Basic procedures for NMR

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Sample Handling

It is a good practice to filter (step 3) NMR solutions directly into the sample tube, or centrifuge them to keep the solution free from dust and other contamination.

Note: The sample tube should always be held by the top!

Typical procedures to prepare a sample might be as follows:

1. For a solid sample using a 5 mm tube dissolve up to 20 mg of the sample in about 0.6 ml of the chosen solvent. Typically for a liquid sample, and when observing protons, dissolve 20% sample in 80% deuterated solvent.

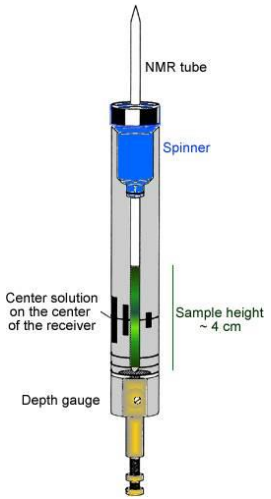
2. If the solvent does not contain already, add a small amount (~0.1%) of reference compound Tetramethylsilane (TMS). Make sure the TMS signal is smaller than the most intense sample or solvent signal (otherwise the Signal to noise ratio is wasted because of low receiver gain).

3. If needed, filter the solution into the sample tube through a Pasteur pipette containing a small plug of glass wool.

4. Close the tube with a cap, seal the top with parafilm **to reduce evaporation** and **label the tube** near the top. Be careful to ensure that the cap, parafilm and label are concentric or otherwise they will adversely affect sample spinning

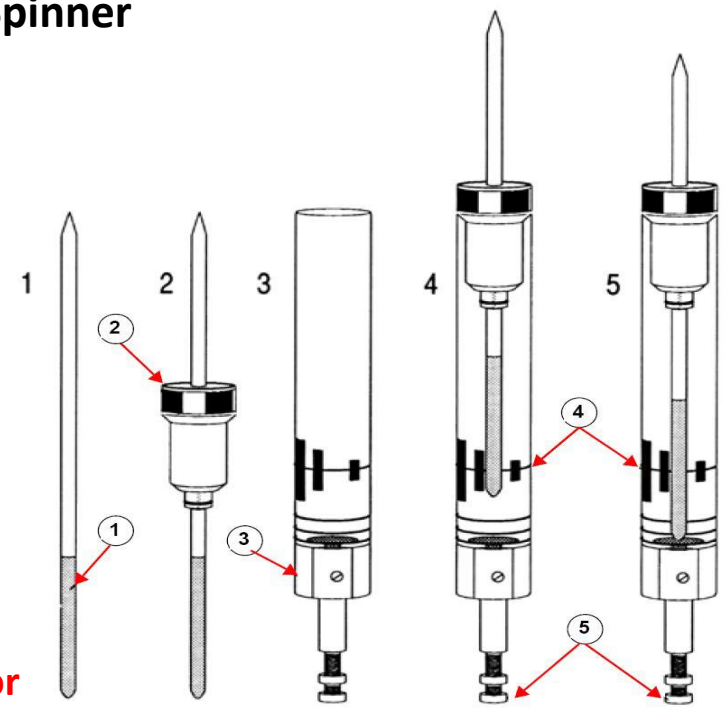
Sample Preparation for measurement

- Use clean and dry sample tubes (wash tube with EtOH).
- Use medium to high quality sample tubes.
- Always filter the sample solution.
- Always use the same sample volume or solution height.
- Filling volume of a 5 mm tubes is **0.6 ml** or 5 cm.
- Use the sample depth gauge to adjust the sample depth (1.8 cm for older style probes, 2.0 cm for newer style probes).



Inserting the Sample into the Spinner

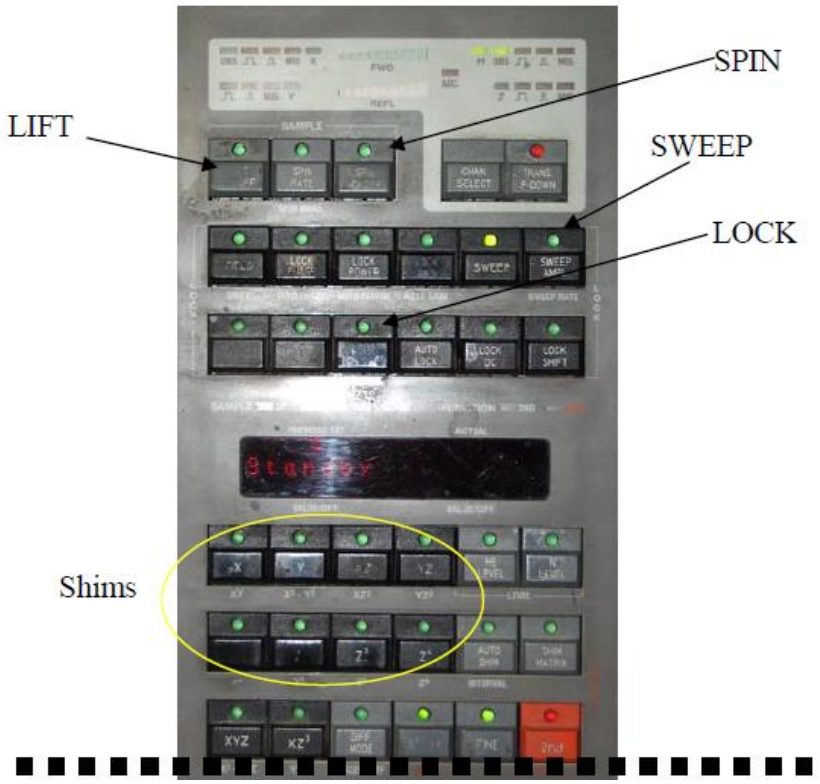
1. Sample	4. Center Line
2. Spinner	5. Depth Adjustment Screw
3. Depth Gauge	



- Follow the recommended procedure as shown in the laboratory demonstration.
- Adjust proper position of the sample
- The sample tube should sit tightly inside the spinner.
- Wipe the sample tube clean with EtOH before inserting into magnet or autosampler

Improper adjustment leads to no detectable signals or breaking the NMR tube inside the detector.

Insertion of NMR sample (with spinner) to the magnet:



The Keypad: Located next to the console Computer. The layout consists of a matrix of buttons

1. Press "LIFT" button on the keypad (see description below).
2. A standby sample, if any, will slowly rise up to the top of the magnet.
3. Replace the sample with yours. Before releasing it, ensure the tube & spinner are supported by the compressed air from the magnet entry port.
4. Press the "LIFT" button again to lower your sample into the magnet

- By pressing a specific button, a certain function can be modified by turning the wheel-knob.
- The LED flashes if a selected function fails.
- The value of adjustment is shown on the center LED bar.

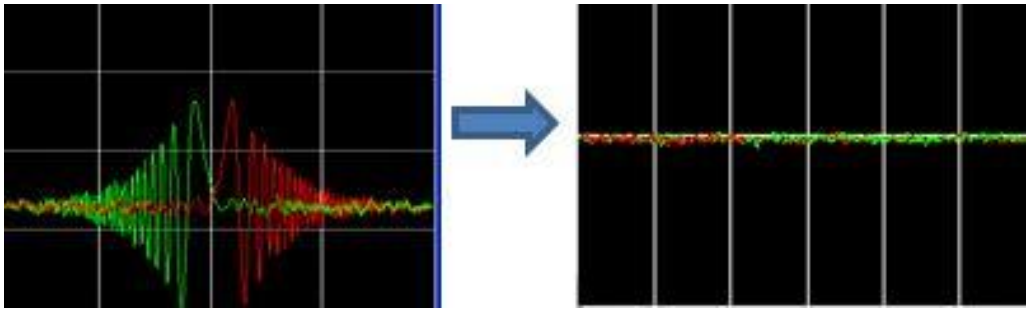
Three major groups:

1. Mechanical control on NMR sample.
2. Field controls (lock, sweep).
3. Shim gradient adjustments

Locking the Sample

Lock: for automatic locking

Select solvent

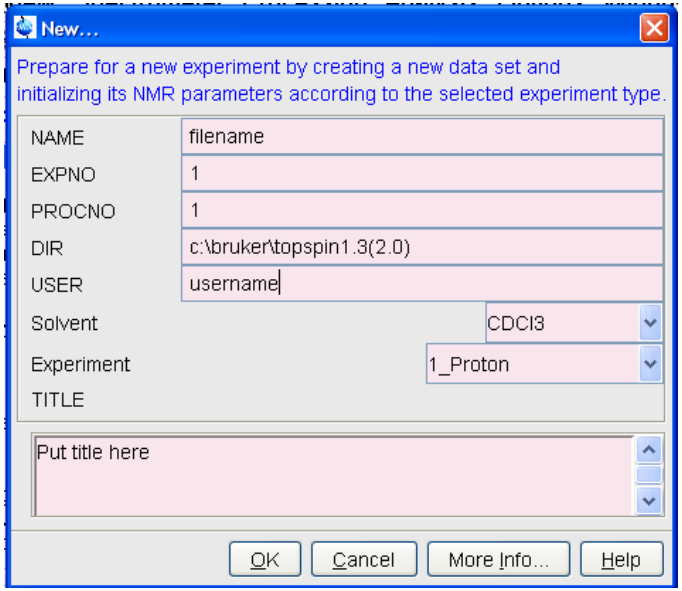


The lock signal can be controlled **manually** by adjusting Phase, Power, Gain, and Field (with lock off). The Field parameter is Z0.

Note: If lock has been achieved, the signal should be of the form of a horizontal line with some associated noise or ripples (see figure below). The height of this line is called the lock level.

