Introduction to TopSpin

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TopSpin Features

- Easy (drag-and-drop) opening of data from Bruker spectrometers
- Excellent multiple display mode for comparing spectra
- Easy processing of 2D data, including integration and peak-picking
- Permits multiple windows for multiple projects

...and on the other hand,
- Power = big (can run a spectrometer but takes up much disk space)
- Historical artifacts can be confusing (eg disk storage)

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TopSpin is the successor to a program called XWinNMR, which appeared in the mid-90s (I believe) and was used to run Bruker spectrometers until it was replaced by TopSpin in 2005 or so. While much development of TopSpin has focused on acquisition, processing has always been a major component of the software and now TopSpin can be used to open data from the three major NMR manufacturers (Varian/Agilent, JEOL, and Bruker) and data stored in the standard format JCAMP-DX (as generated, for example, by some of the low-field NMR manufacturers). It can be used in native format under Windows, Mac, and Linux. Thus, TopSpin is both very powerful (scripts can be written in C to do many forms of batch and advanced processing), and somewhat demanding on workstations.
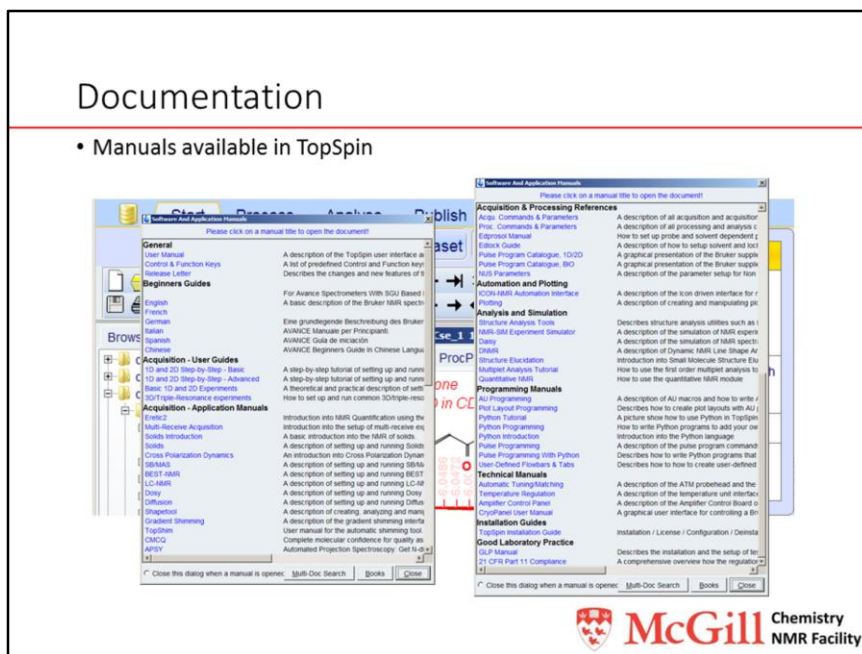
Software manuals are accessible from within TopSpin under the ⓘ icon. A good place to start is with the Beginners Guide, which gives a brief introduction to the NMR experiment and NMR hardware and then instructions about how to run some frequently used experiments. Also useful are the 1D and 2D Step 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.
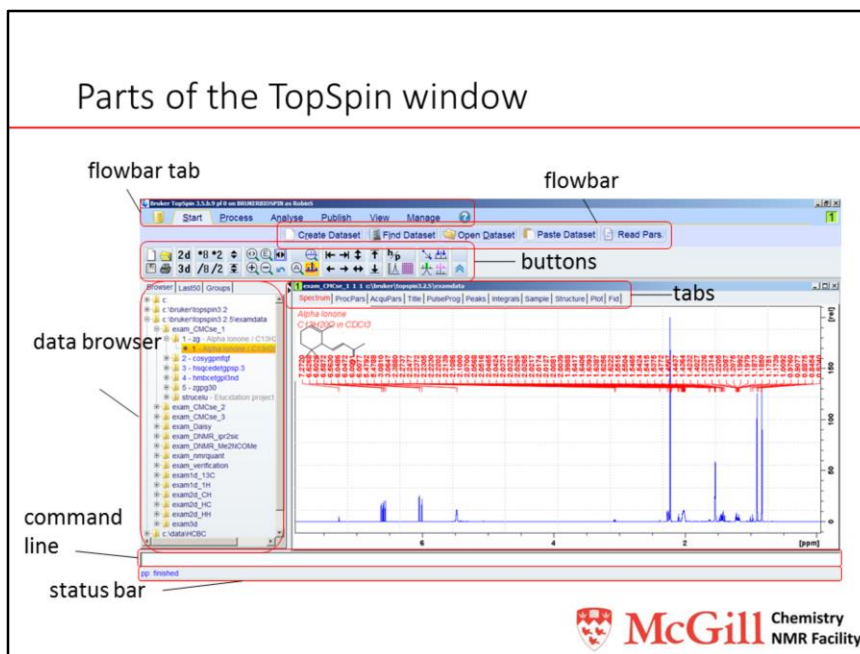
The TopSpin 3 window is based around the concept of flowbars, which itself was borrowed from the ribbons in MS Word 2007 and later. Each flowbar contains a different function and they can also be customized. The rest of the screen shows the datasets (data browser), the spectrum itself with various parameters accessible in tabs, the command line (where commands can be entered) and the status bar, where information is returned after some commands have been executed. Various icons indicating the status of different parts of the spectrometer (lock, VT, etc.) can be shown in the status bar, although they aren't very useful for offline processing!

If you want more space for the spectrum window, push F11 to maximize the spectrum window space. Remove the data browser by typing Ctrl-D; bring it back by typing Ctrl-D again.

