

# **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
- Nikon AX: NIS-Elements FLIM Examples
- Nikon AX: Phasor-Based Structural Separation Using FLIM
- NovaFLIM Tutorials
- 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

# 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
- 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

# Samples

• Diamond NV Centers

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