LifeFit Documentation

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CHAPTER 1

What is LifeFit

LifeFit is a Python package to analyze **time-correlated single-photon counting (TCSPC)** data sets, namely **fluorescence lifetime** and **time-resolve anisotropy** decays.

CHAPTER 2

Webserver

You can run LifeFit directly in your browser: https://tcspc-lifefit.herokuapp.com/

CHAPTER 3

Installation

There are different options how to install LifeFit.

3.1 Conda

Install the package into your conda environment

```
conda install -c fdsteffen lifefit
```

3.2 PyPI

Alternatively, you can install the latest release of with pip

```
pip install lifefit
```

3.3 Install from source

Finally, you can also get the latest development version directly from Github

```
pip install git+https://github.com/fdsteffen/Lifefit.git
```

CHAPTER 4

Dependencies

Lifefit depends on the following Python packages:

- numpy
- scipy
- uncertainties

CHAPTER 5

Tutorial

For an introduction into the functionality of LifeFit visit the *tutorial*. The Jupyter Notebook can be downloaded `here`.

CHAPTER 6

Bug reports

Please report any *bugs* via the issue tracker on Github.

6.1 Lifefit Tutorial

[1]:
```
# import modules
import lifefit as lf
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
import os

# seaborn settings
sns.set_style('white')
sns.set_context("notebook")
sns.set(font='Arial')

# plot settings
def set_ticksStyle(x_size=4, y_size=4, x_dir='in', y_dir='in'):
    sns.set_style('ticks', {'xtick.major.size': x_size, 'ytick.major.size': y_size,
↪'xtick.direction': x_dir, 'ytick.direction': y_dir})
```

6.1.1 Lifetime

First, define the path to the data

[23]:
```
atto550_dna_path = lf._DATA_DIR+'/lifetime/Atto550_DNA.txt'
irf_path = lf._DATA_DIR+'/IRF/irf.txt'
```

Next, we read in our datafile for the fluorescence decay and the instrument response function (IRF). Instead of using the `lf.read_decay()` function we can define a custom import function that outputs a two-column array containing numbered channels and intensity counts.

[24]:
```
atto550_dna, timestep_ns = lf.tcspc.read_decay(atto550_dna_path)
irf, _ = lf.tcspc.read_decay(irf_path)
```

Next we instantiate a `Lifetime object` by rpoviding the data arrays of the fluorescence decay and the IRF along with the timestep between two channels

[25]:
```
atto550_dna_life = lf.tcspc.Lifetime(atto550_dna, timestep_ns, irf)
```

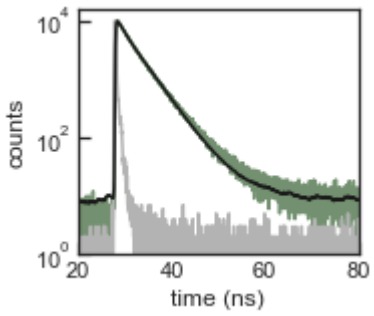
Fit the fluorecence decay by iterative reconvolution with the IRF

[26]:
```
atto550_dna_life.reconvolution_fit([1,5])
```

```
========================================
Reconvolution fit with experimental IRF
tau0: 1.01 ± 0.01 ns (29%)
tau1: 3.89 ± 0.01 ns (71%)
mean tau: 3.61 ± 0.01 ns

irf shift: 0.11 ns
offset: 1 counts
========================================
```

