

**NMR is FUN**

**MAKING PRETTY**

**PICTURES WITH**

**TOPSPIN**

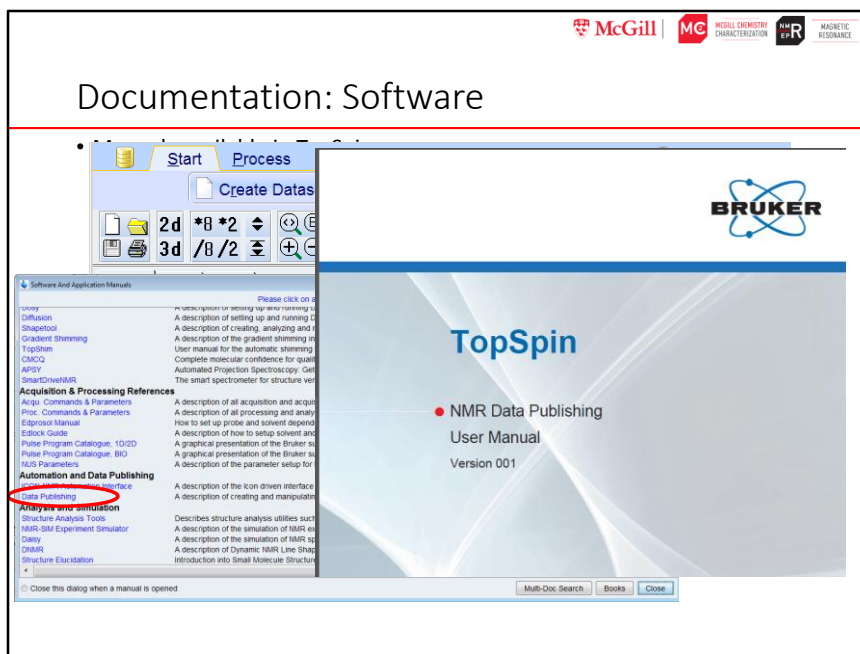



## Content

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- Getting data organized
- Good data processing
- Plotting

## Documentation: Software



Software manuals are accessible from within TopSpin under the  icon. A good place to start is with the Beginners Guide, which gives a brief introduction to the NMR experiment and NMR hardware and then instructions about how to run some frequently used experiments. Also useful are the 1D and 2D Step by Step – Basic manual and the Processing reference manual. Also, if you type the name of a command followed by a question mark into TopSpin (e.g. efp?), then you will be sent to a help page explaining the command.

## How data is stored on disk by TopSpin

- `<directory>\<dataset name>\<expno>\pdata\<procno>`

- *Example:* `c:\users\rstein21\data\RSS_01234\1\pdata\1`

or

- `<directory>\data\<identifier>\nmr\<dataset name>\<expno>\pdata\<procno>`

- *Example:* `c:\data\AV400\nmr\RSS_5683b\3\pdata\1`

- Every FID (raw data) has a different expno
- Each spectrum (processed data) has a different procno
- In IconNMR, you choose the dataset name
- You may also choose the expno, but you may also accept the default
- On the spectrometer PCs, the directory is `c:\data\<your username>\nmr`

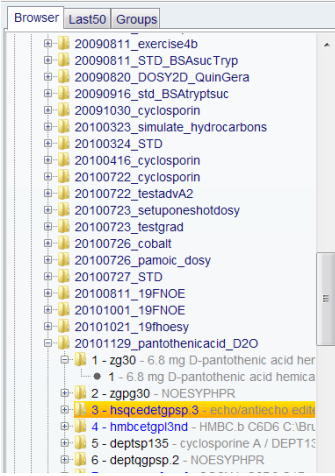
Bruker is somewhat strict about how data is stored on disk. This is because its data browser can only display data stored in certain locations.

Specifically, each piece of raw data has its own expno (experiment number), which is a directory; each expno directory contains a directory called pdata which can hold one or multiple pieces of processed data in different processing numbers (procno's).

Every expno must be contained within a dataset name. Thus, for the dataset called "RSS\_01234", for example, there could be a 1H experiment in expno 1, an HSQC in expno 2, and an HMBC in expno 3. Within `RSS_01234/3/pdata/1` would be the processed HMBC, and `RSS_01234/3/pdata/99` might contain a projection from the 2D HMBC dataset.

Above the expno is the directory. The directory appears in the data browser. However, if the directory has the form `<directory>\data\<identifier>\nmr`, then only the part of the directory before `\data` appears in the data browser at the top level.

## View data: Data browser



The screenshot shows the 'Data browser' window in TopSpin. The left pane shows a tree view of directories and datasets. The right pane shows a list of datasets with their names and experiment numbers. The dataset '3 - hsqcedetgppsp3' is highlighted in yellow.

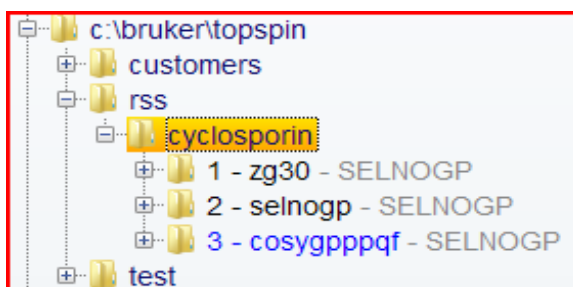
- Once you have found the data in the data browser, open it by:
  - double-clicking
  - clicking and dragging
  - right-clicking and selecting
    - display
    - display in new window
- Close and open the databrowser by typing Ctrl-D (this is especially useful with small screens)
- The data browser can be made into a separate window in the browser preferences (right-click in the data browser). After activating this preference, quit TopSpin and restart it to make the data browser float separately in its own window.

After copying data to another computer with access to TopSpin, use the data browser to open it.

The data browser lists all the datasets. A directory containing data can either be of the form <directory>/<name>/<expno> or <directory>/data/<user>/nmr/<name>/<expno>. In the first case, opening up a directory (by clicking on the + sign next to the directory name) gives a list of dataset names, beneath which are the experiment numbers, and within those the processed data numbers.

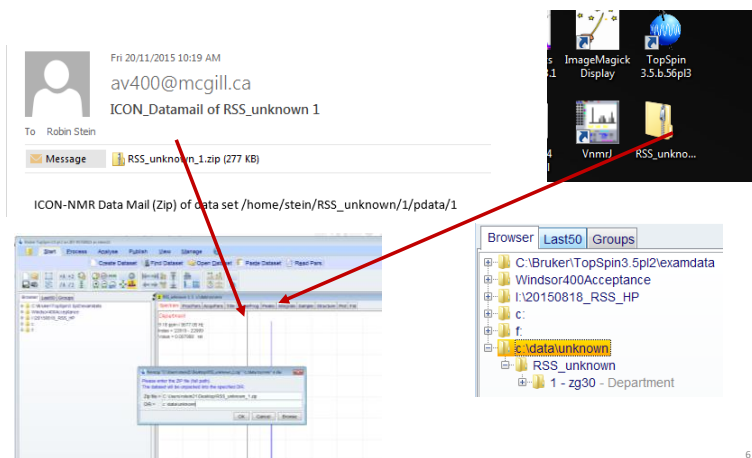


In the second case, opening up a directory gives a list of user names, beneath which are the dataset names, and then the experiment numbers come below these:



## Opening Bruker data in TopSpin

- Drag and drop zip file from local Outlook email or Desktop to TopSpin:




If you use Outlook 2013 or similar on your computer (that is, Outlook not on the Internet), you can simply drag and drop the zip file into the main TopSpin window. If you aren't using Outlook, you can save the zip file to your Desktop (or anywhere else on your computer), and drag and drop it to the main TopSpin window. A window will pop up that asks for the directory (DIR) to save the data to.

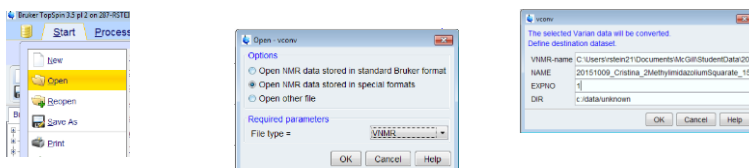
I recommend choosing `c:\data\.` (case A) If you prefer to sort your data by instrument or project name, use `c:\data\<<project or instrument name>\nmr` (case B).

Then, right-click in the data browser (to the left of the main part of the TopSpin window), and select `Add new data dir...` Under `DIR`, if you previously chose a directory whose path did *not* follow the form `<directory>\data\<<project or instrument name>\nmr` (case A), enter the full directory name here (for example, `c:\data`). However, if you previously chose a directory whose path *did* have the form `c:\data\<<instrument or project name>\nmr`, (case B), then enter only the part of the directory before "data" (for this example, enter `c:\`). Then your data will be displayed along the left side of the window. Left-click on the plus icons (+) to open the data browser tree and show the data within it. If you right-click in the data browser and choose `Scroll to Active Dataset`, the currently active dataset will be highlighted in the

tree.

## Getting Varian data onto your own computer

- Getting data from computer onto disk
  - You will receive a .zip file in your email. Save this file to somewhere on your disk where you will remember the location
  - Browse to that location, right-click on it, and unzip it (note: Macs often unzip it for you)
  - You will end up with a directory whose name ends in .fid with four files in it
- Getting data into TopSpin
  - In TopSpin, click on the  icon or type **vconv**
  - If needed, choose Open NMR data stored in special formats and file type = VNMR
  - Browse to the directory ending in .fid, then choose an EXPNO (1) and a directory (for example, c:\data\VNMR500\nmr, or simply c:\data)



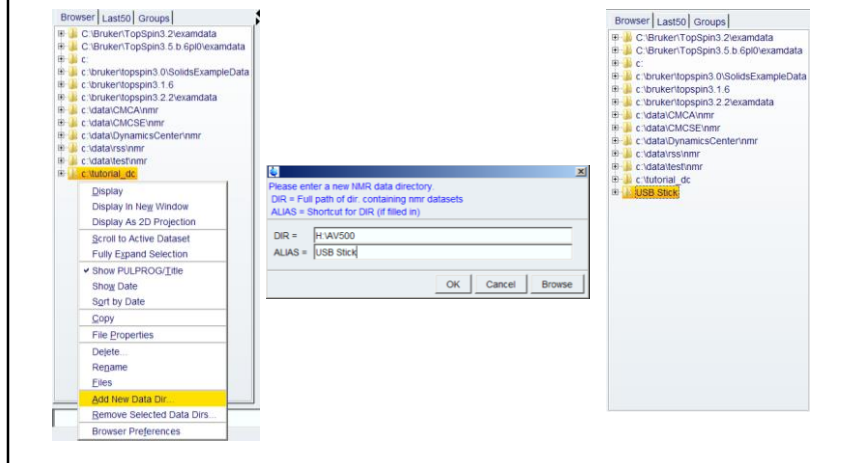
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After you open the data, right-click in the data browser (to the left of the main part of the TopSpin window), and select Add new data dir... Under DIR, enter the directory you just chose (for example, c:\data\unknown). Then your data will be displayed along the left side of the window. Left-click on the plus icons (+) to open the data browser tree and show the data within it. If you right-click in the data browser and choose Scroll to Active Dataset, the currently active dataset will be highlighted in the tree.



## Adding new directories to data browser

- Right-click in data browser to add new directories

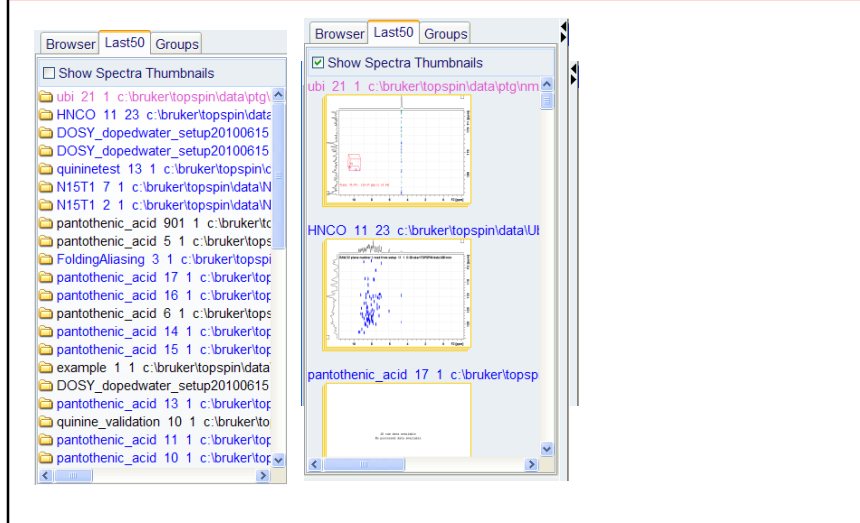


As shown previously, the directory being added can be part of the path with form <directory>/<name>/<expno> or <directory>/data/<user>/nmr/<expno>. The alias chosen for the directory shows up in the data browser. (By the way, TopSpin 1 and 2 only allowed this second style of data storage.)