To add a button, for example to run a command frequently, right-click to the right of the other buttons, press "1D-nD", and fill in the information requested. The tool tip text is the information that appears when you hover over the button with the mouse cursor.
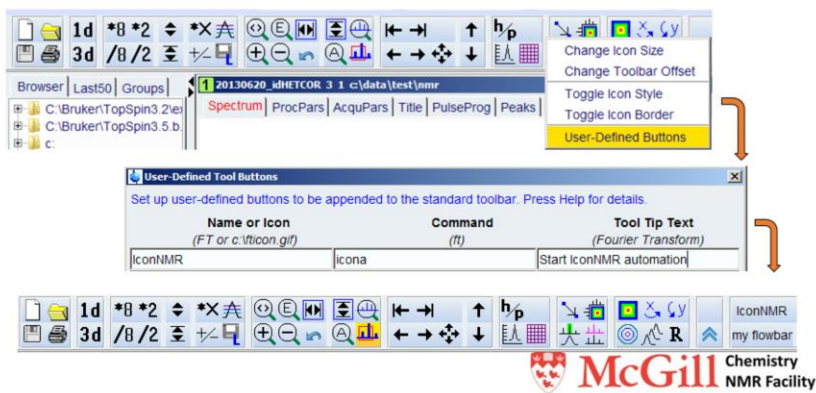
The three fields in the User-defined Tool Buttons window are the text or icon that appear on the button, the command that pushing the button executes (in this case, **icona** starts IconNMR in automation), and the description (the tool tip) that appears when the mouse cursor hovers over the button.
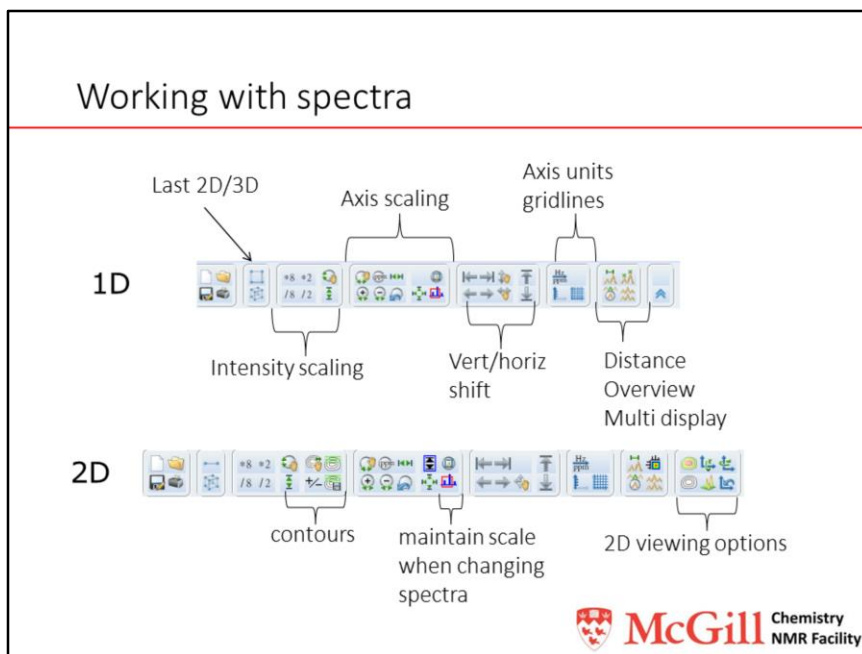
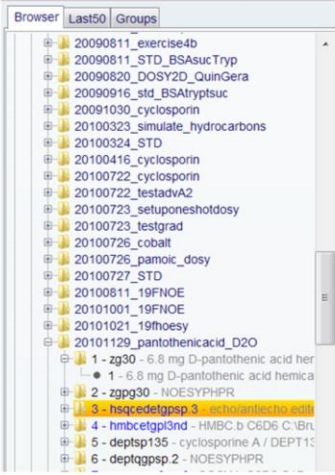A tool tip giving information about the function of a button appears when you hover over it.

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:

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 whch are the experiment numbers,and within those the processed data numbers.
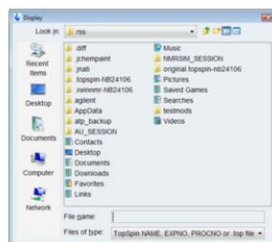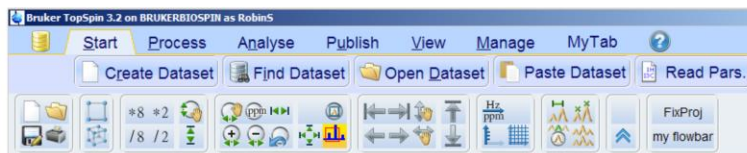

In the second case, opening up a directory gives a list of user names, beneath which are the dataset names, and then the experiment numbers come below these:
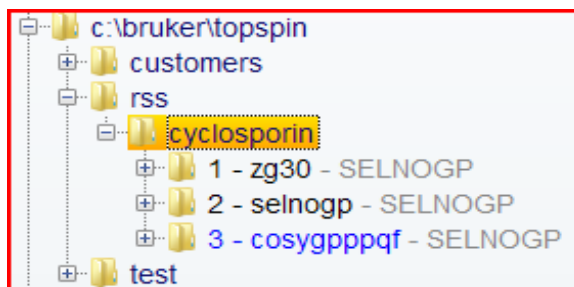
Unless you want to start an acquisition in TopSpin, the first thing to do in TopSpin is to open an old dataset for viewing and processing. Options for opening a dataset are available under the Start Flowbar. The search for the dataset is fairly straightforward, and opening a dataset allows for the use of the standard Windows File Manager to find data.

Also, it is possible to open a particular experiment by typing re or re followed by an experiment number (for example, re 2).

Another option for opening a dataset is dragging and dropping a dataset (name, expno, or procno) into the main TopSpin window, or by dragging and dropping a zip file created with the TopSpin command tozip. (The command tozip creates a zip file of the FID and/or the SER file from a single expno or from all expnos in a dataset.)

TopSpin 1 and TopSpin 2 do not have flowbars, but the File menu can be used to open files, and the fromzip command can open data zipped by TopSpin.

Data acquired with any version of TopSpin can be processed with any other version of TopSpin (though some parameters might not display correctly in plotting).