Plot the IRF, the fluorescence decay and the fit

[27]:
```
with sns.axes_style('ticks'):
    set_ticksStyle()
    f, ax = plt.subplots(nrows=1, ncols=1, figsize=(2.5,2.25), sharex=False,␣
↪sharey=True, squeeze=False)

    ax[0,0].semilogy(atto550_dna_life.fluor[:,0], atto550_dna_life.fluor[:,2],␣
↪color=[0.45, 0.57, 0.44])
    ax[0,0].semilogy(atto550_dna_life.irf[:,0], atto550_dna_life.irf[:,2], color=[0.7,
↪0.7,0.7])
    ax[0,0].semilogy(atto550_dna_life.fluor[:,0], atto550_dna_life.fit_y, color='k')

    ax[0,0].set_ylabel('counts')
    ax[0,0].set_xlabel('time (ns)')
    ax[0,0].set_xlim((20,80))
    ax[0,0].set_ylim(bottom=1)
```



6.1.2 Anisotropy

Read the four different fluorescence decays and generate a `lifetime object` from each channel

[29]:
```
atto550_dna_path = {}
atto550_dna = {}
atto550_dna_life = {}
for c in ['VV','VH','HV','HH']:
    atto550_dna_path[c] = lf._DATA_DIR+'/anisotropy/{}.txt'.format(c)
    atto550_dna[c], fluor_nsperchan = lf.tcspc.read_decay(atto550_dna_path[c])
    atto550_dna_life[c] = lf.tcspc.Lifetime(atto550_dna[c], fluor_nsperchan, irf)
```

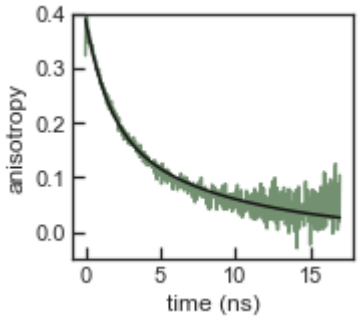
Compute an `anisotropy object` from the lifetime objects and fit a two-rotator model to the anisotropy decay

[30]:
```
atto550_dna_aniso = lf.tcspc.Anisotropy(atto550_dna_life['VV'], atto550_dna_life['VH
↪'], atto550_dna_life['HV'],atto550_dna_life['HH'])
atto550_dna_aniso.rotation_fit(p0=[0.4, 1, 10,1], model='two_rotations')
```

```
====================
Anisotropy fit
model: two_rotations
r0: 0.19 ± 0.01 ns
b: 0.00 ± 0.02 ns
tau: 8.50 ± 0.45 ns
tau2: 1.57 ± 0.14 ns
====================
```

Plot the anisotropy decay with the fit

[31]:
```
with sns.axes_style('ticks'):
    set_ticksStyle()
    f, ax = plt.subplots(nrows=1, ncols=1, figsize=(2.5,2.25), sharex=False,␣
↪sharey=True, squeeze=False)

    ax[0,0].plot(atto550_dna_aniso.time, atto550_dna_aniso.r, color=[0.45, 0.57, 0.
↪44])
    ax[0,0].plot(atto550_dna_aniso.time, atto550_dna_aniso.fit_r, color='k')
    ax[0,0].set_ylim((-0.05,0.4))
    ax[0,0].set_xlabel('time (ns)')
    ax[0,0].set_ylabel('anisotropy')
```



6.2 LifeFit package description

Fit lifetime decays

class lifefit.tcspc.**Anisotropy**(*VV*, *VH*, *HV*, *HH*)

Bases: `object`

Create an Anisotropy object with four polarization resolved lifetime decays

Parameters

- **VV** (*ndarray*) – vertical excitation - vertical emission
- **VH** (*ndarray*) – vertical excitation - horizontal emission
- **HV** (*ndarray*) – horizontal excitation - vertical emission
- **HH** (*ndarray*) – horizontal excitation - horizontal emission

Example

```
>>> lf.tcspc.Anisotropy(decay['VV'], decay['VH'], decay['HV'],decay['HH'])
```

static G_factor(*HV*, *HH*)

Compute G-factor to correct for differences in transmittion effiency of the horizontal and vertical polarized light

Parameters

- **HV** (*ndarray*) – horizontal excitation - vertical emission
- **HH** (*ndarray*) – horizontal excitation - horizontal emission