Directories can be removed from the list by right-clicking in the data browser and selecting “Remove Selected Data Dirs.” This does not remove the data; it only shortens the list displayed.

To delete data, one method is to choose the data (name, expno, or procno), right-click, and choose “Delete...”

## Recently viewed datasets/groups

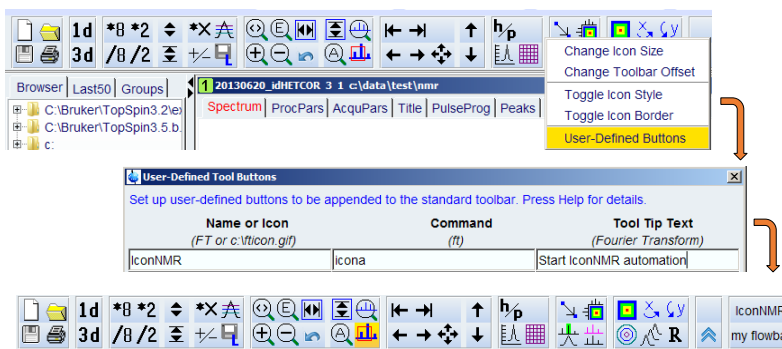


The “Last50” tab of the data browser shows the 50 most recent datasets used; thumbnails showing the spectra can be displayed, if desired. To display a dataset from the last 50, use the same techniques as for displaying data from the main data browser (double-clicking, right-clicking, or clicking and dragging). 2D datasets are indicated in blue, 3D in pink.

Groups of datasets can be defined under the “Groups” tab. Open a series of datasets that you would like to have access to easily (for example, a  $^1\text{H}$ , a  $^{13}\text{C}$ , an HSQC, and other spectra of a molecule that you study frequently). Then right click and select “Add new dataset group...”. This just creates a group, but it has no datasets associated with it. To add datasets to it, right-click and select “All open data windows.” Now all those datasets can be recalled more easily.

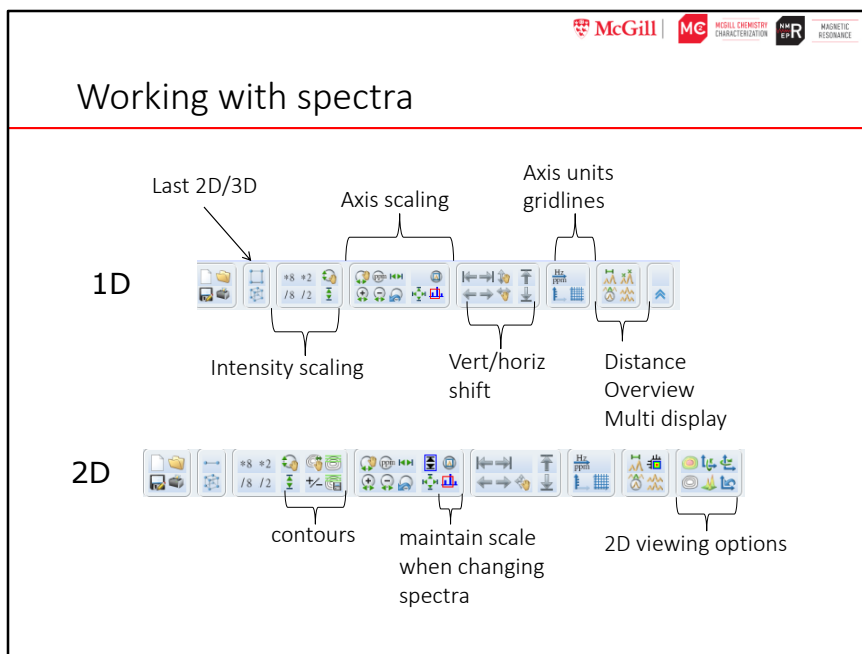
## User-defined buttons

- To create a shortcut to a command that you use frequently, right-click next to the buttons or between the buttons to add a user-defined button



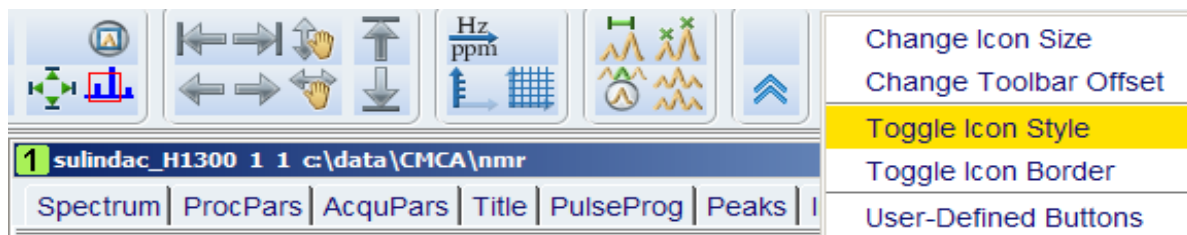
The three fields in the User-defined Tool Buttons window are the text or icon that appear on the button, the command that pushing the button executes (in this case, **lcona** starts IconNMR in automation), and the description (the tool tip) that appears when the mouse cursor hovers over the button.

## Working with spectra



A tool tip giving information about the function of a button appears when you hover over it.

To change the look of icons to those of previous versions of TopSpin, right-click to the right of the icons and choose "Toggle Icon Style."

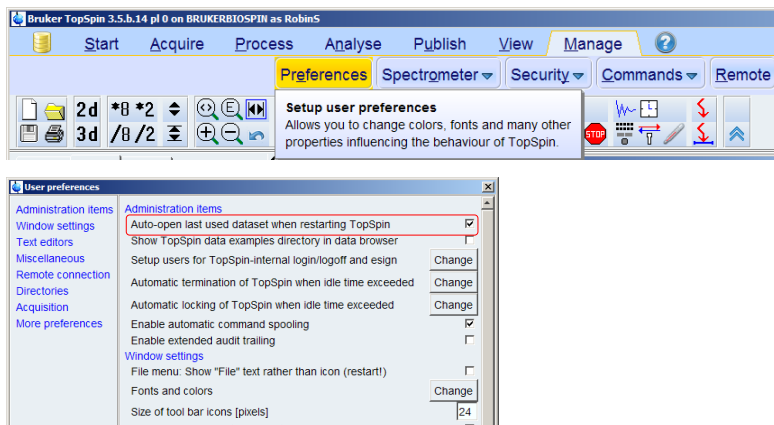


If you have a small screen, you can remove the space around the border using "Toggle Icon Border." After changing the icon style and removing the border, the buttons look like:








## Setting preferences

- Type **setres** or go to Manage / Preferences



Auto-open last used dataset when restarting TopSpin: this makes it easier to pick up your work where you left off.



## How TopSpin works: Parameters and Commands

- Commands tell TopSpin to do something (for example, load data, process data, or acquire data)
- Parameters describe how actions will be performed
- Status parameters show the parameters used to acquire or process the spectrum as it currently exists

TopSpin commands can be input either by typing them on the command line or by using the Flowbars to select the command. For example, use File / Open to open a dataset, or type **efp** on the command line to process a 1D dataset.

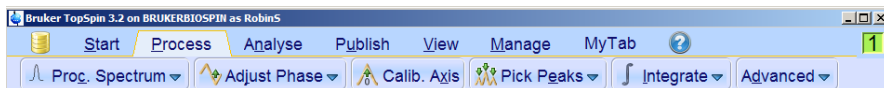
Most commands that are executed depend on the values of certain parameters. For example, the command **efp** applies exponential line-broadening according to the parameter **lb** in Hz. If **lb** is small, little line-broadening will be applied; if it is large, a lot of line-broadening will be applied. To set a command, either type its name on the command line or go to the ProcPars tab for processing parameters (such as **lb** for line-broadening) or to the AcqPars tab for acquisition parameters (such as **ns** for the number of scans to acquire).

TopSpin also keeps track of the parameters that were actually used by a command. The parameters that were actually applied are called status parameters. After a command is executed, the values of the parameters it used are updated as status parameters. So, to see what line broadening you have already applied, type **s lb** (the **s** refers to status). Or, to see how many scans were actually acquired for an experiment, type **s ns**.

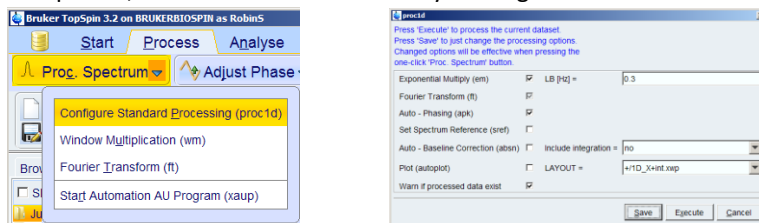
The plot layout is a parameter – one way to set it is to type layout.

## Process flowbar / Basic automated processing

- The process flowbar contains the basic processing commands



- Clicking on Proc. Spectrum starts a basic automated processing sequence, which can be customised by clicking on the arrow on the



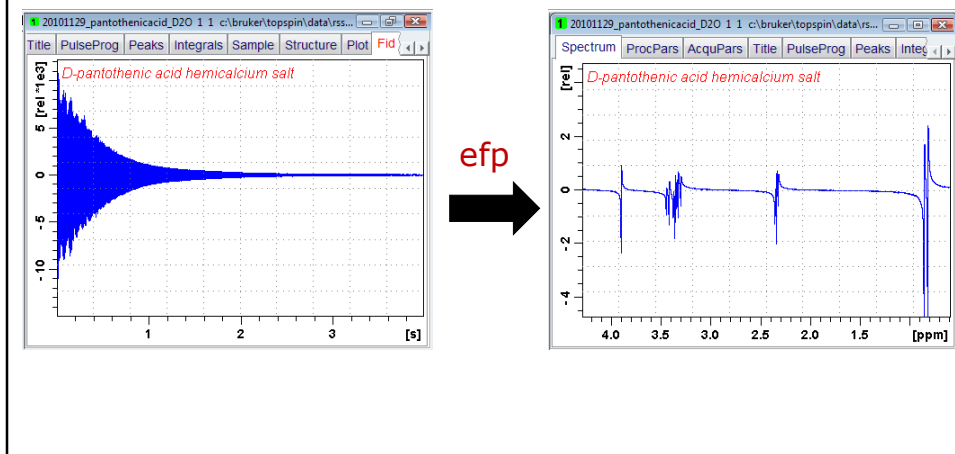
Basic 1D processing includes several steps, which can be performed by clicking on Proc. Spectrum or by typing commands:

- applying line broadening to the raw data (FID) to smooth away noise at the end of the decayed data. The larger the amount of line broadening, the less noise there is, but also the less resolution. Splitting from J coupling is harder to see when there is more line broadening. Usually, between 0.1 and 1.0 Hz of linebroadening is applied; 0.3 Hz is normally the default setting.
- performing a Fourier transform, to convert the raw data (FID) to processed data (a spectrum).
- phasing the spectrum, so that peaks appear positive.
- referencing the spectrum, to put TMS at exactly 0 ppm (if there is no TMS in the sample, an error message is generated, which can be ignored).
- correcting the baseline to make it perfectly flat.
- integrating the spectrum.
- plotting the spectrum.



## efp: Basic 1D Fourier transform command

- Manually performing the steps of the Proc. Spectrum tab show what data processing actually does



The command **efp** performs three processing commands sequentially: multiplication by an **e**xponential linebroadening function followed by a **F**ourier transform, followed by an application of the **p**hase parameters present in the dataset.

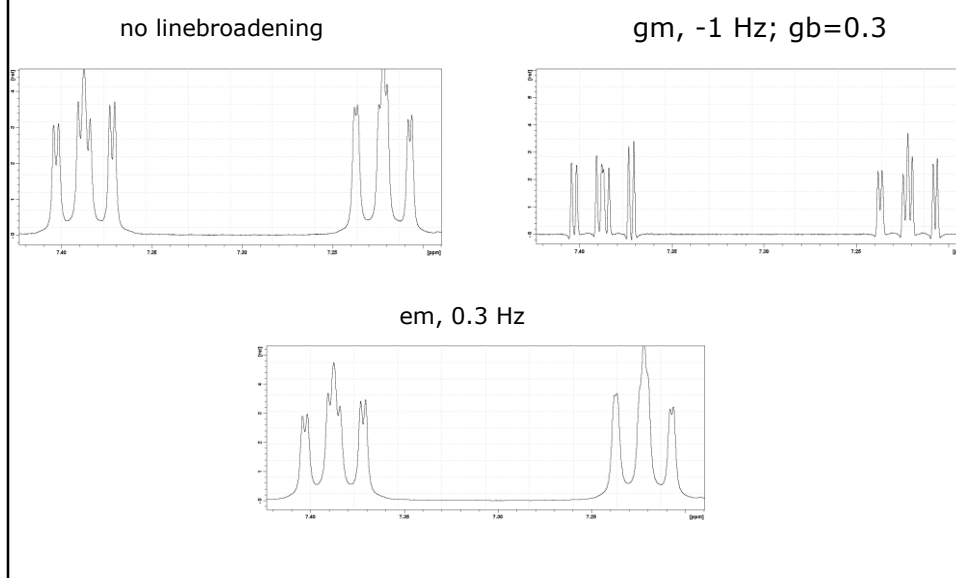
Multiplication by an exponential linebroadening function improves the signal-to-noise ratio of the spectrum by multiplying the FID (the raw data) by a decaying function. This means that the noise at the end of the FID is multiplied by a very small number so as to minimize the noise in the spectrum. To change the amount of linebroadening, type **lb** and enter a number (usually between 0.1 and 1.0 Hz), before typing **efp**.

$$y = e^{-\frac{(i-1) \cdot LB \cdot \pi}{2 \cdot SWH}}$$

The Fourier transform is the mathematical transformation that changes the FID into the spectrum.

