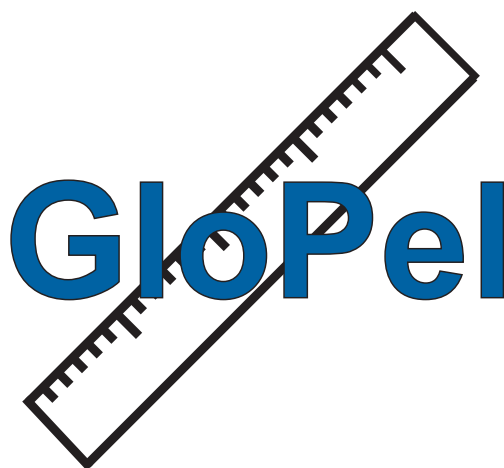

GloPel User Manual

Version 1.0.1



by

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Contents

1	Introduction	4
1.1	Installation Instructions	6
1.1.1	Run from source	6
1.1.2	Use compiled executables	6
2	Using GloPel	8
2.1	Loading data	8
2.2	Data (pre)-processing	9
2.2.1	Phase correction	10
2.2.2	Normalization and zero-time	10
2.2.3	Background correction	11
2.3	Analysis	12
2.3.1	Analysis using Tikhonov regularization	13
2.3.2	Model-based fitting	16
2.4	Inspection and Validation	19
2.5	Validation	19
2.6	Save the results	21
3	Global analysis	23
3.1	Data processing of two time traces	23
3.2	Global analysis of two time traces	24

Preface

GloPel has been developed in the group of Prof. Dr. Stefan Weber at the University of Freiburg, Freiburg im Breisgau, Germany, during the last couple of years as a software for advanced global analysis of PELDOR/DEER spectra.

This manual describes the general usage of GloPel and gives an overview of the maths behind that has been implemented.

If you use GloPel for your own research and publish results accordingly, please give credits citing the appropriate reference :

Stephan Rein, Philipp Lewe, Susana L. Andrade, Sylwia Kacprzak, and Stefan Weber. Global analysis of complex PELDOR time traces. *Journal of Magnetic Resonance*, 295 (2018) 17–26

Some parts of this Manual were taken from the publication [1] of which the author was responsible for program development and for writing most parts of the manuscript.

A number of people have helped shaping GloPel and the ideas behind. First and foremost, Prof. Dr. Stefan Weber and Dr. Sylwia Kacprzak (now Bruker Biospin) were for years the driving force behind GloPel due to the need for advanced analysis of PELDOR/DEER data with limited signal-to-noise ratio. Dr. Till Biskup (now Saarland University) contributed ideas for programming and details of the implementation that make all the difference between a program useful for a larger audience and a simple in-house solution.

Freiburg, October 2019

Stephan Rein

Disclaimer

GloPel is distributed under a GPLv3 license. The software is provided "as is", without warranty of any kind, express or implied, including but not limited to the warranties of merchantability, fitness for a particular purpose and noninfringement.

1 Introduction

GloPel is a platform-independent GUI application, designed for advanced and global analysis that can reliably analyze PELDOR data from samples that cannot be measured with high signal-to-noise ratio in sufficiently long observation time windows. The here-presented global-analysis approach employs Tikhonov regularization of the solution to the inverse problem. Alternatively, a model-based (sum of multiple Gaussian functions) fit may be used.

GloPel is currently restricted to the global analysis of two time traces, for the sake of keeping the GUI as simple as possible. It is recommended to acquire two experimental time traces, one with a longer acquisition time and a poorer signal-to-noise ratio and one with a short acquisition time and a good signal-to-noise ration. It is highly recommended to keep all spectrometer settings constant and only change the acquisition time and PELDOR step size. Especially taking the sample out between the two experiments should be avoided. The standard procedure in GloPel, explained in detail in the following sections, for a single analysis are:

1. Loading the raw experimental data set (as .txt or BES3T Bruker file format)
2. Preprocessing of the raw data (settings for background function, background dimension, cutting of data, ...)
3. Analysis of the data with either Tikhonov regularization or multi-Gaussian fitting
4. Checking the results on the Residual Inspection submodule
5. Validation of the results (in the case of Tikhonov regularization) using the Validation submodule
6. Saving all the analysis results

For a global analysis the steps are:

1. Analysis of data set 1 (the PELDOR time time trace with longer acquisition time)
2. Loading data set 2
3. Data preprocessing of data set 2 using the background function of data set 1
4. Analysis of data set 2
5. Global analysis of both data sets
6. Residual inspection and validation of the results (in case of global Tikhonov regularization)
7. Saving the global analysis results (the analysis of the individual analysis is saved automatically)

For details of the individual steps and installation details, read the following sections.

1.1 Installation Instructions

GloPel is available free of charge and open source (see license). We recommend everybody with a recent Python installation on their computers to download the source code via pip and use it. In this case, no real installation is necessary, and starting GloPel is as simple as typing the following command into a terminal:

1.1.1 Run from source

Install GloPel in a Python3 environment

```
pip install glopel
```

Once installed, the program can be started by just calling **GloPel** from the console by just giving:

```
glopel
```

If you want to use it withing a python script/console give:

```
python3
>>> from GloPel.GloPel import run
>>> run()
```

1.1.2 Use compiled executables

However, GloPel can be downloaded as executable binary for Windows and several Linux flavors. It was tested on various systems including Windows 7 (32-bit and 64-bit architecture), Windows 10 (32-bit and 64-bit architecture), and Ubuntu 16.04.

Please note that these versions are much harder to debug if something goes wrong, therefore, they are provided as is without any further support.

A note on how these binaries have been created: Thanks to PyInstaller, it is rather straightforward to create binary distributions of Python programs, both as directories

with all the necessary files contained as well as single-file executables. PyInstaller allows to define additional options in a “spec” file. This file is included in the sources available. To get a one-file binary, it was called simply as follows:

```
pyinstaller --onefile --noconsole GloPel.spec
```

1. Binaries for Linux have been built with Ubuntu 16.04 (Xenial Xerus) and a Python installation consisting of Python 3.5, matplotlib 3.0.2, PyQt 5.11.3, NumPy 1.16.1, SciPy 1.2.0, reportlab 3.5.19, cvxopt 1.2.3, osqp 0.6.1
2. Binaries for Windows have been built with Windows7 (32-bit architecture) and a Python installation consisting of Python 3.5 or, matplotlib 3.0.2, NumPy (+MKL) 1.11.3, SciPy 1.2.0, PyQt 5.11.3, osqp 0.6.1

2 Using GloPel

2.1 Loading data

GloPel has a load button (Figure 1), which automatically opens your file browser. GloPel can read either text files (as .txt or .dat MATLAB format) or the Bruker BES3T file format (.DTA, .DSC). For text formats, GloPel expects either three columns (time, real, imaginary) for data recorded with quadrature detection or two columns (time, real) for signals which were recored without imaginary part. If BES3T file format data is imported, both, the .DTA (binary file) and .DSC (description file) file is expected.

