

## Specifications

|  |   |
|--|---|
| <b>Excitation Source (optional)<sup>1)</sup></b> |   |
| Light source                                     | Picosecond Laser Diode Heads (LDH Series)   |
| Wavelengths                                      | 375 - 510 nm, 530 nm, 635 - 900 nm  |
| Repetition rate                                  | up to 40 MHz (optional 80 MHz)  |
| <b>Scanning by Physik Instrumente (optional)</b> |   |
| Controller                                       | E-710 digital PZT controller  |
| 2D scanning stage                                | P-733 piezo-positioner  |
| Effective range/resolution                       | 80 µm × 80 µm range with less than 10 nm resolution   |
| Z scanning                                       | P-721 PIFOC objective nano-positioner   |
| Effective range/resolution                       | 80 µm range with less than 10 nm resolution   |
| <b>Objectives</b>                                |   |
| Standard   | PL 20x3 PlanAchromat, NA 0.4, air spaced, 400 - 750 nm<br>PL 40x PlanAchromat, NA 0.65, air spaced, 400 - 750 nm<br>UPLSAPO 60x PlanApochromat, NA 1.2, water immersion, 400 - 900 nm   |
| Optional   | oil immersion, apochromatic correction, air spaced,<br>IR/UV-enhanced or long working distance, TIRF objectives   |
| <b>Detectors</b>                                 |   |
| Type <sup>1)</sup>                               | τ-SPAD ..... SPAD (PDM Series)  |
| Spectral range                                   | 400 - 1000 nm ..... 400 - 1000 nm   |
| Dark counts (at 20°C, typ. value)                | < 100 cps ..... < 250 cps   |
| <b>TCSPC Data Acquisition</b>                    |   |
| Type   | PicoHarp 300 ..... HydraHarp 400  |
| Time resolution (bin width)                      | 4 ps ..... 1 ps   |
| Dead time  | < 95 ns ..... < 80 ns   |
| Discriminator level, zero cross adjust           | software adjustable   |
| <b>TTTR Mode</b>                                 |   |
| Output data format (Channel/Macrotime)           | 12/16 bits  |
| Macro timer resolution                           | equals synch period   |
| Sustained data throughput                        | up to 5 million counts/second ..... up to 9 million counts/second   |
| Peak data throughput (burst)                     | up to 10 million counts/second ..... up to 12 million counts/second   |
| <b>Software Features<sup>2)</sup></b>            |   |
| General concept                                  | use of versatile TTTR file format for data acquisition, data archiving in workspace, time gating for all methods, separation of up to four detector signals, scripting language for user-defined analysis procedures  |
| Point measurements                               | data conversion to: MCS trace, F(C)CS calculation and fitting, FLCS, TCSPC histogram, on/off-state histogram, burst size analysis, (PIE-)FRET histogram, photon counting histogram, lifetime histogram, antibunching plot   |
| Fluorescence Lifetime Imaging (FLIM)             | data conversion to: fluorescence intensity images, fluorescence lifetime images, time gated analysis, TCSPC histogram for region of interest, decay analysis  |
| <b>Operational and Electrical</b>                |   |
| Operating environment (recommended)              | Quad-core CPU > 3 GHz, RAM >= 4 GB  |
| Power requirements                               | 110/230 V, 50/60 Hz   |
| <b>Dimensions (w × d × h)</b>                    |   |
| Laser combining unit                             | 600 × 400 × 200 mm (without laser driver)   |
| Microscope and main optical unit                 | 1150 × 600 mm (2 detection channels)  |
| 19" electronic rack (typical)                    | 500 × 550 × 400 mm  |
| Table  | Maximum spacing between each unit is 1.5 m. It is recommended to allow 0.5 m access around all sides of the system.<br>Breadboard optionally available from PicoQuant:<br>1600 × 600 × 50 mm, M6 holes, 25 mm grid<br>4 ft × 2 ft × 2 inch, ¼ - 20 holes, 1 inch grid |


<sup>1)</sup> lasers, other detectors and cooling available upon request <sup>2)</sup> for details please see our SymPhoTime data sheet



For more details please refer to our brochure.  
Please contact us for a free copy.

