FFS DATA PROCESSOR – SOFTWARE TOOL FOR GLOBAL ANALYSIS OF FLUORESCENCE FLUCTUATION SPECTROSCOPY DATA

Victor V. Skakun[1], Anatoli V. Digris[1], Eugene A. Barsukov[1],

Ruchira Engel[2], Antonie Visser[2]

[1] Department of Systems Analysis, Belarusian State University, Minsk, Belarus

[2] MicroSpectroscopy Center, Wageningen University, Wageningen, The Netherlands

e-mail: info@sstcenter.com; WEB: http://www.sstcenter.com

Analysis methods:

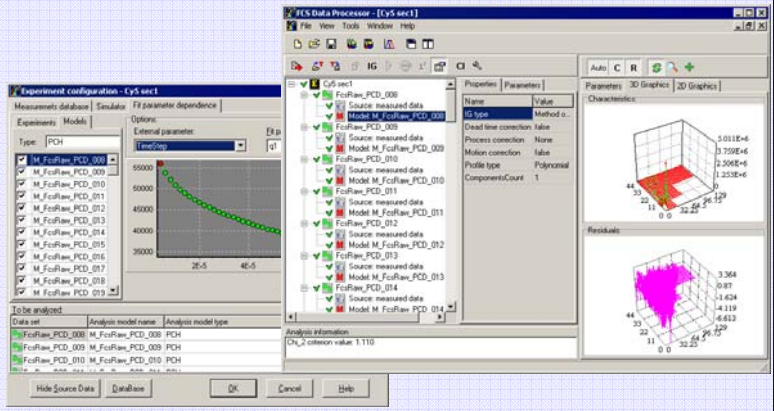
- ✓ **Fluorescence (Cross)Correlation Spectroscopy (FCS, FCCS)**
  - **supported motions: 2D/3D free, anomalous and confined diffusion, flow**
  - **supported processes: triplet, conformation, protonation**
  - **supported correction to: background, brightness difference**
- ✓ **Photon Counting (Multiple) Histogram (PCH, PCMH)**
  - **supported PSFs: polynomial, 3D Gaussian, 3D Gaussian with out-of-focus correction**
  - **supported correction to: 3D free diffusion, triplet, dead-time**
- ✓ **(Time Integrated) Fluorescence Cumulants Analysis (FCA, TIFCA)**
  - **supported PSFs: polynomial, 2D/3D Gaussian, 2D/3D Gaussian with out-of-focus correction, Gauss-Lorenzian, Gauss-Lorenzian with out-of-focus correction**
  - **supported correction to: 3D free diffusion**

Analysis features:

- ✓ **Global fit: several datasets are combined and simultaneously fitted**
- ✓ **Sequential fit: independent analysis of datasets one by one with building dependencies of obtained fit parameters**
- ✓ **Quality of fit is judged by χ^2 criterion and visual inspection of residuals**
- ✓ **Automatically generated initial guesses for parameters**
- ✓ **Parameter fixing, constraints and linkage**
- ✓ **Confidence intervals by exhaustive search and standard errors**
- ✓ **Easy extendable models library**
- ✓ **User – defined models**
- ✓ **Built-in simulator of correlation functions**

Interface features and data management:

- ✓ **Multi-document interface**
- ✓ **Advanced parameter management for sorting, quick visual linkage and easy navigation through the parameters space**
- ✓ **2D and 3D graphical data representation**
- ✓ **Templates allow to prepare analysis settings in seconds**
- ✓ **Saving and loading experimental data and analysis results from databases**
- ✓ **Import of external data & export of analysis results**
- ✓ **Compatible with ConfoCor™ and ConfoCor2™ (Carl Zeiss, Jena) and supports data formats of other manufacturers**
- ✓ **Support for building and previewing auto(cross)correlation, photon counting distribution, cumulants and other characteristics from raw data**
- ✓ **Bursts counting and rejecting**