Phasing is an adjustment of the appearance of the spectrum to give positive lines with a good lineshape. This comes from adjusting the real and imaginary parts of the spectrum so that the absorptive part of the spectrum (the part with a good lineshape) is displayed as the real part.

## Other kinds of linebroadening



The basic linebroadening method is exponential multiplication, which tends to improve the S:N ratio in spectra. On the other hand, to increase resolution, the simplest thing to do is just omit the exponential multiplication by processing with the command **fp**. For further resolution enhancement, the Lorentz-Gauss transform is used to change the shape of the lines and make them sharper. This involves multiplying the data by an *increasing* exponential, to avoid smoothing away the resolution, while at the same time multiplying by a Gaussian function to make the FID go to zero at the end. This can help to resolve small couplings.

$$y = e^{-\pi \cdot LB \cdot \frac{i-1}{SWH} - \left(\frac{LB \cdot \pi}{2 \cdot GB \cdot AQ}\right) \left(\frac{i-1}{SWH}\right)^2}$$

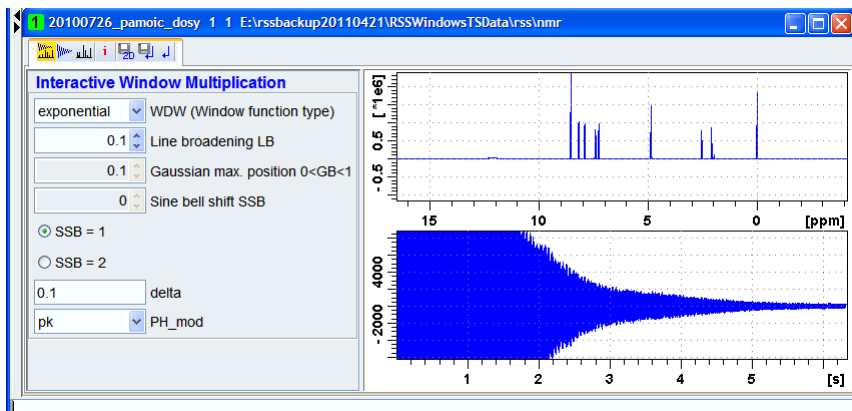
The parameters that control the line broadening commands are **lb**, which must be set to a negative value for the Lorentz-Gauss transform (to approximately the width of the uncoupled lines), and **gb**, which controls the position of the maximum of the Gaussian function and must be between 0 (meaning maximum at the start of FID) and 1 (maximum at the end of the FID).

To do this, set the parameter **lb** to the negative of the peak width measured following the command **fp**, and set **gb** to ~0.3. Then process with the command **gfp**, instead of **efp**. In the above example, the peak width is about 1 Hz, so **lb** has been set to -1 Hz.

The cost of this resolution enhancement is sensitivity: the middle of the FID is enhanced at the expense of the beginning. The spectrum with no LB has an S:N ratio of 3900:1, that with 0.3 Hz LB has 11600:1, and that processed with gfp has 1300:1.

# Examining effects of linebroadening:

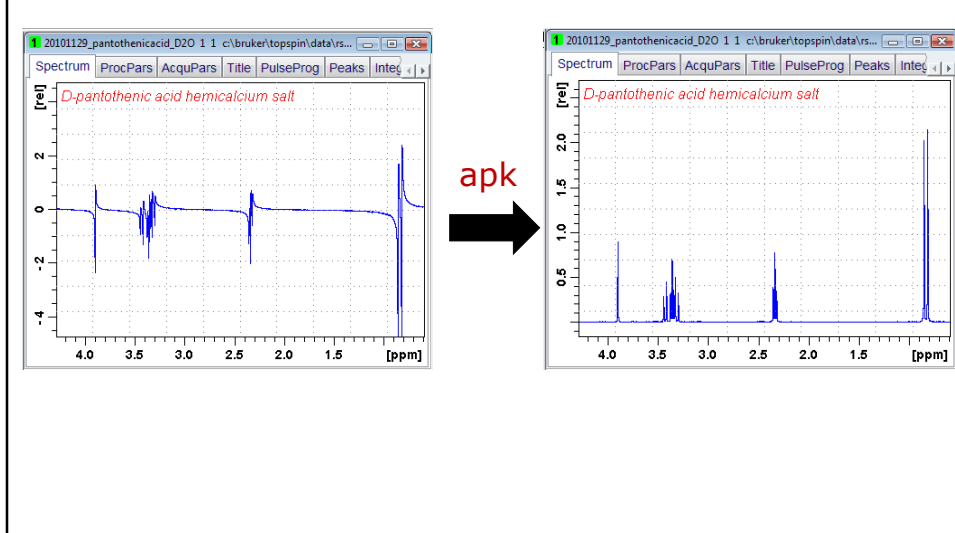
.winf



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The **.winf** command allows you to interactively examine the effect of window functions on a spectrum. Note that the default settings will be not always be those expected, so if you have been applying Gaussian multiplication with gfp you may find that the spectrum looks odd, since by default it is processed in the interactive window multiplication window with em, despite the negative lb. It may be necessary to set the phase mode, PH\_mod, to pk so that the correct phasing appears.

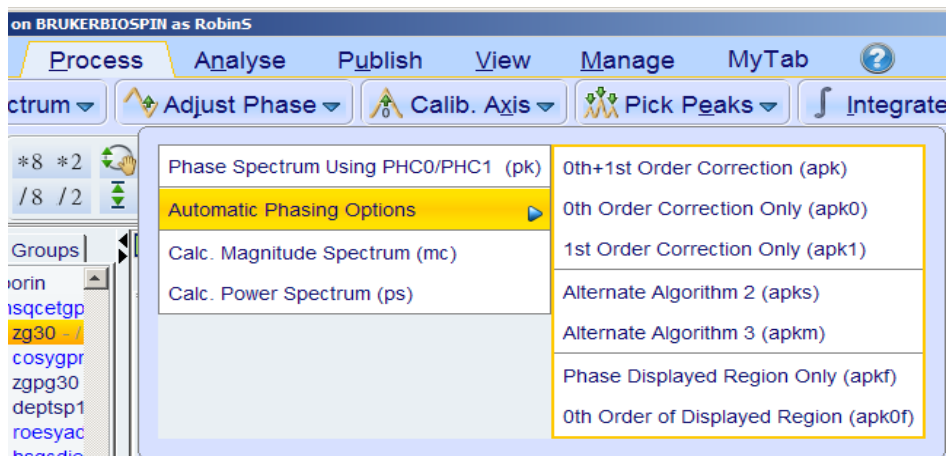
## apk: Automatic phase correction



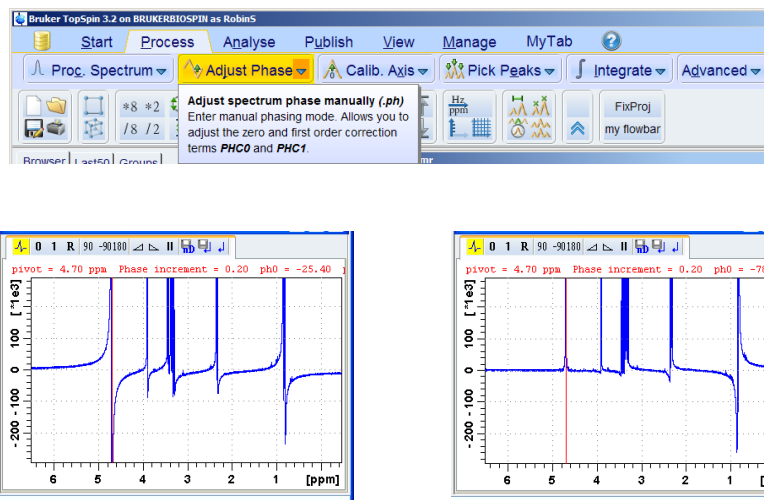
Phasing creates an entirely absorptive lineshape in the spectrum. Two parameters are adjusted to create this: `phc0`, which adjusts the phase of all parts of the spectrum evenly, and `phc1`, which affects the spectrum linearly across it. These two parameters are adjusted to maximize the positive signal in the spectrum automatically by typing `apk`.

For many spectra, the command `apk0` is more appropriate than `apk`, because only zero-order phasing (the parameter `phc0`) should need to be adjusted to give a flat baseline and absorptive lineshape with a modern spectrometer.

The various automatic phasing options can also be accessed from the Process flowbar:



# Manual phasing

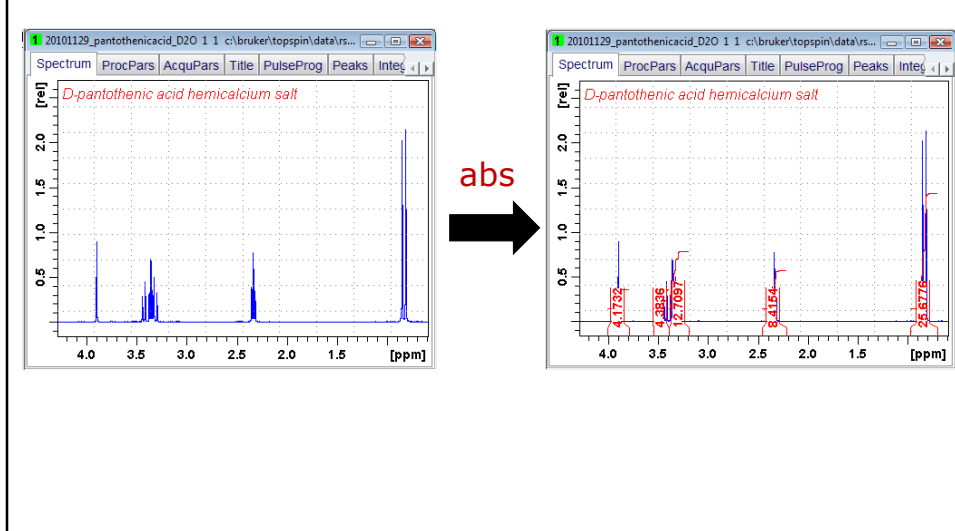


Manual phasing is used to correct the phasing when automatic phasing does not do a satisfactory job. Click on the Adjust Phase button to enter manual phasing.

To phase manually, start by using 0<sup>th</sup> order phasing to make the peak with the pivot point (red line) perfectly phased. Do this by clicking and dragging on the button marked **0**. To change which peak is the pivot point, right-click on the peak and choose Set Pivot **0** int. Sometimes it helps to use a peak at the edge of the spectrum rather than the largest peak, which TopSpin chooses by default. The sensitivity of the mouse while clicking and dragging can be increased or decreased by clicking the triangle buttons. After 0<sup>th</sup> order phasing, use 1<sup>st</sup> order phasing to bring the other peaks into phase: do this by clicking and dragging on the button marked **1**.

For very crowded spectra where all peaks overlap **1** remember that the baseline should be one continuous flat (or smoothly curved) line. Looking at the baseline regions outside the spectral peaks (say less than 0 and greater than 10 ppm) can therefore also be a guide when phasing.

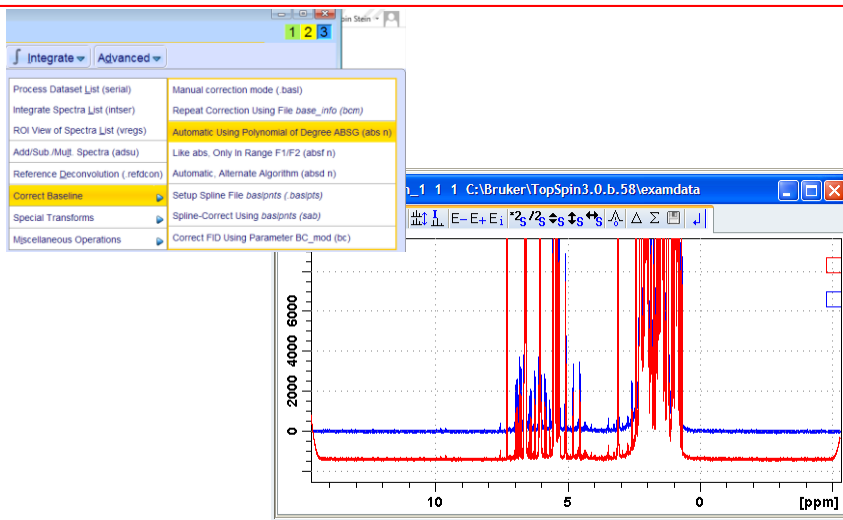
# abs: Automatic baseline correction and integration



The command **abs** does two things to the spectrum: it corrects the baseline, and it does its best to integrate the spectrum. Use **abs n** to correct the baseline without integrating the spectrum.

