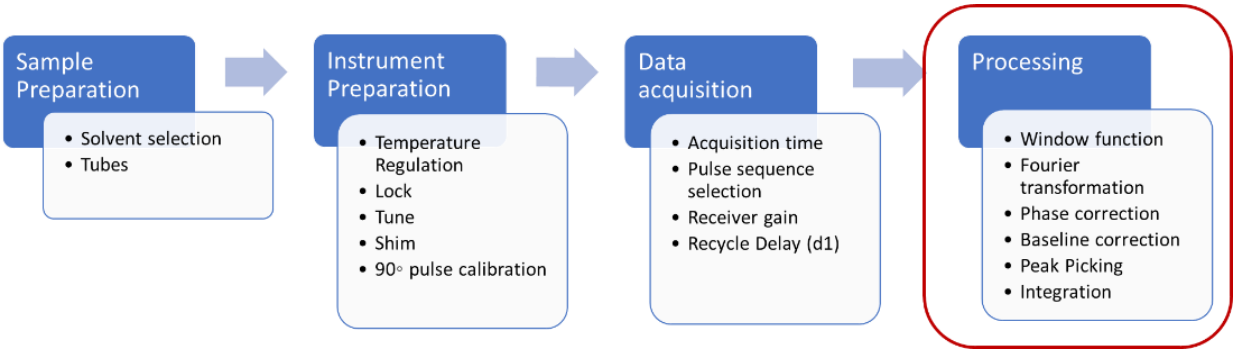
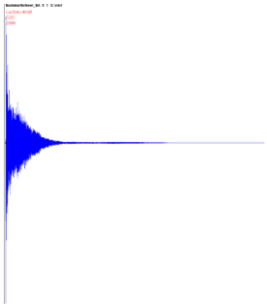
NMR HANDS-ON PROTOCOLS – PROCESSING

Workflow NMR experiments



Processing in NMR: from FID to spectrum

Raw data

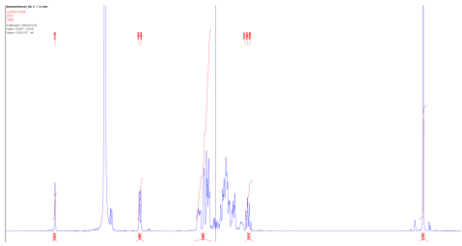


Step 1: FID manipulation (Window function & Fourier Transform)

Step 2: Phase correction

Step 3: Baseline correction

Spectrum



Step 4: calibration, integration, peak picking, ….

Window function (WDW) and Fourier transformation (FT)

Window functions are mathematical functions applied to a dataset, typically in the time or frequency domain. When you have a signal that contains both desired information and unwanted noise, window functions can be used to "window" or select a specific portion of the signal for further analysis. This process helps isolate the relevant information while reducing the impact of noise from the surrounding data.

The free induction decay (**FID**) is multiplied by window function (**WDW**) to follows the natural decay of the signals. To transform a time domain into a frequency domain we used the Fourier transformation (**FT**).

The most used WDW are **exponential multiplication (EM)** function and **Gaussian multiplication (GM)**. The **exponential multiplication (EM)** function leads an improvement of signal/noise ratio (**sensitivity enhancement**). However, it leads to a decrease in resolution.

The **Gaussian multiplication (GM)** leads to a resolution enhancement of the signals, however a in this case the sensitivity is reduced.

Depending on the WDW selected other parameter should be defined: the **line broadening** (**lb**).

For Gaussian multiplication (**GM**) you should consider **lb** and **gb** parameters.

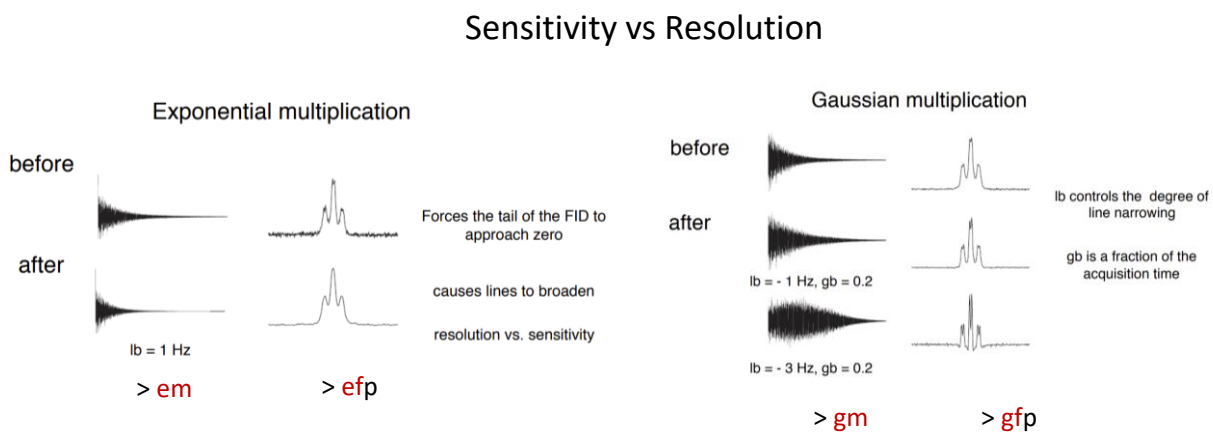


Figure 1: Effect of the window functions on the 1H NMR spectrum processing.

WDW by default is **EM** with **lb** of 0.3.

To check the parameters click on PROCPARS in the tab bar OR in the command line type

> **edp** <ENTER>

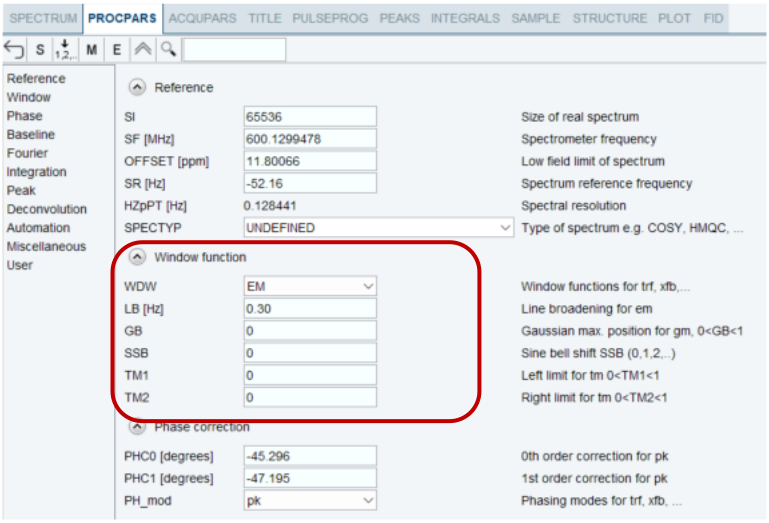


Figure 2: Processing parameter bar in Topspin highlighting the window function related parameters.