Create a DATA file:
Create new experiment

Click or type **edc**



a. **Name:** Define the name to identify your sample. e.g. sample813

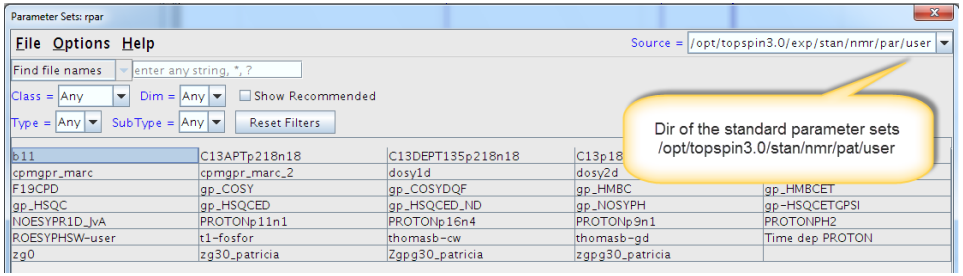
b. **EXPNO:** "experiment number"…. Any integer [1 ….. 999].

c. **PROCNO:** 1

d. **Dir:** C:\Bruker\Topspin 1.3\data\username

e. **USER:** Mandatory format "Your-name-month". where month is when the data sets are created.

f. **Solvent:** click and select the solvent in your sample.



wrpa: Copy a data set, raw and processed data

Re: Read data of specified name or expno

rpar**:** Load experiment parameters

getprsol: Get probe and solvent dependent parameters

NOTE**: Must do getprosol before probe tuning**

Shimming

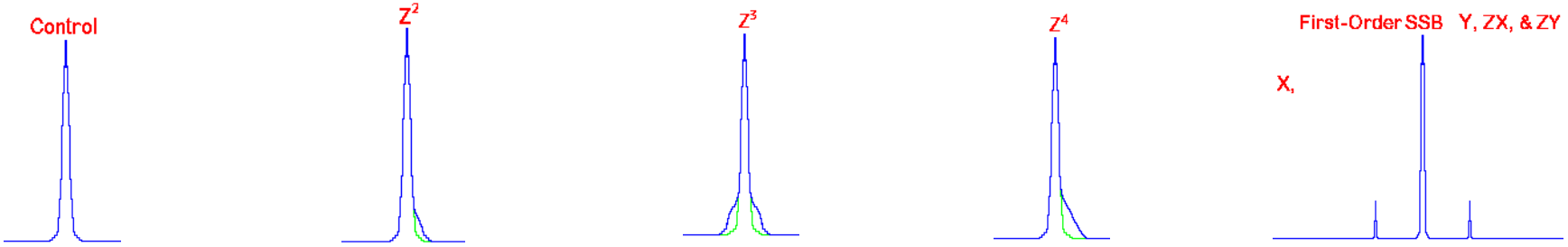
Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to reshim each time a sample is changed, especially when the sample solvent changes. The system manager has stored appropriate shim values (in so called shim files) that are frequently updated and will greatly reduce the shimming time required whenever shimming is needed.

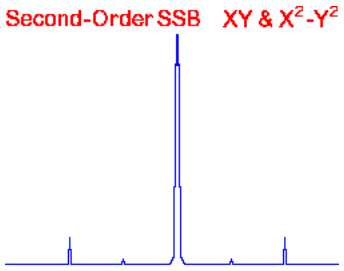
Broad lines, asymmetric lines, and a loss of resolution are indications that a magnet needs to be shimmed. The shape of an NMR line is a good indication of which shim is misadjusted. Consider a single narrow NMR line. If we zoom in on this line we might see the following shape. The following series of spectra depict the appearance of this spectral line in the presence of various inhomogeneities.

rsh: read a recent shimfile

wsh: write shims

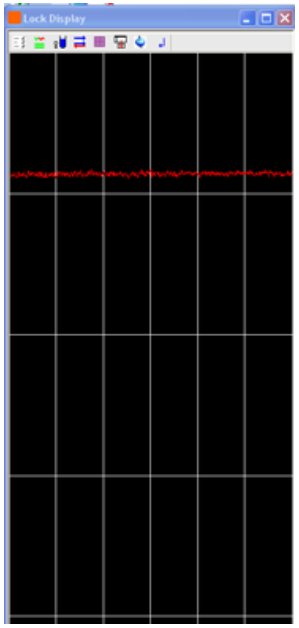
Shimming -errors





You can check the line shape with a quick single scan to obtain a ^{1}H spectrum after any shimming

Manual Shim



1. Take note of the lock level in the lock display as the initial reference level.
2. Start with the functions **Z and Z^2, Z^3**
3. Readjust **Z and Z^2**
4. For the first sample that you do, adjust also functions **X** and **Y** then **XZ** and **YZ** (if there is a large change return to X and Y), then **XZ^2** and **YZ^2 (**if there is a large change return to **X, Y, XZ and YZ)** and then **XY** and **X^2-Y^2**
5. Then you may adjust **Z^3 and return to adjust Z and Z^2**
6. Finally, collect a quick one scan proton spectrum and review for sharp and symmetrical line shape

For best resolution, adjust the X, Y and XY shims to obtain the maximum lock level.

For most simple samples, steps 1-3 will be sufficient to obtain a good spectrum

Tuning and Matching the Probe

Tuning involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately. If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly

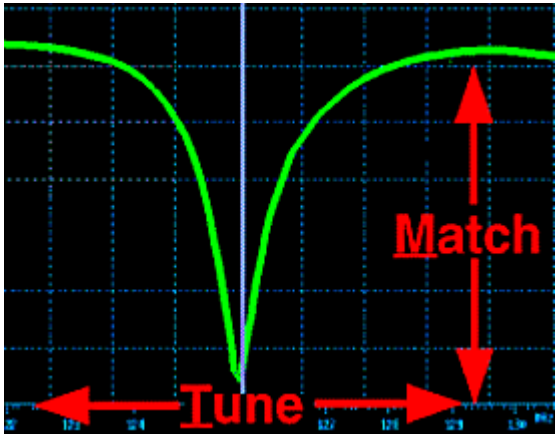
Matching
involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).

Note: **Bruker offers two different types of tuning and matching adjustments**

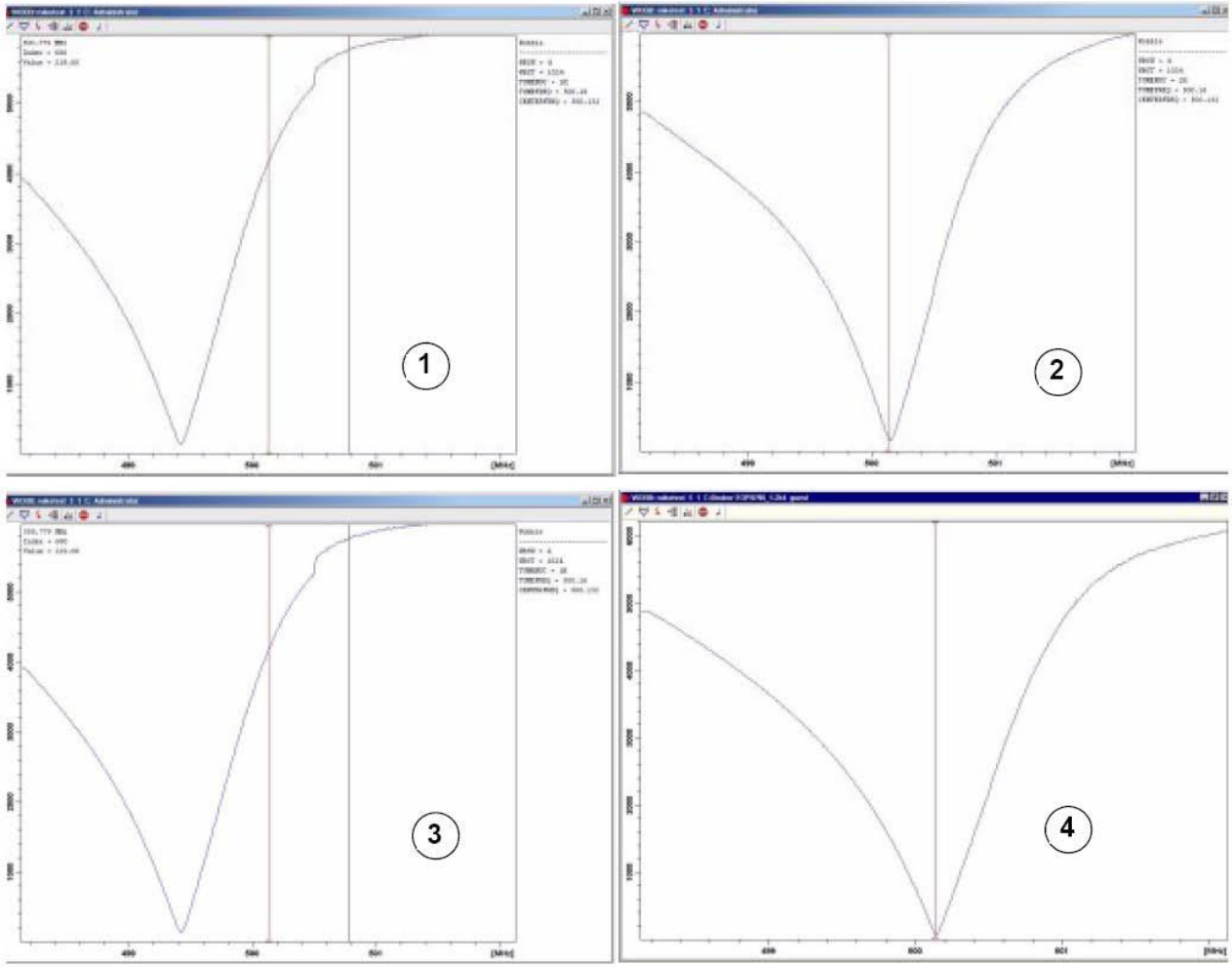
atma: for probe with automatic tuning **THIS is used on the 300 MHz**

atmm: Tune/match ATM probe manually

atma exact: will determine the optimum tuning and matching more precisely than atma without an argument and will therefore be slower



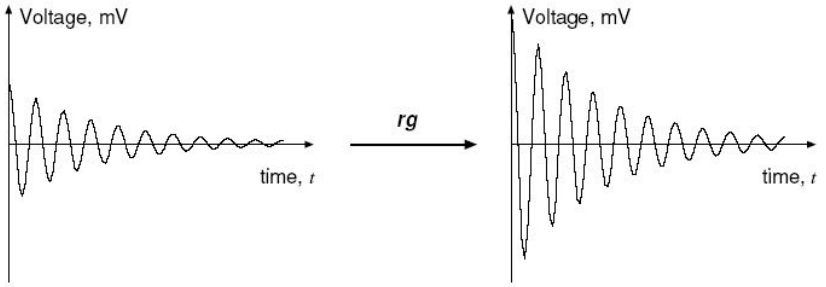
Examples of Wobble Curves with Different Tuning and Matching



1.	Bad matching and tuning.	3.	Good matching, bad tuning.
2.	Bad matching, good tuning.	4.	Good matching and tuning.

Receiver Gain

The receiver gain is a very important parameter that is used to match the amplitude of the FID to the dynamic range of the digitizer. The gain is set by clicking rg and providing a suitable value



The NMR-signal received from the resonant circuit in the probehead needs to be amplified to a certain level before it can be handled by the computer.

The detected NMR-signals vary over a great range due to differences in the inherent sensitivity of the nucleus and the concentration of the sample.

Rg values: 0-200

Rga: automatic receiver gain optimization

In all cases, the automatic receiver gain command should be used on the 300 MHz

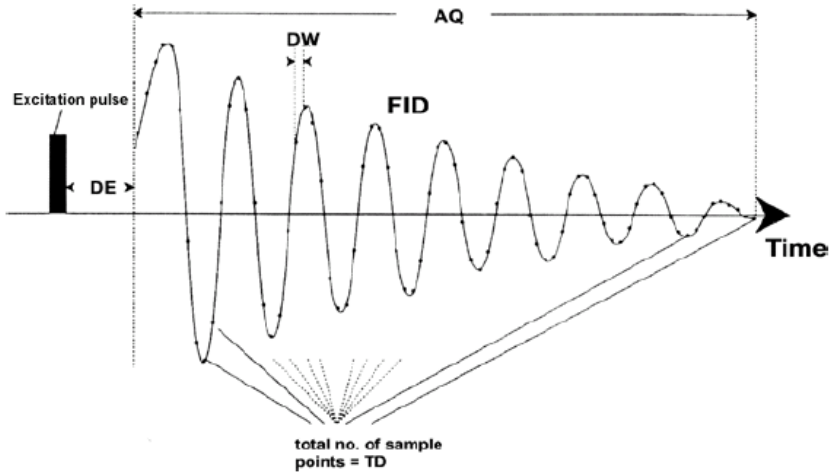
Acquisition parameters

d1 – relaxation delay for proper integration.

td –the number of points to be sampled and digitized to form the FID.

