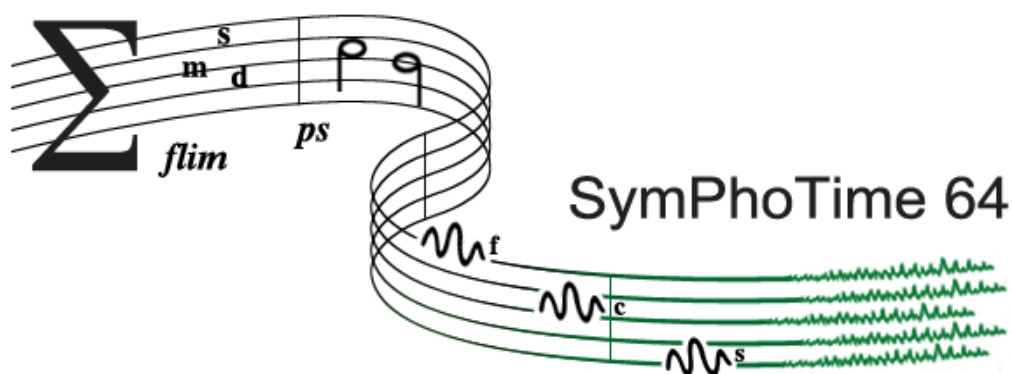


SymPhoTime 64



PICOQUANT
Unternehmen für optoelektronische
Forschung und Entwicklung

Confocal TCSPC
Data Acquisition
and Analysis Software



Installation Manual and Technical Data

Version 2.8

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1. Introduction

1.1 SymPhoTime 64 in a Nutshell

Originally designed for use with PicoQuant's MicroTime 200 confocal microscope, the software from the SymPhoTime family with its latest version, SymPhoTime 64, has evolved into a versatile tool applicable from laser scanning microscopy to cuvette measurements.

Of pivotal importance are the Time–Tagged Time–Resolved (TTTR) measurement modes of the PicoQuant TCSPC devices, which allow the performance of vastly different measurement tasks based on one single data format, yet without any sacrifice of information available from each single photon. The TTTR data of all supported sources are stored in the same, brand-new PicoQuant Unified Tag File Format, which allows all measurement data to be handled in a standardised yet flexible way.

The SymPhoTime 64 software is designed with almost unlimited flexibility for integration of virtually all algorithms and methods for the analysis of fluorescence dynamics that users may require. Based on the powerful TTTR data collection and by application of STUPSLANG, our underlying scripting engine, users can perform an unlimited number of analysis steps without losing track of the interdependence and origin of their measurement and analysis data. Results can be obtained through a vast set of analysis tools, such as intensity time trace, burst analysis, lifetime histogramming, fluorescence correlation spectroscopy, lifetime imaging, to name only a few.

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Getting Started

1.2 Requirements

The SymPhoTime 64 software is suitable for PCs running the Windows 10 x64 OS. It demands a reasonable performance of the PC. For routine work, a machine with x64 quad-core CPU, incl. SSE2 and Intel 64 or AMD64 extension with at least 2.2 GHz CPU clock and a minimum of 4 GB RAM is necessary. For improved performance in data acquisition or during complex analysis tasks like FLIM or FCS, a CPU with more cores and more RAM (e.g. 16 GB) is recommended, for extensive data files as for example acquired with rapidFLIM, at least 64 GB RAM is recommended, preferably 96 GB

The software takes approximately 100 MB, however, not including the storage space for data files. To use the software efficiently, a full HD screen resolution is needed. For daily work, a screen resolution of at least 1680 × 1050 pixels is recommended. Even better is a dual display set-up.

The SymPhoTime 64 package is protected by a HASP protection module (dongle) that must be connected to the USB port of the PC during operation. In order to recognize its presence and to use the HASP protection module, a software driver is automatically installed with the SymPhoTime 64 software package.


















