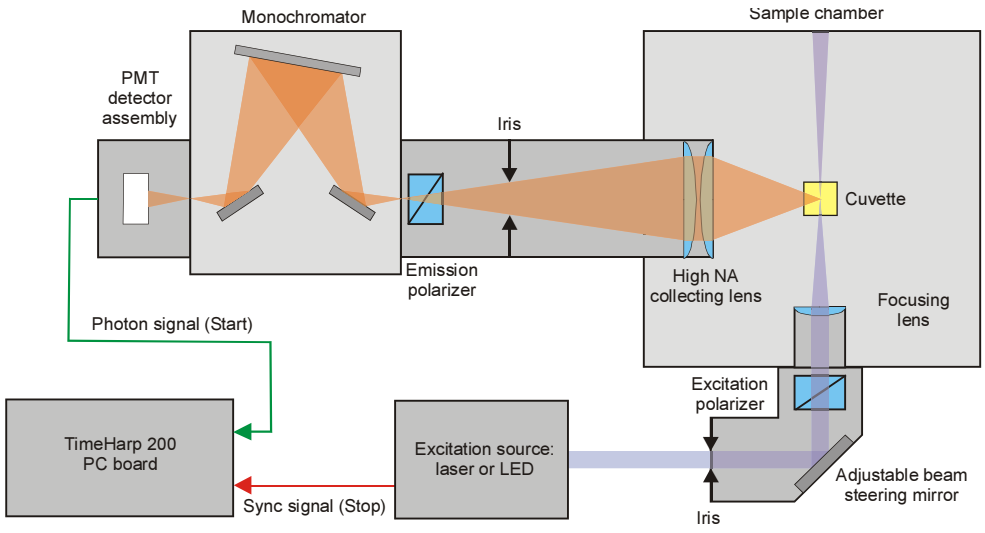
Data acquisition is done by compact yet versatile electronics. The TimeHarp 200 PC board and the PicoHarp 300 stand-alone system represent the state-of-the-art TCSPC instrumentation developed and manufactured by PicoQuant. Depending on the excitation source and photon detector, instrument response function (IRF) as short as 30 ps can be achieved with the PicoHarp. However, not all the samples require such high time resolution. The NanoHarp 250 photon counter/multiscaler is the data acquisition board of choice for measuring decays on a microsecond to millisecond time scale.

A complete FluoTime 200 contains all the optics, electronics and software for recording luminescence decays. The system combines versatility and ease of use with high performance excitation sources and detection electronics in a modular, robust setup.

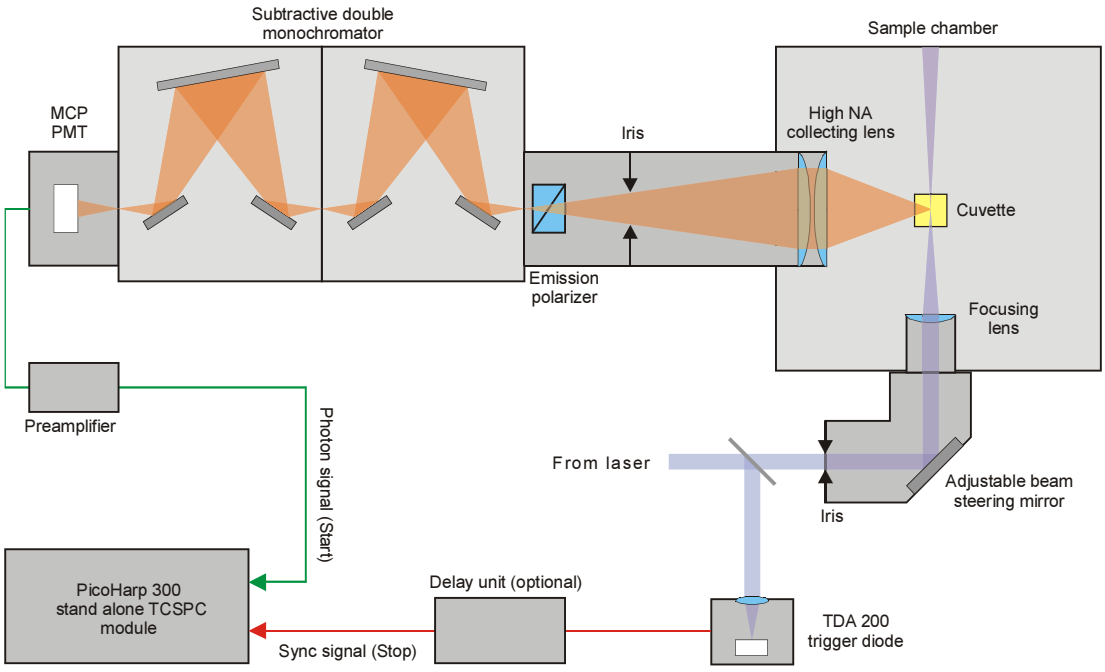
Details of the underlying data acquisition methods can be found in various Technical Notes available upon request from PicoQuant. For further studies we recommend the literature listed in chapter *5*.

2. Spectrometer Layout

The optical system of the FluoTime 200 is based on a standard L–geometry. The schematic layout of a FluoTime 200 Basic with TCSPC detection is shown in the illustration below.



The FluoTime 200 TISSA model is recommended for applications demanding the highest achievable time resolution by TCSPC. Its typical set-up is shown below.



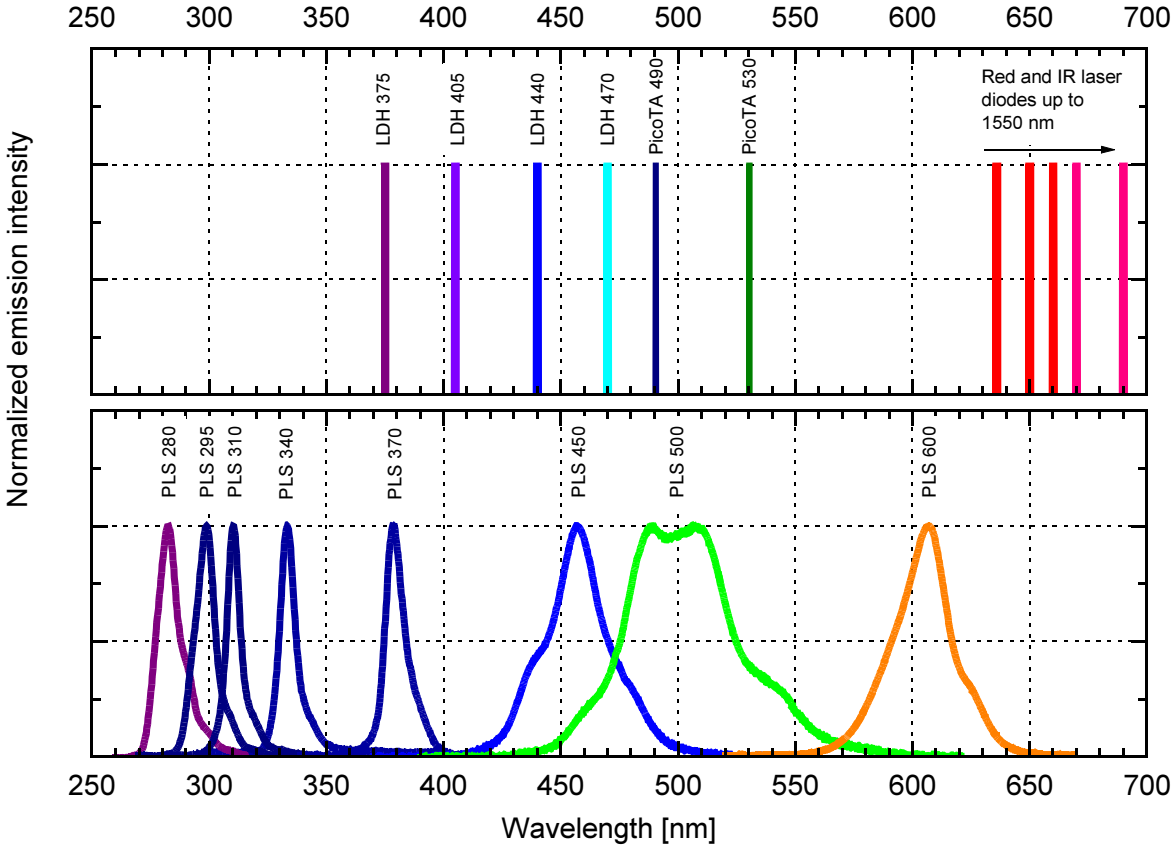
The excitation radiation from a suitable pulsed light source is guided into the sample chamber via a laser coupling module. The fluorescence response of the sample is collected by adjustable collection optics, passes through an optional polariser, monochromator and reaches the single photon sensitive detector. The synchronisation signal from the excitation source and the signal from the detector is routed to and processed by the TCSPC electronics. Due to its modular concept, an upgrade to T– or X–geometry is possible.

2.1 Excitation Arm

2.1.1 Pulsed Excitation Sources

The FluoTime 200 is designed to primarily utilize modern, high repetition rate excitation sources from PicoQuant, such as gain-switched picosecond laser diodes or sub-nanosecond pulsed light emitting diodes (LED). The wavelength, degree of polarization, pulse duration and pulse energy depends on the particular excitation source. If your system contains a PicoQuant laser or LED, please refer to the manual of the PDL 800-B, PDL 808 or PDL 828 driver for detailed information.

The family of PicoQuant diode lasers and LEDs is continuously growing. The up-to-date list of LED/laser heads is available on the PicoQuant website `www.picoquant.com`. The current spectral coverage (as of June 2006) is shown in the picture below:

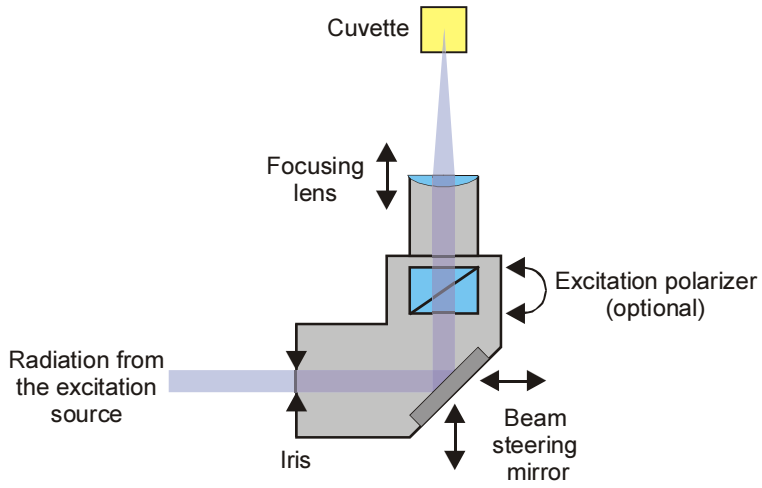


For investigation of slow luminescence decays, like phosphorescence or lanthanide emission, the FluoTime can be equipped with a xenon flash lamp. It emits approximately 400 ns broad flashes at up to 300 Hz repetition rate. In this case, a dedicated ScienceTech 9030 monochromator is used to select the desired excitation wavelength from the broad xenon emission spectrum. The flash lamp requires only 12 V power and an external trigger signal. The latter can be generated by e.g. the NanoHarp 250 multiscaler board, which is typically used in connection with the flash lamp.