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# MicroTime 200



## Time-resolved Confocal Fluorescence Microscope

- Complete system with laser combining unit, inverted microscope body and multichannel detection unit
- Multicolor excitation, laser wavelengths from 375 to 900 nm
- Multiple detector options for up to 4 truly parallel detection channels
- Picosecond temporal resolution
- Diffraction limited optical resolution of less than 0.5 µm
- XYZ-scanning piezo stage for 2D- and 3D-lifetime imaging



## Applications

- Time-resolved microscopy in biology, chemistry and material science
- Fluorescence Lifetime Imaging (FLIM)
- Single molecule imaging and spectroscopy
- Intensity and lifetime based Förster Resonance Energy Transfer (FRET) studies
- Fluorescence Correlation Spectroscopy: FCS, FCCS, FLCS, 2-focus FCS
- Semiconductor testing and analysis

# Configuration and Standard Components

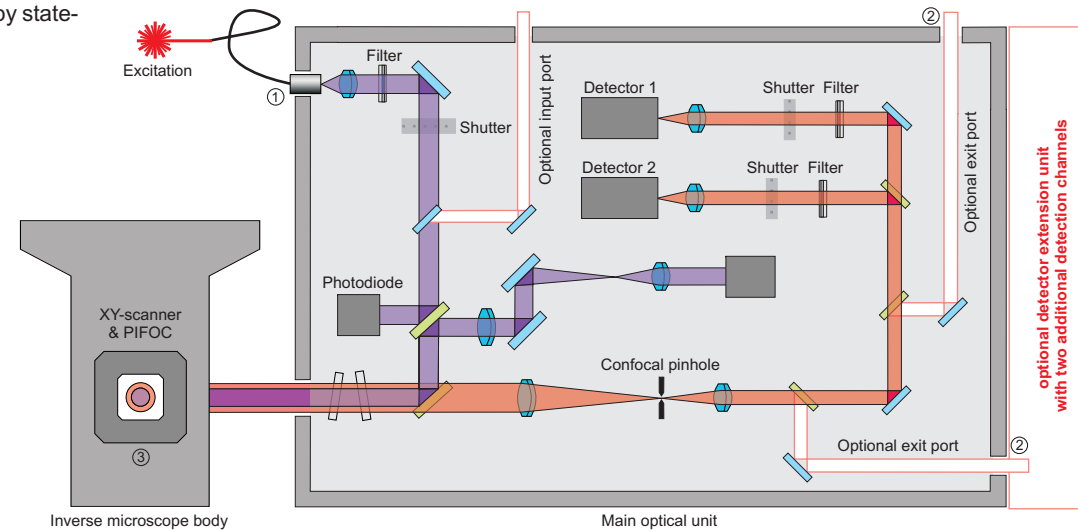
The MicroTime 200 time-resolved fluorescence microscope system is a powerful instrument capable of Fluorescence Lifetime Imaging (FLIM) with single molecule detection sensitivity. It contains the complete optics and electronics needed for recording virtually all aspects of the fluorescence dynamics of microscopic samples or femtoliter volumes. The instrument gains its exceptional sensitivity and flexibility in combination with an unprecedented ease-of-use from a unique fusion of miniaturized and highly sophisticated state-of-the-art technologies. For the first time, these technologies enable to run an instrument of comparable complexity and power to be operated in routine work, without having to spend more time on instrument maintenance than on original scientific content. The underlying key technologies are the proven picosecond diode lasers and the Time-Correlated Single Photon Counting (TCSPC) electronics developed by PicoQuant, complemented by state-of-the-art piezo-scanning technology and optics from industry leaders.

## Main Optical Unit

All optics needed to achieve confocal excitation, detection and beam/focus diagnostics are installed together with the detectors in the self-contained main optical unit. The coupling to the inverted microscope body is achieved through the infinity beam port of the IX 71 microscope body. This design allows an easy coupling of external lasers and integration of various optical elements for beam diagnostics and power monitoring in the main optical unit. Still compact, the open design offers various customer specific modifications such as exit ports for the addition of e.g. spectrographs.

