LIPSPIN USER MANUAL

(v1.0)

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This manual does not have any warranty. Do not hesitate to contact the author if you have comments on any aspect of this manual.

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Installation

LipSpin is released as original m-files and standalone versions. In order to execute LipSpin, follow the instructions depending on the version you have received.

M-files

M-files coding LipSpin can be downloaded from github repositories and loaded in the MATLAB IDE. Matlab scripts and functions have been developed with MATLAB v7.10 and compatibility with other versions cannot be guaranteed. Note that some functionalities of LipSpin require external toolboxes commonly supplied with most MATLAB versions such as Statistics, Optimization and Signal toolboxes. In order to execute LipSpin in the MATLAB IDE follow the next steps:

1. Download LipSpin folder and copy it in your local computer.
2. Copy the above directory in the MATLAB path variable (include subdirectories).
3. Type “lipspin” in the MATLAB command window.

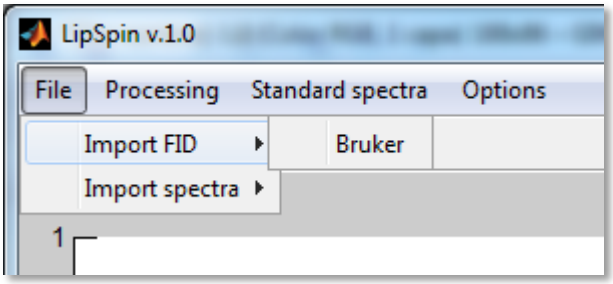
Standalone version

LipSpin can also be provided as a standalone version on demand, which can be run in Microsoft Windows OS without having MATLAB installed. Standalone LipSpin only requires Matlab Compiler Runtime (MCR) to be installed in your local computer. MCR contains all the necessary MATLAB libraries called by LipSpin. MCR version is optimised for each LipSpin compilation, consequently, be sure of using the right version of the MCR by asking the developer of your compiled LipSpin standalone version.

1. Verify if the required version of the MATLAB Compiler Runtime (MCR) is installed in your computer.
2. If the MCR is not installed, run MCRInstaller.exe provided with your LipSpin version. Now, MCR should have created a folder in “Program Files” folder and a new variable in the windows PATH environment variable.
3. Run LipSpin.exe.

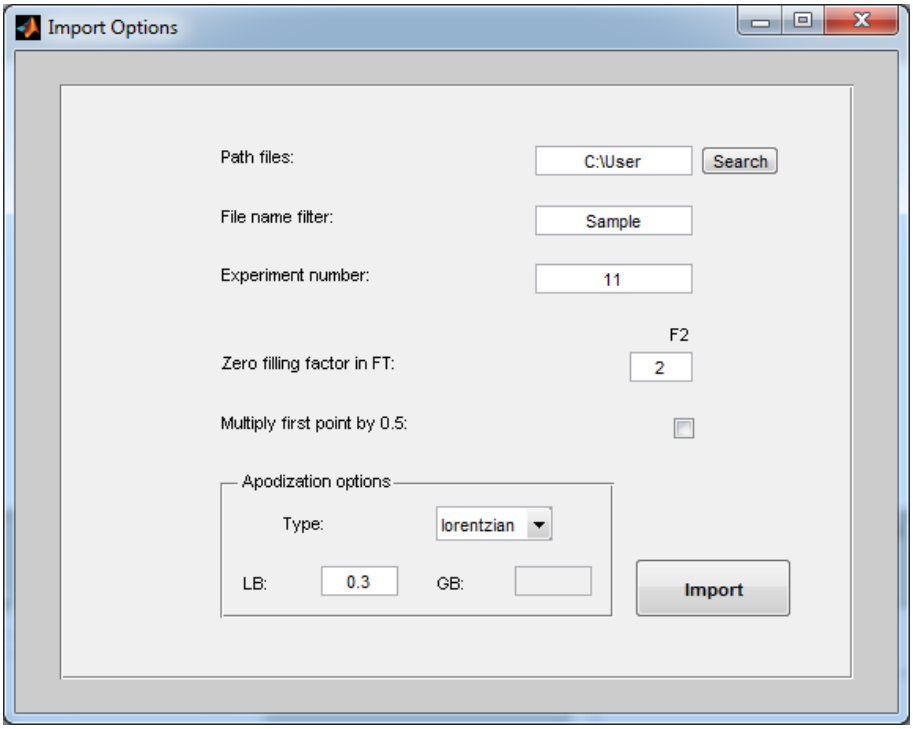
Import NMR data

NMR data can be imported as either time-domain “free induction decays” (FID) or Fourier transformed 1D NMR spectra from the tab “File” in the menu bar of the main screen. LipSpin allows loading multiple NMR data files at once, providing that all share the same time or spectral axis scale. Only Bruker files are supported in current versions of LipSpin but other NMR manufacturer formats are expected to be included in future releases.

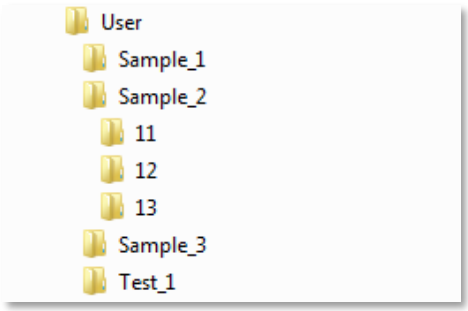


Import FID

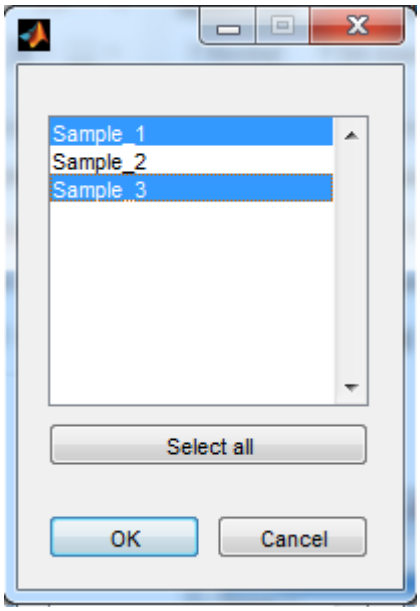
By converting NMR spectra from FID, the user can load raw data directly from NMR acquisition while avoiding the use of additional software other than LipSpin for data processing. The Import window includes the following options:



- ***Path files***: full path of the directory where the sample folders are located. In the example below, it refers to the full path of *User*, (for example *C:\User*).



