



BrightEyes-FFS GUI fit models

1 Introduction

The BrightEyes-FFS GUI is a graphical user interface built together with the BrightEyes-FFS python package available on PyPi and GitHub. The software is designed for analyzing fluorescence fluctuation spectroscopy data obtained with small array detectors, such as the SPAD array detector and the Zeiss Airyscan.

The BrightEyes-FFS GUI can

- Load raw FFS intensity traces from .h5, .tiff, and .czi files
- Calculate various correlation curves with various algorithms
- Load correlation curves from .csv files
- Filter out bad chunks of data automatically
- Fit correlation curves to various models
- Calculate the diffusion law to analyze anomalous diffusion
- Calculate and plot diffusion asymmetry and flow maps
- Save/load entire analysis sessions
- Load analysis sessions in Python for plotting or further analysis
- Generate automatically Jupyter Notebooks from a GUI session for quick plotting or repeating the analysis in Python
- Customizing the GUI by adding correlation types and fit models

2 Calculating correlations

Correlations from time traces can be calculated in the time domain using *multiptetau* and in the Fourier domain using the *Wiener-Khinchin* theorem. For time-correlated single-photon data, an arrival time-based algorithm *TT2Corr* is available. Note that *TT2Corr* still needs as an input a time-trace (with 0s for no photon detection and 1 for a photon detection). For calculating *All cross-correlations*, the GUI automatically uses a sparse-matrices calculation in the time-domain, ignoring the algorithm chosen by the user.

3 Fit functions

Fitting is performed with the least squares optimizer from Scipy. The following fit functions are by default available. The meaning of the fit parameters are:

$\langle N \rangle$	Average number of molecules in the focal volume
τ_D	Diffusion time (s)
τ_t	Triplet time (s)
τ_{ab}	Antibunching time (s)
c_{ab}	Antibunching amplitude
τ_{conf}	Time scale of conformational dynamics (s)
c_{conf}	Conformational dynamics amplitude
τ_{rot}	Time scale of rotational diffusion (s)
c_{rot}	Rotational dynamics amplitude
T	Fraction in triplet state
F	Fraction of species 1
F_{eq}	Fraction in equilibrium
k_{off}	Rate of unbinding (/s)
ω_0	Lateral beam waist (m)
z_0	Axial beam waist (m)
SP	Shape parameter of the focal volume, i.e., z_0/ω_0
α	Anomalous diffusion parameter
β	Relative brightness
G_0	Offset in the autocorrelation function
R	Radius of the orbital scan (m)
T_0	Period of the orbital scan (s)
a, b	Detector afterpulsing properties

3.1 Free diffusion 1 component

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{1 + \frac{\tau}{\tau_D}} \frac{1}{\sqrt{1 + \frac{\tau}{SP^2 \tau_D}}} + G_0 \quad (1)$$

3.2 Anomalous diffusion 1 component

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{1 + \left(\frac{\tau}{\tau_D}\right)^\alpha} \frac{1}{\sqrt{1 + \left(\frac{\tau}{\tau_D}\right)^\alpha \frac{1}{SP^2}}} + G_0 \quad (2)$$

3.3 Free diffusion 1 component with flow

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{1 + \frac{\tau}{\tau_D}} \frac{1}{\sqrt{1 + \frac{\tau}{SP^2 \tau_D}}} \exp\left(-\frac{(\rho_x - v_x \tau)^2 + (\rho_y - v_y \tau)^2}{\omega_0^2 (1 + \frac{\tau}{\tau_D})}\right) + G_0 \quad (3)$$

3.4 Free diffusion 2 components (with triplet state)

$$G(\tau) = \frac{1}{\langle N \rangle (F + \beta(1 - F))^2} \left(1 + \frac{T \exp(-\tau/\tau_t)}{1 - T} \right) \times \left(\frac{F}{1 + \frac{\tau}{\tau_{D1}}} \frac{1}{\sqrt{1 + \frac{\tau}{SF^2\tau_{D1}}}} + \beta^2 \frac{1 - F}{1 + \frac{\tau}{\tau_{D2}}} \frac{1}{\sqrt{1 + \frac{\tau}{SF^2\tau_{D2}}}} \right) + G_0 \quad (4)$$

3.5 Free diffusion 2 components with afterpulsing

$$G(\tau) = \frac{1}{\langle N \rangle (F + \beta(1 - F))^2} \left(1 + \frac{T \exp(-\tau/\tau_t)}{1 - T} \right) \times \left(\frac{F}{\left(1 + \frac{\tau}{\tau_{D1}}\right) \sqrt{1 + \frac{\tau}{SF^2\tau_{D1}}}} + \beta^2 \frac{1 - F}{\left(1 + \frac{\tau}{\tau_{D2}}\right) \sqrt{1 + \frac{\tau}{SF^2\tau_{D2}}}} \right) + a\tau^b + G_0 \quad (5)$$

