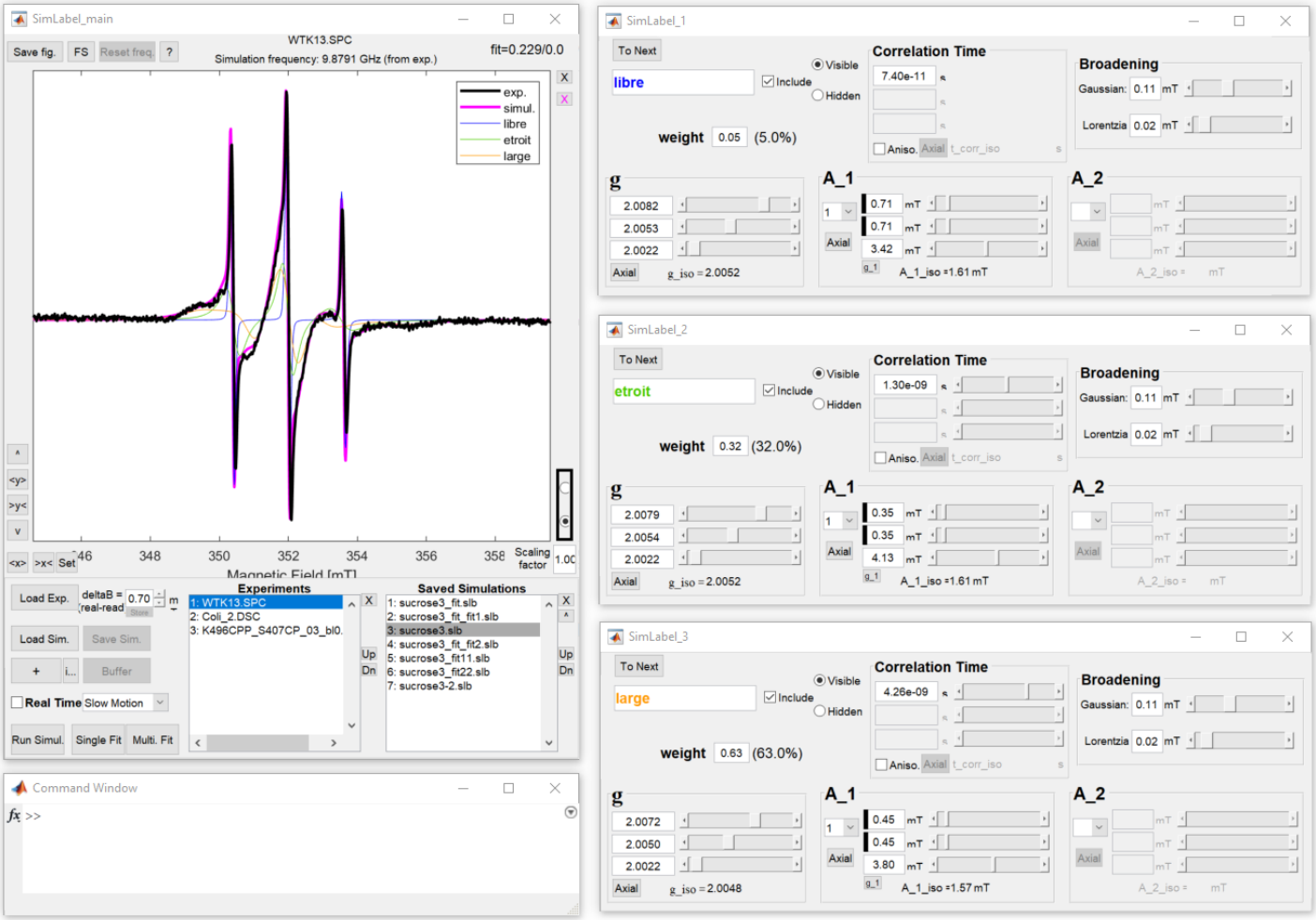
SimLabel 2025

Simulations of cw EPR spectra from Spin **Label**ing

Documentation



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Synopsis

SimLabel[1] is a Graphical User Interface (GUI) developed in MATLAB. Thanks to some **EasySpin functions**[2], SimLabel provides an easy way, **without coding,** of **visualizing, simulating and fitting continuous wave (cw) Electronic Paramagnetic Resonance (EPR) spectra resulting especially from Site Directed Spin Labeling experiments**. It consists of a mixing board, with sliders, and is as intuitive as possible. Basic EasySpin knowledge is recommended but not mandatory.

SimLabel actually enables to run the appropriate EasySpin function with the appropriate inputs without coding. These inputs refer first to the "experimental" parameters that are the microwave frequency and the magnetic field range. If available, these parameters are automatically picked up from the parameters file for the frequency and from the display for the magnetic field range. Other inputs are the magnetic parameters characterizing the spin Hamiltonian, namely the principal values of the g and hyperfine tensors. These parameters are provided by the user.

A multi-component simulation results from the weighted sum of single simulations of n components (n≥1). n is theoretically unlimited, but should not exceed 4 for reliable simulations. To each component corresponds a window containing all the parameters needed by SimLabel for running the simulation. The spectrum of each individual component can be displayed.

A *real time* mode enables the user to observe the simulated spectrum evolving when a parameter is changing.

Each simulation can be saved as *.slb files and reloaded for future sessions. Saved simulations can also be compared to each other. Each saved simulation can be exported in ASCII files.

Automatic least squares fitting to an experimental spectrum is available for every saved simulation. EasySpin already provides a GUI for automatic fitting through the *esfit* function. This GUI could directly be started from SimLabel. SimLabel manages the data exchange between these two GUI (SimLabel and esfit GUI), so that the results exported from the *esfit* GUI are imported in SimLabel.

SimLabel was first designed to facilitate the simulations of slow motion multicomponent nitroxide cw EPR spectra recorded at room temperature. Actually all the **simulations of cw EPR spectra resulting from one unpaired electron (S=1/2), with small g-anisotropy, coupled to one or two nuclei** with nuclear spins ranging from I=1/2 to 5/2, in the slow motion regime or in the solid state can be performed!

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A. Requirements, install and launch

Very basic knowledge of MATLAB is necessary. MATLAB is a Mathworks product.

EasySpin is a toolbox (=set of functions) of MATLAB. It has to be installed and present in your MATLAB path (see EasySpin download). Thus every EasySpin functions can be called (*e.g* by typing their name in the command window) whatever is the MATLAB local folder.

SimLabel is contained in the file *simlabel.m*. The local folder of this file has to be added to the MATLAB's path: *File/Set Path*… Choose the folder to add with *add folder* and save before closing. This documentation has to be saved in the same folder so as to be opened via SimLabel.

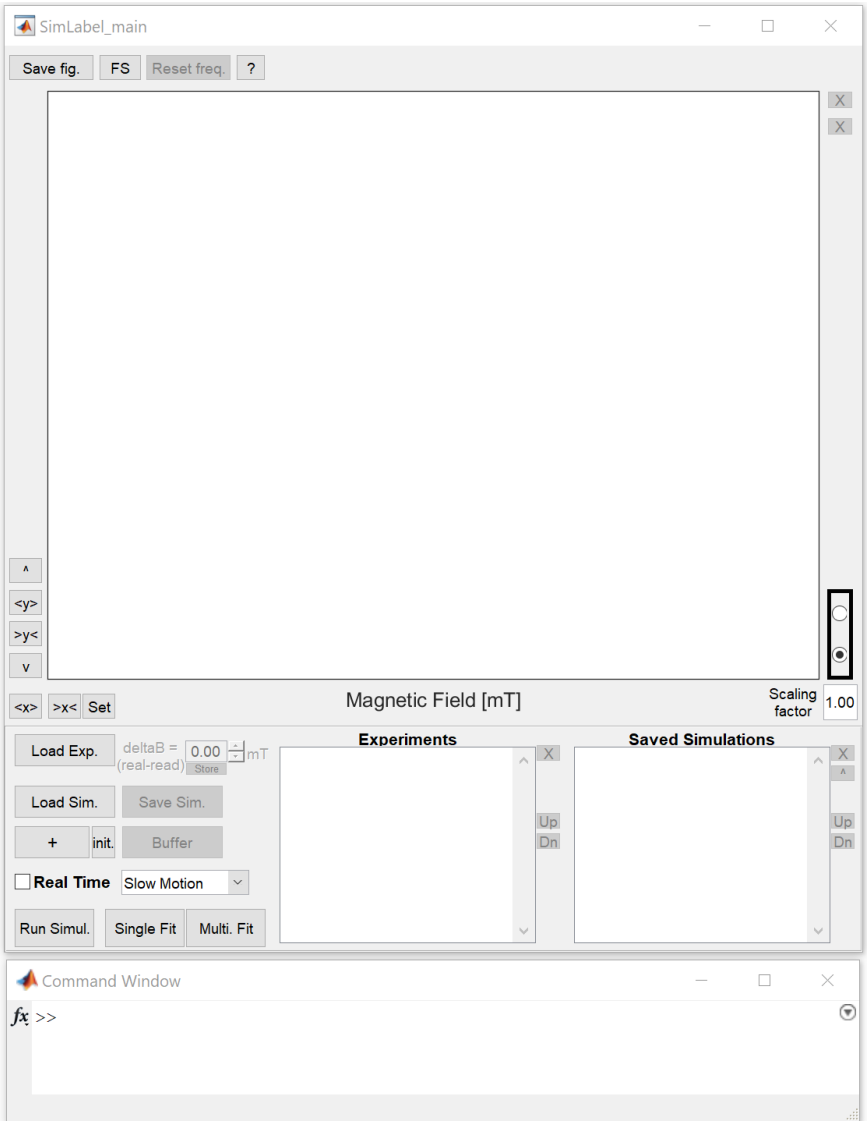
Select the local folder for your SimLabel session in the MATLAB main window. **You must be allowed to write in this directory** (see F. for more details). The directory of your spectra is advised.

