

## **HowTo's and Tutorials**

### Instrumentation

#### **Fluorometer**

- EasyTau
- Interfacing a time-resolved spectrometer (FluoTime 300) with a microscope (MicroTime 100)
- Measuring the Quantum Yield with the Integrating Sphere Assembly for the FluoTime 300

## **Microscopy**

- Configuring SymPhoTime64
- FLIM Measurement Using a Nikon A1 with a FLIM and FCS Upgrade
- FLIM Measurement Using a Zeiss LSM710/LSM780/LSM880 with a FLIM and FCS Upgrade
- FLIM Measurements Using an Olympus FV3000 with a PicoQuant FLIM Upgrade
- FLIM-FRET Measurement Using an Olympus FV1200 with a FLIM and FCS Upgrade
- How to Avoid the Pile-up Effect in FLIM Measurements
- How to Check the Overlap of Different Color Confocal Volumes
- How to exchange the main dichroic of the MicroTime 200
- How to Measure the Instrument Response Function (IRF)
- How to Perform Antibunching Measurements
- · How-to select the correct pinhole size
- Image on the beam diagnostics camera during focusing
- MT200 Daily Alignment
- MT200 FCS Measurement
- MT200 Fundamental Alignment
- Performing a FCS measurement with an Olympus FV1200 Upgrade Kit
- Recording a Fluorescence Lifetime Image (FLIM) Stack with a LSM Upgrade Kit on a Nikon A1

# SymPhoTime64 / Analysis

The SymPhoTime tutorials rely on the sample workspace downloadable here (1.5GB).

#### General

- Antibunching Analysis
- Determination of the Focal Width
- How to create a time-gated Image in SymPhoTime
- How to Work with Instrument Response Functions (IRFs) Measured with a Microscope

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- Intensity Time Trace Analysis
- Registering New Scripts in the SymPhoTime 64
- Static Anisotropy Analysis for Images
- SymPhoTime 64 Analysis Tips and Tricks
- SymPhoTime Lifetime Fitting

#### **FLIM**

- Lifetime Fitting Using the FLIM Analysis
- Lifetime-Fitting Using the FLIM Analysis (updated for SymPhoTime V 2.5 and above)
- Lifetime-Fitting Using the Rapid Reconvolution Model
- Pattern Matching
- Phasor Analysis
- ROI Fitting Using the FLIM Analysis
- ROI Fitting Using the FLIM Analysis (updated for SymPhoTime v2.5)
- Visualizing Dynamics Using the Multi Frame FLIM Analysis
- Visualizing Dynamics Using the Multi Frame FLIM Analysis (updated for SymPhoTime v2.5 and above)

#### **FRET**

- Calculate Ratiometric FRET Images
- Calculate Ratiometric Single Pair FRET Distributions
- Calculate Ratiometric Single Pair FRET Distributions Using PIE-FRET
- FLIM-FRET Calculation for Multi Exponential Donors
- FLIM-FRET Calculation for Single Exponential Donors

#### **FCS / Correlation**

- Calculate and Fit FCS Traces with the FCS Script
- Calculate FCCS Traces with the Grouped FCS Script
- Calibrate the Confocal Volume for and with FCS
- Separation of 2 Species with Different Lifetimes Using FLCS
- Spectral Crosstalk Removal via FLCCS

# EasyTau2 / Analysis

Measurement results obtained by FluoTime series spectrometers controlled with EasyTau 2 software are
directly stored in a workspace in .etc data containers. This is also the case when TimeHarp260,
 PicoHarp300, HydraHarp400 or MultiHarp150 is controlled via EasyTau 2. Such .etc files can be opened



- It is also possible to use the native software of the timing electronics for data acquisition, instead of control via EasyTau 2. Recent versions of TimeHarp260.exe, PicoHarp.exe, HydraHarp.exe and MultiHarp.exe, started from Windows Start menu, store the histograms .phu data format. Older software versions of various timing electronics may use other data file formats. Possible file name extensions are .thd, .nhd, .phd and .hhd In order to analyze such files, they have to be imported into an already existing EasyTau2 workspace.
- Data file import
- Reconvolution fit

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# **Samples**

• Diamond NV Centers

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