Optionally, various excitation sources of other manufacturers can also be coupled to the FluoTime 200. These can be mode-locked lasers (e.g. Titanium:Sapphire, dye lasers, etc.), diode pumped solid state Q-switched lasers or flash lamps. Consult the relevant documentation for the excitation source and contact PicoQuant for support.

2.1.2 Coupling Optics

The laser-coupling module consists of a folded tube with an iris, a beam steering mirror, optional replaceable polariser module and a focusing lens. The typical optical layout of the laser-coupling module is shown in the picture below.

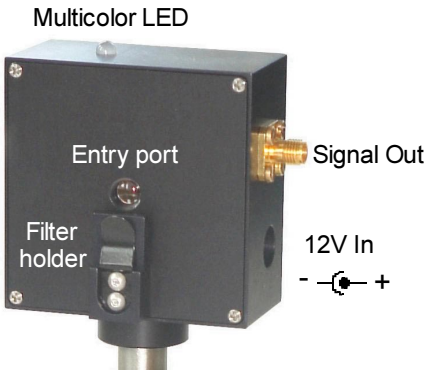


The iris allows the adjustment of the excitation intensity and avoids the penetration of ambient light into the sample chamber. The beam is directed towards the sample by an adjustable steering mirror. An optional prism polariser, placed in the optical path, selects the desired polarization plane of the non-polarized radiation. If the excitation source emits polarized radiation, the inserted prism can be used as a variable attenuator of the excitation intensity. The prism holder can be replaced with an empty one by loosening the spacer and pulling out the holder from the mount. Finally, the beam is focused by an adjustable lens onto the sample.

2.1.3 TDA 200 Photodiode

This device is used for synchronizing the timing electronics if the external laser system cannot provide an appropriate electrical SYNC signal. The TDA 200 is built around a silicon pin photodiode with an active area of 0.5 mm diameter and can be used from 350 to 1100 nm with peak sensitivity at around 800 nm. When a fraction of the excitation beam is directed towards the TDA 200 unit, it responds to each excitation pulse with a fast negative output pulse (typical rise time 250 ps, duration approximately 500 ps). This can be directly coupled to the timing electronics for synchronization purposes.

The multicolour LED is a pulse height indicator independent from laser repetition rate. It facilitates the adjustment of the light intensity reaching the photodiode, in order to meet the requirements of the installed timing electronics. Refer to the relevant manual for the SYNC (or trigger) input voltage limits. Further information about the trigger diode assembly can be found in chapter 3.3.



2.2 Sample Chamber

The sample chamber contains a versatile sample holder and two microswitches to secure the detector. Opening the chamber lid releases the microswitches and closes the shutter in front of the detector to avoid damage by excessive light.

The standard sample holder is designed to accommodate a 1 cm path length (or smaller) cuvette. To suppress parasitic reflections, it can be rotated around its vertical axis as necessary. If requested, the cuvette holder can be equipped with a magnetic stirrer or replaced by a front-face sample holder.

Temperature control of the sample is possible by means of a circulating liquid and an external thermostat (which is not part of the system). The necessary tubing inside the chamber is pre-installed and terminated at the outside wall of the sample chamber by blue tubing glands with push-in fittings. These are easily accessible at the outside wall and you can use the delivered 4 mm outside diameter tube to make the necessary connections. It is assumed that the cooling medium will be water. Note that the cuvette holder is made from aluminium and the tubing from polyurethane (manufacturer: FESTO, type: PUN-4×0.75 SW, part no. 159661, see `www.festo.com`).

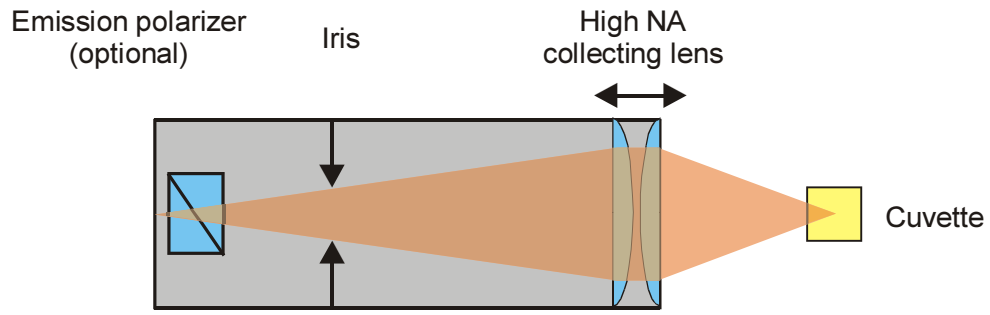
The third gland at the sample chamber wall can be used for e.g. dry gas purge.

The sample chamber is large enough to accommodate an Oxford Instruments OptistatDN™ series cryostat. This optional component allows low temperature photoluminescence measurements to be made. Precise control of the sample temperature is possible with various cryostat types from 2.3 to 500 K. Detailed information can be found at `www.oxinst.com`. If your system is fitted with this device, please refer to the attached manuals of the cryostat and its controller unit.

2.3 Emission Arm

2.3.1 Detection Optics

The radiation emitted by the sample (fluorescence hereafter for simplicity) can be spectrally filtered inside the sample chamber by placing a suitable filter close to the cuvette.



The fluorescence is collected by a high numerical aperture UV grade lens. The collection focus is set manually by turning the handle ring outside the sample chamber. The fluorescence then passes through an adjustable iris (the second handle ring outside the sample chamber).

An optional prism polariser placed in the optical path selects the desired polarization plane of fluorescence that reaches the monochromator. Similar to the excitation arm's coupling optics, the prism holder can be replaced by an empty one by loosening the spacer and pulling out the holder from the mount.

The spectrometer is not light tight unless the polariser holder is properly inserted and mounted. To protect the photon detector, always open the sample chamber lid before disassembling he mount!

2.3.2 Monochromator

The FluoTime 200 in basic configuration uses the ScienceTech Model 9030 monochromator, a compact, 100 mm focal length instrument with low temporal dispersion, high throughput and low stray light level. This Seya-Namioka type monochromator has a 32 mm × 32 mm aberration corrected concave holographic grating with 1200 lines/mm blazed at 450 nm and an aperture of f/3.2. The spectral (linear) dispersion is 8 nm/mm. The delivered set of interchangeable slits with 0.5, 1.0 and 2.0 mm width therefore provide 4, 8 and 16 nm spectral bandwidth, respectively.

The spectrometer is not light tight unless the slits are properly inserted. To protect the photon detector, always open the sample chamber lid before changing the slits!

The standard grating is recommended for a spectral range of 350–800 nm; other concave holographic gratings for up to 3200 nm are also available.

Gratings for ScienceTech 9030 and 9030DS monochromators

Grating lines/mm	Spectral range (nm)	linear dispersion (nm/mm)	Counter multiplication factor
1200	200–800 blaze 250	8	1
1200 (Standard)	350–800 blaze 450	8	1
800	300–1200	12	1.5
600	400–1600	16	2
300	800–3200	32	4

Systems with MCP PMT detectors and ultrafast excitation sources can exploit the advantages of the ScienceTech 9030DS. In this double subtractive monochromator, the two gratings operate in a subtractive dispersion mode. The first monochromator selects a bandpass and the second monochromator then removes the temporal and angular aberrations (dispersions). The result is superior stray light rejection and ultimate time resolution.

The monochromator is wavelength calibrated by PicoQuant. The simplest spectrometer configuration provides manual wavelength selection. Use the turning knob with the counter displaying the wavelength in nm units on the top of the monochromator. When using gratings of different groove densities (lines/mm), the counter reading must be multiplied by an appropriate factor (see the table above). In case of a motorized monochromator, the wavelength setting and readout is provided by the control software of the data acquisition electronics.

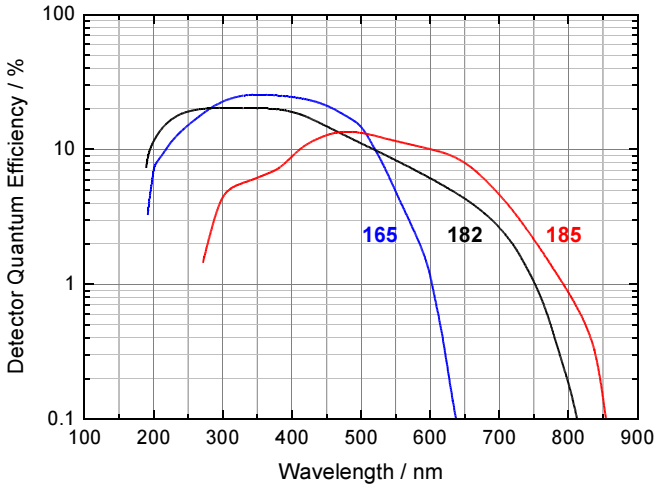
The optional ScienceTech Model 9055 direct drive monochromator has an asymmetrical Czerny-Turner optical layout with an aperture of f/3.5. It contains up to 3 gratings in a turret, consequently covering a broad wavelength range with resolution better than 0.2 nm (depending on the installed gratings). Input and output slits are continuously adjustable by micrometer screws. This monochromator can be used only in a computer controlled scanning mode.

For further information about the installed monochromator, please refer to the enclosed monochromator manual or to the ScienceTech website `www.sciencetech-inc.com`

2.3.3 Photon Detectors

The single photon sensitive detector (usually a photomultiplier, PMT) is mounted directly to the exit slit of the emission monochromator. Note that such a detector is an extremely sensitive device and even dimmed ambient light can permanently damage it. The detector is protected against overexposure to light when the sample chamber is open, by means of a built-in shutter that is interlocked with the chamber lid. However, the user is responsible for controlling the light intensity reaching the PMT during any manipulation when the lid is closed.

PicoQuant offers three proprietary photon detector assemblies, PMA-165, -182 and -185, that provide high sensitivity, wide dynamic range and accordingly low dark noise even without cooling. These detectors are based on the Hamamatsu H5783 photosensor modules and are recommended for the majority of applications. The detection spectral range from 185 nm to 850 nm is covered by various photocathode materials. Each unit has a built-in high voltage power supply and signal preamplifier, both preset to optimal timing performance. These devices are built in a gold-plated iron housing to achieve the highest level of protection against electromagnetic interference. With fast TCSPC electronics (e.g. PicoHarp) and short pulse laser diode, instrument response function (IRF) as short as 200 ps can be achieved with this detector type.