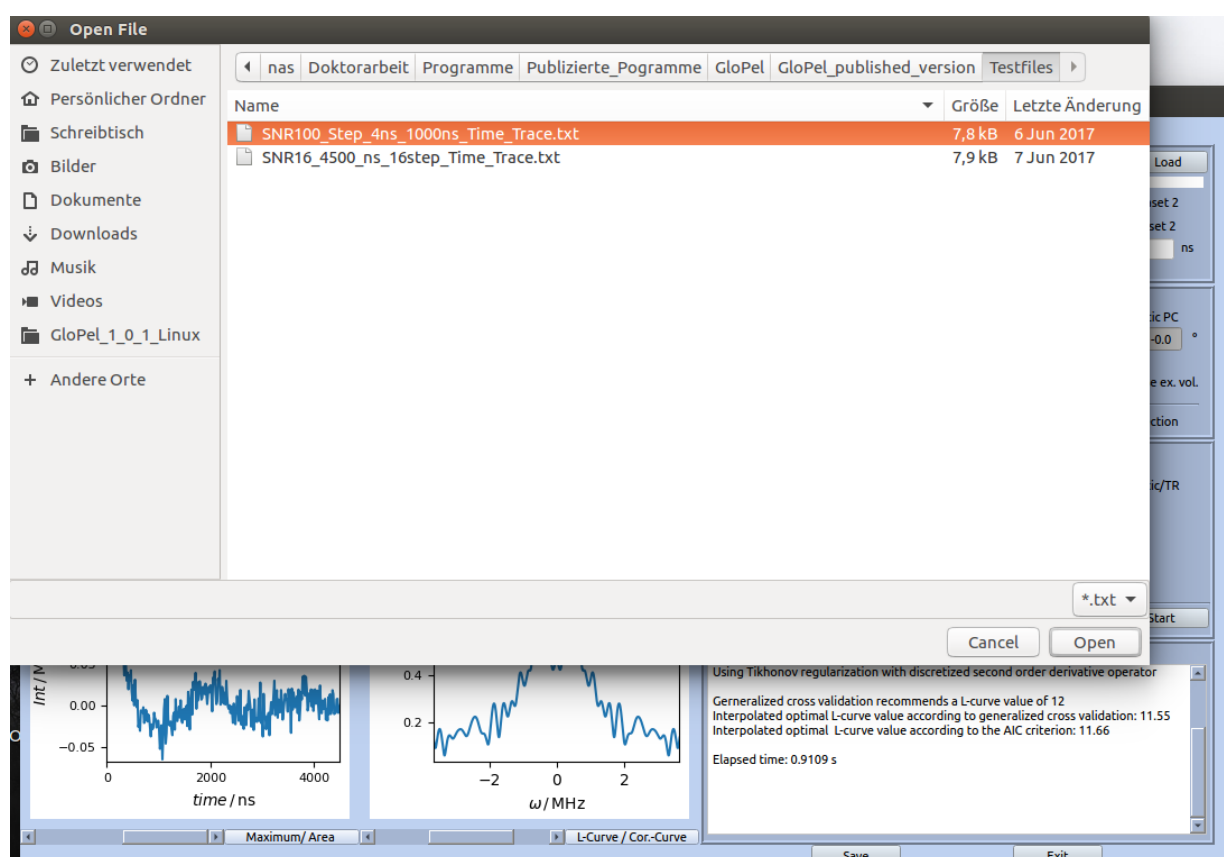


Figure 1: Loading experimental data in GloPel. The default file format is .txt but the format can be altered to either .dta (Bruker file format) or .dat (MATLAB file format).

Other file formats are currently not supported.

2.2 Data (pre)-processing

Data processing, prior to analysis, includes:

- Phase correction (automatically done)
- Normalization (automatically suggested)
- Zero-time determination (automatically suggested)
- Optional cutting of the data
- Determination of the background function (start and background dimension)

The main GUI of GloPel, with data processing function highlighted in red, is shown in Figure 2. For phase correction, GloPel uses an iterative algorithm to minimize the least-

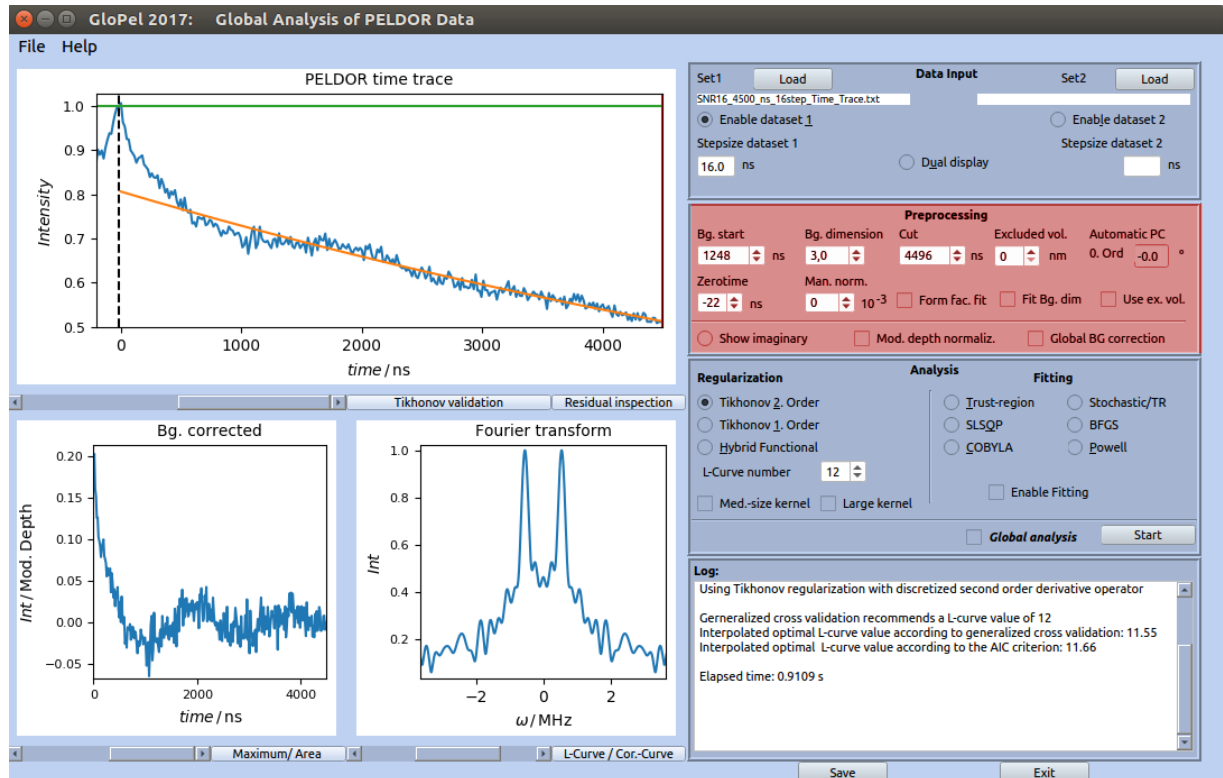


Figure 2: GloPel main GUI. The box with the data processing options is highlighted in red.

squares deviation of the imaginary part of a time trace from a zero line using a zeroth-order shift,

2.2.1 Phase correction

$$V_{\text{pc}}(T) = \exp(i \gamma_0) \cdot V_{\text{raw}}(T) ., \quad (1)$$

γ_0 is the phase angle in the complex plane. Phase correction is automatically performed upon loading the raw data and cannot be changed manually.

2.2.2 Normalization and zero-time

in GloPel A model function is fitted to the locally symmetric PELDOR time trace around time zero (Figure 3):

$$F(T) = a_1 \left(1 - a_2 \cdot (T - a_0)^2 \cdot \exp \left[-a_3 \cdot |T - a_0| \right] \right) . \quad (2)$$

The parameters a_0 , a_1 , a_2 and a_3 are fitted by nonlinear optimization. $F(T)$ is symmetric about the axis located at a_0 , reflecting the symmetry of the PELDOR time trace at the symmetry axis located at $T = 0$ of the experiment. The normalization can be manually

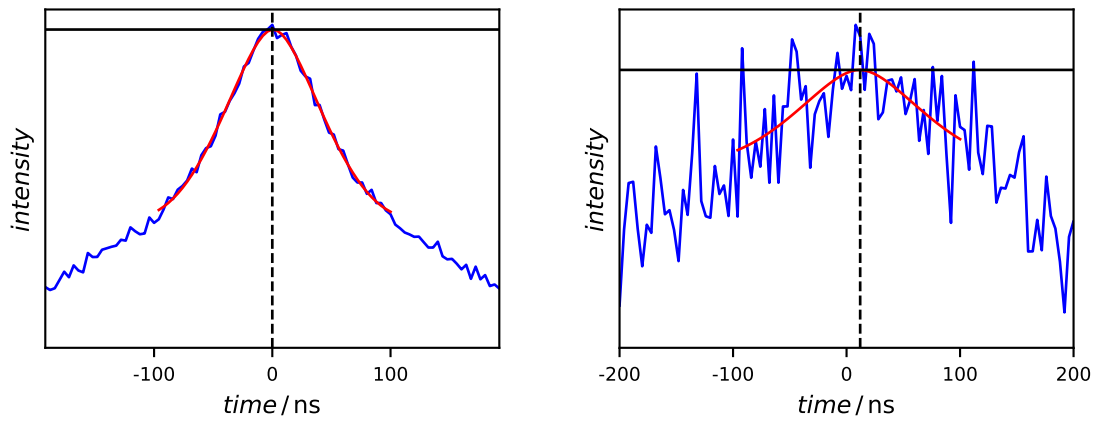


Figure 3: Two time traces (blue) with the corresponding fit functions (red). The zero-time, determined using Equation 2 is shown as vertical dotted line. The normalization line is shown as horizontal solid black line.

shifted by the user. The zero-time can be altered by the user as well.