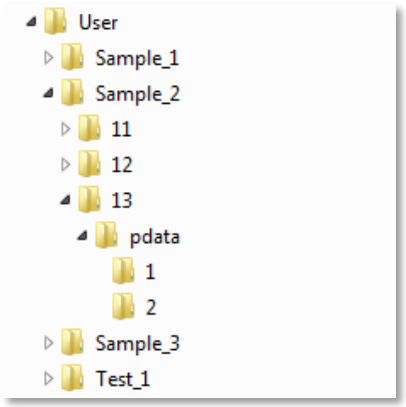
- ***File name filter:*** filters the list of samples to those having this string in their folder names. In the example above, entering "Sample" will remove *Test_1* from latter selection.
- ***Experiment number:*** following the Bruker folder structure, LipSpin requires the experiment number where the *fid* file is located (in the example above: *11, 12* or *13*).
- ***Zero filling:*** increases the spectral resolution of the NMR spectra by multiplying the data length by 2^n, where n is a natural number included in the "F2" field. ***Note: Increasing the data size will increase RAM demand and could slow down the program and the OS execution. Recommended values: 0-2.*
- ***Multiply first point by 0.5:*** this option is aimed to reduce the DC offset in the NMR spectra. In most of the cases, it produces negligible effects.
- ***Apodization:*** allows applying none, Gaussian or Lorentzian windowing to FID before Fourier transformation (FT). Gaussian is aimed to increase peak resolution and could be suitable for peak identification in large overlapped regions. If Gaussian window is applied, LB should be a value close to the negative of the peak width in Hz (measured at half height, e.g: -2) and a good starting point for GB could be 0.2 to 0.4. Lorentzian is aimed to increase the S/N ratio and is the common choice for quantitative spectral analysis. Common values for LB using Lorentzian window range between 0.3 and 1.
- ***Import:*** opens a selection window that lists the samples in "Path files" after filtering by "File name filter". Selected samples will be loaded into the main window.



Import spectra

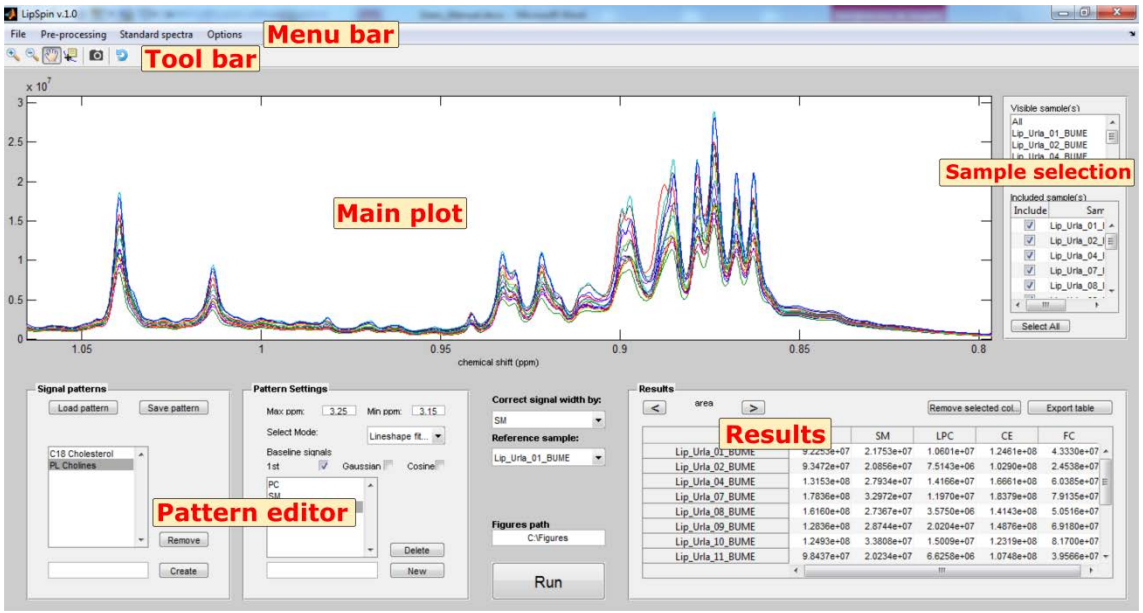
This option allows loading spectra that have been previously converted from FID using third-party software. Refer to the previous section “Import FID” for explanation of ***Path files***, ***File name filter*** and ***Experiment number*** fields. Additionally, this window includes:

- ***Processing number:*** folder name of processed spectra (*1D* file). In the example below, folders *1* and *2* contains different processed spectra from the same FID. Note that LipSpin requires the processing folders to be in a *pdata* folder.

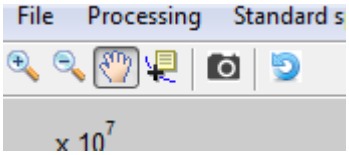


Main LipSpin Window

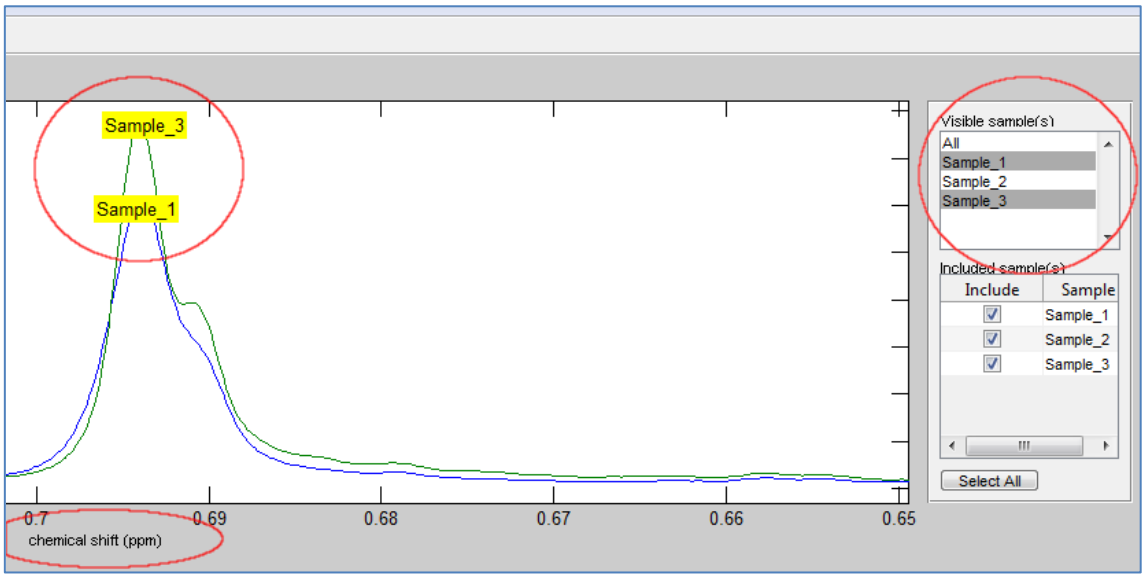
Once the spectra have been imported, they are displayed in the main LipSpin window.



