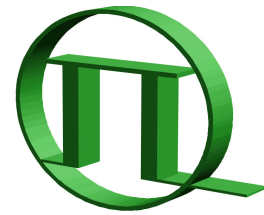
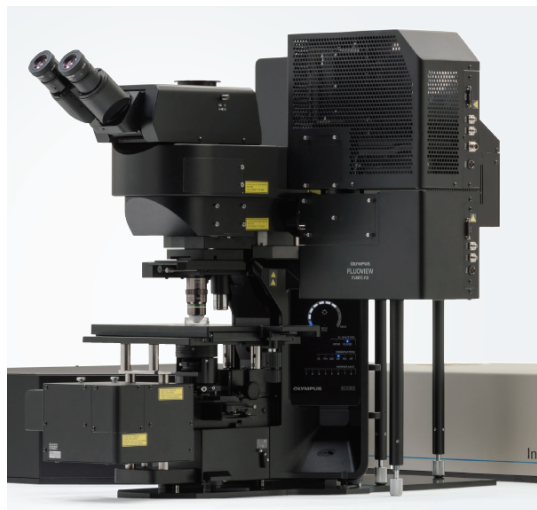


LSM Upgrade Kit

Compact Lifetime and FCS
Upgrade Kit for
Olympus FVMPE-RS Laser
Scanning Microscopes



PICOQUANT GmbH
Unternehmen für optoelektronische
Forschung und Entwicklung



User's Hardware Manual and Technical Data

Version 1.2

Table of Contents

1. Introduction.....	3
2. Operation.....	5
2.1. Safety Instructions.....	5
2.2. Starting Hardware and Software Equipment.....	5
2.3. FLIM Data Acquisition.....	6
2.3.1. Starting Point.....	6
2.3.2. Changing from internal FVMPE-RS PMTs to external TCSPC detectors.....	6
2.3.3. Set suitable scan parameters.....	7
2.3.4. Optimize the detected photon count rate.....	8
2.3.5. Start FLIM data acquisition.....	10
2.3.6. Resulting raw data file and documentation.....	11
2.3.7. Measure an instrument response function (IRF).....	13
2.3.8. Adjusting the threshold level for laser pulse detection.....	14
2.3.9. Remarks.....	15
3. Troubleshooting.....	16
3.1. Hardware configuration gets lost or software needs to be installed again.....	16
3.2. Instrument is losing sensitivity.....	16
3.3. No image is displayed during measurement.....	16
4. Recommended literature.....	18
5. Abbreviations.....	19
6. Support and Warranty.....	20

1. Introduction

This manual describes the basic procedures to acquire NDD FLIM - images using the LSM Upgrade Kit in combination with a LSM.

More detailed explanation about the technical details of the setup as well as software analysis can be found in the additional documentation:

- Detection Unit - Manual:** This document contains information about the detector and how to change filters. The PMA Hybrid detectors are equipped with an automatic overload shutter to prevent too high detection count rates which could damage the detector. Overload is signalled with a beep sound. If an overload was detected, the shutter in front of the detector closes. After some seconds, the shutter opens automatically again. Please make sure that the cause for the over-illumination is eliminated as fast as possible.

Excessive light will reduce the sensitivity of the PMA Hybrid detector over time.



Fig. 1.1: Detection Unit for LSM NDD FLIM Upgrades: The Single (left) or Multi channel PMA detection unit (right).

- PicoHarp 300 - Manual:** Here you find all information about the Time Correlated Single Photon Counting (TCSPC) device. This manual also includes an introduction about single photon counting.

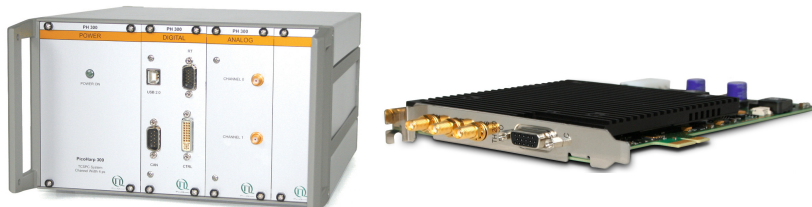


Fig. 1.2: PicoHarp 300 and TimeHarp 260

- Router (PHR800):** The router is needed in systems equipped with a PicoHarp 300 and more than one detector.



Fig. 1.3: PHR 800 router. The configuration for a Multi-PMT setup is displayed.

- **Software Manual (SymPhoTime 64 - Manual):** Here you find all information about the software installation. The SymPhoTime 64 software contains a detailed, context sensitive online help function.
- **Pre-Installation Requirements:** This is an important document that already should have been considered before installation. If you want to change the configuration of your LSM, you'll find detailed information, if the desired change can be realized and whether additional parts are necessary. In case of doubt, contact a PicoQuant representative.
- **System Specifications:** The System Specifications contain specific information about your individual LSM FLIM Upgrade, including a description of the included parts, filter handling and a cable plan, which helps to restore the configuration after disassembly.

2. Operation

2.1. Safety Instructions



LASER Warning!

Lasers for two photon excitation deliver infra-red emission which is in most cases not visible for the eye. These lasers emit light of laser class 4 / IV. Laser class 3B / IIIb and 4 / IV requires to wear special laser safety glasses. **Do not remove any items of the NDD adapter before switching the microscope and laser completely off.** The installation room of the LSM Upgrade kit must be labeled as laser area. For class 4 / IV lasers, a laser safety officer has to be announced to meet the laser safety regulations.

Lasers can be hazardous and have unique safety considerations. Permanent eye injury and blindness is possible if lasers are used incorrectly. Pay close attention to each safety REMARK and WARNING statement in the user manual. Read all instructions carefully BEFORE operating this device.

Never connect or disconnect any cable while the data acquisition and control electronics are ON. Charged signal cables can destroy the devices!

Protect the photon detectors (PMA Hybrid) as much as possible, particularly from excessive light intensities, e.g. room light, the microscope illumination lamp, unattenuated backscattered excitation, etc.

