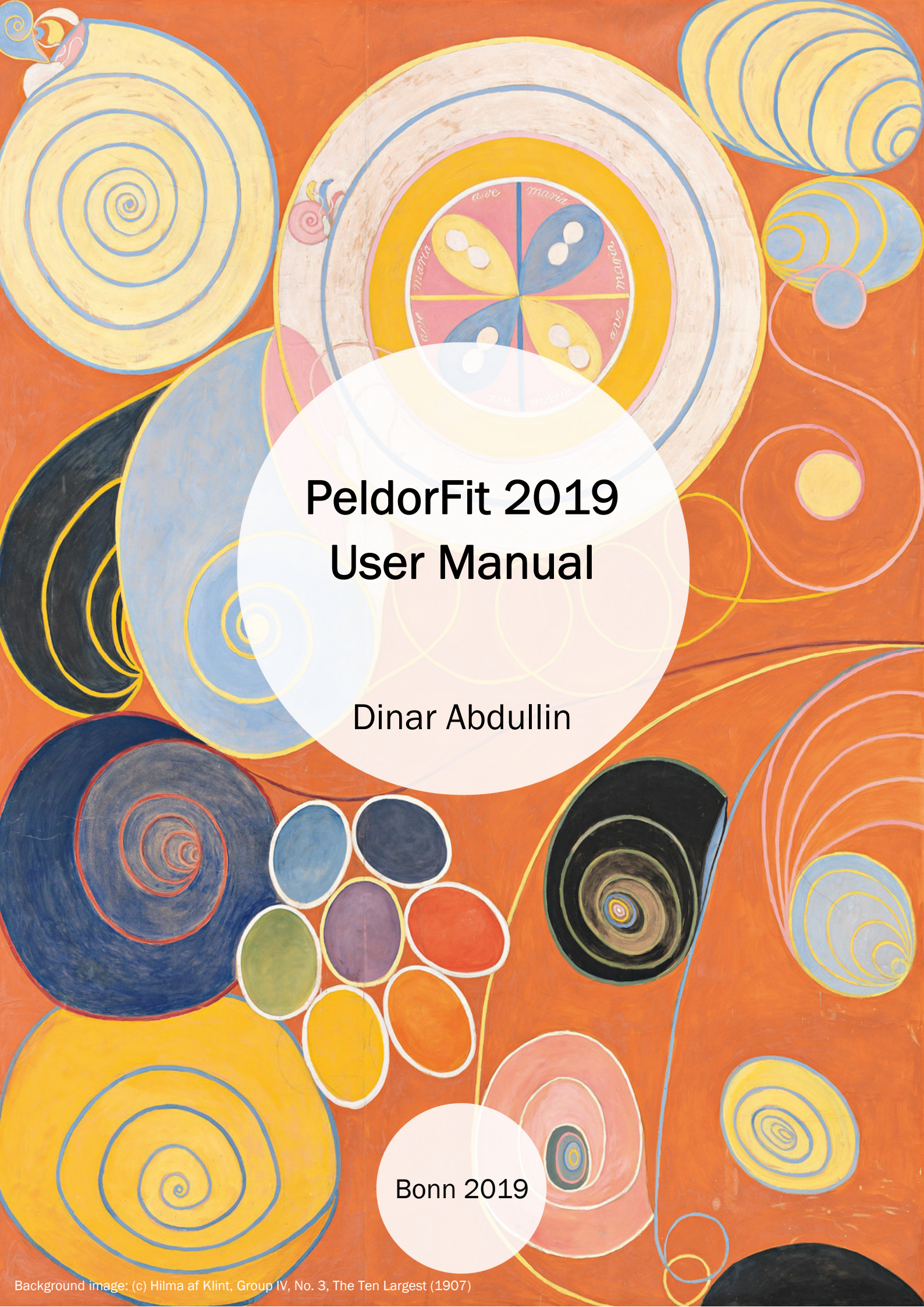

PeldorFit 2019
User Manual

Dinar Abdullin

Bonn 2019

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1 Introduction

1.1 What is it for?

A program PeldorFit is developed for analysis of orientation-selective Pulsed ELectron-electron Double Resonance (PELDOR[1] or DEER[2]) data. PELDOR data is called to be orientation-selective if it is depends on the relative orientation of electron spin centers involved in the PELDOR experiment. Thus, orientation-selective PELDOR data encodes information about the distances and relative orientations between electron spin centers. In order to extract this information, several PELDOR time traces with different pump/detection positions are usually acquired and then analyzed all together. The latter analysis is the subject of PeldorFit.

The idea underlying PeldorFit is a fitting of PELDOR time traces using a geometric model of a spin system. The geometric model consists of a defined set of parameters, which describe the distances and relative orientations between electron spin centers. The detailed description of these parameters is given in Chapter 1.2. As the parameters of the geometric model are essentially fitting parameters, their values are optimized by PeldorFit until the simulated PELDOR time traces provide the best agreement with the experimental ones. This optimization procedure is done by means of a genetic algorithm. More details about the optimization procedure can be found in Ref. [3].

1.2 Geometric models of a spin system

Since there is an infinite number of possible spin system geometries, it is impossible to describe all spin systems with a single geometric model. Therefore, PeldorFit is restricted to three particular cases:

1) a two-spin system with a unimodal distance and angle distributions (Model 1),
2) a two-spin system with a bimodal distance and angle distributions (Model 2),
3) a three-spin system with a unimodal distance and angle distributions (Model 3).

Each of these cases has its own model, which is described in the following.

Model 1. A first model used to describe the geometry of a two-spin system is shown in Figure 1.1. It consists of two coordinate frames associated with the *g*-tensor axes of two spin centers, named here spin A and spin B. Both spins are assumed to be point objects, i.e., spin delocalization is neglected. The reference coordinate system of the model is set to be coincident with the *g*-tensor axes of spin A. The orientation of the *g*-tensor axes of spin B is given by three Euler angles (α, β, γ) in accordance with the *z-x'-z"* convention. The vector connecting two spins is described by three spherical coordinates: a length *r,* a polar angle ξ, and an azimuthal angle φ. Thus, the relative orientation of the spins is described by six parameters (r, ξ, φ, α, β, γ). In order to account for the flexibility of a spin-carrying molecule, all six parameters are allowed to have either a uniform distribution or a Gaussian distribution. In both cases, the distributions are described by two parameters, a mean value and a width (Figure 1.2). In the case of the Gaussian distribution, a standard deviation is used as a width parameter. Thus, in total 12 parameters are used to describe a non-rigid spin pair, ($\langle r \rangle$, Δr, $\langle \xi \rangle$, $\Delta \xi$, $\langle \varphi \rangle$, $\Delta \varphi$, $\langle \alpha \rangle$, $\Delta \alpha$, $\langle \beta \rangle$, $\Delta \beta$, $\langle \gamma \rangle$, $\Delta \gamma$). Note that the model neglects the correlation between the values of individual geometric parameters. Strictly speaking, this assumption does not necessary hold for every molecular system. However, the correlation between individual geometric parameters is usually unknown and, therefore, is omitted in the PeldorFit analysis.

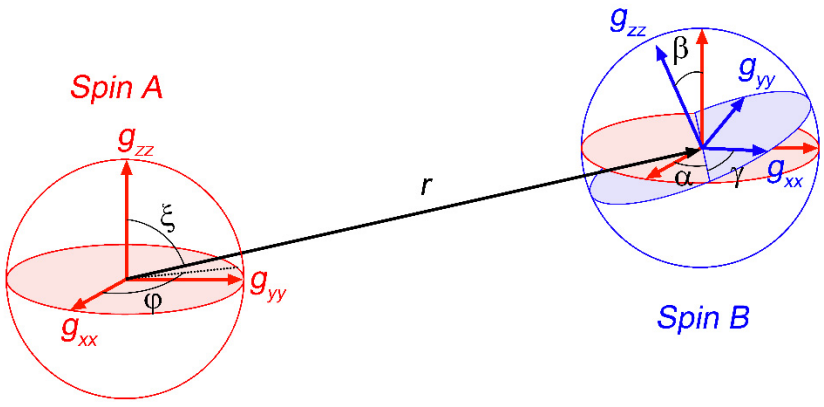

Figure 1.1. A PeldorFit model of a two-spin system.

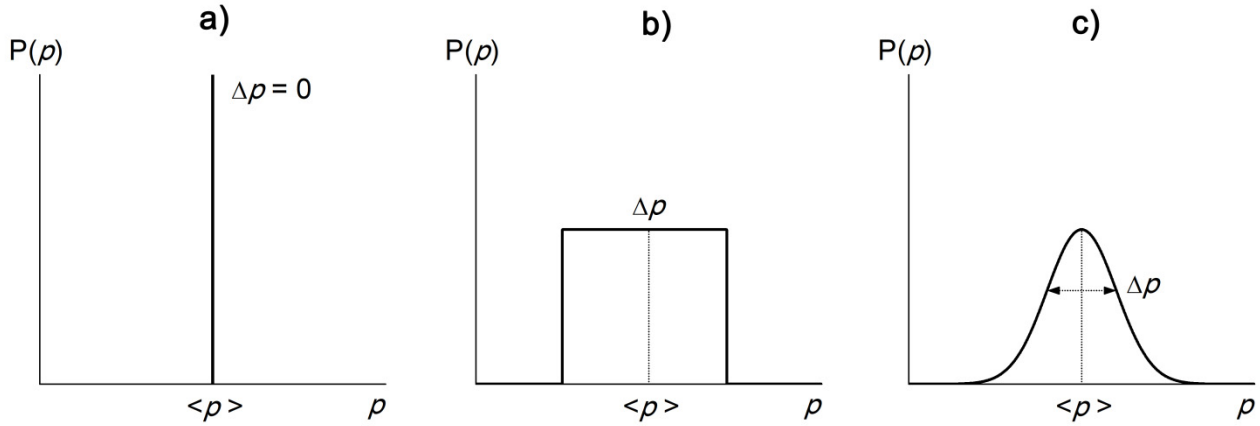

Figure 1.2. The types of distributions used for the PeldorFit parameters. a) A single value <*p*>. b) Uniform distribution with a mean value <*p*> with a width Δ*p*. c) Normal distribution with a mean value <*p*> and a standard deviation Δ*p*. Here, *p* denotes one of six geometric parameters (*r, ξ, φ, α, β, γ*).