Final number of accumulated scans = NS +DS

Typically TD is set to 16, 32 or 64K for standard 1D FID's. Although increasing the size of TD will improve the FID resolution, it does so at the expense of a longer acquisition time.



ns: number of scans. Increasing the number of scans increases signal-to-noise ratio, but also increases overall experiment time.

ds-number of dummy scans. Several sets of pulses which are identical to those used for acquisition are sometimes transmitted to the sample before any signals emitted by the sample are allowed to enter the receiver. **This is to allow the sample to reach a steady state or equilibrium.** The number of such dummy scans depends on the sample relaxation time and susceptibility to heating. Typical values for standard experiments are 4 or 8. The pulse program can be consulted in other cases.

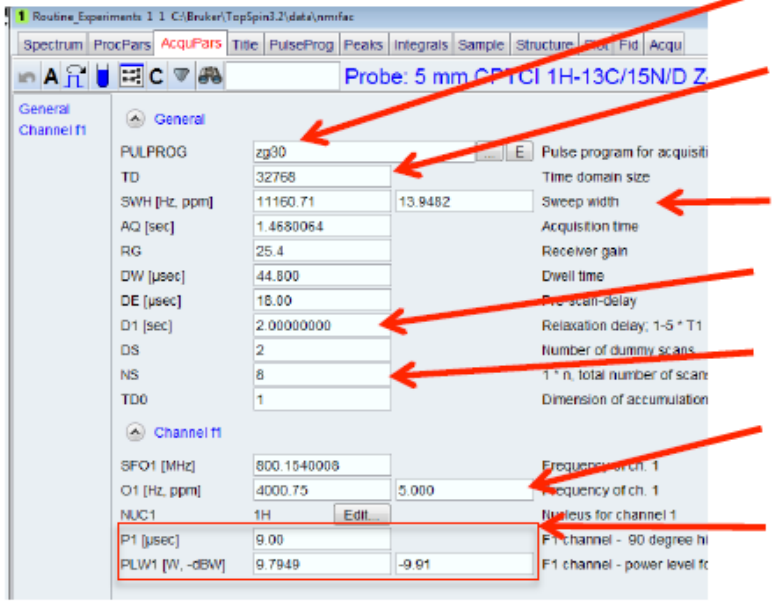
o1**:** Transmitter Frequency **O**ffset for Channel F1 in **Hz**. This is the frequency used to excite the observe nucleus (logical channel F1) and will be at the center of the spectrum. It may be thought of as the central frequency in the window through which the spectrum is observed

o1p: Transmitter frequency offset for channel F1 in **ppm**

Check your acquisition parameters

ased- check and optimize experimental setup (Display important parameters)

Modify acquisition parameters if desired.



Pulse Program. zg30 means 30° hard pulse, and acquire

Number of points collected. Usually 32k or 64k

Sweep Width, in Hz and in ppm

Relaxation Delay, usually 2 seconds for

Number of scans

Carrier Frequency in ppm. Center of your spectrum

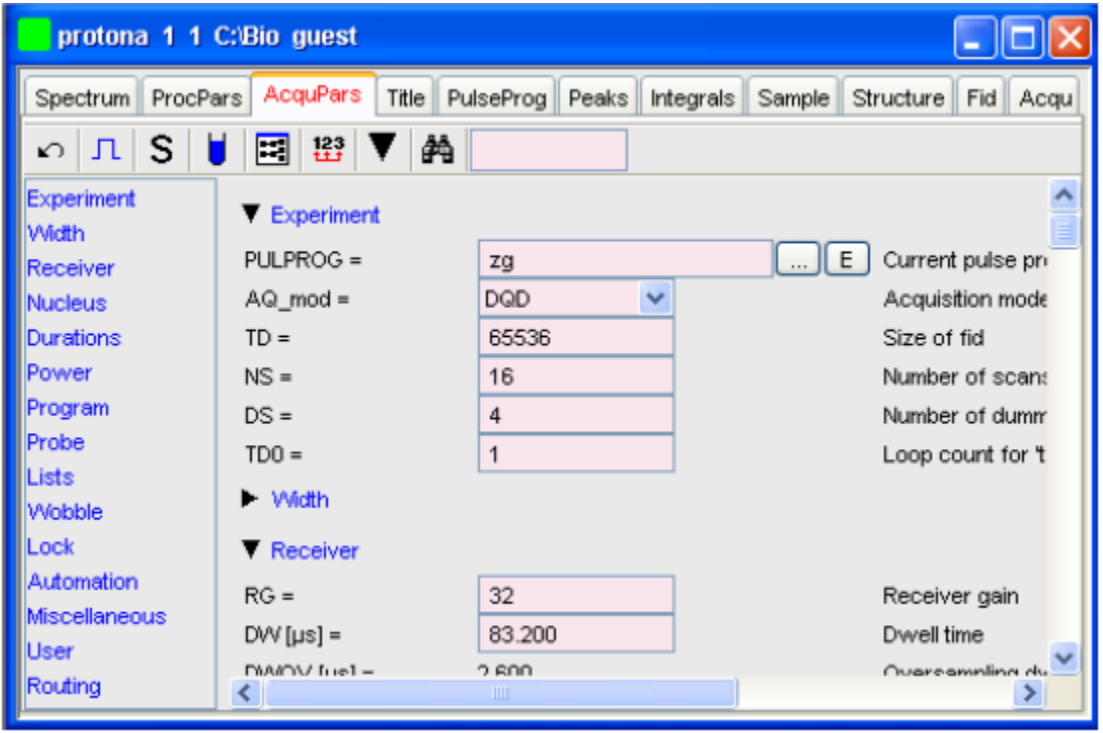
90° pulse length and power. The **getprosol** command will populate these values for you.

You can also use the command **pulsecal**, which will find the accurate 90° pulse

eda - To access all of the acquisition parameters

Note: The disadvantage of eda is that it shows many parameters, most of which do not have to be changed for a standard experiment. Generally, it is more convenient to use ased to set the acquisition parameters. This command only shows the parameters which are actually used for the current experiment

Click the AcquPars tab to display the acquisition parameters



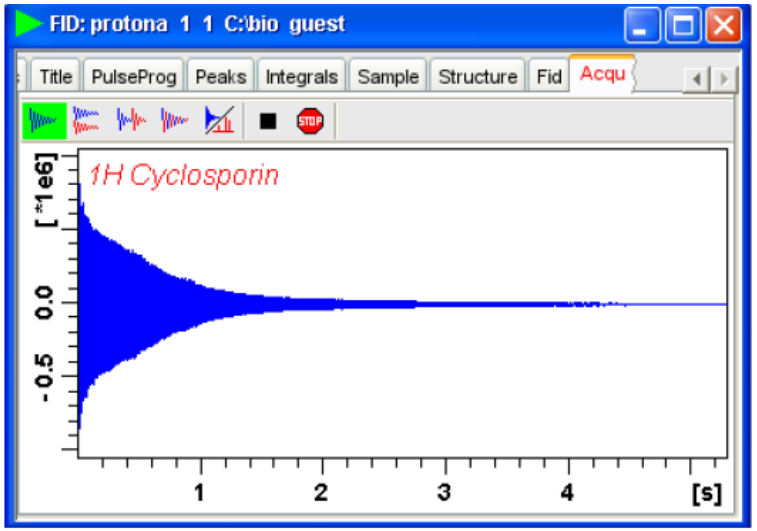
show the pulse program parameters only

button to read the prosol parameters.

To start the acquisition:

– Click ▶ in the upper toolbar or enter **zg on the command line.**

The data window toolbar will automatically switch to the Acqu tab and the FID display window will appear:

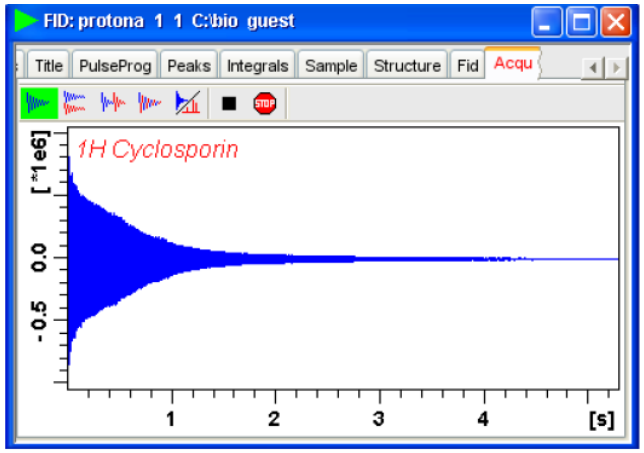


Acquire data

zg-record a spectrum

multizg.-(for # experiments) It will ask you for the number of experiments to be performed. If this number is higher than the number of experiments you have set up, it will repeat the last one for the remaining experiments.

expt: display the experiment time



The buttons in the toolbar have the following functions:

- Show FID in shuffled mode.
- Show FID in unshuffled mode, horizontally arranged.
- Show FID in unshuffled mode, vertically arranged.
- Show FID in unshuffled mode, interleaved.
- Switch between FID and spectrum.
- Stop the acquisition [**stop**].
- Halt the acquisition [**halt**].

