# Software Package for Multiparameter Fluorescence Spectroscopy, Full Correlation and Multiparameter Fluorescence Imaging

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**Trial Package is available**

**Abstract**

We present a software package for fluorescence data analysis in Multiparameter Fluorescence Detection (MFD) [1]. With this technique time-resolved observation of intrinsic properties of chromophores (i.e. spectral properties of absorption and fluorescence, fluorescence quantum yield, fluorescence lifetime, and anisotropy) is recorded. Selective analyses of molecular subensembles and time dependent parameter traces from single molecule are possible [2].

Subsequent software-correlation yields full correlation curves covering more than 12 orders of magnitude in time [3]. An advanced time-correlated single photon counting (TCSPC) technique simultaneously provides traditional fluorescence autocorrelation (FCS) or cross correlation (FCCS) from selected molecules.

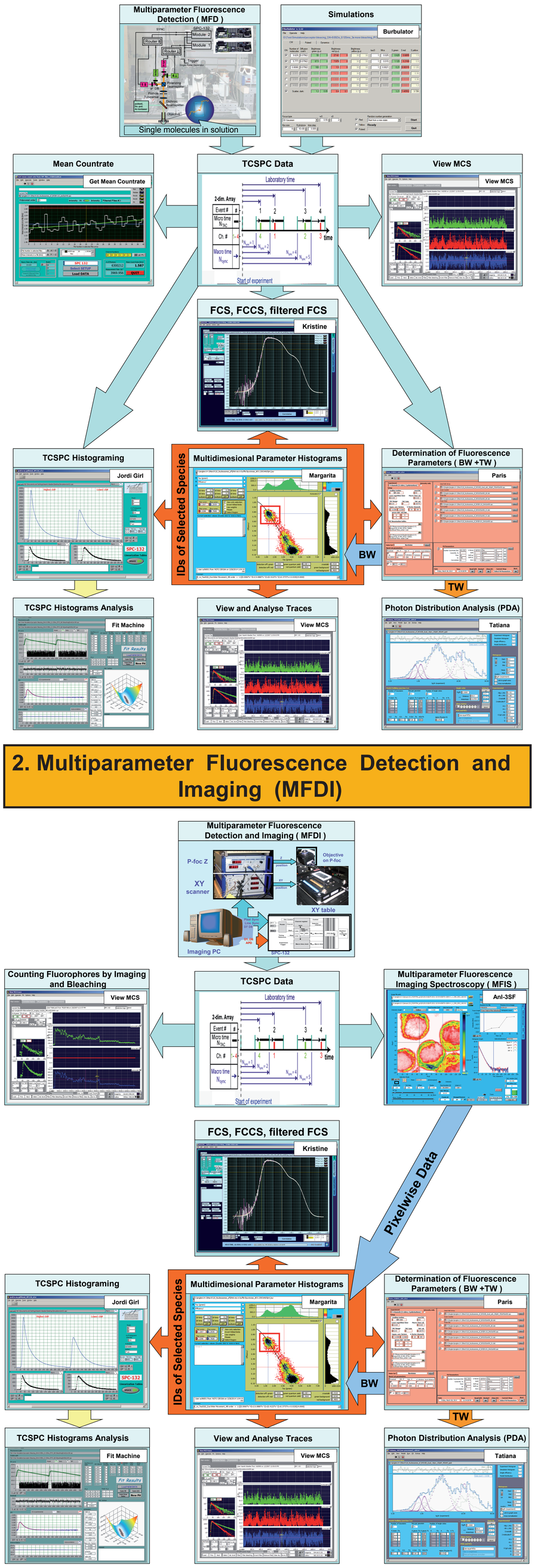
Software allows for all potential applications of techniques using pulsed or cw laser excitation: antibunching effects, rotational diffusion and fluorescence resonance energy transfer (FRET) as are discussed.

The combination of FCS and fluorescence lifetime (FLCS) [4] is implemented into the software correlator. Direct generation of necessary filters for polarisation dependent signals is possible.

Software for probability distribution analysis (PDA) is presented for quantitative and precise description of the photon counting histograms (PCH) from fluorescence resonance energy transfer experiments (FRET) [5]. An accurate description of the histogram profile based on the statistical distributions of fluorescence and background signals is sensitive to small changes in fluorescence signal even when signal counts are low. The PDA formalism allows monitoring the changes in the emission spectra of single molecules, dynamical changes of the system, etc. PDA harbours the potential for Molecular Ångstrom Optics.