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

▷ 7		1	Queued				
			Queued	RSS_02351	1	CDCl3	chloroform-d	N PROTON
▷ 8			Available				

- On the spectrometer PCs, the directory is \home\<your username>

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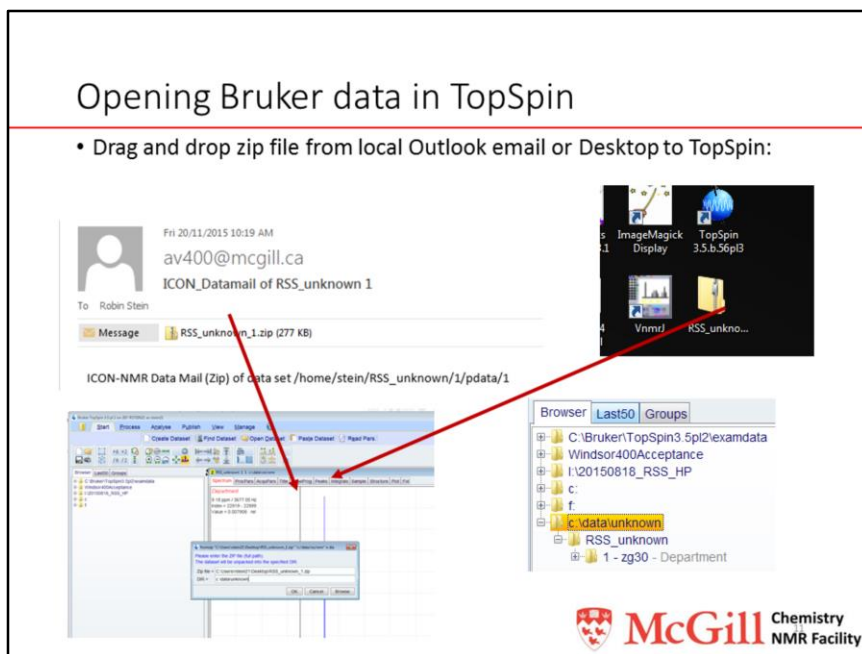
If you use Outlook 2013 or similar on your computer (that is, Outlook not on the Internet), you can simply drag and drop the zip file into the main TopSpin window. If you aren't using Outlook, you can save the zip file to your Desktop (or anywhere else on your computer), and drag and drop it to the main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

- If you are using the 300:
  - You will receive a .tgz file in your email. Save this file to somewhere on your disk where you will remember the location
  - Install the program 7-zip or Winzip
  - Use the program 7-zip or Winzip twice to unzip the dataset
  - You will end up with a directory whose name ends in .fid with four files in it
  - On Macs, double-clicking on the .tgz file will unpack it
- On the 400 and 500:
  - Data is usually saved in /home/<your username>/vnmrsys/data
  - You can copy the data directory to a USB stick; it will be a directory whose name ends in .fid and which contains four files
  - On either the 400 or the 500, you can FTP from your local computer to:
    - merc400.chem.mcgill.ca
    - dante.chem.mcgill.ca (the 500)
    - Use your own username and password
    - You must be on the McGill network

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You can download 7-zip, Winzip for free. WinSCP and Filezilla, FTP programs, for free.

Copying Varian data in TopSpin: vconv
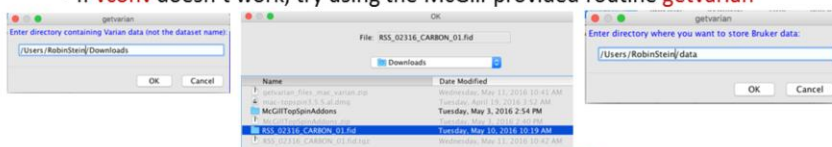
After you open the data, right-click in the data browser (to the left of the main part of the TopSpin window), and select Add new data dir… Under DIR, enter the directory you just chose (for example, c:\data\unknown). Then your data will be displayed along the left side of the window. Left-click on the 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.

On Macs, the **vconv** command does not always work, and a routine can be obtained called **getvarian**, which does work. The routine must be saved to <topspin home>/exp/stan/nmr/au/src/user, and then the command getvarian can be typed into TopSpin.
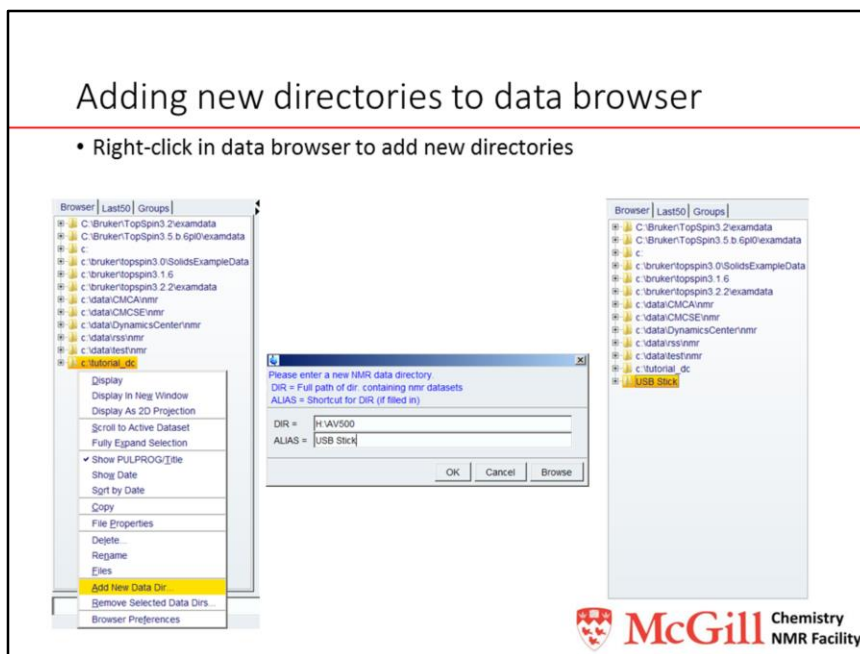
As shown previously, the directory being added can be part of the path with form
<directory>/<name>/<expno> or <directory>/data/<user>/nmr/<expno>. The alias
chosen for the directory shows up in the data browser. (By the way, TopSpin 1 and 2
only allowed this second style of data storage.)