The baseline correction is done by fitting a polynomial function  $y = a + bx + cx^2 + dx^3 + \dots$  to the parts of the spectrum without peaks in them.

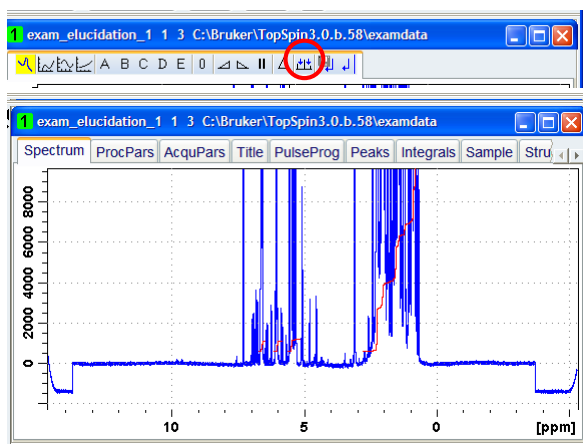
## Baseline correction: **abs** command



If the baseline is not at 0, use the command **abs** to adjust it automatically. This helps make integrals more correct. The command **abs** also integrates the spectrum; the command **abs n** adjusts the baseline without also integrating the spectrum.

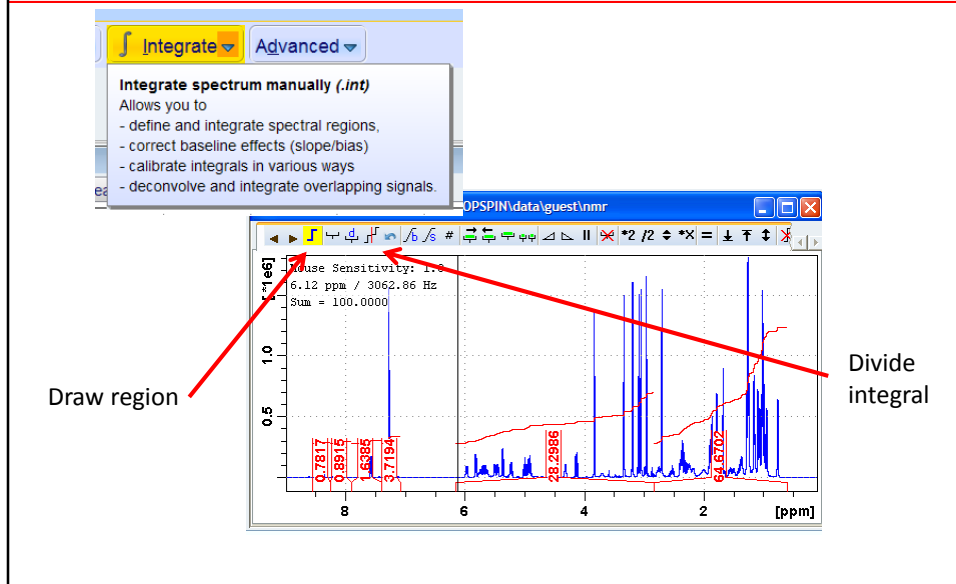


## Manual baseline correction



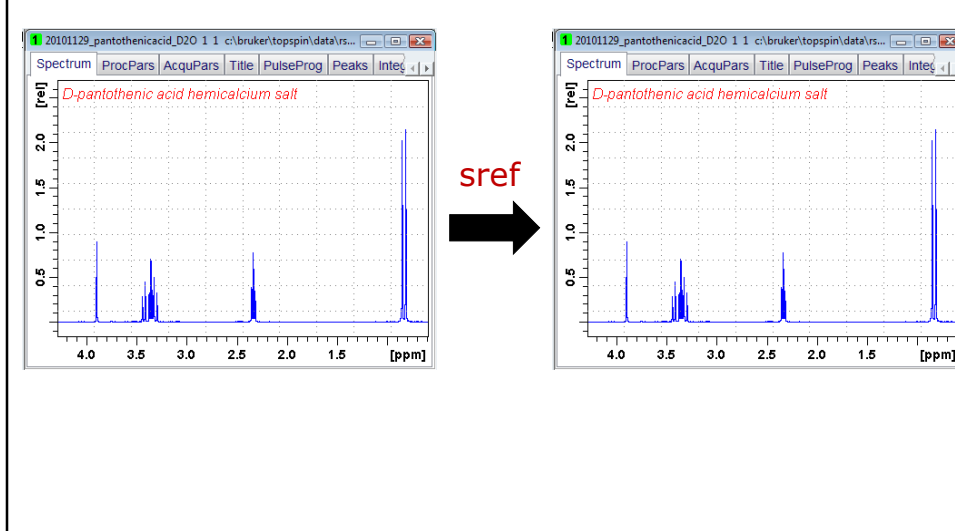
In manual baseline correction mode, you can adjust the parameters of polynomial subtraction manually, and you can choose points for spline baseline correction. The most useful manual baseline correction option is probably the spline baseline correction. This allows you to define a set of points that lie on the baseline which are then fit to a cubic spline curve through these points. This can be used to subtract background signals. To do this, select the “setup spline file baslPnts” option, left-click on the points on the baseline, and save. If this produces an odd result, it may be that points you have selected fall at the top and bottom of the noise. To get around this, average over regions of points centered on the selected points. Do this by setting the parameter **nzp** to 20 or more, and reapply the correction by redoing **efp** and then **sab**.

# Manual integration



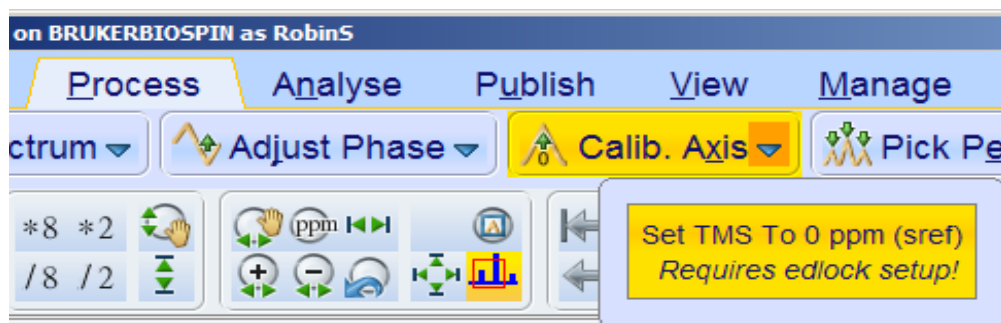
To get into manual integration mode, just click on the Integrate button in the Process flowbar. You can use the manual integration mode to define integrals from scratch, or to modify those generated by **abs**. In this mode, right-clicking on an integral gives options for calibrating the integral, normalising the sum of all integrals, and deconvoluting integrals.

# sref: Automatic referencing of spectrum to TMS/TSP (if present)

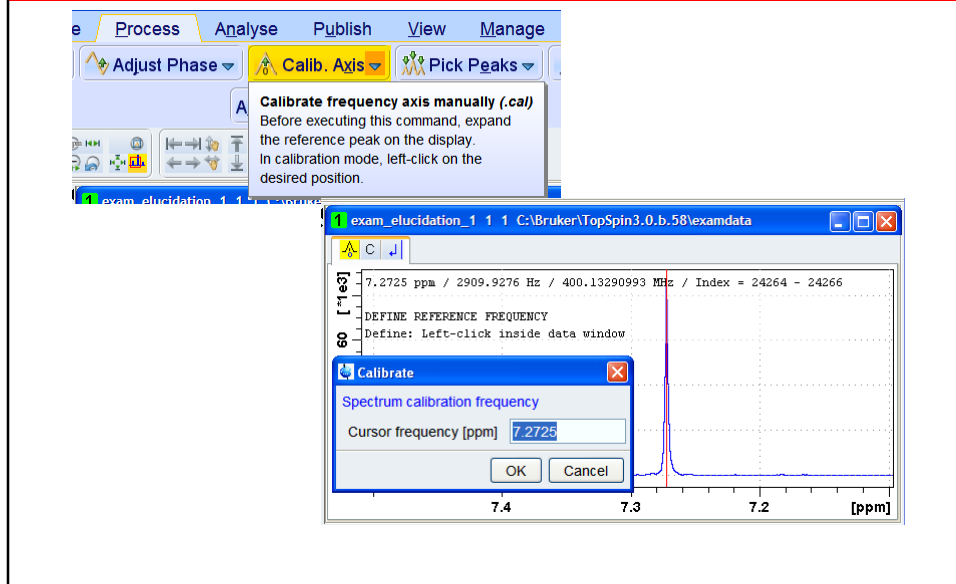


Automatic referencing of TMS is performed using the **sref** command. If TMS is not present, the command **sref** sets the calibration parameter *sr* to 0. This puts the solvent peak in the correct location.

Automatic referencing can also be obtained by clicking on the arrow next to Calib. Axis in the Process toolbar:

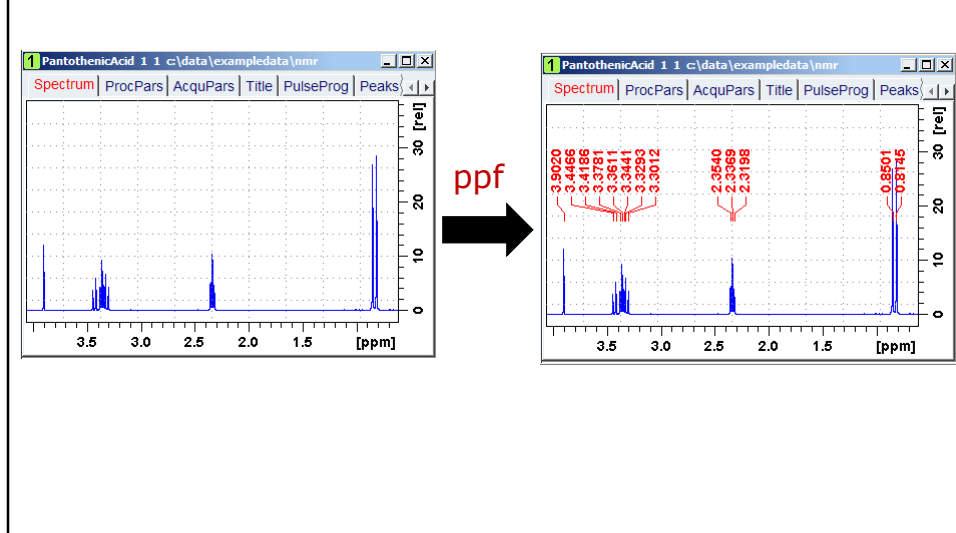


# Referencing



When spectra are acquired with locking (which is the standard when a deuterated solvent is used), then the chemical shifts should be almost exactly right, even without further referencing. However, if there is a compound in the sample whose chemical shift is known, its peak can be used for more precise referencing. TMS (or TSP/DSS in water samples) will be automatically found by *sref*, but if there is no TMS we could use the solvent peak. Calibrate it by left-clicking on the peak to reference to, then enter its exact chemical shift.

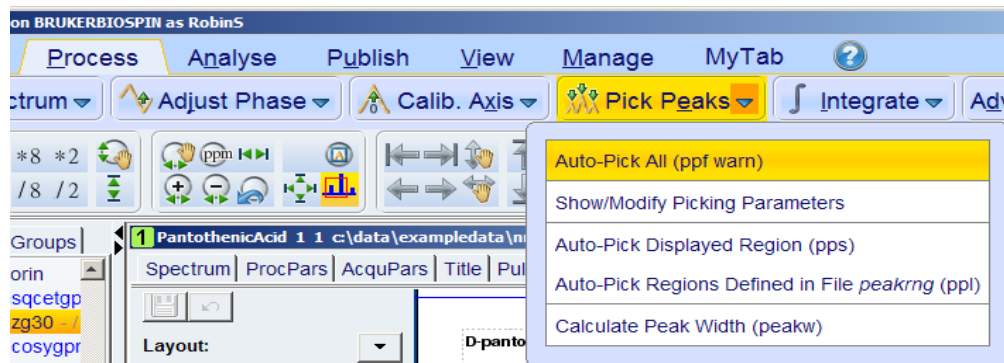
# ppf: Peak picking







The command **ppf** automatically picks peaks on the entire spectrum. The command **pps** may also be used; it picks peaks only the displayed spectrum region.

Peaks are found according to the parameters **pc**, **mi**, and **maxi**. **Pc** is the peak picking constant: a point is only considered a peak if it is a local maximum and bigger than **pc** \* a noise factor – i.e. if **pc** is small, all peaks will be found, but so might spikes. **Mi** is the minimum intensity or threshold, and **maxi** is the maximum intensity of peaks. Usually, typing **mi** and increasing it to 0.1 or greater before running **ppf** or **pps** helps with automatic peak picking.

