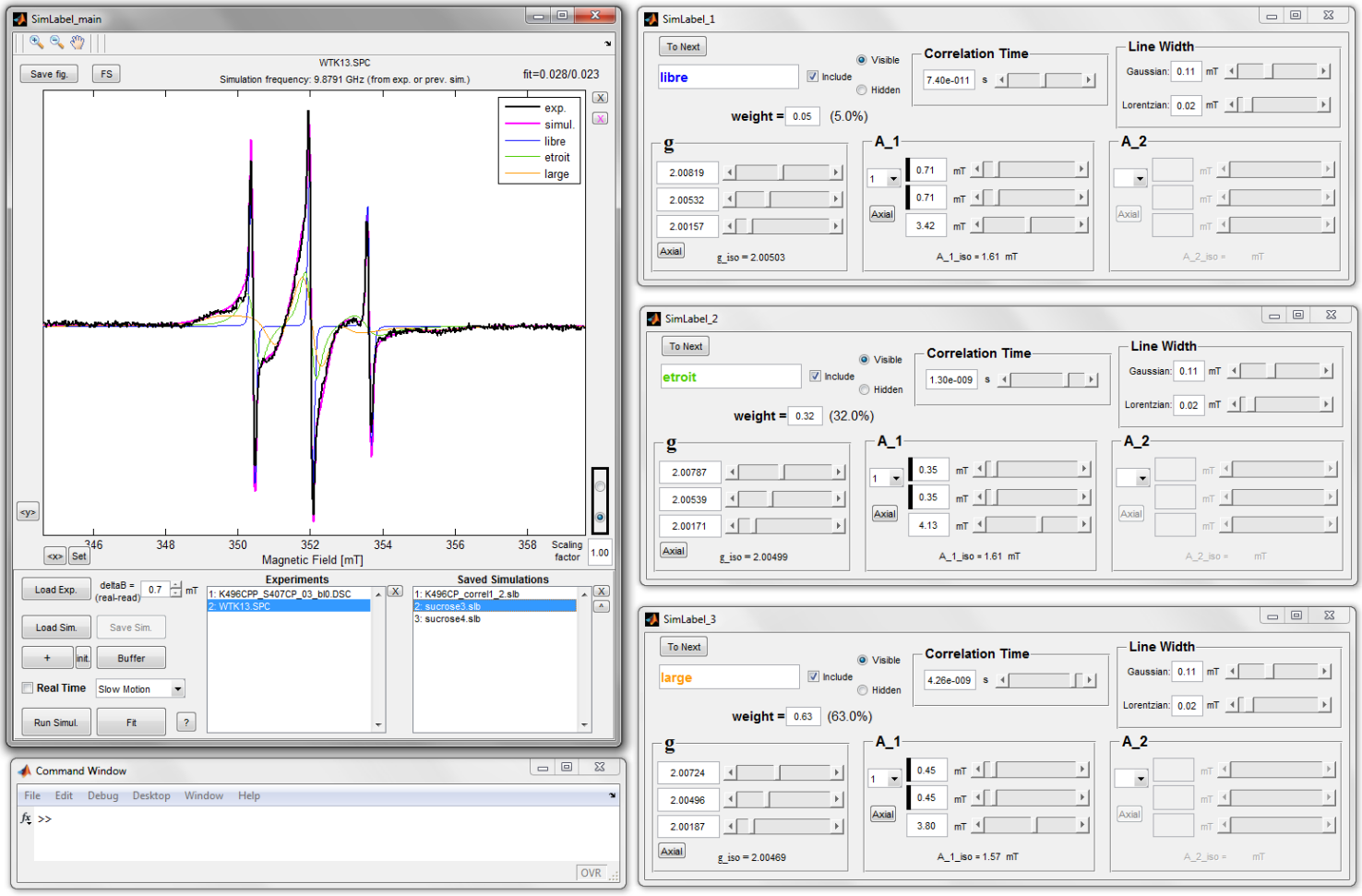
# SimLabel

## **Sim**ulations of cw EPR spectra from Spin **Label**ing

## Documentation





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## Synopsis

SimLabel is a Graphical User Interface (GUI) developed in MATLAB. Thanks to some **EasySpin functions** (Stoll S., J. Magn. Res.,178, 42-45, 2006), 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 is unlimited and n≥1). 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. The data exchange between these two GUI (SimLabel and *esfit* GUI) is managed by SimLabel, 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) 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.