Model 2. The next model also describes the geometry of a two-spin system but, in contrast to Model 1, it assumes that the corresponding distance and angle distributions are bimodal. This model might be useful in cases when a spin-carrying molecule has two different conformations or when one of the spin centers, e.g., a spin label, has two different conformations. In order to describe two different conformations of a spin pair, Model 1 needs to be extended by introducing the second set of geometric parameters *r, ξ, φ α, β,* and *γ*. Thus, Model 2 consist of twelve geometric parameters, (r_1, ξ_1, φ_1, α_1, β_1, γ_1, r_2, ξ_2, φ_2, α_2, β_2, γ_2), and one additional parameter *w*, which describes the relative weight of both conformations (Figure 1.3). In complete analogy to the Model 1, each of the geometric parameters of Model 2 can have a single value, a uniform distribution, or a normal distribution (Figure 1.2). Thus, the total number of parameters can be up to 25.

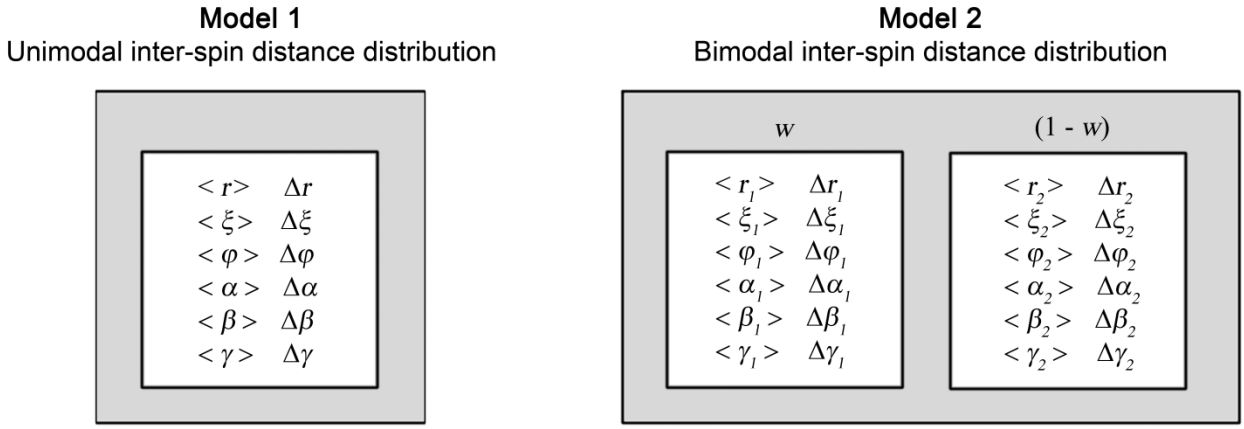

Figure 1.3. The set of geometric parameters used by Model 1 and Model 2.

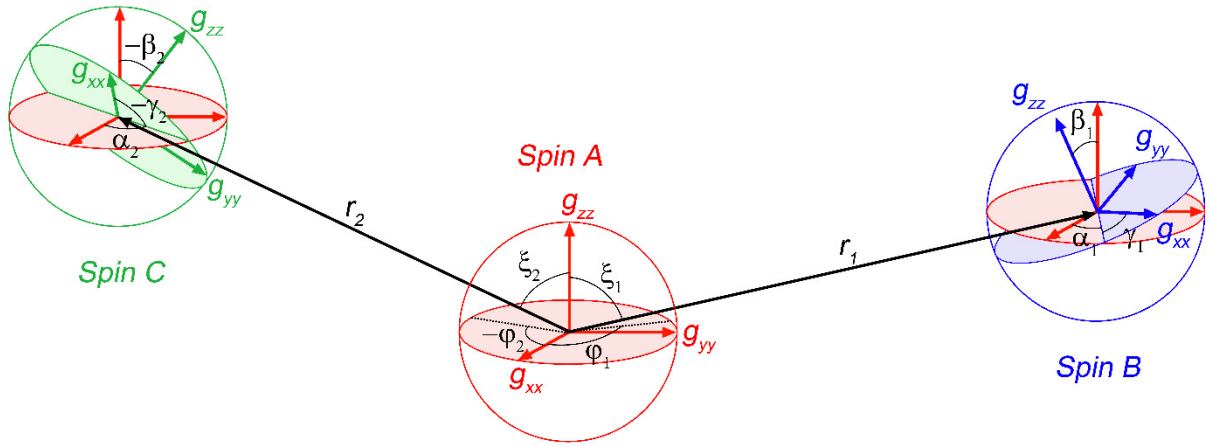

Figure 1.4. A PeldorFit model of a three-spin system.

Model 3. To date, PELDOR studies were applied not only to two-spin systems but also to systems with more than two spins. However, determination of distance distributions from the PELDOR data of multi-spin systems becomes increasingly difficult as the number of spins *per* molecule increase. Therefore, PeldorFit is restricted only to the case of three-spin systems. The geometric model for three-spin systems is depicted in Figure 1.4. This model uses the same set of parameters and assumptions as Model 1. The only difference to Model 1 is the third spin, called here spin C. The reference coordinate system of the model is set again to be coincident with the *g*-tensor axes of spin A. The position and orientation of spins B and C are described by two sets of parameters, (r_1, ξ_1, φ_1, α_1, β_1, γ_1) and (r_2, ξ_2, φ_2, α_2, β_2, γ_2), respectively. As before, each of these parameters can have either a single value, a uniform distribution, or a normal distribution (Figure 1.2). As a result, the model might include up to 24 parameters.

It is important to note that the values of the geometric parameters of Model 3 may depend on the assignment of spin centers to spins A, B, and C, respectively. This can be readily demonstrated on the example of three spin centers that are located at different corners of an irregular triangle. As can be seen from Figure 1.5, the same geometry of the three-spin system can be described in six different ways using the same geometric parameters (only r_1 and r_2 are shown). PeldorFit provides only one of those geometries, assuming that other geometries can be readily deduced when needed.

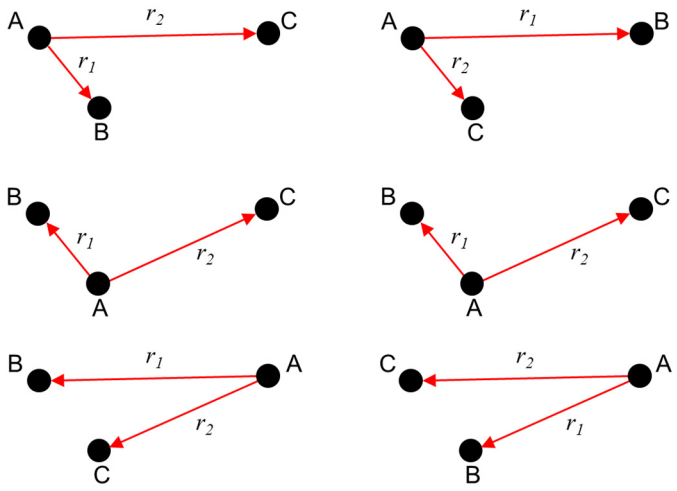

Figure 1.5. Different ways of assignment of three spin centers to spins A, B, and C, respectively.

1.3 Symmetry considerations

For simplicity, symmetry considerations will be discussed with regard to Model 1. Note that the same considerations are also valid for the more complex Models 2 and 3. Depending on the symmetry of the magnetic tensors (*g*- and *A*-tensors) of spins A and B, all five angular parameters (ξ, φ, α, β, γ) or only a subset of them are required to simulate PELDOR time traces. A summary of different spin center's symmetries and corresponding angular parameters is given in Table 1.1. For example, if the magnetic tensors of both spins are rhombic, the full set of angles is required. In contrast, three angles are sufficient when the magnetic tensors of both spins are axial. In this case, the angles φ and γ can be excluded from consideration. Thus, if the magnetic tensors of spins A and B are axial or isotropic, the number of fitting parameters in PeldorFit can be reduced.

In addition, due to the inversion symmetry of the magnetic tensors, there is a number of relative orientations of spin centers, which provide identical PELDOR time traces. If each spin is

Table 1.1. Number and ranges of the angular parameters ξ, φ, α, β, and γ required to simulate the PELDOR time traces in the cases of isotropic, axial, and rhombic spins A and B. Adapted from Ref. [3].

Spin A	Spin B	ξ	φ	α	β	γ
axial	isotropic	[0°, 90°]	-	-	-	-
axial	axial	[0°, 90°]	-	[0°, 180°]	[0°, 90°]	-
rhombic	isotropic	[0°, 90°]	[0°, 180°]	-	-	-
rhombic	axial	[0°, 90°]	[0°, 180°]	[0°, 180°]	[0°, 90°]	-
rhombic	rhombic	[0°, 90°]	[0°, 180°]	[0°, 180°]	[0°, 90°]	[0°, 180°]

considered as a point object, the 180° rotation of one of its *g*-tensor axes does not influence the shape of PELDOR time traces. In total 16 combinations of such axes rotations are possible for a two-spin system. Thus, each set of angles (ξ, φ, α, β, γ) has 15 symmetry-related sets of angles, which cannot be distinguished by fitting PELDOR data. In order to account for this, PeldorFit calculates all 16 symmetry-related sets of angles in every fitting run.

Finally, if both spins are spectroscopically identical, e.g., if they are both nitroxides, there is an additional uncertainty in assignment of these spins to spin A and spin B. Depending on which of the identical spins is assigned to spin A, two different sets of angles (ξ, φ, α, β, γ) can be obtained. These sets of angle are however inter-convertible, when one exchanges spin A with spin B and *vice versa*. Therefore, PeldorFit calculates only one of those sets.

1.4 Technical information

PeldorFit is a console C++ application. The source code of the program can be found at

https://github.com/dinarabdullin/PeldorFit2019

The Linux and Windows executables of the program can be found at

https://github.com/dinarabdullin/PeldorFit2019/releases

The program is free and can be distributed under GNU General Public License.

To speed up the calculations, it is recommended to run the program on a hardware with few hundreds CPUs. All examples in this manual were calculated using a 64-core workstation from sys-Gen GmbH with 2.3 GHz processor frequency and 132 GB RAM.

2 Installation

Download the zip-archive "PeldorFit2019.zip" from the web page

https://github.com/dinarabdullin/PeldorFit2019/releases

and unzip it to a directory where the program will be stored. The archive contains two folders with the compiled executable files for Linux and Windows. That is it!

3 Running the program

1. Open Terminal (Linux) or Command Prompt (Windows).
2. Go to the directory in which the PeldorFit executable file is stored:
   cd .../PeldorFit2019/Linux (for Linux)
   cd .../PeldorFit2019/Windows (for Windows)
3. Set the permission properties (only for Linux):
   chmod 755 peldorfit
4. Prepare a configuration file (Chapter 4) and run the program by the following command:
   sh PeldorFit.sh .../config.cfg (for Linux)
   PeldorFit.exe .../config.cfg (for Windows)
   The examples of the configuration file can be found in the *Examples* folder of the program. The description of these examples is given in Chapter 6.