Peak picking can be done through the Process toolbar:

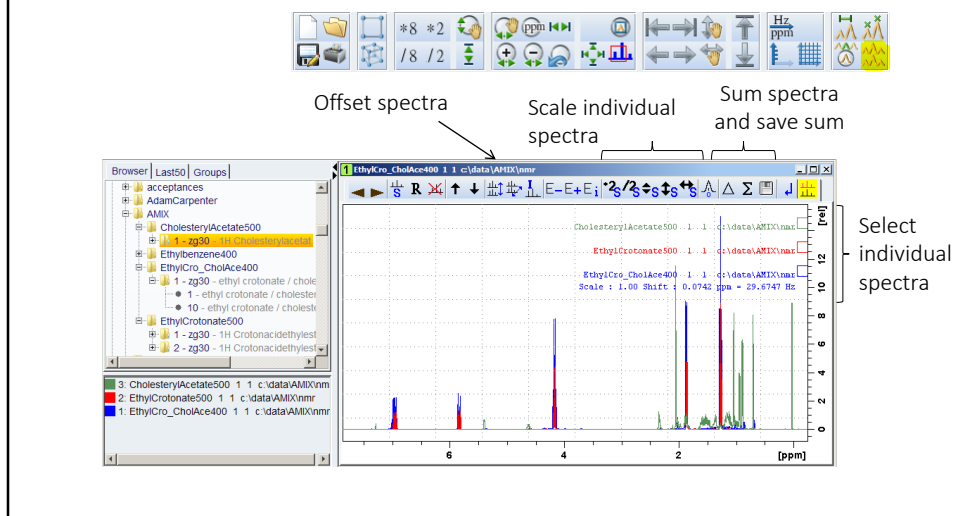


# Manual peak picking

In manual peak picking mode, peaks in a region can be picked by clicking and dragging a box; any peak whose top is inside that box will be picked. Peaks can also be defined manually, either by picking the peak top (  ) or by selecting the p  to the right of the cursor (  ). Manual mode can also  sed to modify the peaks picked automatically by pps/ppf. For example, a peak can be deleted by right- clicking on a peak and choosing “Delete peak under cursor.”

## Multiple display mode

- Use the multiple display icon to display multiple spectra simultaneously



Multiple display mode offers a way to view multiple spectra simultaneously. Once in the mode, any command used to display a new spectrum (eg using the **re** command to read another spectrum or dragging and dropping a spectrum into the main part of the window) will display it on top of the spectrum already present.

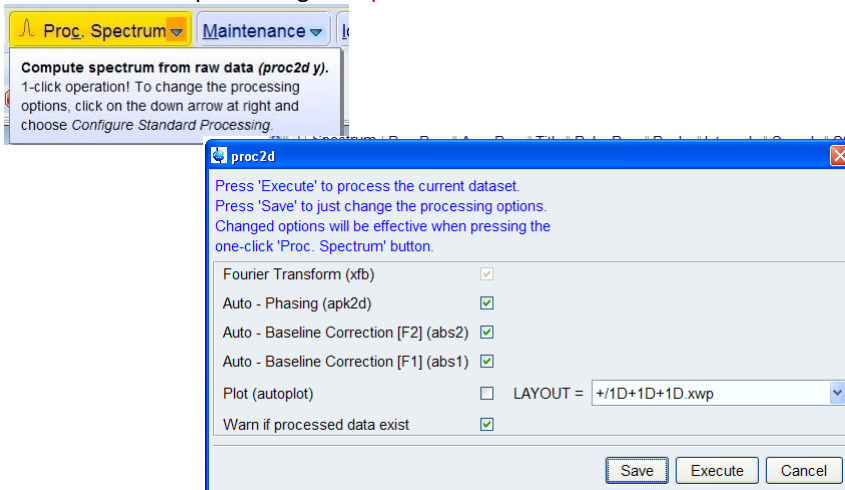
Individual spectra can be selected in the lower left or on the right and scaled and shifted individually. Sums and differences of spectra can be taken. These can be saved with the floppy disk icon, which then prompts for a procno to save the new sum or difference spectrum in.

The spectra can be displayed on top of each other (the default, shown in the picture here) or offset from each other.

When a 2D spectrum is present, multiple display mode gives the option of viewing and extracting individual rows and columns or of viewing 1D spectrum superimposed on the 2D spectrum.

## Processing 2D spectra

- 2D: automated processing with **proc2d**



Like 1D spectra, 2D spectra can be processed in a semi-automatic fashion in TopSpin 3. The processing can be customised using the **proc2d** window, accessible from the menu next to the Proc. Spectrum tab.

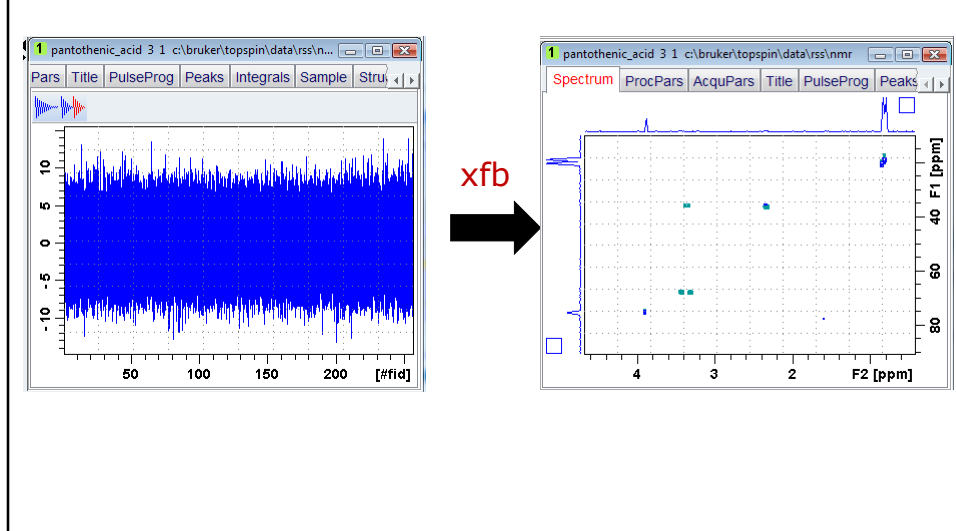
Often, errors will appear regarding phasing and/or phase-sensitive data: these occur when the option of “auto-phasing” is selected, but the data is not phase-sensitive. In this case, it doesn’t make sense to phase the data. The error can be ignored.

HMBC <sup>13</sup>C spectra must be processed with the command **xf2m** after the automatic processing.



## Processing 2D spectra

- **xfb**: Basic 2D processing command

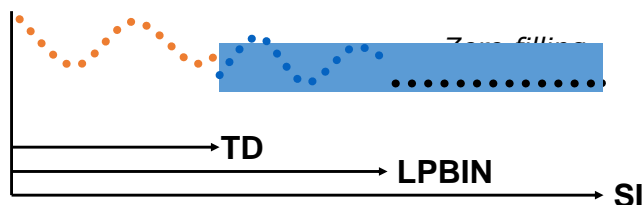


The basic 2D processing command is **xfb**. This command always performs a Fourier transform of both dimensions. It applies window multiplication (as long as the parameter **wdw** is not set to “no”) and it applies phasing according to the parameter **ph\_mod**. (A value of **ph\_mod** of “no” means that no phasing will be applied, “pk” means that phases given by **phc0** and **phc1** will be applied, “mc” means that the data will be processed in magnitude (non-phase sensitive) mode, and “ps” means that it will be processed in power mode. In magnitude mode, the intensity plotted at any point (where a point is defined by a frequency in the F1 and a frequency in F2 dimensions) is the square root of the sum of the squares of the measured real and imaginary components at that point. In power mode, the intensity plotted is the sum of the squares of the measured real and imaginary components at that point.) Usually, either pk or mc are used.

Note that the standard  $^{13}\text{C}$  HMBC implemented on the Bruker spectrometers is phase-sensitive in F1 only. So, you must use two commands to process these spectra: **xfb**, then **xf2m**.

## Linear prediction

- To keep experiment time down, 2D data is often restricted (truncated) in f1. To improve the quality of the spectrum, linear prediction can be used to extrapolate the data:



- In **F1** column set:
  - ME\_mod** = LPfc
  - NCOEF** = about 40
  - LPBIN** = size (0 means double TD)

The parameter **ME\_mod** stands for “maximum entropy” mode, a different name for linear prediction. The parameters to define linear prediction (the options for **ME\_mod**) are:

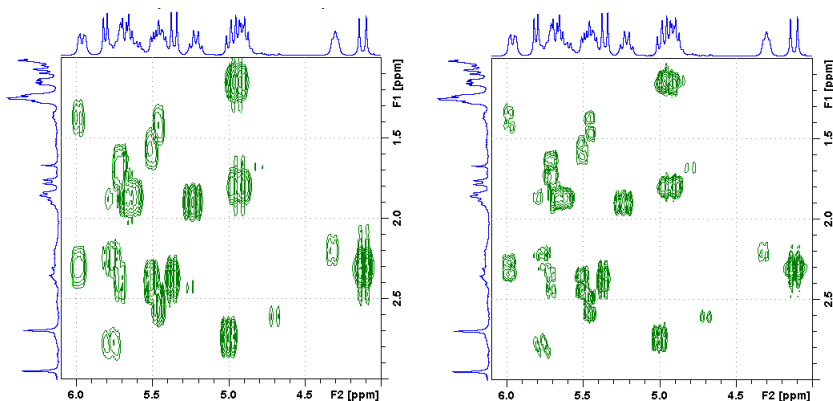
**LPfc** = Linear prediction, forward, complex (the “complex” does not matter as the software automatically determines the type of data: LPfb will work just the same)

**NCOEF** = number of coefficients. Broadly speaking, this is the number of “frequencies” to try and find in the signal for extrapolation; it should be greater than the number of points expected. Increasing this number excessively will increase computation time, and usually will not make the spectrum any better.

**LPBIN** = the total number of points in the extrapolated fid, so if **TD > LPBIN** no extrapolation is done. The more data is extrapolated the more unreliable the answer will become, and in general a doubling of **TD** is reliable. This is indicated by the special case of **LPBIN=0**. However, if signal to noise is good, you may be able to get good results using a bit more linear prediction (e.g. **LPBIN = 4 \* TD**).

After any linear-prediction, the FID is then zero-filled to a total of **SI** points and is ready for Fourier transformation.

## Linear prediction example



TD(1) = 128  
No linear prediction

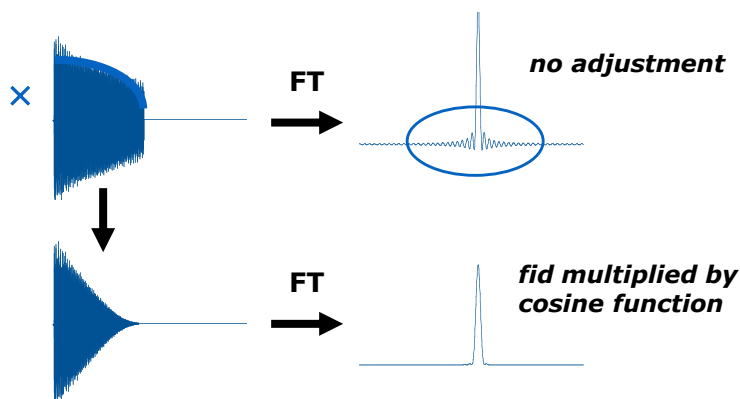
TD(1) = 128  
**Linear prediction to 256**

After linear prediction the peaks are better resolved in the vertical (f1) dimension. As with zero filling in 1D, there is nothing to be gained by linear predicting too many points – the errors in the calculations become more and more significant until the peak shape deteriorates and no information is gained.

Processing 2D spectra

### 2D truncation

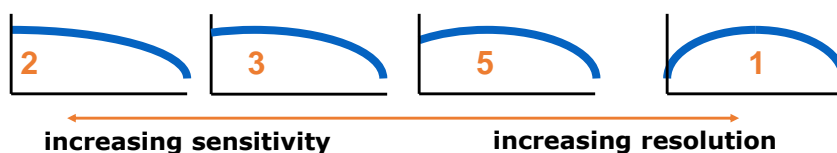
- Truncation artifacts can be reduced by using window functions that force end of fid to zero:



Linear prediction is usually not sufficient to keep 2D data from being truncated or adding enough points in the FID to define the points in the spectrum properly. So, zero-filling is usually necessary. However the “step” that occurs between the end of the fid and the added zeros give rise to so-called “sinc wiggles” in the spectrum. If the FID is smoothly taken down to zero at the end, then this artifact is removed, although this broadens the peaks slightly.