2.2. Starting Hardware and Software Equipment

To **start** the LSM itself, its detectors and lasers please refer to your FVMPE-RS manual. In the following we **assume a running Laser Scanning Microscope** with the sample already in place and in focus. The target region of interest for FLIM is already identified. The LSM upgrade kit can be started completely separate from the FVMPE-RS itself.

The LSM FLIM Upgrade hard- and software should be started in the following order:

1. Start the external **PicoHarp 300** TCSPC device, and if applicable also the **PHR 800** router
2. Start the **PC**.
3. Start the **SymPhoTime 64 software**.
4. **Open** an already existing **workspace** or create a new one.
5. Place correct **fluorescence filters** in the corresponding filter holder position of the external PicoQuant detector unit and the confocal microscope.
6. **Make sure that no light reaching the detector(s) (room light off? Shielding closed?) and power** them on. Prior to data acquisition, the detector should be operated for 5 minutes to stabilize.
7. If you want to work with user specific settings, load your **User Configuration**.

The FLIM Upgrade is now ready to use.

2.3. FLIM Data Acquisition

Scope:

Acquire a FLIM image using the Olympus FVMPE-RS scan head with appropriate scanning and dichroic settings and external PicoQuant TCSPC NDD detectors.

2.3.1. Starting Point

1. **NDD detection is very background light sensitive.** In general you need to cover the sample stage very well and/or keep the room dark.
2. **Make sure that in the NDD detection unit the right filters are inserted.**
3. **FVMPE-RS:** Choose and pre-scan with the FVMPE-RS (its laser and PMTs) the correct region where you want to perform a FLIM measurement. For the ad hoc inspection make sure that you are using a suitable excitation wavelength, a corresponding excitation dichroic mirror, a useful microscope objective and suitable detection filtering for the internal FVMPE-RS PMTs. Finally stop the scanning process.
4. All components (laser, detectors, software) of the PicoQuant upgrade kit are already running. For details about powering on see chapter 2.2.
5. **SymPhoTime 64 software:** The desired **User Profile** of the SymPhoTime 64 has been loaded. The SymPhoTime 64 allows configuring the view of the acquisition window. Thus the user can preconfigure the online analysis he wants to see during acquisition, e.g. images (also of the different spectral channels if more than one detector is present), a time trace, a decay (TCSPC) histogram. Several online analyses can be displayed simultaneously.
6. **SymPhoTime 64 software:** A workspace has been loaded or created. The workspace defines where the data acquired should be stored to.

2.3.2. Changing from internal FVMPE-RS PMTs to external TCSPC detectors

1. **FVMPE-RS:** Set the PQ NDD slider above the objective (upright) or below (inverse microscope) to the FLIM position (Fig. 2.1 (upright microscope) and Fig. 2.2 (inverse microscope)). This means sliding the slider in direction of the microscope objective. **Please read also the corresponding NDD manual.**

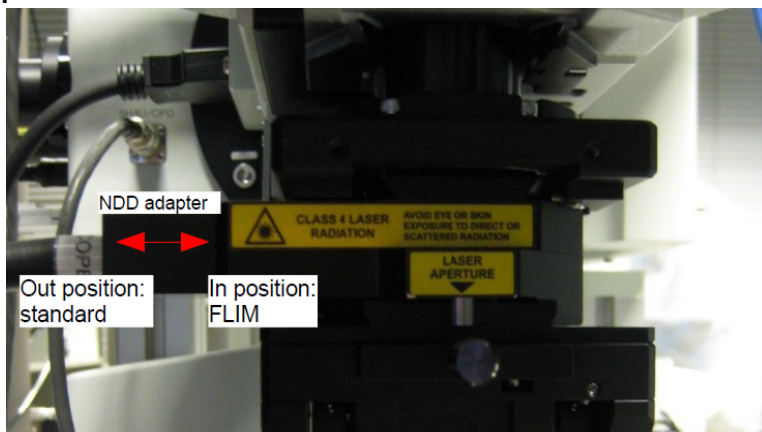


Fig. 2.1: Upright microscope: NDD adapter mounted at the pigtail holder of the microscope stand. The inset can be moved in (to the left) for FLIM imaging and out (to the right) for standard imaging (red arrow).

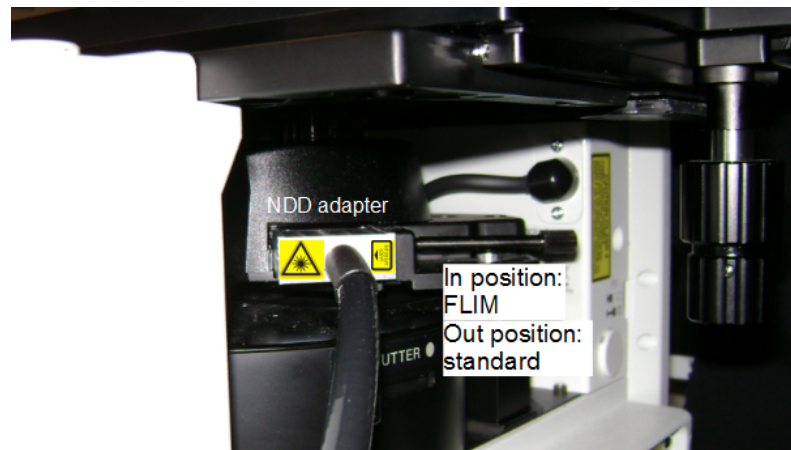


Fig. 2.2: Inverse microscope: NDD adapter mounted at the DIC slider slot of the microscope stand. The NDD adapter can be moved in for FLIM imaging and out for standard imaging.

2. **PicoQuant Detection Unit:** Make sure you have **suitable fluorescence detection filters** (bandpass / long-pass) in the corresponding filter holders of the detection unit. When using a dual channel detection unit, make sure that the correct beam splitter / filter combination is chosen (see your detection unit manual for details).