4 Configuration file

A PeldorFit configuration file contains all input parameters of the program. It has a usual ASCII data format and a *.cfg* extension. The configuration file can be created and edited with common text editors, such as Notepad++. Since the number of input parameters in a single configuration file is typically quite large, it is highly recommended to use the configuration files from Examples as a template to build your own configuration file. This will save time and reduce the probability of mistakes.

Each PeldorFit configuration file consists of five main parts:

1) experimental data and settings,
2) parameters of the spin system,
3) optimization parameters,
4) optimization settings,
5) output settings.

Each of these parts is described in detail below. Note that comment lines in the configuration file are indicated by symbols # or //. The comment lines, as well as empty lines, are ignored by PeldorFit. Here is an example of comment lines:

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
#---------------------- PeldorFit2018 Configuration File ----------------------


# Experimental parameters
#------------------------
// filename          : name of the data file
// detPiLength       : length of the detection pi-pulse (ns)
// detPiHalfLength: length of the detection pi/2-pulse (ns)
// pumpPiLength   : length of the pump pi-pulse (ns)
// detFreq           : detection frequency (GHz)
// pumpFreq          : pump frequency (GHz)
// magnField         : magnetic field (T)
```

4.1 Experimental data and settings

The first part of the configuration file specifies PELDOR data and corresponding experimental settings. This information is combined together in a list called experimentals:

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
experimentals = (
        {filename = "Examples/unimodal_nitroxide_nitroxide/bisnitroxide_XX.dat";
        detPiLength = 14;
        detPiHalfLength = 7;
        pumpPiLength = 14;
        detFreq = 93.999600;
        pumpFreq = 93.930000;
        magnField = 3.3415; },

        {filename = "Examples/unimodal_nitroxide_nitroxide/bisnitroxide_YY.dat";
        detPiLength = 14;
        detPiHalfLength = 7;
        pumpPiLength = 14;
        detFreq = 93.999600;
        pumpFreq = 93.930000;
        magnField = 3.3443; },

        ...
);
```

In this list, information about each individual PELDOR experiment is enclosed in curly brackets. Within the curly brackets, the following parameters have to be defined:

filename	A path to a data file which contains a background-subtracted PELDOR time trace. The first column of this file should contain time points in microseconds. The second column should contain the values of a signal normalized to 1.
detPiLength	The length of a detection π-pulse in [ns].
detPiHalfLength	The length of a detection π/2-pulse in [ns].
pumpPiLength	The length of a pump pulse in [ns].
detFreq	The frequency of detection pulses in [GHz].
pumpFreq	The frequency of a pump pulse in [GHz].

magnField The value of a magnetic field in [T].

Note that all commas and semicolons appearing in experimentals are important for reading out the configuration file by PeldorFit.

4.2 Parameters of the spin system

The second part of the configuration file specifies spectroscopic parameters of spin centers. First, one has to set the number of spins to either 2 or 3. In case of three-spin systems, one has to specify additionally whether the multi-spin effects[4,5] need to be taken into account or not. The multi-spin effects can be usually neglected when the modulation depths of all PELDOR time traces are low.[6]

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
nSpins = 2;
multiSpinEffects = 0;
```

Here,

nSpins A number of spin centers. It can be either 2 or 3.

multiSpinEffects If set to 0, the multi-spin effects will be neglected.
If set to 1, the multi-spin effects will be taken into account for three-spin systems.

Next, the spectroscopic parameters of each of the spins are defined. For definiteness, the spins are denoted as spinA, spinB, and spinC (required only for three-spin systems):

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
spinA:
{
        g = [2.0104, 2.0073, 2.0033];
        gStrain = [0.0004, 0.0003, 0.0001];
        n = [1];
        I = [1.0];
        A = [8.0, 6.0, 96.0];
        AStrain = [0.0, 0.0, 12.0];
        lwpp = 22.4;
};

spinB:
{
        g = [2.0104, 2.0073, 2.0033];
        gStrain = [0.0004, 0.0003, 0.0001];
        n = [1];
        I = [1.0];
        A = [8.0, 6.0, 96.0];
        AStrain = [0.0, 0.0, 12.0];
        lwpp = 22.4;
};
```

PeldorFit assumes that the resonance frequencies of spin centers are determined by a Zeeman interaction, a hyperfine interaction of electron spins and nuclear spins, and an inhomogeneous line broadening. The unresolved anisotropy of *g* and hyperfine interaction (*A*) tensors can also be taken into account via *g*-strain and *A*-strain, respectively. The naming of spectroscopic parameters is consistent with the program EasySpin (www.easyspin.org):

g — *g*-factor. It should always to contain 3 components:
g = [g_{xx}, g_{yy}, g_{zz}] in the case of orthorhombic g-factor;
g = [$g_{\perp}$, $g_{\perp}$, $g_{\parallel}$] in the case of axial g-factor;
g = [g_{iso}, g_{iso}, g_{iso}] in the case of isotropic g-factor.

gStrain — *g*-strain (the unresolved anisotropy of the g-factor). It should always to contain 3 components: gStrain = [Δg_{xx}, Δg_{yy}, Δg_{zz}]. If not needed, the notation gStrain = [] should be used.

n — A number of equivalent magnetic nuclei coupled to electron spin. Up to two different types of equivalent nuclei can be used:
n = [] : no nuclear spins are coupled to the electron spin.
n = [n_1] ($n_1 > 0$) : n_1 nuclear spins with I_1 and A = [A_{1xx}, A_{1yy}, A_{1zz}] are coupled to the electron spin.
n = [n_1, n_2] (n_1, $n_2 > 0$) : n_1 nuclear spins with I_1 and A_1 = [A_{1xx}, A_{1yy}, A_{1zz}] and n_2 nuclear spins with I_2 and A_2 = [A_{2xx}, A_{2yy}, A_{2zz}] are coupled to the electron spin.

I — A nuclear quantum number(s) of a nucleus (nuclei) coupled to an electron spin. This parameter is connected to n:
I = [] if n = [];
I = [I_1] if n = [n_1]
I = [I_1, I_2] if n = [n_1, n_2]

A — *A*-tensor in [MHz]. It should consist of 0, 3 or 6 components in case of 0, 1 or 2 two types of equivalent nuclei, correspondingly. This parameter is connected to n:
A = [] if n = [];
A = [A_{1xx}, A_{1yy}, A_{1zz}] if n = [n_1]
A = [A_{1xx}, A_{1yy}, A_{1zz}, A_{2xx}, A_{2yy}, A_{2zz}] if n = [n_1, n_2]

AStrain — *A*-strain in [MHz] (the unresolved anisotropy of the A-tensor). It applies only to the first specified sort of nuclei and should contain 3 components: AStrain = [ΔA_{1xx}, ΔA_{1yy}, ΔA_{1zz}]. If not needed, the notation AStrain = [] should be used.

lwpp — A peak-to-peak linewidth in [MHz].

4.3 Optimization parameters

The third part of the configuration file specifies which geometric model of a spin system (see Chapter 1.2) will be used and which parameters of the chosen model will to be optimized. For this information, the configuration file contains a list called parameters:

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
parameters = (
   {opt = 1;  mode = 1;  range = [ 1.50,  2.50],  value = 0.0},     // Parameter 1 : r1 mean
   {opt = 1;  mode = 1;  range = [ 0.00,  0.10],  value = 0.0},     // Parameter 2 : r1 width
   {opt = 1;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 3 : xi1 mean
   {opt = 1;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 4 : x1 width
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 5 : phi1 mean
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 6 : phi1 width
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 7 : alpha1 mean
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 8 : alpha1 width
   {opt = 1;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 9 : beta1 mean
   {opt = 1;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 10: beta1 width
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 11: gamma1 mean
   {opt = 1;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 12: gamma1 width
   {opt = 0;  mode = 1;  range = [ 1.50,  2.50],  value = 0.0},     // Parameter 13: r2 mean
   {opt = 0;  mode = 1;  range = [ 0.00,  0.10],  value = 0.0},     // Parameter 14: r2 width
   {opt = 0;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 15: xi2 mean
   {opt = 0;  mode = 0;  range = [  0.0,  90.0],   value = 0.0},    // Parameter 16: xi2 width
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 17: phi2 mean
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 18: phi2 width
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 19: alpha2 mean
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 20: alpha2 width
   {opt = 0;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 21: beta2 mean
   {opt = 0;  mode = 0;  range = [  0.0,  90.0],  value = 0.0},     // Parameter 22: beta2 width
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 23: gamma2 mean
   {opt = 0;  mode = 0;  range = [  0.0, 180.0],  value = 0.0},     // Parameter 24: gamma2 width
   {opt = 0;  mode = 0;  range = [ 0.00,  1.00],  value = 0.0},     // Parameter 25: ratio between r1 and r2
   {opt = 0;  mode = 0;  range = [ 0.00,  5.00],  value = 0.0},     // Parameter 26: J mean
   {opt = 0;  mode = 0;  range = [ 0.00,  5.00],  value = 0.0},     // Parameter 27: J width
   {opt = 1;  mode = 0;  range = [ 0.85,  0.95],  value = 0.0}      // Parameter 28: scale factor for mod. depth
);
```

In this list, each text line corresponds to one of the generalized parameters of all three available geometric models. For definiteness, all generalized parameters are numbered in a pre-defined manner and enter the parameters list in accordance to their serial number (the user cannot change the order). Table 4.1 lists the serial numbers of all generalized parameters and provides the description of each parameter. Let's briefly go through the definitions of all parameters. The parameters no. 1-12 correspond to the mean values and distribution widths of r_1, ξ_1, φ_1, α_1, β_1, and γ_1. These geometric parameters are used by all three geometric models of PeldorFit (Chapter 1.2). The parameters no. 13-24 correspond to the mean values and distribution widths of r_2, ξ_2, φ_2, α_2, β_2, and γ_2. These parameters are relevant only for Models 2 and 3 (Chapter 1.2). The parameter no. 25 is needed only for Model 2 and describes the weight of the first conformation relative to the second conformation (w in Figure 1.3).

Table 4.1. The generalized parameters / optimization parameters of PeldorFit.

No. of parameter	Parameter description[a]	Units	Ranges	Modes[b]
1	$<r_1>$	nm	Any reasonable ranges inside [1.5, 8.0]	
2	Δr_1	nm	Any reasonable ranges inside [1.5, 8.0]	
3	$<\xi_1>$	degrees	[0, 90]	
4	$\Delta\xi_1$	degrees	[0, 90] for mode = 0 [0, 30] for mode = 1	
5	$<\varphi_1>$	degrees	[0, 180]	
6	$\Delta\varphi_1$	degrees	[0, 180] for mode = 0 [0, 60] for mode = 1	
7	$<\alpha_1>$	degrees	[0, 180]	
8	$\Delta\alpha_1$	degrees	[0, 180] for mode = 0 [0, 60] for mode = 1	
9	$<\beta_1>$	degrees	[0, 90]	
10	$\Delta\beta_1$	degrees	[0, 90] for mode = 0 [0, 30] for mode = 1	
11	$<\gamma_1>$	degrees	[0, 180]	
12	$\Delta\gamma_1$	degrees	[0, 180] for mode = 1 [0, 60] for mode = 2	mode = 0: uniform distribution mode = 1: normal distribution
13	$<r_2>$	nm	Any reasonable ranges inside [1.5, 8.0]	
14	Δr_2	nm	Any ranges inside [1.5, 8.0]	
15	$<\xi_2>$	degrees	[0, 90]	
16	$\Delta\xi_2$	degrees	[0, 90] for mode = 0 [0, 30] for mode = 1	
17	$<\varphi_2>$	degrees	[0, 180]	
18	$\Delta\varphi_2$	degrees	[0, 180] for mode = 0 [0, 60] for mode = 1	
19	$<\alpha_2>$	degrees	[0, 180]	
20	$\Delta\alpha_2$	degrees	[0, 180] for mode = 0 [0, 60] for mode = 1	
21	$<\beta_2>$	degrees	[0, 90]	
22	$\Delta\beta_2$	degrees	[0, 90] for mode = 0 [0, 30] for mode = 1	
23	$<\gamma_2>$	degrees	[0, 180]	
24	$\Delta\gamma_2$	degrees	[0, 180] for mode = 1 [0, 60] for mode = 2	
25	w		(0, 1)	
26	$<J>$	MHz	Any reasonable ranges	mode = 0: uniform distribution
27	ΔJ	MHz	Any reasonable ranges	mode = 1: normal distribution
28	η		(0, 1]	mode = 0: η is the same for all simulated PELDOR time traces mode = 1: η is different for each PELDOR signal time traces

[a] For more details see Chapter 1.2.

[b] The modes are relevant only if the parameter is optimized (opt = 1) or has a fixed value (opt = 2).

In addition to the geometric parameters, PeldorFit allows to account for exchange coupling between spin centers. Thus, the parameters no. 26 and 27 correspond to a mean value and a distribution width of an exchange coupling constant *J*. Note that *J* can have the same types of distribution as all geometric parameters of the model (see Figure 1.2). The last parameter with no. 28 is a scale factor for the modulation depth η ($0 < \eta \leq 1$). This parameter is often very useful for reproducing experimental modulation depths of PELDOR time traces, because it allows taking into account imperfections of a pump pulse, incomplete spin labelling, etc.

Now let's return to the discussion of the parameters list. Each line of this list corresponds to one of the generalized parameters and contains four settings called opt, mode, range, and value. The most important setting is opt, since it determines whether the corresponding parameter will be included into the final model or not:

opt
: If opt = 0, the parameter is excluded from the final model.
: If opt = 1, the parameter is included to the final model and will be optimized.
: If opt = 2, the parameter is included to the final model and will have a constant value (will not be optimized).

Using the opt setting, one can select the set of parameters that are relevant for PELDOR simulations and exclude all other parameters.

In the case of opt = 0, the values of other three settings (mode, range, and value) are irrelevant. The value of the corresponding parameter will be set automatically to 0.

In the case of opt = 1, the values of mode and range settings have to be specified in accordance to Table 4.1. For all geometric parameters and the *J* coupling constant, the statement mode = 0 sets the distribution type of the corresponding parameter to a uniform distribution, and the statement mode = 1 to a normal distribution. This definition differs, however, for the scale factor η: Here, mode = 0 means that a single value of η will be optimized for all PELDOR time traces, whereas in case of mode = 1 the value of η will be optimized for each PELDOR time trace separately. The range setting sets the lower and the upper bounds of the corresponding optimized parameter. The user is free to choose the bounds for each of the parameters. However, it is recommended to use the bounds given in Table 4.1.

Finally, in the case of opt = 2, the corresponding parameter will be set to a constant value, which is specified in value. Before typing the value of the fixed parameter into the value field, make sure that this value is within the parameter's bounds given in Table 4.1.

4.4 Optimization settings

Optimization of a selected model and corresponding parameters is performed by a genetic algorithm. This algorithm has been shown to be very efficient when one deals with a large number of optimization parameters and needs to find a global minimum.[7–9] Importantly, a genetic algorithm has its own internal parameters, which determine its ability to find a global minimum. Optimal values of these parameters may vary depending on a particular PELDOR data set. Therefore, the configuration file allows user to set these values manually. However, it is highly recommended to use the internal parameters from the configuration files given in Examples (at least as starting values), because the latter ones were obtained after extensive tests of the genetic algorithm on several PELDOR data sets.

The intrinsic parameter of the genetic algorithm are stored in the list genetic:

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
genetic:
{
        num_generations_max = 500;
        size_generation = 192;
        prob_crossover = 0.5;
        prob_mutation = 0.01;
        merit_function = 1;
        num_averages = 100000;
};
```

Here,

num_generations_max — The total number of optimization steps (generations). This parameter determines the convergence of the genetic algorithm to a global minimum. The convergence can be monitored by plotting a goodness-of-fit vs. optimization step (see the output file score.dat). The values of a merit function have to be constant for the last 100 optimization steps. Otherwise, the value of num_generations_max has to be increased.

size_generation — The number of parameter sets (chromosomes) that are evaluated simultaneously by the genetic algorithm. A good practice is to set this parameter to 10 x (the number of fitting parameters).

prob_crossover — A crossover rate.

prob_mutation — A mutation rate. If the convergence of the genetic algorithm is achieved but the fit to PELDOR time traces is not satisfactory, one can try to increase this parameter.

merit_function	A merit function, which is used to judge how good simulated PELDOR time traces fit to corresponding experimental PELDOR time traces (i.e., to calculate a goodness-of-fit). If merit_function = 1, the merit function is a root-mean-square-deviation (RMSD). If merit_function = 3, the merit function is a Pearson product moment correlation coefficient (PCC). If merit_function = 2, the merit function is RMSD/PCC.
num_averages	The number of Monte-Carlo averages used to simulate PELDOR signals. It determines how accurate the powder averaging will be done. (This value should not be lower than 10^5.) This parameter also effects the duration of optimization.

4.5 Output settings

The last part of the configuration file specifies what information will be output by PeldorFit after the fitting is completed. The output settings are given in a list called output:

Taken from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
output:
{
        directory = "Examples/unimodal_nitroxide_nitroxide/Results/";
        record_spectrum = 0;
        record_score = 1;
        record_parameters = 1;
        record_fit = 1;
        record_symmetric_solutions = 1;
        record_error_plot = 1;
        error_plot_variables = ([1,2],[3,4],[5,6],[7,8],[9,10],[11,12]);
};
```

The output list contains the following settings:

directory	A path to a disk space where the output data will be stored.
record_score	If set to 1, a goodness-of-fit is recorded in dependence of optimization step. Then, this dependence is saved into score.dat.
record_fit	If set to 1, experimental and simulated PELDOR time traces are saved into fit.dat.
record_parameters	If set to 1, optimized parameters of a geometric model are saved into parameters.dat.
record_symmetric_solutions	If set to 1, symmetry-related sets of angular parameters (see Chapter 1.3) are saved into symmetric_parameters.dat.

record_error_plot — If set to 1, a goodness-of-fit is recorded in dependence of fitting parameters specified in error_plot_variables. Here, this dependence is denoted as an error plot, because it is usually depicted graphically and provides error estimates for optimized fitting parameters. Usually, each error plot is recorded just for one or two fitting parameters, whereas all other fitting parameters are set to their optimized values. All error plots are then saved into error_plot_X.dat or errorplot_X_Y.dat, where X and Y are replaced by the serial numbers of variables. For example, error_plot_1.dat will contain the dependence of a goodness-of-fit on the parameter $<r_1>$, and error_plot_3_4.dat will contain the dependence of a goodness-of-fit on the parameters $<\xi_1>$ and $\Delta\xi_1$.

error_plot_variables — The variables of error plots. The variables of each individual error plot are given is square brackets. To specify the variables, the serial numbers of generalized parameters have to be used (see Table 4.1). For example, a notation error_plot_variables = ([1], [3], [5]) means that three error plots will be recorded for the parameters $<r_1>$, $<\xi_1>$, and $<\varphi_1>$, respectively. The notation error_plot_ variables = ([1, 2], [3, 4]) means that two error plots will be recorded for two pairs of parameters, ($<r_1>$, Δr_1) and ($<\xi_1>$, $\Delta\xi_1$), respectively.

error_plot_size — The number of points in a single error plot.

record_spectrum — If set to 1, an EPR spectrum of a spin system is calculated using the parameters of SpinA, SpinB, and SpinC (if included). The spectrum is computed in the frequency domain (in GHz), and the magnetic field is set to the magnField value of the first experiment listed in experimentals. After the calculation, the spectrum is saved into spectrum.dat.

In order to switch off the recording of particular output data, the corresponding parameter should be set to 0.

Additionally, the program PeldorFit has an operation mode, which is called error_plot_only. This mode can be useful in cases, when the user has already done the fitting of PELDOR time traces and now he just wants to use the previous results to record the error plots for the optimized parameters. Usually, one would need to create a new configuration file with the error plot included in it and to run the whole optimization ones again. The error_plot_only mode allows to avoid repeating the optimization procedure (which is very time-consuming) and, instead, allows using the results of previous optimization to calculate the required error plots. Here is how one activates the error_plot_only mode:

Taken with some modifications from Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg

```
error_plot_only:
{
        enable = 0;
        error_plot_variables = ([1,2]);
        error_plot_size = 1000;
        input_directory = "Examples/unimodal_nitroxide_nitroxide/Results/parameters.dat";
        output_directory = "Examples/unimodal_nitroxide_nitroxide/Results/";
};
```

where

enable	The switch, which inactivates the usual “optimization mode” and activates the “error plot only” mode: 0 = disable, 1 = enable
error_plot_variables	Same setting as error_plot_variables in the output list.
error_plot_size	Same setting as error_plot_size in the output list.
input_directory	A path to a disk space where a file with the optimized values of fitting parameters (parameters.dat) is stored.
output_directory	A path to a disk space where the output data will be stored.

5 Output data

Depending on the output settings of the configuration file, some or all of the following output files are generated by PeldorFit:

a) score.dat

This file stores the values of the merit function for each of the optimization steps (generations). The first column of the file lists to the numbers of optimization steps. The second column contains the corresponding values of a goodness-of-fit, e.g., RMSD values.

b) fit.dat

This file stores the best fits to the experimental PELDOR time traces. For each PELDOR time trace specified in the configuration file, PeldorFit writes three columns into the file. The first column corresponds to the time values in microseconds. Other two columns contain the values of the experimental signal and its fit, respectively.

c) parameters.dat

This file stores the optimized parameters of a geometric model. The first column of the file contains the names of optimization parameters. The second column provides the optimized values of these parameters.

d) symmetric_parameters.dat

This file stores the 16 symmetry-related sets of optimized angular parameters. The first column of the file contains the names of angular parameters. The second column contains the optimized values of angular parameters, which are identical to the ones listed in *parameters.dat*. Next 15 columns list the values of the symmetry-related angular parameters. Additionally, the RMSD values corresponding to all 16 sets of angular parameters are given in the last line of the file.

e) errorplot_X.dat or errorplot_X_Y.dat where X, Y = 1 - 28

This file stores an error plot for a single fitting parameter *X* (e.g., $<r_1>$) or for a pair of fitting parameter *X* and *Y* (e.g., $<r_1>$ and Δr_1). Depending on the number of variables *N*, first *N* columns of the file contain the values of the fitting parameters chosen for the error plot. The last column of the file contains the corresponding values of a goodness-of-fit, e.g., RMSD values.

f) *spectrum.dat*

This file stores the simulated ESR spectrum of a spin system. The first column of the file contains values of microwave frequency in gigahertz. The second column contains the corresponding intensities of the spectrum normalized to 1.

5.1 Plotting the output data by means of Python scripts

To enable graphical representation of the PeldorFit output data, the common tools like Origin, Matlab, or matplotlib can be used. Here, several Python scripts were developed for this purpose:

a) plotScore.py plots the data of score.dat;

b) plotFit.py plots the data of fit.dat;

c) plotDistDistr.py plots the distance distribution based on the data of parameters.dat;

d) plotErrorPlot.py plots the data of errorplot_X.dat and errorplot_X_Y.dat.

In order to use these scripts, one needs to install Python 2.7 and two additional Python libraries called numpy and matplotlib (all are free available). All scripts can be found in the PeldorFit directory /PeldorFit2018/Visualization/. To run these scripts:

1. Open Terminal (Linux) or Command Prompt (Windows).
2. Go to the directory in which the Python scripts are stored:
   cd .../PeldorFit2019/Visualization
3. Set the permission properties (only for Linux):
   chmod 755 [name of the script]
4. Run the script by the following command:
   python [name of the script] [full path of the output file] [optional arguments]
   In particular, the user has the following options:

[name of the script]	[full path of the output file]	[optional arguments]
plotScore.py	full path to score.dat	--fontsize (or -fs): set the font size of the figure
plotFit.py	full path to fit.dat	--fontsize (or -fs): set the font size of the figure
plotDistDistr.py	full path to parameters.dat	--rmin: set the lower bound of the distance --rmax: set the upper bound of the distance --fontsize (or -fs): set the font size of the figure
plotErrorPlot.py	full path to errorplot_X.dat or errorplot_X_Y.dat	--cmin: set the lower bound of the colormap --cmax: set the upper bound of the colormap --fontsize (or -fs): set the font size of the figure

6 Examples

This chapter provides the examples of using PeldorFit for analysis of orientation-selective PELDOR data-sets of three different model systems.

6.1 Two-spin system with a unimodal distance distribution

The first example corresponds to the W-band PELDOR data-set of a bis-nitroxide model compound **1**[10] (Figure 6.1). This data-set consists of six PELDOR time traces, which were acquired using six different pump/detection positions across the nitroxide spectrum (Figure 6.1b). The details of the PELDOR experiments can be found in Ref. [10]. Now, let's apply PeldorFit to determine the distance distribution and the relative orientation of nitroxide spin centers in **1**.

Step 1. Preparation of the configuration file

Before one runs the program, a configuration file with all input data of the program has to be prepared. The detailed description of how one creates such file and what information has to be provided is given in Chapter 4. Make sure that you have read that chapter! For **1**, the configuration file can be found in the following directories:

/PeldorFit2019/Linux/Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg (Linux)

/PeldorFit2019/Windows/Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg (Windows)

As follows from bisnitroxide_config.cfg, the nitroxide spin centers of **1** are described by a two-spin model with a unimodal distance distribution (Model 1 in Chapter 1.2). As described in

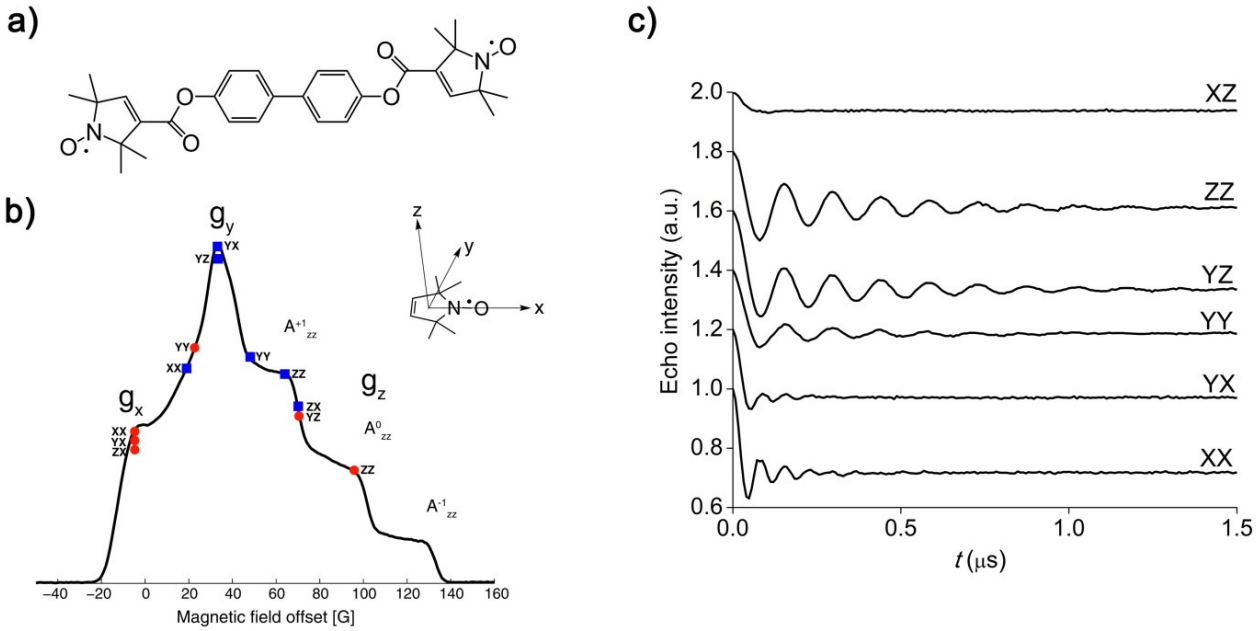

Figure 6.1. a) Lewis structure of bis-nitroxide model compound **1**. **b)** The echo-detected W-band EPR spectrum of **1**. Red dots and blue squares show the positions of the detection and pump pulses, respectively. The dots and squares are labelled with their respective PELDOR experiment. The inset shows a nitroxide and the relative orientation of the *g*-tensor indicated by arrows. **c)** The W-band PELDOR time traces of **1**. Adapted from Ref. [10].