Software for confocal Multiparameter Fluorescence Imaging (MFI) is developed. Each pixel of MFI corresponds to "single burst" in solution experiments and all MFD type analysis are applied. Intensity, lifetime, anisotropy, correlation times, correlation amplitudes or any MFD parameter images can be reconstructed [6,7].

## 1. Multiparameter Fluorescence Detection (MFD)




## 2. Multiparameter Fluorescence Detection and Imaging (MFDI)




## PARIS ( Analyses )

- ✓ Primary analysis program for reading SPC data, selecting single molecule bursts, and calculating lifetime, anisotropy, and intensity for each separate burst.
- ✓ For all or selected single molecule bursts time window analysis with shifted intervals option can be applied.

## MARGARITA ( MFD )

- ✓ Reads the files produced by PARIS, and generates user specified 2D and 1D fluorescence parameter histograms.
- ✓ Burst coordinates (burst ID) for selected species can be exported for further analysis.
- ✓ A third fluorescence parameter can be used for species selection.

## KRISTINE (FCS, FCCS, filtered FCS)

- ✓ Full correlation from picoseconds to seconds for TCSPC data.
- ✓ TAC gated FCS.
- ✓ Countrate filter can be applied to SPC files.
- ✓ Species selective FCS (Burst ID correlation).
- ✓ Time-resolved FCS or component selective correlation (with Fluorescence Lifetime Filters).
- ✓ Generates Fluorescence Lifetime Filters.
- ✓ Generates TAC linearization tables.
- ✓ More than 100 fitting functions with up to 32 parameters and 32 constants are implemented.
- ✓ Global fitting is possible.

## TATIANA ( PDA )

- ✓ Probability distribution analysis ( PDA ) method for the analysis of fluorescence resonance energy transfer (FRET) signals to determine with high precision the originating value of a shot-noise-limited signal distribution. PDA theoretical distributions are calculated explicitly including crosstalk, stochastic variations, and background and represent the minimum width that a FRET distribution must have. In this way an unambiguous distinction is made between shot-noise distributions and distributions
- ✓ Simultaneously and effectively extracts highly resolved information from FRET distributions.
- ✓ Gaussian distribution of Donor-Acceptor distances is modeled in the PDA method.
- ✓ Corresponding fit routines for single state, N states, N Gaussian distributed states, Model Free states (Maximum of Entropy) and two states with
- ✓ Rate constants can be recovered.
- ✓ Steady state anisotropy is derived with very high precision from single molecule data.

## Anl - 3SF ( TCSPC IMAGING )

- ✓ Finds the proper photons for each pixel of 2D and recovers the image from binary files.
- ✓ Calculates correlation curve and TAC histogram for each pixel or for the selection of the pixels.
- ✓ Calculates for each pixel lifetime, anisotropy, intensity, correlation diffusion time, correlation amplitude.
- ✓ Exports the calculated parameters for Margarita.
- ✓ Exports pixel coordinates in format of burst ID. Most of above mentioned software can analyze the pixel photons like photons from bursts, so all fluorescence parameters can be calculated for each pixel and all types of fluorescence parameter images can be constructed.
- ✓ Generates movies from the series of frames, saving for each movie frame all the calculated parameters as well as raw data.

## 3. Full correlation curve of Rh110

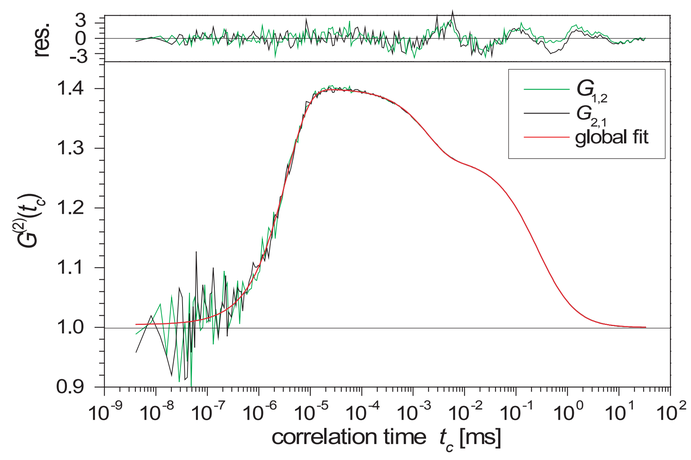



Figure 1. Correlation curves $G_{1,2}$ and $G_{2,1}$ of Rhodamine 110 aqueous solutions. Excitation at 496 nm, measured with PMT detectors. Recording time 16 min. Fit results are: N=3.5, $z_0/w_0$=1.5, $t_D$=0.3ms, $T_{eq}$=0.29, $t_T$=1.9µs, m=1.02, $t_A$=3.5ns.