Launch SimLabel by typing in the command window:

```
>> simlabel
```

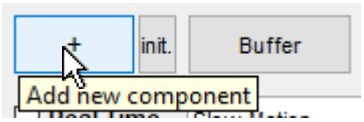
(or `SimLabel` or `SIMLABEL` or `SIMlabel` or…)

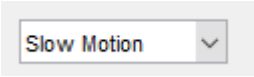
Press "enter". The *SimLabel_main* window opens. It is recommended not to hide the command window whether any message occurs.



B. Getting started without experimental spectrum

When the user hovers the pointer over a graphical control (like buttons, popup menus, sliders …), without clicking it, a tooltip may appear with information about the item being hovered over.



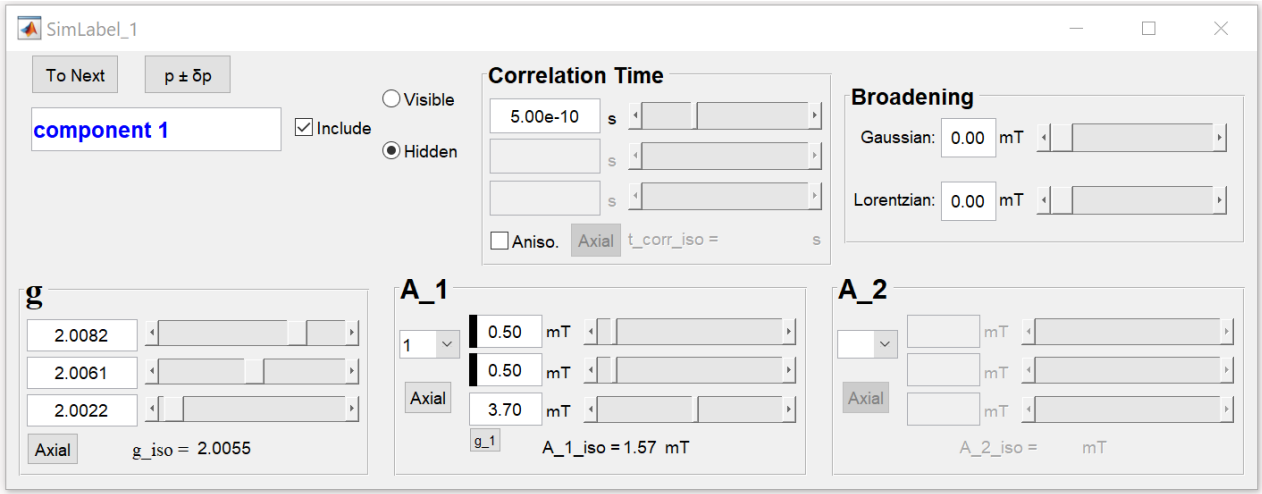


The default type of simulation is *Slow Motion* (see the popup menu on the bottom left corner of the *SimLabel_main* window – *Frozen Solution* is also available). This simulation type is performed using the EasySpin function *chili*. The *chili* algorithm is based on Freed's works[3].

Note: The *Frozen Solution* simulations are performed using the EasySpin function *pepper*.

1) - **Add a new component** by clicking 

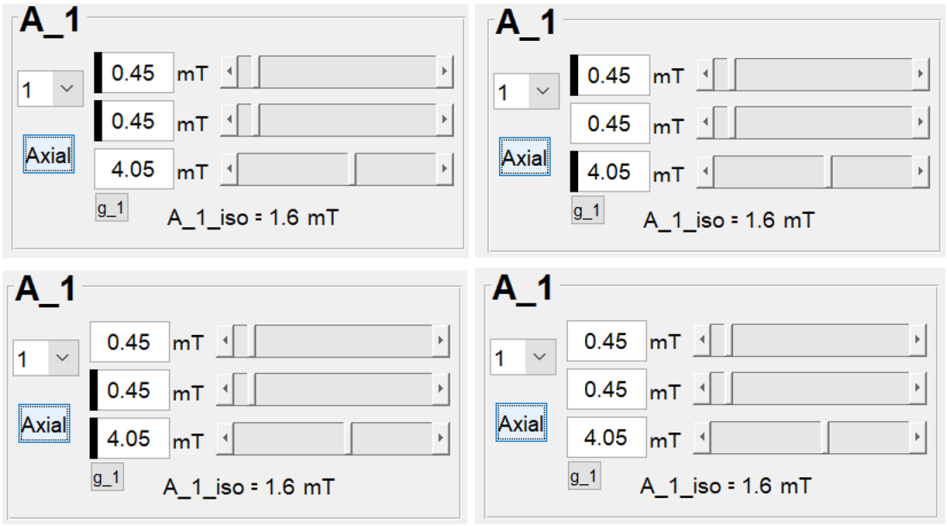
in the *SimLabel_main* window.
- The *SimLabel_1* window opens with default parameters (see C. for more details).



- The default name of this first component is "component 1". The user can modify this name.
- The *include* box must be checked , 

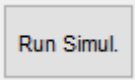
so that the corresponding component will be included in the simulation.
- The default correlation time is isotropic. If the *Aniso.* box is checked, anisotropic correlation time is considered. This tensor can be axial (see below).
- A second hyperfine coupling can be added by selecting a nuclear spin value in the popup menu of the *SimLabel_1* window, *A_2* panel. The simulation run considers the hyperfine coupling only if the three principal values of the A tensor are given.
- g and/or A tensors can be axial. Successively clicking 

defines the different sets of equivalent directions, which are highlighted with black markers (see C. for more details).



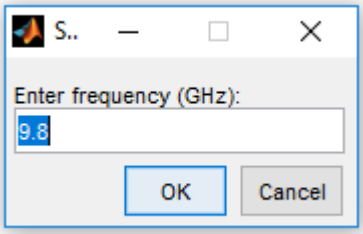
Note: - Before running a simulation in the slow motion regime, anisotropy has to be introduced for at least one of the two tensors (g or A). This is a requirement for using the EasySpin *chili* function.

- The sliders can be used or new values directly entered in the edit boxes. If one of the new values is out of the slider range, this value is well considered even if the slider disappears.

2) - **Run simulation** by clicking 

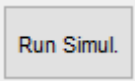
in the *SimLabel_main* window.

- Enter the microwave frequency given in GHz in the dialog box (default value 9.8 GHz) and press OK.

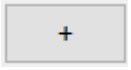


- The default range can be modified with 

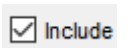
.

- If the range is modified, click 

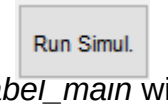
again for the simulation range to match with the display.

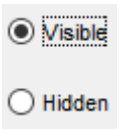
3) - For **multiple components**, add new components by clicking 

in the *SimLabel_main* window.

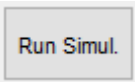
- When a new component is included by checking 

in the *SimLabel_X* window, a new modifiable parameter called *weight* appears. This is a relative weight. The corresponding percentage is also given.

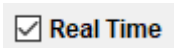
- If 

is clicked, each included component spectrum is visible in the *SimLabel_main* window by selecting 

in the *SimLabel_X* window.

Note: 

performs a global simulation: when clicked, every component spectra are calculated and summed.