Zero filling (ZF)

The raw NMR data is collected in the form of discrete data points, often called data points or complex points, which represent the signal intensity at specific time intervals. These data points are typically collected for a finite duration of time, which corresponds to a certain range of frequencies in the resulting NMR spectrum.

Zero-filling is a technique used to increase the number of data points in the time-domain signal. This is achieved by appending extra data points with values of zero to the end of the original data set. By adding "zero" intensity points at the end of the fid leads to an increase of resolution. By zero filling no new information is added (unlike linear prediction (LP)) but it is used, and it allows to obtain a better definition of the frequency function derived from the time function.

- **SI = ½ TD : we are not adding any zeros**
- **SI = TD : we are adding "TD" number of zero intensity points**

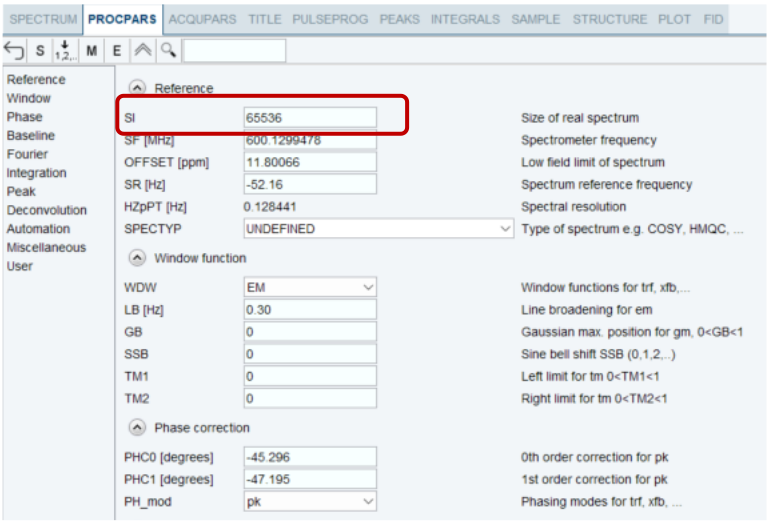


Figure 3: Processing parameter bar in Topspin highlighting the zero filing parameter.

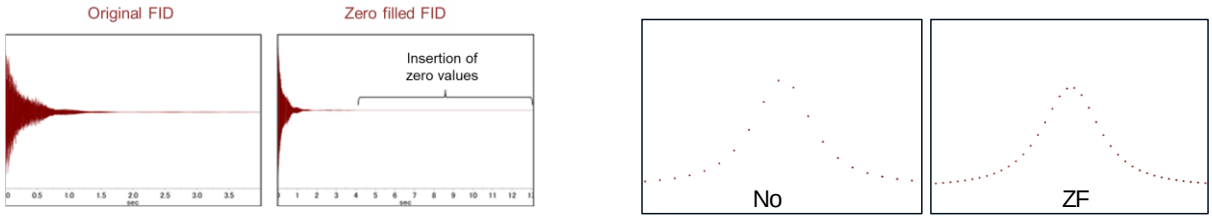


Figure 4: Zero filing effect on the spectrum raw data and FT transformed.

However, if your goal is to report data without any manipulation, then you need to process with the following conditions:

SI= ½ TD Since the FT process splits the total points acquired (td) into real and imaginary points. We only plot 1 set, the real points and disregard the imaginary points.

WDW = no

No apodization, multiplication of the time domain data

For these conditions you should type

> ft <ENTER> (Fourier transform (1D))

However, if you want to enhance sensitivity you should applied an exponential multiplication before Fourier transformation and type

> ef <ENTER> (**E**xponential window multiplication + Fourier transform (1D)) (**This is the most commonly used**)

If you wish resolution, you should type

> gf <ENTER> (Gaussian window multiplication + Fourier transform (1D))

Fourier Transform (FT)

The Fourier Transform (FT) is a mathematical operation used to convert the NMR data from its original time-domain format, where we collect signals over time, into a frequency-domain spectrum. This transformation is like taking a complex musical chord played over time and decomposing it into its individual musical notes. In NMR, it separates the overlapping signals from different nuclei in the sample into distinct peaks in the spectrum, each corresponding to a specific resonance frequency, simplifying the spectrum analysis.

Fourier Transform 1D

TDeff : TD points used in FT – default is 0. This means that all time domain points are used by default in FT.

ACQUPARS

PROCPARS

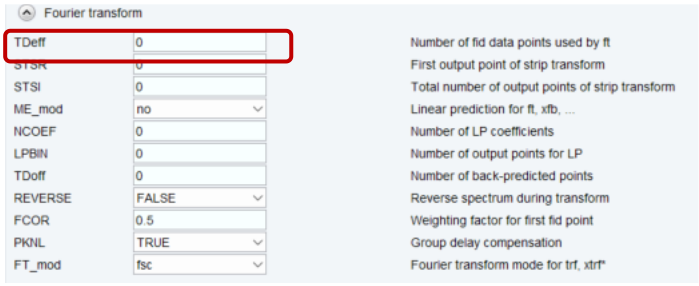


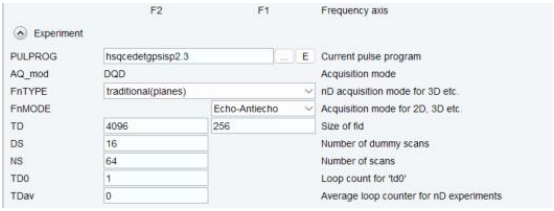
Figure 5

Fourier Transform 2D

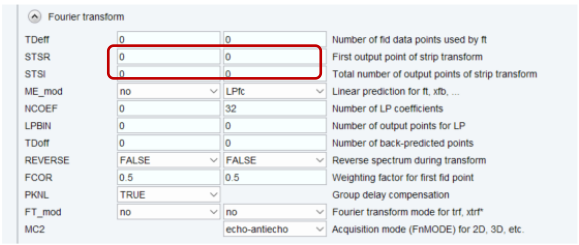
In the 2D spectra you have multiple FID organized into a 2D matrix. The command to for FT a 2D spectrum is