Chapter 1.2, this model consists of six geometric parameters r_1, ξ_1, φ_1, α_1, β_1, and γ_1. The distribution of distance parameter r_1 is set to a Gaussian distribution, whereas the distributions of all angular parameters, ξ_1, φ_1, α_1, β_1, and γ_1, are set to uniform distributions. Thus, the inter-nitroxide distance distribution is described by a mean distance $<r_1>$ and its standard deviation Δr_1. Each of the angular distributions is also described by two values, a mean angle $<a>$ and a uniform width Δa (a = ξ_1, φ_1, α_1, β_1, and γ_1). In addition to the geometric parameters, the scaling factor for the modulation depth, η, is set to be a fitting parameter too. Since the PELDOR time traces of **1** were acquired on a cavity-free spectrometer and, therefore, the pump pulse efficiency was the same for all of them, a single value of η is used for all time traces.

Step 2. Running the program

As soon as the configuration file is prepared, one can run PeldorFit. To do this, first open Terminal (Linux)/Command Prompt (Windows) and navigate to the directory

cd .../PeldorFit2018/Linux (for Linux) or

cd .../PeldorFit2018/Windows (for Windows).

Next, run the program by the following command:

sh PeldorFit.sh Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg (for Linux) or

PeldorFit.exe Examples/unimodal_nitroxide_nitroxide/bisnitroxide_config.cfg (for Windows).

Step 3. Operation of the program

Now the program is running. First, the program reads out the configuration file and pops up the message:

Loading input data from the config file...

Number of PELDOR signals is 6

Input data is loaded!

The last message appears only when the configuration file does not contain any syntactic mistakes. Next, the fitting of the PELDOR time traces begins. During the fitting, the parameters of the selected geometric model are optimized until the simulated PELDOR time traces provide the best fit to the experimental PELDOR time traces. The optimization is done by means of the genetic algorithm and proceeds through 500 iteration steps (see Chapter 4.4). Note that this procedure may take a long time, so please be patient! During the optimization, the program pops up the following progress messages:

Optimization of the spin geometry via genetic algorithm... Please be patient!

Optimization step 1 / 500

...

Optimization step 500 / 500

In the end, the program saves the results of the fitting. The following messages appear:

Recording the goodness-of-fit vs optimization step... Done!

Recording the optimized values of fitting parameters... Done!

Recording the fits to the PELDOR signals... Done!

Recording the symmetry-related sets of fitting parameters... Done!

Recording the error plot... Done!

Finished! The optimization took 4.77 hours.

Step 4. The fitting results

All results of the fitting are saved into the directory

Examples/unimodal_nitroxide_nitroxide/Results/,

which is specified in the configuration file. The output files include

- score.dat
- fit.dat
- parameters.dat
- symmetric_parameters.dat
- errorplot_1_2.dat, errorplot_3_4.dat, errorplot_5_6.dat, errorplot_7_8.dat, errorplot_9_10.dat, errorplot_11_12.dat

Let's briefly discuss the content of these files. First, it is important to make sure that the genetic algorithm has converged to the global minimum. This information is contained in the file called score.dat. The content of this file is depicted in Figure 6.2. As can be seen, the RMSD between the experimental PELDOR time traces and their fits falls gradually down during the first 350 optimization steps and, after this, does not change anyhow significantly during the last 150 optimization steps. This shows that the global minimum was reached and that optimal geometry of the spin system was found.

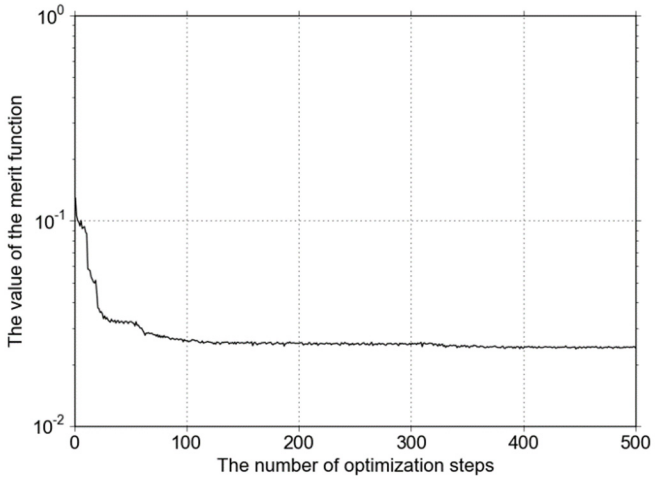

Figure 6.2. The goodness-of-fit, given by the RMSD between the experimental PELDOR time traces of **1** and their fits, is depicted in dependence of optimization step. Generated by means of plotScore.py (see Section 5.1).

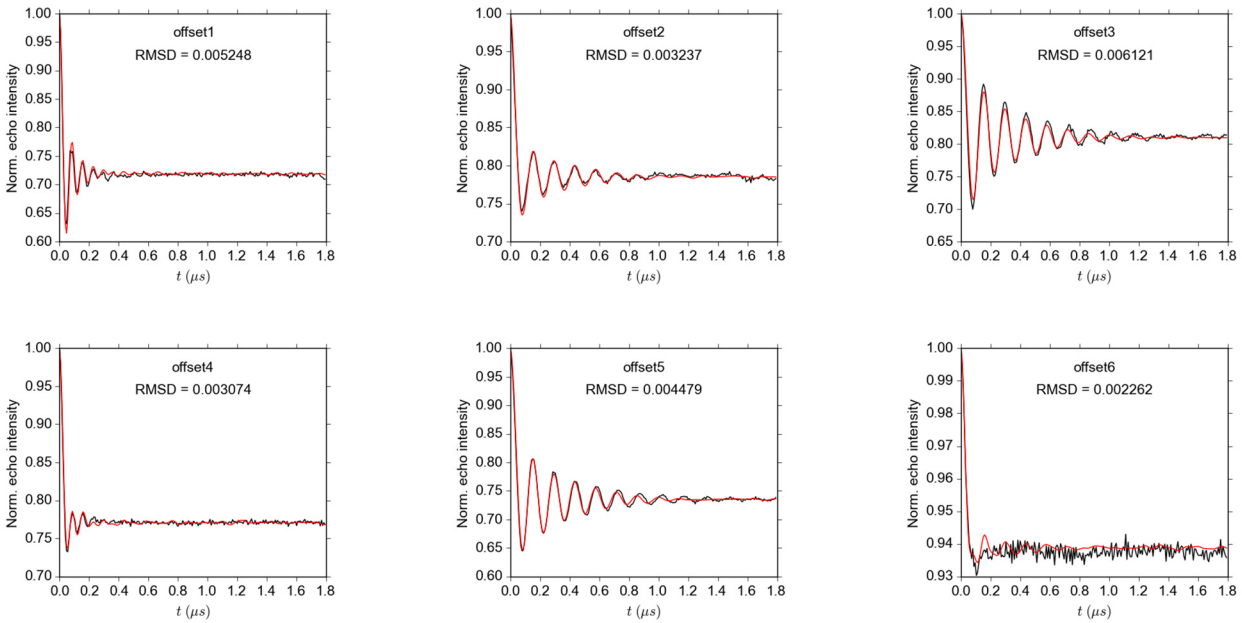

Figure 6.3. The PELDOR time traces of **1** (black lines) are overlaid with the corresponding fits (red lines). Generated by means of plotFit.py (see Section 5.1).

Although PeldorFit kept track of goodness-of-fit by calculating the RMSD value, an additional check of the obtained fit by eye is advisable. The fit to the PELDOR time traces are stored in the file called fit.dat. Figure 6.3 depicts content of this file for **1**. As can be seen, the shapes of all PELDOR time traces is well reproduced by the corresponding fits. Thus, one can conclude that the optimized geometric model provides a good agreement with the PELDOR data.

Now, let's consider the optimized geometric model and, in particular, its parameters stored in the file called parameters.dat. As was mentioned above, the parameters of the model include the mean values and the distributions widths of r_1, ξ_1, φ_1, α_1, β_1, and γ_1, as well as the scaling factor η. The optimized values of these parameters are listed in Table 6.1. The inter-nitroxide distance distribution corresponding to the optimized $<r_1>$ and Δr_1 is depicted in Figure 6.4.

Table 6.1. Optimized parameters of the geometric model of **1**.

Parameter	Value
$<r_1>$	1.94 nm
Δr_1 (standard deviation)	0.03 nm
$<\xi_1>$	90°
$\Delta\xi_1$ (uniform width)	12°
$<\varphi_1>$	156°
$\Delta\xi_1$ (uniform width)	55°
$<\alpha_1>$	145°
$\Delta\alpha_1$ (uniform width)	33°
$<\beta_1>$	52°
$\Delta\beta_1$ (uniform width)	84°
$<\gamma_1>$	162°
$\Delta\gamma_1$ (uniform width)	50°
η	0.93

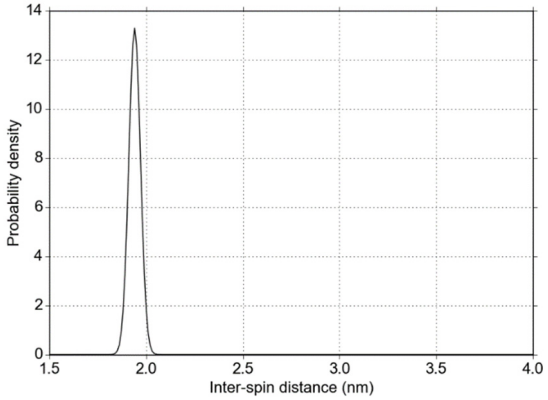

Figure 6.4. The inter-nitroxide distance distribution in **1**. Generated by means of plotDistDistr.py (see Section 5.1).

Table 6.2. Summary of the symmetry-related sets of angles for **1**.

Symmetric transformation[a]	$<\xi_1>$,°	$<\varphi_1>$,°	$<\alpha_1>$,°	$<\beta_1>$,°	$<\gamma_1>$,°	RMSD
Fitting result	90	156	145	52	162	0.026
180° rotation about g_{xx}^B	90	156	325	128	18	0.026
180° rotation about g_{yy}^B	90	156	325	128	198	0.025
180° rotation about g_{zz}^B	90	156	145	52	342	0.026
180° rotation about g_{xx}^A	90	204	35	128	342	0.028
180° rotation about g_{xx}^A and g_{xx}^B	90	204	215	52	198	0.026
180° rotation about g_{xx}^A and g_{yy}^B	90	204	215	52	18	0.024
180° rotation about g_{xx}^A and g_{zz}^B	90	204	35	128	162	0.027
180° rotation about g_{yy}^A	90	24	215	128	342	0.026
180° rotation about g_{yy}^A and g_{xx}^B	90	24	35	52	198	0.027
180° rotation about g_{yy}^A and g_{yy}^B	90	24	35	52	18	0.026
180° rotation about g_{yy}^A and g_{zz}^B	90	24	215	128	162	0.026
180° rotation about g_{zz}^A	90	336	325	52	162	0.027
180° rotation about g_{zz}^A and g_{xx}^B	90	336	145	128	18	0.026
180° rotation about g_{zz}^A and g_{yy}^B	90	336	145	128	198	0.026
180° rotation about g_{zz}^A and g_{zz}^B	90	336	325	52	342	0.025

[a] g_{xx}^A, g_{yy}^A, and g_{zz}^A denote the principal components of the *g*-tensor of spin A; g_{xx}^B, g_{yy}^B, and g_{zz}^B denote the principal components of the *g*-tensor of spin B.