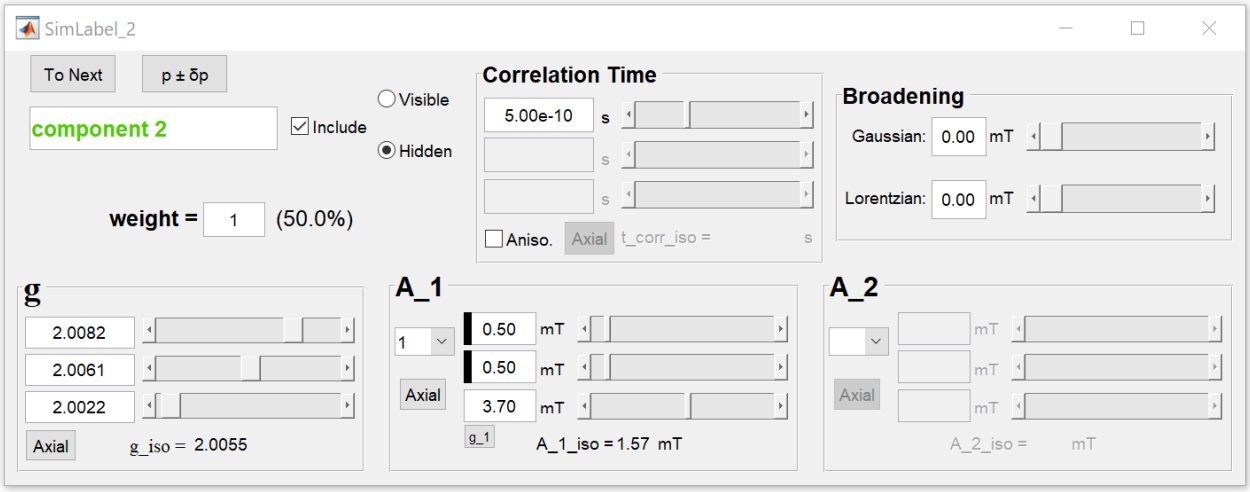
4) If 

is checked on the *SimLabel_main* window, the user can see in real time how evolves the simulated spectrum when a chosen parameter is varying.

C. SimLabel_X window

The *SimLabel_X* windows appear when a new component is added or when a saved simulation is loaded (X=1,2,3,…). Each of them contains all the parameters needed for using a simulation function of EasySpin. In other words, they define the spin Hamiltonian of the component X.

In the *Slow Motion* mode, adding successively two components yields two *SimLabel_X* windows with the default parameters. Below is an example of the *SimLabel_2* window.



- The user can change the name of the component ***component 2***. The color enables to readily identify the corresponding spectrum when visible.

- The component can be included or not included in the simulation, just by checking/unchecking *include*.

- If included, and once the simulation is done, the component spectrum can be shown on the *SimLabel_main* window by selecting V*isible*. The default choice is *Hidden*.

- When several components are considered, the *weight* parameter enables to weight the current component contribution in the final simulated spectrum. The sum of all the component weights can differ from 1, since they are relative. The corresponding percentage is automatically calculated.

- The *Correlation Time* is the rate of isotropic rotational diffusion in seconds, and is linked to the paramagnetic species mobility[3]. The longer the *Correlation Time* is the less mobile is the paramagnetic label. This parameter is only available in the *Slow Motion* mode and can be isotropic (default) or anisotropic. When *Aniso.* is checked, the average value *t_corr_iso* is automatically given. The *Axial* button enables considering an axial tensor. Successive clicks define the different sets of equivalent directions, which are labeled with black markers. After selecting the equivalent directions, one of the concerned parameters has to be changed to make it effective.

- The *Line Width* (or *Broadening* in *Slow Motion* mode) stands for the full width at half maximum of an isotropic convolutional broadening, which can be pure gaussian, pure lorentzian or voigtian (mix of gaussian and lorentzian). The broadening is defined by

the parameters *Gaussian* and *Lorentzian* (see E.4. for more details). In *Slow Motion* mode, the default values are zero, because a line width is still generated by the chili function.

- The principal values of the g tensor are set in the *g* panel. The average g value *g_iso* is automatically given. The *axial* button enables considering an axial tensor. Successive clicks define the different sets of equivalent directions, which are labeled with black markers. After selecting the equivalent directions, one of the concerned parameters has to be changed to make it effective.

- For one species, two different hyperfine couplings *A_1* and *A_2* are available. Each nuclear spin is set by the value of the popup menu and range from 1/2 to 5/2. If no value is set for the nuclear spin, the corresponding hyperfine coupling is not considered. These tensors, given by their principal values in mT (in MHz in the tooltip), can also be axial thanks to the *axial* button. Successive clicks define the different sets of equivalent directions, which are labeled with black markers. The averaged values *A_1_iso* and *A_2_iso* are automatically given if available. The button *g_1* in the *A_1* panel sets the g_1 value to f(A1_3) where f(x)=-0.0025*x+2.0175 (x in mT) (correlation established for nitroxides[4-5]).

- The button *To Next* stores the parameters of the current component for the next added components. The initial default values can be restored with the *init.* button of the *SimLabel_main* window (see D.).

- The button ***p ± δp*** opens (or closes) a new window, called *SimLabel_X_info*, summarizing the parameters of the current component. If these parameters result from an automatic fitting and if uncertainties were estimated during the automatic fitting process (from EasySpin 6.0.0 and later), these uncertainties are also displayed.

Note: - g and A tensors axes are taken parallel in SimLabel. The single axe of the top single value of g and the single axe of the top single value of A are parallel, and so on.

- The default values of g and A are those of the well-known methanethiosulfonate (**MTSL**). Depending on the polarity and proticity of the MTSL environment[4], we have:

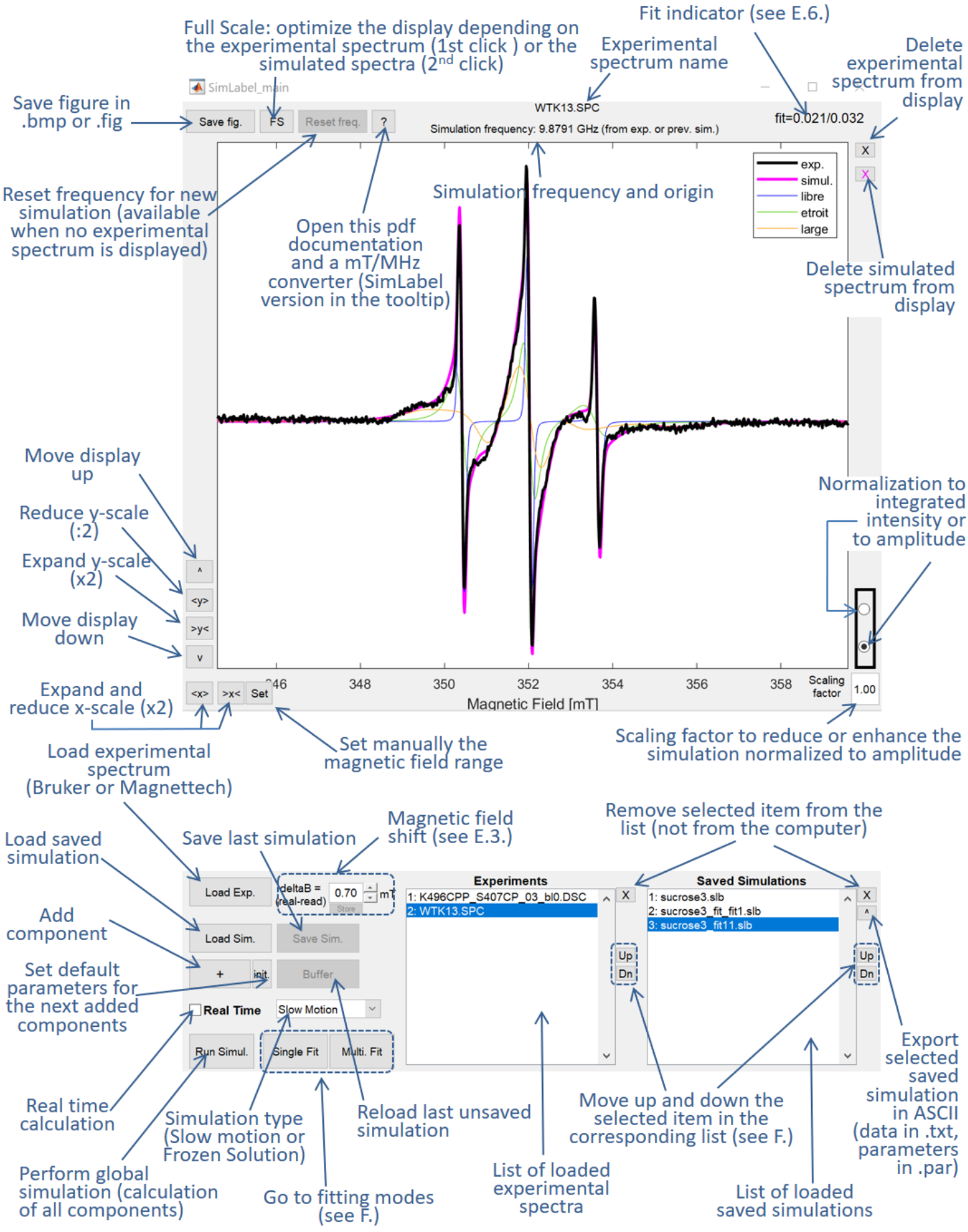
g_x in [2.00800, 2.00940], ie g_x=2.00870(70), g_y = 2.00610(5) and g_z = 2.00220(5).
A_{xy} in [0.35, 0.55] mT (A_{xy} = 0.45 ± 0.10 mT)
A_{xy} in [9.8, 15.4] MHz (A_{xy} = 12.6 ± 2.8 MHz)
and A_z in [3.25, 4.85] mT (A_z = 4.05 ± 0.80 mT)
A_z in [91.1, 135.9] MHz (A_z = 113.5 ± 22.4 MHz)