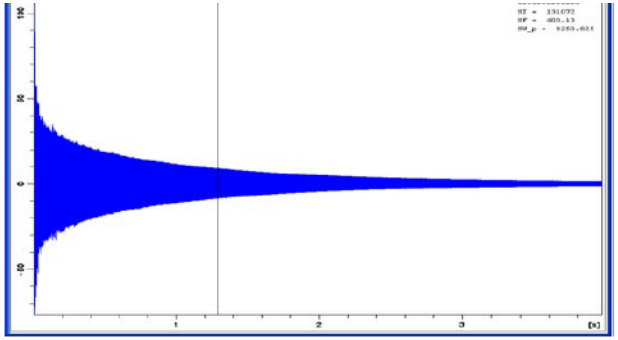
tr- optional step. to preview an on-going experiment (such as a C-13), before its completion

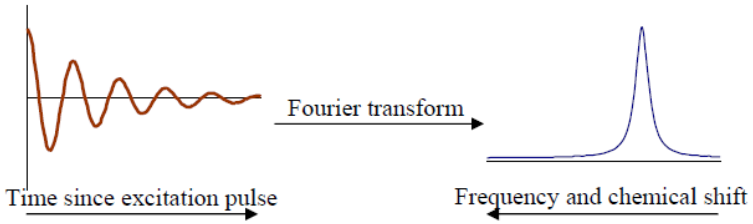
halt: To halt the measurement ***before it completes the NS scans***

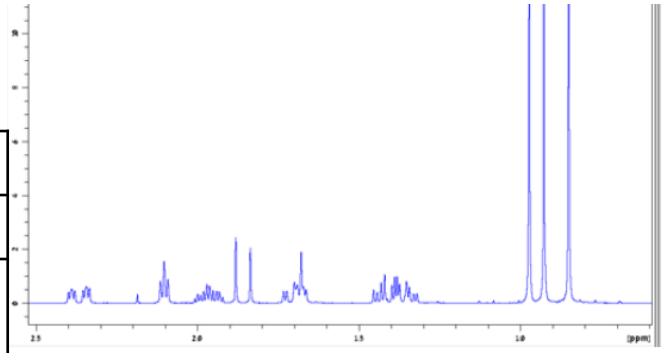
DO NOT TYPE STOP! **Stop** command stops the acquisition immediately without writing the data to disk

Kill: kill a specific process

Processing 1D data

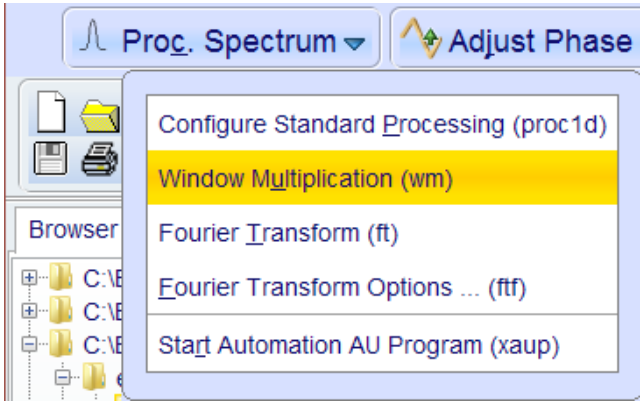






Action	Command	Comment
exponential multiply	em	lb is linebroadening in Hz
fourier transform 1D	ft fp ef efp	fourier transform only ft+pk em+ft em+ft+pk
Automated phase correction	apk	Not always sufficient
Baseline correction	abs	Automatic baseline correction and integration on a 1D-spectrum
	absn	Add the letter 'n' after the command to avoid the automatic setting of integral limits.
Automatically calibrate the spectrum	sref	calibrates the TMS peak to 0 ppm

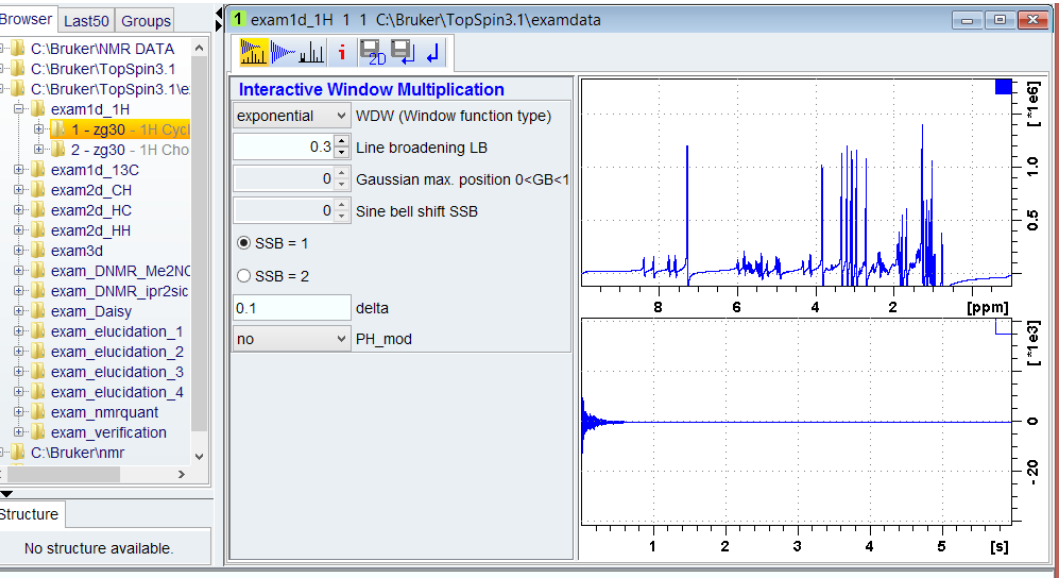
1D Interactive Window Multiplication Procedure



You can perform interactive window manipulation as follows:

1. Select the window function (parameter WDW).

2. Set the corresponding parameter(s), e.g.
– LB for exponential.
– LB and GB for Gaussian.
– SSB for sine bell and squared sine.



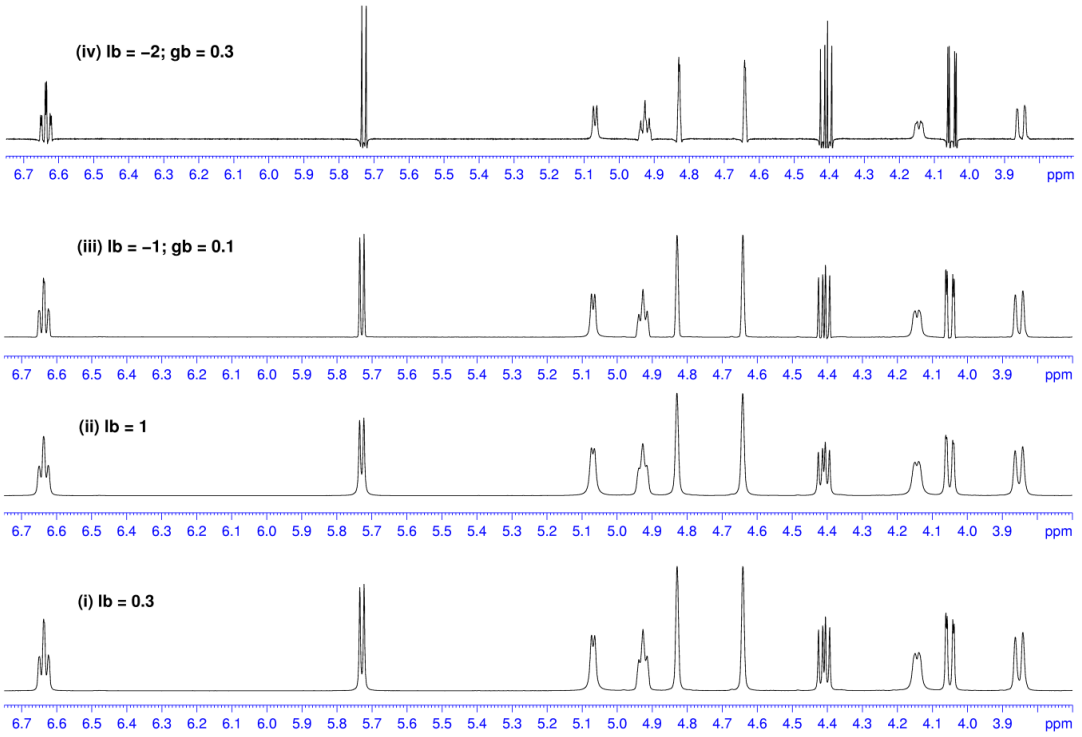
The displayed spectrum and/or FID will be automatically adjusted as you change the window function and parameters.

3. Click the Save button to store the window settings and return. Now you can perform further processing steps like Fourier transform, phase correction etc.

Exponential multiplication (em): can improve S/N. The default lb is 0.3 Hz; employing a higher line broadening value can yield better S/N, but at the expense of resolution.

Gaussian multiplication (gm): used for resolution enhancement using lb as a negative value e.g. -1, -2 Hz and gb (Gaussian broadening; 0 to 1); typically 0.2-0.3.
This reduces linewidths, making multiplets clearer, but at the expense of S/N and may also lead to peak-shape artifacts

examples



How to use the Toolbar (1D)

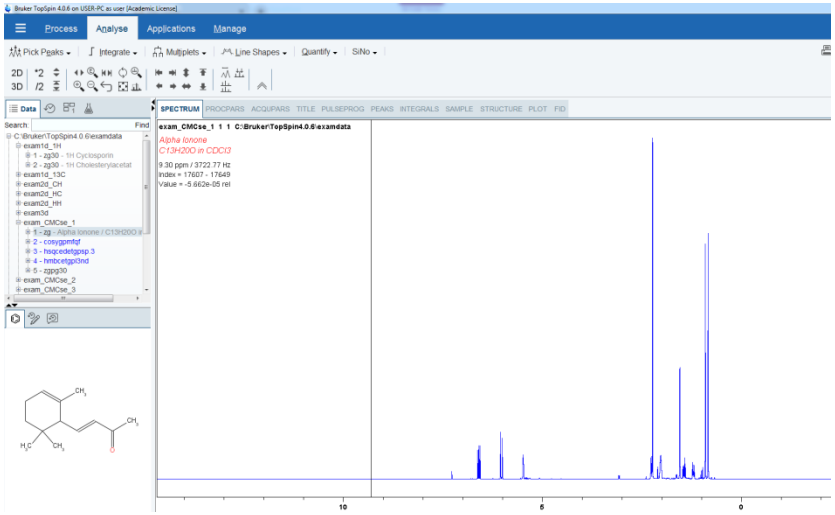
The lower toolbar contains buttons for display functions.

Buttons for vertical scaling (intensity)

Increase the intensity by a factor of 2 [***2***].

Decrease the intensity by a factor of 2 [***/2***].

Change the intensity smoothly.



Buttons for horizontal shifting

Shift to the left, half of the displayed region [**.sl**].

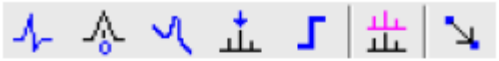
Smoothly shift to the left or to the right.

Shift to the right, half of the displayed region [**.sr**].

Shift to the extreme left edge of the spectrum [**.sl0**].

Shift to the extreme right edge of the spectrum [**.sr0**].

Buttons for interactive functions



The functions of the individual buttons are:

Enter phase correction mode.

Enter calibration mode.

Enter baseline correction mode.

Enter peak picking mode.

Enter integration mode.

Enter multiple display mode.

Buttons for vertical shifting

Shift the spectrum baseline to the middle of the data field [**.su**].

Smoothly shift the spectrum baseline up or down.