2.2.3 Background correction

For the background function $B(T)$, the well-known homogeneous distribution of inter-molecular isolated spin pairs is implemented in GloPel’s current version,

$$B(T) = \exp(-k T) , \quad (3)$$

with k being proportional to the concentration of spins and the modulation depth [2, 3]. However, GloPel also provides the possibility to use exponential decays with fractional dimensions of the form $B(T) = \exp(-k T^{N/3})$, [4]. The background dimension N can be varied manually from 1 to 6 in steps of 0.1. Hence, other dimensions than the default homogeneous background decay ($N = 3$) may be chosen, *e.g.*, $N \approx 2$ for membrane proteins, or $N = 6$ for a Gaussian decay. Alternatively, the background dimension can be fitted, similar as in DeerAnalysis [5] and GLADD [6]. The user can set the time T_s , where

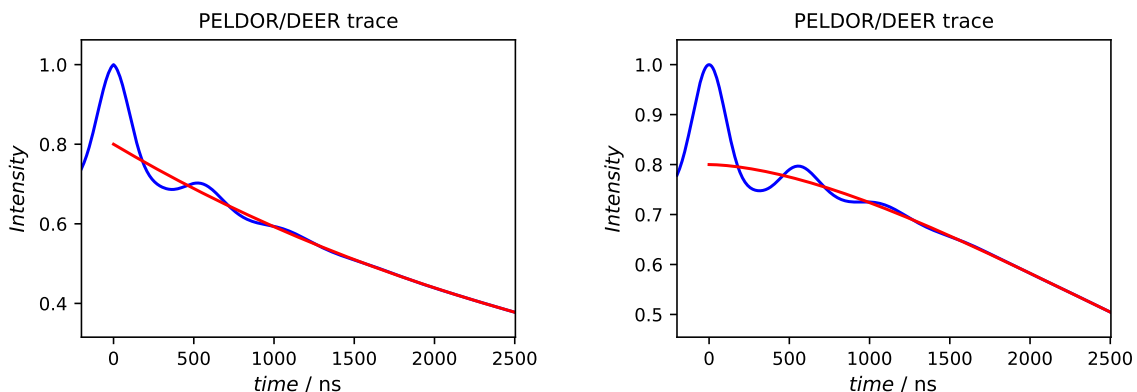


Figure 4: Simulated time traces with background function with different dimensionality. (Left) $N = 3$. (Right) $N = 5$. The background functions are illustrated in red.

the background function is fitted to the experimental data with $T \geq T_s$. The choice of T_s and the choice of the dimensionality N are two critical factors for a reliable PELDOR analysis. The shape of the Fourier transform of the background corrected signal can be used to approximate the correct “start” of the background function T_s . The Fourier transform is supposed to look like a pake pattern. Examples for Fourier transforms of background corrected time traces, using a different starting time for fitting the background function, are shown in Figure 5.

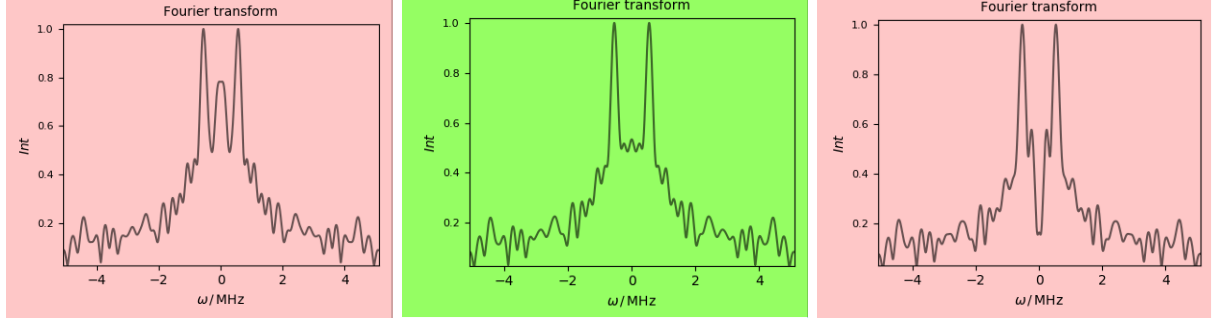


Figure 5: Fourier transforms of the background corrected time trace, using different starting time for the the background function. The Fourier transform shown in the middle resembles the pake pattern and is the best choice for the background start.

2.3 Analysis

GloPel provides Tikhonov regularization as well as multi-Gaussian fitting as analysis tools to extract the distance distribution from the background-corrected time trace (Figure 6).

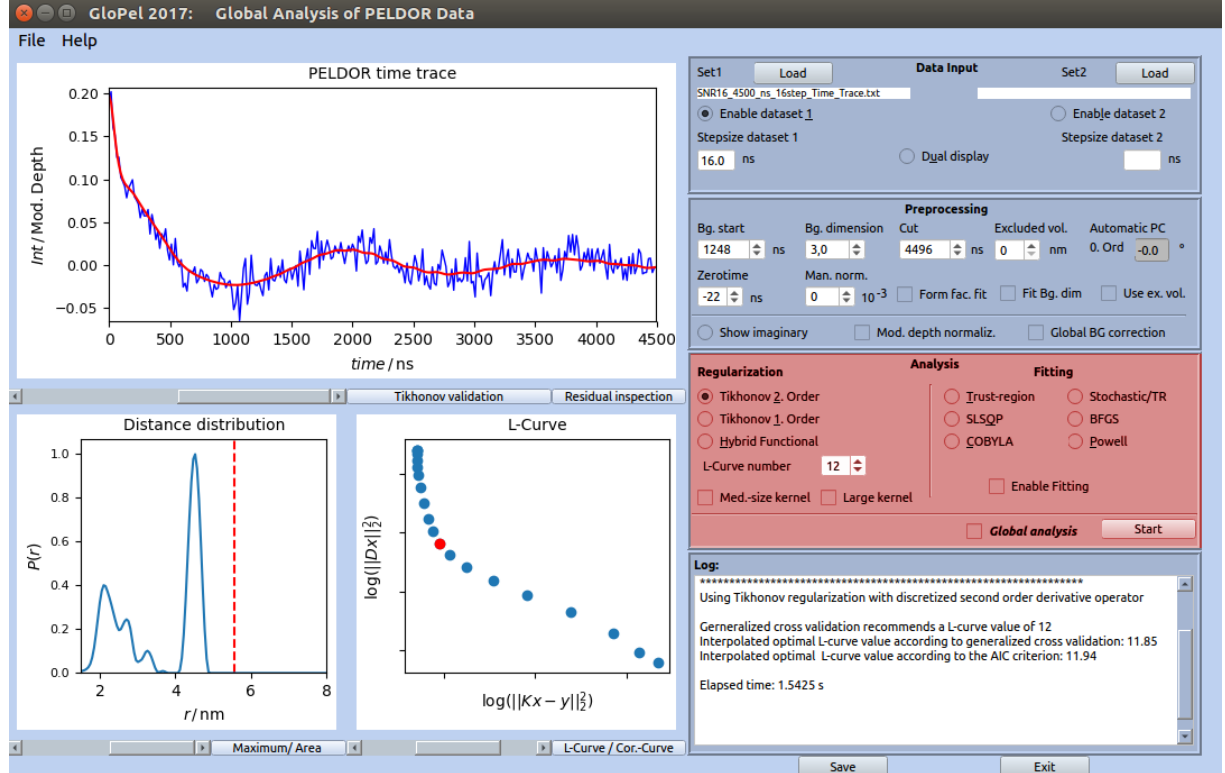


Figure 6: GloPel main GUI. The box with the analysis options is highlighted in red.

2.3.1 Analysis using Tikhonov regularization

GloPel provides Tikhonov regularization as analysis method to approximate the inverse solution of the PELDOR problem [5, 7]. The Tikhonov functional:

$$\min \left[||\mathbf{K} \mathbf{P} - \mathbf{S}||^2 + \alpha^2 ||\mathbf{L} \mathbf{P}||^2 \right], , \quad (4)$$

is minimized by using the quadratic programming solver [8] from the CVXOPT or OSQP Python package for a given regularization parameter α . \mathbf{K} is the kernel matrix, \mathbf{P} is the distance distribution vector and \mathbf{S} is the experimental PELDOR time trace vector.

Functional and kernel size

The matrix kernel size \mathbf{L} can be altered by the user. Three different kernel sizes, namely, normal (131×131), medium-size (261×261), and large (521×521) are available. Increasing the kernel size to a medium-size kernel or a large kernel might be necessary if the distance distribution is broad with a high probability density at short distances. The penalty of the The matrix \mathbf{L} is in GloPel either the discrete second-derivative, the first-derivative operator or a hybrid operator (where \mathbf{E} is the unit matrix)

$$\mathbf{L} = \frac{\Delta^2 P(r)}{\Delta r^2} \quad \text{or} \quad \mathbf{L} = \frac{\Delta P(r)}{\Delta r} \quad \text{or} \quad \mathbf{L} = \beta \frac{\Delta^2 P(r)}{\Delta r^2} + \gamma \mathbf{E} \quad (5)$$

The user can choose between the three penalty expressions. If no special problem has to be considered (e.g. a distance distribution with small and broad distance peaks), the standard second derivative operator is recommended.