## Data Analysis

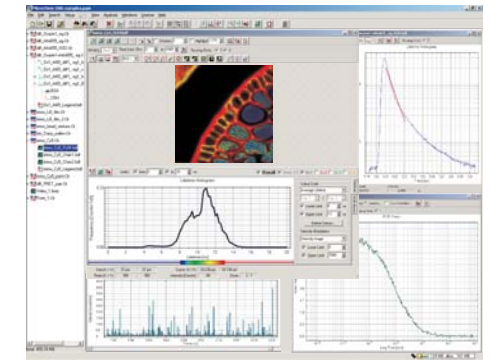
Based on this clear concept of data handling, the operating software of the MicroTime 200 (SymPhoTime) was designed with almost unlimited flexibility for integration of virtually all algorithms and methods for the analysis of fluorescence dynamics that users may require. Based on the powerful but generic TTTR data collection, users can perform an unlimited number of analysis steps without losing track of the interdependence and origin of their measurement and analysis data. This is made possible by a workspace concept, in which the TTTR file is the origin of each data analysis. Derived results can be obtained through a vast set of analysis tools, such as intensity time trace, burst analysis, lifetime histogramming, Fluorescence (Lifetime) Correlation Spectroscopy (F(L)CS) and lifetime imaging, to name a few. All derived data is maintained in the workspace, including a log file, keeping track of all measurement and analysis steps. Image data can be processed further in an easy to use image calculator or exported to standard formats. A newly developed scripting language interface allows to modify and expand the analysis routines according to the individual needs towards e.g. static anisotropy imaging, multi-parameter burst analysis or FLIM-FRET analysis.



## TCSPC Data Acquisition

For data acquisition the outstanding Time-Correlated Single Photon Counting (TCSPC) systems PicoHarp 300 or HydraHarp 400 are utilized. These highly integrated devices provide several measurement modes. One especially powerful mode is of pivotal importance for the design of the MicroTime system: in Time-Tagged Time-Resolved (TTTR) measurement mode each photon is recorded individually. The data stream is

recorded continuously allowing online display of the fluorescence lifetime image, correlation curves (auto- and cross-correlation), intensity time traces or fluorescence decays during data acquisition. Each photon record contains a picosecond timing of the photon relative to the laser pulse and a coarser nanosecond timing with respect to the start of the experiment. This combination provides the performance of vastly different measurement tasks based on one fundamental data format, without any sacrifice of information available from every detected photon. It also allows all measurement data to be handled in a standardized and yet very flexible way. Due to the independent measurement channels of the TCSPC units, it is also possible to store the absolute arrival time of each detected photon with picosecond resolution. This permits to calculate correlations from picoseconds to seconds or coincidence correlations ("antibunching").



## Sample Holder

The MicroTime 200 can be equipped with two scanner configurations: Sample or objective scanning. Using sample scanning, the sample holder is designed either to accommodate 20 x 20 mm<sup>2</sup> microscope cover slips or microscope slides. Objective scanning allows free access to the stationary sample, e.g. for live cell investigations in bulky sample compartments or applications using a cryostat.

## Detector

The MicroTime 200 can be equipped with up to four truly parallel detection channels. Each detector channel has its own dedicated filter holder and mechanical shutter. Two types of single photon sensitive detectors are available: Single Photon Avalanche Diodes (SPAD) and Photomultiplier Tubes (PMT).

## Options and Accessories

### Laser coupling via polarization maintaining singlemode fiber ①

The excitation subsystem consists of a pulsed diode laser driver, laser head(s) and optical components to attenuate and couple the laser's output into a polarization maintaining single mode optical fiber. The Laser Combining Unit (LCU) can host up to 5 laser heads. With a special multichannel laser driver, Pulsed Interleaved Excitation (PIE) can be performed.



### Exit ports for custom detection schemes ②

Up to two exit ports with optional fiber coupling can be installed to increase the versatility of the MicroTime 200. The beam from the basic confocal unit is then split and directed to the exit port(s), which utilize variable beam splitters. This way, the detection system can be easily upgraded and adapted to the user's needs. For example, spectral and polarization-resolved data acquisition (e.g. two colour, two mutually perpendicular polarization orientations) can be achieved by adding two other detection channels. Spectrographs and cooled CCD camera can be attached as well.