> **xfb** <ENTER>

ACQUPARS



PROCPARS



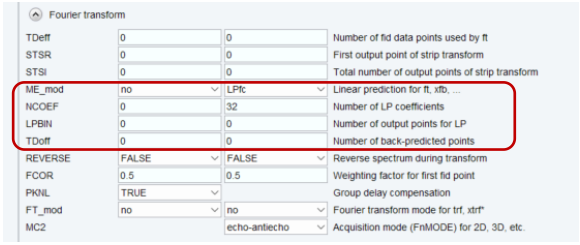


Figure 6: Acquisition and processing tab in a 2D NMR experiment.

Notes in PROCPARS

STSR / STSI – strip transform – default is 0 all frequency domain points are used in FT by default.

ME_mod : linear prediction forward lpfc by default. All TD points in F1 are used in predicting another TD points in F1. default - for 2D LPfc in F1 with NCOEF =32

In the case of $^{1}H,^{15}N$-HSQC usually **change** STSI in F2 for 1024 from 2048.

PROCPARS

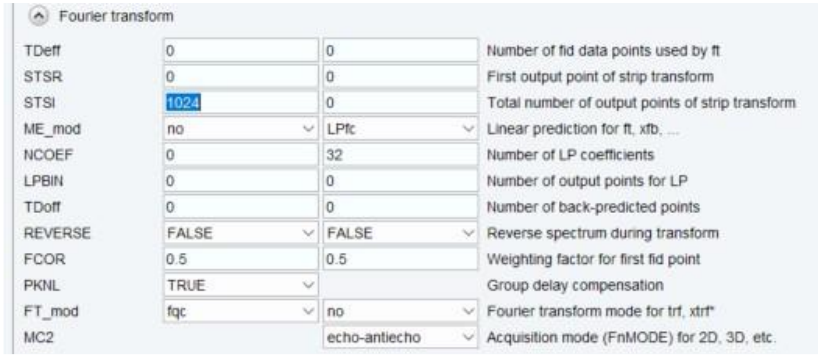


Figure 7

Phase correction

When NMR spectra are initially acquired, they often contain phase distortions or shifts that can obscure meaningful information about the sample's chemical composition and structure. These distortions can result from imperfections in the NMR instrument or experimental conditions. Phase correction addresses these issues by adjusting the phases of the NMR signals to align them properly. Phase-corrected NMR spectra are not only visually cleaner but also provide more reliable information about chemical shifts, coupling patterns, and interactions between nuclei in the sample.

Phase correction 1D

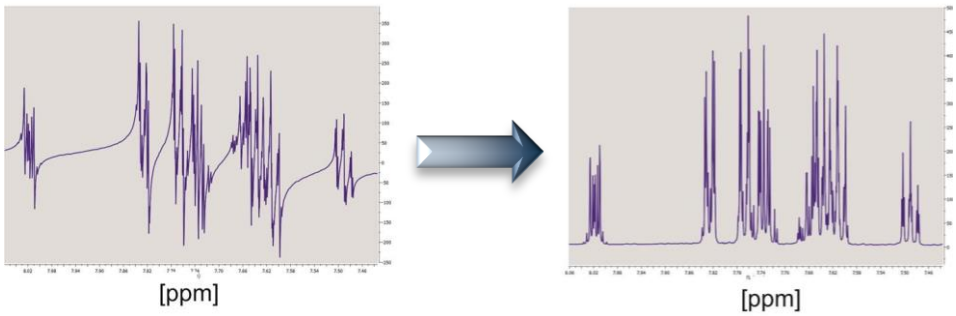


Figure 8: Effect of the phase correction in the transformed 1H NMR spectra (modified from 10.1016/j.jmr.2017.05.014)

The FT splits the total points acquired (TD) into real and imaginary points, however in the spectrum we only observe the real points.

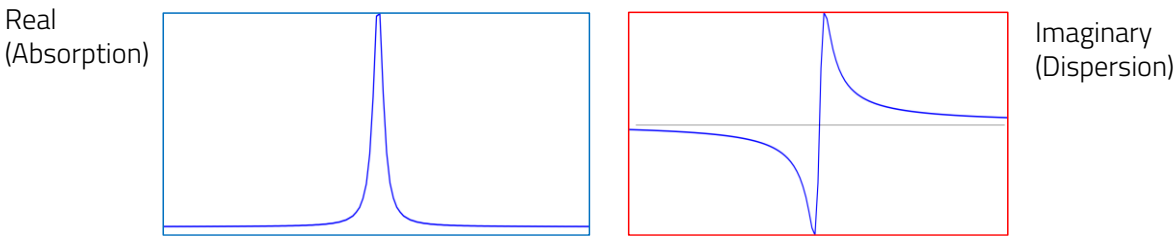


Figure 9: Decomposition of the FID data by the FT showing the real and imaginary components.

After FT, the spectrum must be phased to pure "absorption" mode (pure up or down peaks). Phase is done applying zero order and first order correction.

Automatic mode is usually enough.

> apk <ENTER> OR **> apks** <ENTER>

You can also perform phase manually (if needed).

Select **pk** in **PH_mod** in the PROCPARS

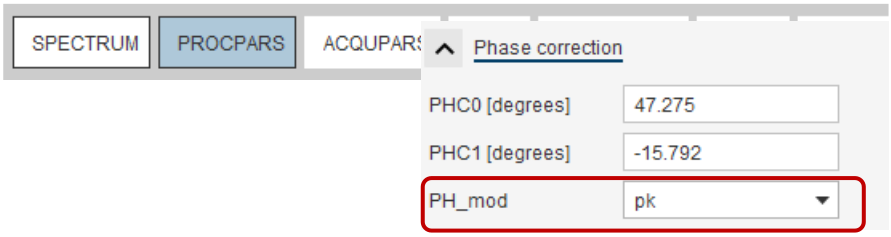


Figure 10: Phase correction parameters in the PROCPARS tab.