2.3.3. Set suitable scan parameters

1. In the **FVMPE-RS - software**, set the correct scan pattern:
 - Use a **mono-directional scan**. Using less pixels (e.g. 256x256) pixels is recommended, because in this way, more photons can be collected in each pixel. This results in better fits of the lifetime data. However, you can also “bin” the pixels of the image in the analysis section later in order to calculate and analyse an image with less pixels. This will result in no loss of the acquired fluorescence. When using image sizes larger than 512x512 pixels, the online preview in the SymPhoTime 64 software might become slow. In this case, switch off the online previews in the SymPhoTime 64 software. See Fig. 2.4 for how to set the pixel size and scanning mode in the FluoView software.
 - **Average and Sequential Scan:** can only be used in conjunction with the „Frame“ option. „Line“ option will scan every line several times which cannot be correctly interpreted by the SymPhoTime software (see Fig. 2.3).

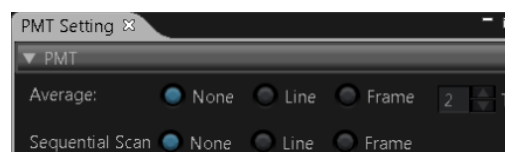


Fig. 2.3: Use **Average** or **Sequential Scan** only with the “Frame” selection when capturing FLIM images.

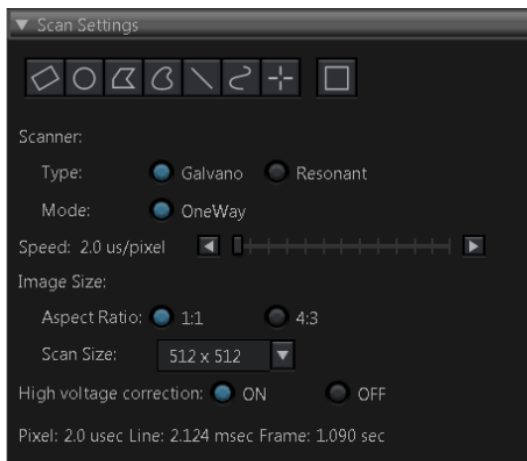


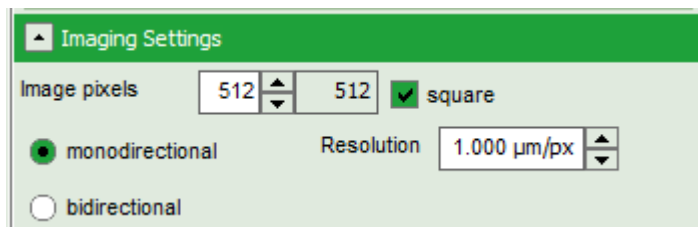
Fig. 2.4: LSM scanning settings in the FVMPE-RS software. Recommended settings are:

- Type: Galvano
- Mode: OneWay
- Speed: around 20µs / pixel
- Size: 512x512

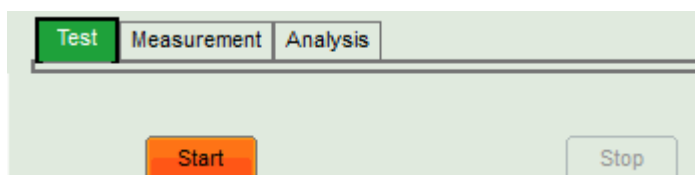
2.3.4. Optimize the detected photon count rate

1. **SymPhoTime 64 software:**

To set up the right parameters for data acquisition, go to the main “Test” - tab of the SymPhoTime 64 software. In the “Imaging” drop-down-menu, enter the selected image size and the selected scanning pattern, bi- or mono-directional scanning, and the image dimensions (in µm/pixel).



2. **SymPhoTime 64 software:** Press the Start-button.



2. **FVMPE-RS software:** Start the scanning process.

3. **SymPhoTime 64 software:**

The measurement preview is displayed, together with a time trace (Fig. 2.5). Make sure that the Peak Count Rate (marked in red) is below 4000 kcps by adjusting the laser intensity. The Peak Count Rate is determined from the maximum in the time trace. The “Time trace binning” should be set to 1 ms. Here you can watch the changes of the count rate over time. A red line additionally indicates a threshold of 10% of the repetition rate. The intensity should always remain below this value. If only very tiny lifetime-

changes are expected, stay even well below this number. There is also a “Max count rate” indicator (see Fig. 2.5). This value should stay below 4000 kcps (kilocounts per second) for accurate lifetime results also in the brightest pixels. By following this rule the so called “pile up” effect is reduced. Pile up can lead to a decrease of the determined lifetime. By staying below 4000 kcps the lifetime reduction will be less than 5%.

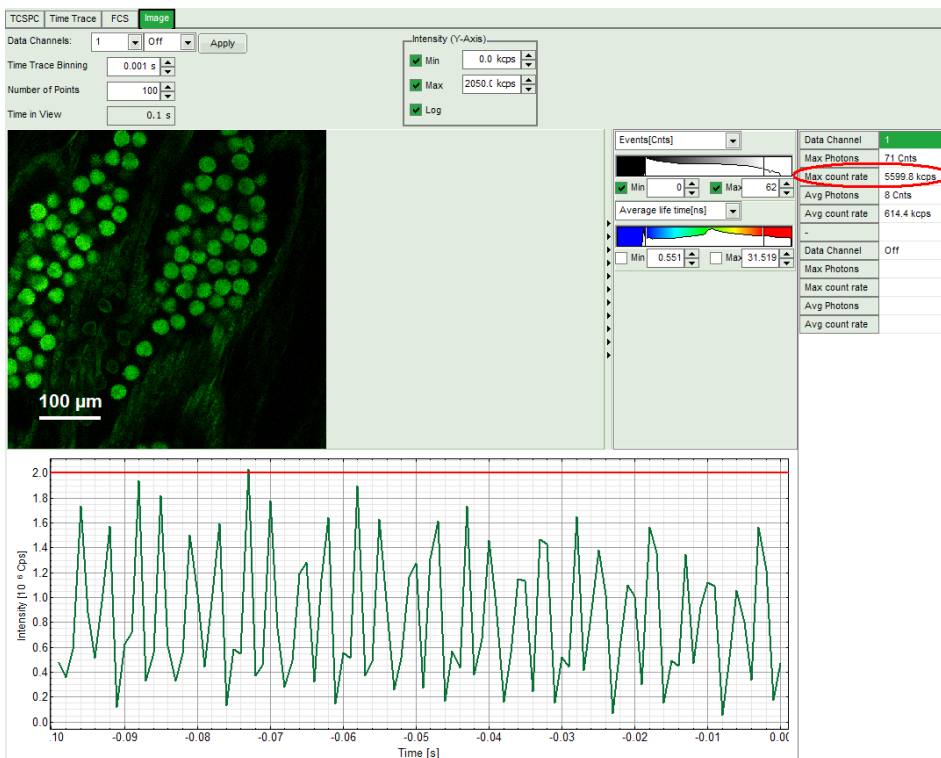
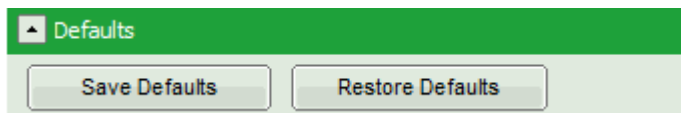