The FluoTime 200 Tissa version is usually equipped with the Hamamatsu R3809U-5X series microchannel-plate photomultiplier (MCP PMT), in order to achieve the highest possible time-resolution with the TCSPC method. With ultrafast laser sources, the IRF is less than 50 ps. This detector has an external high voltage supply (FUG HCP 14-3500) and is used with an appropriate preamplifier (PicoQuant PAM 102-M, see below). The gain of the amplifier allows the detector to be operated at a reduced signal current, in order to extend the lifetime of the expensive MCP PMT. Furthermore, the gain helps to reduce noise pick-up in long signal cables.

Various other detectors can be interfaced with the FluoTime 200 optical hardware. Later detector upgrade is also possible. For example, a liquid nitrogen cooled Ge single photon avalanche diode or the Hamamatsu H9170 series NIR-PMT module were already incorporated into FluoTime 200 systems. Please consult your specific needs with PicoQuant.

The specifications for the installed photon detector can be found in the appendix of this manual. For further details, please refer to the PicoQuant website `www.picoquant.com`

2.3.4 Signal Preamplifier

The PAM 102 series preamplifiers are specially targeted at timing sensitive applications with fast photon detectors. Such devices produce very small output pulses with extremely short pulse widths, usually too small for direct interfacing with TCSPC electronics. The pulse rise times (or fall times) are typically 150 to 500 ps, and the pulse widths are correspondingly small. Therefore, an amplifier with an appropriately fast rise time is needed between the detector and the timing discriminator of the TCSPC system. The rise time of an amplifier

is directly related to its bandwidth. Best timing resolution is usually achieved when the amplifier rise time is comparable to the detector's rise time. For common detectors this corresponds to an optimum amplifier bandwidth of 1 to 2.5 GHz. This is why the PAM 102 is designed for a bandwidth in this range. The PAM 102 is available in three different versions. The 25 dB gain, non-inverting P-version (for PMTs) and M-version (for MCP-PMTs) differ in the threshold level for the overload protection signal. Another option is the 20 dB inverting version for PMTs (T-version) to be used in combination with the TimeHarp 200. All PAM 102 units have standard 50 Ohms SMA signal input and output connectors, gold plated housing for maximum RF immunity and contain an overload indicator.

2.4 Timing Electronics

Currently, three types of compact and high performance time-resolved photon counting solutions are available from PicoQuant. The FluoTime 200 utilizes the TimeHarp 200 TCSPC board as a standard, but upon request it can be equipped with the NanoHarp 250 board or PicoHarp 300 system too.

All functions of these state-of-the-art data acquisition systems are controlled by an easy to use 32-bit Windows based software. Proper understanding of the control software is essential to operate the FluoTime. For detailed information about the timing electronics and for operating instructions please refer to the dedicated manual. The control software provides functions such as the setting of measurement parameters, display of results, loading and saving of measurement parameters and data curves. Important measurement characteristics such as count rate, count maximum, position and peak width are displayed continuously.

Other software features include: full integration with Windows device management, and preservation of individual settings on multi-user platforms. Optionally, driver libraries (DLL) for 32-bit Windows program development are available to build custom applications in C/C++, Delphi, Visual Basic and LabVIEW. Demo code is provided for an easy start.

2.4.1 TimeHarp 200

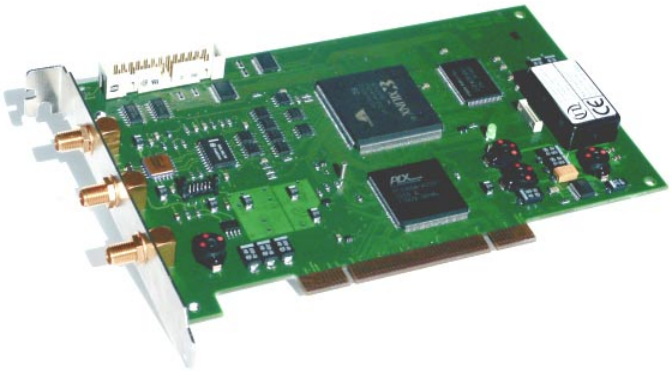
The TimeHarp 200 is a short PCI slot data acquisition board that contains the complete electronics for time-correlated single-photon counting (TCSPC). The TimeHarp is ideally suited for fluorescence lifetime measurements with the modern pulsed diode lasers or pulsed LEDs as excitation sources. Owing to the reverse start-stop mode, synchronisation with fast repetition rate excitation and consequently high sustained measurement rates are possible.

Both signal inputs are programmable for a range of signal levels. The start input has a built in constant fraction discriminator suitable for common photon detectors. The system provides a channel resolution better than 40 ps. In combination with a pulsed diode laser and a PMA series detector, total IRF widths of about 300 ps FWHM can be obtained. This permits measurement of sub-nanosecond fluorescence lifetimes.

Conventional histogramming is only one of the various measurement modes of the versatile TimeHarp 200. For example, in a so-called “Continuous mode”, the histograms are repetitively accumulated and saved to the hard disk without interruption and dead time in between. This mode makes possible to follow the evolution of the decay kinetics on a macroscopic time scale, from milliseconds to hours.

2.4.2 NanoHarp 250

The NanoHarp 250 is a short PCI slot data acquisition board for multichannel scaling and photon counting. It is an ideal instrument for acquisition of slower luminescence decays. The board's multi-stop capability allows efficient recording of long-lived fluorescence decays up to the millisecond time range with correspondingly slow excitation rates.



Both signal inputs have programmable level discriminators. The timing circuitry provides two time bin resolutions of either 4 ns or 32 ns, which can be binned on board resulting in a maximum usable time span of 1.04 ms or 2.15 s, respectively. On-board histogram memory allows for collection of 262144 counts per bin without overflow or software intervention.

The board is additionally equipped with a programmable trigger output, which can generate pulse periods between 0.1 and 1600000 µs, corresponding to repetition frequencies between 0.625 Hz and 10 MHz. This feature can e.g. be used to control external lasers, flash lamps, etc.

2.4.3 PicoHarp 300

The PicoHarp 300 is a stand alone, high-end plug and play TCSPC system. It is connected to the PC through a state of the art USB 2.0 high speed interface. A new design approach provides identical, independent input channels. In connection with a FluoTime system they are used as a pair of start and stop inputs for TCSPC.

The PicoHarp allows a forward start-stop operation even at full repetition rate of mode locked lasers up to 84 MHz. A highly stable, quartz calibrated time resolution of 4 ps is well matched to the fastest photon detectors today today, e. g. SPADs of the PDM series or micro-channel plate photomultiplier tubes (MCP-PMT). Overall IRF widths down to 50 ps can be achieved even with pulsed diode lasers. Experiments with low excitation repetition rate benefit from the PicoHarp's multi-stop capability.

2.4.4 Optional Delay Unit

The ORTEC Model 425A Nanosecond Delay is an optional component of the timing electronics. It provides a variable delay for the input signal in 1 ns steps from 0 to 63 ns. The input and output impedances of the delay line are 50 Ω, making it fully compatible with the other electronic components. The delays are accomplished by coaxial cables and no power is required to operate the instrument. The main use of the ORTEC 425A is to properly delay the SYNC pulses (i.e. the STOP signal in reversed mode) so that they reach the TCSPC electronics within the selected time range of interest. Fine adjustment of the delay allows to optimally place the fluorescence decay in the instrument's measurement range.

2.5 FluoFit Software

Photon counting is in principle a statistical measurement. Numerical analysis of the raw data is an integral part of the method. To accomplish this task, the FluoTime series spectrometers are supplied with the FluoFit software package. This a powerful analysis software for fluorescence decay and anisotropy measurements. Running FluoFit simultaneously with the measurement software allows for “almost on-line” data analysis.

Tail fitting as well as a numerical reconvolution algorithm to account for the instrument response function (IRF) can be applied. The decay data can be fitted to exponential decay models up to 4th order or alternatively to different lifetime distribution models (Gaussian, Lorentzian and stretched exponential). Global fitting as well as batch mode fitting is supported for all models. The software allows to freely vary the number of fit parameters, including IRF and signal background as well as time shift. Start parameters for the fitting algorithm can be determined automatically or entered manually. The fitting limits are easily adjusted with graphical sliders. Reduced chi-square, weighted residual and autocorrelation trace are shown for assessment of the goodness of fit. An advanced error analysis using different methods is also possible. User preferences are widely adjustable and can be automatically stored and retrieved. The 32-bit software is available for Windows ME/2000/XP and features a modern and easy to use graphical user interface. Results can be printed, saved and exported for later reference. The program supports data files from the PicoHarp 300, TimeHarp 200, NanoHarp 250 and some other photon counting systems as well as ASCII data files. Instrument response and fluorescence decay may be loaded from different sources. A comprehensive on-line help library is provided for ease of use.

The FluoFit software is available in two versions to meet different needs. The main features of both versions (Basic and Professional) are shown in the following table:

	FluoFit Basic	FluoFit Professional
Data import from PicoHarp, TimeHarp, NanoHarp, SPC, MSA and ASCII	✔	✔
Exponential decay models	✔	✔
Lifetime distributions models		✔
Anisotropy analysis		✔
Global analysis and batch fitting		✔
Goodness of fit assessment	✔	✔
Error analysis		✔
Export of analysis results	✔	✔
Storage of user preferences	✔	✔

Please note, that the FluoFit software package is protected by a Hardlock protection module (dongle) that must be connected to the appropriate port of the PC (or server, if it is a network dongle) during operation. The FluoFit will not work without it.

We recommend to register as a FluoFit user. You will receive e-mail notifications of new versions and upgrade possibilities.

3. Assembling and Setting Up the Spectrometer

3.1 Space Considerations

Configuration:	Base plate dimensions (width × length):
Single monochromator with PMA series or uncooled MCP PMT detector	400 mm × 650 mm (15.8 in × 25.6 in)
Double monochromator with PMA series or uncooled MCP PMT detector	400 mm × 750 mm (15.8 in × 29.5 in)
Xe flash lamp with excitation monochromator, single emission monochromator with PMA series detector	650 mm × 850 mm (25.6 in × 33.5 in)
Single monochromator with cooled MCP detector (see the image on the cover - top)	400 mm × 1000 mm (15.8 in × 39.4 in)
Double monochromator with cooled MCP detector	400 mm × 1100 mm (15.8 in × 43.3 in)
Custom configuration with 9055 monochromator (an example, see the image on the cover – bottom)	820 mm × 710 mm (32.3 in × 28.0 in)

The instrument base plate is 61 mm (2.40 in) thick and has approximately 29 mm (1.14 in) long adjustable feet. The optical axis is exactly 122.1 mm (4.807 in) from the upper surface of the baseplate, that is approximately 212 mm (8.35 in) from the surface of the desk or breadboard on which the FluoTime spectrometer is placed.