To enter manual phase mode, go to **PROCESS TAB** and click **Adjust Phase**. OR **.ph** <ENTER> in the command line.

From a "**pivot**" point, used as reference, start to phase the PH0 (zero order phase) and then PH1 (first-order phase) if needed. This is done by clicking and dragging the "0" button to adjust the phase of the pivot point (usually the tallest peak, automatically selected by Topspin). After that is phased, click, and drag the "1" to adjust the phase of peaks far from the tallest peak. You can also right click on another signal in the spectrum and click "Set Pivot Point" to make a different peak the pivot.

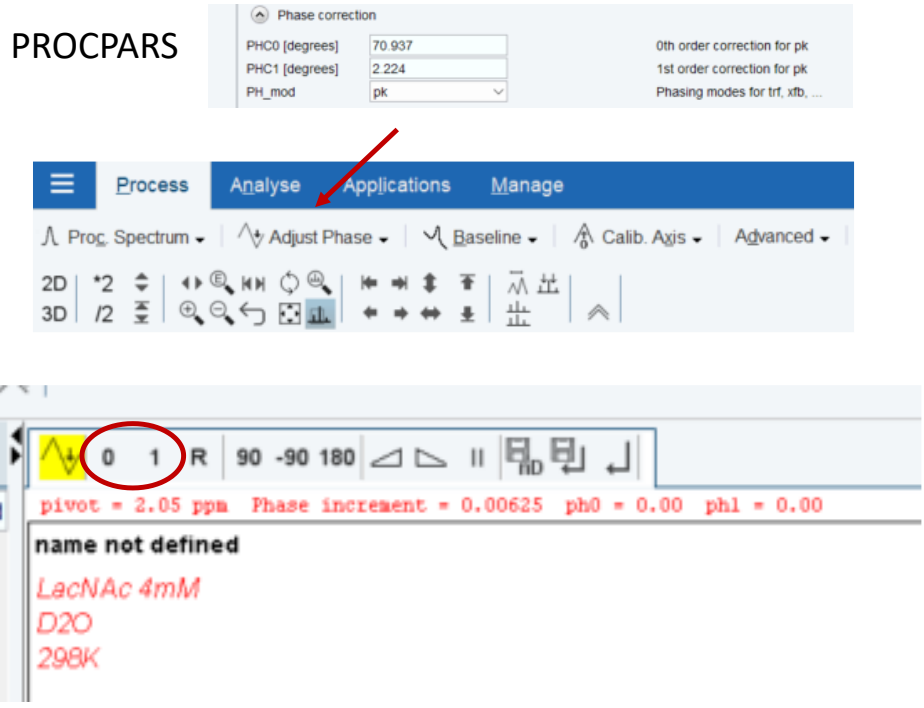


Figure 11: Manual phase correction dialog box in Topspin.

You recognize the need for PH1 correction by observing the dissimilarity of the peak shape distortions across the spectrum from one side to the other.

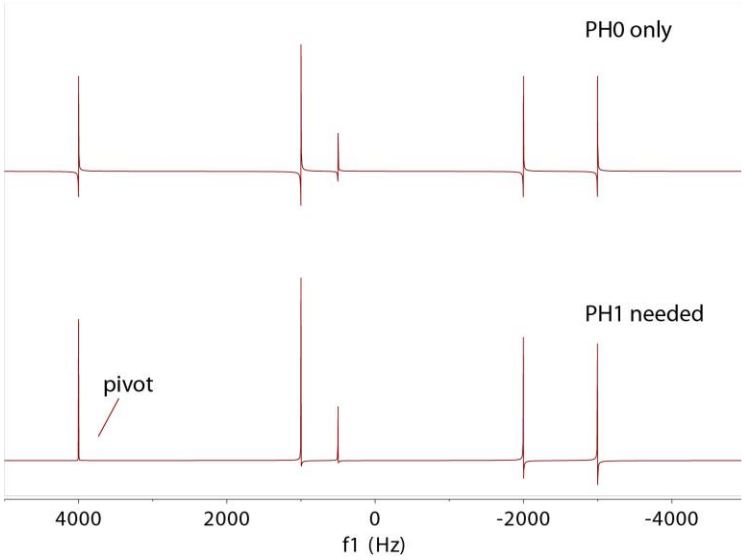


Figure 12: Effect of PH0 and PH1 corrections on the spectra (adapted from https://nmr.chem.ucsb.edu/education/part1.html#phasing)

At the end save

If you already have some phase parameters that you want to use just apply **> efp** `<ENTER>` or **> gfp** `<ENTER>` depending on an exponential or gaussian window.

Phase correction 2D

For automatic phase you can use **> apk2d** `<ENTER>` This command makes correction in both dimensions.

However, adjustments can be performed also by manual phasing. Thus, start manual phase dialog box as previously described.

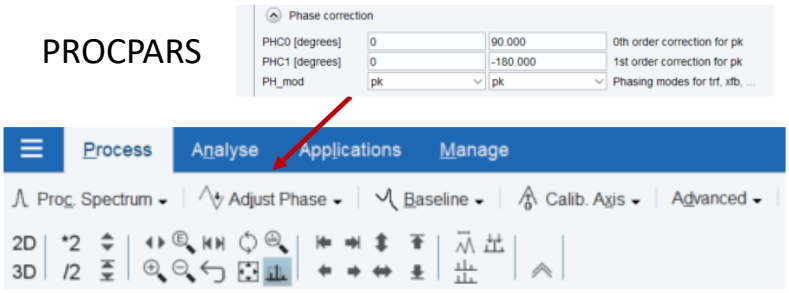


Figure 13

Place the mouse on one of the major correlations (for homonuclear experiments select the autocorrelations) signal, and right click and select "**Add**" to choose peaks spanning the width and height of the spectrum to phase. Repeat the same procedure another correlation peak preferably far away from the previous, right click and "**Add**" again. Repeat if needed.

Select **R** icon (rows) and adjust the phase using 0 and 1 (like the 1D phasing). Click Save. Repeat for the **C** icon (columns) if needed.