Fig. 2.5: Online monitoring of the photon count rate in the SymPhoTime 64 preview window. On the left, a FLIM image is displayed. The intensity time trace is shown below. An intensity of 10% of the repetition frequency is indicated by the red line in the graph. The peak count rate is also indicated on the right (red circle).

- **SymPhoTime 64 software:** The Image **preview settings** can be pre-set, when no measurement is running. Defaults can be saved using the “Save Defaults” button in the Default drop down menu.



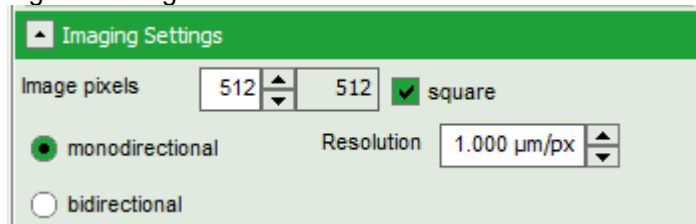
To finally save the settings in a User profile, select “Settings” => “Save as User Profile” in the main menu bar. This way, all settings can be saved in a user specific profile and loaded with the next start of the software.

- **Adjust the photon count rate** by changing the intensity of the pulsed laser
- Using the “Image” tab of the “Test” preview window you will see a **preview** of the **FLIM image**. The lifetime displayed in the image by the color code is calculated by the mean arrival time of the fluorescence photons after the last laser pulse. The image is updated roughly each second. It allows the optimization of the field of view and focus setting.

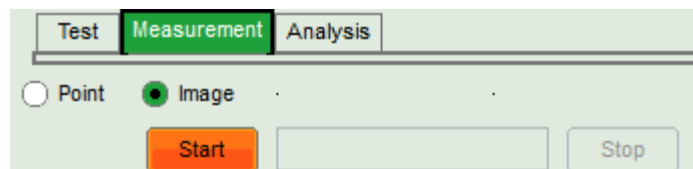
2.3.5. Start FLIM data acquisition

1. SymPhoTime 64 software:

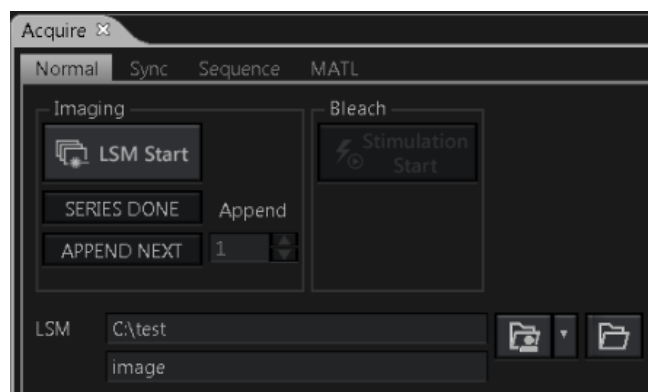
To set up the right parameters for data acquisition, go to the main “Measurement” - tab of the SymPhoTime 64 software. In the “Imaging” drop-down-menu, enter the selected image size and the selected scanning pattern, bi- or mono-directional scanning and the image resolution (in $\mu\text{m}/\text{pixel}$). Note that the entered dimensions become a part of the raw data file and therefore cannot be changed after image recording.



2. SymPhoTime 64 software: Make sure that the “Image” Measurement mode is selected and press the “Start” button.



3. FVMPE-RS software: Start the scanning process.



4. SymPhoTime 64 software: During LSM data recording the chosen online analyses are displayed, e.g. one or – in case of several detectors - several FLIM -images. When scanning several frames, all frames are integrated over time. This display allows you to evaluate the quality of the FLIM data captured. Please note that the FLIM preview image is just integrated for the display, the stored raw data still allows you to analyze the data separately frame by frame (Multi Frame FLIM Analysis).

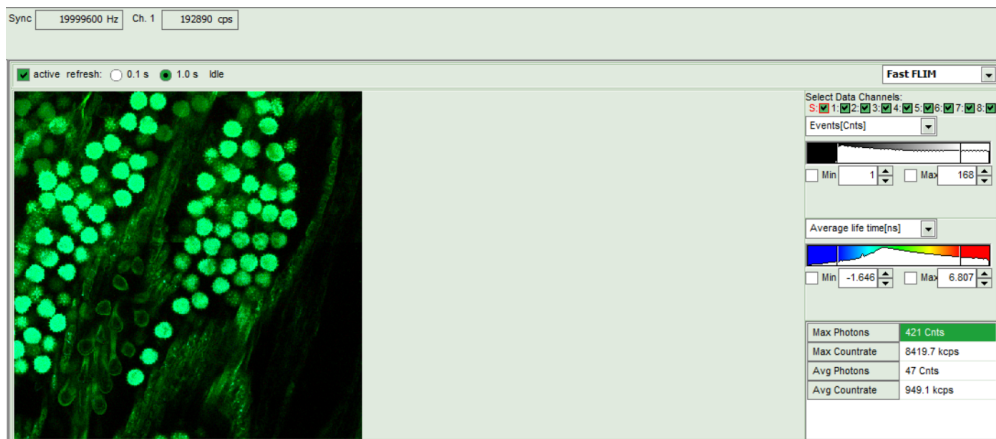
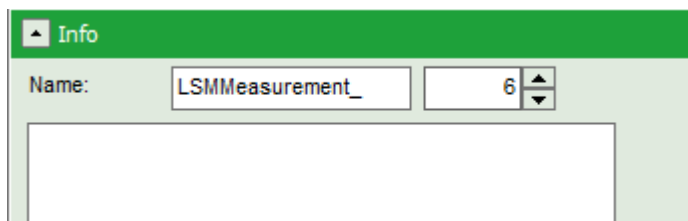