## 4. Probability Distribution Analyses

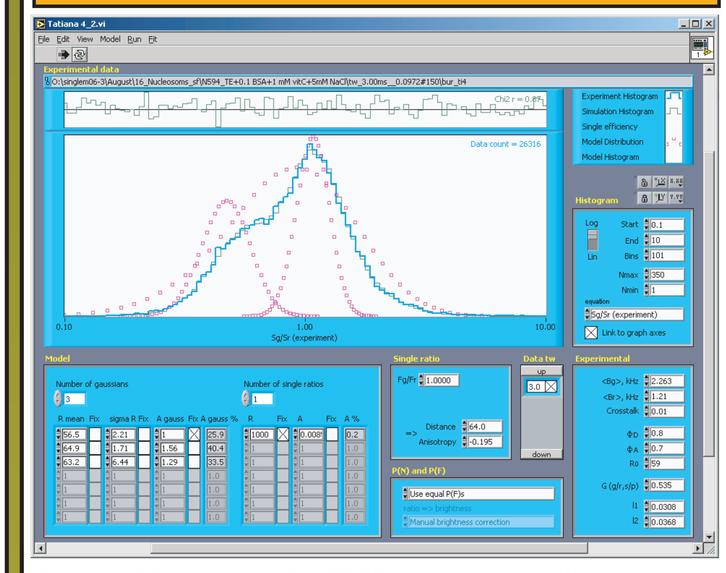



Figure 2. PDA analysis of spFRET measurements of pure internally labelled 601-170-med-N594 DNA (length 170 bp, 92 bp distance between Donor-Alexa 488 and Acceptor-Alexa 594) and nucleo-somes at 5 mM NaCl. Model of three Gaussian distributed distances and one fixed ratio (corresponding to D-only species) is used. Two bound (HF-25.9 %, LF-40.4 %) and loosely bound (33.5

## 5. Force Spectroscopy + MFD

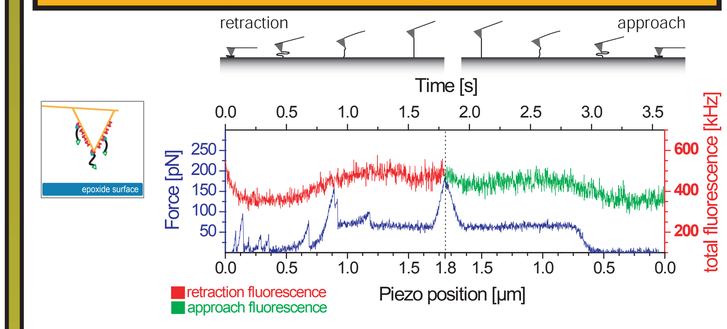



Figure 3. Combined simultaneous force and fluorescence spectroscopy (SFFS) data for a single λ-DNA. Selective analysis of fluorescence trace can be realized in ELKE or MARCELLE software. Several fluorescence parameters can be displayed together with force on 2D histograms (Fig.4).

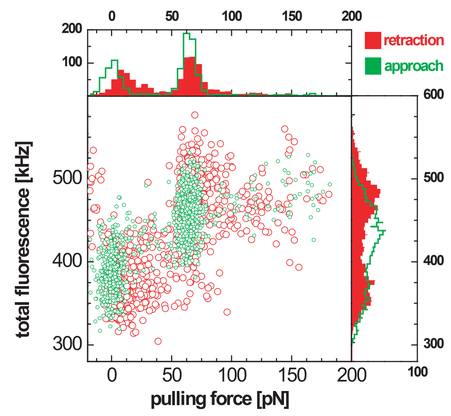



Figure 4. 2D histogram representation of the SFFS data (Fig.3). Pulling force is an additional parameter to MFD. It allows us to correlate fluorescence information with mechanical properties of macromolecules and provides a way to understand structure and functions of macromolecules.

**References:**

[1] Kühnemuth et al., Single Molecules, 2, 251 (2001).
[2] Widengren J., et al., Anal. Chem. 78, 2039 (2006).
[3] Felekyan S., et al., Rev. Sci. Instr. 76, 083104 (2005).
[4] Böhmer M., et al., Chem. Phys. Lett. 353, 439 (2002).
[5] Antonik M., et al., J. Phys. Chem. B 110, 6970 (2006).
[6] Gaiduk et al., Chem.Phys.Chem. 5, 976 (2005).
[7] Kudryavtsev et al., Anal.&Bioanal.Chem., 387, 71 (2007).
[8] Gaiduk A. et al., Microscopy Research Techniques (in press).