Regularization parameter

The optimal regularization parameter α is automatically determined by GloPel using generalized cross validation. In addition to the value obtained by using generalized cross validation the result obtained by the corrected Aikake information criterion (Figure 10).

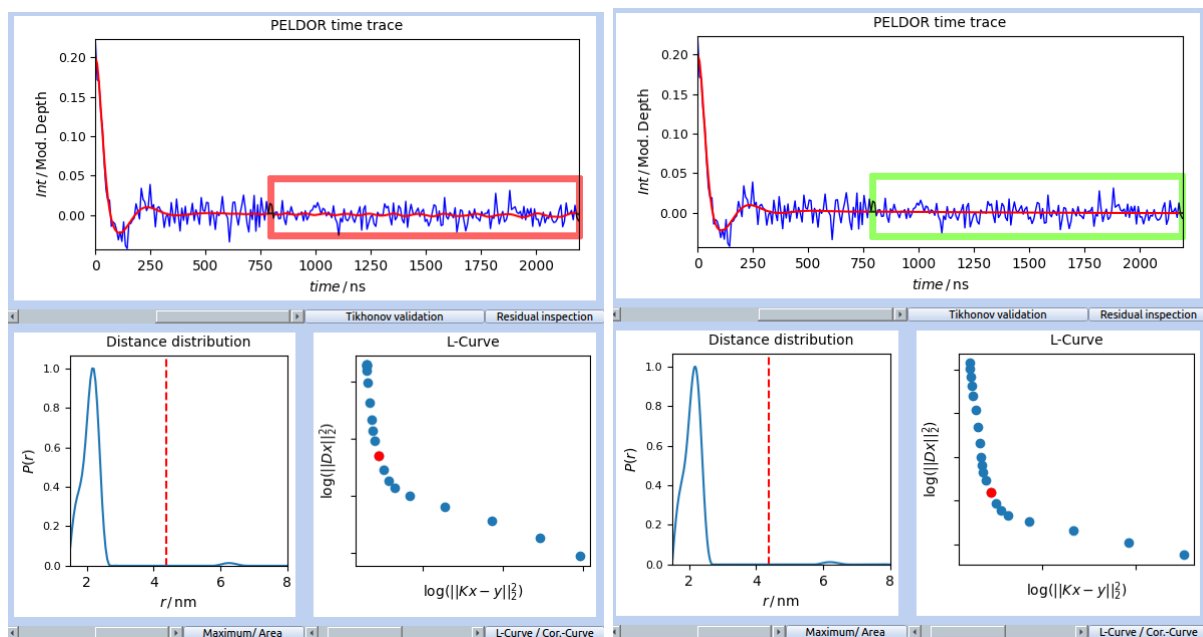


Figure 7: Analysis of a PELDOR trace using a normal kernel (left) or a large kernel (right). For broad distance distributions, with small central distances, the standard kernel size might be insufficient.

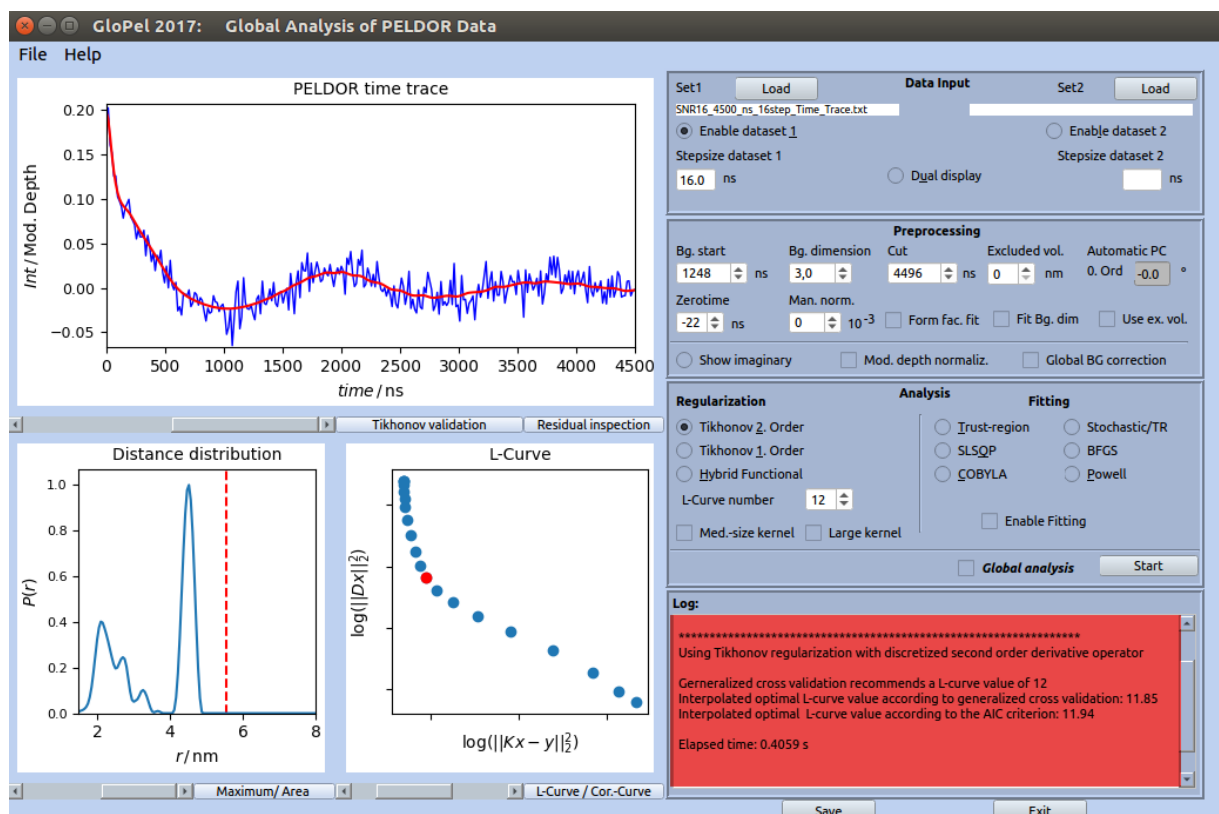


Figure 8: GloPel main GUI. The log output after applying Tikhonov analysis is highlighted in red.