1.3 Feature Overview

Data Acquisition	Features
TCSPC-Based ^[1-10]	PicoHarp 300 & 330.....by import or direct measurement HydraHarp 400.....by import or direct measurement TimeHarp 260.....by import or direct measurement MultiHarp 150 & 160.....by import or direct measurement
Supported Configurations	MicroTime 200 100 × 100 (× 100) μm Piezo Scanning Stage 100 × 75 mm Scanning Stage FLIMbee galvo scanner Atomic Force Microscopes (AFM) MicroTime 100 Laser Scanning Microscopes (LSM) Stand-Alone TCSPC
Routing	1 to 16 Detectors
FLIM ^[22-24]	Online FLIM Calculation and Preview
Fluorescence Time Trace	Diffusion Single Molecule Detection Interactive Molecule Selection (Click and Drag) Online Time Trace and FCS Calculation and Preview
Automated Measurements	Z-Stacks Time Stacks Multi Point/Stitching

Analysis ^[17-21]	Features
General Features	TCSPC Fitting Multi-Exponential Decay (1 to 5 Exponentials) Least-Squares Fitting, MLE Fitting Global Analysis IRF Reconvolution Tailfit Fast Pattern Matching Pattern Matching rapid FLIM Cyclic Excitation User Scripting (STUPSLANG) User-Defined Analysis / Equations User-Defined Fitting Multi-Parameter Filtering
Imaging	(Time-Gated) Fluorescence Intensity Imaging FLIM Lifetime Histogram FRET Imaging Pattern Matching
Diffusion	FCS ^[28-46] FCCS FLCS ^[45, 46] PIE-FCS Fitting ^[28-30] Global Analysis Diffusion Constants Triplet state Conformational Protonation Gaussian PSF Confidence Intervall Estimation ^[31-35] Fluorescence Intensity Traces Count Rate Histogram, PCH, Burst Size Histogram Fluorescence Lifetime Traces Lifetime Histogram BIFL (Burst Integrated Analysis)
FRET ^[25-27]	PIE (Pulsed Interleaved Excitation) Bleedthrough Correction
Steady-State Anisotropy	Objective correction factors included
Single Molecule Detection	Fluorescence Intensity Traces Blinking (On / Off Histogramming) Count Rate Histogram, PCH Intensity-Gated TCSPC Fluorescence Lifetime Traces Lifetime Histogram

1.4 Installation Procedure

The software is supplied pre-installed and on DVD, together with a copy protection module (HASP). On the installation DVD you will find the following files and directories:

	SymPhoTime64_Setup.exe	self-extracting installation file
	Readme.txt	installation notes
	techdocs	Various application notes
	other products	Information on other PicoQuant products
	LSM_Remote_Control	Remote Control setup and developer informations for LSM
	Samples.sptw	Workspace with example data
	Developer	Developer informations for SymPhoTime 64
	FileDemos	Demos for implementing reading of ptu files
	ScriptingDemoCode	Example STUPSLANG Scripts for SymPhoTime 64
	MT200	drivers and diagnosis software for the MicroTime 200 hardware:
	IDS-uEye	MT200 side-port camera driver setup
	MT200MainBoard	MT200 shutter control (connected via USB) drivers
	NanoCapture	PI E-710 Scanner diagnosis and calibration software setup
	PCAN	MT200 shutter control (connected via CAN) drivers
	PI-E725	PI E-725 Scanner driver setup
	PILine-WRS	Wide range scanner (100 mm x 75 mm) driver
	Video	Frame grabber driver and associated software installation files

If this software is purchased as part of a complete system, it will be pre-installed upon delivery. Usually there will be no need for re-installation. The most probable situation where a user may need to perform an installation, will be on a PC used for data analysis only. In this case, or if the software was purchased as a standalone product, install the software by running `SymPhoTime64_Setup.exe` and follow the instructions of the installer program.

If the controlling PC of a MicroTime 200 microscope needs to be re-installed, make sure that the drivers of all devices are correctly installed. (The drivers for all possible components are located in the appropriate subfolders of the `MT200` directory.) Then run `SymPhoTime64_Setup.exe` selecting the "MicroTime" installation.



As the complete installation for a microscope system is complex and difficult, it is strongly recommended to contact the support, when the necessity of a re-installation occurs.

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1.5 License Information

The functionality of the software package depends on the purchased license. To show the license configuration choose Help | *About...* from the main menu and press the *Get Support Information* button of this dialog. The serial number of the HASP module, the location of the current license and the available software packages are shown. Currently, there are four packages available within the SymPhoTime 64 software, called "FCS Analysis", "Image", "FCS", "Complete" and STED.

"Complete" includes the functions of both "FCS" and "Image". "FCS Analysis" limits the "FCS" license to the analysis functions for evaluating TTR files recorded by the MultiHarp, TimeHarp, PicoHarp or HydraHarp software.

1.6 System Configuration Using Configure Mode



The configure mode is only for experienced users, who are building their own experimental setup using PicoQuant equipment together with the SymPhoTime 64 software.