As described in Chapter 1.3, the angles $<\xi_1>$, $<\varphi_1>$, $<\alpha_1>$, $<\beta_1>$, $<\gamma_1>$ can not determined uniquely and have symmetry-related values. Therefore, PeldorFit calculates all 16 symmetry-related sets of angles and stores them in the file called symmetric_parameters.dat. The content of this file is summarized in Table 6.2. Note that all sets of angles provide an identical fit to the PELDOR time traces, which is reflected by almost the same RMSD values determined for different sets of angles.

Finally, one has to quantify of the precision of the optimized geometric parameters. Since PeldorFit deals with a large number of fitting parameters (13 in this case) and they are interdependent, the error estimation is not trivial. In PeldorFit, this task is approached by exploring the dependence of goodness-of-fit on a single fitting parameter or a pair of fitting parameters. While doing this, all other fitting parameters are set to their optimized values. The obtained dependences are called error plots, because they allow estimation of errors of individual parameters. In the present example, the error plots were recorded for six pairs of geometric parameters: $<r_1>$ and Δr_1 (errorplot_1_2.dat), $<\xi_1>$ and $\Delta\xi_1$ (errorplot_3_4.dat), $<\varphi_1>$ and $\Delta\varphi_1$ (errorplot_5_6.dat), $<\alpha_1>$ and $\Delta\alpha_1$ (errorplot_7_8.dat), $<\beta_1>$ and $\Delta\beta_1$ (errorplot_9_10.dat), $<\gamma_1>$ and $\Delta\gamma_1$ (errorplot_11_12.dat). All of them are depicted in Figure 6.5.

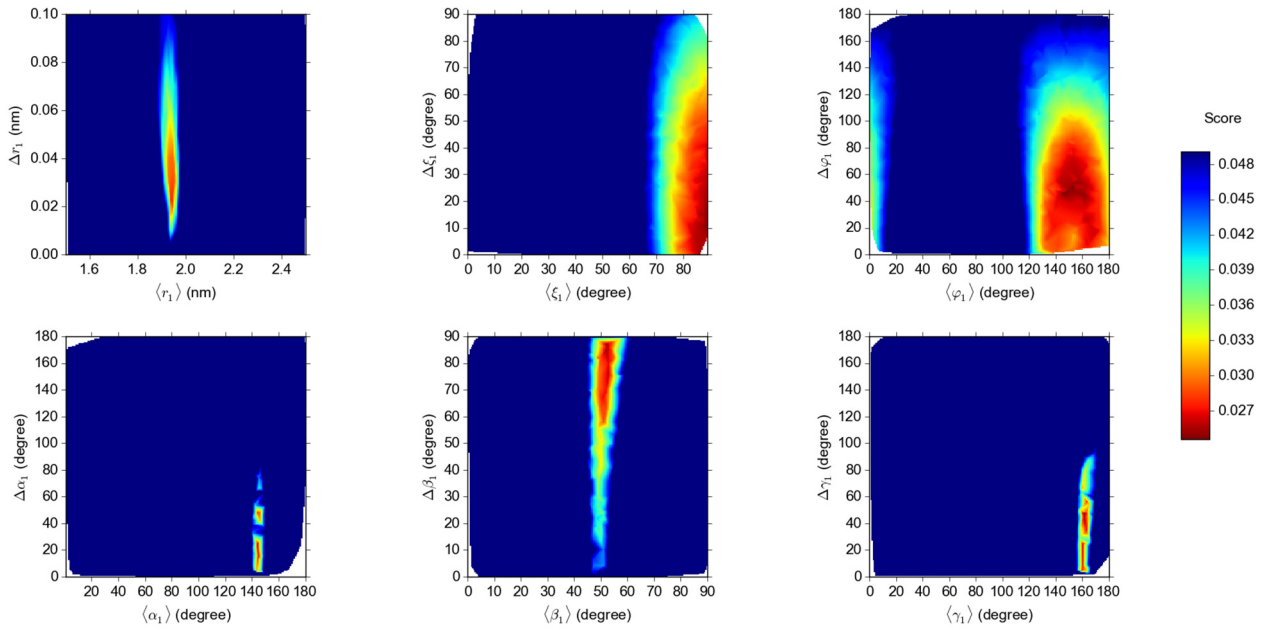

Figure 6.5. The error plots obtained for **1**. Generated by means of plotErrorPlot.py (see Section 5.1).

6.2 Two-spin system with a bimodal distance distribution

The second example deals to the X-band PELDOR data-set of a nitroxide-labelled single cysteine mutant of azurin T21R1[11] (Figure 6.6). T21R1 contains two paramagnetic centers, the Cu^{2+} ion and the nitroxide center. The Cu^{2+} ion has a well-defined binding site in azurin and, therefore, its spin center has a fixed orientation with respect to the protein structure. The R1 side chain is intrinsically flexible and, in the given example, adapts two different conformations[12,13] shown in Figure 6.6a. Importantly, these two conformations have different distances to the Cu^{2+} center, giving rise to a bimodal inter-spin distance distribution.

The PELDOR time traces of T21R1 were recorded using four different frequency offsets. The pump pulse was always applied at the maximum of the nitroxide spectrum, whereas the detection pulses were set to be in resonance with different spectral components of Cu^{2+} (Figure 6.6b) The details of the PELDOR experiments can be found in Ref. [11]. Now, let's apply PeldorFit to determine the distance distribution and the relative orientation of Cu^{2+} and nitroxide spin centers in T21R1.

Step 1. Preparation of the configuration file

The configuration file for T21R1 can be found in the directories:

/PeldorFit2018/Linux/Examples/bimodal_copper_nitroxide/bimodal_config.cfg (Linux)

/PeldorFit2018/Windows/Examples/bimodal_copper_nitroxide/bimodal_config.cfg (Windows)

To account for the two different conformations of the R1 side chain, a two-spin geometric model with a bimodal distance distribution (Model 2 in Chapter 1.2) is specified in the configuration file bimodal_config.cfg. As described in Chapter 1.2, this model consists of two sets of geometric parameters (r_i, ξ_i, φ_i, α_i, β_i, γ_i), where i is 1 or 2. Each set of parameters corresponds to one of the R1 conformers. The relative weight of these conformers is described by the parameter w. The distance parameters r_i are set to have Gaussian distributions with

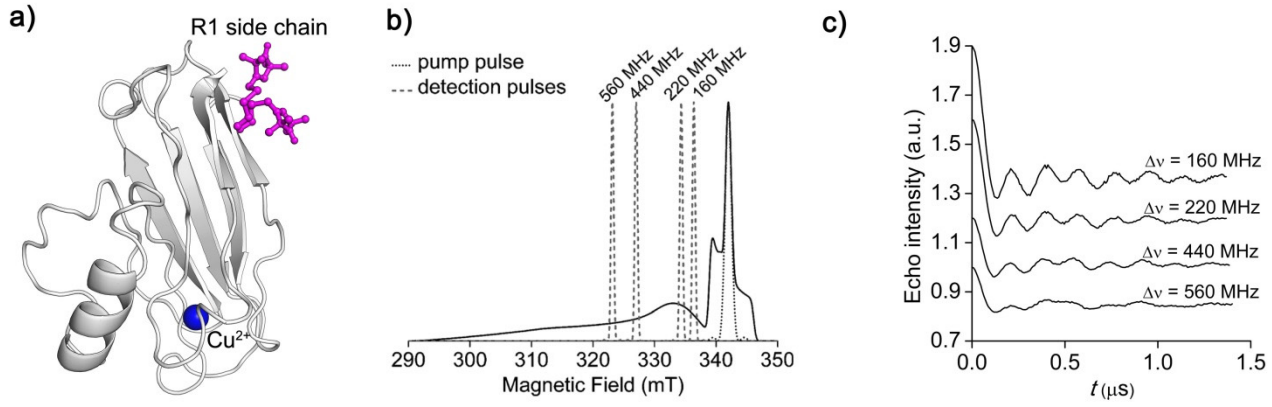

Figure 6.6. a) The crystal structure of the nitroxide-labelled azurin mutant T21R1 (PDB 4BWW). **b)** The echo-detected X-band EPR spectrum of T21R1 shown together with the simulated excitation profiles of the pump (dotted line) and the detection (dashed lines) pulses. **c)** The X-band PELDOR time traces of T21R1. Adapted from Ref. [11].

mean values $<r_i>$ and and standard deviations Δr_i. Due to the significant rigidity of the Cu^{2+} center and both R1 conformers, all angular parameters are assumed to have unique values and no distributions. Thus, only the mean angles but not their widths are included in the list of fitting parameters. Moreover, due to the axial symmetry of the Cu^{2+} *g*-tensor, the PELDOR time traces of T21R1 are insensitive to the angles φ_i (see Table 1.1). Therefore, both φ_i angles are excluded from consideration. Taken together, the final geometric model consists of the 13 parameters: $<r_1>$, Δr_1, $<\xi_1>$, $<\alpha_1>$, $<\beta_1>$, $<\gamma_1>$, $<r_2>$, Δr_2, $<\xi_2>$, $<\alpha_2>$, $<\beta_2>$, $<\gamma_2>$, and *w*. In addition, the scaling factor for the modulation depth, η, is set to be a fitting parameter too. In contrast to the first example, the PELDOR time trace of T21R1 were acquired with different pump efficiencies and, therefore, η is optimized for each PELDOR time trace separately.

Step 2. Running the program

To run PeldorFit, first open Terminal (Linux)/Command Prompt (Windows) and navigate into the directory

cd .../PeldorFit2019/Linux (for Linux) or

cd .../PeldorFit2019/Windows (for Windows).

Next, run the program by the following command:

sh PeldorFit.sh Examples/bimodal_copper_nitroxide/bimodal_config.cfg (for Linux) or

PeldorFit.exe Examples/bimodal_copper_nitroxide/bimodal_config.cfg (for Windows).

Step 3. Operation of the program

Now the program is running. First, the program reads out the configuration file and pops up the message:

Loading input data from the config file...

Number of PELDOR signals is 4

Input data is loaded!

Next, the fitting of the PELDOR time traces begins. During the fitting, the parameters of the selected geometric model are optimized by the genetic algorithm. The optimization proceeds through 500 iteration steps. During the optimization, the program pops up the following progress messages:

Optimization of the spin geometry via genetic algorithm... Please be patient!

Optimization step 1 / 500

...

Optimization step 500 / 500

Finally, the program saves the results of the fitting. The following messages appear:

Recording the goodness-of-fit vs optimization step... Done!

Recording the optimized values of fitting parameters... Done!

Recording the fits to the PELDOR signals... Done!

Recording the symmetry-related sets of fitting parameters... Done!

Recording the error plot... Done!

Finished! The optimization took 1.30 hours.

Step 4. The fitting results

All results of the fitting are saved into the directory

Examples/bimodal_copper_nitroxide/Results/,

which is specified in the configuration file. The output files include