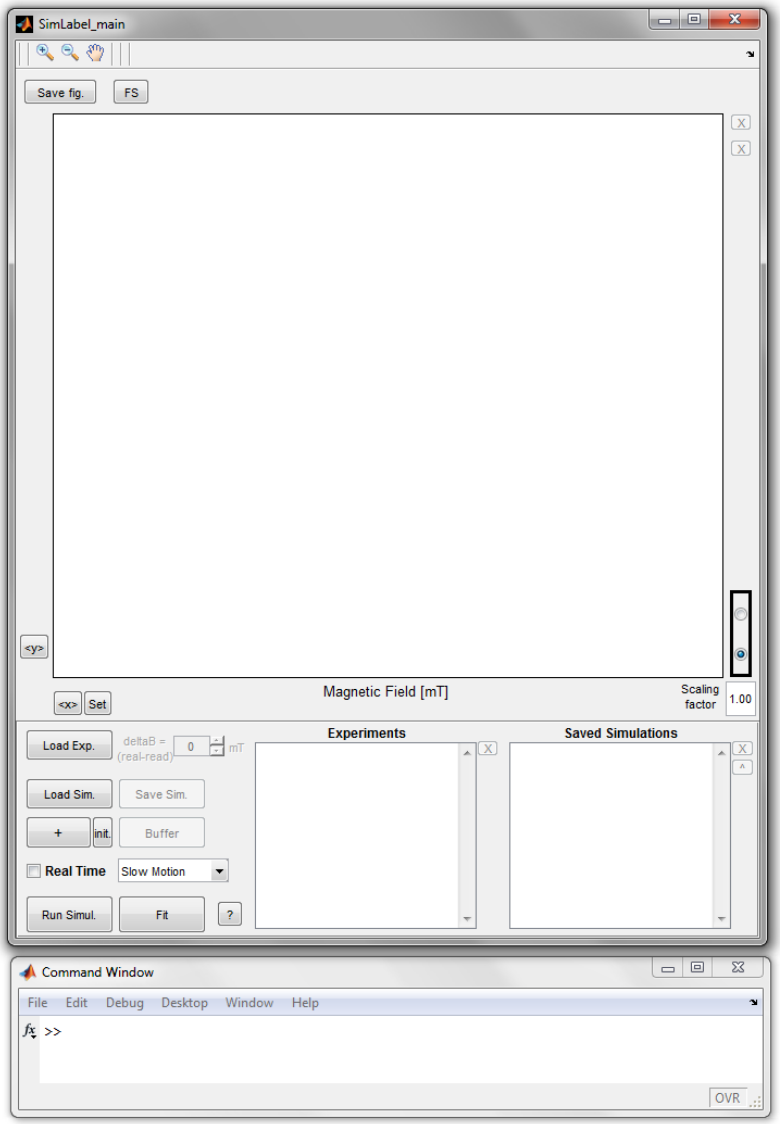
Select the local folder for your SimLabel session in the MATLAB main window. **You must be allowed to write in this directory** (see F. for more details). The directory of your spectra is advised.

Launch SimLabel by typing in the command window:

```
>> simlabel
```

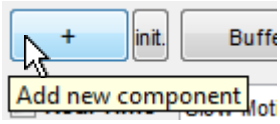
(or `SimLabel` or `SIMLABEL` or `SIMlabel` or…)

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



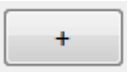
## B. Getting started without experimental spectrum

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.

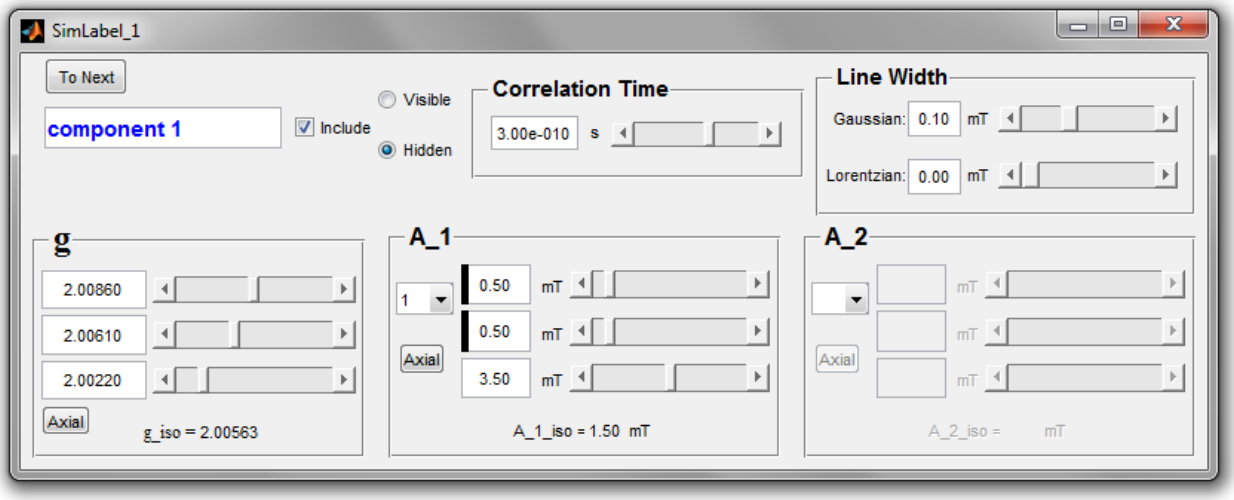


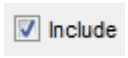

The default type of simulation is *Slow Motion* (see the popup menu on the bottom left corner of the *SimLabel_main* window – *Frozen Solution* is also available). This simulation type is performed using the EasySpin function *chili*. The *chili* algorithm is based on Freed's works 

(Schneider and Freed, Biol. Magn. Res., 8, 1-76, 1989).