### 2D or 3D Fluorescence Lifetime Imaging (FLIM) ③

For 2D or additionally 3D imaging at sub-micrometer resolution, the system incorporates a piezo scanning stage and a PiFoc, driven by a high performance closed-loop digital position controller from Physik Instrumente GmbH. The scan controller, as well as all other components, including shutters and monitoring detectors are seamlessly integrated in the SymPhoTime software. A wide range scanner for cm scanning ranges is additionally available upon request.



### Free space excitation port

In addition to the fiber coupling port, the MicroTime 200 can be equipped with a free space excitation port for external lasers, e.g. Ti:Sa laser system.

### One or two detection channels

The main optical unit can host up to two detectors of choice. Each detector is secured by its own safety shutter which can be controlled manually or by the SymPhoTime software.

### Upgradeable to up to 4 detector channels

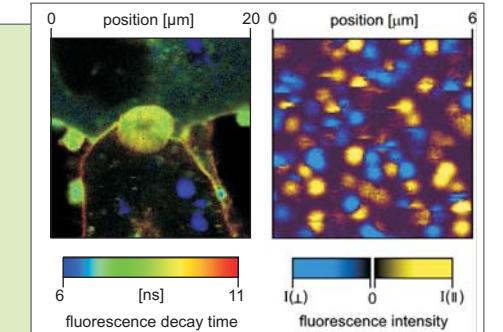
Further preferred detectors can be added through an additional detector unit coupled to the main optical unit via the additional exit ports.

### CCD camera for wide field imaging

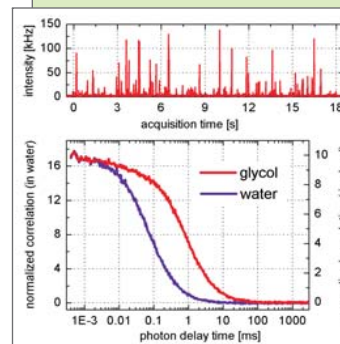
The IX 71 microscope body still provides freely accessible ports, which can be equipped with e.g. a CCD camera for wide field imaging.

## Measurement Examples

Raster-scanned images of two different samples: On the left there is a fluorescence lifetime image of a part of the membrane of a living hepatocyte cell, stained with the dye NBD (7-nitrobenz-2-oxa-1,3-diazole), whose lifetime is depending on the hydrophobicity of the environment (courtesy of Prof. Herrmann, Humboldt-University Berlin). Every pixel shows the result of an exponential fit to the fluorescence decay built from all photons contained in this pixel. The sample was excited with a picosecond diode laser at 467 nm using a 100x, 1.3 N.A. oil immersion microscope objective. The image consists of 300 x 300 pixels with an acquisition time of 2 ms/pixel. The right hand side shows the polarization-resolved fluorescence of isolated, single Cy5 molecules on top of a standard glass cover slip. The collected



fluorescence light was divided with a polarizing beam splitter cube and simultaneously detected with two SPAD detectors. The image contains all molecules which exhibit either a predominant parallel (yellow) or perpendicular (blue) polarized emission. Excitation was carried out with a picosecond diode laser at 638 nm through an 100x, 1.3 N.A. oil immersion objective. The data acquisition time was 5 ms for each of the 200 x 200 pixels.



On the left: Time-resolved fluorescence measurements of freely diffusing ATTO 655 molecules in different solvents (about 100 pmol/l): The partial view of the MCS trace in the upper half depicts the typical fluorescence bursts of single molecules which diffuse through the confocally defined investigation volume. The lower half shows the related FCS curve of ATTO 655 molecules in two solvents with a different viscosity, which affects the mobility of the fluorophores. The fluorescence intensity auto-correlation was directly calculated from the TTTR raw data. Atto 655 was excited with a picosecond diode laser at 640 nm via an 100x, 1.3 N.A. oil immersion objective.

On the right: Intensity and lifetime fluctuations of a single Cy5 molecule adsorbed on a glass coverslip surface. The MCS trace in the upper half depicts spontaneous decreases and total interruptions of the fluorescence emission (photons binned in 100 ms). The corresponding fluctuations of the fluorescence lifetime in the lower half was extracted from successive fractions (1.25 s each) of the emitted photons, which are collected in a TCSPC histogram and fitted with an exponential decay. Two representative histograms are shown on the right side and indicate that the same molecule can exhibit a fast (0.6 ns) and a slow (1.65 ns) fluorescence decay at different times of the acquisition.