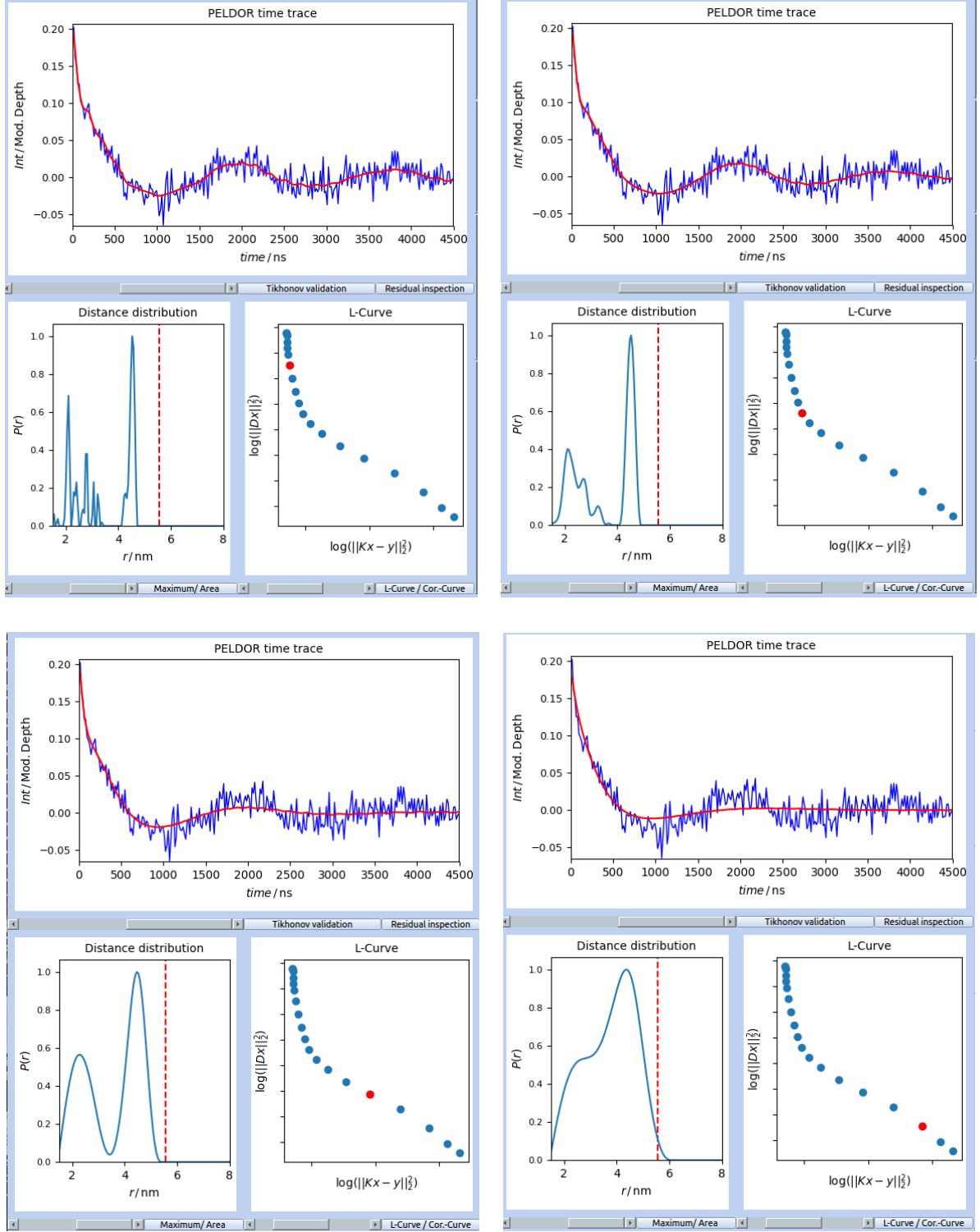


Figure 9: Different choice of α (highlighted as red point). The choice of α show in the top-right panel was automatically determined by GloPel by using generalized cross validation.

Nevertheless, the Tikhonov solution is calculated for 20 discrete points of α and the L-Curve is shown with the optimal regularization parameter indicated in red. The regularization parameter α can be altered by the user. This might be necessary if the background

function was not properly determined and the suggested regularization parameter is far away from the “corner” of the L-Curve. Different values of α for the same PELDOR time trace are shown in Figure 9.

2.3.2 Model-based fitting

As an alternative to Tikhonov regularization, a multi-Gaussian model can be fitted to the experimental data. Two models are automatically compared within a fitting procedure, a 5-Gaussian model with one overall standard deviation and a 4-Gaussian model with individual standard deviation for each Gaussian function.

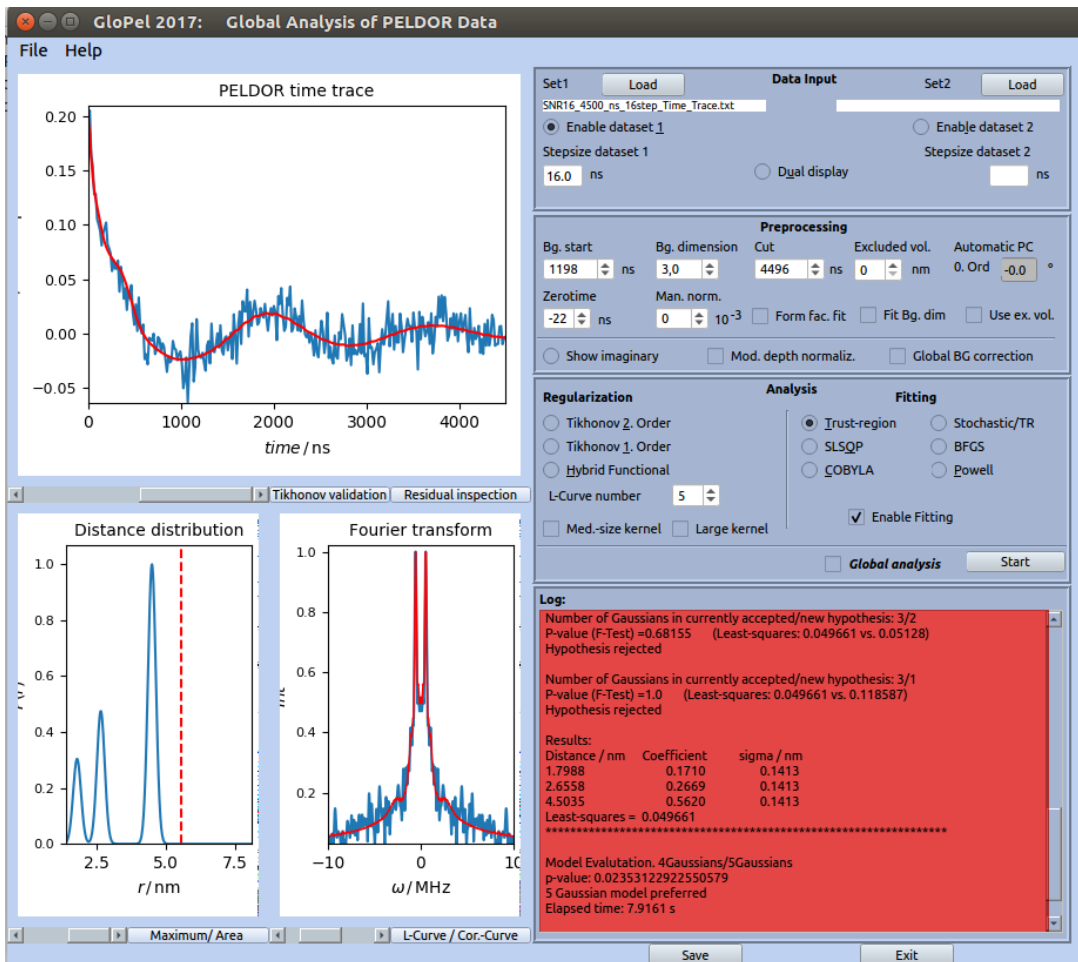


Figure 10: GloPel main GUI. The box with the information about the fitting is highlighted in red.

The full expression of the fitting function $V_{\text{fit}}(T)$ used in GloPel is given by

$$\begin{aligned}
V_{\text{fit}}(T) = & \\
& \lambda \sum_{k=1}^{4(5)} f_k \sum_{i=1}^j I(r_{k,i}) \int_0^{\pi/2} \cos\left((1 - 3 \cos^2 \phi) \omega_{k,i} T\right) \sin \phi \, d\phi \\
& \text{with } I(r_{k,i}) = \frac{1}{\sigma_k \sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{r_{k,0} - r_{k,i}}{\sigma_k} \right)^2 \right] \\
& \text{and } \sum_{k=1}^{4(5)} f_k = 1 \quad \text{with } f_k \in \mathbb{R}_0^+ .
\end{aligned} \tag{6}$$

In a non-linear optimization, using any of the above-mentioned routines, the central positions of the four (five) Gaussian distributions (r_k), the corresponding linear coefficients (f_k), and the single width σ (in case of five Gaussians) or the individual widths σ_k (in case of four Gaussians) are fitted. Both models are calculated subsequently, without the necessity for the user to provide any startup parameters, and the superior model, according to the statistical F-test, is taken as fit solution. The full log console output of a fitting procedure is shown in Script 1.

Script 1: Full output on the log console after applying a multi-Gaussian fit, using the trust-region algorithm.

Algorithm: Trust-region-reflective

Number of Gaussians in currently accepted/new hypothesis: 4/3

P-value (F-Test) =0.0 (Least-squares: 0.04902 vs. 0.04903)

Hypothesis accepted

Number of Gaussians in currently accepted/new hypothesis: 3/2

P-value (F-Test) =0.00235 (Least-squares: 0.04903 vs. 0.050109)

Hypothesis accepted

Number of Gaussians in currently accepted/new hypothesis: 2/1

P-value (F-Test) =1.0 (Least-squares: 0.050109 vs. 0.118569)

Hypothesis rejected

Results:

Distance / nm	Coefficient	sigma / nm
---------------	-------------	------------

```

2.6635          0.4576          0.3210
4.5099          0.5424          0.1139
Least-squares = 0.050109
*****

*****

Algorithm: Trust-region-reflective

Number of Gaussians in currently accepted/new hypothesis: 5/4
P-value (F-Test) =0.0          (Least-squares: 0.049535 vs. 0.049526)
Hypothesis accepted

Number of Gaussians in currently accepted/new hypothesis: 4/3
P-value (F-Test) =0.0          (Least-squares: 0.049526 vs. 0.049661)
Hypothesis accepted

Number of Gaussians in currently accepted/new hypothesis: 3/2
P-value (F-Test) =0.68155      (Least-squares: 0.049661 vs. 0.05128)
Hypothesis rejected

Number of Gaussians in currently accepted/new hypothesis: 3/1
P-value (F-Test) =1.0          (Least-squares: 0.049661 vs. 0.118587)
Hypothesis rejected

Results:
Distance / nm    Coefficient    sigma / nm
1.7988           0.1710           0.1413
2.6558           0.2669           0.1413
4.5035           0.5620           0.1413
Least-squares = 0.049661
*****

Model Evalutation. 4Gaussians/5Gaussians
p-value: 0.02353122922550579
5 Gaussian model preferred
Elapsed time: 7.9161 s

```

In GloPel, six different optimization routines can be used: (1) the trust-region-reflective algorithm, (2) sequential least-squares programming (SLSQP), (3) Powell's conjugate direction method, (4) the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm, (5) constrained optimization by linear approximation (COBYLA), as implemented in the Python package Scipy, and (6) a semi-stochastic algorithm.