Fig. 2.6: SymPhoTime Measurement Preview being displayed during LSM data acquisition. In this preview, the FLIM image is displayed on the upper left. The display parameters are shown on the right.

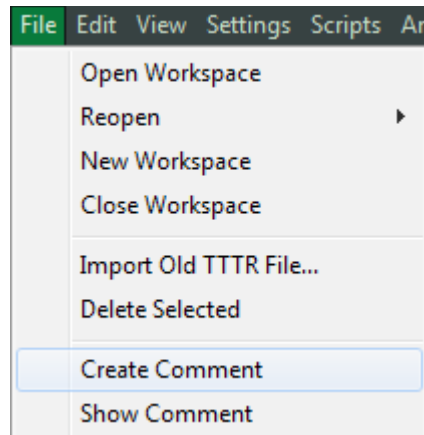
5. **FVMPE-RS software:** The **LSM scanning process stops** either after having scanned the pre - set number of frames or by manually interrupting the scan.
6. **SymPhoTime 64 software:** The data recording stops by just manually interrupting the data acquisition (press “stop”).
7. **SymPhoTime 64 software:** The recorded raw - data - file (*.ptu) is displayed in the workspace menu on the left and can now be opened for analysis.

2.3.6. Resulting raw data file and documentation

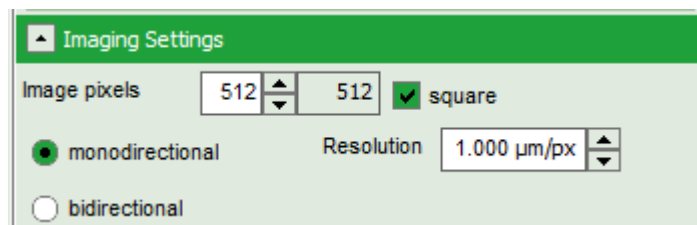
- **SymPhoTime 64 software:** The measurement result is a .ptu-raw data file, containing for every detected photon the maximum available information (temporal, spatial, detection channel information), together with the online analysis files “.pqres”. If e.g. a online Fast FLIM image is to be processed, highlight the raw data (.ptu) file and open this file with e.g. the FLIM analysis option in the “Analysis” tab. If you double-click on the “OnlineFastFLIM_... .pqres-file”, the FLIM analysis is directly opened.
- Every raw date file is stored together with the actual settings. It contains all actual measurement parameters known by the SymPhoTime software. To access, double click on the raw (“.ptu”)-file. The raw data file contains all actual measurement parameters known by the SymPhoTime software; individual information (measurement type, sample, etc.) can be entered **before** the measurement via the “Info” drop down menu.



- **Note** that the raw data file **cannot be changed after** the measurement; if you need to add information after the measurement, you can add a comment file via the main menu bar.



- Also the **image dimensions** that are used for internal distance calculation have to be entered as “Resolution” in the “Imaging Settings” of the **SymPhoTime 64 - software** before the start of the measurement .



To find the pixel size of the image in the **FVMPE-RS software**, look at the button of the image display (Fig. 2.5). If you want to change the x,y-axis dimensions from pixels to microns in the SymPhoTime 64 software enter the **pixel size (in microns) in the window of the SymPhoTime 64** software before the start of the measurement. Unfortunately, it can not be changed after image acquisition.

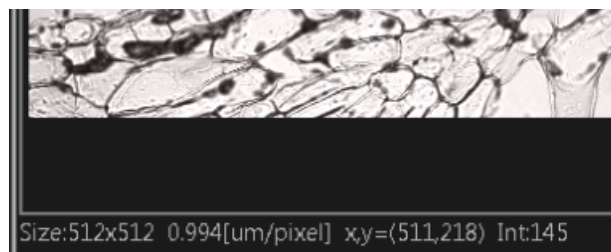


Fig. 2.7: FVMPE-RS scan information giving the pixel size in microns per pixel.

SymPhoTime 64 software: Specific recorded frames can be selected for analysis in the FLIM analysis. Highlight the raw data file, go to the main “Analysis” tab, and select the “FLIM”-analysis from the “Imaging” drop down menu. The FLIM analysis window pops up. The frames chosen for analysis can be entered in the field “Frame” (from “First Frame” up to “Last Frame”).

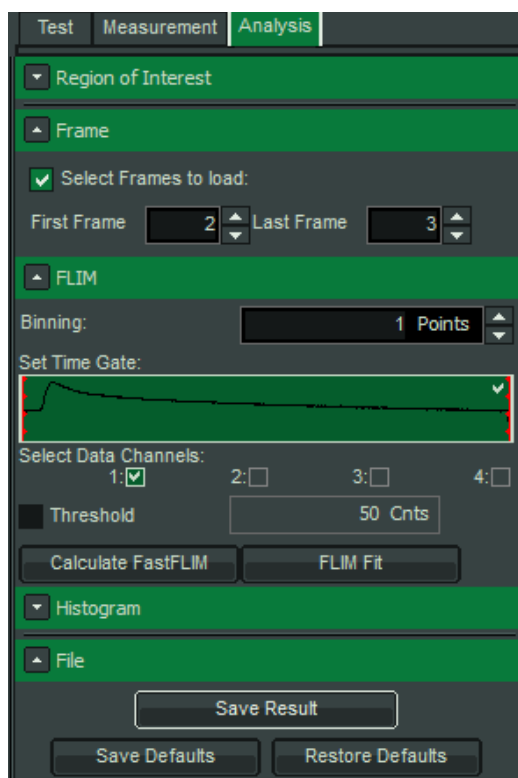


Fig. 2.8: Changing the selection of frame numbers for analysis in the SymPhoTime 64 Software.