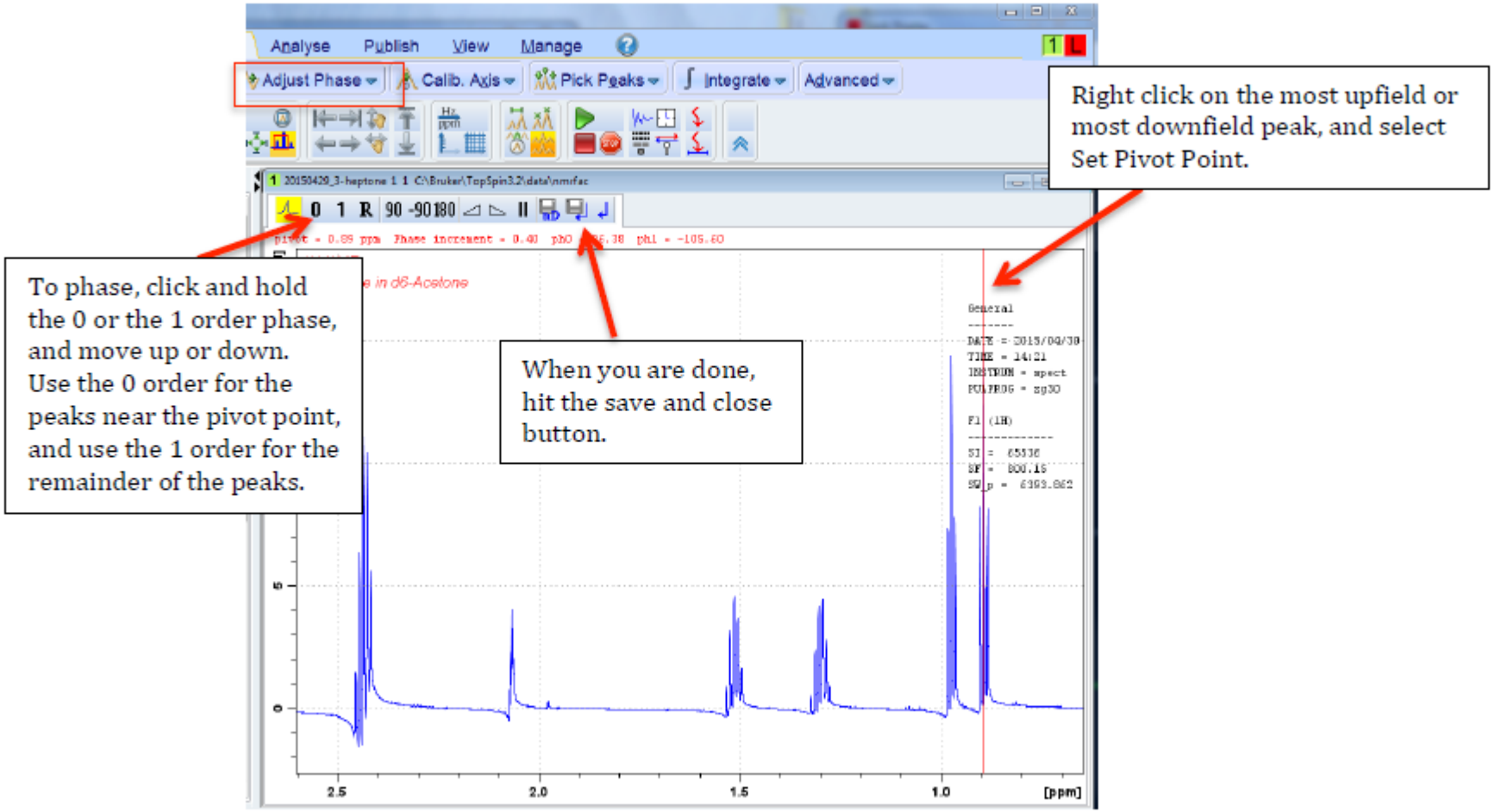
Shift the spectrum baseline to the bottom of the data field [**.sd**].

Phase correction

pk: apply phase correction **(**apply **phc0 and phc1** to spectrum)

apk: automated phase correction

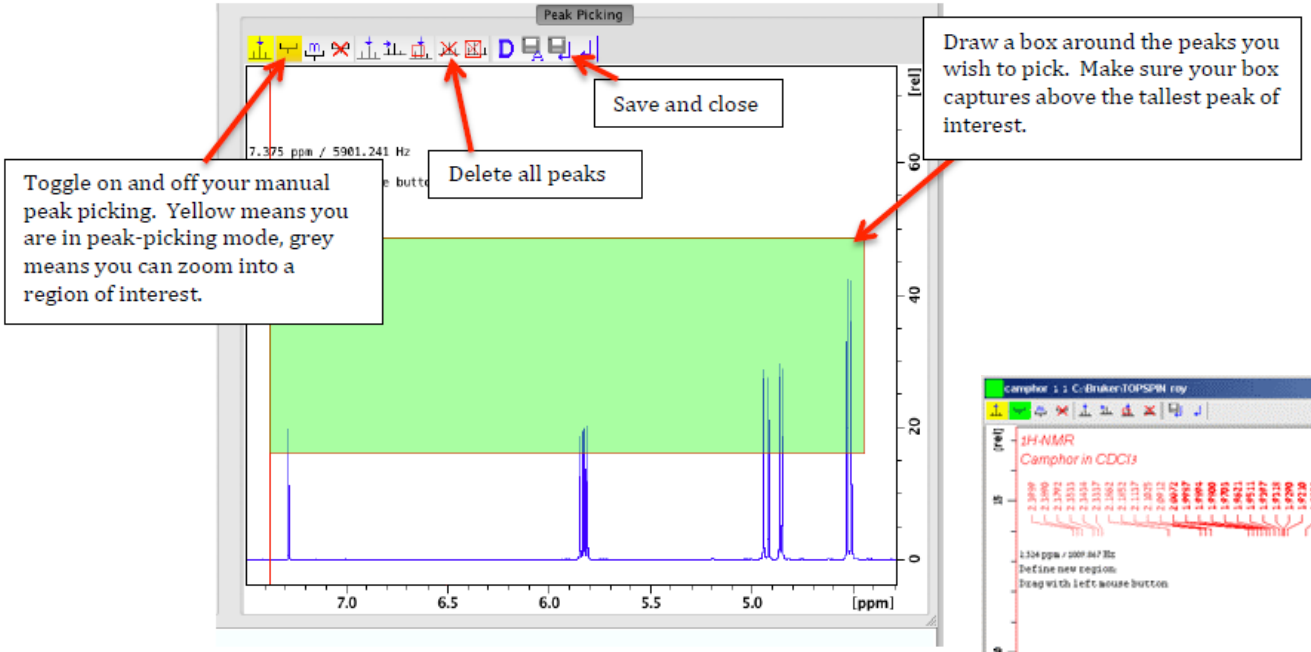
Manual phase correction



Peak picking

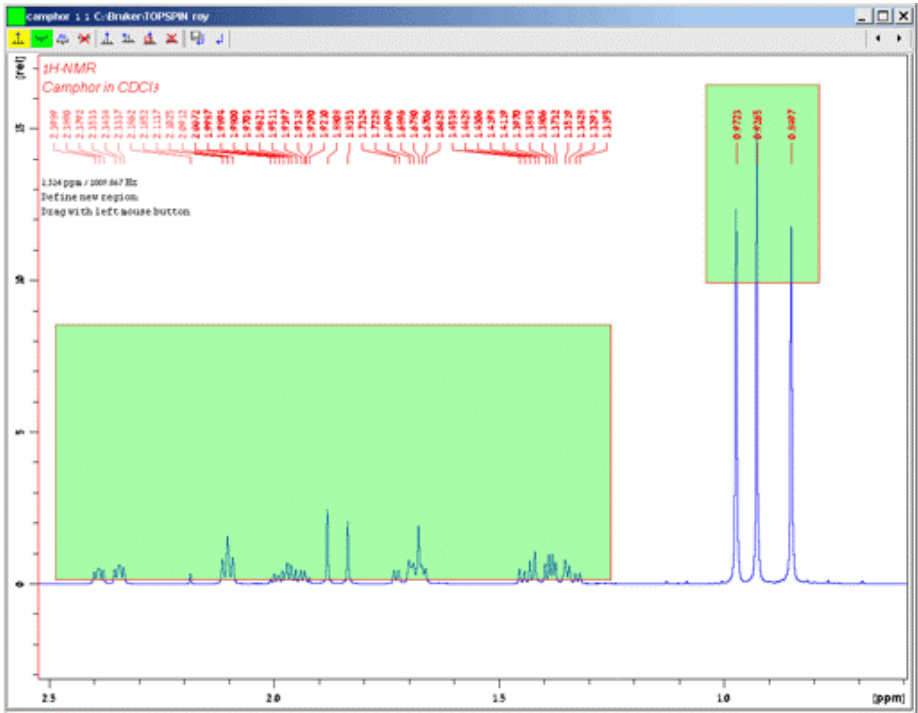
pp- Perform peak picking

Or select the Peak Picking button under the Process tab  The peak picking window will open.