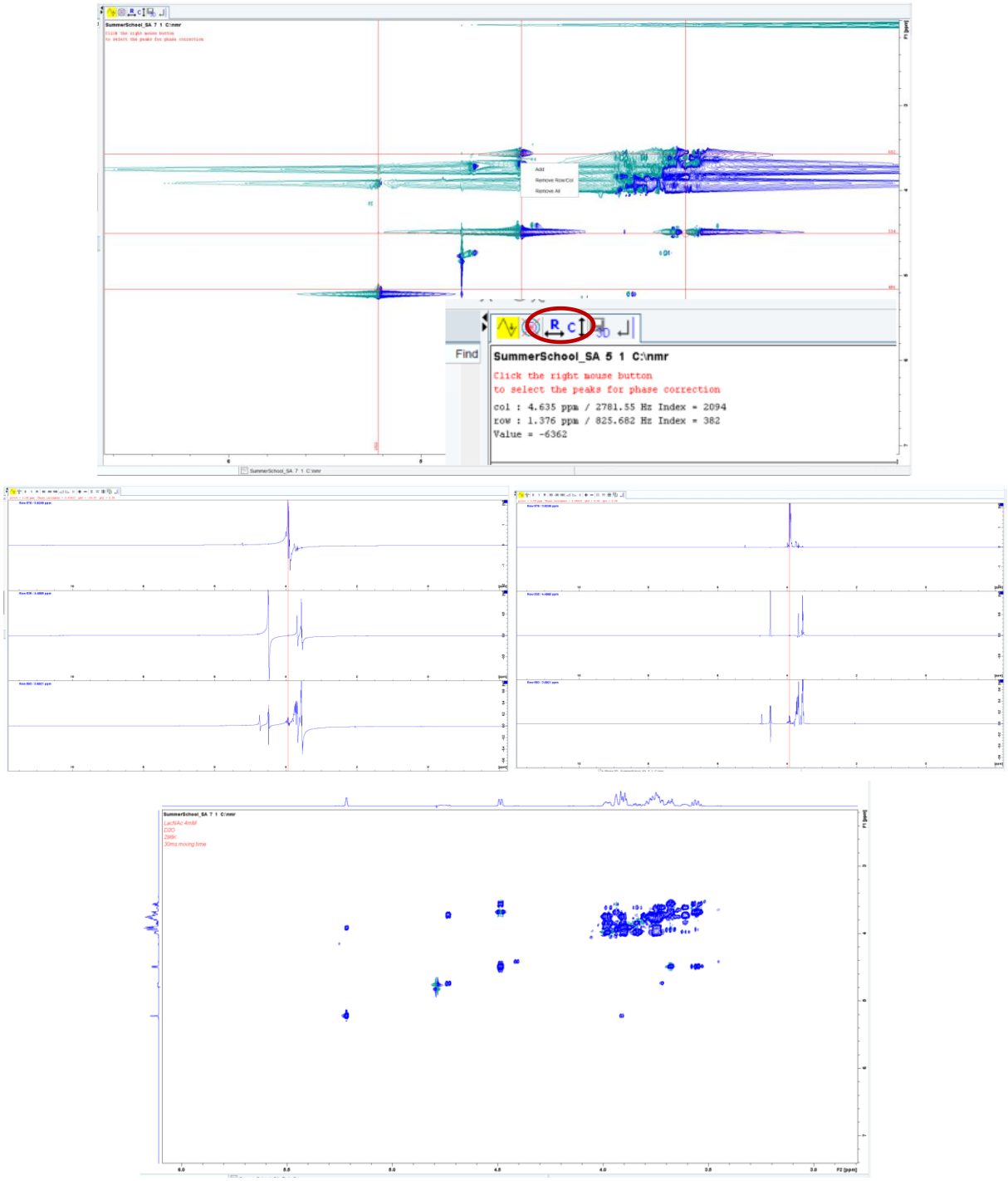


Figure 14: Manual phase correction process for a 2D NMR spectra.

Baseline correction

This baseline is caused by various factors such as instrument imperfections, solvent signals, or other low-intensity interference that can obscure the true spectral peaks of interest. Baseline Correction should be done after other FID

processing including window function selection, Zero-Filling/Linear prediction, and phase correction. The main goal when performing baseline correction is to obtain a visually flat baseline.

Note: For an accurate integration a baseline correction should be firstly performed.

Baseline correction 1D

For automatic **baseline correction WITHOUT integration** write > **absn** <ENTER>

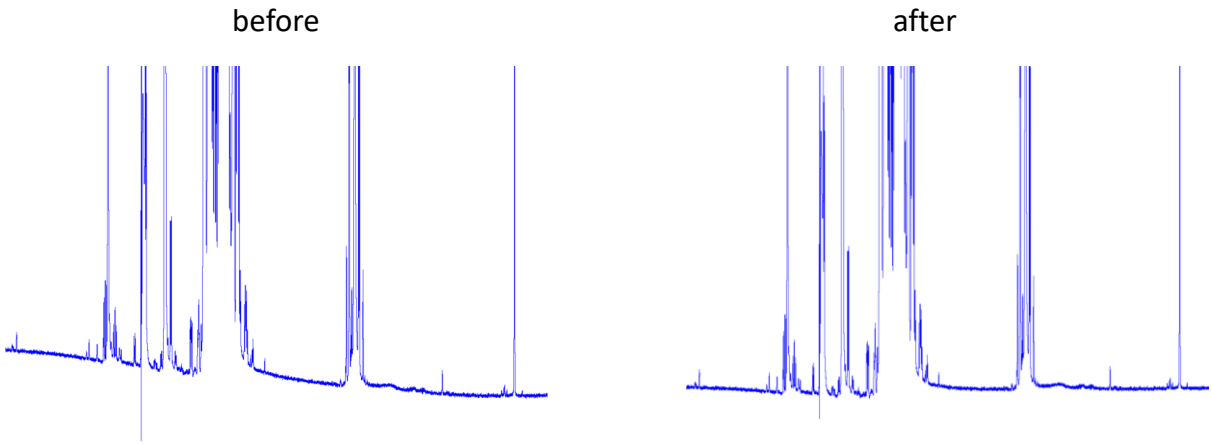


Figure 15: Before and after baseline correction in a ^{1}H NMR spectra.