Returns **G** (*float*) – G-factor

Notes

The G-factor is defined as follows:

$$G = \frac{\int HV}{\int HH}$$

static aniso_decay(*VV*, *VH*, *G*)

Parameters

- **VV** (*ndarray*) – vertical excitation - vertical emission
- **VH** (*ndarray*) – vertical excitation - horizontal emission
- **G** (*float*) – G-factor

Returns **r** (*ndarray*) – anisotropy decay

Notes

The anisotropy decay is calculated from the parallel and perpendicular lifetime decays as follows:

$$r(t) = \frac{I_{\mathrm{VV}} - GI_{\mathrm{VH}}}{I_{\mathrm{VV}} + 2GI_{\mathrm{VH}}}$$

export(*filename*)

static hindered_rotation(*time*, *r0*, *tau_r*, *r_inf*)

Hindered rotation in-a-cone model

Parameters

- **time** (*array_like*) – time bins

- **r0** (*float*) – fundamental anisotropy
- **tau_r** (*float*) – rotational correlation time
- **r_inf** (*float*) – residual anisotropy at time->inf

Returns *ndarray* – hindered rotation anisotropy decay

static local_global_rotation(*time*, *r0*, *tau_rloc*, *r_inf*, *tau_rglob*)
Local-global rotation in-a-cone model

Parameters

- **time** (*array_like*) – time bins
- **r0** (*float*) – fundamental anisotropy
- **tau_rloc** (*float*) – local rotational correlation time
- **r_inf** (*float*) – residual anisotropy at time->inf
- **tau_rglob** (*float*) – global rotational correlation time

Returns *ndarray* – local-global rotation anisotropy decay

static one_rotation(*time*, *r0*, *tau*)
Single rotator model

Parameters

- **time** (*array_like*) – time bins
- **r0** (*float*) – fundamental anisotropy
- **tau_r** (*float*) – rotational correlation time

Returns *ndarray* – two-rotation anisotropy decay

rotation_fit(*p0=[0.4, 1], model='one_rotation', manual_interval=None, bounds=(0, inf), verbose=True, ns_before_VVmax=1, signal_percentage=0.01*)
Fit rotation model to anisotropy decay.

Parameters

- **p0** (*array_like*) – start values of the chosen anisotropy fit model
- **model** (*str*) – one of the following anisotropy models: {'one_rotation', 'two_rotations', 'hindered_rotation', 'local_global_rotation'}
- **manual_interval** (*2-tuple of float, optional*) –
- **bounds** (*2-tuple of float or array_like*) – lower and upper bounds for each parameter in p0. Can be either a tuple of two scalars (same bound for all parameters) or a tuple of array_like with the same length as p0. To deactivate parameter bounds set: *bounds=(-np.inf, np.inf)*
- **verbose** (*bool*) – print anisotropy fit result
- **ns_before_VVmax** (*float, optional*) – how many nanoseconds before the maximum of the VV decay should the search for r0 start
- **signal_percentage** (*float, optional*) – percentage of the VV decay serving as a treshold to define the end of the anisotropy fit interval

Example

```
>>> obj.rotation_fit(p0=[0.4, 1, 10, 1], model='two_rotations')
```

serialize()

static two_rotations(*time*, *r0*, *b*, *tau_r1*, *tau_r2*)
Two-rotator model

Parameters

- **time** (*array_like*) – time bins
- **r0** (*float*) – fundamental anisotropy
- **b** (*float*) – amplitude of second decay
- **tau_r1** (*float*) – first rotational correlation time
- **tau_r2** (*float*) – second rotational correlation time

Returns *ndarray* – two-rotation anisotropy decay

class lifefit.tcspc.**Lifetime**(*fluor_decay*, *fluor_ns_per_chan*, *irf_decay=None*, *gauss_sigma=None*, *gauss_amp=None*)