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

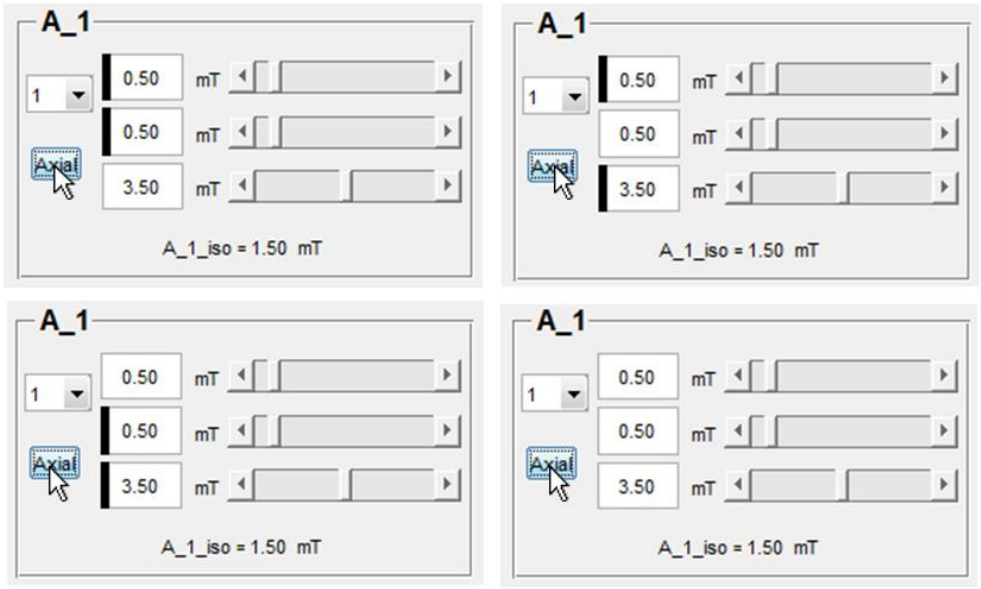
in the *SimLabel_main* window.
- The *SimLabel_1* window opens with default parameters (see C. for more details).




- The default name of this first component is "component 1". This name can be modified by the user.
- The *include* box must be checked 

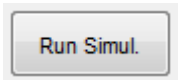
, so that the corresponding component will be included in the simulation.
- A second hyperfine coupling can be added by selecting a nuclear spin value in the popup menu of the *SimLabel_1* window, *A_2* panel. The simulation run takes the hyperfine coupling into account only if the three principal values 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)..



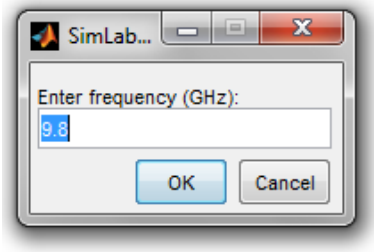

*Note*: - Before running a simulation in the slow motion regime, anisotropy has to be introduced for at least one of the two tensors (g or A). This is a requirement for using the EasySpin *chili* function.

- 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 

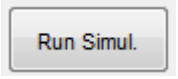
in the *SimLabel_main* window.

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

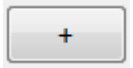



- The default range can be modified with 

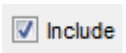
.

- If the range is modified, click 

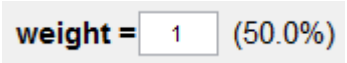
again for the simulation range to match with the display.

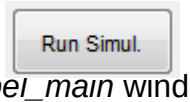
3) - For **multiple components**, add new components by clicking 

in the *SimLabel_main* window.

- When a new component is included by checking 

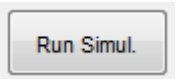
in the *SimLabel_X* window, a new modifiable parameter called *weight* appears. This is a relative weight. The corresponding percentage is also given.



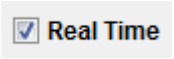
- If 

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.

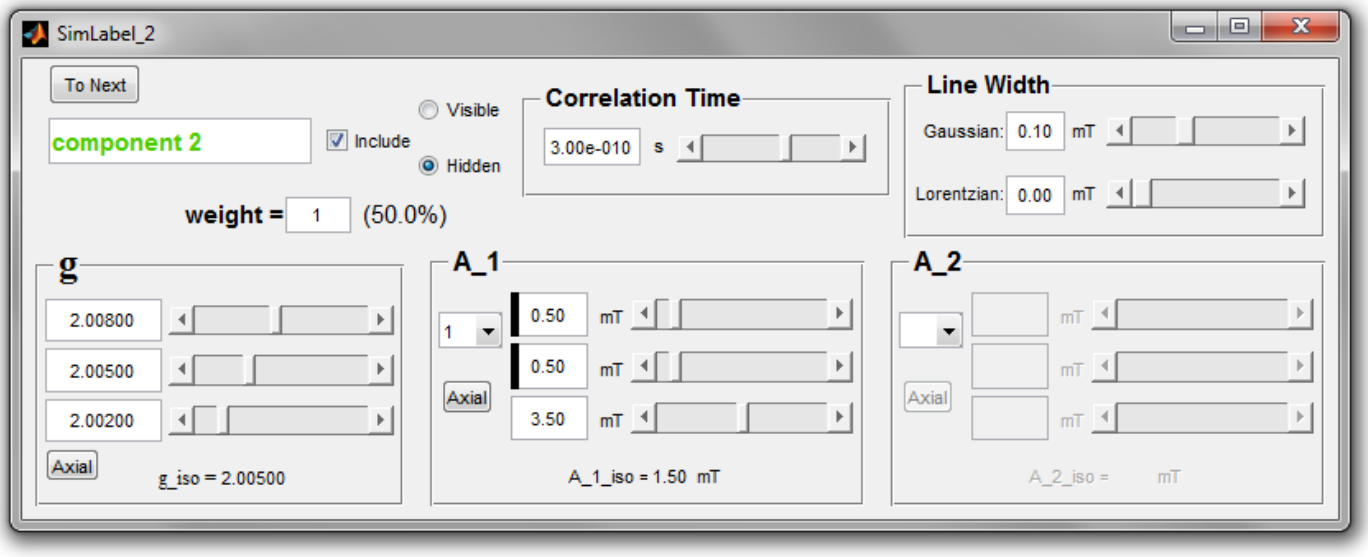
4) If 

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 name of the component ***component 2*** can be changed by the user. 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 (Schneider and Freed, Biol. Magn. Res., 8, 1-76, 1989). The longer the *Correlation Time* is, the less mobile is the paramagnetic label.
- The *Line Width* stands for the full width at half maximum of the isotropic convolutional broadening, which can be pure gaussian, pure lorentzian or voigtian (mix of gaussian and lorentzian). Thus the broadening is defined by the parameters *Gaussian* and *Lorentzian* (see E.4. for more details).