BC_mod: function subtracted from the fid (**default is quad**), which means the average intensity of the last quarter of the fid. A ABSG of 5 is usually selected meaning that a polynomial of 5th order is applied for baseline correction.

PROCPARS

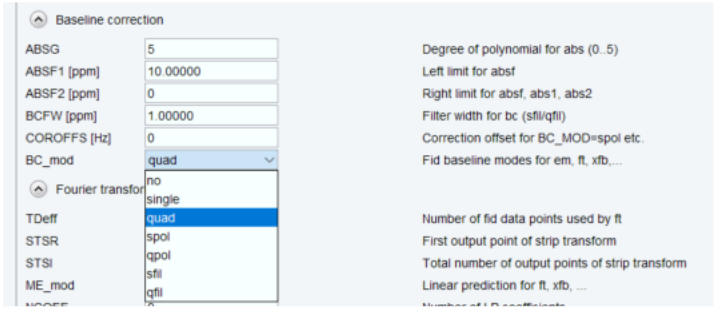


Figure 16

There are other BC_mod options for residual on resonance signal:
qpol: polynomial function of degree 5 or
qfil: gaussian function with the width of **BCFW 0.05**.

Baseline correction 2D

The 2D spectra may have baseline offsets in one or the other dimension.

The > **abs2** <ENTER> corrects F2 direction and the > **abs1** <ENTER> corrects F1. You can also do > **abs2d** <ENTER> (in both dimensions).

Calibration (SR)

Calibrate your spectra using an internal reference.

Reference compound for samples in D_2O or H_2O/D_2O is usually the TSP or TMSP **1** (sodium trimethylsilyl propanoic acid) or DSS **2** (sodium trimethylsilylpropane sulfonate). TSP/TMSP is usually deuterated TMSP-d_4.

The trimethylsilyl group of DSS is usually less sensitive to pH changes than TSP/TMSP. The deuterated version of DSS is expensive thus in the ^{1}H-NMR proton it is usually observed resonances from 3 – 0.6 ppm at lower intensity than the reference resonance at 0 ppm.

Calibration 1D

Type > **.cal** <ENTER>

Or using the tab menu.

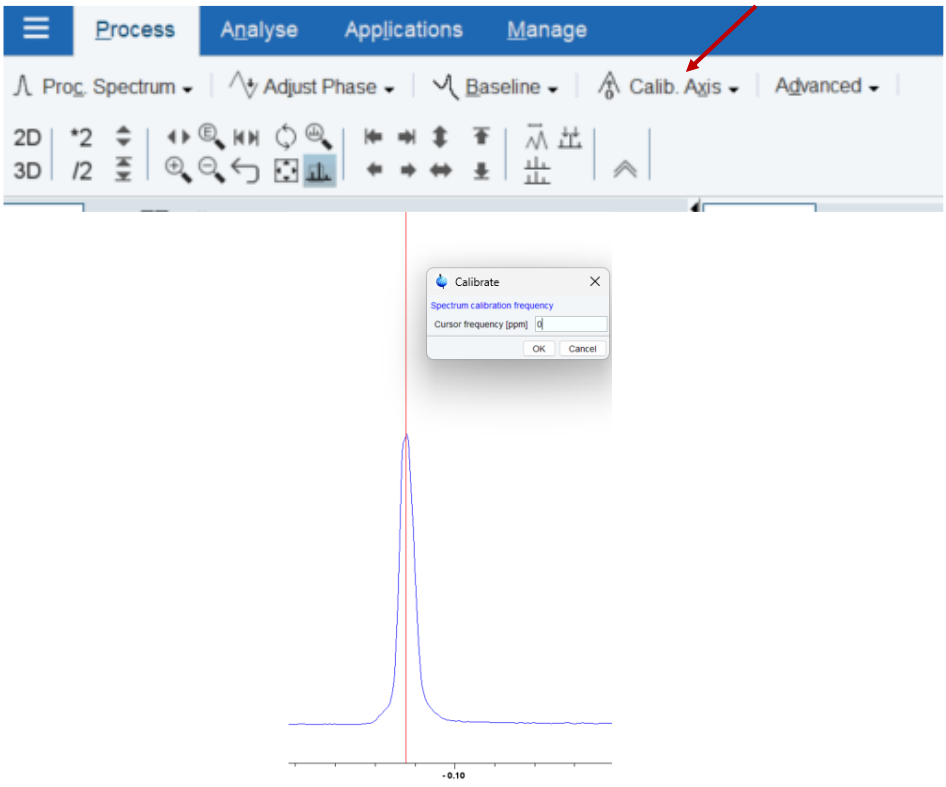


Figure 17: Calibration of a 1D spectra.

Choose a peak, usually the solvent or the reference (e.g. TSP), place the cursor in the signal, click, type the value and click OK. This gives you an "SR" value, which can be handy to know for referencing 2D spectra. The SR parameter can be found in the ProcPars tab (Figure 18) or by typing > **sr** <ENTER> in the command line.

Upon calibration the spectrum reference frequency changes.

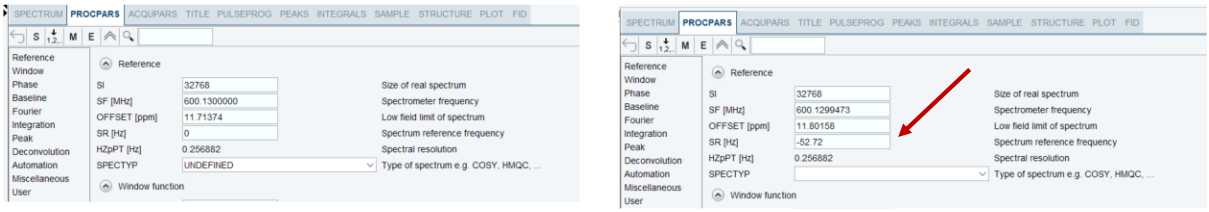


Figure 18: Changes in the calibration values in the PROCPARS.