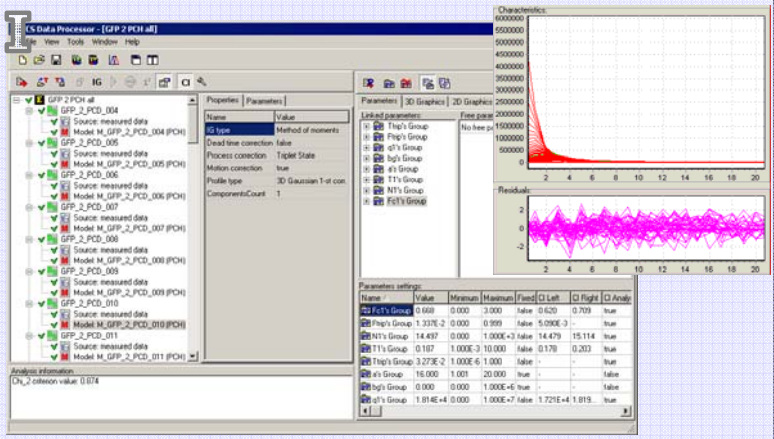


Example:

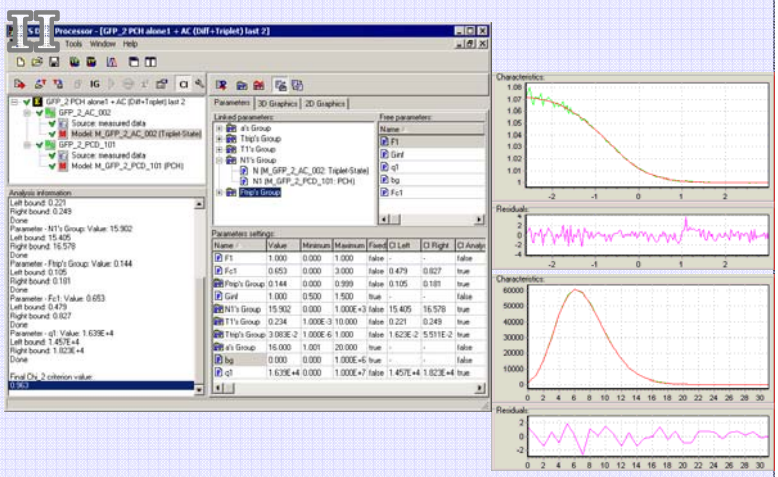
The analysis of raw FFS data of enhanced GFP (green fluorescent protein) is presented. Dynamic and brightness properties of the sample were obtained:

I. by global analysis of photon counting distributions (PCD) calculated at different bin times from single raw data trace.
Fifty one PCD calculated at different bin times (from 5E-6 sec to 1E-3 sec) were analyzed globally by one component PCH model with 1st order brightness profile, diffusion and triplet state corrections. All corresponding parameters of individual PCH models were linked.

II. by global analysis of single PCD and single autocorrelation function.
Single autocorrelation function and single PCD were analyzed globally. The autocorrelation function was fitted by one component triplet-state model. PCD was fitted by one component PCH model with 1st order brightness profile, diffusion and triplet state correction. Average number of molecules, diffusion time, structural parameter and triplet-state parameters of both models were linked.



The global analysis of PCD only (first approach) is almost insensitive to triplet parameters. This is indicated by undefined (>100%) confidence intervals (see table below). The comparable quality fit can be obtained without correction to any fast blinking process. In contrast the second approach produces more accurate estimations for diffusion and triplet parameters and is much faster. Better estimations of dynamic parameters in the second approach are achieved due to the presence of correlation function. In turn accurate estimations of dynamic parameters allow to get unbiased and more accurate values for brightness parameters. The last row in the table shows brightness recalculated so that ($q = \gamma_2 q_{app}/(1+F_{corr})$) it corresponds to the brightness available from FCS analysis.



	AC	Global PCH (fig I)	Global AC+PCH (fig II)
F trip	0.145 [0.112; 0.176]	0.013 [0.005; —]	0.144 [0.105; 0.181]
T trip, μs	30.9 [18.4; 50.0]	32.7 [—; —]	31.0 [16.0; 55.0]
T diff, ms	0.234 [0.223; 0.246]	0.187 [0.178; 0.203]	0.234 [0.221; 0.249]
F corr	—	0.67 [0.62; 0.71]	0.65 [0.48; 0.83]
N	15.9 [15.5; 16.4]	14.5 [14.47; 15.1]	15.9 [15.4; 16.6]
q_{app}, **cpsm**	—	18140 [17210; 18190]	16390 [14570; 18230]
***q*, cpsm**	3506	3841	3512