- We have chosen to show

- EasySpin does not define hyperfine coupling with nuclear spin but with the nucleus type. Thus SimLabel uses this corresponding table:

Nuclear Spin	Nucleus sent to EasySpin
1/2	^{1}H
1	^{14}N
3/2	^{7}Li
2	^{36}Cl
5/2	^{17}O

D. SimLabel_main window



E. How SimLabel works

1) *Global simulation vs real time mode*

When *Run Simul.* is clicked, a global simulation is performed. A global simulation means that the spectrum of every included and weighted component is calculated. The sum of these spectra is then done to provide the resulting spectrum.

In contrast, in R*eal Time* mode, when a parameter is changed, only the corresponding component spectrum is calculated. It is then added to the other unchanged component spectra to update the resulting spectrum.

Note: - A simulation is made of 1024 points. However Simlabel can manage experimental spectra with more or less point.
- Hyperfine couplings are considered as soon as three principal values are given.
- The *Real Time* feeling depends on the calculation speed of the computer that is running SimLabel.
- In *Real Time* mode, the possibilities of resizing the abscissa, changing the experimental spectrum or reloading a simulation are not allowed. The corresponding buttons are disabled. This avoids the magnetic field ranges and/or the frequencies to mismatch.

2) *Experimental parameters*

The spectra simulations are performed by the EasySpin functions *chili* or *pepper*, depending on the chosen simulation type *Slow Motion* or *Frozen Solution* respectively. Concerning the “experimental” parameters, the EasySpin functions for spectral simulations need two parameters: the magnetic field range and the microwave frequency.

If a spectrum is displayed in the graphical window (an experimental spectrum or a previous simulation), the magnetic field range is picked up from the display, otherwise a large 100mT default range is chosen.

If an experimental spectrum is loaded, its frequency is picked up. If not and if a previous simulation is displayed, the previous frequency is picked up, unless the *Reset freq.* (reset frequency) button was pressed. Otherwise the user is asked to set the frequency. The frequency of the displayed simulation is shown above the graph with the origin of this frequency if available.

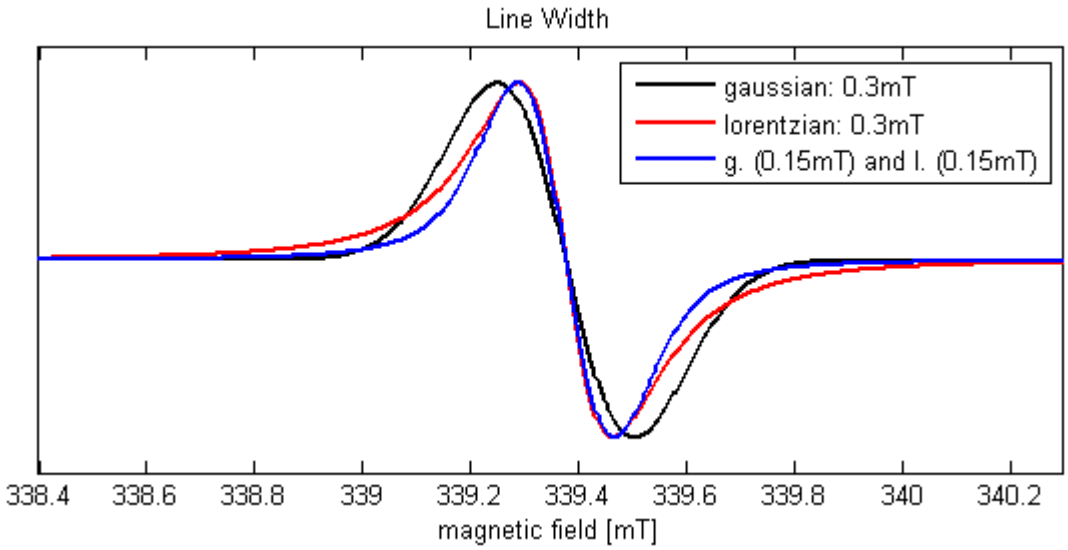
3) *Magnetic field offset*

In the *SimLabel_main* window, the user can give a magnetic field offset *deltaB* for the experimental spectrum. The knowledge of this value is essential for determining accurate g values. When modified, this *deltaB* is automatically taken into account. The user can store it by pressing the *store* button for future SimLabel sessions. If *experiment.** is the experimental spectrum file, *deltaB* is saved by SimLabel in the file *experiment.mat* located in the same folder. If the file *experiment.mat* exists, the *deltaB* is automatically considered anytime the corresponding experimental spectrum is loaded.

Note: In EPR spectrometers, the magnetic field is in most cases measured by a Hall-effect sensor or an NMR teslameter. Because the sample and the sensor are not at the same position, the real magnetic field experienced by the sample and the measured magnetic field do not match exactly. Some spectrometers can consider this offset when recording, but its value changes as soon the resonator is slightly moved in the magnet air gap. The magnetic field offset can be estimated thanks to a standard sample of well-known g-value.

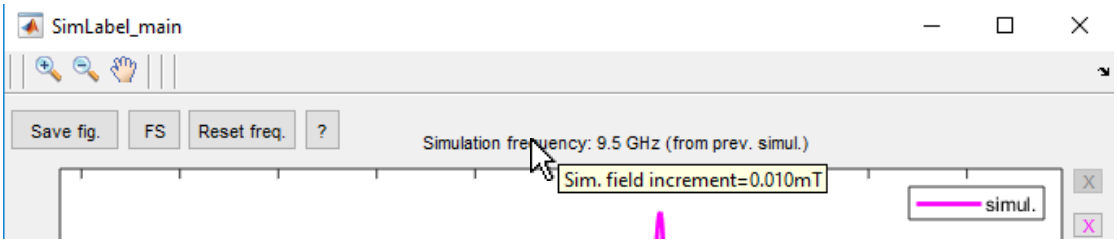
4) Broadening line widths

Whatever the simulation type is, line broadening is an isotropic convolutional broadening with either a pure Gaussian, or a pure Lorentzian or a Voigtian (mix of Gaussian and Lorentzian) line shape. The shape of these profiles is shown in the figure below. No physical model is assumed to cause the broadenings. **This phenomenological method is suitable for S=1/2 system with small g anisotropy**, that is the case of nitroxides. The *Gaussian* and *Lorentzian* line widths (fwhm=full width at half maximum) are given in the *SimLabel_X* window (see C.).



Note: - In the *Slow Motion* mode, the *Gaussian* and *Lorentzian* parameters should be first set to zero (default value), because the EasySpin *chili* function algorithm already generates broadening. In the Frozen Solution mode, the *Gaussian* and/or *Lorentzian* parameters is/are required.

- In the case of multicomponent simulation with different broadenings in slow motion, the broadening linewidths (lw) should not be less than twice the magnetic field increment (dB) (**lw>2dB**), otherwise numerical errors on relative amplitude can be generated. In this particular case, the user should use same broadenings for all the components (lw=0 is possible). For information, the field increment of the simulation is given in the tooltip of the simulation frequency.



- If a voigtian broadening is considered,

$$\tfrac{3}{4}\,(fwhm_{gauss}+fwhm_{lor}) < fwhm_{voigt} < fwhm_{gauss}+fwhm_{lor}$$

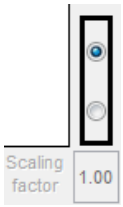
where $fwhm_{gauss}$ $fwhm_{lor}$ and $fwhm_{voigt}$ are the full width at half maximum of the gaussian part, the lorentzian part and the resultant voigtian profile, respectively.