The toolbar contains typical MATLAB navigation tools that allow zooming, panning and displaying data point coordinates. A png snapshot can also be saved from the “Save Figure” tool. The undo button allows reverting last action (only once).



For the aim of aiding sample identification, right-clicking in a spectral line will show/hide a tag with its sample name. Users can also restrict visualization to only selected samples in the “Visible sample(s)” list.

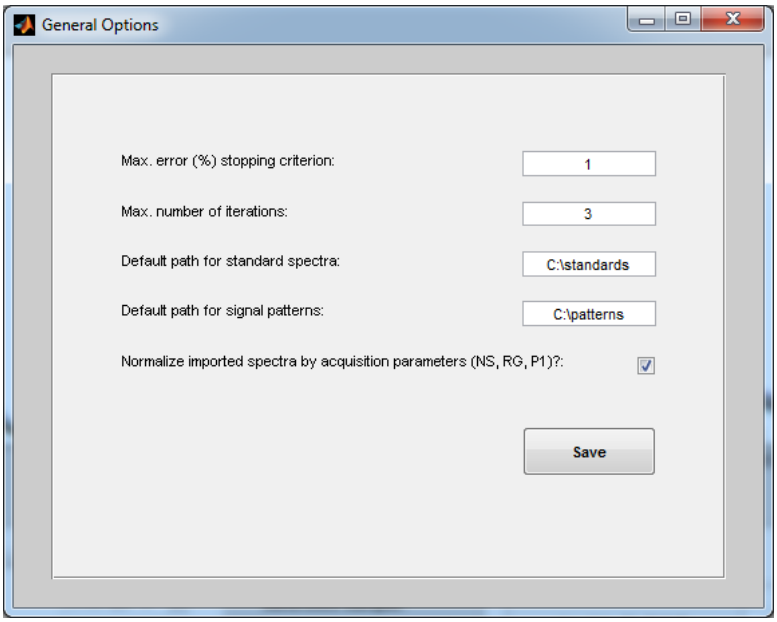


Right-clicking the tag “chemical shift (ppm)” below the x-axis will swap the axis scale in ppm for the axis scale in Hz. This feature is interesting for determining J-coupling constants and line widths.

Finally, the checkbox list in the “included sample(s)” panel allows indicating the samples that will be included if a spectral pre-processing or lineshape fitting is carried out.

General options

The General options window is accessible from the menu bar and allows setting the general options of LipSpin. These options are permanently saved in the *options.nmrcfg* file which should be located in the same folder of LipSpin script (m-files) or LipSpin.exe (standalone version).



- ***Max. error (%) stopping criterion:*** threshold that will stop lineshape fitting iterations based on minimum %RMSE.
- ***Max. number of iterations:*** maximum allowed iterations of lineshape fitting algorithm without fulfilling the maximum %RMSE stopping criterion.
- ***Default path for standard spectra:*** directory with “.nmrstd” files to be automatically loaded in the current session.
- ***Default path for signal patterns:*** directory with “.nmrsgnl” files.

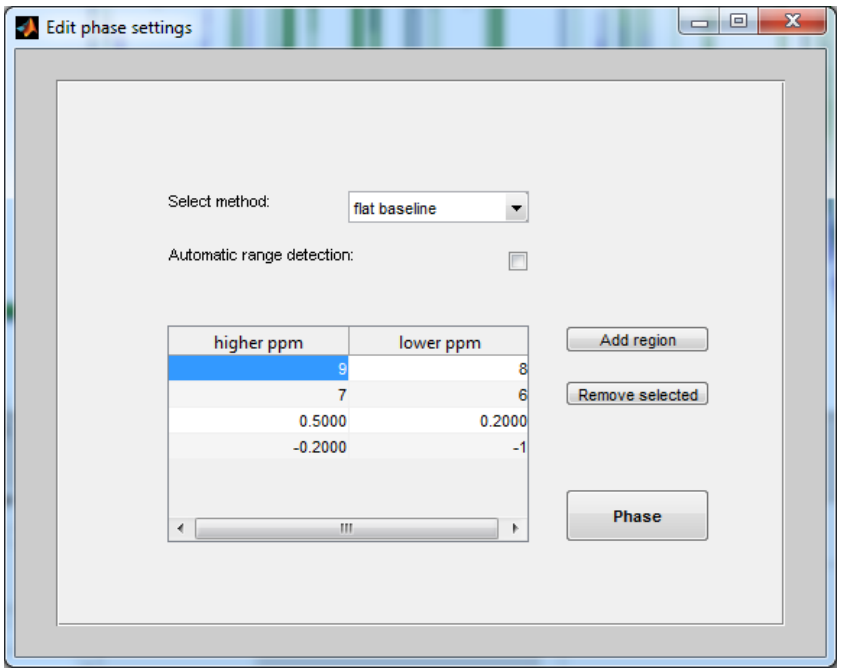
- ***Normalise imported spectra by acquisition parameters (NS, RG, P1):*** checking this box will normalise spectra base on their specific acquisition conditions: number of scans (NS), receiver gain (RG) and 90º pulse length (P1). ***Note: this is mandatory for quantitative inter-sample comparison if the spectra are not normalised using internal standards.*

Preparing the NMR spectra

Preparing the NMR spectra for quantitative analysis implies several processing steps including phase correction, baseline correction, shift reference, spectral alignment and line-shape enhancement.