If you have purchased a MicroTime 200, MicroTime 100 or an LSM upgrade kit, the delivered system is already configured correctly. Usually, changes of the configuration will not be necessary. Please contact PicoQuant before changing settings in configure mode. Inadequate settings may cause serious problems.

To work properly with any experimental set-up, the SymPhoTime 64 software needs to know its hardware configuration. The SymPhoTime 64 stores this configuration data on two levels. The first level constitutes a kind of "known good" configuration for the system. These settings are called *Factory Settings*. They are the same for all user accounts, therefore they are stored in a single configuration file (*Settings.pfs*). To retrieve this configuration, a user can select *Settings | Use Factory Configuration* from the main menu.

The second level of configuration data storage allows individual users to customise the system and the software. These customisations are saved in user named files with the extension "**.pus*" for each individual user account.

Some hardware-related settings are critical for the performance of the system and must not be changed in day-to-day routine. These settings can only be edited in configure mode. Changes to these settings and their individual date of change are stored in the *Factory Settings*. All conflicting user settings older than their factory date of change are ignored (factory override) but changing the user settings later on will replace the values again (user override).

The software can be started in configure mode from the Windows Start menu entry of the SymPhoTime 64 by using the link *SymPhoTime Configure Mode*. This link is only applicable if the user is member of the administrators group or if he starts the software with administrator privileges.



You need to explicitly start the SymPhoTime 64 with administrator privileges to change the factory configuration. The provided `ConfigureModeStarter.exe`, started by use of the link *SymPhoTime Configure Mode* is elevating or asking for an administrator name and password.



Before changing any settings in configure mode, a backup of the current configuration file `Settings_BAK<nnn>.pfs` is saved, where `<nnn>` is a subsequential number.

If it should become necessary to fall back to the settings from this backup file, they may be recovered by simply substituting the file `Settings.pfs` with the latest backup file.

1.7 Setting up LSM scanner configuration in SymPhoTime 64

To configure the system for a given LSM scanner follow these steps:

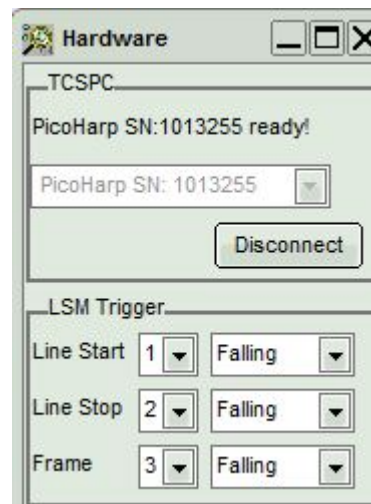
1. Open the dialogue “Settings | Hardware Setup”
2. Type in the values as shown below
3. Press “Save Defaults” afterwards

Below you will find the informations for the different LSM companies and LSM types for the **SPT64**. Please note that these settings are for the **PicoHarp300**.

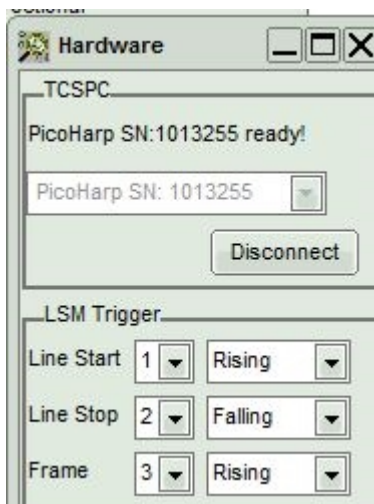
Olympus FV 300:



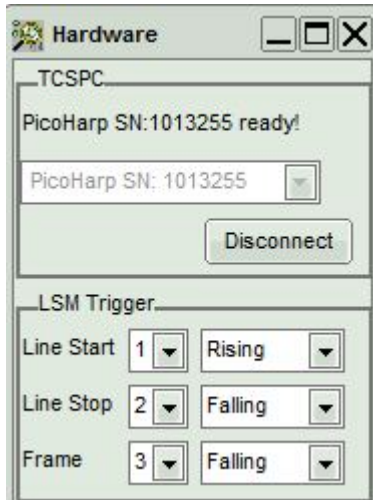
Olympus FV 1000:



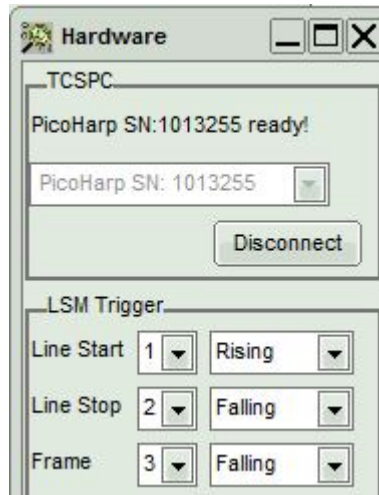
Olympus FV 3000:



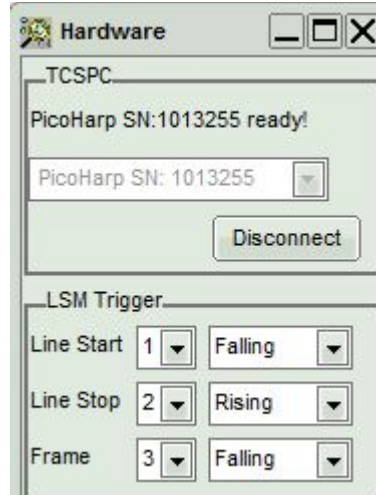
Leica SP2 / SP5 / SP8:



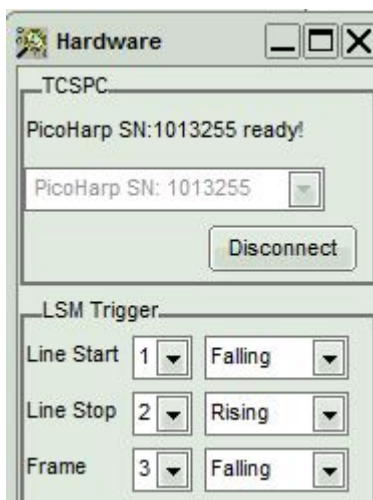
Zeiss LSM 510:



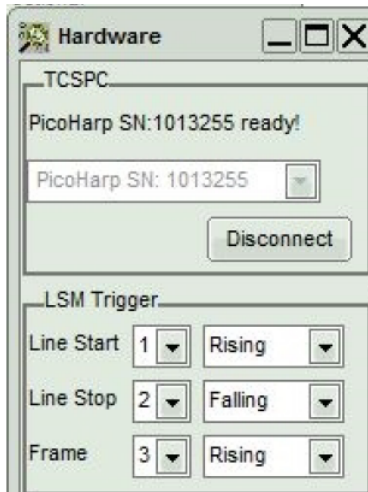
Zeiss 710 / 780 / 980



Nikon C1 / C1si / C2 / C2si / A1:



Scientifica HyperScope ScanImage:



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PicoQuant maintains a database of publications mentioning PicoQuant devices. It can be found at our website at <https://www.picoquant.com/scientific/references/>. It is a valuable source if you would like to know which laboratories are using PicoQuant products or how broad the field of various applications is. Furthermore, numerous measurement examples are published on the PicoQuant website. Please visit the MicroTime 200 section of https://www.picoquant.com/scientific/technical-and-application-notes/category/technical_notes_microscopy.

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3. Appendix

3.1 Technical Reference Data

Data Acquisition

TCSPC devices:

for direct measurements.....PicoHarp 300 & 330, HydraHarp 400, TimeHarp 260, MultiHarp 150 & 160
 data import only !!!..... TimeHarp 200

Supported Configurations..... 100 × 75 mm Scanning Stage,
 100 × 100 (× 100) μm Piezo Scanning Stage,
 FLIMbee galvo scanner
 Atomic Force Microscopes (AFM), Laser Scanning Microscopes (LSM),
 Stand-Alone TCSPC

Detectors..... 1 to 16 detectors

Measurement Modes:

for direct measurements TTTR: t2, t3
 data import only !!! TTTR: t3r

Analyses

Supported Methods.....FLIM, FRET, Anisotropy, PIE, FCS, FCCS, FLCS, SMD, PCH, Lifetime Histogram,
 Fluorescence Time/Lifetime Traces, BIFL, On / Off Histogram, Burst Size Histogram,
 TCSPC Lifetime Fitting, FCS Fitting, User Scripting (STUPSLANG).