5) <u>Scaling spectra</u>

Overlaying the experimental spectrum and the simulated spectrum requires a previous scaling. SimLabel offers two possibilities for scaling spectra: a normalization to the integrated intensity or to the maximum amplitude. The user can switch from one normalization to the other with the radio buttons on the bottom right of the *SimLabel_main* graph window. This selection determines the nature of the multiplying factor for rescaling spectra.

If y is the raw data array (from experiment or simulation) and if and y_{norm} is the normalized data array, we have:

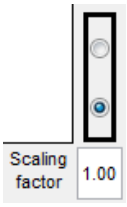
$y_{norm} = {}^{y}/_{h}$, in which *h* is the normalization factor.



The top selection displays spectra normalized to their integrated intensity. In this case, h is calculated by numerical double integration of the raw derivative spectrum:

$$h = \iint y \cdot dB$$

This method is the most rigorous one, but the more sensitive to the experimental noise.

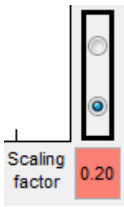


The bottom selection displays spectra normalized to their peak-to-peak amplitude. For the experimental spectrum, we define:

$$h = max(y) - min(y) \quad \text{thus} \quad max(y_{norm}) - min(y_{norm}) = 1$$

For the simulated spectrum, we have

$$h = \big(max(y) - min(y)\big)/\alpha \quad \text{thus} \quad max(y_{norm}) - min(y_{norm}) = \alpha$$



where α is the scaling factor that could be modified by the user. The α default value is 1. It could be useful to decrease α, for example, for adjusting a one-component simulation to a specific visible component of a multicomponent experimental spectrum. When α is not 1, the box is colored to be noticeable.

6) Fit indicator

When an experimental spectrum and a simulated spectrum are displayed and if the frequencies and the magnetic field ranges match, two indicators of the fit relevance, fit_1 and fit_2, are calculated and displayed in the upper right corner of the graphical window:

$$fit=fit_1/fit_2$$

These fit indicators fit_1 and fit_2 are calculated with the derivative and the absorption spectra, respectively. The fit_n indicator is calculated according to:

$$fit_n = \frac{\sum\left(y_{sim,i}^n - y_{exp,i}^n\right)^2}{\sum\left(y_{exp,i}^n\right)^2}$$

Where (x_{sim}^n, y_{sim}^n) and $\left(x_{exp}^n, y_{exp}^n\right)$ are the simulated and the experimental spectra, respectively (n=1: derivative spectra, n=2: absorption spectra). Obviously, $x_{sim}^n = x_{exp}^n$.

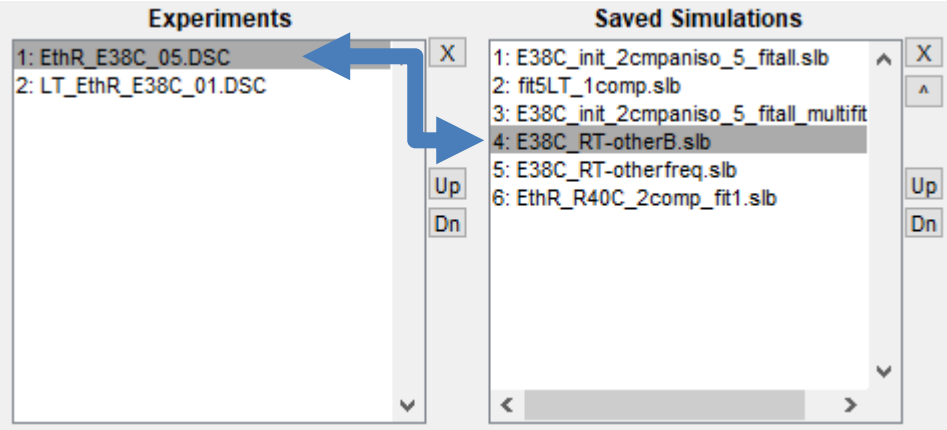
These indicators are inspired from a chi-square.

F. Automatic fittings

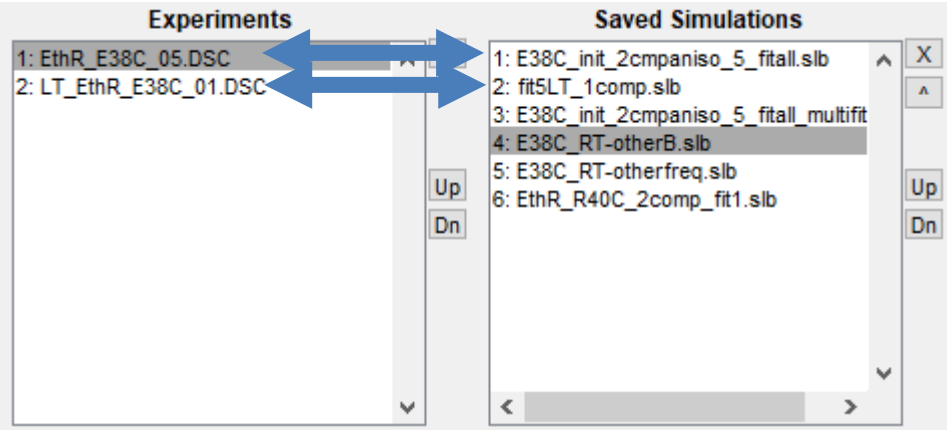
A GUI for automatic fitting a simulated spectrum to an experimental spectrum, using least-squares fitting techniques, is already available in EasySpin (*esfit* function). This GUI can be launched from SimLabel, which automatically manages the interfacing between these GUIs. For that, SimLabel requires experimental spectrum and corresponding saved simulation (same frequencies and same magnetic field ranges).

Two different modes of automatic fittings are available:

- The *Single Fit* mode enables to fit one experimental spectrum at a time. This mode is available when an experimental spectrum and a corresponding saved simulated spectrum are selected in the lists (displayed), whatever their position in the lists.



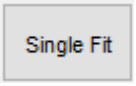
- The *Multi Fit* mode enables to fit several experimental spectrum from the same sample with common parameters in the same time. For example, multi-frequency spectra at room temperature and low temperature. For this mode, experimental spectra and corresponding saved simulated spectra have to be at the same position in the lists, whatever the displayed (selected) spectra. The user can use the *Up* and *Dn* (down) buttons to place them correctly. All the experimental spectra in the list are considered.



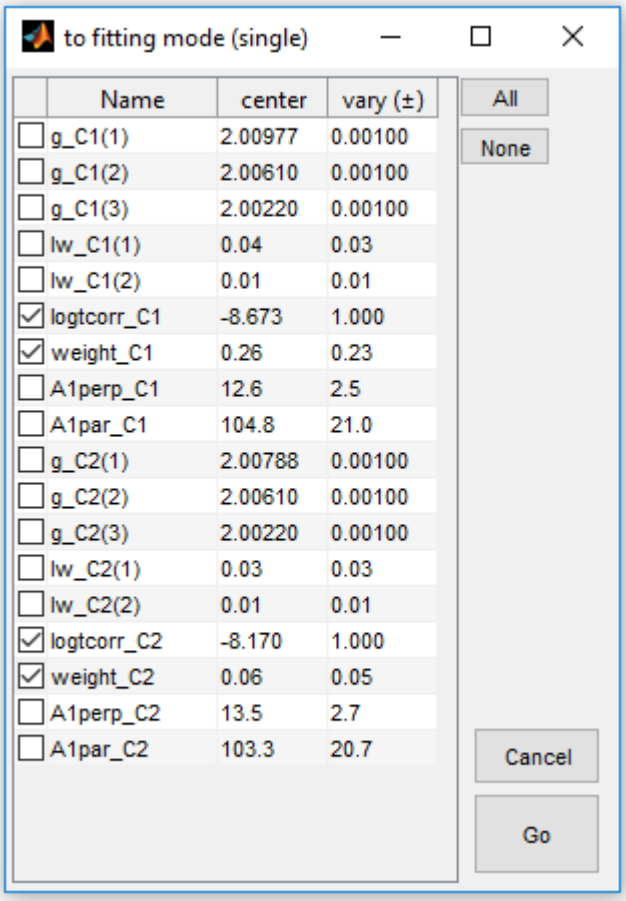
Note: The user must ensure to have **permissions to write in the starting folder** whose explicit path is given in the Matlab main window. Indeed SimLabel generates automatically and temporarily a new file called *temporary_func4esfit.m* or

temporary_func4multifit.m in the current folder when automatic fitting is running. SimLabel deletes this file when the user quits the fitting mode.