Bases: object

Create lifetime class

Parameters

- **fluor_decay** (*ndarray*) – n x 2 array containing numbered channels and intensity counts of the fluorescence decay
- **fluor_ns_per_chan** (*float*) – nanoseconds per channel
- **irf_decay** (*ndarray, optional*) – n x 2 array containing numbered channels and intensity counts for instrument reponse function (IRF) if *None*, then IRF is approximated by a Gaussian

Variables

- **ns_per_chan** (*float*) – nanoseconds per channel
- **fluor** (*ndarray*) – n x 4 array containing time, channel number, intensity counts and associated Poissonian weights of the fluorescence decay
- **irf** (*ndarray*) – n x 3 array containing time, channel number and intensity counts of the IRF
- **irf_type** (*str*) – type of IRF: {'Gaussian', 'experimental'}
- **fit_param** (*ndarray*) –
- **fit_param_std** (*ndarray*) –

Example

```
>>> fluor, fluor_nsperchan = lf.tcspc.read_decay(pathToFluorDecay)
>>> irf, irf_nsperchan = lf.tcspc.read_decay(pathToIRF)
>>> lf.tcspc.Lifetime(fluor, fluor_nsperchan, irf)
```

static average_lifetime(*a*, *tau_val*, *tau_std*)
Calculate average lifetime according to[1]

Parameters

- **a** (*array_like*) – weighting factors of tau
- **tau_val** (*array_like*) – fluorescence lifetimes
- **tau_std** (*array_like*) – standard deviation of the fluorescence lifetimes

Returns **av_lt** (*tuple*) – average lifetime and associated standard deviation

References

static convolution(*irf*, *sgl_exp*)
Compute convolution of irf with a single exponential decay

Parameters

- **irf** (*array_like*) – intensity counts of the instrument reponse function (experimental of Gaussian shaped)
- **sgl_exp** (*array_like*) – single-exponential decay

Returns **convolved** (*ndarray*) – convoluted signal of IRF and exponential decay

static exp_decay(*time*, *tau*)
Create a single-exponential decay

Parameters

- **time** (*array_like*) – time bins
- **tau** (*float*) – fluorescence lifetime

Returns **sgl_exp** (*array_like*) – single-exponential decay

export(*filename*)

classmethod from_filenames(*fluor_file*, *irf_file=None*, *fileformat='HORIBA'*, *gauss_sigma=None*, *gauss_amp=None*)
Alternative constructor for the Lifetime class by reading in filename for the fluorophore and IRF decay

Parameters

- **fluor_file** (*str*) – filename of the fluorophore decay
- **irf_file** (*str*) – filename of the IRF decay
- **fileformat** (*str, optional*) – currently implemented formats: {'HORIBA'}

Example

```
>>> lf.tcspc.Lifetime.from_filenames(pathToFluorDecay, pathToIRFDecay)
```

static gauss_irf(*time*, *mu*, *sigma=0.01*, *A=10000*)
Calculate a Gaussian-shaped instrument response function (IRF)

Parameters

[1] J. Lakowicz, *Principles of Fluorescence*, 3rd ed., Springer, **2010**.

- **time** (*ndarray*) – time bins
- **mu** (*float*) – mean of the Gaussian distribution
- **sigma** (*float, optional*) – standard deviation of the Gaussian distribution
- **A** (*float, optional*) – amplitude of the Gaussian distribution

Returns **irf** (*ndarray*) – Gaussian shaped instrument response function (IRF)

nnls_convol_irfexp (*x_data*, *p0*)

Solve non-negative least squares for series of IRF-convolved single-exponential decays. First, the IRF is shifted, then convolved with each exponential decay individually (decays 1,. . . ,n), merged into an m x n array (=A) and finally plugged into scipy.optimize.nnls(A, experimental y-data) to compute *argmin_x || Ax - y ||_2*. This optimizes the relative weight of the exponential decays whereas the curve_fit function optimizes the decay parameters (tau1, taus2, etc.)