## 2D window functions

- Use WDW = SINE (sine-type) or QSIN (sine squared)
- Parameter SSB determines shift of function
  - SSB = 2 for maximum sensitivity (cosine function)
  - SSB = 3, 4, 5, 6 ... shifts function to increase sharpness
  - SSB = 1 for maximum sharpness (sine function, emphasises centre of fid)



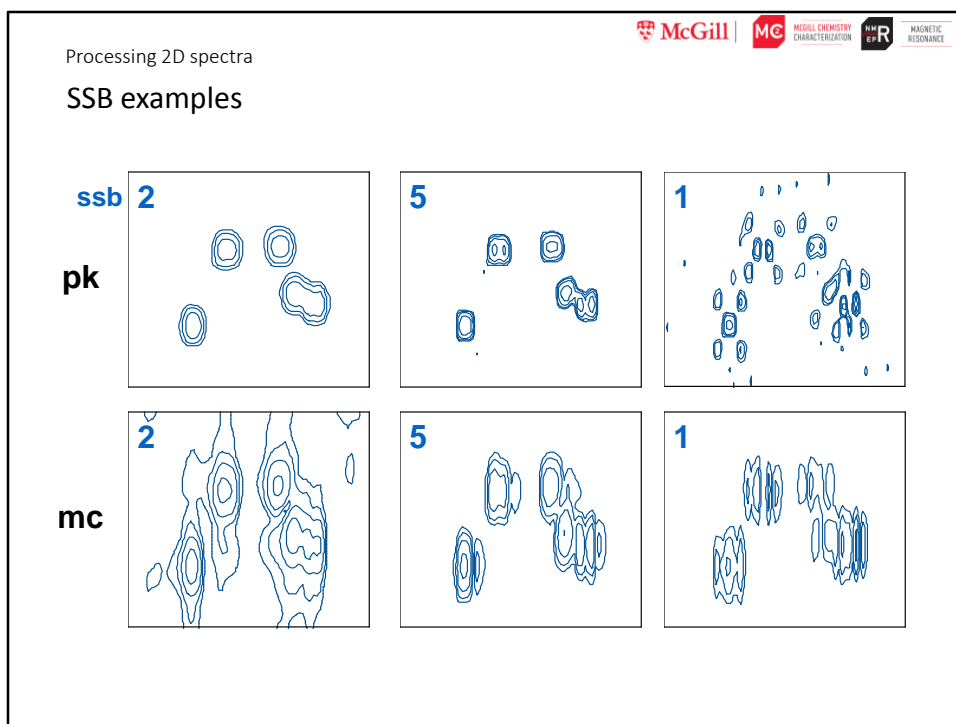
Usually, this parameter (WDW) is set correctly. But, if you read Varian data in with TopSpin, it may not be set correctly.

All the “sine” based functions explicitly end at zero, avoiding truncation.

By shifting the function from a cosine function (**SSB** = 2 and first point = 1) towards a sine function (**SSB** = 0 or 1 and first point = 0), the function additionally provides resolution enhancement (emphasising the middle of the fid) at the cost of worse sensitivity (the beginning of the fid is suppressed). Note that setting **SSB** = 0 is the same as 1 (i.e. maximum sharpness).

Other window functions, such as the traditional Gaussian or exponential multiplication, can still be used with 2D datasets, but they are not as generally applicable and usually require more fine tuning to get good results.


As a rule, use **SSB** = 2 with phase-sensitive experiments whose first points have non-zero intensity and that give intrinsically sharp (Lorentzian) peaks. Use **SSB** = 0 with non-phase-sensitive experiments whose first points are 0 and which give broader peaks.




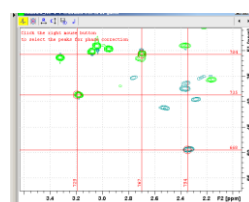
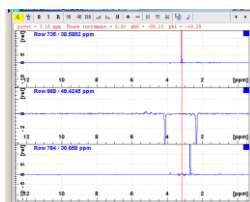
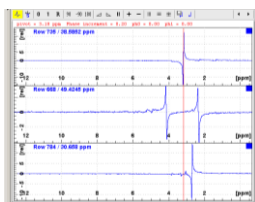
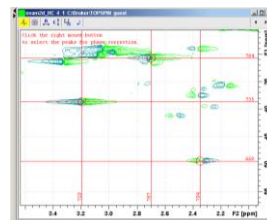
The difference between the two examples above is the result of the intrinsic lineshape difference between a phased spectrum (normal absorption Lorentzian lineshape) and the much broader magnitude-mode lineshape (a mixture of the absorption and dispersion lineshapes). If you want to illustrate this just type “mc” in a normal 1D spectrum (normal 1D spectra are phase-sensitive; they can be phased). Hence, for magnitude mode spectra, resolution enhancement is an important component of processing.

Typically, for phase-sensitive experiments (HSQC, DQF-COSY, NOESY, ..), start with **SSB=2**, and then increase it you need extra resolution and have enough signal. For magnitude mode experiments (HMQC, COSY, ...), start with **SSB=1**. If more sensitivity is needed, try a very large number, and then decrease it.

## Manual 2D phasing

- Start phase mode:  Adjust Phase
- Then right click on a few peaks (choose peaks that span the width and height of the spectrum to phase) and select “Add” to choose them for phasing

- Click rows or columns to show traces 
- Phase all rows first, using 0 and 1, then save
- Then phase all columns and save



Phasing a 2D experiment is very similar to a 1D, except that there are 2 dimensions: both f1 (vertical) and f2 (horizontal) can be phase corrected.

Keep in mind how the spectrum should look after phasing: NOESY spectra should have the diagonal negative and crosspeaks positive. DEPT-edited HSQC spectra should have peaks either positive (CH<sub>3</sub> and CH) or negative.

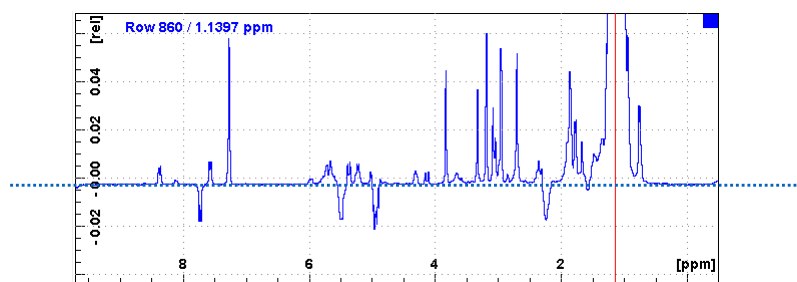
Rows and columns are extracted from the 2D for phasing, because it would be too slow to interactively phase the whole 2D matrix. The aim is to extract 1D traces that, taken together, contain peaks across the width of the spectrum (if you are phasing a NOESY with many cross peaks, this may only require one trace, but for an HSQC with single discrete peaks it may be necessary to use three or more traces to cover the left, middle, and right of the spectrum).

It may occasionally be necessary to repeat the phasing after the first attempt for best results. Remember that, as for 1D spectra, it can be helpful to try to get a flat baseline as well as look at peak shapes.

If, after phasing, the data are reprocessed using the command `xfb`, the stored phase parameter will be re-applied if the **PH\_mod** parameters are set to “pk”.

## Exact 2D phasing


- In some cases phasing requires more attention!
- For experiments such as NOESY/ROESY, the large diagonal peaks and small cross peaks make exact phasing important, but negative peaks can make the lineshape less obvious
- In general, phase for a smooth baseline:

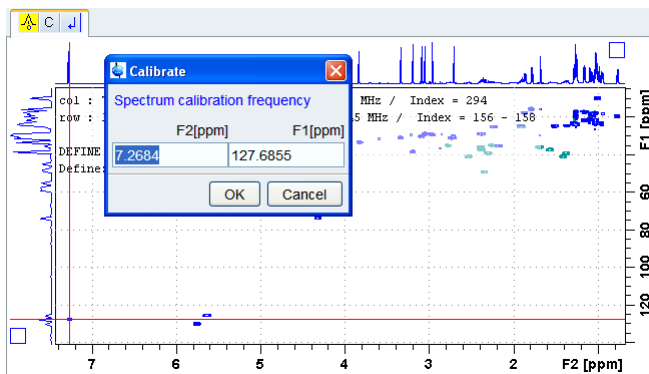


When phased correctly, the bulk of the baseline should usually be flat (even if not at zero) or at least a smooth continuous curve. You might see deviations at the extreme edges of the spectrum due to digital filters.



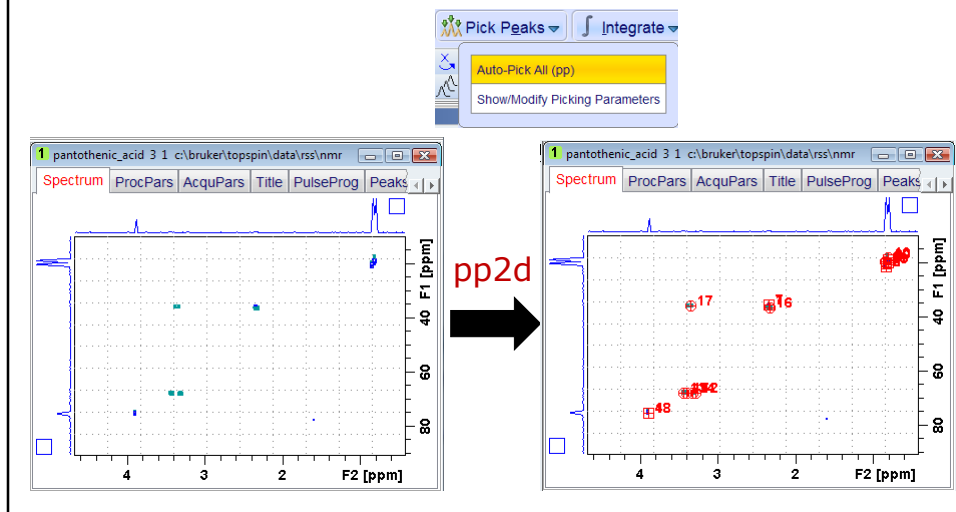
## Referencing 2D spectra

- either `sref` (which puts TMS at 0 ppm, if found)
- or manually  `Calib. Axis`
- then click on a peak and enter the correct chemical shift when prompted



## pp2d: peak picking in 2D spectra

- After setting the contour levels, automatic peak picking can give the chemical shifts of all peaks:



The **pp2d** dialogue box lets you set the threshold based on the contour levels. Set the contours so you can see (only) the peaks of interest and save them before starting **pp2d**. Then, under Minimum intensity / Set to, choose “Lowest contour level.”

Peak picking [ \_pp2d ]

**Options**

- Append peaks to list
- Discard new peak(s) if already in list
- Export results as XWinNMR peak list

**Parameters**

**Region**

	From (F1P)	To (F2P)	Set to ▶
F2 [ppm]	1.8650	0.7760	
F1 [ppm]	23.4088	11.7044	

**Sensitivity**

Minimum intensity [rel] (MI)	0.1000	Set to ▶
Maximum intensity [rel] (MAXI)	1.0000	
Diagonal gap [points] (PPDIAG)	0	
Resolution [points] (PPRESOL)	1	

**Miscellaneous**

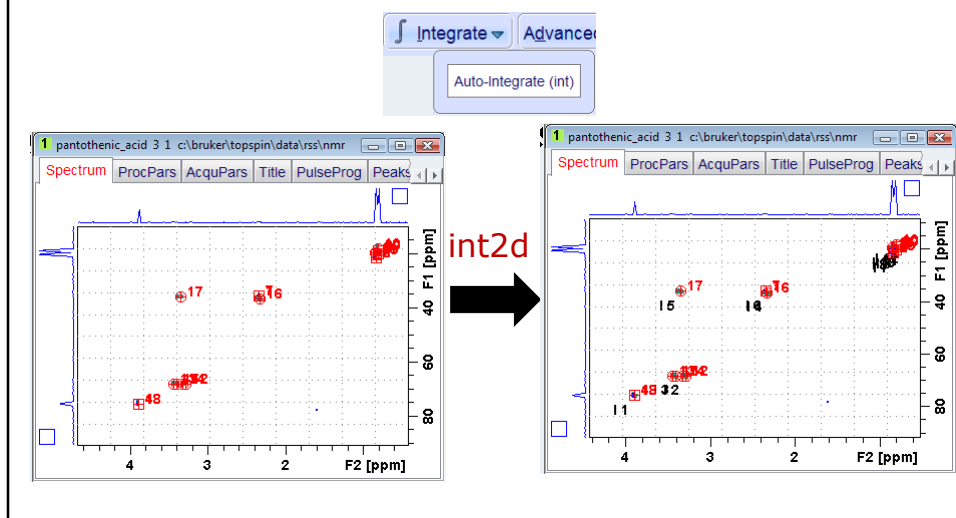
Maximum # of peaks (PPMPNUM)	100
Interpolation type (PPIPTYP)	None
Pick peaks of sign (PSIGN)	Positive