2.4 Inspection and Validation

2.5 Validation

GloPel provides a validation of the distance distribution obtained using Tikhonov regularization, similar to that implemented in DeerAnalysis [5]. In this routine, the impact of pre-processing parameters, such as the values of the background fitting parameter T_D and the background dimension N , as well as added noise, is evaluated for a user-defined symmetric range around the original pre-processing values. The Validation module can be opened in a separate sub-GUI (Figure 11) The results obtained by the validation pro-

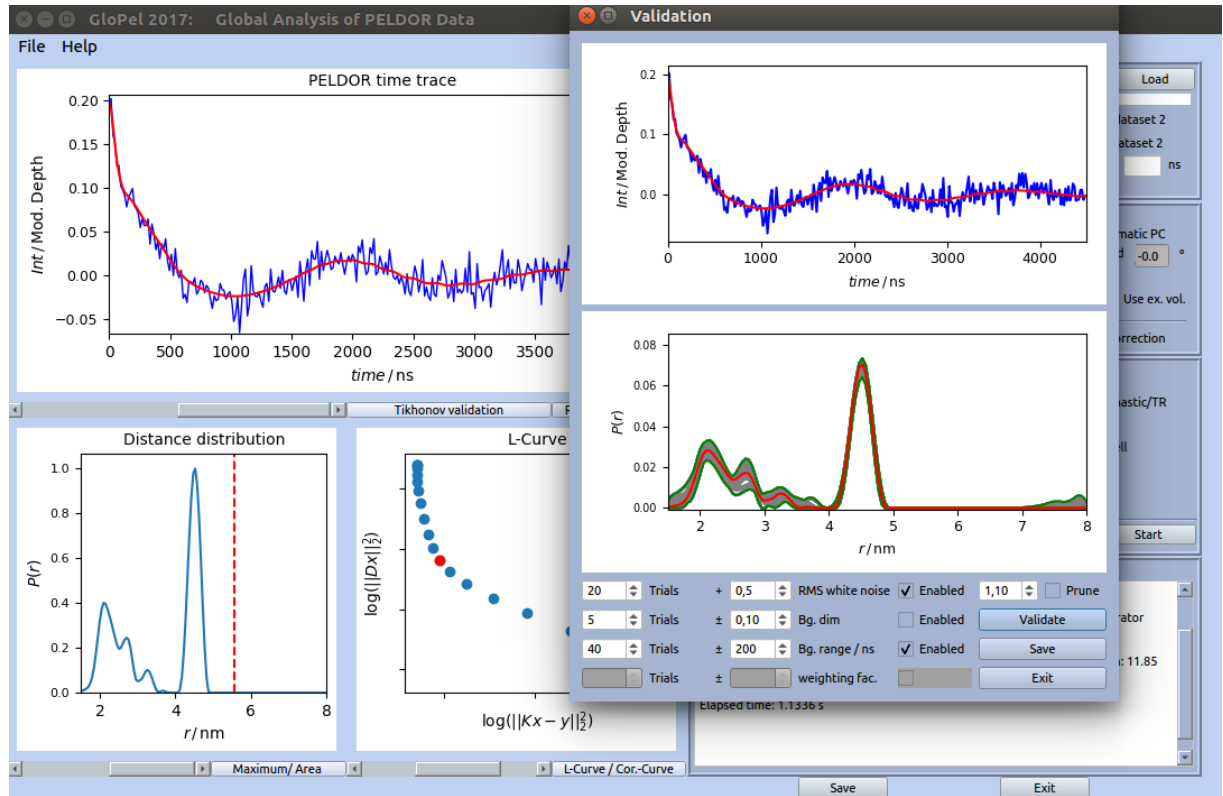


Figure 11: GloPel validation sub-GUI. The validation results can be directly saved.

cedure can be directly saved. In addition to the standard text format output, a PDF report is generated.

In addition to the Tikhonov validation module, the residuals of the Tikhonov or fit solution can be inspected (Figure 12 and Figure 13). Different options are available to inspect the residuals. The residuals can be displayed directly. Alternatively, the autocorrelation

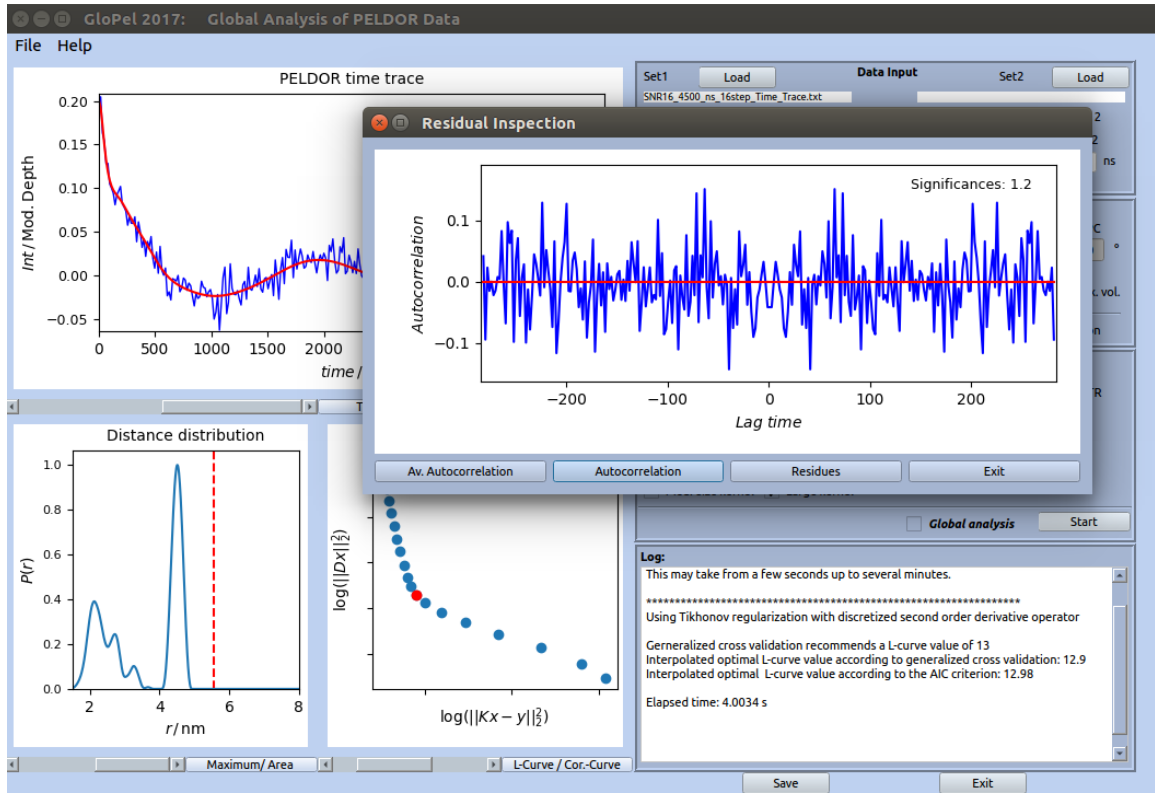


Figure 12: GloPel residual inspection sub-GUI.