Parameters

- **x_data** (*array_like*) – array of the independent variable
- **p0** (*array_like*) – start values for the fit model

Returns

- **A** (*ndarray*) – matrix containing irf-convoluted single-exponential decays in the first n columns and ones in the last column (background counts)
- **x** (*ndarray*) – vector that minimizes || *Ax - y* ||_*2*
- **y** (*ndarray*) – fit vector computed as *y = Ax*

reconvolution_fit (*tau0=[1], tau_bounds=(0, inf), irf_shift=0, sigma=None, verbose=True*)

Fit the experimental lifetime decay to a series of exponentials via interative reconvolution with the instrument reponse function (IRF).

Parameters

- **tau0** (*int or array_like*) – start value(s) of the fluorescence lifetime(s)
- **tau_bounds** (*2-tuple of float or 2-tuple of array_like, optional*) – lower and upper bounds for each parameter in tau0. Can be either a tuple of two scalars (same bound for all parameters) or a tuple of array_like with the same length as tau0. To deactivate parameter bounds set: *bounds=(-np.inf, np.inf)*
- **irf_shift** (*int, optional*) – shift of the IRF on the time axis (in channel units)
- **sigma** (*array_like , optional*) – uncertainty of the decay (same length as y_data)
- **verbose** (*bool, optional*) – print lifetime fit result

Example

```
>>> obj.reconvolution_fit([1,5])
```

serialize()

lifefit.tcspc.**fit** (*fun, x_data, y_data, p0, bounds=([0, 0, 0], [inf, inf, inf]), sigma=None*)

Wrapper for the curve_fit function of the scipy.optimize module The curve_fit optimizes the decay parameters (tau1, tau2, etc.) while the nnls weights the exponential decays.

Parameters

- **fun** (*callable*) – The model function f(x,...) taking x values as a first argument followed by the function parameters
- **x_data** (*array_like*) – array of the independent variable
- **y_data** (*array_like*) – array of the dependent variable
- **p0** (*array_like*) – start values for the fit model
- **bounds** (*2-tuple of float or 2-tuple of array_like, optional*) – lower and upper bounds for each parameter in p0. Can be either a tuple of two scalars (same bound for all parameters) or a tuple of array_like with the same length as p0. To deactivate parameter bounds set: *bounds=(-np.inf, np.inf)*
- **sigma** (*array_like, optional*) – uncertainty of the decay (same length as y_data)

Returns

- **p** (*ndarray*) – optimized fit parameters
- **p_std** (*ndarray*) – standard deviation of optimized fit parameters

lifefit.tcspc.**parseCmd**()
Parse the command line to get the experimental decay and instrument reponse function (IRF) file.

Returns

- **fluor_file** (*str*) – filename of the fluorescence decay
- **irf_file** (*str*) – filename of the IRF (if None then the IRF is approximated by a Gaussian)

lifefit.tcspc.**parse_file**(*decay_file*, *fileformat='Horiba'*)
Parse the decay file

Parameters

- **decay_file** (*StringIO*) –
- **fileformat** (*str, optional*) – currently implemented formats: {'HORIBA'}

Returns

- **decay_data** (*ndarray*) – n x 2 decay containing numbered channels and intensity counts for instrument reponse function (IRF)
- **ns_per_chan** (*float*)

lifefit.tcspc.**read_decay**(*filepath_or_buffer*, *fileformat='Horiba'*)
Read TCSPC decay file from HORIBA or another data format

Parameters

- **filepath_or_buffer** (*str, os.PathLike, StringIO*) – filename of the decay or StringIO object
- **fileformat** (*str, optional*) – currently implemented formats: {'HORIBA'}

Returns

- **decay_data** (*ndarray*) – n x 2 decay containing numbered channels and intensity counts for instrument reponse function (IRF)
- **ns_per_chan** (*float*)

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