Reset all to ▶

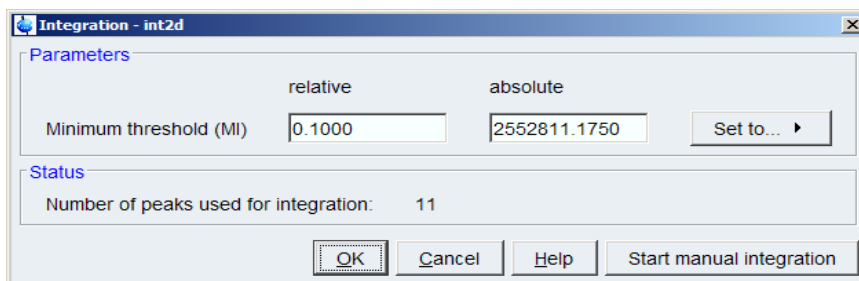
OK Cancel Help Start manual picker

## int2d: automatic integration of 2D spectra

- After peak-picking, peak integration is simple:

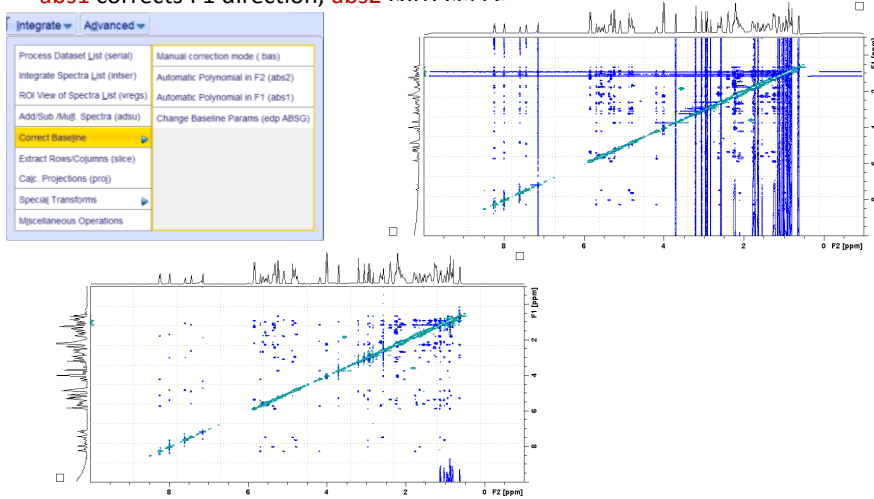


The command `int2d` allows for integration of the peaks chosen with the `pp2d` command.



## Baseline correction of 2D spectra

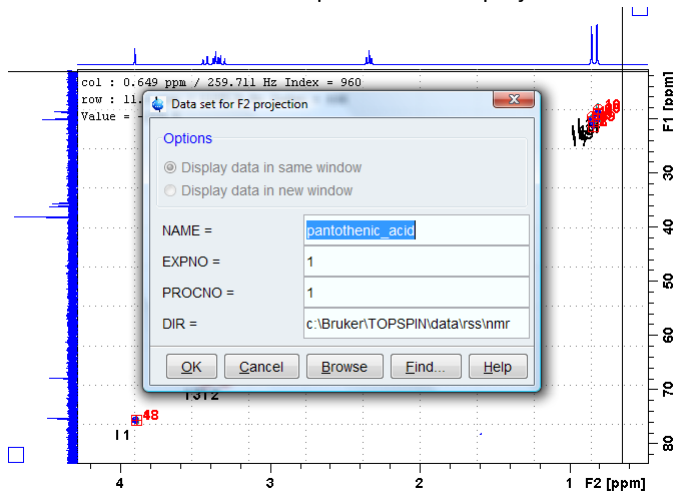
- 2D spectra may have baseline offsets in one or other dimension
- **abs1** corrects F1 direction, **abs2** corrects F2



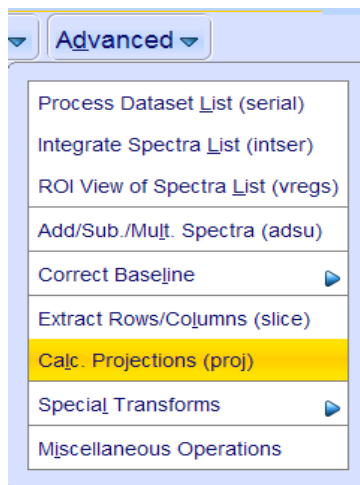
NOESY/ROESY are usually the worst spectra for needed baseline corrections, especially in t1, because of t1 noise.

## Setting projections

- Right-click above or to the left of spectrum to set projection

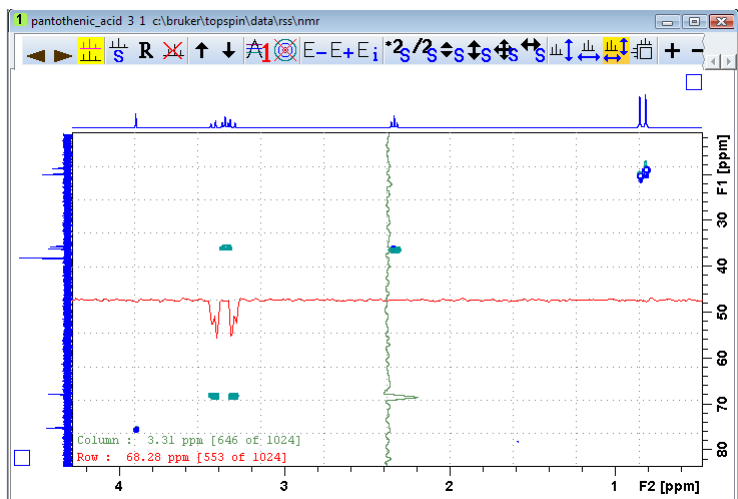



Right-click on the projection above or to the left of the 2D dataset to set an actual spectrum to be displayed in place of the projection. If you want to calculate a projection (or row/column sum) and store it in another procno, the command **proj** can be used. The projections should be defined in the standard spectrum viewing window before plotting is done.



Another option is to right-click on a spectrum listed in the data browser and choose Display as 2D Projection

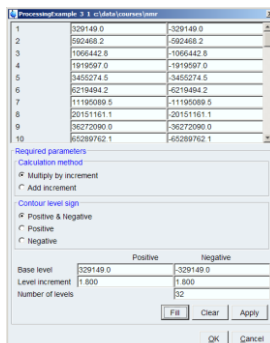
## Using multiple display mode to view and extract rows or columns



To view individual rows and/or columns, go into the multiple display mode by clicking on the icon . The scan rows/columns buttons within multiple display mode allow you to look at individual rows/columns of a 2D spectrum. If you want to do more with a row/column, like compare it in multiple display mode with another spectrum, you can save it to its own procno by right-clicking while the desired row and/or column is displayed.

## Contour levels

- Right-click on the spectrum and choose Edit contour levels...



- Or try the command **clev**



## Tidying up the spectrum

- To further improve spectra:
  - Baseline correction for samples in water: **abs2.water**. Also consider using **BC\_mod=qfil** in f2 to suppress the central noise stripe.
  - Fine-tune contour levels (manually, with the mouse button and/or by setting **nlev** to the number of levels, then running **levcalc** or **clev**, or with **edlev**)
  - Symmetrization (for experiments such as COSY): **sym** or **syma** (for phase-sensitive experiments like NOESY), but use this with caution
  - Reducing t1-noise: **t1away** (an AU program available upon request)

When symmetrizing, watch out for generating false peaks where noise bands correlate. Only symmetrize after applying noise-reducing techniques such as **abs1/2** and t<sub>1</sub>-noise subtraction first, and after adjusting levels so that only real peaks are displayed. Then perform **sym** (or **syma** for phase-sensitive data). Remember that this loses or creates information, so it must only be applied with caution. An automatic t<sub>1</sub> noise subtraction is done by the AU program **t1away** (ask me for a coyp) which sets the lowest intensity peaks in each column of the 2D spectrum to 0, allowing peaks to be displayed.

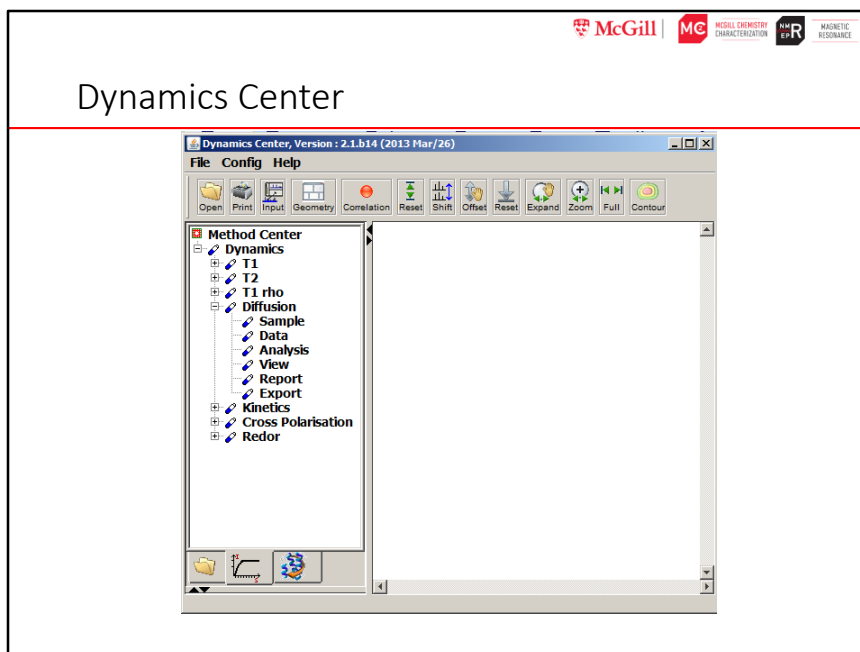
## Pseudo-2D data

- 1D data: Processed data has two frequency axes (raw data has two time axes)
- 2D data: Processed data has two frequency axes (raw data has two time axes)
- Pseudo-2D data: Processed data has one frequency axis and one axis that is not frequency
  - The non-frequency axis may be time between experiments, relaxation delay, diffusion coefficient, or some other variable
  - Raw data has at least one time axis, in the direct dimension
- To process the time axis in the direct dimension only, use the command `xf2`

Most NMR data is a function of frequency (chemical shift). However, some data, such as relaxation measurements, diffusion spectroscopy measurements, or kinetics measurements, have a second axis which is not a chemical shift axis. In this case, the main or direct dimension has to be processed using the command `xf2`. Then the other dimension can be processed afterwards.

The direct dimension can be phased using standard 2D phasing methods, using the most intense row of the pseudo-2D dataset. Baseline correction is done using the `abs2` command to baseline correct the direct dimension. Traces can be extracted for further analysis, if desired, using the multiple display mode.

## Dynamics Center



The Dynamics Center is Bruker's approach to processing the non-frequency dimension of non-frequency 2D datasets. After doing the  $xf_2$  transform to process the frequency dimension of relaxation, diffusion, or similar datasets, the Dynamics Center is used to process the second dimension.

After doing  $xf_2$ , type `expl` to open a Windows Explorer browser. If this does not work, the workaround is:

Use a text editor like Notepad to open the text file called

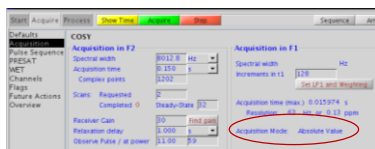
**`c:/users/<username>/topspin-<pc>/prop/globals.prop`**

Add the line **`FILE_EXPLORER_PATH=c:/Windows/explorer.exe`** at the bottom.

Drag the file `2rr` over the appropriate box in the Dynamics Center window and follow through the prompts to finish processing the data and output a report.

## Varian 2D and pseudo-2D data in TopSpin

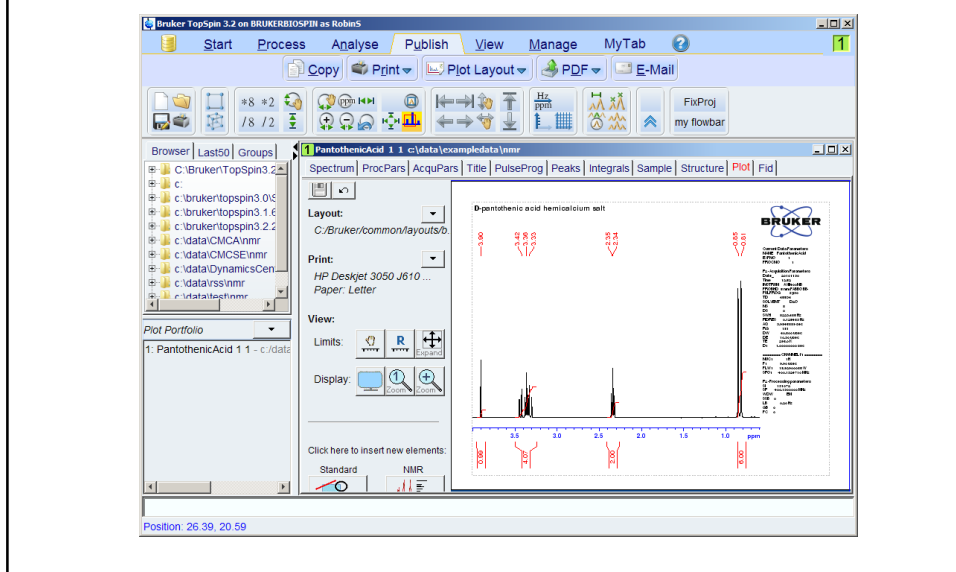
- Several processing parameters tend to be set wrong
- They are (generally) set correctly for non-phase sensitive spectra (eg COSY), but are wrong for phase-sensitive spectra



- For 2D, phase sensitive datasets
  - MC2 in F1: try echo-antiecho
  - ph\_mod in both dimensions: PK
  - SSB in both dimensions: 2 (also try setting WSW to QSIN)
  - Set ME\_mod in F1 to Lpfc
  - Then redo **xfb** and phase with **apk2d** and/or manual phasing
- For pseudo-2d datasets
  - SI in F2: a large number (32k or 64k)
  - WDW: EM
  - LB: 0.3 Hz or so

These changes are needed to get the right lineshape for Varian 2D datasets. If you do this frequently, the changes can be automated.