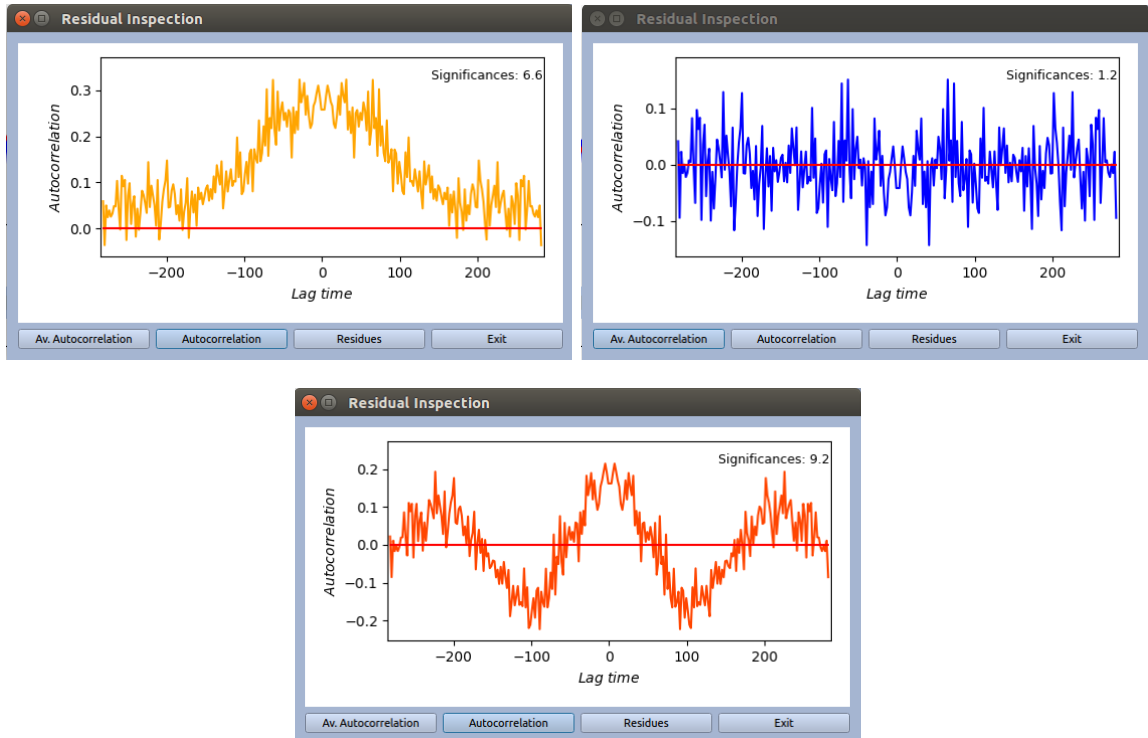


Figure 13: Autocorrelation functions of residuals for different situations. (Top, left) Wrong background choice. (Top, right) Good background choice and correct choice of the regularization parameter. (Bottom) Wrong choice of the regularization parameter (too large α).

function of the residuals (the δ -peak excluded) or the Gaussian-averaged autocorrelation function can be displayed. The shown significances are an arbitrary measure for the deviation of the autocorrelation function of residuals from a normal distribution and should be small for a proper analysis (≤ 5). Especially in a global analysis the residuals should be inspected to check if the used overall background function was a good choice.

2.6 Save the results

The results can be saved after applying an analysis (Tikhonov or model-based fitting). The results are saved in text format files. For a single analysis three different files are save: `_distr.txt`, `_fit.txt`, `_infosheet.txt`. The `_distr.txt` file contains the distance distribution, the `_fit.txt` file the background-corrected time trace as well as the time domain fit. The `_infosheet.txt` file contains information about the analysis and pre-processing settings (Script 2).

Script 2: Information sheet for a data set analyzed using Tikhonov regularization.

```
Infosheet for SNR16_4500_ns_16step_Time-Trace.txt
```

```
Analyzed at: 2019-09-20 10:08:20
```

```
Analyzed with: GloPel Version 1.0.3a
```

```
*****Analysis results*****
```

```
Regularization method: Second order Tikhonov regularization
```

```
L-curve number: 13
```

```
Obtained least-square-deviation = 0.04899378
```

```
*****Presettings*****
```

```
Used zerotime = -22 nm
```

```
Used background start = 1198 nm
```

Used background dimension = 3

Obtained background decay: $k = 0.09982$

Obtained modulation depth: $\lambda = 0.196417$

Manual normalization = $0E-3$

No global background function used

No modulation depth normalization used

Time trace was cut at: 4496.0 nm (Raw data maximum time: 4512.0 nm)

3 Global analysis

GloPel was initially developed for analyzing multiple time traces simultaneously in a global analysis. However, as for the signal-to-noise problem the analysis of more than two time traces brought no further improvement, the GloPel GUI is limited to the global analysis of two time traces. Applications of global analysis are cases, where long and short spin-label distances simultaneously modulate the primary time-domain curves. In these situations we recommend to record echo decays that comprise (i) data points with sufficiently narrow spacing (small step-sizes) within a shorter observation window and good signal-to-noise ratio, and (ii) data with a wider spacing (larger step-sizes) within a wide observation window to probe also longer distances and to provide a reliable background curve for subtraction from both decays.

3.1 Data processing of two time traces

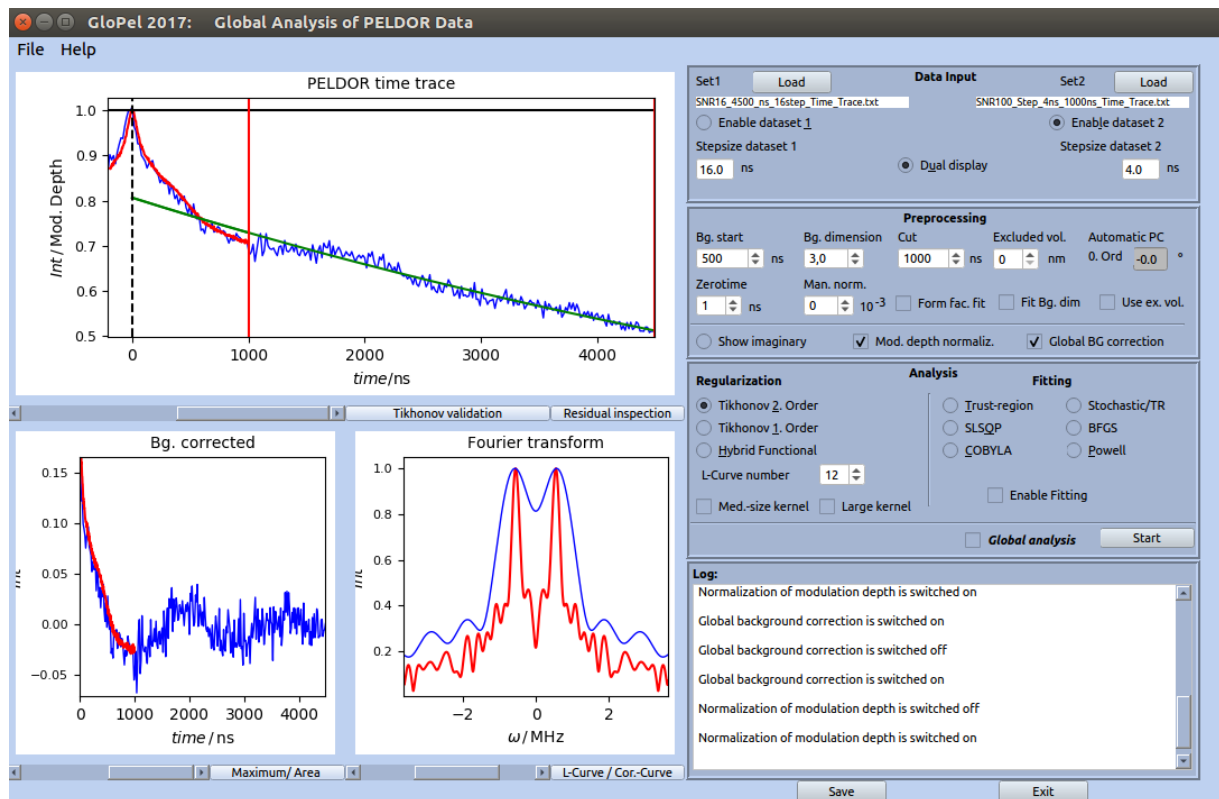


Figure 14: GloPel GUI with enabled dual display.

Two data sets can be loaded into the GloPel GUI and visualized by using the dual display

option (Figure 14). The data processing includes the same steps as for a single time traces with the difference that the modulation depth of the two should be normalized to each other and that the background function should be determined using the time trace with longer acquisition time (global background function or overall background function). A comparison of individual background functions and overall background function is shown in Figure 15.