2.3.7. Measure an instrument response function (IRF)

For accurate measurements of lifetimes close to the timing resolution of the instrument, the instrument response function (IRF) should be taken into account. The width of the IRF displays the timing resolution of the instrument. An IRF can be obtained following these steps:

1. Place a sample with an **negligible short lifetime** (e.g. Erythrosin B or Fluorescein, either dissolved in a saturated KI-solution. For fluorescein, a slight basic pH value is necessary to dissolve a sufficient amount of dye) in the laser focus on the microscope stage. The dye chosen should have a similar emission range as the sample that should be measured afterwards. Potassium iodide is a strong quencher and reduces the fluorescence lifetime to a few picoseconds. See e.g. [Applied Spectroscopy, Vol.63, p.0363-0368 \(2009\)](#). Also second harmonic generation may be used to measure the IRF.
1. Place a suited **emission filter** in the filter holder in front of the PQ detector, ideally the same as used later for the measurements.
2. Set the **FVMPE-RS** settings in the same way as for a **FLIM measurement**. Start the scanning process.
3. **SymPhoTime 64 software:** You can now check the decay behavior of your sample using the TCSPC preview of the "Test" tab. Activate the TCSPC Histogram-tab, press the "Start" button and monitor the fluorescence decay as shown in Fig. 2.9. In the "TCSPC settings" drop down menu, also the time width per channel can be set. Adjust the count rate to approximately 50 kCounts/s.

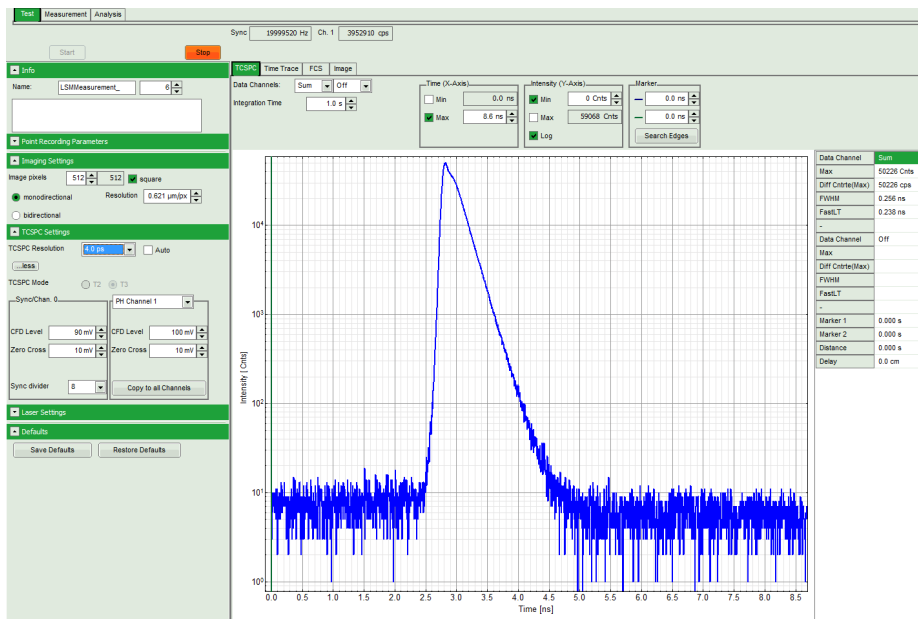
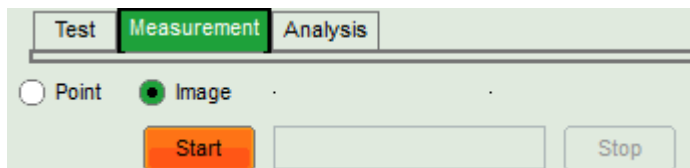


Fig. 2.9: TCSPC-preview window. The time width per channel can be set with the “TCSPC Settings” drop-down menu. The count rate is displayed over the preview graphs for each channel separately.

4. **SymPhoTime 64 software:** Stop the test measurement by pressing “Stop”.
5. **SymPhoTime 64 software:** Switch to the “Measurement” tab and start the data acquisition pressing “start”.



6. When the decay histogram in the TCSPC preview has reached 10000 counts in the peak channel, **stop** the image acquisition in the SymPhoTime and Olympus FluoView software. You can open the TCSPC histogram of the captured image, using the “TCSPC histogram” - analysis of the SymPhoTime 64 software. It contains the IRF.
7. Replace the IRF solution with your sample again.

2.3.8. Adjusting the threshold level for laser pulse detection

In general, for the detection of the laser pulse sequence of a femtosecond laser, the internal trigger diode of the laser is needed. Normally the system is already set to detect the trigger signal (SYNC) by PicoQuant. If the laser does not have an internal photodiode for pulse sequence detection, an external photodiode (like the TDA from PQ) is used. The photodiodes are not always sensitive to all wavelengths, especially beyond 1000 nm. The trigger settings of the laser pulse can be adapted to the real signal in the SymPhoTime 64 software. This CFD level of the SYNC input can in the TCSPC drop down menu.