TCSPC Fitting

Methods.....STUPSLANG scripted: FLIM, fluorescence time traces, BIFL, lifetime histogramming

Models..... 1 to 5 exponentials, iterative reconvolution

Optimisation.....least squares, MLE, Marquardt–Levenberg, Monte Carlo, Global Analysis

Error test/assessment..... χ^2 , distribution weighted residuals

Corrections.....rapid FLIM, cyclic excitation

Error analysis.....Bootstrap

FCS Fitting

Models...STUPSLANG scripted: pure diffusion, triplet–state, conformational, protonation; 2D/3D Gauss. PSF

Optimisation.....least squares, Marquardt–Levenberg, Monte Carlo, Global Analysis

Error test / assessment..... χ^2 , distribution weighted residuals

Error analysis.....Bootstrap

User Interface

Graphical user interface.....Windows® GUI, menu or mouse driven, STUPSLANG scripted

Preferences.....saved in factory / user settings data files

Supported Data Formats

Input for Analyses.....ptu and pqres files: PicoQuant Unified Tag File Format

Output.....pqres files: PicoQuant Unified Tag File Format; ASCII, TIFF, BMP, BIN-Export

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Operating Environment

Required PC:

- CPU with SSE2 and EMT64 or AMD64 extension; recommended: quad-core or better
- CPU clock minimum 2.2 GHz; recommended: >= 3 GHz
- RAM minimum 4 GB; recommended: >= 16 GB
- Disk space >= 100 MB (except data storage)
- Display(s) with Single Display: Full HD; with Dual Display: 2 × 1680 × 1050 or better
- Operating system Windows 10 x64, Windows 11 x64
- Protection Module (HASP) Port USB

3.2 Abbreviations

BIFL	Burst Integrated Fluorescence Lifetime
BNC	British Naval Connector or Bayonet Nut Connector or Bayonet Neill Concelman
CAN	Controller Area Network
CCD	Charge-Coupled Device
CFD	Constant Fraction Discriminator
cps	Counts per Second
FCS	Fluorescence Correlation Spectroscopy
FIFO	First In, First Out (buffer type)
FLIM	Fluorescence Lifetime Imaging
FRET	Förster Resonance Energy Transfer
FWHM	Full-Width at Half-Maximum
IO	Input / Output
IRF	Instrument Response Function
LED	Light Emitting Diode
LSM	Laser Scanning Microscope
MCS	Multichannel Scaling
OD	Optical Density
PC	Personal Computer
PCI	Peripheral Component Interconnect
PIE	Pulsed Interleaved Excitation
PMT	Photomultiplier Tube
RGB	Red-Green-Blue (colour scheme)
ROI	Region of Interest
SMA	Sub-Miniature version A (connector type)
SMD	Single Molecule Detection
SPAD	Single Photon Avalanche Diode
STUPSLANG	S ymPhoTime U ser P rogramming S cript L ANGuage
SYNC	Synchronisation (signal)
TCSPC	Time-Correlated Single Photon Counting
TTL	Transistor-Transistor Logic
TTTR	Time-Tagged Time-Resolved

3.3 Support

If you observe any errors or bugs, please try to find a reproducible error situation. Please e–mail us a detailed description of the problem and of any relevant circumstances, together with the data in question. **When you contact the support, please bring up the *Help | About...* dialog from the main menu) and press the *Get Support Information* button of this dialog. Select the complete information shown e.g. by *Select All* from the context menu and copy and paste it to a text file. Please attach this file (and include the S/N of your system, if applicable) when you mail your support request to support@picoquant.com. This will help us with the diagnosis of the problem. Thank you very much in advance. Your feedback will help us to improve the product and its documentation.**

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

Of course, we also appreciate good news: If you have obtained exciting results with this software or published scientific papers, we would also like to know! Please send an e–mail to info@picoquant.com containing the appropriate citation. Gain additional publicity! PicoQuant maintains a database of publications mentioning PicoQuant devices and / or written by us.

It can be found at our website at <https://www.picoquant.com/scientific/references/>. It is a valuable source if you want to know which laboratories are using PicoQuant products or how broad the field of various applications is.

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

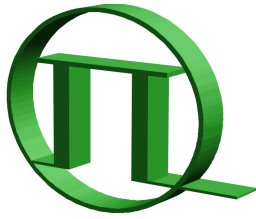
Retraction of old devices

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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PICOQUANT
Unternehmen für optoelektronische
Forschung und Entwicklung

PicoQuant GmbH
Unternehmen für optoelektronische Forschung und Entwicklung
Rudower Chaussee 29 (IGZ), 12489 Berlin, Germany

Telephone: +49 / (0)30 / 1208820 0
Fax: +49 / (0)30 / 1208820 90
e-mail: info@picoquant.com
WWW: <http://www.picoquant.com>