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…"
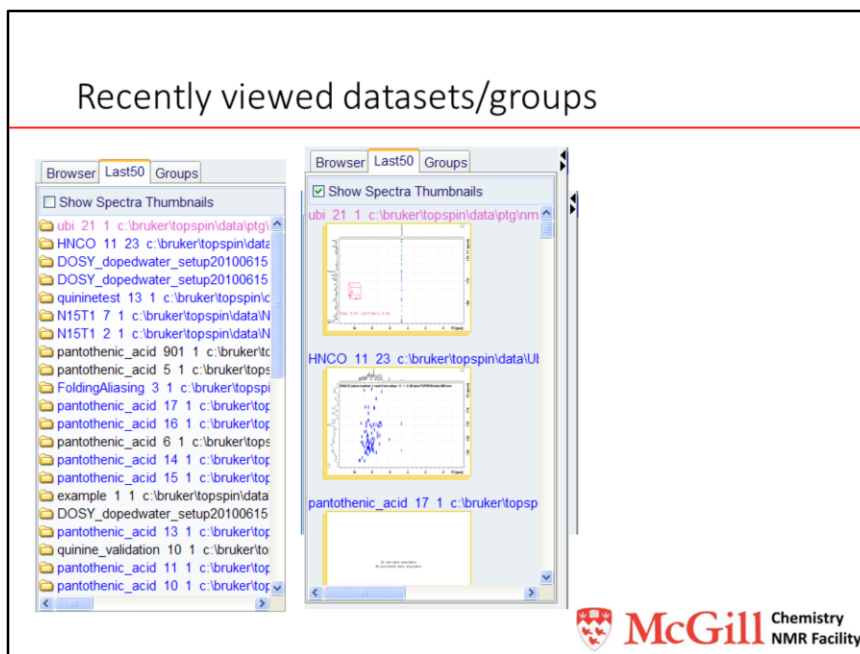
The "Last50" tab of the data browser shows the 50 most recent datasets used; thumbnails showing the spectra can be displayed, if desired. To display a dataset from the last 50, use the same techniques as for displaying data from the main data browser (double-clicking, right-clicking, or clicking and 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}H, a ^{13}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.

Two important options available under Manage / Preferences (or the command **setres**) are highlighted.

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

Enable automatic data processing will process any dataset when it is dragged from the data browser into the spectrum window. Sometimes imported Varian data will not use exactly the same processing parameters as were saved with the dataset, so it is worth checking the parameters listed under the ProcPars tab (for example, the line broadening, **lb**, is often set to 0.3 Hz, but sometimes a larger value is better).

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

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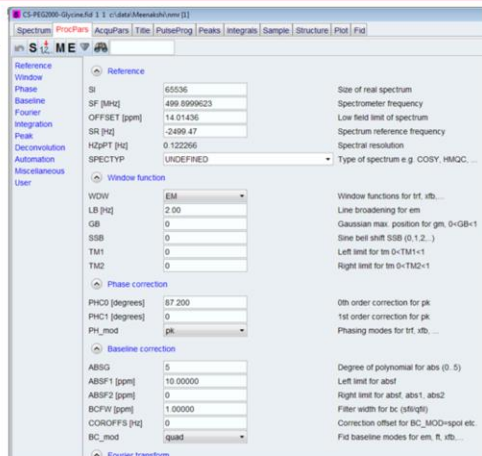
TopSpin commands can be input either by typing them on the command line or by using the Flowbars to select the command. For example, use File / Open to open a dataset, or type **efp** on the command line to process a 1D dataset.

Most commands that are executed depend on the values of certain parameters. For example, the command **efp** applies exponential line-broadening according tot 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 AcquPars 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. In most cases, these parameters are imported correctly from Varian data.

Other useful parameters: `holder` (the sample changer position number, for the Bruker 500) and `date` (the date and time the experiment was run at).

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 flat.
- integrating the spectrum.
- plotting the spectrum.
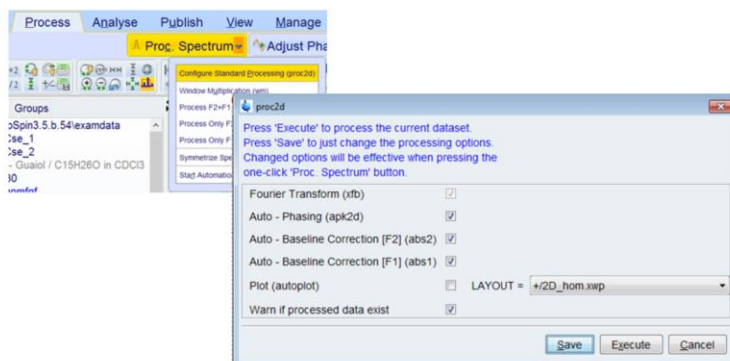
Basic 2D processing includes several steps, which can be performed by clicking on Proc. Spectrum or by typing commands:

- applying a window function to account for the truncation of data that usually occurs in 2D datasets (resolution is lost, so J couplings can't usually be obtained from 2D experiments, but time is saved)
- performing a Fourier transform in each dimension, to convert the raw data (FID) to processed data (a spectrum)
- phasing the spectrum, if appropriate, so that peaks appear positive and/or negative, according to the spectrum (a DEPT-edited HSQC has both positive and negative peaks, while most COSY are not phase-sensitive at all)
- 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 flat in both dimensions, reducing the appearance of noise

These changes are needed to get the right lineshape for Varian 2D datasets. If you do this frequently, the changes can be automated.
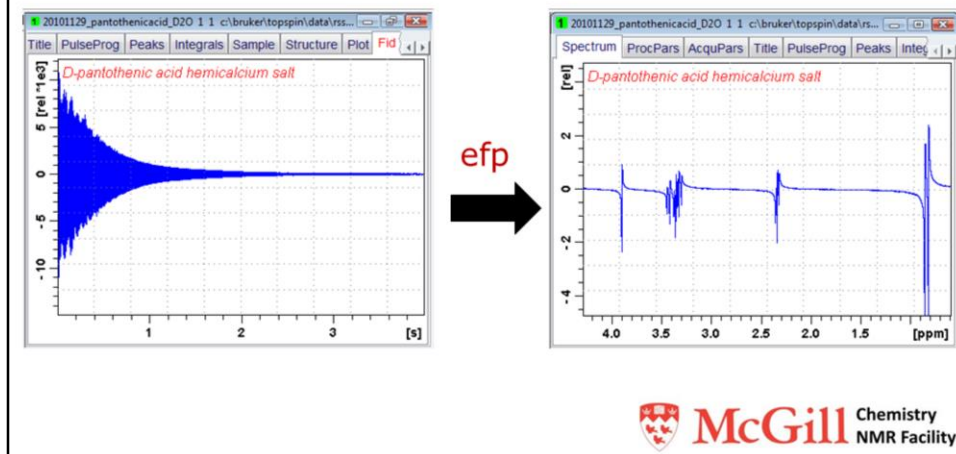
The command efp performs three processing commands sequentially: multiplication by an **e**xponential linebroadening function followed by a **F**ourier transform, followed by an application of the **p**hase parameters present in the dataset.