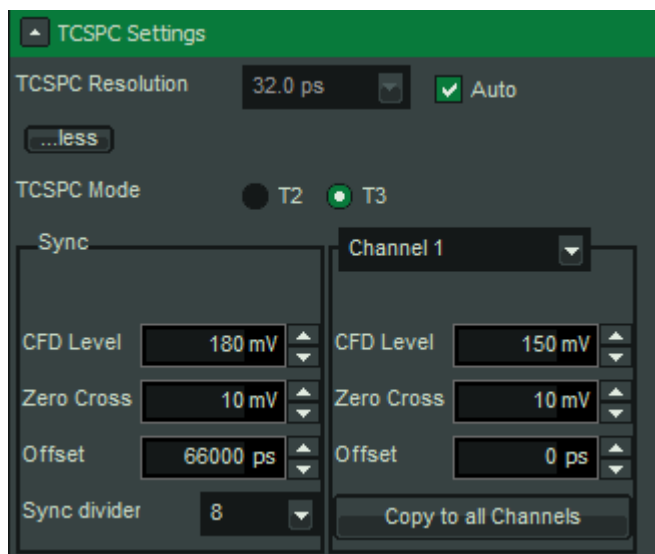


Fig. 2.10: Settings of the input trigger levels of the PicoHarp. On the left side, the laser synchronization threshold settings are displayed (CFD Level), on the right side, the detector settings. Zero Cross can always stay at 10 mV.

2.3.9. Remarks

- **Bidirectional scanning:** With bidirectional scanning the images are often distorted, therefore mono-directional scanning is preferred.
- The SymPhoTime software also allows to perform post-acquisition **pixel binning** for optimization of the best compromise between lifetime accuracy and image resolution.

3. Troubleshooting

In general, make sure that all components of the Olympus system as well as of the FLIM upgrade kit are switched on.

3.1. Hardware configuration gets lost or software needs to be installed again

The actual **hardware configuration** of your LSM FLIM Upgrade (LSM trigger signal configuration, name of the TCSPC device and its settings, number of detection channels, predefined view settings....) is saved in a **settings.pfs-file**. Restoring the configuration from the .pfs-file is also useful, if the software has to be installed again.

- Use “restore factory defaults” in the “settings” main menu bar. Apart from the designed folder location of the SymPhoTime, the original settings of the LSM-FLIM Upgrade are stored on the external USB memory storage delivered with the instrument.

In case the **hardware equipment is changed**, the *.pfs file needs to be changed in order to adapt the software to the respective hardware changes. Please contact PicoQuant in this case. As the system is delivered already configured, it is not recommended to change settings without PicoQuant's supervision.

For storing individual user settings (e.g. incl. previews), the software can be started in the user mode and saved via “Settings” / ”Save User Settings as”. This creates a *.pus – file, which contains the user specific settings. When changing from one user setting to another, the software needs to restart.

3.2. Instrument is loosing sensitivity

Decreasing Detection Sensitivity

- The PMA Hybrid detector can loose sensitivity with time, especially when exposed to high light conditions (e.g. room light) over longer time.

3.3. No image is displayed during measurement

- Is the pulse repetition rate correct? The Pulse frequency is displayed in a control window over the preview windows. It must display around 80000000 Hz for most systems.

When starting a TCSPC measurement in the test mode, dark counts should be displayed (see Fig. 2.9). A peak must not necessarily appear, but at least background noise should be displayed. If the repetition rate is 0, check whether the detector is switched on and the filter(s) inserted.

- Check, whether **correct emission filters** are correctly placed. If you have a 2 - Detector unit, make sure that the beam splitter is set to the correct position.
- When all detectors are on and background counts are displayed in the oscillator window, place a **fluorescent sample** on the Olympus microscope, set all settings in the Olympus software for FLIM and start scanning, with the “Time Trace” measurement in the “Test”-tab window running. An **increase in the count rate** should be observed, especially when the laser intensity is increased during the scan. If the count rate does not increase, make sure that the **shutters** in front of the detectors are not closed (the filter should sit in the filter holder correctly). Shutters should click softly when opening and closing.

- If the shutter is not the problem, take a FLIM image as described in the “acquire a FLIM image” section. Although no counts are displayed, a .ptu file is generated. **Calculate a time trace** from your FLIM - *.ptu - file by choosing the “Intensity Time Trace” analysis in the time-trace analysis drop-down menu (only available if you have a full software licence including point analysis). The displayed time trace should contain the photon counts. Select “marker 1 - 3” to show **line start, line stop and frame markers** that are passed from the instrument. You may have to select a suited display range in order to display the marker signals. If no marker signals are present, check cable connection between the Olympus FluoView FVMPE-RS and the LSM FLIM Upgrade. Also try to start the FluoView FVMPE-RS and the PicoQuant system new. If no marker signals are displayed in spite of a present connection, contact PicoQuant. If you don't have the Point Measurement Mode included in your license, check cable connections and send the file to PicoQuant.