- 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, 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 *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.)

*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). From Bordignon E. et al., (Appl. Magn. Reson.,37, 391-403, 2010), depending on the polarity and proticity of the MTSL environment, we have:

**$g_x$ in [2.00800, 2.00940], $g_y$ = 2.00610(5) and $g_z$ = 2.00220(5).**

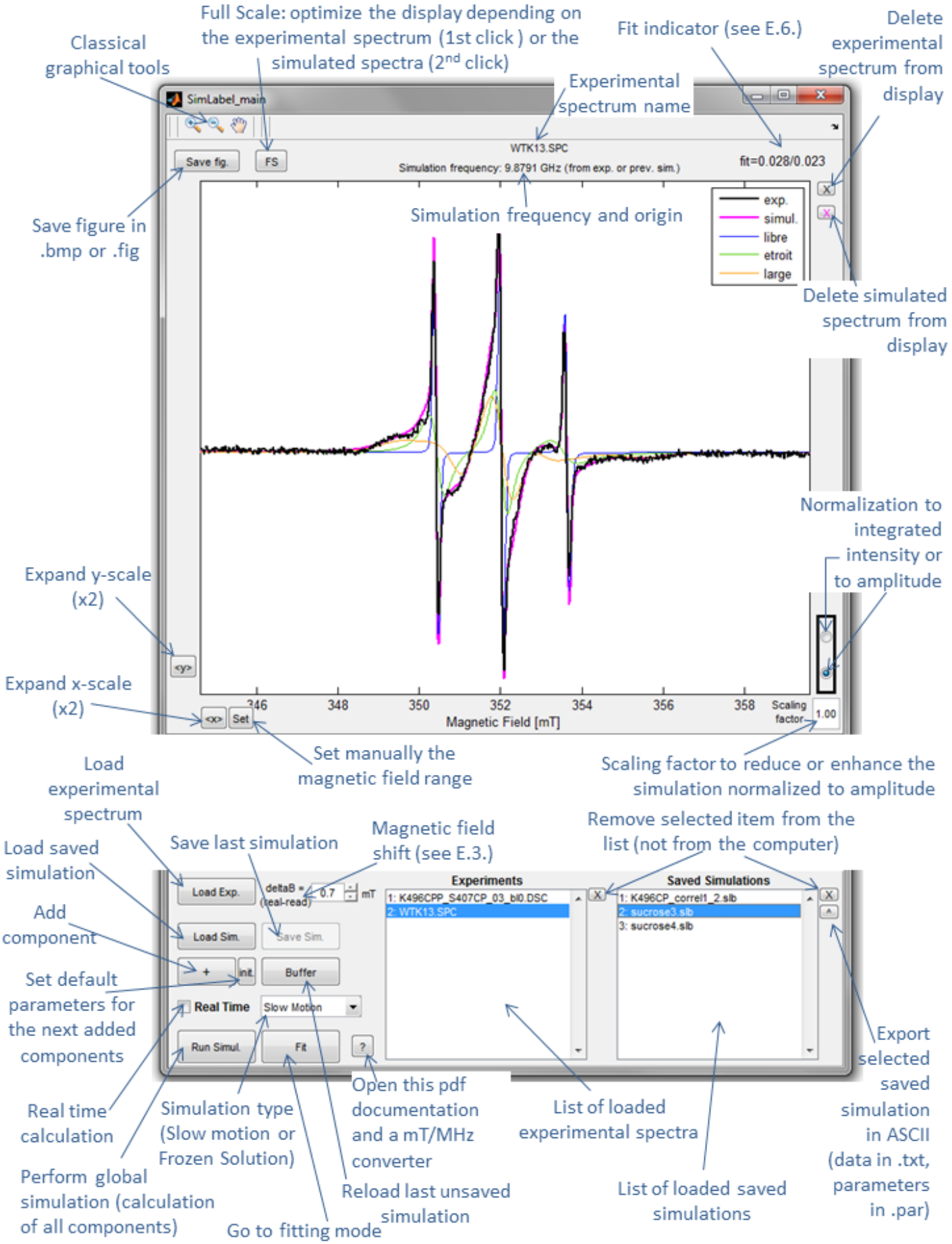
**$A_{xy}$ in [0.35, 0.55] mT and $A_z$ in [3.25, 4.85] mT.**

(see G.5.)

- 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. Experimental spectra with more or less points are still managed by SimLabel.
- Hyperfine couplings are considered as soon as three principal values are given.
- The R*eal 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. Otherwise the user is asked to set the frequency. The frequency of the displayed simulation is displayed above the graph with the origin of this frequency if available.