3.6 Anomalous diffusion 2 components (with triplet state)

$$G(\tau) = \frac{1}{\langle N \rangle (F + \beta(1 - F))^2} \left(1 + \frac{T \exp(-\tau/\tau_t)}{1 - T} \right) \times \left(\frac{F}{1 + \left(\frac{\tau}{\tau_{D1}}\right)^{\alpha_1}} \frac{1}{\sqrt{1 + \left(\frac{\tau}{\tau_{D1}}\right)^{\alpha_1} \frac{1}{SP^2}}} + \beta^2 \frac{1 - F}{1 + \left(\frac{\tau}{\tau_{D2}}\right)^{\alpha_2}} \frac{1}{\sqrt{1 + \left(\frac{\tau}{\tau_{D2}}\right)^{\alpha_2} \frac{1}{SP^2}}} \right) + G_0 \quad (6)$$

3.7 Free diffusion circular scanning

$$G(\tau) = \frac{1}{\langle N \rangle \left(1 + \frac{4D\tau}{\omega_0^2}\right) \sqrt{1 + \frac{4D\tau}{\omega_0^2 SP^2}}} \exp \left(-\frac{4R^2 \sin^2 \left(\frac{\pi}{T_0} \tau \right)}{\omega_0^2 + 4D\tau} \right) + G_0 \quad (7)$$

3.8 Free diffusion pair-correlation

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{1 + \frac{\tau}{\tau_D}} \frac{1}{\sqrt{1 + \frac{\tau}{SP^2\tau_D}}} \exp \left(-\frac{\rho^2}{\omega_0^2 \left(1 + \frac{\tau}{\tau_D}\right)} \right) + G_0 \quad (8)$$

3.9 2D free diffusion 2 components (with flow and afterpulsing)

$$G(\tau) = \frac{1}{\langle N \rangle (F + \beta(1 - F))^2} \left(1 + \frac{T \exp(-\tau/\tau_t)}{1 - T} \right) \times \left(\frac{F}{1 + \frac{\tau}{\tau_{D1}}} + \beta^2 \frac{1 - F}{1 + \frac{\tau}{\tau_{D2}}} \right) + a\tau^b + G_0 \quad (9)$$

3.10 Nanosecond FCS

$$G(\tau) = A \left(1 - c_{ab} \exp\left(-\frac{\tau}{\tau_{ab}}\right) \right) \left(1 + c_{conf} \exp\left(-\frac{\tau}{\tau_{conf}}\right) \right) \times \left(1 + c_{rot} \exp\left(-\frac{\tau}{\tau_{rot}}\right) \right) \left(1 + c_t \exp\left(-\frac{\tau}{\tau_t}\right) \right) \times \left(\left(1 + \frac{\tau}{\tau_D} \right) \sqrt{1 + \frac{\tau}{SP^2\tau_D}} \right)^{-1} \quad (10)$$

3.11 Uncoupled reaction and diffusion analysis

$$G(\tau) = \frac{A}{\left(1 + \frac{\tau}{\tau_D}\right) \sqrt{1 + \frac{\tau}{SP^2\tau_D}}} + (1 - F_{eq}) \exp(-k_{off}\tau) \quad (11)$$

Here, the time scale of binding is assumed to be much slower than the diffusion time.

4 Other analysis options

4.1 Asymmetry heat map

Radial plot of 4 (for a 5×5 detector) or 6 (for the Airyscan) pair-correlations between the central element and the nearest neighbours. The color represents the cross-correlation value at a given lag time, for increasing lag times at an increasing distance from the center. The asymmetry heat map requires the calculation of *Asymmetric diffusion analysis*.

4.2 Flow heat map

Four each of the four directions (up, down, left, right), six pair-correlations (between detector elements and their nearest neighbours in the respective direction) are calculated and their average is taken, leading to four correlations: G_{up} , G_{down} , G_{left} , G_{right} . The differences $\pm(G_{up} - G_{down})$ and $\pm(G_{left} - G_{right})$ are plotted on a horizontal and vertical line in a polar plot. For the other directions, a linear combination of these two curves is calculated. The flow heat map requires the calculation of *Cross-correlation for flow analysis*.

4.3 Maximum entropy method free diffusion

Fit with N components for a large N (*e.g.*, $N = 200$), each component assumed to be freely diffusing, Eq. 1, while at the same time preferring the broadest possible distribution of diffusion times.