The minimal height of the instrument is approx. 320 mm (12.6 in), however additional vertical space is necessary for manipulation with the sample chamber. Cooled PMT housings can increase the nominal height up to 420 mm (16.5 in), even more vertical space (approx. 800 mm or 31.5 in) is needed for the cryostat.

There are several external devices that belong to the system. The most space consuming is usually the dedicated computer with keyboard, mouse and monitor. Other, optional components are: PDL 800-B, PDL 808 "Sepia" or PDL 828 "Sepia II" diode laser driver, high voltage supply for MCP PMT, vacuum pump and controller for the NIR PMT, digital controller of the cryostat. These are relatively small units, but their distance from the spectrometer is obviously limited by cables or tubes.

3.2 Cable Connections, Wiring and Safety Instructions

All components of the FluoTime 200 spectrometer are factory preset to operate on the standard power outlet line voltage of the country of delivery. Please check that the actual line voltage corresponds to the local standard.

The 12 V power adapter used to supply the spectrometer hardware is a universal switching type device that operate from 100 to 240 V AC, 50 or 60 Hz. The FluoTime basic hardware has no main power switch. The spectrometer is powered ON when its 12 V adapter is attached and plugged in. Unless the system contains a special detector, we strongly recommend to keep the FluoTime with power ON all the time in order to preserve the top performance of the built in PMA detector unit. The power consumption is minimal.

The signal cables are RG223/U double shielded coaxial wires with SMA male connectors and labeled at both ends appropriately. PMA units have a built-in preamplifier. An optional photon detector like an MCP PMT, a NIR PMT or a Ge-SPAD is connected through an external PAM 102 series preamplifier mounted on the spectrometer base plate. In this case, an additional short RG223/U cable connects the detector's direct output to the preamplifier input.

While the standard PMA series detectors have a built in high voltage (HV hereafter) supply and need no special accessories, MCP detectors require dedicated HV supply unit. The FUG HCP 14-3500 stabilized HV source is connected to the MCP PMT with a special coaxial cable. The optional thermoelectrically cooled housing has its own controller (Hamamatsu C4878) with separate cable connection. The Hamamatsu H9170 series NIR PMT assembly contains a stand-alone controller that integrates the HV source and the current supply for the Peltier cooling. All the necessary extra cables have special connectors that cannot be confused. Please refer to the relevant component's manual and follow the safety instructions.

If the spectrometer uses PDL 800-B, PDL 808 "Sepia" or PDL 828 "Sepia II" based excitation source together with the TimeHarp, then there is a single SYNC cable. FluoTime systems which utilize an external laser setup usually come with TDA 200 photodiode and an optional ORTEC delay unit. In this case, one of the signal cables (usually the SYNC line) is composed by two pieces. Please observe the information on cable labels.

PicoHarp and NanoHarp based systems require yet another cabling. In any case, the final cable lengths are optimized by PicoQuant for correct timing. Please refer to the Appendix of the manual.

Note that in sub-nanosecond time-resolved spectroscopy the signal cable length is very important; any alteration of it affects the timing. Modification of the wiring requires some experience. Always use high quality double shielded coaxial cables terminated with proper connectors. The use of various BNC/SMA coaxial adapters as well as chaining several cable pieces is discouraged because every junction is a potential source of signal distortion and noise pick-up.

Motorized ScienceTech 9030 or 9030DS monochromators are controlled through a CAN interface. Attach the magenta cable with two 9-pin D-sub connectors to the appropriate port of the spectrometer and to the CAN plug-in board depicted on the picture below.

Beware, CAN is a serial interface and uses the same connector type as the standard serial (COM) port. Do not confuse these ports!

Contrary to the 9030 series, the optional ScienceTech 9055 direct drive monochromator has a built-in command interpreter. The grating movement is controlled by ASCII commands sent through the PC's serial interface. Connect the monochromator control cable to the appropriate COM port of the PC. FluoTime 200 systems with this type of monochromator have no CAN interface.

3.3 Getting the SYNC Signal

For time resolved photon counting measurements, a precise synchronization signal from the pulsed excitation system is crucial. In the following, it is assumed that the user is familiar with the basic use of the control software of the data acquisition electronics. Instructions how to use the control software can be found in the appropriate manual. Turn on the PC and start the relevant application.

PDL series drivers provide a suitable electrical SYNC signal. For compatibility purposes, it is a rectangular, 6 ns long NIM pulse with amplitude < -800 mV into 50 Ω. Set the *TRIGGER SOURCE* switch on the PDL 800-B to *INT* position. (On "Sepia" driver, set the *TRIGGER* switch located on the SOM 808 oscillator to *Internal*.)

TimeHarp users:

The LTT 100 signal adapter should be attached to the driver's SYNC output before the SYNC cable. LTT 100 transforms the relatively long SYNC pulse into a pair of sharp peaks. The first, falling peak is used by the TimeHarp's level trigger, the second one is ignored. Open the TimeHarp Control Panel, set the *Sync Level* to -100 mV. The *SYNC Hz* section of the main window shows the detected repetition rate of the laser. The displayed value must be stable and exactly corresponding to the selected repetition rate. Fine tune the *Sync Level* if necessary.

PicoHarp users:

In order to avoid signal crosstalk, a 10dB in-line attenuator should be attached to the driver's SYNC output before the signal cable. Open the PicoHarp Control Panel and locate the *Inp.Chan. 0/Sync* section and set *Discr.* to 100 mV. The *ZeroCr.* parameter should be around 10 mV. The *Divider* settings depends on the repetition rate. Best results are obtained with *Divider* = 4 for 2.5 to 20 MHz and *Divider* = 8 for 40 to 80 MHz repetition rates, respectively. Observe the detected SYNC rate in the *Input0 /cps* section of the main window. The displayed value must be stable and exactly corresponding to the real pulse repetition rate. Fine tune the *Discr. mV* and *ZeroCr.* parameter values if necessary.

NanoHarp users:

By convention, here the synchronization signal is called “Trigger”. This may be confusing because in connection with the NanoHarp, the PDL series driver itself is typically triggered by an external signal source. (In this case the *TRIGGER SOURCE* switch on the PDL 800-B is set to *EXT* position; on "Sepia" driver, the *TRIGGER* switch is set to *External*.) It is strongly recommended to use the SYNC output of the PDL driver as a signal for NanoHarp, regardless of the actual trigger source. For example, the NanoHarp can trigger the PDL 800-B, but it cannot simultaneously trigger itself.

To avoid signal crosstalk, use a 10dB in-line attenuator attached to the PDL driver's SYNC output before connecting here the trigger signal cable. Open the Control Panel, set the *Trigger Inp. Level* to -100 mV and select *Falling* edge. Beware, that positive voltage values are also accepted. Here the *Level* must be negative. The *Trg.Inp. /Hz* section of the main window shows the detected repetition rate. The displayed value must be stable and exactly corresponding to the real pulse repetition rate. Fine tune the *Trigger Inp. Level* if necessary.

If the TDA 200 photodiode is used for synchronization, the procedure is a bit more complicated. Connect the TDA 200 output via the ORTEC delay unit to the appropriate input connector of the timing electronics. No signal transformation, nor attenuation is necessary. Power on the TDA 200. This is done by simply attaching the dedicated 12 V power adapter. Make sure that the red light on the adapter is on.

Direct a fraction of the excitation beam towards the entry port of the photodiode (see the picture on page 4). It is strongly recommended to use wedge for this purpose. An ordinary beam splitter plate with parallel surfaces may reflect two pulse images slightly shifted in time, resulting in timing uncertainty.

The output pulse amplitude of TDA 200 depends on the energy of the input pulses. Watch the multicolour LED on the top of the unit, which is a pulse height indicator independent from pulse repetition rate. The green light turns on softly when the peak amplitude of the output pulse reaches -50 mV and turns to red if it exceeds -300 mV. (Note, that the TDA output must be properly terminated, e.g. connected to the input connector, otherwise the colour information is invalid.) Overloading the pin diode does not damage the TDA 200, but it may produce output pulses with more than -1 V peak amplitude, unsuitable for the rest of the electronics. Try to achieve a stable greenish yellow colour. To reduce the intensity, it is usually sufficient to slightly displace the beam. If the light source is too bright, place a suitable filter into the holder in front of the sensor’s entry port. The actual output peak amplitude is not critical but it must be stable. Now watch the detected SYNC rate (or trigger frequency for NanoHarp) in the control software.

TimeHarp users:

Open the TimeHarp Control Panel. In order to find the proper *Sync Level*, change the voltage value in -5 mV steps starting from, say -10 mV. Find the range where the displayed SYNC rate is insensitive to the set voltage level and then select the value in the middle of that range. Ideally, this is somewhere between -50 mV and -150 mV, but other values are acceptable as well. The criterion is the stability of the detected SYNC rate, which should be equal to the real repetition rate.

PicoHarp users:

Open the PicoHarp Control Panel and locate the *Inp.Chan. 0/Sync* section and set the *ZeroCr.* parameter to 5..10 mV. The *Divider* settings depends on the expected repetition rate. Best results are obtained with *Divider* = 4 for 2.5 to 20 MHz and *Divider* = 8 for 40 to 80 MHz repetition rates, respectively. Observe the detected SYNC rate in the *Input0 /cps* section of the main window. In order to find the proper *Discr. mV* parameter, change the voltage value in 5 mV steps starting from 10 mV. Find the range where the displayed *Input0 /cps* is insensitive to the set discrimination level and then

select the value in the middle of that range. Ideally, this is somewhere between 50..150 mV, but other values are acceptable as well. The criterion is the stability of the detected SYNC rate, which should be equal to the real repetition rate.

NanoHarp users:

Open the Control Panel, set the *Trigger Inp. Level* to -10 mV and select *Falling* edge. Beware not to enter a positive value; with the TDA 200 the *Level* voltage must be negative. The *Trg.Inp. /Hz* section of the main window shows the detected trigger frequency. Change the voltage value in -5 mV steps and find the range where the displayed trigger frequency is insensitive to the set voltage. Select the value in the middle of that range. Ideally, this is somewhere around -50..-150 mV and but other values are acceptable as well. The criterion is the stability of the detected trigger frequency.

3.4 Quick Check of the Photon Detector

Having the synchronization signal available and the cable connections completed, a useful initial test is the check of the dark count rate of the detector. Power on the FluoTime. Slightly lift one side of the sample chamber lid. You should hear the “click” of microswitches and the sound as the shutter closes. Repeat this procedure if you are uncertain. Then open the lid completely to make sure that the shutter will be closed during the following steps.