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
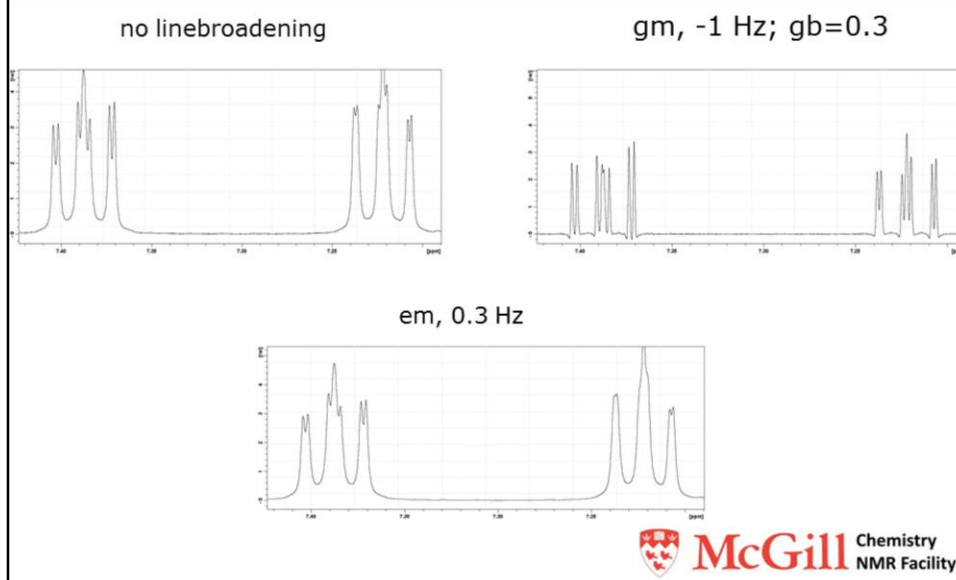
The basic linebroadening method is exponential multiplication, which tends to improve the S:N ratio in spectra. On the other hand, to increase resolution, the simplest thing to do is just omit the exponential multiplication by processing with the command **fp**. For further resolution enhancement, the 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.
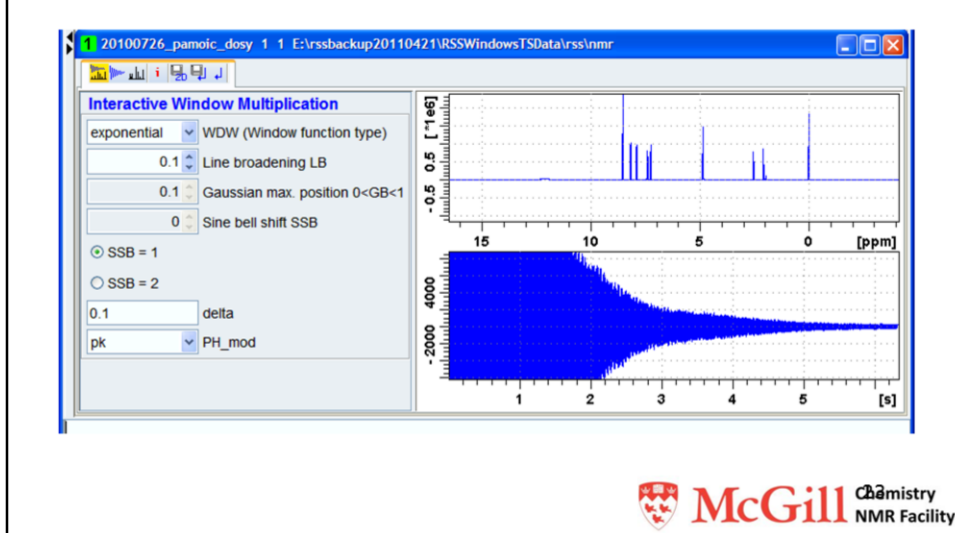
The **.winf** command allows you to interactively examine the effect of window functions on a spectrum. Note that the default settings will be not always be those expected, so if you have been applying Gaussian multiplication with gfp you may find that the spectrum looks odd, since by default it is processed 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.
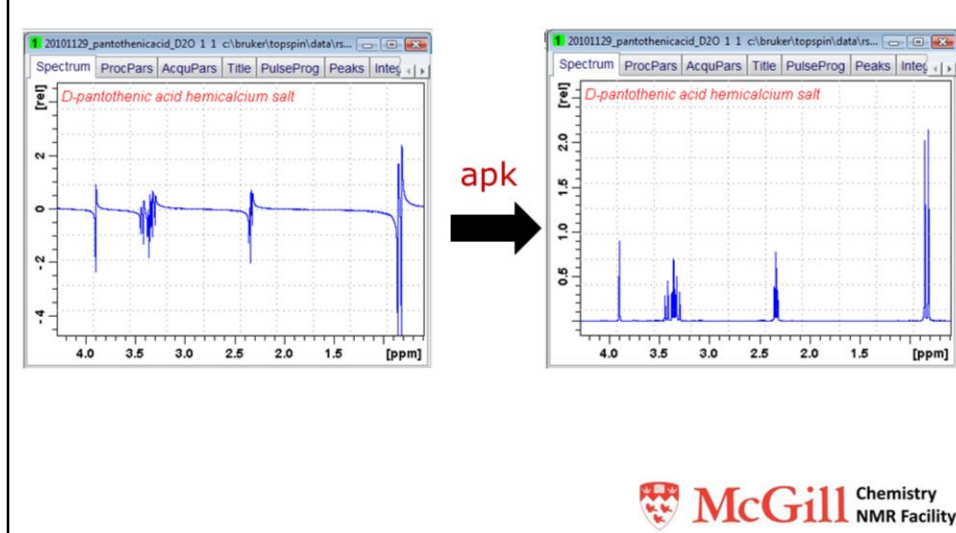
Phasing creates an entirely absorptive lineshape in the spectrum. Two parameters are adjusted to create this: phc0, which adjusts the phase of all parts of the spectrum evenly, and phc1, which affects the spectrum linearly across it. These two parameters are adjusted to maximize the positive signal in 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:
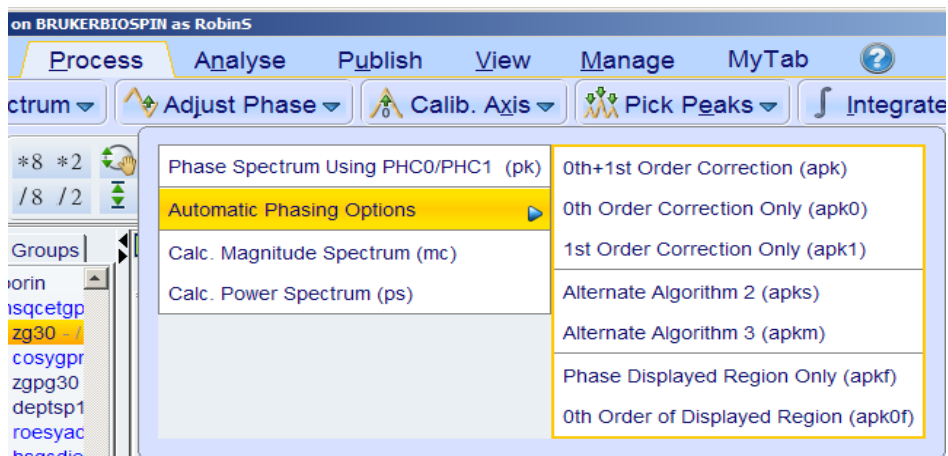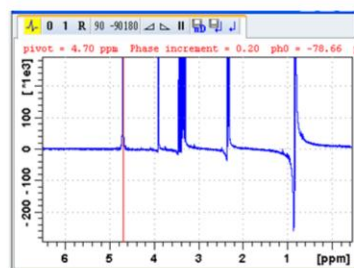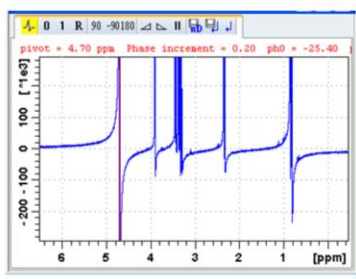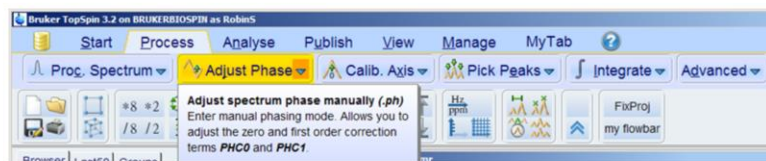
Manual phasing is used to correct the phasing when automatic phasing does not do a satisfactory job. Click on the Adjust Phase button to enter manual phasing.
To phase manually, start by using 0th order phasing to make the peak with the pivot point (red line) perfectly phased. Do this by clicking and dragging on the button marked   . 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 0th order phasing, use 1st order phasing to bring the other peaks into phase: do this by clicking and dragging on the button marked   .