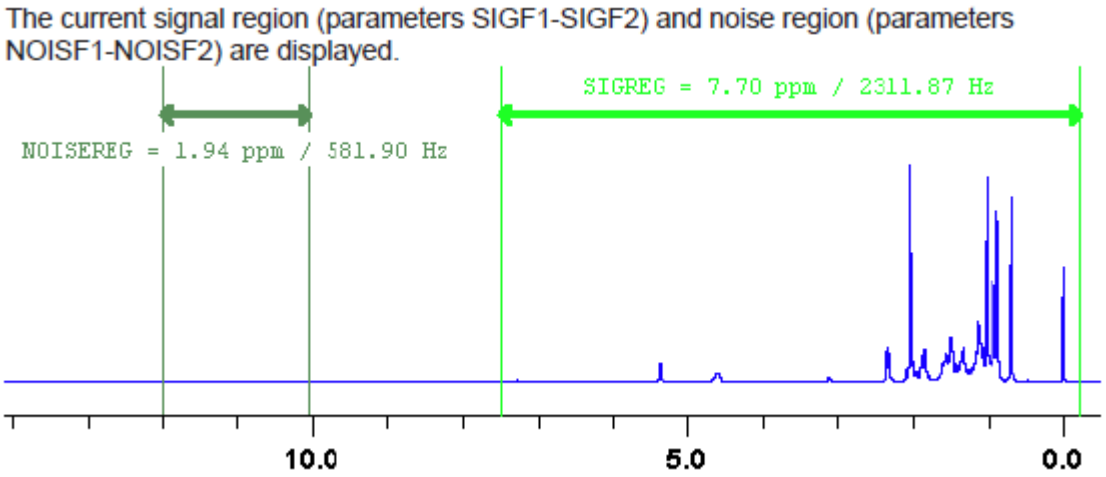


 cancels the selected peaks

 allows individual peak picking

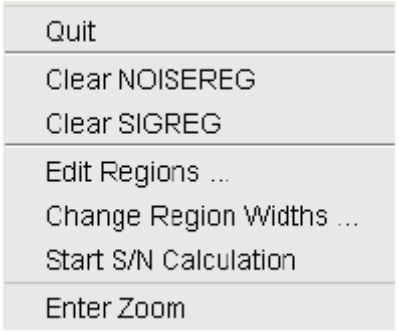


How to Perform Interactive S/N Calculation



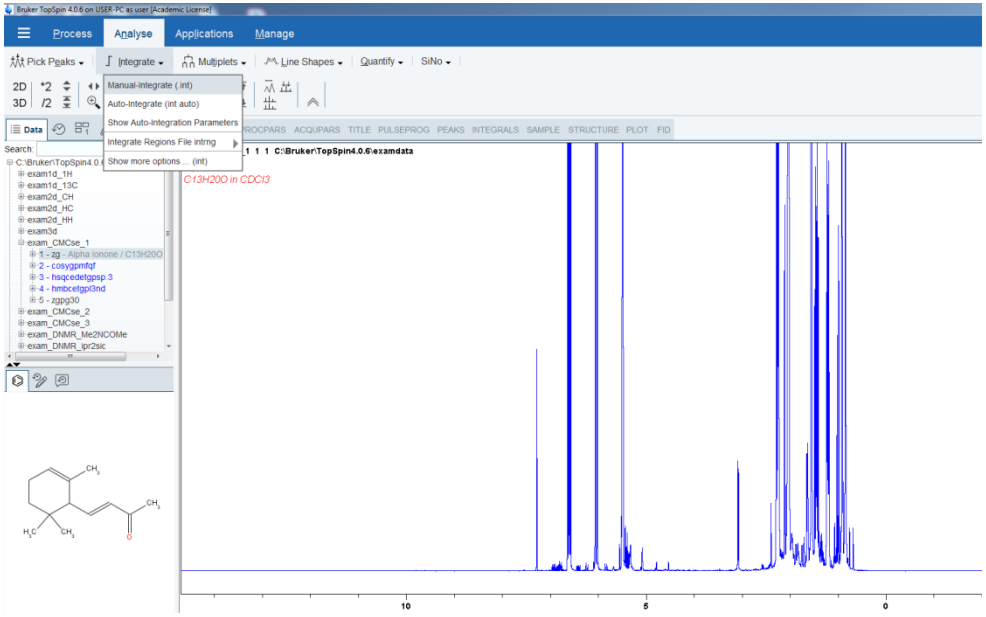
Data window in S/N measurement mode.

2. Move the mouse into the data window.
3. Left-click-hold and drag the mouse from one edge of the *signal region to the other edge.*
A horizontal double-headed arrow will indicate the signal region.
4. Left-click-hold and drag the mouse from one edge of the *noise region to the other edge.*
A horizontal double-headed arrow will indicate the noise region.
5. Right-click any position in the data window. The popup menu as shown will appear



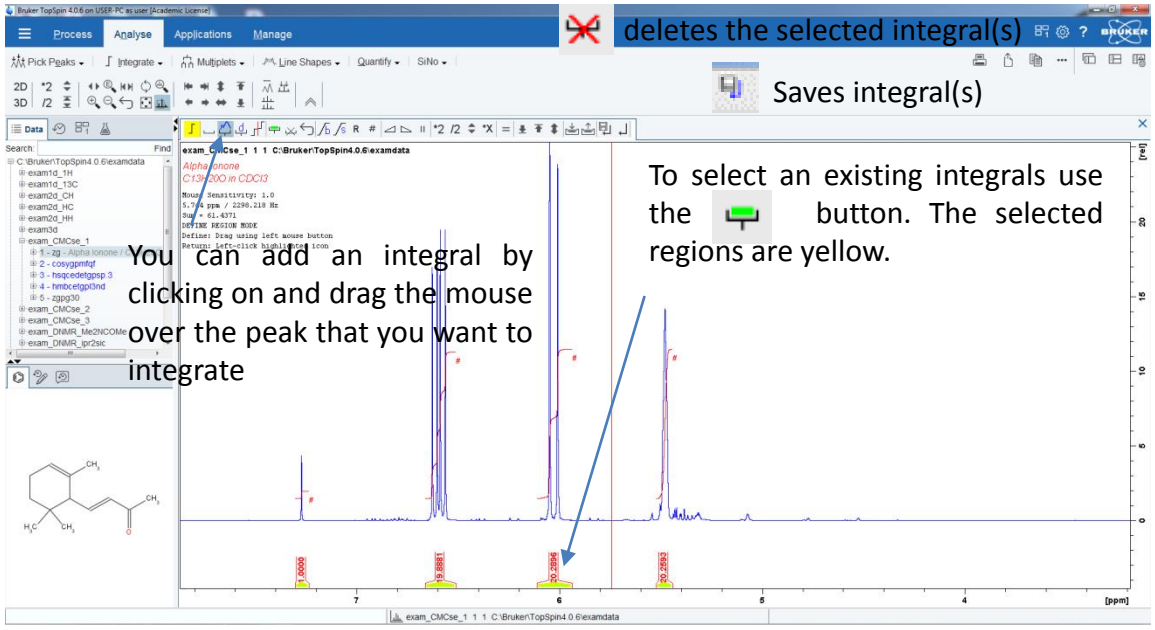
Choose **Start S/N calculation.**
The other entries allow you to redefine or clear the regions. After the S/N calculation has finished, the result will appear on the screen.

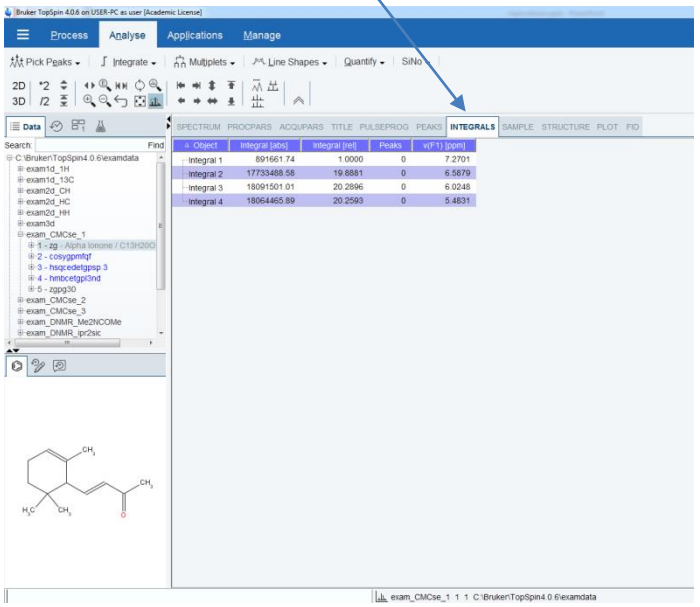
Integration



You can integrate 1D spectra by selecting manual integrate from Analyse tab/integrate

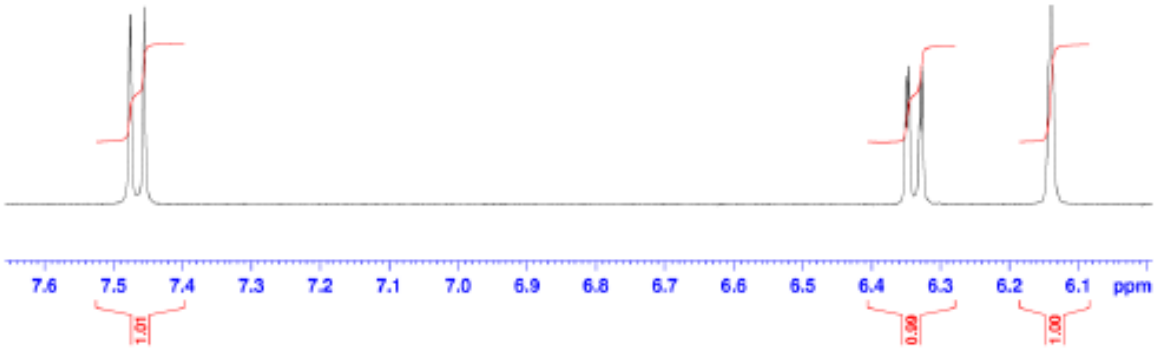
From here you can see all your integrals



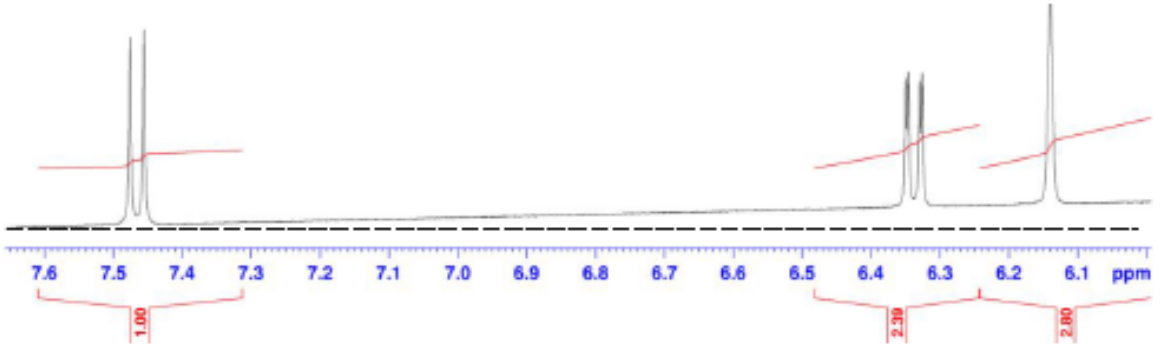


Incorrect Integration

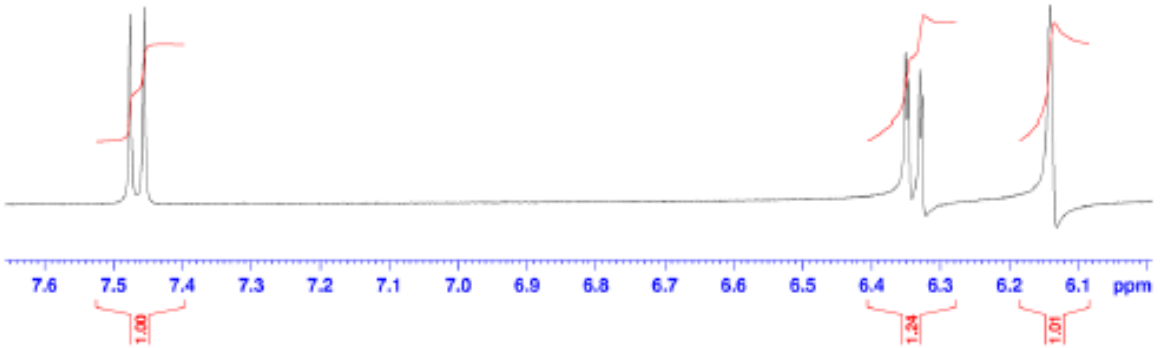
Correct phase and baseline



Incorrect baseline (sloping)