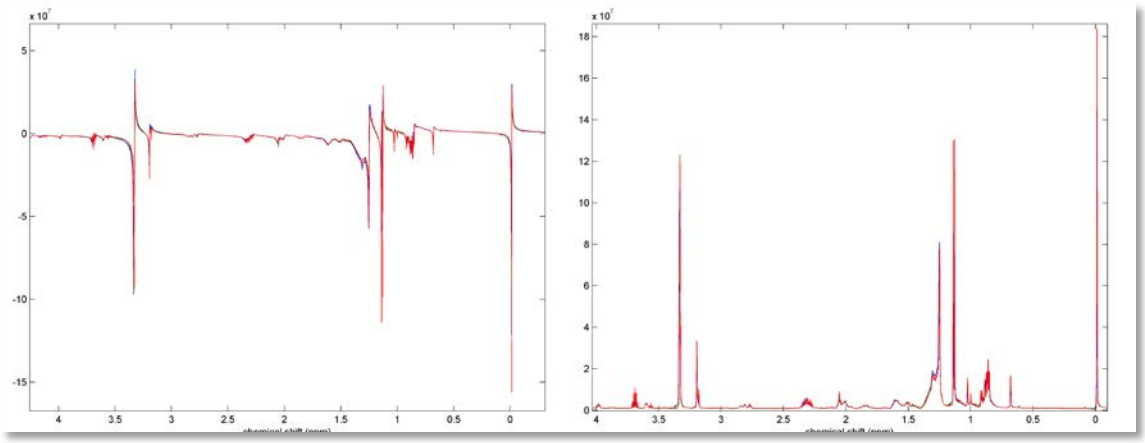
Phase correction

Phase correction is available from the tab "Autophase" in the "Pre-processing" option of the menu bar. Phase correction should be applied before any other processing step and it is an essential requirement for proper performance of the lineshape fitting algorithm. It sets the spectral line in pure absorptive mode.



- ***Select method***: LipSpin provides two different methods to correct zero- and first-order phase:

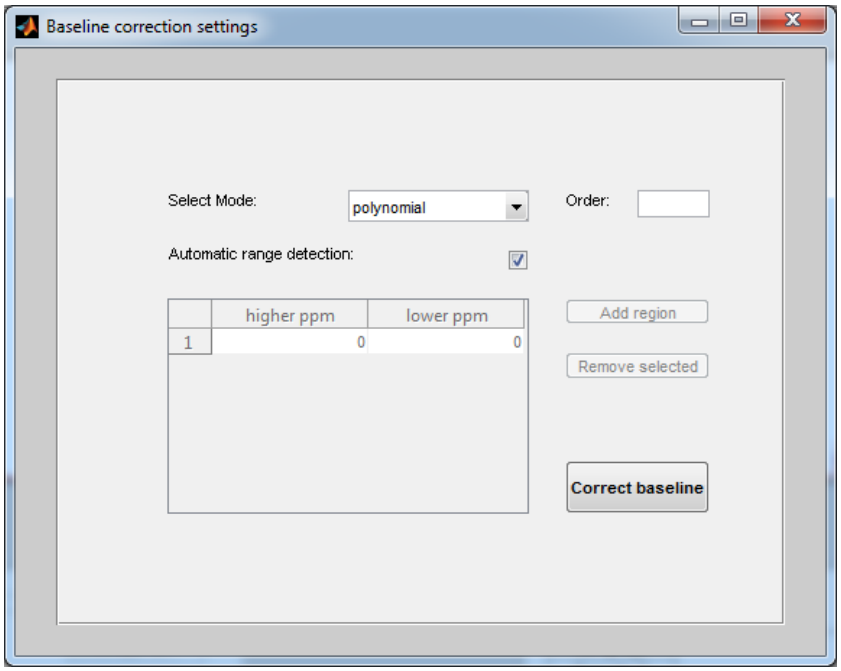
- ***Entropy*:** maximises the entropy of the spectrum. This method provides modest results but it works well for standards and spectra with few peaks. More info in: Chen et al. (2002) (https://doi.org/10.1016/S1090-7807(02)00069-1)
- ***Flat baseline*:** minimises the least-squares differences between a horizontal line and the spectral line for the defined regions, considered to have no peaks. An automatic version of region selection is included for this method (an example of the performance in the figure below). ***Tip: for the best performance, select regions disperse along the whole axis scale and of similar length. Flat regions closer to the intense and separated peaks (chloroform and TMS) are the ones more affected by phase distortions, selecting them will provide the best phase correction.*



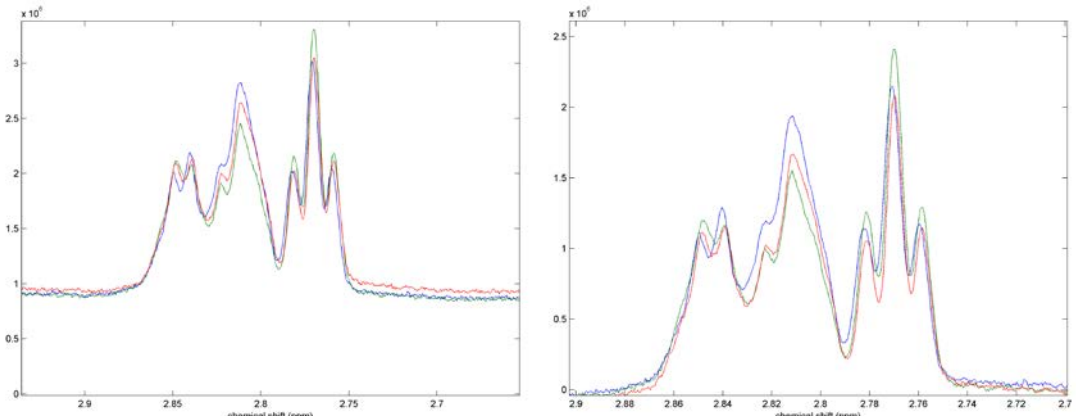
- ***Regions:*** list of regions defined as [high_ppm low_ppm]. This regions are used to find the points used for baseline interpolation.

Baseline correction

Baseline correction is available from the tab “Baseline” in the “Pre-processing” option of the menu bar. Baseline correction interpolates polynomial functions to a set of points within the user- or automatically-defined regions. Baseline correction can be skipped for lineshape fitting as it includes baseline functions, but it should be thoroughly applied when quantification by bucket integration is used.