1) *From SimLabel to esfit GUI: Single Fit*

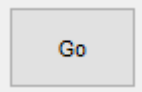
When the user clicks 

in the *SimLabel_main* window, a first window, named *to fitting mode (single)*, appears. It contains a table summarizing the parameters of the simulation components which can be considered in the fitting process. The parameters are renamed in the first column. The name of all parameters referring to the n^{th} component ends with *_Cn*. Then their value called *center* and their default variation range called *vary* are given in the second and third columns, respectively. The *center* and *vary* columns are editable so that the user can change their value. To be variable in the fitting process, a given parameter has to be checked. The variation range *center* ± *vary* will then be explored. The correspondence between the component name and the component number used for renaming the parameters are displayed on the graph of the *SimLabel_main* window.



An example is given in the above window. In this case, we have two components, both with one axial hyperfine tensor A. Only the correlation times and the weights are checked to be variable in the automatic fitting process. The parameters of the first component are:

- *g_C1(1)*: first principal value of g tensor (along x axis, for example),
- *g_C1(2)*: second principal value of g tensor (along y axis),
- *g_C1(3)*: third principal value of g tensor (along z axis),
- *lw_C1(1)*: Gaussian part of the line width, in mT (full width at half maximum, see C and E.4),
- *lw_C1(2)*: Lorrentzian part of the line width, in mT, (full width at half maximum , see C and E.4),
- *logtcorr_C1*: decimal logarithm of the isotropic correlation time (time in s), required in this form by Easyspin,
- *weight_C1*: relative weight of component 1 (should not be too weak compared to the others if checked for being variable),
- *A1perp_C1*: perpendicular value of first (and single) hyperfine tensor, in MHz as required by Easyspin,
- *A1par_C1*: parallel value of first (and single) hyperfine tensor, in MHz as required by Easyspin.

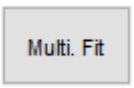
The user has to select the parameters to be variable in the fitting process. The *esfit* GUI for automatic fitting, called *Easyspin Least-Squares Fitting*, opens when clicking 

.

<u>*Note*</u>: For example, if two conformers are present in solution, it could be useful in a first step to fit the two conformers with the same g tensor, the same line width (lw) and/or the same hyperfine coupling(s). These two components are said to be similar.

When two components are equivalent (same g tensor, same line width (lw), same hyperfine coupling(s) (A)), SimLabel automatically informs the user that **similarities** are available. The user must make the choice between considering or not these similarities. Considering these similarities means that the g, lw and A parameters, are equal for both components. Thus, if *Component 1* and *Component 2* are equivalent and are chosen to be similar, the table in the *to fitting mode* window contains all *Component 1* parameters and only *logtcorr_C2* and *weight_C2*. The g and A principal values and lw values of *Component 1* stand also for *Component 2*.

2) <u>From SimLabel to esfit GUI: Multi Fit</u>

When the user clicks 

in the *SimLabel_main* window, a first window, named *to fitting mode (multi.)*, appears. As in the *Single Fit* mode, it contains tables summarizing the parameters of the simulation components that can be considered in the fitting process. The presentation of the data and the way of processing are equivalent as in the previous case.

The difference is that several tabs are present. The first tab, called *Common*, contains the common parameters (g values, hyperfine couplings and weight). The first saved simulation in the list provides them. The second tab, called *Specific(1)*, contains the parameters specific to the spectra in first position (line widths, correlation times). The third tab, called *Specific(2)*, contains the parameters specific to the spectra in second position and so on.

Note: When running with EasySpin prior to 6.0.0, the user can choose the normalization type of the experimental spectra displayed in the *esfit* window in the window *to fitting mode (multi.)*. A normalization to their amplitude or to their integrated intensity can be applied.

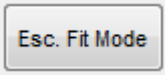
3) esfit GUI

In most cases, the default parameters (*Method*, *Target*, *Scaling*; *Startpoint*) of the *esfit* GUI are convenient, but for more details on *esfit*, visit the related Easyspin web documentation.

Start fitting. Once *stop*ped (manually or automatically), the simulated fitted spectrum has to be exported, so that it could be imported in SimLabel. To do that, first *save parameters* and *export* them. This export is confirmed in the command window. Several fits can be exported before quitting the fitting mode.

Note that for EasySpin 6.0.0 and later, uncertainties are estimated if the fit converges. The uncertainty quantification is skipped if the user stops manually the fitting procedure. Note that a finite maxTime can be set in the algorithm settings.

4) Back to SimLabel

The user can quit the fitting mode at any time by clicking 

in the *SimLabel_main* window.

If one or several fit spectra have been exported from the *esfit* GUI, these spectra can be saved as *.slb files.

If the fitting procedure results in a parameter value equal to the lower or the upper limit of the variattion range, SimLabel notifies the user.

Note: - The starting simulation spectrum sent to the *esfit* GUI has to be saved, because some data in the *.slb file are needed to import the fitting result in SimLabel.

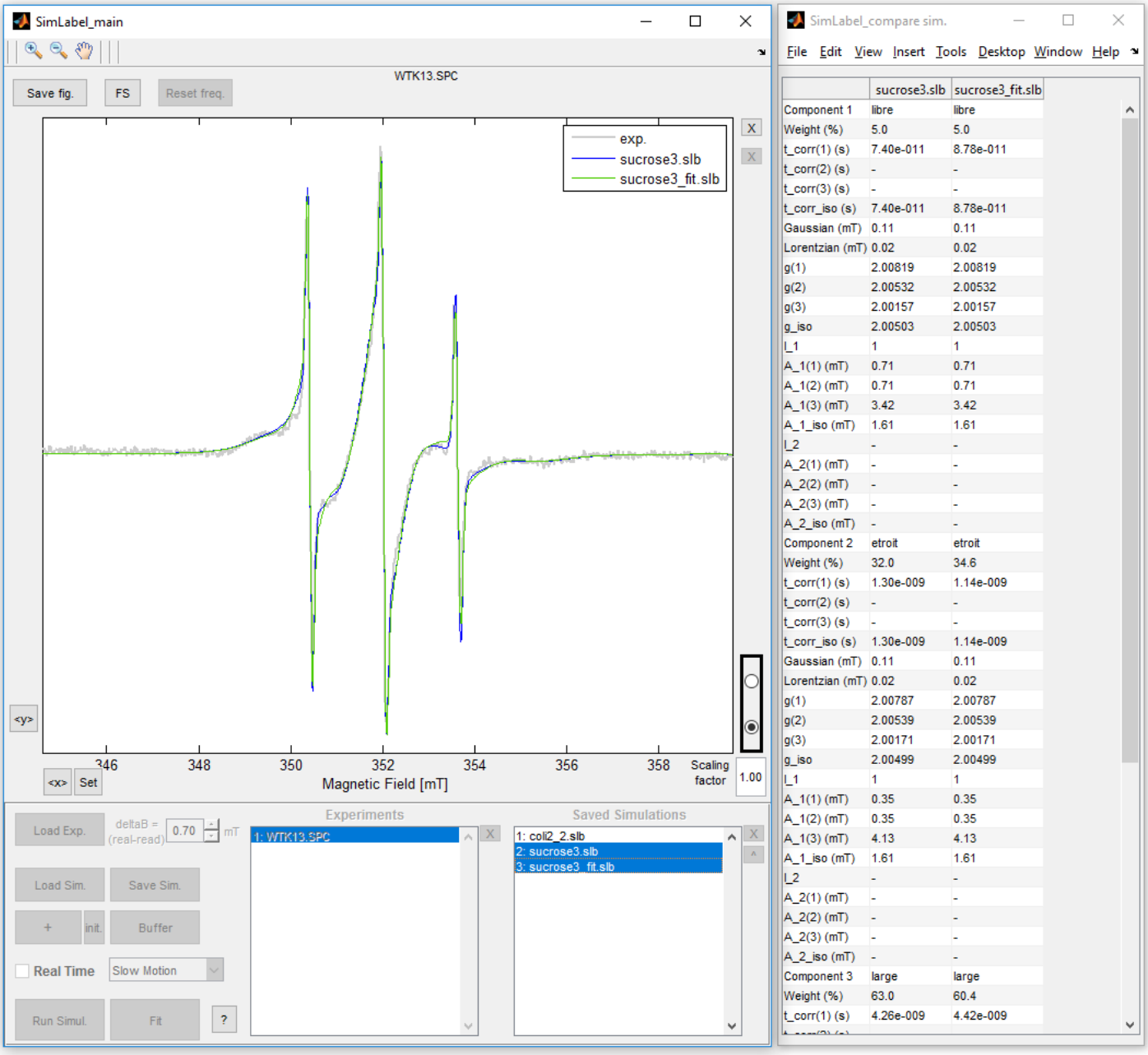
- The uncertainties in esfit are given as the lower and upper values of a confidence interval (95%). In SimLabel (*p ± δp* button of a SimLabel_X window), they are given as p ± δp where p is the considered parameter. These two ways of showing uncertainties are equivalent for all parameters except for the correlation times, as *esfit* works with the logarithm of the correlation times. The uncertainties of correlation times given in SimLabel are then slightly overestimated to keep the best value of esfit as the central value.