If your system has a detector other than PMA series, please refer to the relevant manual and follow the instructions there how to start and operate the unit.

Set the recommended or fail safe values for the second input of the timing electronics. These are the *Discr.* and *ZeroCr.* values for the TimeHarp and PicoHarp, or *Level* and edge (*Falling/Rising*) for the NanoHarp, respectively. In the Appendix of this manual, the optimized parameters are documented. Do not forget to *Apply* the values and observe the dark count rate of the detector.

TimeHarp users:

Using the TimeHarp Control Panel, set oscilloscope mode (*OSC*) with 1 second acquisition time. Start the acquisition by clicking on the  button. Observe the signal intensity as displayed in the *CFD cnts/s* field. The only reason to start the acquisition is to change the *SYNC Hz* rate meter to *CFD cnts/s*. The shape and position of the periodically updated histogram is not important at this moment. Remember that to measure CFD count rates, the SYNC signal must be present at the TimeHarp's SYNC input.

PicoHarp users:

The *Input1 /cps* section of the main window shows the detected count rate.

NanoHarp users:

The *Count.Inp. /Hz* section of the main window shows the detected count rate.

The value fluctuates, but should correspond to the specification listed in the Appendix. If your system has a cooled detector, it takes a while to achieve the reduced dark count rate. Very low count rates (less than 64 cps) cannot be accurately measured by the rate-meter. In this case, increase the acquisition time to 10 seconds or even longer. Locate the *Total Count* section at the bottom of the main window and wait until it is updated. The total number of collected counts divided by the acquisition time gives a more precise value of the average dark count rate.

4. Operation

4.1 Safety Instructions

The FluoTime 200 time-resolved spectrometer may be equipped with one or more diode lasers. To avoid hazardous radiation exposure you should carefully obey the safety instructions that are provided with your diode laser operation manual. If your instrument uses a different excitation system, follow the safety instructions in the relevant manual.

The delivered computer as well as optional components like MCP PMT cooler or HV power supply are preset by PicoQuant to operate on the power outlet line voltage for the country of delivery. Nevertheless, please check that the actual line voltage corresponds to the value set on these instruments! The 12 V power adapters used to supply the spectrometer hardware and the separate TDA 200 photodiode, are universal switching type devices that operate from 100 to 240 V AC, 50 or 60 Hz.

Most of the components have dedicated power switches for regular use, but there is no main power switch on the FluoTime 200. It is powered ON when the 12 V adapter is ON and attached. Unless there is a special detector built in, we strongly recommend to keep the FluoTime powered ON all the time. The power consumption is minimal.

Never connect or disconnect the high voltage supply cable of optional detectors while the HV source is ON! This could destroy the device. The direct output of these detectors must be terminated at the preamplifier input. Charged signal cables are dangerous and can destroy the sensitive electronics of the system.

4.2 Starting the System

From this point forward it is assumed, that the user understands the basics of the time-correlated single-photon counting method and is familiar with the usage of the control software. An explanation of the measurement principles can be found in various Technical Notes available from PicoQuant upon request or in the literature listed in this manual. Please refer to the manual of your data acquisition electronics for detailed instructions how to use the control software.

Turn on the computer and start the control software for the timing electronics. Load any sample data file (delivered with the instrument) into the control software. Alternatively, you can load any other data file saved as a result of a previous successful measurement. This is merely to load reasonable initial setup parameters for the electronics.

Power on the excitation source and select an appropriate repetition frequency. For example, set 10 MHz by turning the frequency divider knob on the PDL series driver and set the switch labeled *SOURCE* to *INT* position. Check the availability and stability of the SYNC (or trigger) signal. (Details can be found in section *3.3 Getting the SYNC Signal* starting on page 13. Locate the beam spot in the sample chamber. You may find a small piece of paper useful during these adjustments. Dim the room light if necessary. The spot should be focused to the center of the cuvette holder. Adjust the beam steering mirror if necessary.

Close the emission iris completely and open the sample chamber lid. Power ON the spectrometer if it is not yet done, i.e. connect the power adapter of FluoTime and make sure that the red light on the adapter is ON.

If your system has a motorized monochromator, you must initialize it before usage, that means the device has to seek its mechanical reference position. Click on the  button to start the appropriate dialog. Position initialization is required each time the software is started. It can be controlled on the *Initialization* page of the dialog. Now the dialog shows the state „not initialized“. This means the software has no information about the current monochromator position. The position initialization is started by the *Initialize* button. When the initialization is running, the caption of the button will change to *Stop* and the LED icon at the bottom of the dialog will start flashing. You should also hear the stepper motor. If the initialization is successful, the string „not initialized“ will change to „Initialization OK“, otherwise it will change to „Initialization failed!“. In this case, check cabling and power supply of the system. This may happen if either the *Stop* button was pressed or an error occurred during the initialization process.

The *Manual* and *TRES* pages will not be available unless the initialization was successfully performed. If for any reason the initialization becomes invalid (e.g. if the monochromator was turned manually and subsequently accidentally reaches the end point switch) the *Initialization* page will be activated and access to the *Manual* and *TRES* pages will be blocked.

With the sample chamber open, check the dark count rate of the detector as described in chapter *3.4 Quick Check of the Photon Detector* on page 15.

At this point, the FluoTime 200 system is ready to perform measurements. The basic measurement procedures are described in the following section.

4.3 Basic Measurement Types

In order to determine the luminescence lifetime, or more generally, the sample response kinetics, one usually needs to perform two consecutive measurements:

1. To record the time evolution of the sample's response to the excitation pulse (decay curve hereafter).

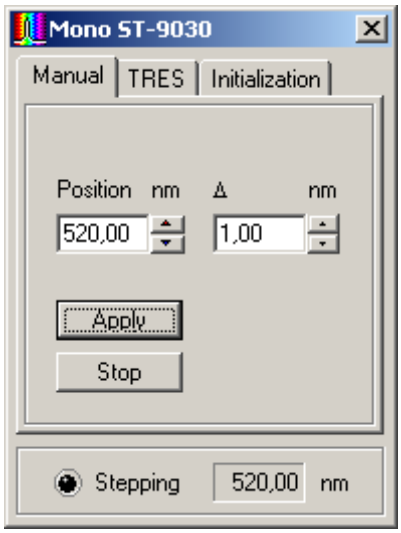
2. To record the time behaviour of the excitation pulse, the so-called instrument response function (IRF hereafter).

These two curves are then analysed with the FluoFit software to get the relevant kinetic parameters (e.g. lifetime) from the raw data. In some cases, typically measuring decays which are very long in comparison to the IRF duration, the IRF is not necessary and the second measurement can be skipped.

4.3.1 Recording a Decay Curve

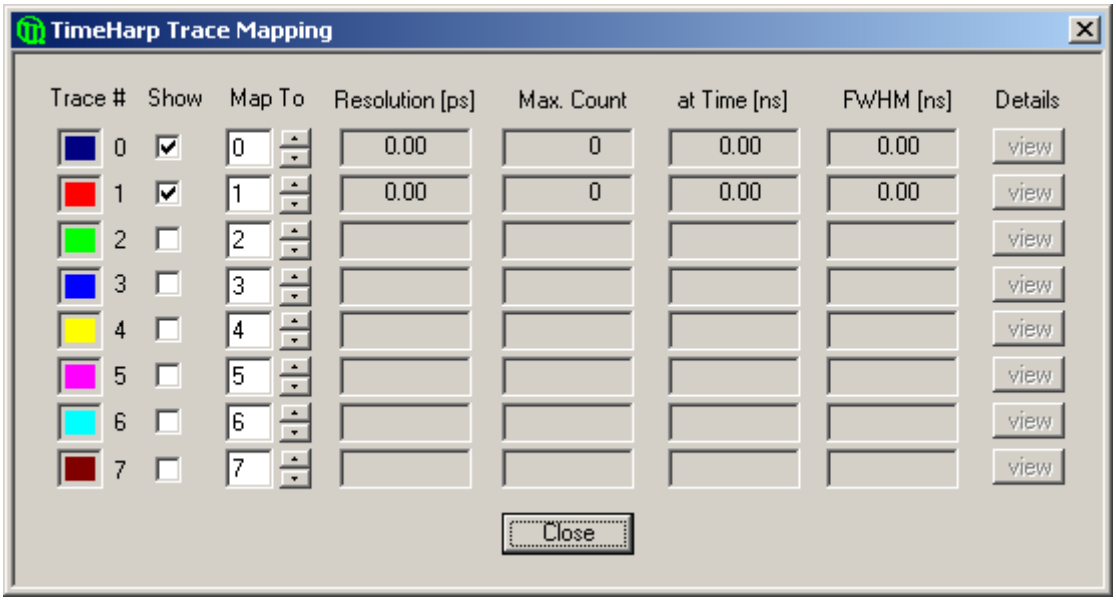
Prepare the FluoTime as described in section 4.2. Set the desired spectral resolution on the monochromator (by changing or adjusting the slits) and the appropriate polarization plane by the emission polariser (if applicable). Place your sample into the sample holder and adjust the position of the excitation beam-spot if necessary.

Select the emission wavelength. In case of manually controlled monochromator this is accomplished simply by turning the knob that provides the wavelength readout as well. If your system is equipped by a stepper motor, click on the button to open the monochromator control dialog.



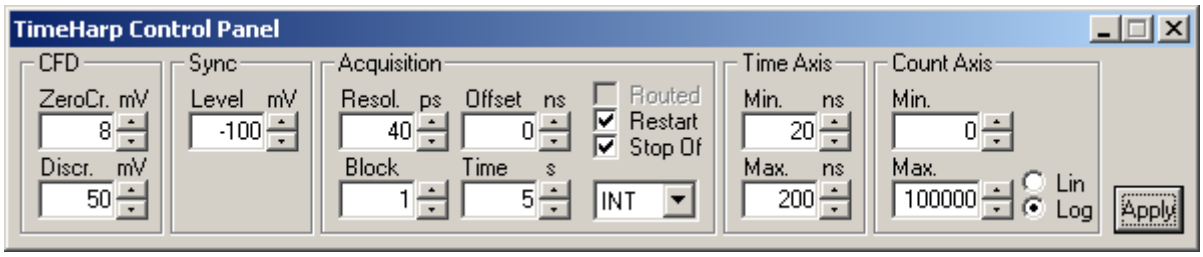
Use the *Manual* page to position the device to a given wavelength. The desired position can be entered in the *Position* edit box. Clicking *Apply* or pressing <ENTER> will cause the monochromator to move to the specified position. Alternatively, the arrow buttons right to the edit box can be used to modify the position. The step width of this action is defined in the Δ (delta) edit box. Pressing arrow buttons will make the change take effect immediately; entering values by using the keyboard require clicking *Apply* or pressing <ENTER> to show an effect. If there is a large difference between the current monochromator position and the position entered, the movement will take some time. If you wish to interrupt this process, click on the *Stop* button. The monochromator will immediately slow down and stop. The deceleration is necessary to preserve the calibration.