For very crowded spectra where all peaks overlap **1** emember 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.
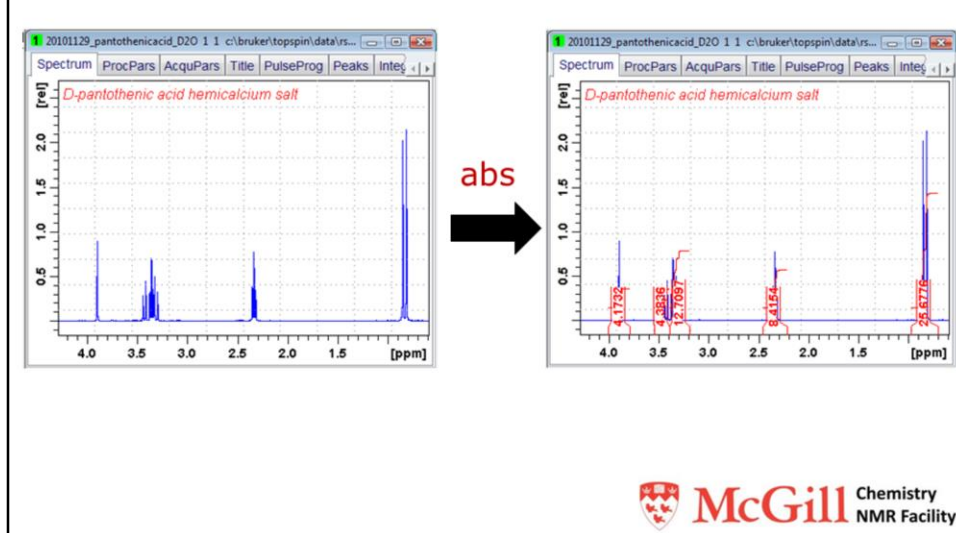
The command abs does two things to the spectrum: it corrects the baseline, and it does its best to integrate the spectrum. Use abs n to correct the baseline without integrating the spectrum.