G. Practical tricks

1) *Compare saved simulations*

It is possible to compare several saved simulations, just by selecting multiple files in the *Saved Simulations* list, basically with the keys *Ctrl* and *Shift*. The spectra are displayed using the selected scaling method (see E.5.). A new table window called *SimLabel_compare sim.* opens. All the data are listed in this table, one component after the other, so that the simulations can be compared in details, component by component, parameter by parameter. This table can also be printed.

To escape, just select one single file in the *Saved Simulations* list or, if enabled, click *Buffer* (see D.).

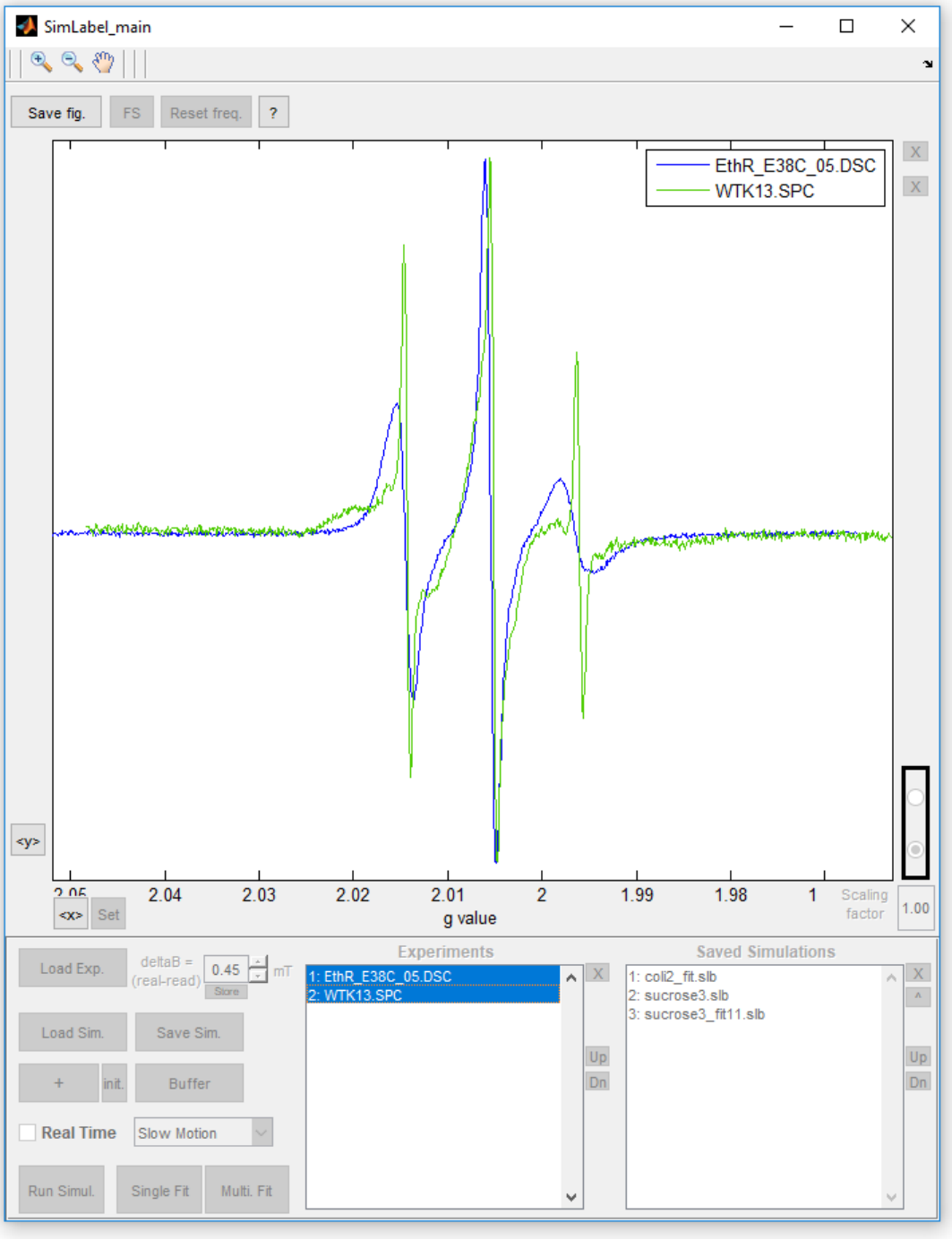


	sucrose3.slb	sucrose3_fit.slb
Component 1	libre	libre
Weight (%)	5.0	5.0
t_corr(1) (s)	7.40e-011	8.78e-011
t_corr(2) (s)	-	-
t_corr(3) (s)	-	-
t_corr_iso (s)	7.40e-011	8.78e-011
Gaussian (mT)	0.11	0.11
Lorentzian (mT)	0.02	0.02
g(1)	2.00819	2.00819
g(2)	2.00532	2.00532
g(3)	2.00157	2.00157
g_iso	2.00503	2.00503
l_1	1	1
A_1(1) (mT)	0.71	0.71
A_1(2) (mT)	0.71	0.71
A_1(3) (mT)	3.42	3.42
A_1_iso (mT)	1.61	1.61
l_2	-	-
A_2(1) (mT)	-	-
A_2(2) (mT)	-	-
A_2(3) (mT)	-	-
A_2_iso (mT)	-	-
Component 2	etroit	etroit
Weight (%)	32.0	34.6
t_corr(1) (s)	1.30e-009	1.14e-009
t_corr(2) (s)	-	-
t_corr(3) (s)	-	-
t_corr_iso (s)	1.30e-009	1.14e-009
Gaussian (mT)	0.11	0.11
Lorentzian (mT)	0.02	0.02
g(1)	2.00787	2.00787
g(2)	2.00539	2.00539
g(3)	2.00171	2.00171
g_iso	2.00499	2.00499
l_1	1	1
A_1(1) (mT)	0.35	0.35
A_1(2) (mT)	0.35	0.35
A_1(3) (mT)	4.13	4.13
A_1_iso (mT)	1.61	1.61
l_2	-	-
A_2(1) (mT)	-	-
A_2(2) (mT)	-	-
A_2(3) (mT)	-	-
A_2_iso (mT)	-	-
Component 3	large	large
Weight (%)	63.0	60.4
t_corr(1) (s)	4.26e-009	4.42e-009

2) Overlay experimental spectra

It is also possible to overlay several experimental spectra, just by selecting multiple files in the *Experiments* list, basically with the keys *Ctrl* and *Shift*. The raw spectra are displayed using a g-value range to avoid frequency conflict.

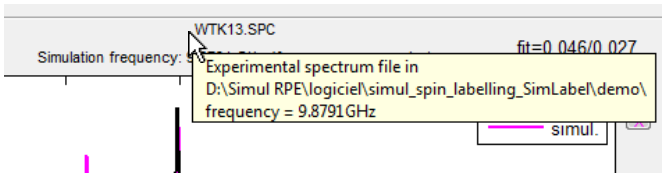
To escape, just select one single file in the *Experiments* list.

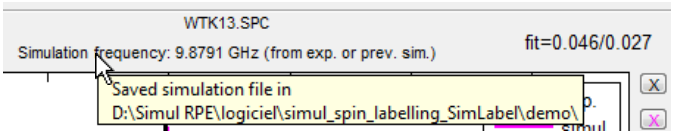


3) Path of the considered files

The path of the experimental loaded spectrum and its record frequency appear in a tooltip when the top label (see figure below) of its name is hovered over. If the simulation is saved, the path of its folder also appears in a tooltip when the *Simulation*

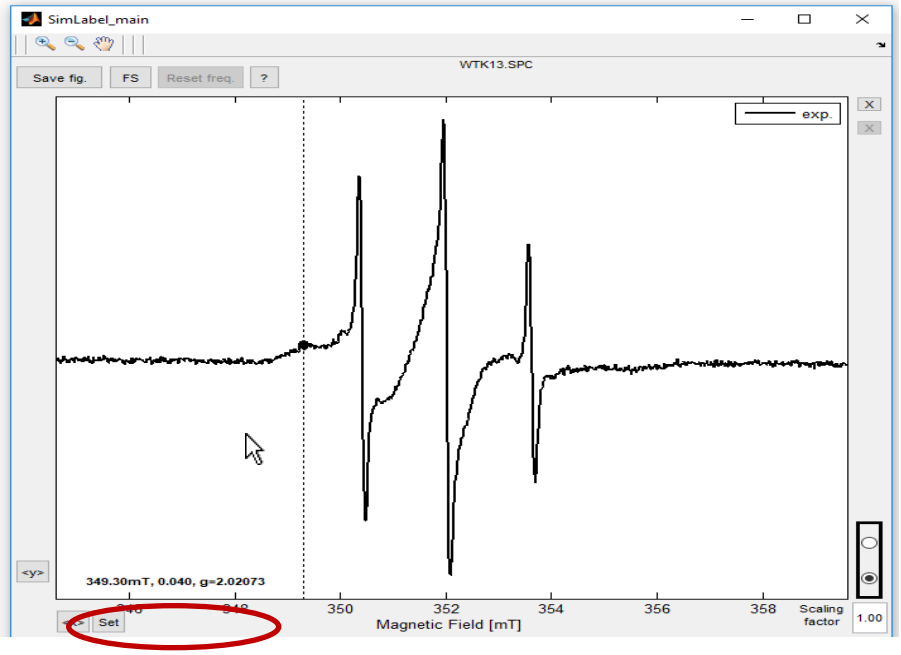
frequency label is hovered over. This could be useful to check the frequencies and to identify a file in case of identical names.