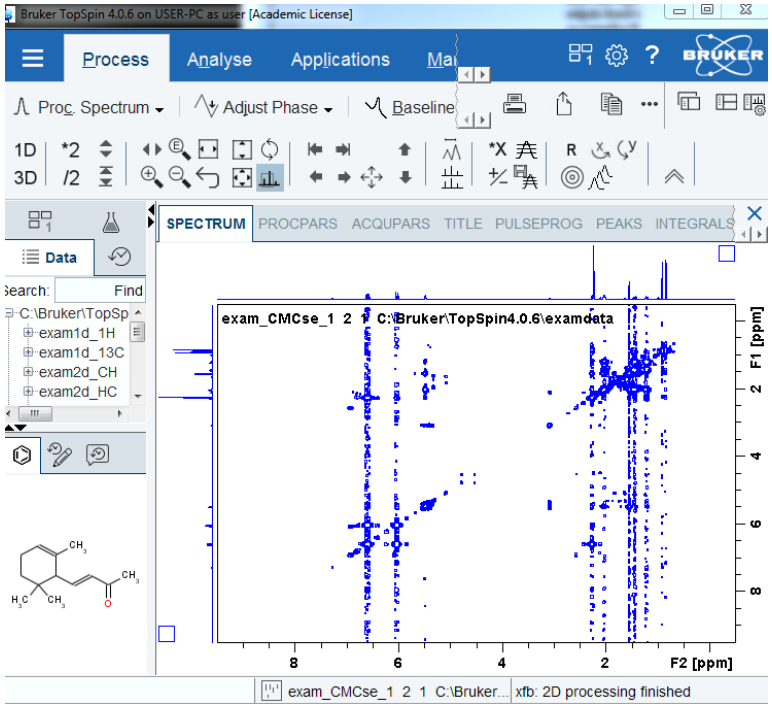


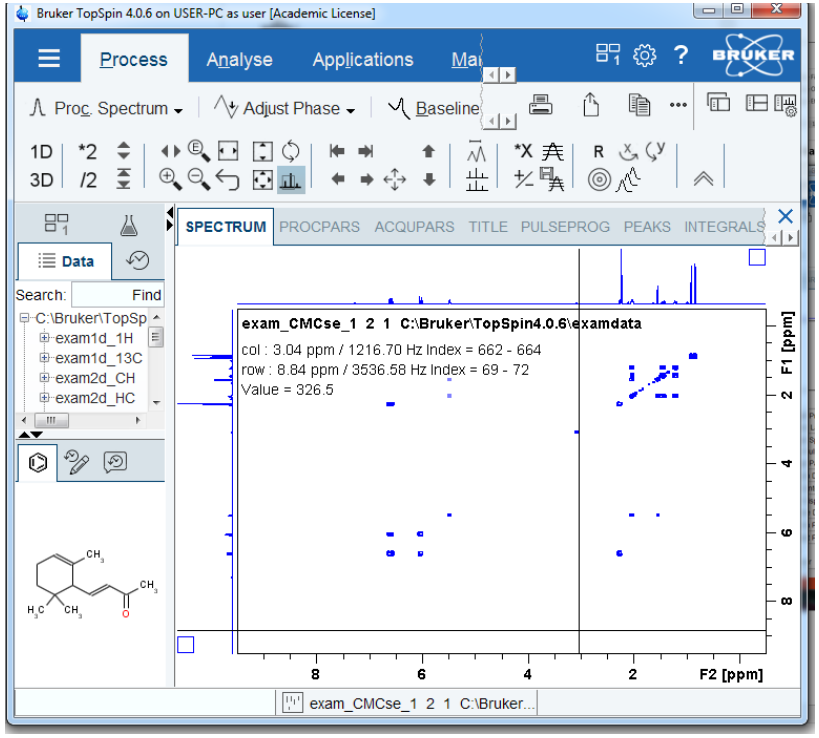
Incorrect phasing



Processing 2D data (auto-commands)

For COSY





xfb: process data including FT in both directions F1 and F2

or xf1: process data including FT in F1 and xf2: process data including FT in F2

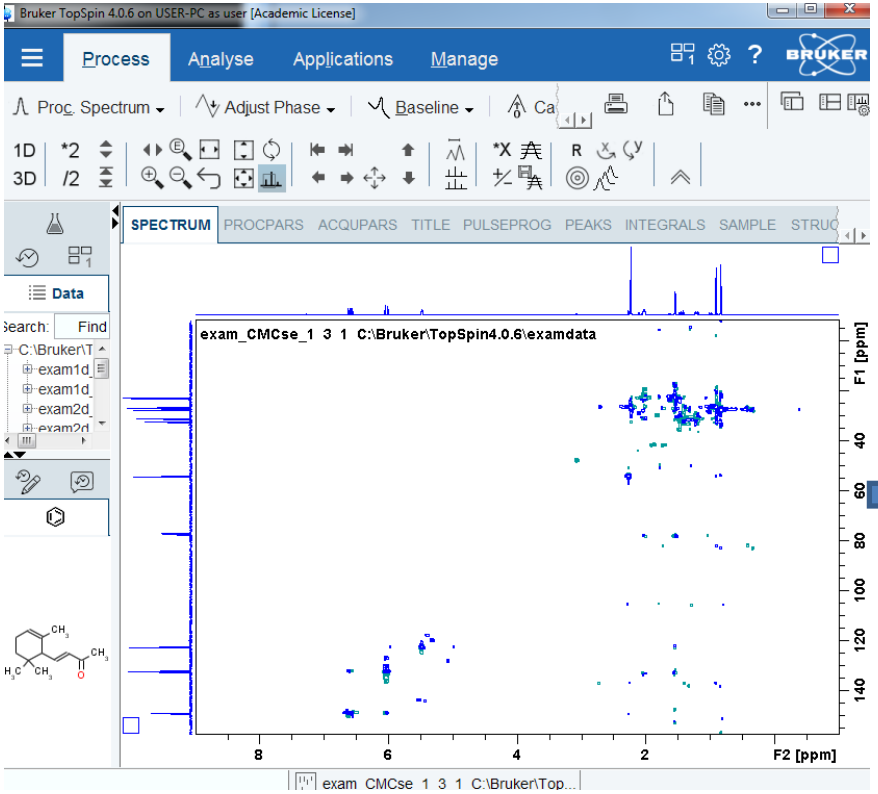
sym: symmetrise spectrum about the diagonal

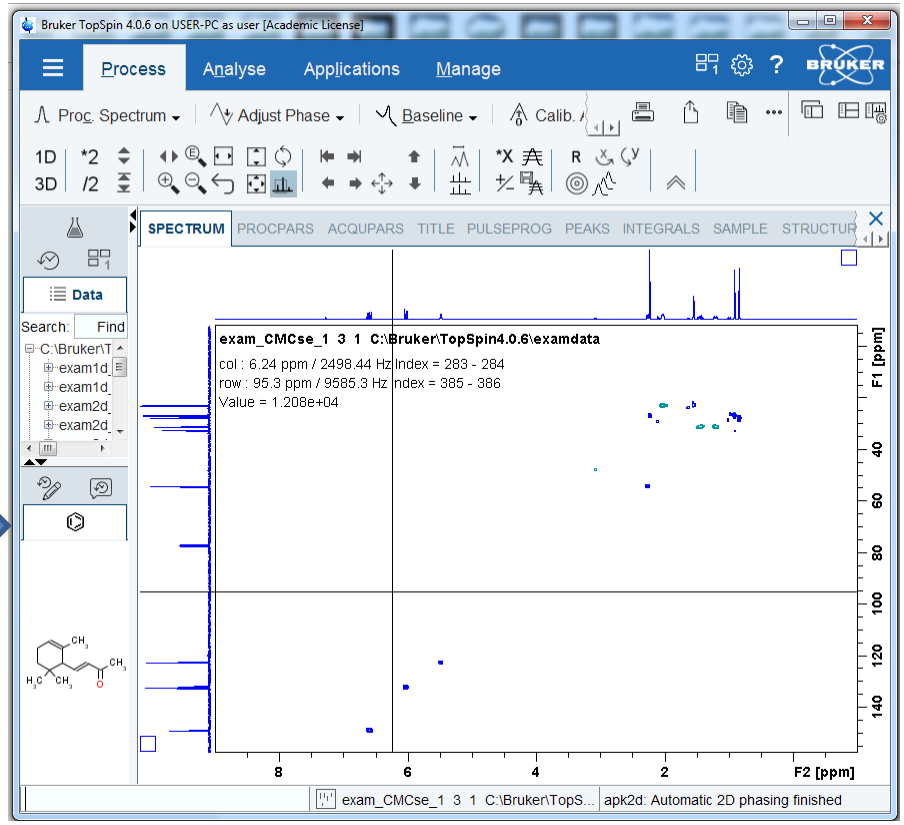
abs1: automatic baseline correction in F1

abs2: automatic baseline correction in F2

Processing 2D data (auto-commands)

For HSQC & HMBC





xfb: process data including FT in both directions F1 and F2

or xf1: process data including FT in F1 and xf2: process data including FT in F2

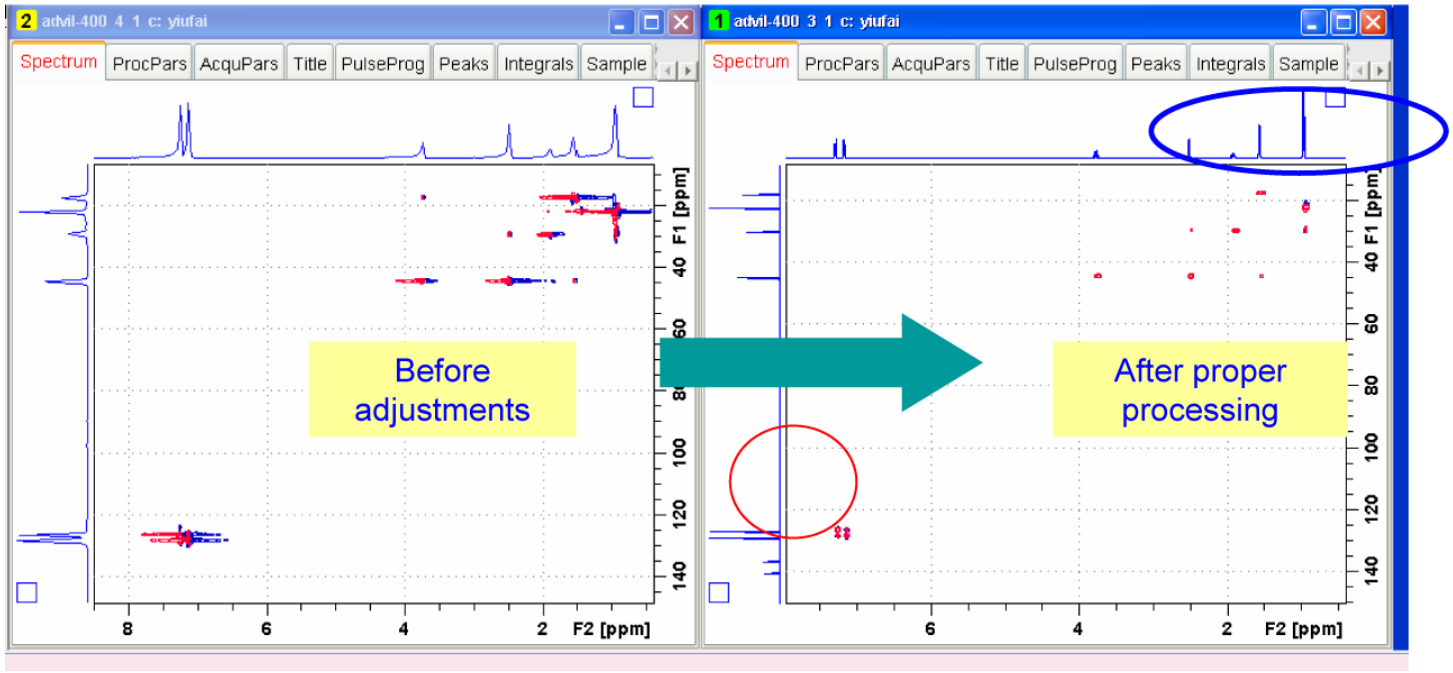
abs1: automatic baseline correction in F1