## Generating a printout quickly: **prnt** or the Plot tab

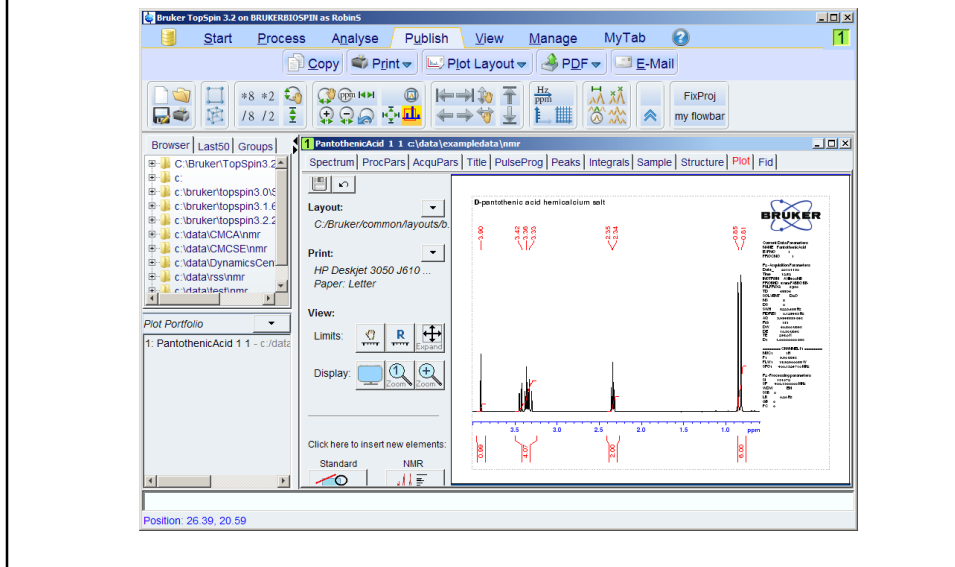


There are several ways to create a printed or PDF output of a spectrum.

Typing **prnt** generates a printout of the active window, exactly as it appears, with text, gridlines and all. A dialog box gives a choice of printers and all the usual printing options.

Clicking on the Plot tab (or going to the Publish flowbar and choosing Plot Layout) opens a plot editor which displays the portion of the spectrum shown in the spectrum tab, according to currently chosen plot layout. The command **prnt** will print the displayed layout, but much more customization is also possible. A PDF can be generated by choosing Publish / PDF; other file formats (JPG, PNG, TIF) can be generated under Publish / PDF / Other formats...

## Customizing the appearance of the plot



The plot tab can be used in two ways: to customize a view of the current spectrum, or to create a more generally useful plot arrangement, known as a layout.

A layout is simply an arrangement of items on the page; the items include the obvious things like spectra, text about the experiment, and titles, and they can even be extended to things like shapes, pictures, or structures.

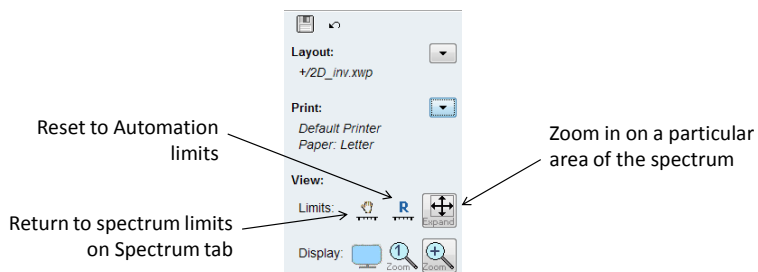
The floppy disk icon can be used to save a modified layout with a new filename for general use, or to save a layout for single use in a particular dataset.

With a small monitor, it can be difficult to see all parts of the left panel. To help, click on the icon that looks like a monitor (🖥️ icon). Alternatively, turn off the Acquisition Status Bar (right-click on the Acquisition Status Bar and choose Acquisition Bar On/Off), reduce the TopSpin icons to one row (🏠 icon), and/or hide the flowbars (right-click on the flowbars and choose Set All Tabs Invisible). To bring things back, click on the Spectrum tab.



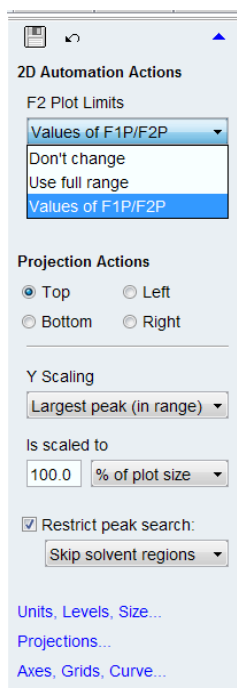
## Plot range

- Usually, the spectrum range shown is whatever was in the spectrum tab before entering the plot tab
- There are several ways to modify the range:



- Automation limits: set in the Automation Actions panel and allow x and y ranges to be set differently depending on the spectrum

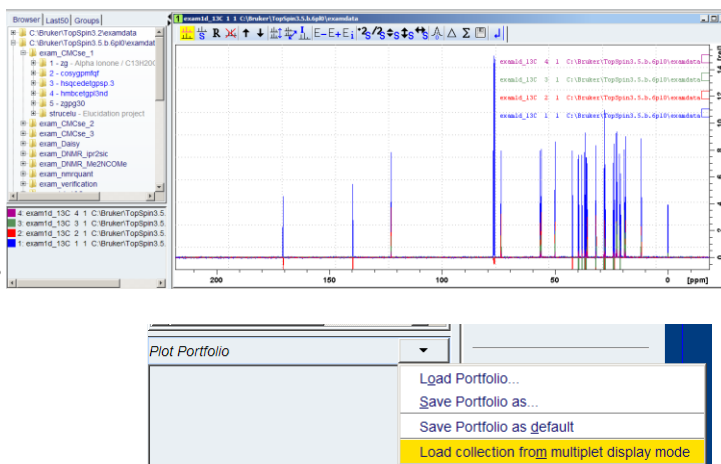
The Automation limits allow various options to be selected if the Reset icon is chosen. This is particularly important for high-throughput or repeated work. For example, if you always want to view the spectrum between 8 and 5 ppm, then set parameter f1p to 8 and f2p to 5, then set the Automation Action for X to be “Values of F1P/F2P”.





## Getting access to multiple spectra for plotting

- Use multiple display (the  icon) to view all spectra for plotting



Use the multiple display mode in the Spectrum tab (the main view of the spectrum), not the plot editor tab. Use the standard techniques for viewing spectra (dragging and dropping from data browser or typing `re <#>`, for example) to load up many spectra.

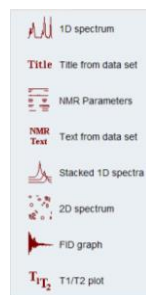
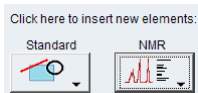
Then, in the plot tab, in the plot portfolio portion of the window (bottom left), use the down arrow to get access to the option “Load collection from multiple display mode.”

In future version of TopSpin, it will be possible to drag spectra from the data browser directly in to the Plot Portfolio window.

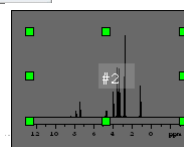
With the stand-alone plot editor, the Data button allows you to add spectra to the portfolio and then to choose which one is displayed where.

## Plotting multiple spectra

- Use “Click here to insert new elements” / “NMR” to add an element to the plot window



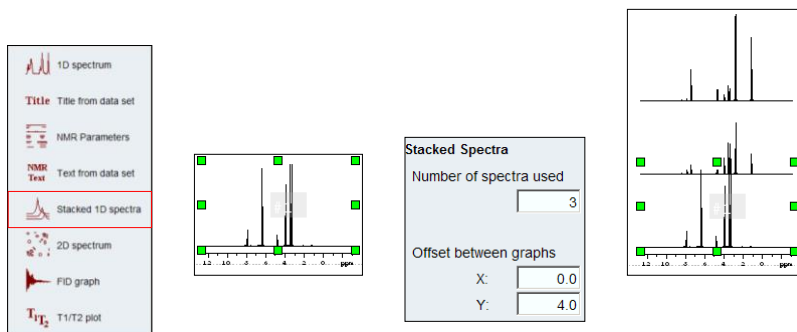
- After selecting an element, click and drag on the plot layout to add it
- Then drag and drop a spectrum from the plot portfolio list onto the new element to display it there (here, spectrum #2 is displayed)



The number displayed over an element when it is selected shows which number spectrum in the plot portfolio list is being plotted. It can be difficult to see, so here a grey box was plotted behind the spectrum so that the highlighted number appears clearly.

## Stacked spectra

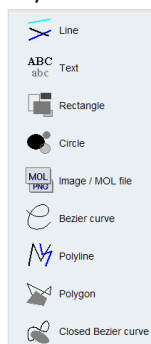
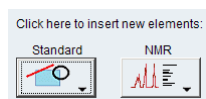
- Use “Click here to insert new elements” / “NMR” to insert a stacked plot
- Draw a box for the first spectrum
- Click on the spectrum, then use “Stacked spectra” to customize stacked plot (including adding more spectra)



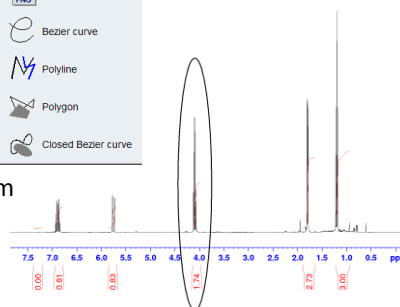
The green box only defines the size of the initial spectrum. The spectrum which appears here (#1 in this example) is taken from the position in the Plot Portfolio; if you wanted to plot spectra 2-5, for example, you would drag spectrum 2 into the stacked spectra region and then plot 4 stacked spectra.

## Adding other objects to the plot

- Use “Click here to insert new elements” / “Standard” to add an element to the plot window



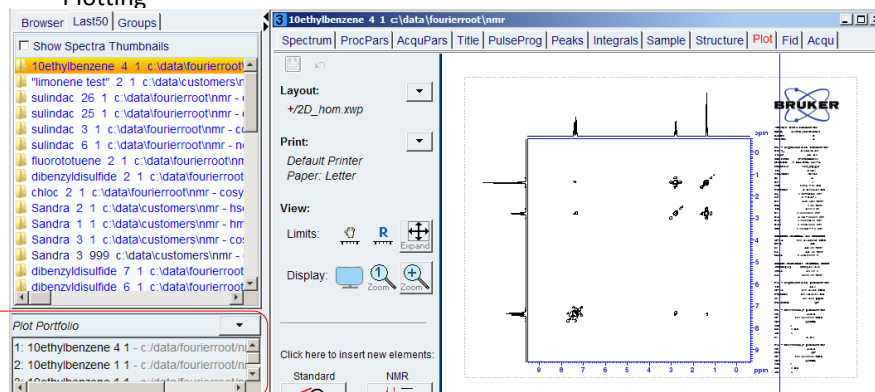
- For shapes, click and drag to place them



In earlier versions of the plot editor, objects were added by selecting them, clicking on the page to place them, and then editing them.

## Plotting 2D spectra

- Plotting



list of spectra (2D and projections), as set as projections, in multiple display mode

Plotting is much the same as for 1D. By default, the spectra set as projections in the spectrum view are used as projections in the plot tab. If the projections are not correct, go back to the Spectrum tab and use Multiple Display mode to bring them in, then in the plot editor, use the arrow icon in the plot portfolio to load the multiple display mode datasets.

## Saving layouts

- Save your layouts so can reuse them
  - Remember: a layout is a set of objects on a page
  - Reusing layouts saves you time and effort
- To reuse a layout:
  - Set the parameter layout to your layout name
  - Or, automate the process
- edau setlayout
 

```
STOREPAR ("layout", "+/my1D_H.xwp")
VIEWDATA_SAMEWIN
QUIT
```

  - Then File/Save
  - Then type setlayout from your dataset and the layout will change
  - You can even make a button