The baseline correction is done by fitting a polynomial function $y = a + bx + cx^2 + dx^3 + ...$ to the parts of the spectrum without peaks in them.
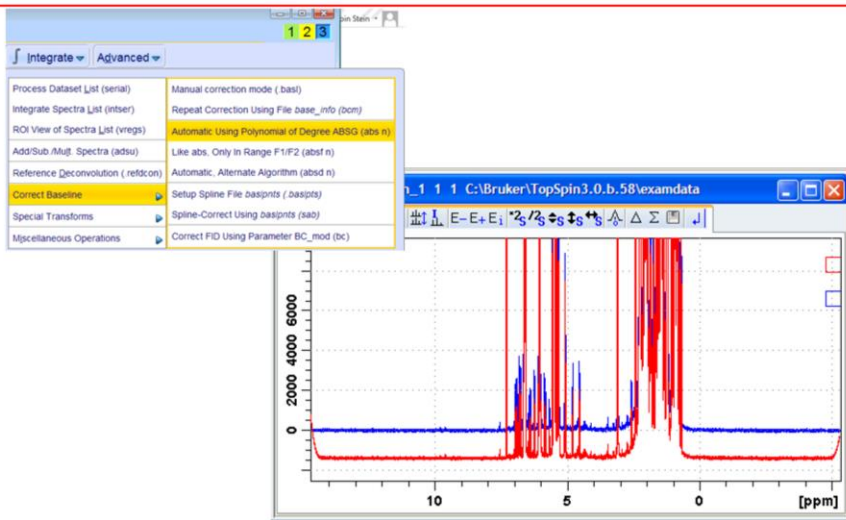
If the baseline is not at 0, use the command abs to adjust it automatically.   This helps make integrals more correct. The command abs also integrates the spectrum; the command abs n adjusts the baseline without also integrating the spectrum.
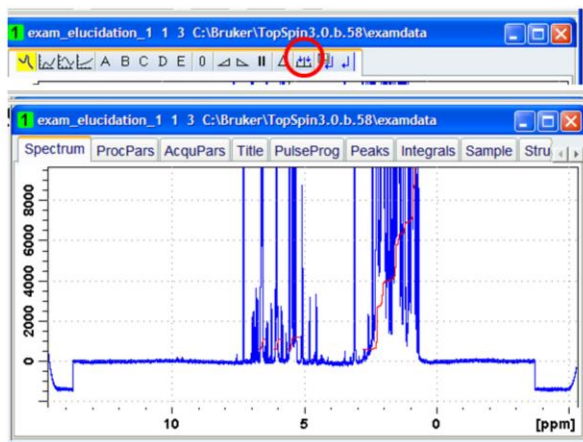
In manual baseline correction mode, you can adjust the parameters of polynomial subtraction manually, and you can choose points for spline baseline correction.
The most useful manual baseline correction option is probably the spline baseline correction. This allows you to define a set of points that 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
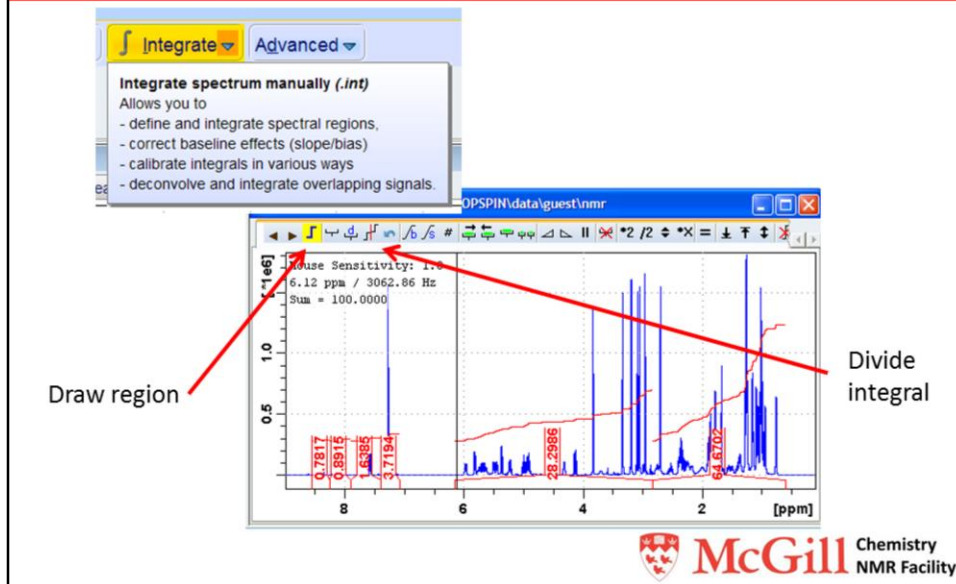
To get into manual integration mode, just click on the Integrate button in the Process flowbar. You can use the manual integration mode to define integrals from scratch, or to modify those generated by abs. In this mode, right-clicking on an integral gives options for calibrating the integral, normalising the sum of all integrals, and deconvoluting integrals.

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:

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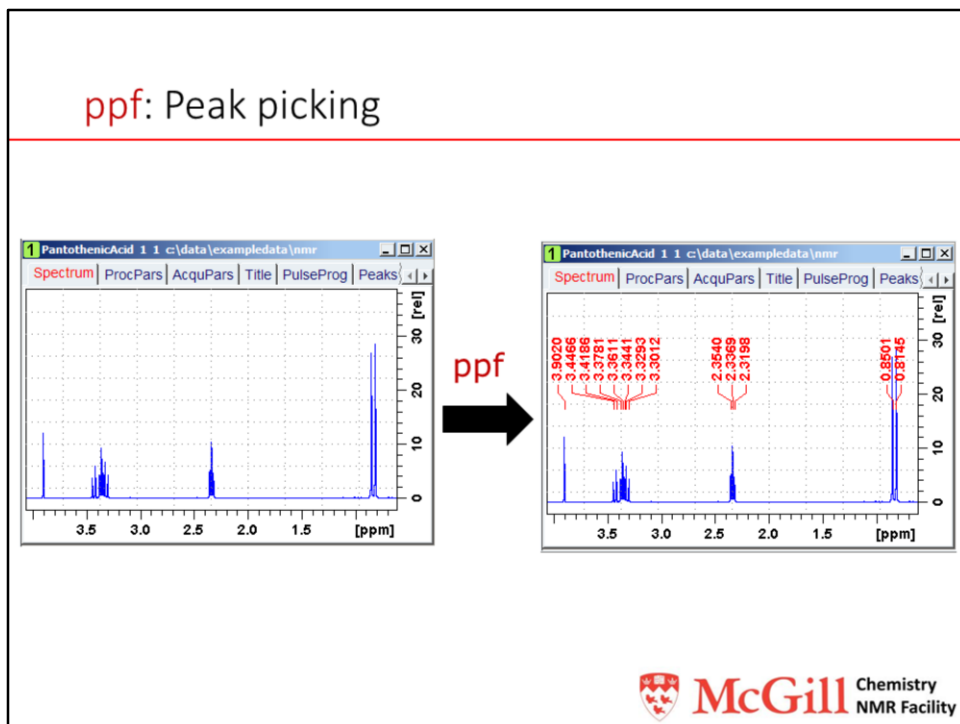
The command ppf automatically picks peaks on the entire spectrum. The command pps may also be used; it picks peaks only the displayed spectrum region.