- score.dat
- fit.dat
- parameters.dat
- symmetric_parameters.dat
- errorplot_1_2.dat, errorplot_3.dat, errorplot_7.dat, errorplot_9.dat, errorplot_11.dat, errorplot_13_14.dat, errorplot_15.dat, errorplot_19.dat, errorplot_21.dat, errorplot_23.dat

The content of the file score.dat is depicted in Figure 6.7. This figure reveals that the RMDS decreased during first 100 optimization steps and, then, reached a plateau during next 400 optimization steps. Based on this, one can be conclude that genetic algorithm has converged to the global minimum after 500 optimization steps. The content of the file fit.dat reveals that the good fit to the PELDOR data was obtained (Figure 6.8) and, consequently the optimized geometric model provides an adequate estimate of the relative orientation of Cu^{2+} and nitroxide spin centers. The parameters of this model, which are stored in the file parameters.dat, are listed in Table 6.3. The distance distribution that corresponds to the parameters $\langle r_1 \rangle$, Δr_1, $\langle r_2 \rangle$, Δr_2 and w is depicted in Figure 6.9. As expected, it contains two well pronounced peaks which correspond to two different conformers of the R1 side chain.

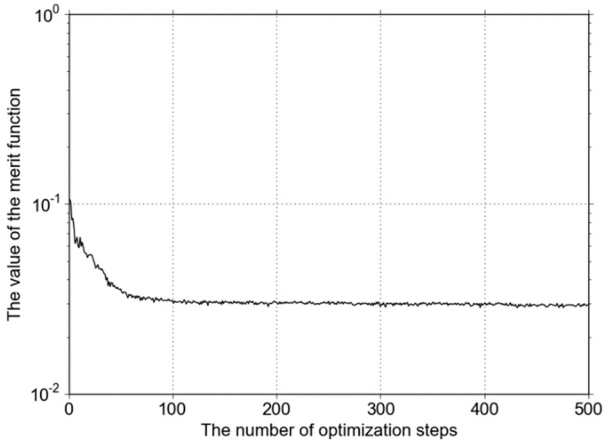

Figure 6.7. The goodness-of-fit, given by the RMSD between the experimental PELDOR time traces of T21R1 and their fits, is depicted in dependence of optimization step. Generated by means of plotScore.py (see Section 5.1).

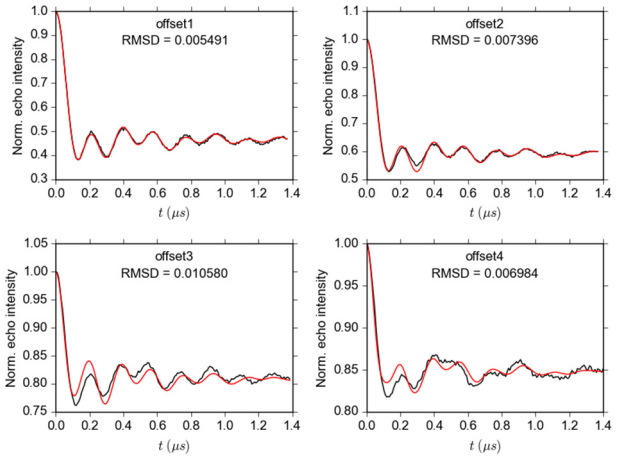

Figure 6.8. The PELDOR time traces of T21R1 (black lines) are overlaid with the corresponding fits (red lines). Generated by means of plotFit.py (see Section 5.1).

Table 6.3. Optimized parameters of the geometric model of T21R1.

Parameter	Value
$\langle r_1 \rangle$	2.15 nm
Δr_1 (standard deviation)	0.03 nm
$\langle \xi_1 \rangle$	32°
$\langle \alpha_1 \rangle^*$	22°
$\langle \beta_1 \rangle$	2°
$\langle \gamma_1 \rangle^*$	87°
$\langle r_1 \rangle$	2.83 nm
Δr_1 (standard deviation)	0.09 nm
$\langle \xi_1 \rangle$	65°
$\langle \alpha_1 \rangle^*$	176°
$\langle \beta_1 \rangle$	3°
$\langle \gamma_1 \rangle^*$	107°
w	0.59

* These parameters are poorly defined.

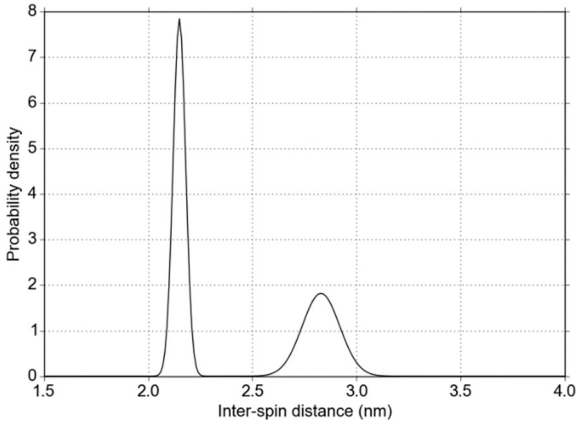

Figure 6.9. The Cu^{2+}-nitroxide distance distribution in T21R1. Generated by means of plotDistDistr.py (see Section 5.1).

The file symmetric_parameters.dat contains 16 symmetry-related sets of angular parameters. These are listed in Table 6.4. Note that all sets of angles provide an identical fit to the PELDOR time traces, which is reflected by almost the same RMSD values determined for different sets of angles.

The last piece of information provided by PeldorFit is the so-called error plots. These plots reveal the dependence of the goodness-of-fit (RMDS) on the following parameters: $\langle r_1 \rangle$ and Δr_1 (errorplot_1_2.dat), $\langle \xi_1 \rangle$ (errorplot_3.dat), $\langle \alpha_1 \rangle$ (errorplot_7.dat), $\langle \beta_1 \rangle$ (errorplot_9.dat), $\langle \gamma_1 \rangle$ (errorplot_11.dat), $\langle r_2 \rangle$ and Δr_2 (errorplot_13_14.dat), $\langle \xi_2 \rangle$ (errorplot_15.dat), $\langle \alpha_2 \rangle$ (errorplot_19.dat), $\langle \beta_2 \rangle$ (errorplot_21.dat), $\langle \gamma_2 \rangle$ (errorplot_23.dat). All of them are shown in Figure 6.10. As can be seen, all distance parameters have a single well-defined minimum, i.e., they can be accurately determined from the PELDOR data of T21R1. To some extent, the same conclusion applies to the angles $\langle \xi_i \rangle$ and $\langle \beta_i \rangle$ (i = 1 and 2). In contrast, the error plots for the angles $\langle \alpha_i \rangle$ and $\langle \gamma_i \rangle$ (i = 1 and 2) do not show any defined minimum. Thus, these angles cannot be reliably determined from the present PELDOR data. The latter result can be well understood if one takes into account that the g-anisotropy of the nitroxide center is fairly low.

Table 6.4. Summary of the symmetry-related sets of angles for T21R1.

Symmetric transformation[a]	<ξ_1>,°	<φ_1>,°	<α_1>,°	<β_1>,°	<γ_1>,°	RMSD
Fitting result	32	0	22	2	87	0.030
180° rotation about g_{xx}^B	32	0	202	178	93	0.032
180° rotation about g_{yy}^B	32	0	202	178	273	0.031
180° rotation about g_{zz}^B	32	0	22	2	267	0.032
180° rotation about g_{xx}^A	148	0	158	178	267	0.035
180° rotation about g_{xx}^A and g_{xx}^B	148	0	338	2	273	0.032
180° rotation about g_{xx}^A and g_{yy}^B	148	0	338	2	93	0.035
180° rotation about g_{xx}^A and g_{zz}^B	148	0	158	178	87	0.032
180° rotation about g_{yy}^A	148	180	338	178	267	0.032
180° rotation about g_{yy}^A and g_{xx}^B	148	180	158	2	273	0.033
180° rotation about g_{yy}^A and g_{yy}^B	148	180	158	2	93	0.032
180° rotation about g_{yy}^A and g_{zz}^B	148	180	338	178	87	0.032
180° rotation about g_{zz}^A	32	180	202	2	87	0.033
180° rotation about g_{zz}^A and g_{xx}^B	32	180	22	178	93	0.032
180° rotation about g_{zz}^A and g_{yy}^B	32	180	22	178	273	0.033
180° rotation about g_{zz}^A and g_{zz}^B	32	180	202	2	267	0.032

Symmetric transformation[a]	<ξ_2>,°	<φ_2>,°	<α_2>,°	<β_2>,°	<γ_2>,°	RMSD
Fitting result	65	0	176	3	107	0.030
180° rotation about g_{xx}^B	65	0	356	177	73	0.032
180° rotation about g_{yy}^B	65	0	356	177	253	0.031
180° rotation about g_{zz}^B	65	0	176	3	287	0.032
180° rotation about g_{xx}^A	115	0	4	177	287	0.035
180° rotation about g_{xx}^A and g_{xx}^B	115	0	184	3	253	0.032
180° rotation about g_{xx}^A and g_{yy}^B	115	0	184	3	73	0.035
180° rotation about g_{xx}^A and g_{zz}^B	115	0	4	177	107	0.032
180° rotation about g_{yy}^A	115	180	184	177	287	0.032
180° rotation about g_{yy}^A and g_{xx}^B	115	180	4	3	253	0.033
180° rotation about g_{yy}^A and g_{yy}^B	115	180	4	3	73	0.032
180° rotation about g_{yy}^A and g_{zz}^B	115	180	184	177	107	0.032
180° rotation about g_{zz}^A	65	180	356	3	107	0.033
180° rotation about g_{zz}^A and g_{xx}^B	65	180	176	177	73	0.032
180° rotation about g_{zz}^A and g_{yy}^B	65	180	176	177	253	0.033
180° rotation about g_{zz}^A and g_{zz}^B	65	180	356	3	287	0.032

[a] g_{xx}^A, g_{yy}^A, and g_{zz}^A denote the principal components of the g-tensor of spin A; g_{xx}^B, g_{yy}^B, and g_{zz}^B denote the principal components of the g-tensor of spin B.

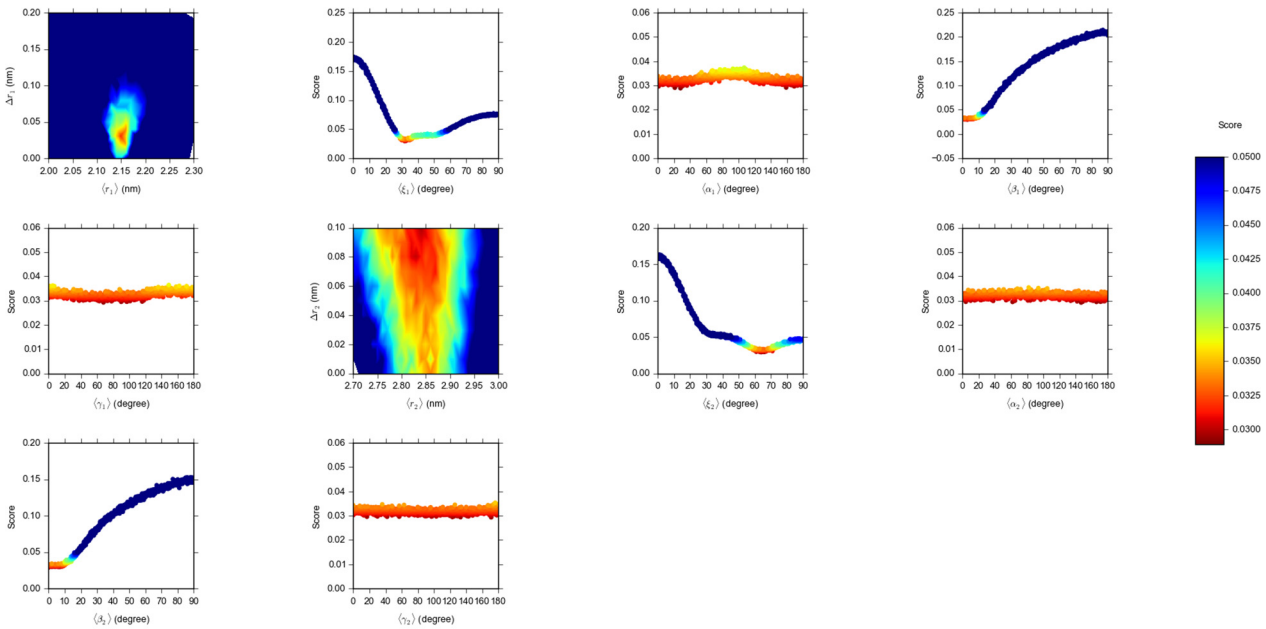

Figure 6.10. The error plots obtained for T21R1. Generated by means of plotErrorPlot.py (see Section 5.1).

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8 Afterword

I would like to thank Prof. Dr. Olav Schiemann, Dr. Gregor Hagelueken, and Prof. Dr. Hideto Matsuoka for the helpful discussions. Also I would like to thank Dr. Andreas Meyer, Dr. Mark Kerzner, and Christine Wuebben for testing the program on their PELDOR data. Finally, I would like to acknowledge the community of www.stackoverflow.com.

If you have any question about the program, I am opened for discussion: abdullin@pc.uni-bonn.de