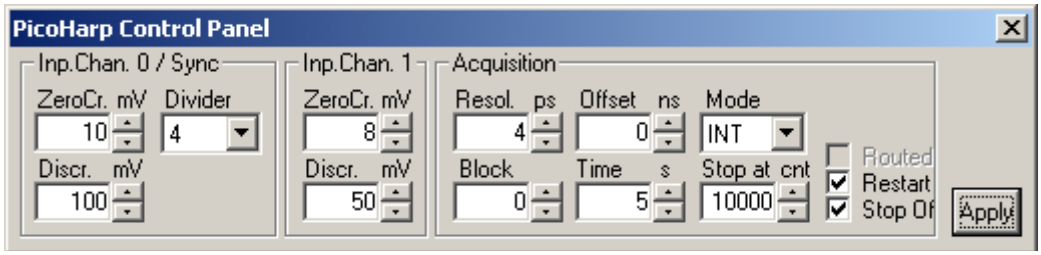
A standard decay measurement with FluoTime means to collect a time-resolved photon counting histogram. This histogram is accumulated in a dedicated memory segment (called block) of the electronics and displayed on the main panel as a trace. To make sure that the trace is visualized during the measurement, open the *Trace Mapping* dialog by clicking on . Select to show *Trace* # 0 and 1 only and check that these traces are mapped to the appropriate block number. A TimeHarp example is shown below; the dialog has the same layout for PicoHarp and NanoHarp as well.

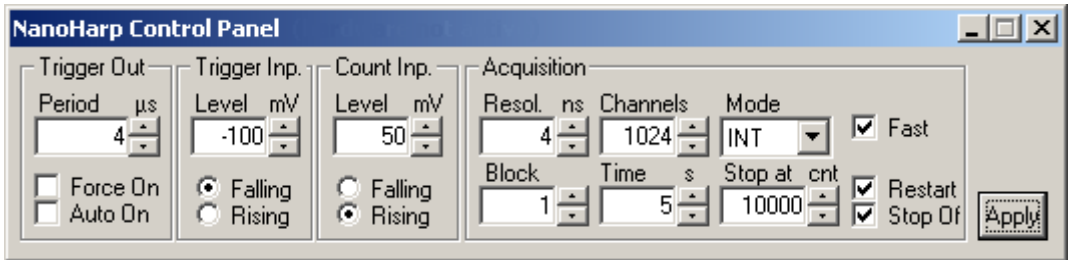


Close the dialog. The next step is to find the optimal light intensity reaching the photon detector. Using the *Control Panel*, set the highest time-resolution (i.e. the lowest *Resol.* value), integration mode (*INT*) to block #1, five seconds acquisition time with *Restart*, and logarithmic *Count Axis*. Start the acquisition by clicking on the Go button. Observe the signal count rate as displayed in the appropriate field at the bottom of the main window. The shape of the periodically rebuilt histogram is not important at this moment.

Examples of the *Control Panels* with the above mentioned acquisition parameters are shown below. Note that the optimal electrical settings for the signal input (left side of the dialog) may be different from the values shown on the screen shots. See the Appendix for the optimal electrical settings.







A fundamental principle of TCSPC states that for a valid measurement the signal count rate should be less than approximately 1% of the excitation repetition frequency. If the signal rate exceeds this limit, the apparent, measured decay would be seriously distorted.

Example: If the excitation repetition rate is 10 MHz, then the signal intensity as displayed by the *CFD cnts/s* value by TimeHarp or *Count.Inp. /Hz* value by PicoHarp must be less than 1.000e+005, preferably around 5.000e+004.

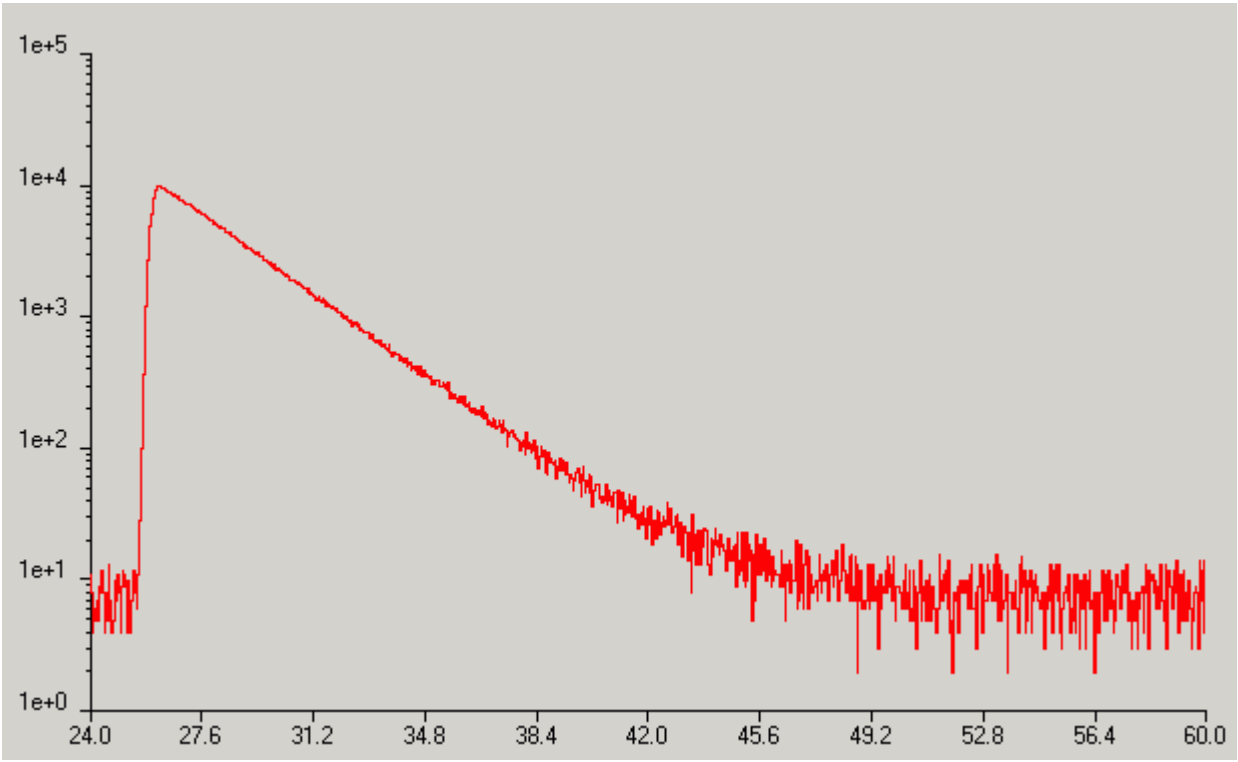
Such a limitation does not apply to NanoHarp the which is based on a different timing principle. However, the maximum photon count rate is still limited by the so-called pulse pile-up. Quite generally, photon counting is the method of choice for weak signals and it is recommended to measure at the lowest count rate that still allows for reasonably fast histogramming.

Optimize the signal intensity. This is an iterative process, which involves opening/closing the emission iris, finding the optimal focus position of the collecting lens as well as the optimal settings of the excitation optics while watching the signal rate.

This procedure can be dangerous (particularly with a new, unknown sample) since accidental over-illumination can damage the photon detector. Always approach the acceptable count rate limit from below. Start with closed emission iris and make your changes slowly. Pay attention because overloading the detector usually manifests itself as a suddenly reduced count rate.

It may happen that the signal intensity will be too low. Then you may want to sacrifice the spectral resolution and use wider monochromator slits. (Do not forget to open the sample chamber when changing the slits!) Another way to increase the signal intensity is to increase the excitation power but this increases the amount of scattered excitation light as well as the Raman scattered light.

The next step is to adjust the acquisition parameters to measure and display the proper histogram. In general, the displayed curve should begin with few channels containing background noise, continue with a more or less steep rise, reaching a maximum and then decaying to the background noise again. An example of such trace is shown below. Your result will look different, as well as the optimized time-axis limits will differ. The curve shape strongly depends on the sample.



TimeHarp and PicoHarp users:

Optimize the time-axis limits to move the histogram's apparent position in the main window. You can do this during the acquisition by clicking on the spin control of the relevant field in the *TimeHarp Control Panel* or using the *PicoHarp Axis Panel*  . If necessary, change the settings of the optional ORTEC delay unit. Do not use the *Offset* feature for this purpose. Long-lifetime decay curves might not fit completely into the time window available for a TCSPC measurement. For example, when the excitation repetition rate is 40 MHz, the sample is re-excited 40 million times every second, which means that the time interval between two subsequent pulses is $1/(4\times10^{7}s^{-1})$ = 25 ns. In this case, any fluorescence response with longer than approximately 3 ns (average) lifetime has no time to decay to

the background noise value. As a rule of thumb, the time interval between the pulses should be at least 10 times the lifetime of the longest decay component. For example, if the expected lifetime is around 10 ns, then the excitation repetition rate should be 1/ (10×10 ns) = 10 MHz or less. Using the TimeHarp, increasing/decreasing the excitation repetition frequency shifts the whole decay curve within (and sometimes out from) the measurement window due to the shorter/longer average time intervals between the detected photon signal and SYNC pulses. Also, changing the repetition rate affects the actual signal intensity and sets a new limit for the optimal signal rate! Measuring long decays may require you to select lower time resolution. This can be done by the *Resol.* spin control in the *Control Panel*.

NanoHarp users:

Optimize the time-axis limits using the *NanoHarp Axis Panel*  . Long-lifetime decays might not fit completely into the time measurement range which is determined by the product of the channel resolution and the number of channels, i.e. *Resol * Channels.* You can extend the time range by selecting a coarser time resolution (preferred) or by increasing the amount of data channels (only rarely useful). However, if the time period between the trigger (excitation) pulses is shorter than the new time range, the histogram will be truncated anyway. For periodical light sources, the maximum available time span equals the reciprocal value of the trigger frequency. If the pulsed source is triggered by the NanoHarp board, the pulse period can be conveniently selected in µs units in the *Trigger Out* section of the *Control Panel*.

Once the signal rate and the displayed time range are optimized, stop the periodically restarting measurement. Set the acquisition time limit long enough to acquire approximately 10000 counts in the channel with the maximum count number (hereinafter referred to as peak channel). See *Max. Count* at the middle bottom of the main panel. Generally, reaching a given *Max. Count* takes longer when measuring at lower signal count rate and/or when measuring a longer lifetime decay. Set the time limit to e.g. several hundred seconds at the beginning. The acquisition can be stopped any time without data loss.