- ***Select mode***: select between four interpolation methods, all admitting automatic range detection.
    - ***Median subtraction:*** in the strict sense, this mode is not an interpolation method. It simply subtracts the median of a set of data points to all spectral data points.
    - **Cubic spline** and **cubic Hermite:** spline interpolations implemented with spline and pchip MATLAB functions where each piece is a third-degree polynomial. **Tip: Cubic Hermite provides best results for baseline correction as it reduces oscillation between data points. The example below shows the spectra before (left) and after (right) the baseline correction showing the intensity differences for the red spectrum and the general offset elimination.



- ***Polynomial:*** fits a polynomial of user-defined order to a set of points. Recommended orders: > 5.

- ***Ranges:*** list of chemical shift ranges defined as [high_ppm low_ppm]. These regions are used to find the points used for baseline interpolation.

Shift reference

Shift reference is available from the tab "Chemical shift reference" in the "Pre-processing" option of the menu bar. This function shifts the spectra to align the most intense peak within a region to the centre of that region. Spectra should be chemical shift referenced so that signal patterns for lineshape fitting are valid between different samples. It requires two parameters:

- ***Chemical shift reference:*** reference ppm where the highest intensity peak has to be positioned.
- ***Tolerance:*** ± ppm around the "chemical shift reference" within the peak is sought.

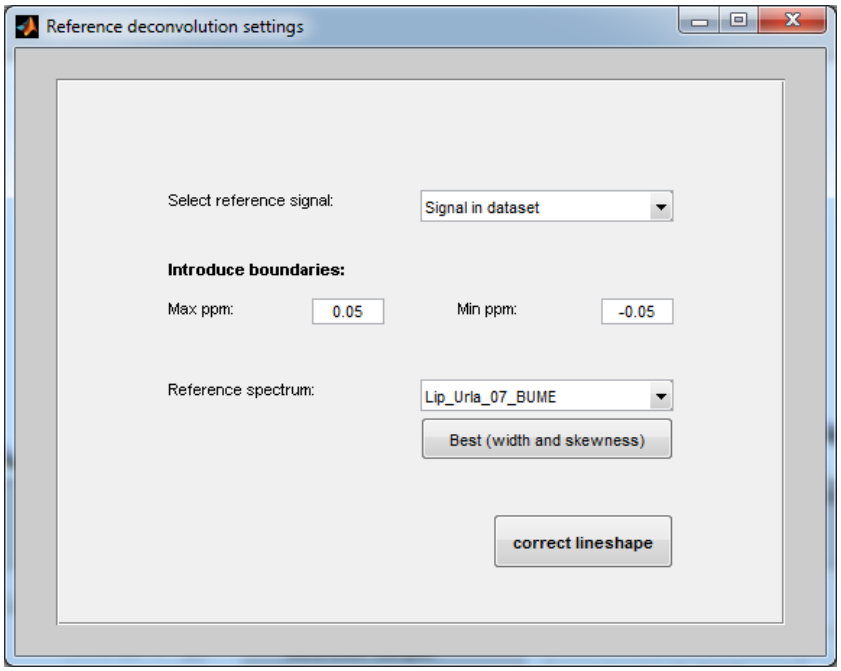
Spectral alignment

Spectral alignment is available from the tab "Align" in the "Pre-processing" option of the menu bar. This function shifts the spectra to maximise correlation (alignment) between samples by using cross-correlation MATLAB function. It allows correcting spectral misalignments of signals from polar groups due to pH or ionic strength discrepancies among samples. It requires three parameters:

- ***Max ppm:*** maximum chemical shift used in spectral alignment.
- ***Min ppm:*** minimum chemical shift used in spectral alignment.
- ***Shift all spectrum [X] / Only region [ ]:*** If checked the calculated shifts are applied to the whole spectrum. Otherwise the algorithm only shifts the region between "Max" and "Min ppm".

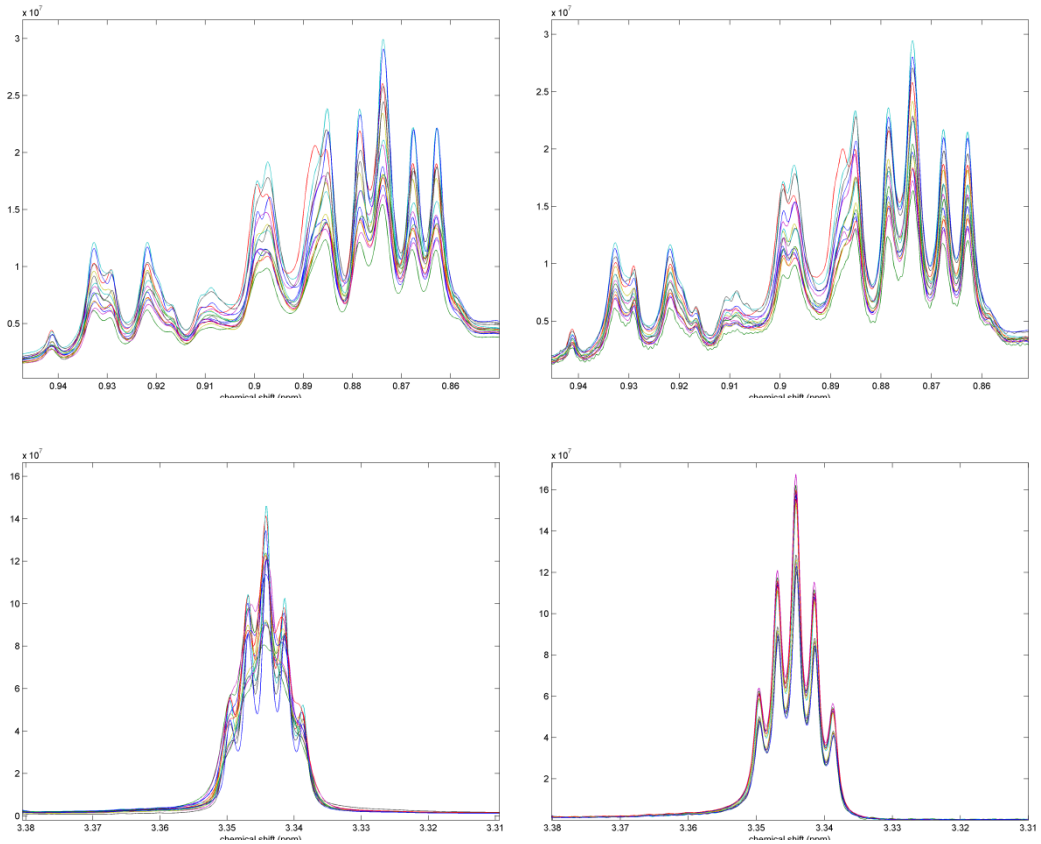
Line-shape enhancement with reference deconvolution

Line-shape enhancement is available from the tab “Reference deconvolution” in the “Pre-processing” option of the menu bar. This optional function allows correcting lineshape distortions due to magnetic inhomogeneities or poor shimming by using a reference peak and providing that this peak has been previously aligned among spectra. ***Note: reference deconvolution could not be recommended for low intensity signal as it decrease S/N and introduce unwanted wiggles in the spectral lines.* More about reference deconvolution in Morris et al. (1997) https://doi.org/10.1016/S0079-6565(97)00011-3.