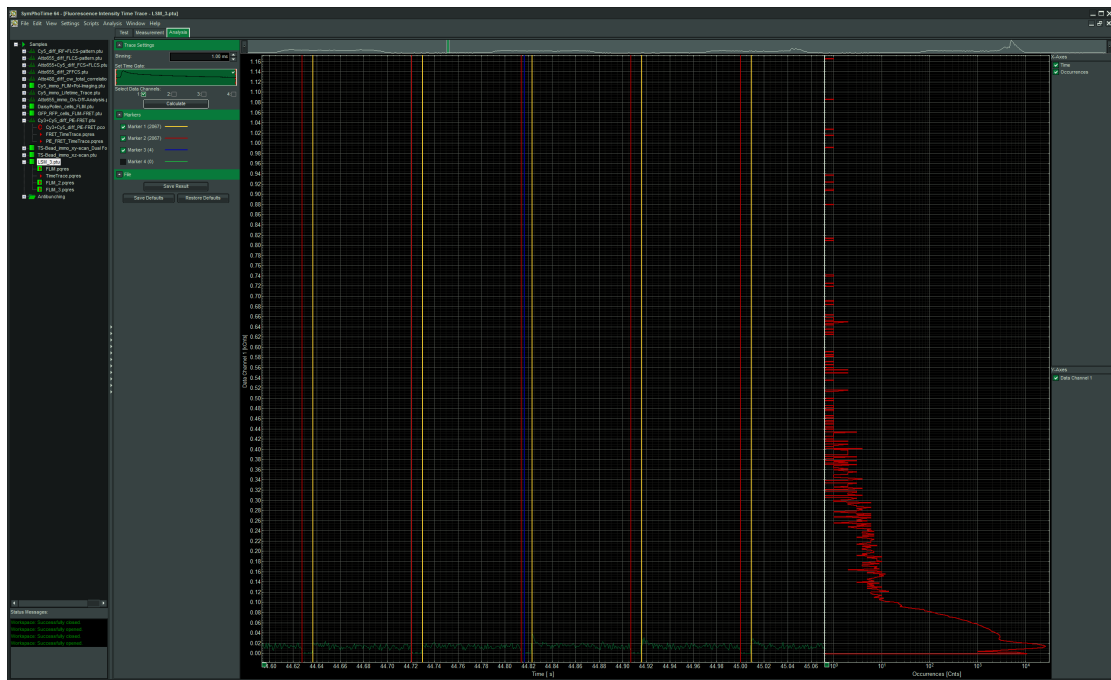


Fig. 3.1: The Time Trace analysis, where the line and frame markers can be displayed.

4. Recommended literature

Publications related to the LSM FLIM upgrade hardware, software, and underlying key technologies:

Wahl M., Koberling F., Patting M., Rahn H., Erdmann R.: *Time-resolved confocal fluorescence imaging and spectroscopy system with single molecule sensitivity and sub-micrometer resolution*. Current Pharmaceutical Biotechnology, Vol.05, p.299-308 (2004)

Koberling F., Wahl M., Patting M., Rahn H.-J., Kapusta P., Erdmann R.: *Two-channel fluorescence lifetime microscope with two colour laser excitation, single-molecule sensitivity, and submicrometer resolution*. Proceedings of SPIE, Vol.5143, p.181-192 (2003)

Ortmann U., Dertinger T., Wahl M., Rahn H., Patting M., Erdmann R.: *Compact TCSPC upgrade package for laser scanning microscopes based on 375 to 470 nm picosecond diode lasers* Proceedings of SPIE, Vol.5325, p.179 (2004)

Benda A., Hof. M., Wahl M., Patting M., Erdmann R., Kapusta P.: *TCSPC upgrade of a confocal FCS microscope*. Review of Scientific Instruments, Vol.76, 033106 (2005)

Further literature can be found in the help menu of the SymPhoTime software.

The following application and technical notes are available from PicoQuant upon request:

Buschmann V., Bleckmann A., Bülter A., Krämer B., Nikolaus J., Schwarzer R., Korte T., Ziolkowska J., Herrmann A., Koberling F.: *Polarization Extention Unit for LSM Upgrade Kits*

Buschmann V., Krämer B., König M., Koberling F., Orthaus S., Bülter A., Erdmann R.: *Compact FLIM NDD Upgrade Kit for Olympus FluoView FVMPE-RSMPE*

Koberling F., Schuler B.: *FRET analysis of freely diffusing molecules using the MicroTime 200*

Krämer B., Buschmann V., Koberling F.: *Region of Interest (ROI) Scanning and Bleaching using Pulsed Lasers with the FluoView FVMPE-RS*

Krämer B., Buschmann V., Koberling F.: *Coupling of pulsed laser sources into the Olympus FluoView FV300 / FVMPE-RS via the IR port using a polarization splitter*

Krämer B., Koberling F.: *Lifetime based hydrophobicity analysis of hepatocytes using the MicroTime 200*

Krämer B., Koberling F., Tannert A., Korte T., Hermann A.: *Lifetime based analysis of lipid organization in hepatocytes using the MicroTime 200*

Orthaus S., Buschmann V., Bülter A., Fore S., König M., Erdmann R.: *Quantitative in vivo imaging of molecular distances using FLIM-FRET*

Wahl M.: *Time-correlated single photon counting in fluorescence lifetime analysis*

Wahl M.: *Time tagged time resolved fluorescence data collection*

Numerous measurement examples are published on the PicoQuant website. Please visit the LSM FLIM Upgrade section of <http://www.picoquant.com/>

5. Abbreviations

BNC	British Naval Connector or Bayonet Nut Connector or Bayonet Neill Concelman
CCD	Charge-Coupled Device
CFD	Constant Fraction Discriminator
cps	Counts per Second
cw	Continuous wave (not pulsed)
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
LCU	Laser Combining Unit
LED	Light Emitting Diode
LSM	Laser Scanning Microscope
MCS	Multichannel Scaling
OD	Optical Density
PC	Personal Computer
PCI	Peripheral Component Interface
PIE	Pulsed Interleaved Excitation
PMT	Photomultiplier Tube
RGB	Red-Green-Blue (colour scheme)
ROI	Region of Interest
SMA	SubMiniature version A (connector type)
SMD	Single Molecule Detection
SPAD	Single Photon Avalanche Diode
SYNC	Synchronization (signal)
TCSPC	Time-Correlated Single Photon Counting
TTL	Transistor-Transistor Logic
TTTR	Time-Tagged Time-Resolved

6. Support and Warranty

If you observe any errors or bugs, please try to find a reproducible error situation. E-mail a detailed description of the problem and relevant circumstances to **info@picoquant.com**. In case of software problems, attach the log file of the measurement. The log - file to any measurement can be generated under *Help -> About → Get Support* in the SymPhoTime 64 Software. Store the generated text as a text file and mail it to PicoQuant. Your feedback will help us to improve the product and documentation.


Questions about the software can also be pasted to the SymPhoTime user forum (see <http://forum.picoquant.com/>).

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

Of course, we also appreciate good news: If you have obtained exciting results with the LSM FLIM Upgrade or published scientific papers, we would also like to know! Please send us 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 http://www.picoquant.com/_scientific.htm. 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.

Thank you very much in advance for your kind cooperation!

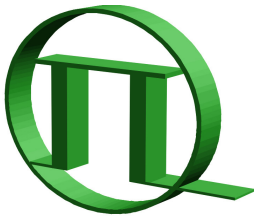
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PICOQUANT
GmbH
 Unternehmen für optoelektronische
 Forschung und Entwicklung

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>