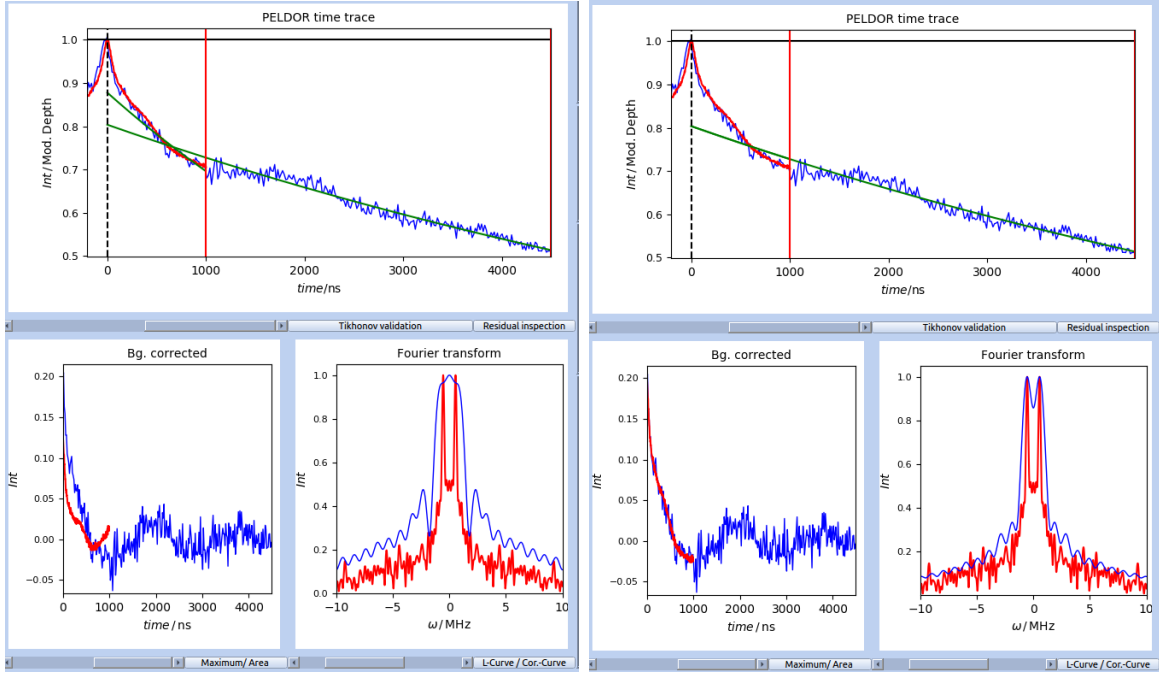


Figure 15: Comparison of data processing using either individual background functions (left) or an overall background function (right). The background function(s) is/are illustrated as green curve(s) in the panel with the PELDOR time traces. The reduced form factor is shown in the panel with the background corrected traces.

3.2 Global analysis of two time traces

Before running a global analysis both time traces need to be analyzed individually. This is necessary because the sum of least-squares from the individual analyses are needed to calculate a weighting factor w for the global analysis. In GloPel a global analysis can be carried out using either Tikhonov regularization or model-based fitting. The global

Tikhonov functional in GloPel is given by:

$$\min \left[\mathbf{P}^T (\mathbf{K}_1^T \mathbf{K}_1 + w \mathbf{K}_2^T \mathbf{K}_2 + \alpha^2 (1 + w) \mathbf{L}^T \mathbf{L}) \mathbf{P} - 2 \mathbf{P}^T (\mathbf{K}_1^T \mathbf{S}_1 + w \mathbf{K}_2^T \mathbf{S}_2) \right] \quad (7)$$

with $P_i \geq 0 \quad \forall i$.

\mathbf{K}_1 and \mathbf{K}_2 are the kernel matrices, \mathbf{L} is the penalty matrix, \mathbf{P} the distance distribution vector, \mathbf{S}_1 and \mathbf{S}_2 the PELDOR time traces, and w the weighting factor. An example for a global Tikhonov regularization is shown in Figure 16. Tikhonov validation for a global

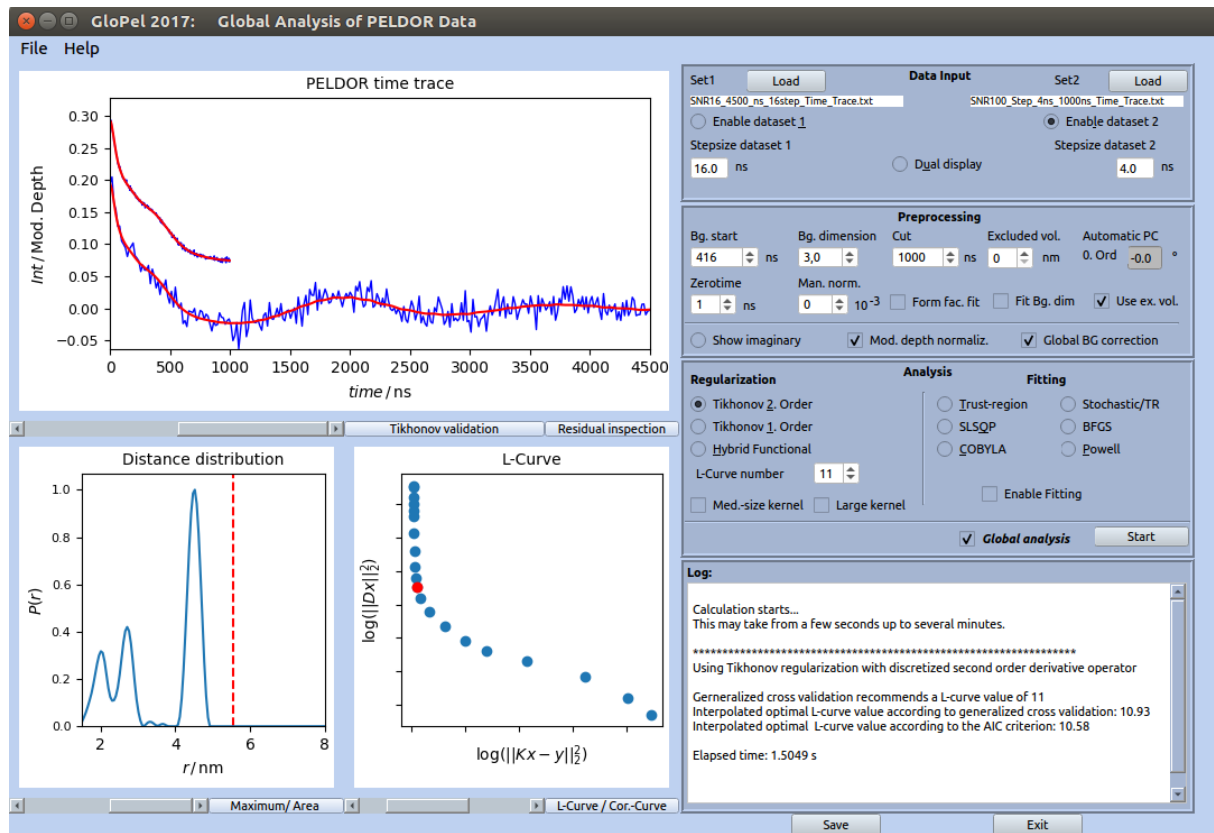


Figure 16: Global Tikhonov regularization carried out with GloPel.

regularization works analog to the Tikhonov validation of an individual analysis except that for a global analysis the weighting factor can be varied in the validation procedure. The procedure for model-based global fitting is identical to the one described for an individual analysis. It is recommended to use the residual inspection after applying a global analysis (Figure 17)

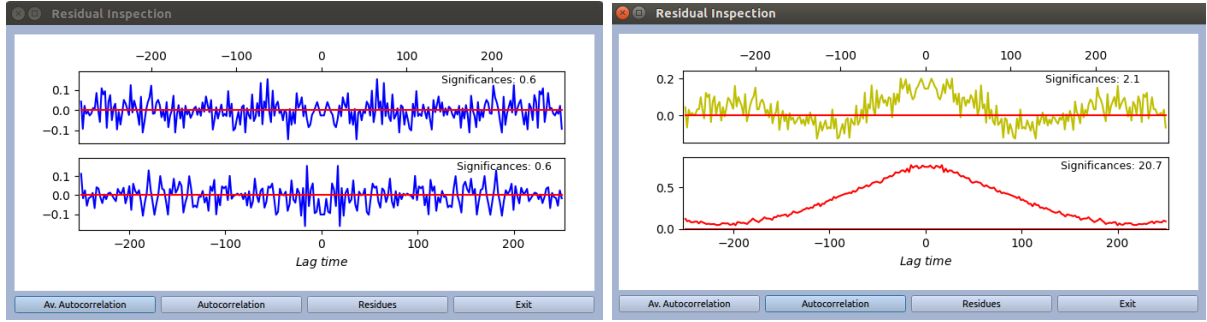


Figure 17: Comparison of the autocorrelation functions of residuals obtained by a global Tikhonov regularization using different background correction functions. (Left) Proper choice of the overall background function. (Right) Bad choice of the overall background function.

References

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