### 3) *Magnetic field offset*

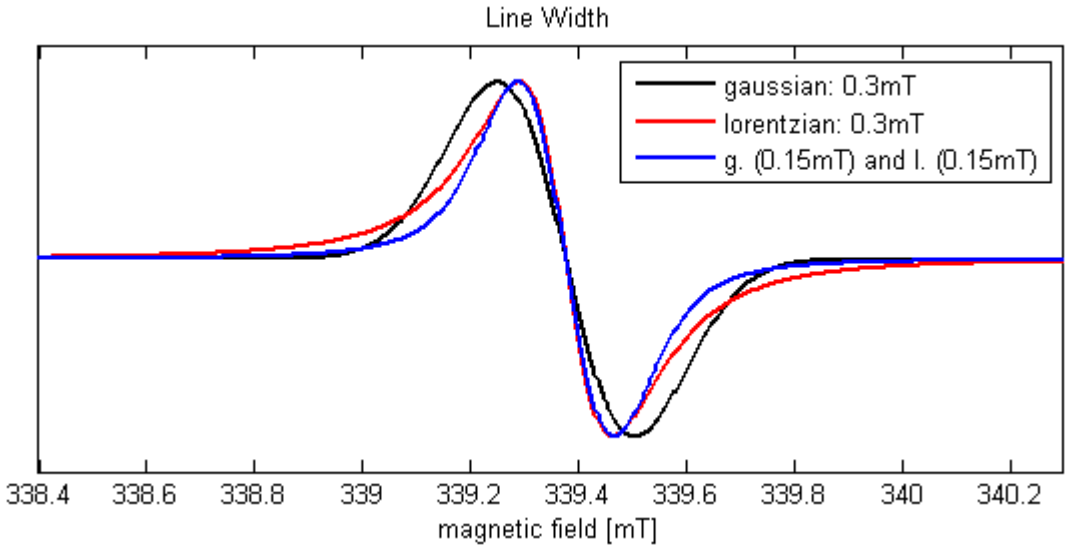
A magnetic field offset *deltaB* for the experimental spectrum can be given by the user in the *SimLabel_main* window. The knowledge of this value is essential for determining accurate g values. When modified, this *deltaB* is automatically taken into account and stored in memory for the next SimLabel sessions. If *experiment.** is the experimental spectrum file, *deltaB* is saved by SimLabel in the file *experiment.mat* located in the same folder. Thus this *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 easily measured thanks to a standard sample of well-known g-value (like a weak pitch).

### *4) 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 (full width at half maximum) are given in the *SimLabel_X* window (see C.).




A particular attention is recommended for line broadenings. When several components are considered, it is highly recommended to use the **same broadening for all similar components**, because no physical sense is associated with these broadenings and because other parameters can influence the line width. **Keeping the line widths constant for the different similar components guarantees meaningful comparisons of correlation times of these components**.

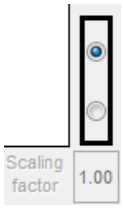
### *5) Scaling spectra*

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:

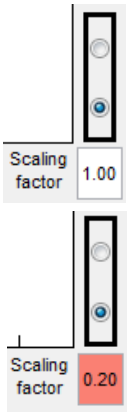
$$y_{norm} = {}^{y}/_{h}$$ , in which *h* is the normalization factor.




The top selection displays spectra normalized to their integrated intensity. In this case, $h$ is calculated by 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 $\alpha$ is the scaling factor that could be modified by the user. The $\alpha$ default value is 1. It could be useful to decrease $\alpha$, for example, for adjusting a one-component simulation to a specific component of a multicomponent experimental spectrum. When $\alpha$ 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:

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

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

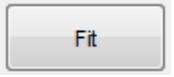
These indicators are inspired from a chi-square.

## F. Automatic fitting

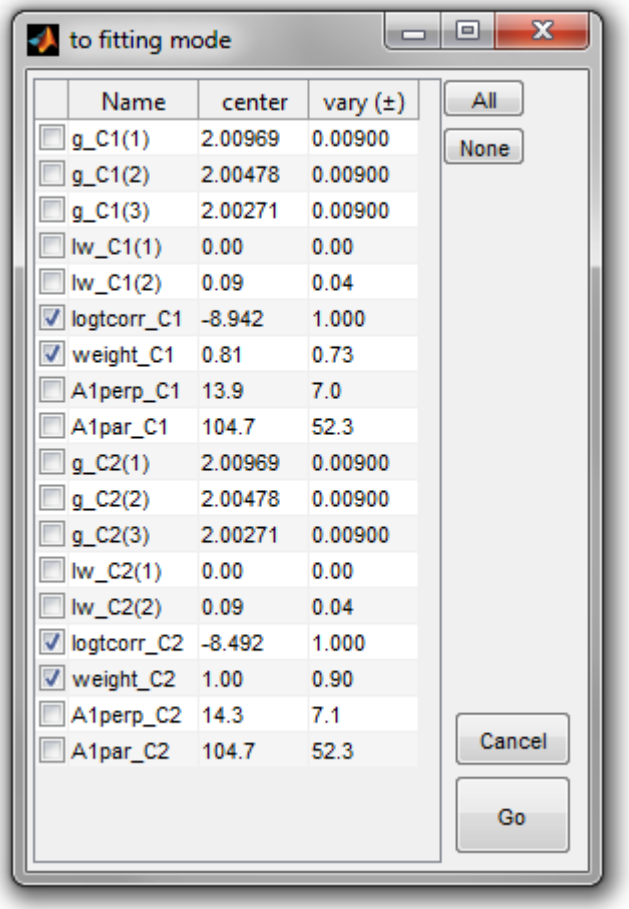
Automatic fitting is available when an experimental spectrum and a saved simulated spectrum are displayed and when the frequencies and the magnetic field ranges match. A GUI for automatic fitting a simulated spectrum to an experimental spectrum, using least-squares fitting techniques, is already available in EasySpin (*esfit* function). This GUI can be launched from SimLabel. The interfacing between these GUIs is automatically managed by SimLabel, but some explanations are however required.

*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 a new file called *temporary_func4esfit.m* is automatically and temporarily generated by SimLabel in the current folder when automatic fitting is running. This file is deleted when the user quits the fitting mode.

### 1) *From SimLabel to esfit GUI*

When the user clicks 

in the *SimLabel_main* window, a first window, named *to fitting mode*, 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 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.