You can also type > **sref** <ENTER> (This calibrates the spectrum by setting the TMS/TSP/DSS signal of a spectrum to exactly 0 ppm. It works on 1D and 2D spectra)

Calibration 2D

For homonuclear ^{1}H experiments (e.g., COSY, NOESY, ROESY) the calibration is straightforward with the F1 and the F2 dimension being equal use the same values obtained in the 1D ^{1}H NMR. Type > **sr** <ENTER> and enter the values for both dimensions.

If you are carrying out an and heteronuclear experiment (e.g., HSQC then the value for SR in F2 and F1 should correspond to the SR value you recorded earlier for the 1D proton and the sr of the other nuclei, respectively. This step ensures that both of your dimensions are properly referenced in chemical shift. We can use the sr value obtained in a 1D X spectra or you can calibrate the other nuclei (^{15}N and ^{13}C) using only the DSS or TSP signal.

- Reference the ^{1}H chemical shift (see above)
- Use the following equation (e.g. ^{15}N):

$$SR_{15N} = (SF_{15N} * 10^6)\left(\frac{((SF_{1H} * 10^6) - (O1_{1H} - SR_{1H})) * \gamma_{15N}}{10^6}\right)$$

Where:

SF_{1H} is the spectrometer ^{1}H frequency plus the O1 (in Hz) (e.g.: at our 600MHz, the spectrometer frequency is 600.13 MHz, assuming a O1 of 2788.08 Hz, SF_{1H} would be: 600.13 + 2788.08); δ_{15N} is the gyromagnetic ration of ^{15}N; SR_{1H} is the calibration value for ^{1}H; SF_{15N} is the spectrometer ^{15}N frequency plus the O3 (O3 is the central frequency in the indirect dimension).

- Change the SR value accordingly in the PROCPARS or type > **sr** <ENTER>

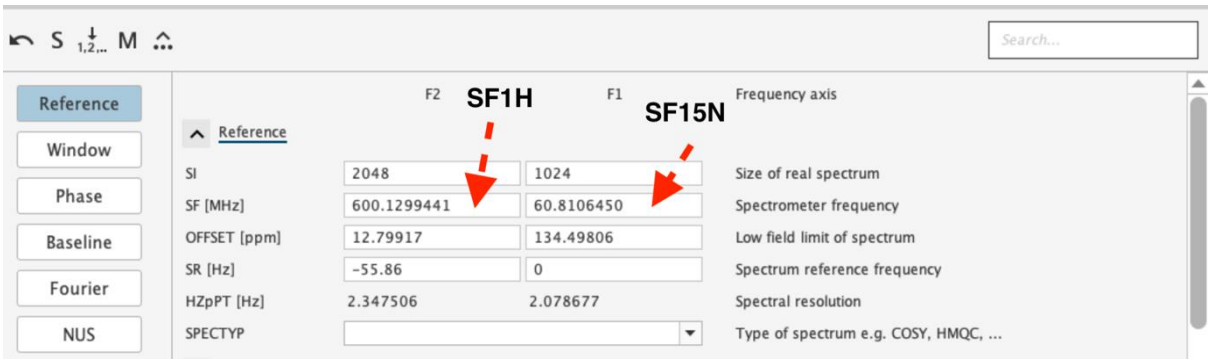


Figure 19: Changes in the SR values in the PROCPARS of a 2D experiment.

Peak Picking

This can be done automatically or manually.

Peak Picking 1D

The automatic peak picking can be done in the command line:

> **ppf** <ENTER> (picks peaks on the entire spectrum)

> **pps** <ENTER> (picks peaks only the displayed spectrum region)

In the automatic mode the peaks are found according to the parameters:

- **pc** (peak detection sensitivity, i.e., the amount of noise rejected; low values pick more peaks);
- **mi** (minimum intensity of peak to be picked);
- **maxi** (highest intensity of peaks to be picked).

Usually, typing > **mi** <ENTER> and increasing it before running **pp** for **pps** helps with automatic peak picking. This can also be done through the Process toolbar:

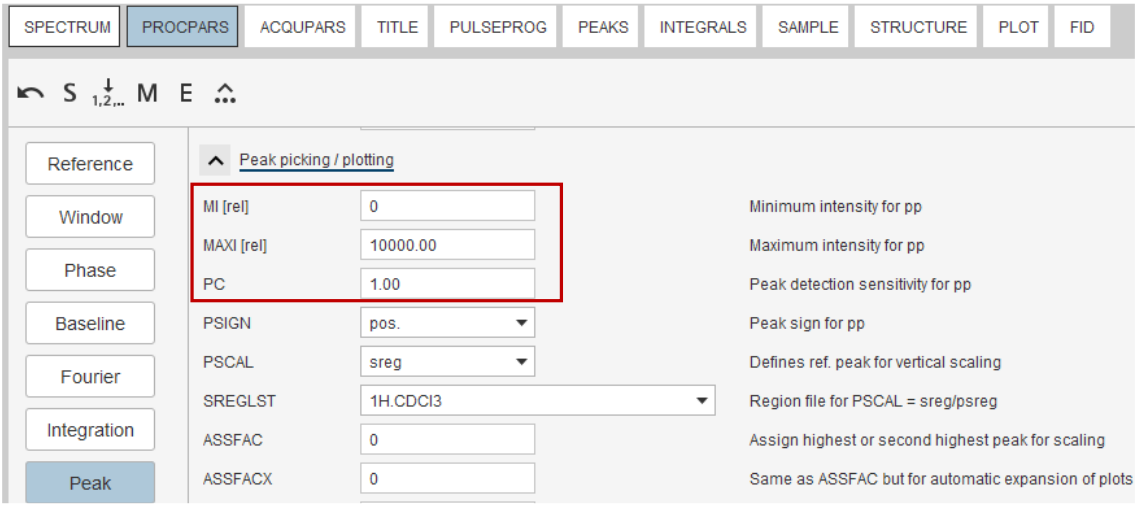


Figure 20: Peak picking parameters in the PROCPARS that control the automatic peak picking mode.

Sometimes is preferable to manually perform the peak picking. This can be done in the command line (> .**pp** <ENTER>) or on the menu bar, click **Analyse.** click **Pick Peaks** on the workflow button bar.

By default, the "Define new peak picking range" button is selected.

Press and drag the left mouse button over the peaks of interest to draw a region of peaks you wish to pick. A highlight region will form. When you are happy with your selection region, release the left mouse button. If necessary, you can repeat the process. When finished, click "**Return, save region**" to store the peak values.