- Select reference signal:
    - ***Signal in dataset:*** select this option if spectral lineshapes will be corrected by using a signal (preferably solvent signals or singles) within a specific spectrum a as a reference. In such a case, select the spectrum with the signal of lowest line width and symmetrical shape in the “Reference spectrum” list after defining ppm boundaries.
    - ***Synthetic TMS signal:*** generates a synthetic TMS signal to be used as a reference peak. Figures below show the effect of using reference deconvolution with synthetic TMS signal in the methyl region (top) and the chloroform signal (bottom).

- ***Boundaries:*** chemical shift limits (in ppm) for the spectral region used for reference deconvolution.
- ***Reference spectrum (only for "signal in dataset" mode):*** spectrum in the dataset used as reference. The button below allows the automatic selection of the spectrum with the best signal based on lowest line width and skewness.



Standard library

The standard library comprises spectra of chemical standards of lipids that have been previously conditioned and saved with LipSpin to be used as reference templates in lineshape fitting. Using templates is recommended for complex signals such as high-order coupling patterns and multiplets that do not follow the multiplicity rules of first-order coupling (i.e. singlets, doublets, triplets, etc.)

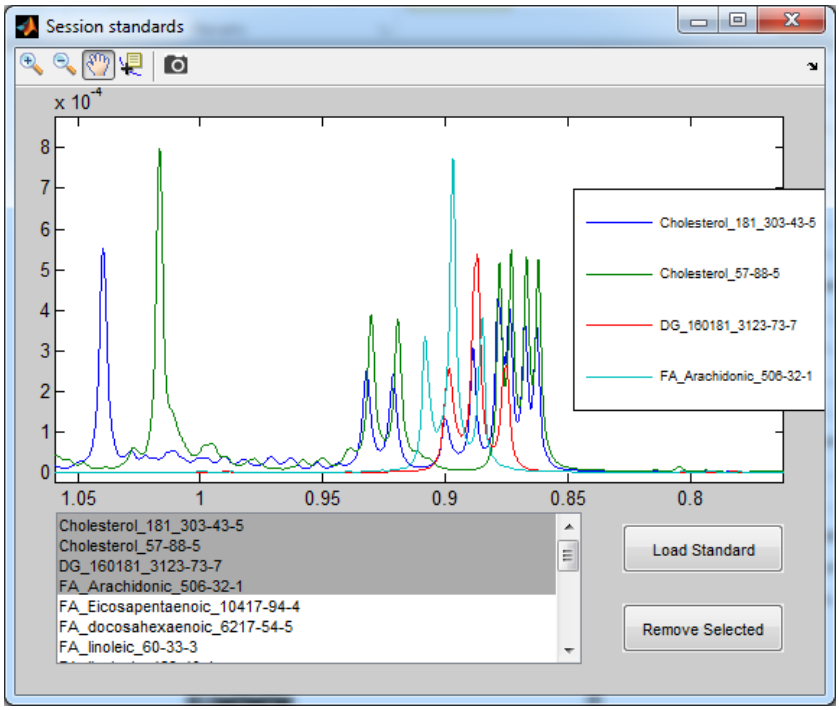
Saving a standard spectrum

Any spectrum imported in LipSpin can be saved as a standard spectrum by pressing “Save standard” in the tab “Standard spectra” of the menu bar. It requires only one spectrum included in the “Included sample(s)” list.

**Tip: Prepare carefully your standard spectra. Be sure that your new standard spectrum is well phased and does not have baseline offsets in the signals that will be used as templates in lineshape fitting (otherwise baseline will be computed in signal quantification). Be sure they are well-referenced; a good practice is using an internal standard such as TMS in all your standards. Finally, keep in mind that templates from standards are mostly valid for samples acquired under the same experimental conditions (temperature, spectrometer frequency and solvent); otherwise the templates could be no longer valid.

Load standards to current session

Standard spectra need to be loaded before being used in lineshape fitting. By default, all the standards in the “Default path for standard spectra” of the “General options” will be loaded automatically after successfully importing sample spectra. These standards can be inspected from the “Load session standards” in the tab “Standard spectra” of the menu bar.



Additionally, more standard spectra can be loaded and removed from the current session.

Editing signal patterns

Create/load signal patterns

Users can create their own patterns or use patterns from the library of patterns that are supplied with released versions of LipSpin. These patterns were created to be used with lipophilic extracts of human serum and plasma samples but they could be applied to other lipid samples. The "Signal patterns" panel in the main window lists all the patterns that will be applied to the fitting process after clicking the "Run" button. Users can create a new pattern by typing the name in the textbox and clicking the "Create" button.

Setting pattern parameters

The "Pattern settings" panel contains all the parameters that define a signal pattern.

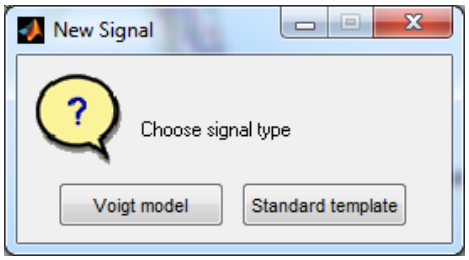
- ***Max ppm:*** left limit (in chemical shift) of the fitting region.
- ***Min ppm:*** right limit (in chemical shift) of the fitting region.
- ***Select mode:***
    - ***Lineshape fitting:*** this mode uses the lsqcurvefit function from the optimization toolbox to adjust the signals defined in the signal pattern to the real spectral line. Baseline parameters are considered in this mode. Use this mode for overlapped signals or isolated signals that may benefit from baseline reduction.
    - ***Integration:*** sums all the point of the spectral line between "Max" and the "Min ppm".
- ***Baseline signals:*** check the signals to add the models to the baseline signal:
    - ***1st poly:*** first-order polynomial (can take negative values).
    - ***Gaussian:*** spatially-distributed broad gaussian lines across the region (only positive).
    - ***Cosine:*** cosine series up to 12th term (only positive).