*Note*: For example, if two conformers are present in solution, only their correlation times make their spectra different. Then it could be useful 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 correlated.

When two components are equivalent (same g tensor, same line width (lw), same hyperfine coupling(s) (A)), SimLabel automatically informs the user that **correlations** are available. The user must make the choice between considering or not these correlations. Considering these correlation 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 correlated, 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*.

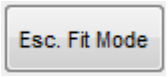
The user has to select the parameters to be variable in the fitting process (30 parameters maximum, even if this maximum should never be reached!). The *esfit* GUI for automatic fitting, called *Easyspin Least-Squares Fitting*, opens when clicking .

*2) 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.

*3) 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.

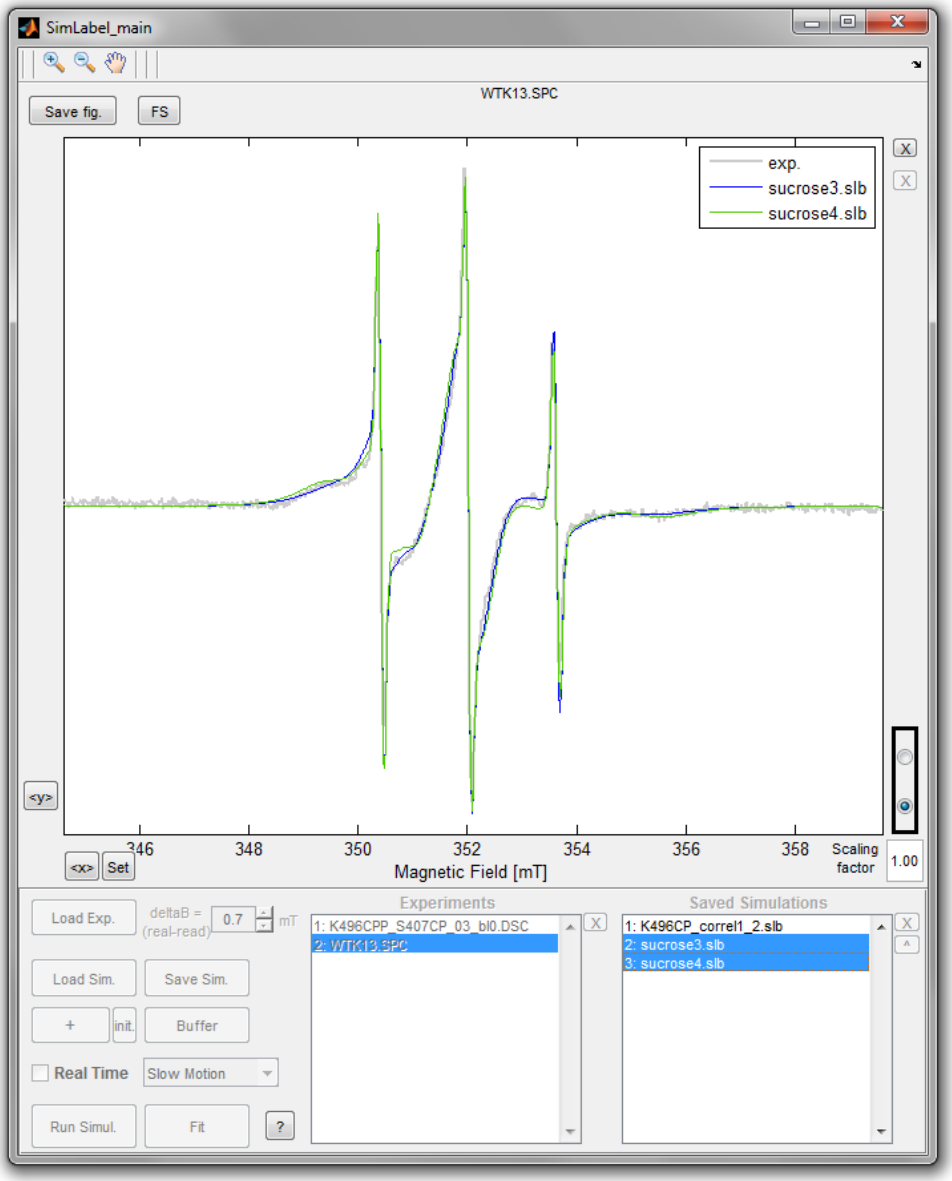
*Note*: The starting simulation spectrum sent to the *esfit* GUI has to be saved, because some data in the *.slb file are needed to import the fitting result in SimLabel.