Figure 21: Manual Peak picking process.

Peak Picking 2D

Peak picking in a 2D dataset is very similar to the 1D procedure.

For automatic peak picking use > **pp2d** <ENTER>

This will open a dialog box that lets you set the threshold based on the contour levels. In the sensitivity section you can define a threshold for sensitivity. Note that MI can also be set interactively with the button **Set to**, to the lowest contour level, the current value of MI or the most recent value stored in the peak list. Click **OK**.

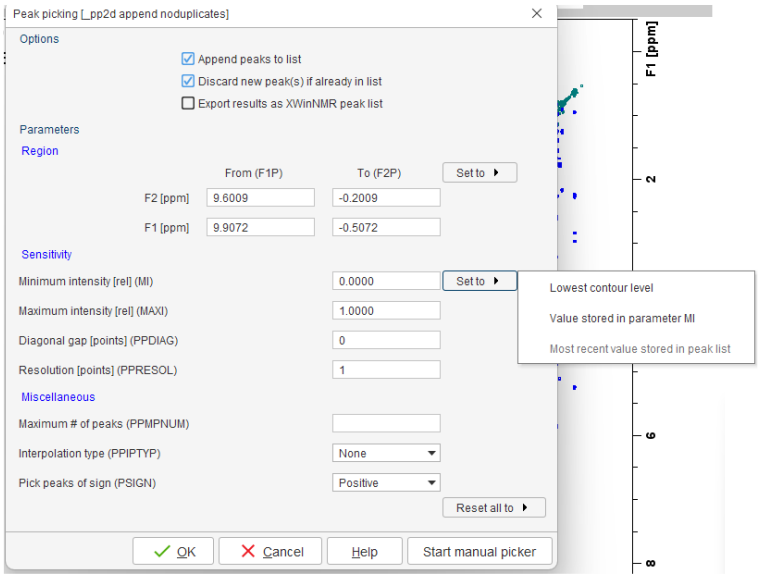


Figure 22: Automatic Peak picking window for 2D dataset.

Manually peak picking process is identical to the 1D.

Integration

Integration 1D

To perform a manual integration, select the **Analyse** menu bar item and click on **Integrate** on the flowbar.

- Make sure the "Define new integral range" button is highlighted.
- Set the cursor line to the left of the first peak to be integrated. Press the left mouse button and drag the cursor line to the right of the peak and then release the mouse button.
- Repeat the last step for all peaks/region of interest.
- On the Integration Tool bar, click **Return, save region** to save the integration regions.

There is also an automatic option for the 1D integration. This is achieved using > **abs** `<ENTER>` a command that does two things to the spectrum: it corrects the baseline, and it does its best to integrate the spectrum. However, most of the time we need to correct or and regions in the spectrum. There is an option in the integration manual mode that allows you to cut previously defined integrals (like the ones obtained from the abs command).

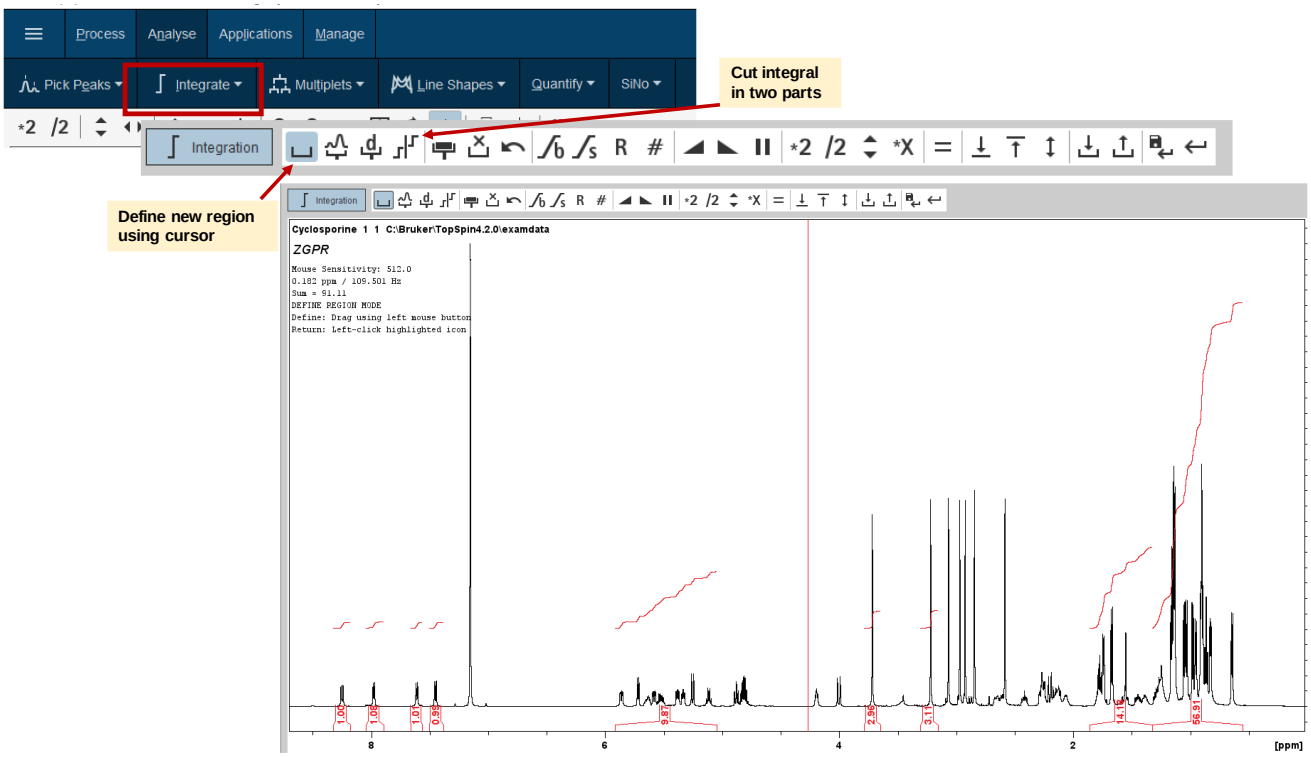


Figure 23: Integration dialog box.