***Note: be careful when using Gaussian and cosine baseline functions as they could model part of non-baseline signals and mislead other signal's quantification. In this case, visual inspection of fitting solutions is a good way of getting insights.*

- ***Signals:*** list with models (Voigt and templates) used for curve fitting the real spectra in the defined region.

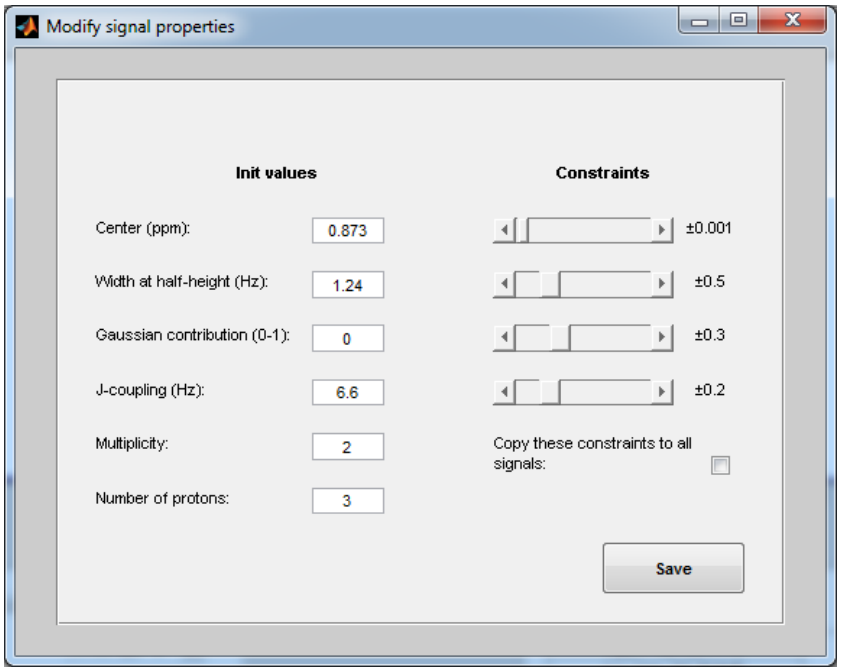
Create and edit signals

Typing a name (not previously used) and pressing "New" will create a new signal. First, a small window will let choosing between creating a new signal based on "Voigt Model" (following first-order coupling patterns) or a "Standard template".



- ***Voigt models***

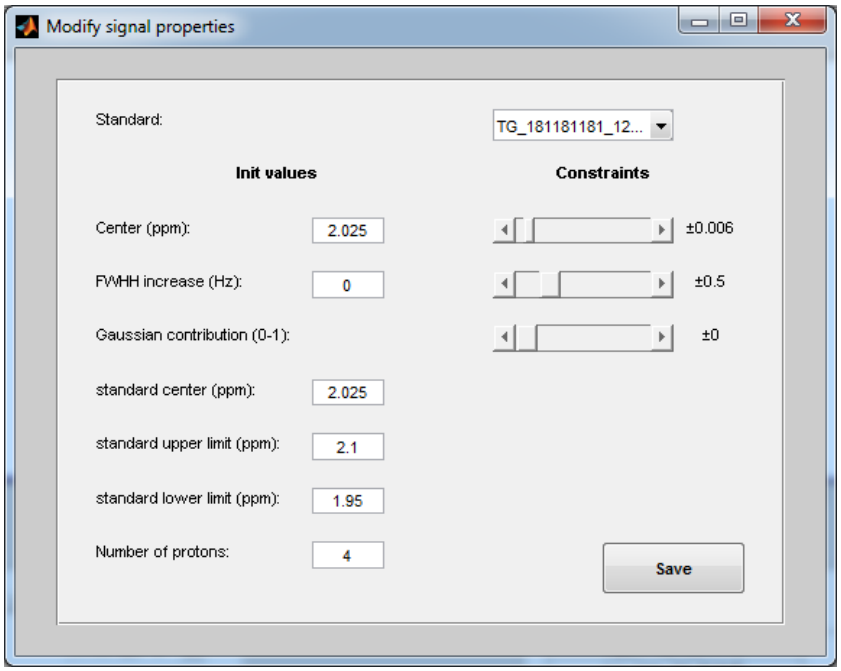
Voigt models are based on complex peak structures of Voigt profiles (combination of Lorentzian and Gaussian profiles) following multiplicity patterns of first-order NMR couplings (i.e. singlets, doublets, triplets, etc), with intensity ratios that follow the Pascal's triangle relations. "Init values" defines the initial values used in the optimization process. "Constraints" sets the limits between which each parameter can oscillate in the optimization process.



- ***Center (ppm):*** Center of the Voigt profile in ppm units.
- ***Width at half height (Hz):*** full width of the Voigt peak measured at its half height (FWHH). This value usually lies about 1 or 2 Hz. ***Tip: switching the axis scale to Hz will help measuring this parameter.*
- ***Gaussian contribution (0-1):*** ratio of Gaussian shape in the Voigt profile.
- ***J-Coupling (Hz):*** distance between peaks in the multiplet.
- ***Multiplicity:*** number of peaks that form the multiplet.
- ***Number of protons:*** number of H's that raise the signal.
- ***Copy these constraints to all signals:*** check and present constraints will be copied to the rest of the signals after pressing "Save".

- ***Standard templates***

Standard templates allow using signals from standard spectra as fitting models. This choice is suitable for resonances with complex coupling patterns not following fist-order multiplicity rules.