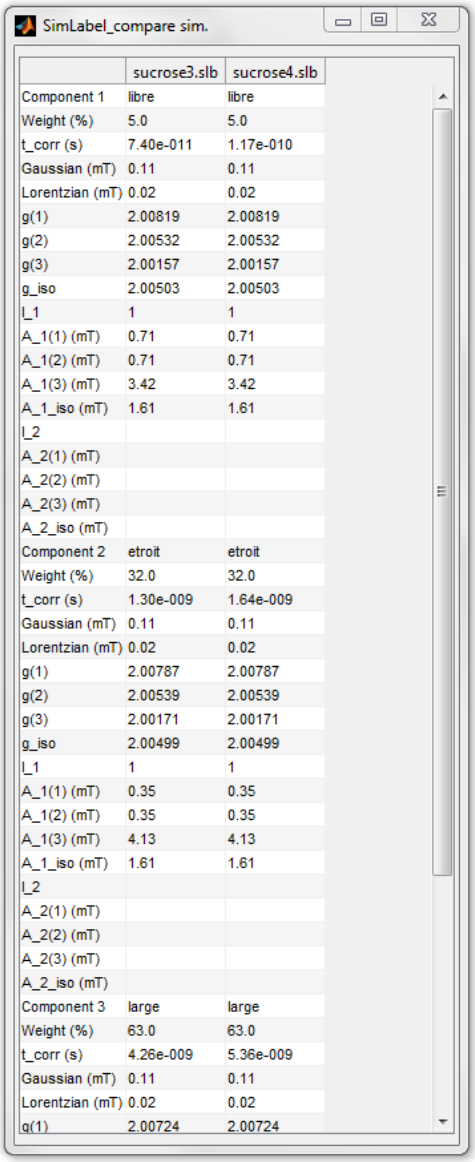
## G. Practical tricks

### 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 simulation can be compared in details, component by component.

To quit this display of multiple saved simulations, just select one single file in the *Saved Simulations* list or, if enabled, click *Buffer* (see D.).



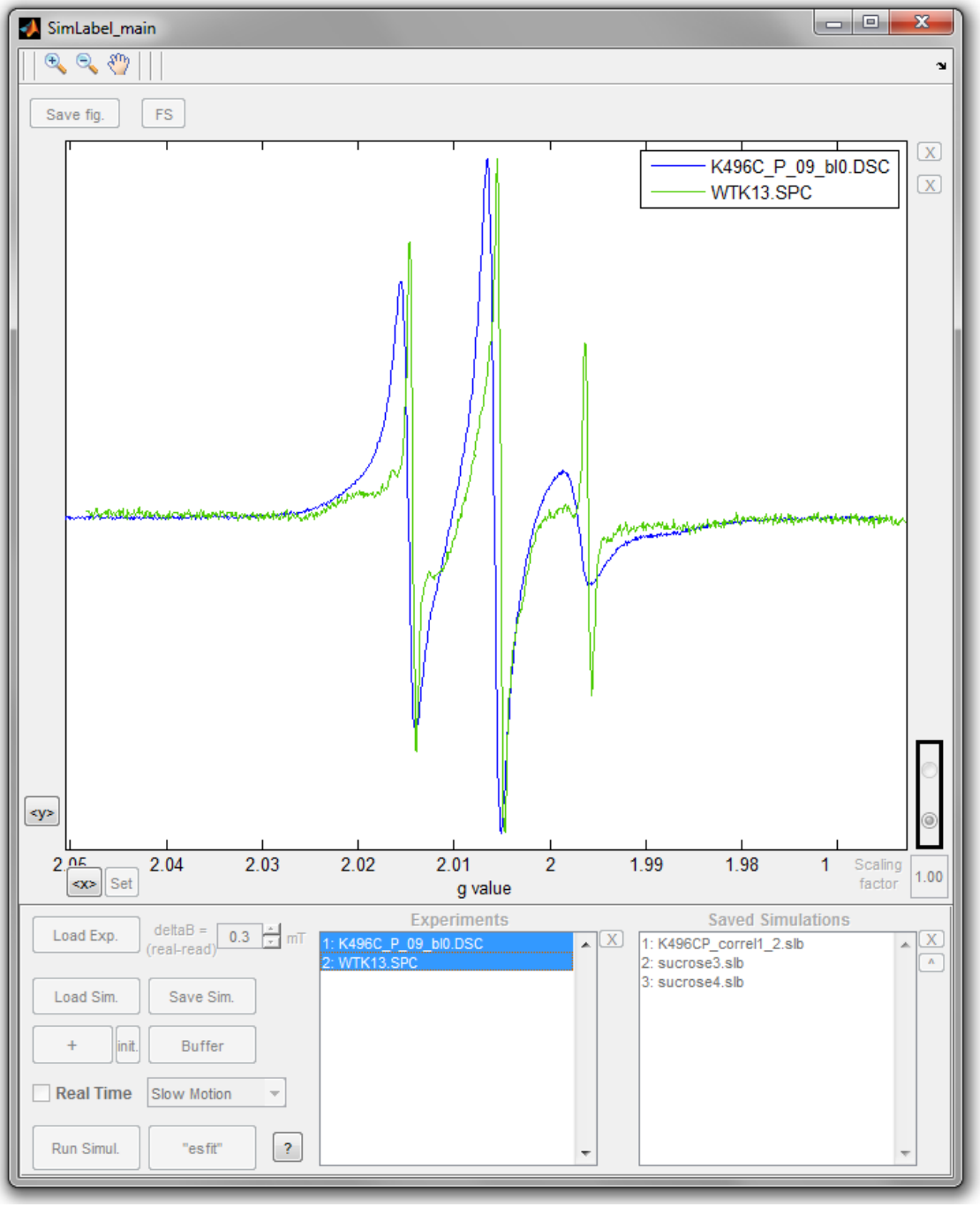




| | sucrose3.slb | sucrose4.slb |
|---|---|---|
| Component 1 | libre | libre |
| Weight (%) | 5.0 | 5.0 |
| t_corr (s) | 7.40e-011 | 1.17e-010 |
| Gaussian (mT) | 0.11 | 0.11 |
| Lorentzian (mT) | 0.02 | 0.02 |
| g(1) | 2.00819 | 2.00819 |
| g(2) | 2.00532 | 2.00532 |
| g(3) | 2.00157 | 2.00157 |
| 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 | 32.0 |
| t_corr (s) | 1.30e-009 | 1.64e-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 | 63.0 |
| t_corr (s) | 4.26e-009 | 5.36e-009 |
| Gaussian (mT) | 0.11 | 0.11 |
| Lorentzian (mT) | 0.02 | 0.02 |
| g(1) | 2.00724 | 2.00724 |

*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.