Deactivate the *Restart* checkbox, start a new measurement and observe the *Max. Count* value. Stop the acquisition once the desired number of counts in the peak channel is reached. The NanoHarp and PicoHarp allows to preset the desired height. Type the value in the *Stop at cnt* field and activate the *Stop Of* checkbox.

You have successfully measured a decay curve. It is in block #1 now. You may want to save your result before continuing.

4.3.2 Recording an Instrument Response Function

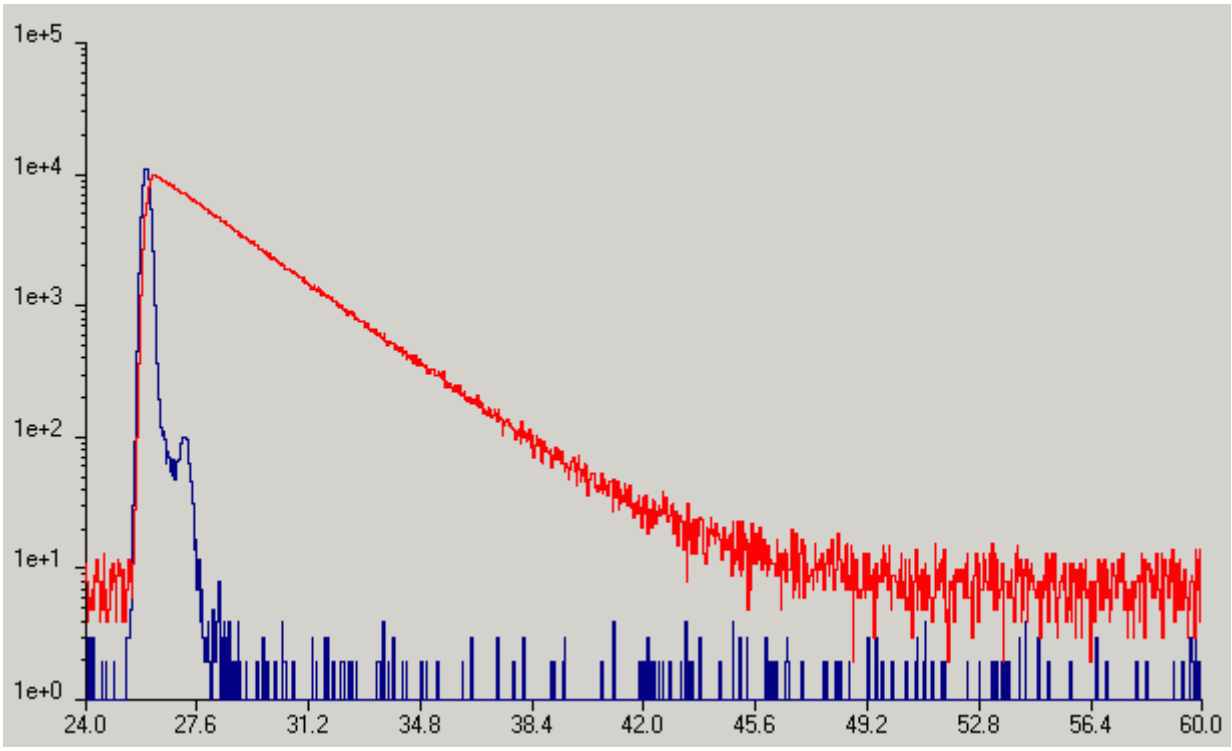
Prepare a suitable light scatterer. This can be accomplished by diluting a drop of DuPont LUDOX solution (delivered with your instrument) with distilled water in a cuvette. Note that the solution should appear completely transparent to the eye. Replace your sample with the scatterer.

Select the memory block #0 for acquisition. Do NOT change the other measurement parameters!

Close the emission iris and set the detection wavelength on the monochromator equal to the excitation wavelength. Start the acquisition and observe the signal intensity. The rules for the optimal signal intensity from the previous section hold for the IRF recording as well. Optimize the signal intensity by opening/closing the iris, but do NOT change the other hardware settings. It may occur that the signal intensity is too high even with a completely closed iris. In this case, dilute the scattering solution or place a neutral density filter into the excitation beam before it enters the sample chamber. It is not recommended to open or close the excitation iris because it may change the IRF.

Once the signal rate is optimized, deactivate the *Restart* checkbox and start a new measurement. Observe the *Max. Count* value at the middle bottom of the main panel. Stop the acquisition once the desired number of counts (e.g. 10000 in the peak channel) is reached. The NanoHarp and PicoHarp allows to preset the desired peak counts. Type the value in the *Stop at cnt* field and activate the *Stop Of* checkbox.

A typical IRF example (blue) in comparison with the decay is shown below:



Your result will look different, the shape strongly depends on the excitation source and on the settings of previous decay curve measurement.

Short signals are more susceptible to pulse pile-up distortion, therefore, record the IRF at even lower count rate than that during a decay measurement. For example, using TimeHarp or PicoHarp detect the scattered photons at maximum 0.5% of the SYNC rate. This is usually not limiting at all because the IRF histogram needs fewer counts in total and it is built up quickly. Since recording the IRF usually takes much less time, it has less noise background. The proper time position is a result of a preservation of the crucial parameters optimized during the previous decay measurement. Note that the curves' rising edges should be close to each other.

You have successfully measured an IRF; it is in block #0 now. You may want to save your results before continuing. Because the decay curve and the corresponding IRF belong to each other, it is a good practice to save them together in a single file.

4.4 Anisotropy Measurements

If the FluoTime is equipped with polarisers, it allows you to perform fluorescence polarization anisotropy measurements. An explanation of the method and the theory behind can be found in the literature listed in chapter 5 at page 28. In this chapter, only basic information relevant for the spectrometer usage is given.

4.4.1 Measurement Principles

The anisotropy kinetics cannot be measured directly. However, the temporal anisotropy information can be calculated (e.g. by the FluoFit software) from experimental data. Thus, one needs to perform consecutive measurements using polarized exciting light and detecting the polarized emission components. These decays are denoted VV, VH, HV, and HH in the following text. V stands for vertical, H for horizontal polarization with respect to the plane of the spectrometer optics. The first letter denotes the polarization of the excitation, the second one that of emission. VV and VH are often referred as parallel and perpendicular polarized decays. When the excitation is V polarized, the anisotropy $r(t)$ can be calculated from the traces VV and VH, channel by channel, in the following way:

$$r(t)=\frac{VV(t)-G\cdot VH(t)}{VV(t)+2G\cdot VH(t)}$$

Here, G is an instrument dependent correction factor. Theoretical treatment of the anisotropy assumes G=1, i.e. that any difference between VV and VH decays is exclusively due to polarization anisotropy. However, with spectrometers using grating monochromators it is practically impossible to measure V and H polarized emissions with equal efficiency. The role of G is then to compensate for the polarization bias of the detection system.

The precise G-factor can be determined either by so-called tail matching of VV and VH decays, or by treating the additionally measured HV and HH decays. The latter procedure is always applicable, but can be time consuming, since it involves two additional measurements. On the other hand, tail matching is applicable only in cases, when the following conditions are met: a) the anisotropy decay is much faster than the intensity decay (i.e. VV and VH curves contain sufficient amount of anisotropy-free intensity decay); b) the residual anisotropy (i.e. anisotropy for large t) is zero.

All the necessary anisotropy calculations can be done by the FluoFit software. Usually one assumes a certain functional form of *r(t)* based on some prior knowledge about the depolarization mechanism in the sample. In this case *r(t)* can be included into a reconvolution analysis of the polarized decays. Please refer to the appropriate section of the FluoFit manual on how to handle and analyse polarized decays.

4.4.2 Experimental Procedures

The radiation emitted by PicoQuant laser diodes is already polarized. The polarization direction of the laser beam is set by rotating the head, though it is still recommended to install and properly set the excitation polariser. An isotropic or partially polarized (e.g. LED) radiation should pass the optional polariser. External laser systems often use a λ/2 (half-wave) plate to set the beam polarization. Please consult the manual of your excitation source in any case.

The sample response to V polarized excitation contains both H and V polarized components. A second polariser is needed in the emission segment to select the decay component of interest for recording.

Set up a decay measurement as described in chapter 4.3.1. Collect the VV curve with at least 30000 counts in the peak channel to the block #1.

Anisotropy investigations require decay data measured with very high statistical precision because the information about the anisotropy dynamics is contained in relatively minor differences of polarized decays. As obvious from the definition formula of *r(t)* above, direct calculation of the anisotropy kinetics also involves division of a small difference by a large sum. To make the difference term statistically significant, a large number of counts should be collected. For example, if a histogram channel contains 10000 counts, the statistical precision (standard deviation) is √10000 = ±100 counts. Hence, the relative error in the intensity

value is 100/10000 = 1%. Such precision is sufficient for lifetime determinations, but not for anisotropy measurements. We recommend to collect more than 30000 counts in the peak channel.

Change the orientation of the emission polariser to H. Do not change the other optical settings! Collect the VH curve to the block #2. Do not change any other acquisition parameter. The VH decay must be collected immediately after finishing the VV measurement and under exactly the same experimental conditions, except of the emission polarizer's orientation.

You may want to record an IRF to block #0. The IRF itself should not depend on the polariser orientation. It is recommended to measure it as VV because the signal intensity is the highest in this case.

If necessary, collect the HV and HH decays to the next available blocks. This procedure is very similar to that for recording VV and VH, but the excitation polarization must be set to horizontal. In case of a non polarized light source, this is accomplished simply by turning the excitation polariser; laser diode heads must be rotated by 90° in addition to that.

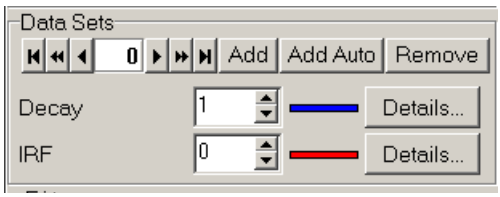
Save your results in a single file and transfer the curves to FluoFit as described in the next section. Subsequent analysis provides the relevant kinetic parameters (e.g. rotational correlation time) from the raw data. Please refer to the FluoFit manual for further instructions.

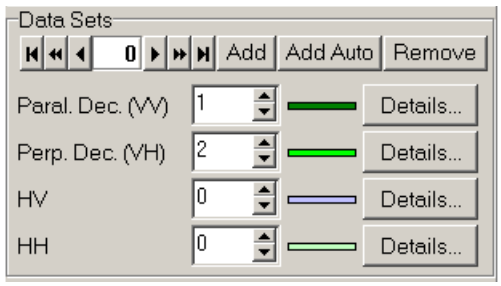
4.5 Rapid Data Analysis

Decay curve analysis is an important aspect of the TCSPC technique. The FluoFit software delivered with your instrument is a powerful tool to obtain the sought after kinetic information from the raw experimental data. It is advisable to run the FluoFit and the control software simultaneously. The Windows clipboard provides a quick way to transfer the curves to FluoFit. Copy the content of the necessary memory blocks into the clipboard. This is accomplished by clicking on the button or by pressing Ctrl+C.