- ***Standard:*** select the standard spectrum from the list of standards in the current session. ***Note: if this field is void after loading a saved pattern indicates that the standard is not loaded in the current session. Press "Run" button in the main window will show the name of the standard for this signal.*
- ***Center (ppm):*** signal position in sample spectra.
- ***FWHH increase (Hz):*** increase the width at half height of the peaks in the template by the indicated factor in Hz. Typical values: 0-1.
- ***Gaussian contribution (0-1):*** allow increasing the Gaussian shape of the spectral template.
- ***Standard center (ppm):*** signal position in the standard spectrum used as a template.
- ***Standard upper limit (ppm):*** left limit of the region of the standard spectrum used as a template
- ***Standard lower limit (ppm):*** right limit of the region of the standard spectrum used as a template.
- ***Number of protons:*** number of H's that raise the signal.

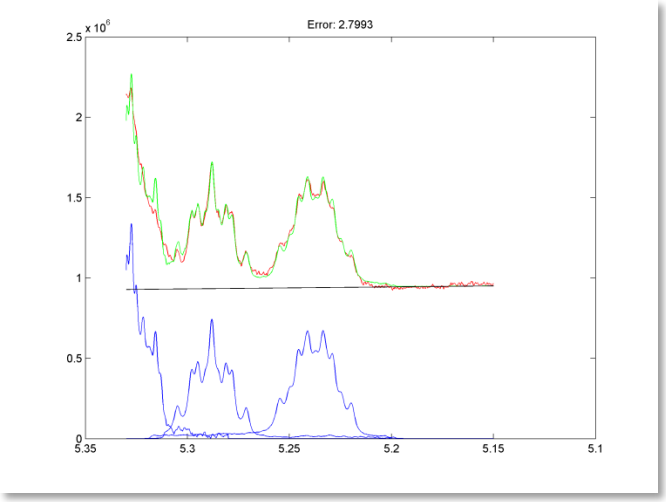
Signal quantification

Once the sample spectra are loaded and properly pre-processed, and when signal patterns have been properly configured and loaded, LipSpin is ready for quantifying the signals defined in the signal patterns for the included samples in the "Included sample(s)" list. Pressing "Run" button runs the quantification process in batch mode comprising all the included samples and for each sample all the signal patterns in the "Signal patterns" list.

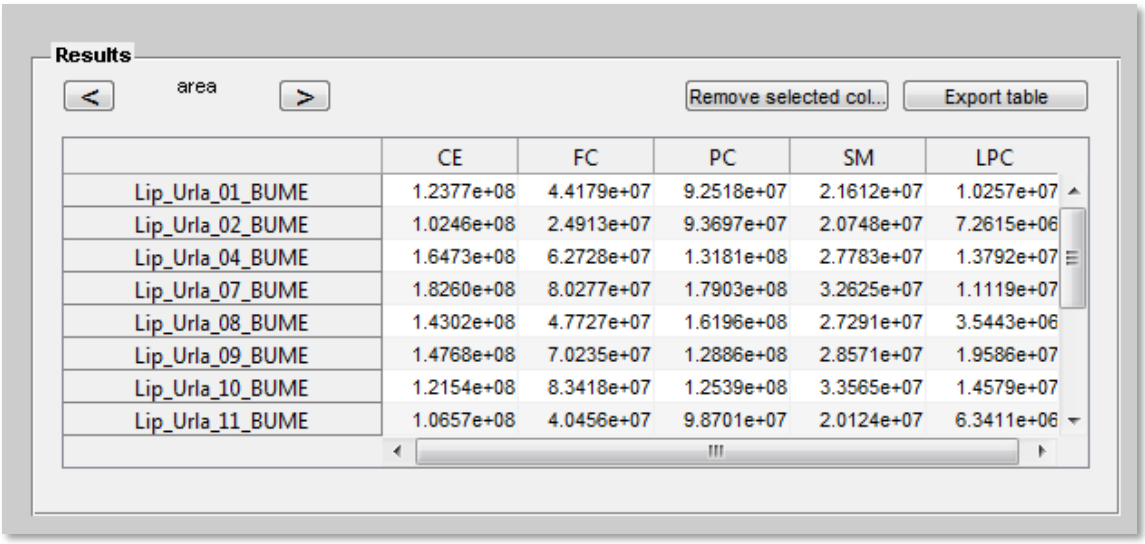
With "***Correct signal width by***", the quantification process can adapt the "Width at half height" of all the signals for each sample according to the width variations of a previously fitted signal obtained from the "Results" table. This tool can be useful for correcting variations of linewidth between samples that affect the whole spectrum (i.e. all the peaks in a spectrum). For instance, if linewidth varies severely between samples it could be better to fit first an isolated singlet (e.g. TMS peak at 0 ppm) and then apply FWHH variations of this signal to the rest of the signals, instead of setting large boundaries in the FWHH constraints. This feature needs a sample to be used as a reference, which should be the sample with narrowest signal.

Inspecting quantification results

If an existing "***Figures path***" is indicated, a .png file containing the graphical solution of the fitting process will be saved. In the example below, the red line indicates the sample spectrum, the green line the fitted spectrum, the blue lines the individual fitted signals and the black line the fitted baseline. The graph also shows the %RMSE of fitting as a goodness of fit indicator.



Once all the samples and patterns have been analysed, the "Results" table is updated reflecting the quantified areas and several parameters related to the fitted solution for each signal (intensity, centre, FWHH, Gaussian contribution and J-Coupling). Inspecting these parameters could help to optimise subsequent analysis if needed.



	CE	FC	PC	SM	LPC
Lip_Urla_01_BUME	1.2377e+08	4.4179e+07	9.2518e+07	2.1612e+07	1.0257e+07
Lip_Urla_02_BUME	1.0246e+08	2.4913e+07	9.3697e+07	2.0748e+07	7.2615e+06
Lip_Urla_04_BUME	1.6473e+08	6.2728e+07	1.3181e+08	2.7783e+07	1.3792e+07
Lip_Urla_07_BUME	1.8260e+08	8.0277e+07	1.7903e+08	3.2625e+07	1.1119e+07
Lip_Urla_08_BUME	1.4302e+08	4.7727e+07	1.6196e+08	2.7291e+07	3.5443e+06
Lip_Urla_09_BUME	1.4768e+08	7.0235e+07	1.2886e+08	2.8571e+07	1.9586e+07
Lip_Urla_10_BUME	1.2154e+08	8.3418e+07	1.2539e+08	3.3565e+07	1.4579e+07
Lip_Urla_11_BUME	1.0657e+08	4.0456e+07	9.8701e+07	2.0124e+07	6.3411e+06

Finally, the current table can be saved in a .csv file by pressing the ***"Export table"*** option.