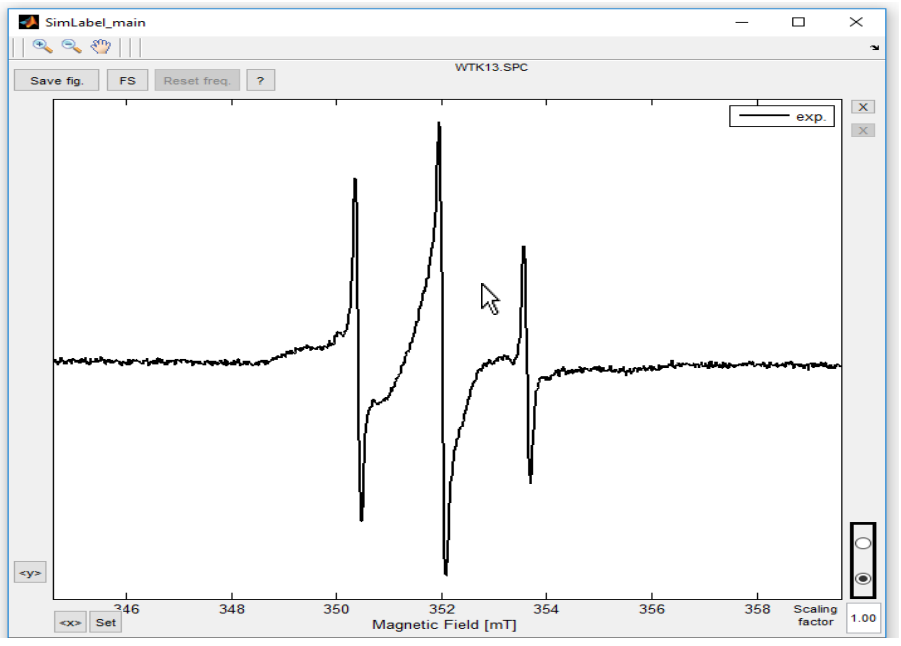


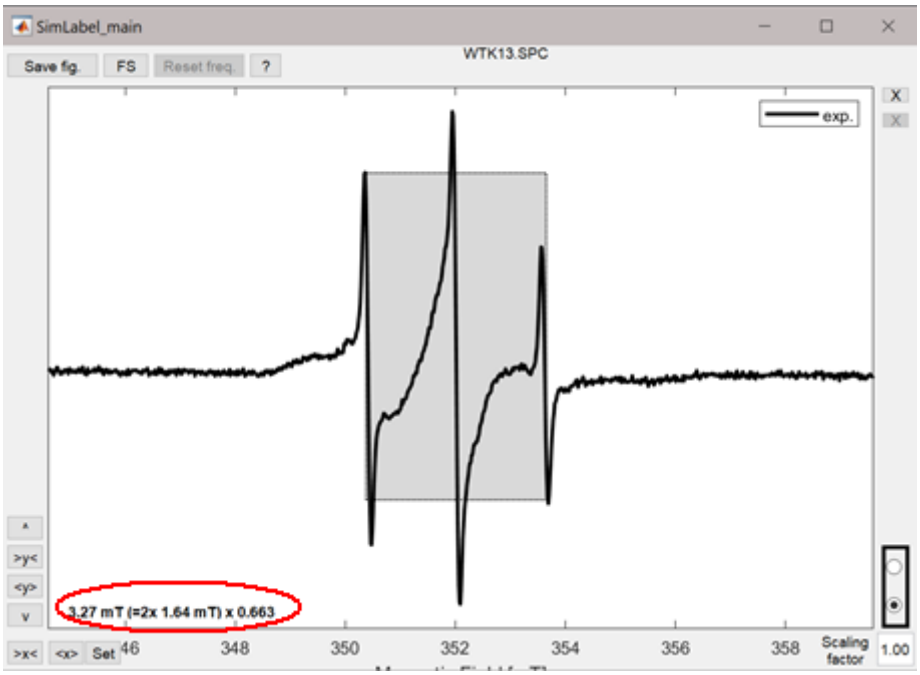


4) <u>Coordinates and g-value of the current point, splitting measurement</u>

When the user clicks a point of a displaid spectrum (experimental or simulated), the coordinates and the g-value are displayed in the graph bottom left. Click on the background to remove this information. If you click another point, a rectangle is displayed, as well as its height and width. Once again, click on the background to remove this information.



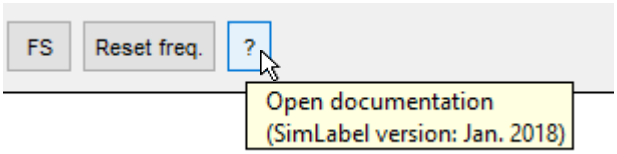




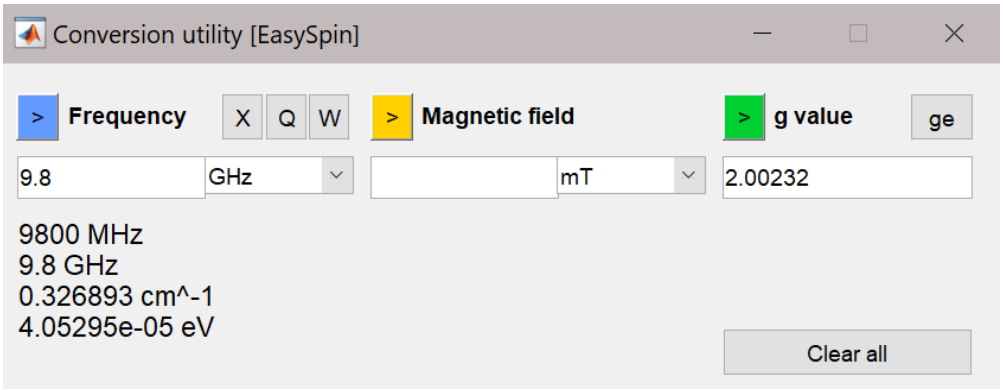
If the magnetic field offset (*deltaB*) is changed, the point and vertical line stay in the same position and informations are automatically updated. It could be useful to determine the *deltaB* value if a g-marker is present.

5) *SimLabel version, documentation and mT/MHz conversion scale*

When the user hovers the pointer over ?, without clicking it, a tooltip may appear with information about the SimLabel current version.



When the user clicks ?, this documentation (if in the same folder than simlabel.m) and the conversion utility from EasySpin open.



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2. Stoll, S.; Schweiger, A., EasySpin, a comprehensive software package for spectral simulation and analysis in EPR. *Journal of Magnetic Resonance* **2006,** *178* (1), 42-55.
3. Schneider, D. J.; Freed, J. H., Calculating Slow Motional Magnetic Resonance Spectra. In *Spin Labeling: Theory and Applications*, Berliner, L. J.; Reuben, J., Eds. Springer US: Boston, MA, 1989; pp 1-76.
4. Bordignon, E.; Brutlach, H.; Urban, L.; Hideg, K.; Savitsky, A.; Schnegg, A.; Gast, P.; Engelhard, M.; Groenen, E. J. J.; Möbius, K.; Steinhoff, H.-J., Heterogeneity in the Nitroxide Micro-Environment: Polarity and Proticity Effects in Spin-Labeled Proteins Studied by Multi-Frequency EPR. *Applied Magnetic Resonance* **2010,** *37* (1), 391-403.
5. Etienne, E.; Pierro, A.; Tamburrini, K. C.; Bonucci, A.; Mileo, E.; Martinho, M.; Belle, V., Guidelines for the Simulations of Nitroxide X-Band cw EPR Spectra from Site-Directed Spin Labeling Experiments Using SimLabel. *Molecules* **2023,** *28* (3), 1348.