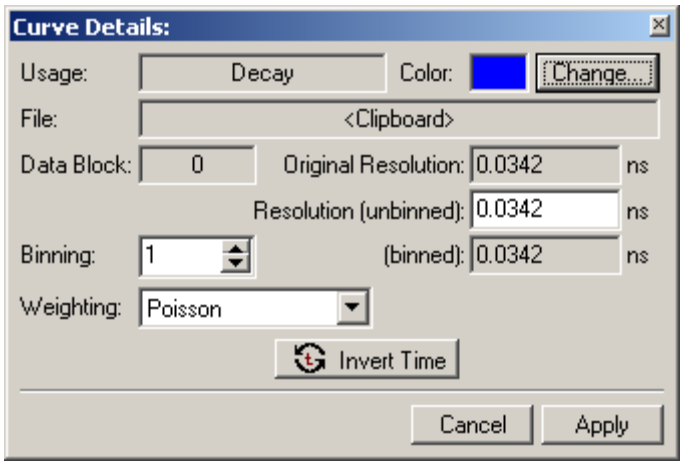
Note: Only the curves actually displayed in the measurement software window are copied. The measurement software can store up to 512 histograms, and 8 of them can be simultaneously displayed on its main window. Use to change their mapping, i.e. to select the desired curves for display (and copy).

Switch to FluoFit. (Use the Windows task bar or press Alt+Tab.) Paste the curves from the clipboard by clicking on the button. When pasting curves, these do not necessarily appear immediately in the main plot, especially when other histograms have already been imported previously. The curve selection and assignment is done after the import using the *Data Sets* section of the *Parameter* panel.





The number which can be edited in the spin edit box to the right of the curve name (e.g. *Decay*), corresponds to the index of the curve in the set of all pasted and/or loaded curves. This may therefore not be identical to its original block number. To show the file name and file index of the currently selected curve, press the corresponding Details... button on the right side. This will open the *Curve Details* dialog which helps to identify the origin of the selected curve:



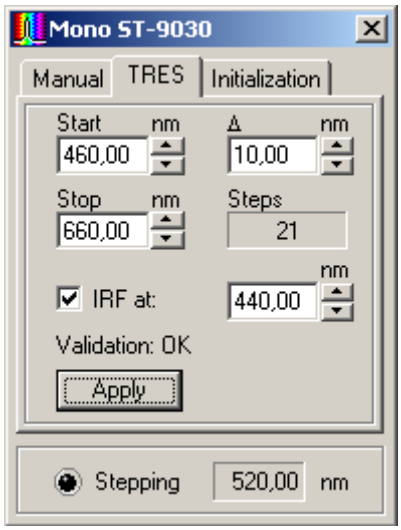
You have now loaded the experimental results into FluoFit. Please refer to the FluoFit manual to learn about data analysis and for further instructions.

4.6 Time-Resolved Emission Spectra (TRES)

In time-resolved fluorescence research, it is often of great interest to observe time-dependent spectral shifts and decay changes in the context of solvent relaxation dynamics and general spectral evolution. This requires recording a set of decays at various emission wavelengths. In principle, it can be accomplished by tedious manual control of the monochromator and subsequent decay measurements, but such a procedure is

inconvenient and time consuming. If your system is equipped with a motorized monochromator, an automated wavelength scan and data collection (referred to as TRES measurement mode) under full software control is possible. Histograms (decays) are saved in consecutive blocks of memory for each wavelength.

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



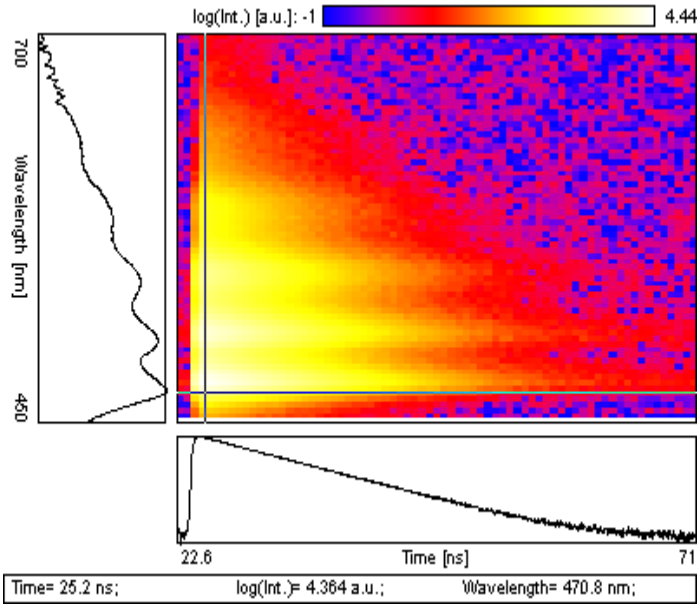
The IRF can be measured automatically as part of the scan, at a separately chosen wavelength. To do so, check the box entitled *IRF at* and enter the wavelength at which to measure the IRF in the corresponding edit box. The monochromator will go to the IRF position at the beginning of the TRES measurement. After the IRF was measured, the actual TRES scan will be performed. The IRF will always be recorded in memory block 0, while the wavelength scan always starts in block 1. Note that starting a TRES measurement will clear and subsequently overwrite all curves in memory that may have been recorded previously. If the curves in memory were loaded from a file, the actual file on disk will not be overwritten until you save the new ones to the file. Use *Save As* and select a new file name if you want to preserve the old file.

The TRES measurement mode (instead of OSC or INT) needs to be selected in the Control Panel, via the drop-down combo box for the measurement mode. A measurement is started or cancelled as usual via the toolbar buttons and . The data acquisition will be performed like a standard Integration Mode measurement, while the block number is incremented for each new wavelength. At the beginning of each TRES run, the active curve (block) will be set to 0, or to 1 if no IRF is collected. All curves that were previously collected and not saved to a file will be overwritten with new data. During a TRES run, the currently collected data will always be shown as Trace #0 (dark blue), even though the block number is actually incremented at each wavelength step. This is to overcome the limitation of only 8 display curves being available. You can watch the block number in the control panel to see the curve currently being collected. Manual entry will be disabled during the run. Also, you can watch the status bar to see the current wavelength and stepping activity. Note that for a TRES measurement the control panel setting *Stop Of* applies as in Integration mode, while *Restart* is meaningless in TRES.

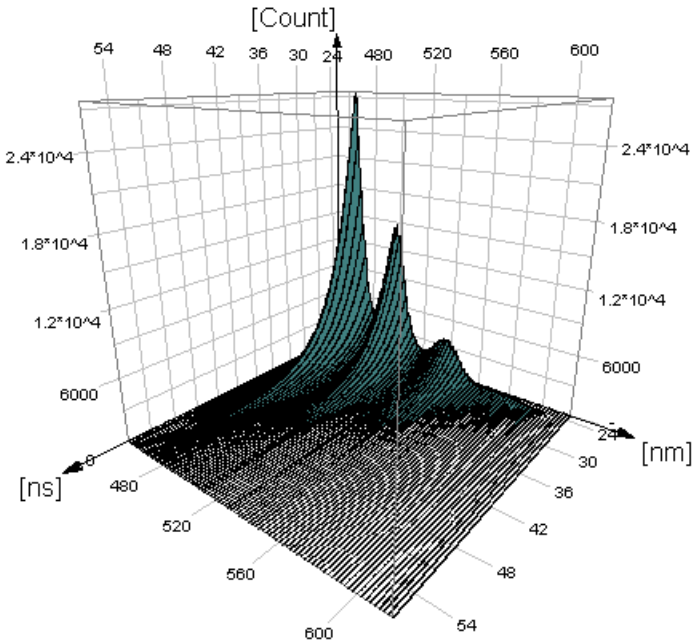
Having collected a complete TRES set you can save it to a regular data file. You can inspect individual curves via the Trace Mapping dialog ( ), or use the PQPlot3D software tool from PicoQuant (see the next chapter).

4.7 TRES Visualization

The PQPlot3D software tool from PicoQuant was written to visualize and analyse the data in various 2D and 3D representations such as *false colour* and *waterfall* plots with a multitude of options for colouring, scaling and changing view aspects in 3D. The figures below show PQPlot3D results from a TRES measurement of naphtacene in hexane.



PQPlot3D is provided on your software installation disk(s). Currently it has no installer. Just copy the files to a hard disk folder and start `PQPlot3D.exe` from there. Note that this is only an inspection tool, free of charge and currently unsupported. Further data analysis may need to be performed by dedicated software, either custom programs or spreadsheet processors.



5. Literature

1) Lakowicz, J. R.: *Principles of Fluorescence Spectroscopy. Second Edition.* Kluwer Academic/Plenum Publishers, New York, 1999; ISBN 0-306-46093-9

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3) O'Connor, D. V., Phillips, D.: *Time-Correlated Single Photon Counting*. Academic Press, London, 1984; ISBN 0-12-524140-2

4) Demas, J. N.: *Excited State Lifetime Measurements.* Academic Press, New York, 1983

5) Birch, D. J. S., Imhof, R. E.: *Kinetic Interpretation of Fluorescence Decays.* Analytical Instrumentation, Vol.14, p. 293-329, 1985

6) Kapusta, P., Erdmann, R., Ortmann, U., Wahl, M.: *Time-resolved Fluorescence Anisotropy Measurements Made Simple*. Journal of Fluorescence, Vol.13, p.179-183, 2003

6. Support

All components of FluoTime 200 system have gone through extensive testing at PicoQuant. The system is very stable and reliable. Nevertheless we will continually make improvements and fix any hardware or software bugs where they can be identified. In this process we strongly depend on your help and qualified feedback.

If you observe any error or malfunction, please try to find a reproducible error situation. E-mail a detailed description of the problem and relevant circumstances to photonics@picoquant.com. Your feedback will help us to improve the product and documentation.

In any case, we would like to offer you our complete support. Please do not hesitate to contact PicoQuant if you need assistance with your system.

Of course, we also appreciate good news: If you have obtained exciting results with one of our systems and published a paper, we would like to know! Please send us an e-mail to photonics@picoquant.com containing the appropriate citation. Gain additional publicity! PicoQuant maintains a large database of publications mentioning PicoQuant devices and/or written by us. It can be found at our website at http://www.picoquant.com/_scientific.htm and it is a valuable source if you want to know which laboratories are using PicoQuant products or how broad is the field of various applications.

Thank you very much in advance for your kind co-operation!

Retraction of old devices

Waste electrical products must not be disposed of with household waste. This equipment should be taken to your local recycling centre for safe treatment.

WEEE-Reg.-Nr.: DE 96457402

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7. Appendix