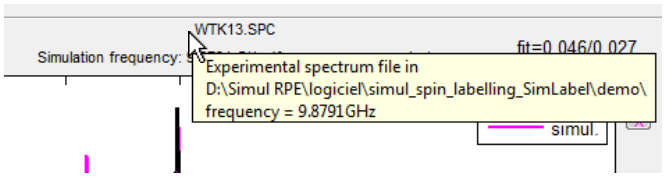
To quit this display, just select one single file in the *Experiments* list.

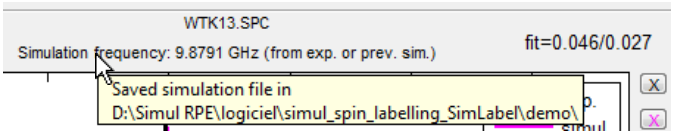



*3) Path of the considered files*

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*

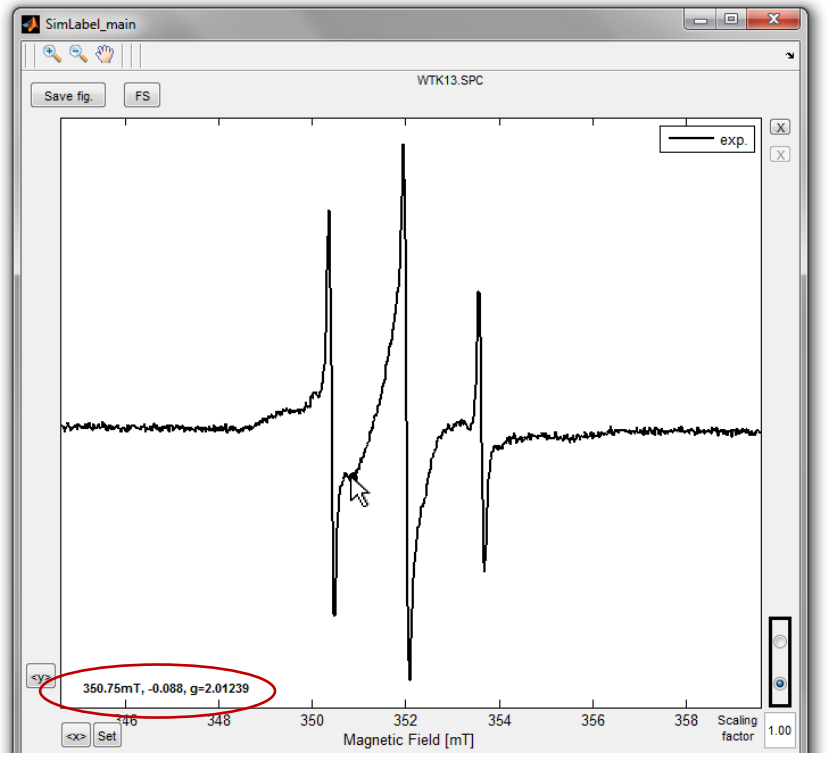
*frequency* label is hovered over. This could be useful to check the frequencies and to identify a file in case of identical names.

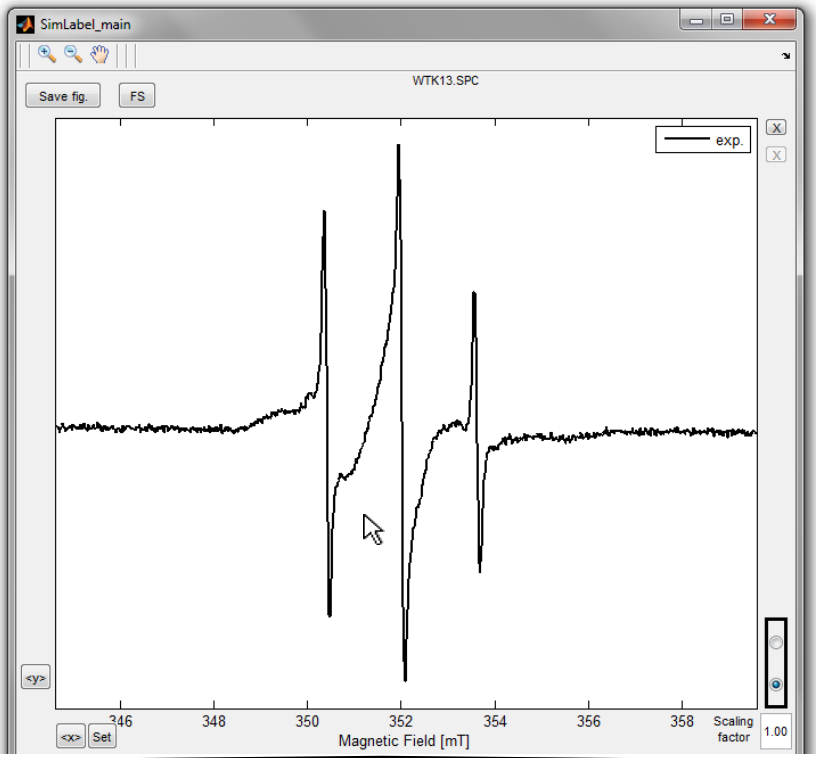



*4) <u>Coordinates and g-value of the current experimental point</u>*

When a point of the experimental spectrum is clicked, the coordinates and the g-value are displayed in the graph bottom left. Click on the background to remove this information.



5) *Documentation and mT/MHz conversion scale*

When the user clicks ? , this documentation and a conversion scale mT/MHz with the typical hyperfine coupling values for nitroxides open.