abs2: automatic baseline correction in F2

apk2d: automatic 2d phasing

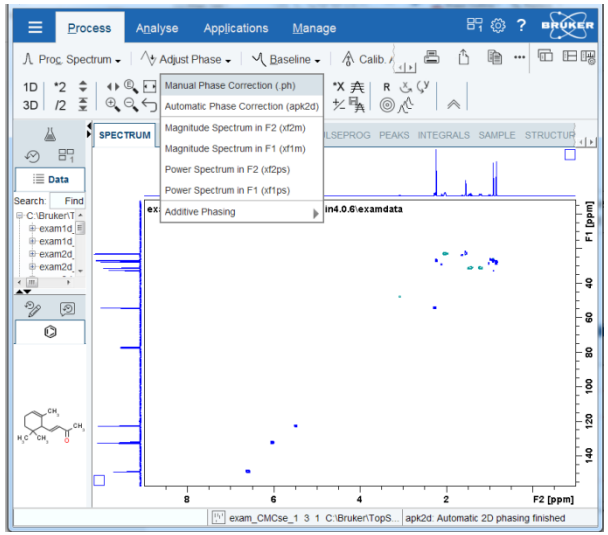
Processing 2D data

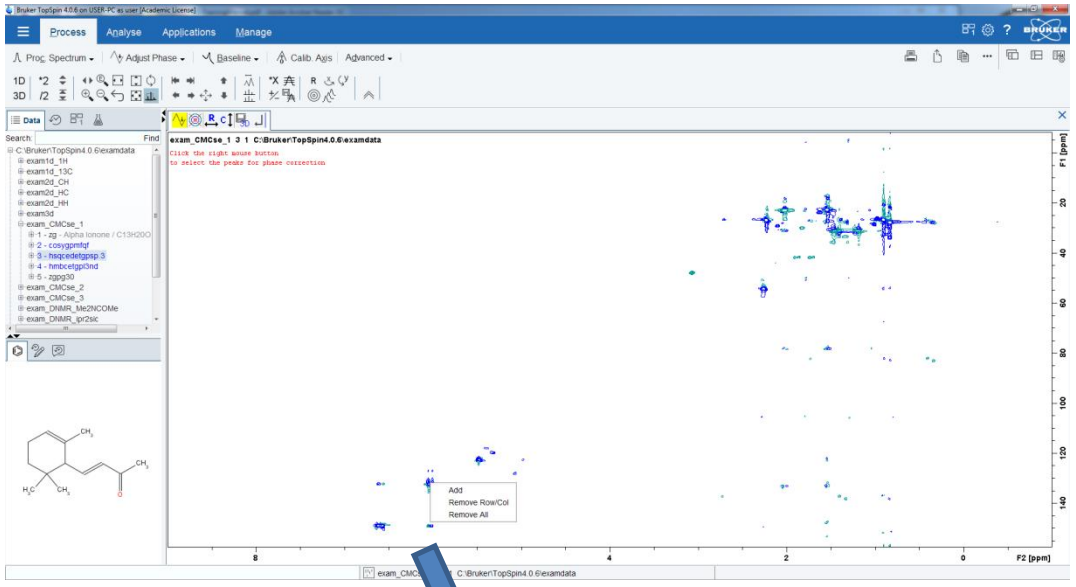
Advanced 2D NMR data processing with TOPSPIN



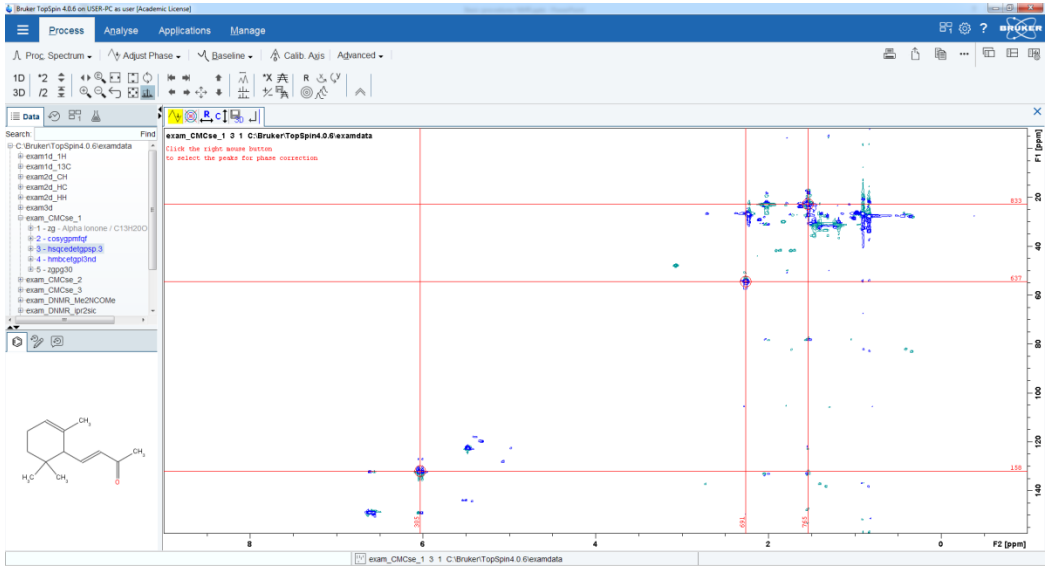
Manual phase correction

Manual phase correction





Select manual phase correction from process tab/adjust phase

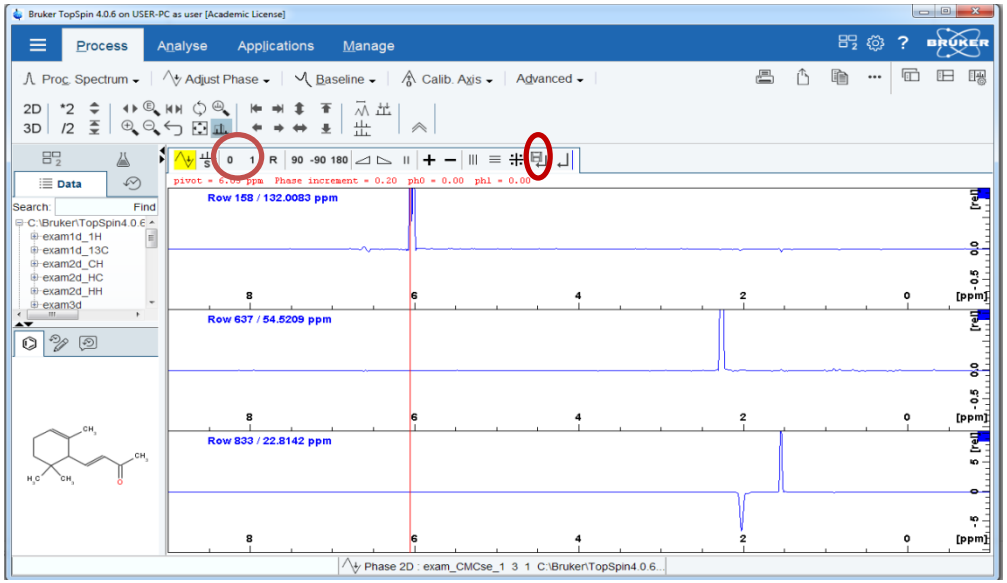


Place mouse to one of the major signals, right click and ‘’add’’

Repeat the same procedure to select another signal far away from the previous one, right click and ‘’add’’

Click  in the new window

2d phase correction on rows

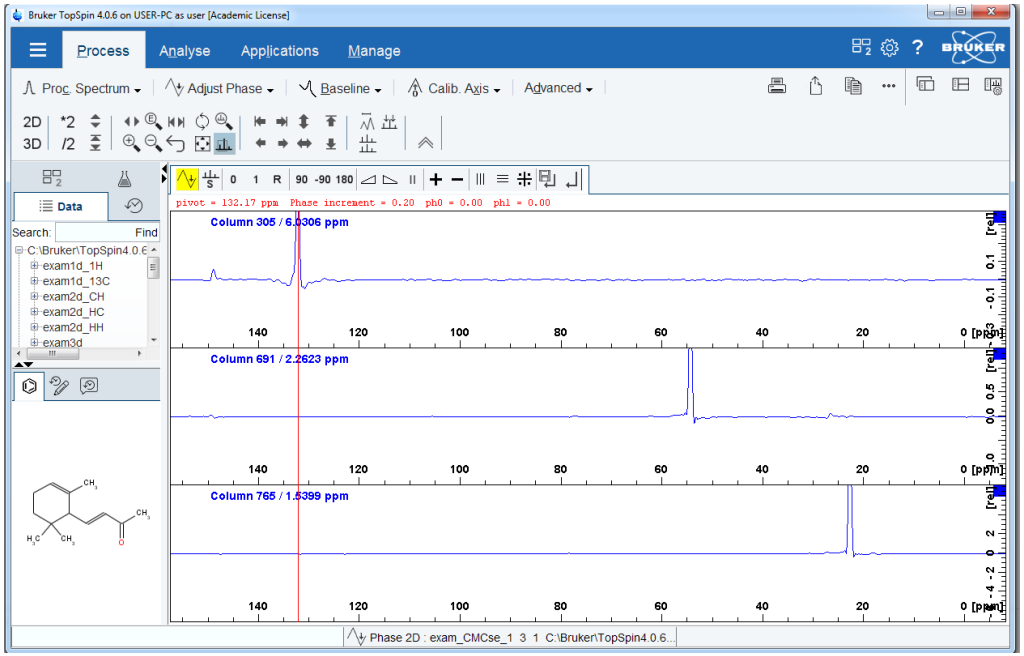


Use 0 and 1 for phase adjustment

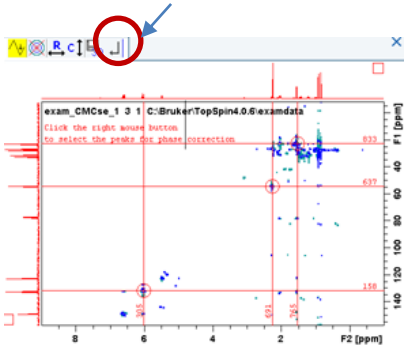
Click save when finished with rows phase adjustment

Repeat same phasing procedure for columns by clicking 

After phasing the column click save and return



Finalize the phase correction



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