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 threshhold, 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:
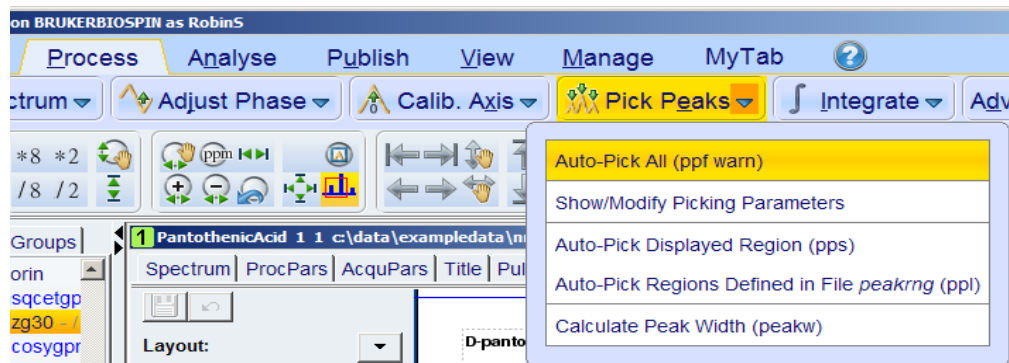
Manual peak picking

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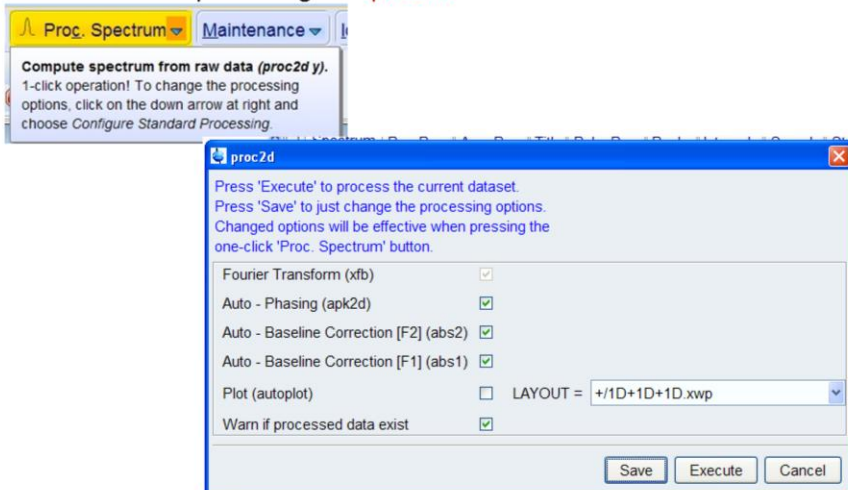
Like 1D spectra, 2D spectra can be processed in a semi-automatic fashion in TopSpin 3. The processing can be customised using the proc2d window, accessible from the menu next to the Proc. Spectrum tab.

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 ^{13}C spectra must be processed with the command xf2m after the automatic processing.

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}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.
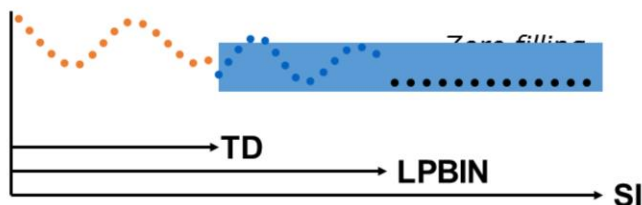
The parameter **ME_mod** stands for "maximum entropy" mode, a different name for linear prediction. The parameters to define linear prediction (the options for **ME_mod**) are:

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
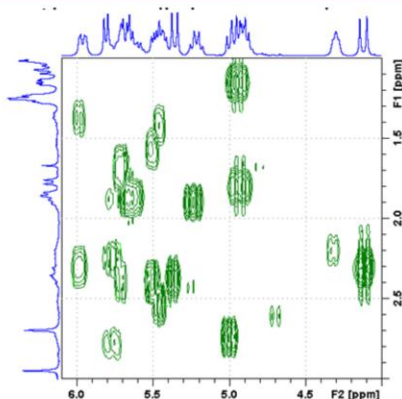
TD(1) = 128
No linear prediction
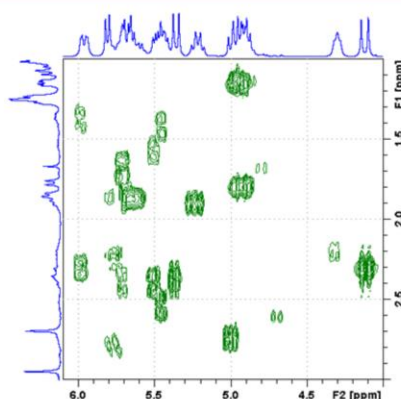
TD(1) = 128
Linear prediction to 256

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.
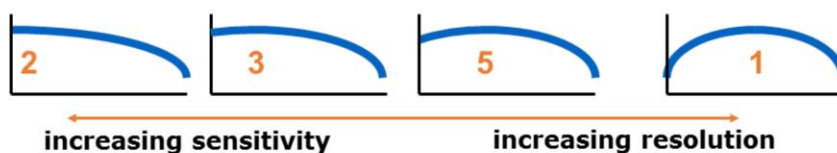
2D Truncation

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

FT — no adjustment

FT — fid multiplied by cosine function

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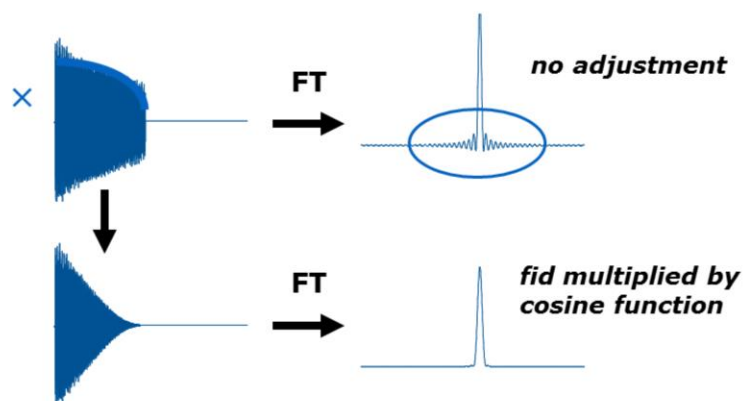
Linear prediction is usually not sufficient to keep 2D data from being truncated or adding enough points in the FID to define the points in the spectrum properly. So, zero-filling is usually necessary. However the "step" that occurs between the end of the fid and the added zeros give rise to so-called "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.

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.
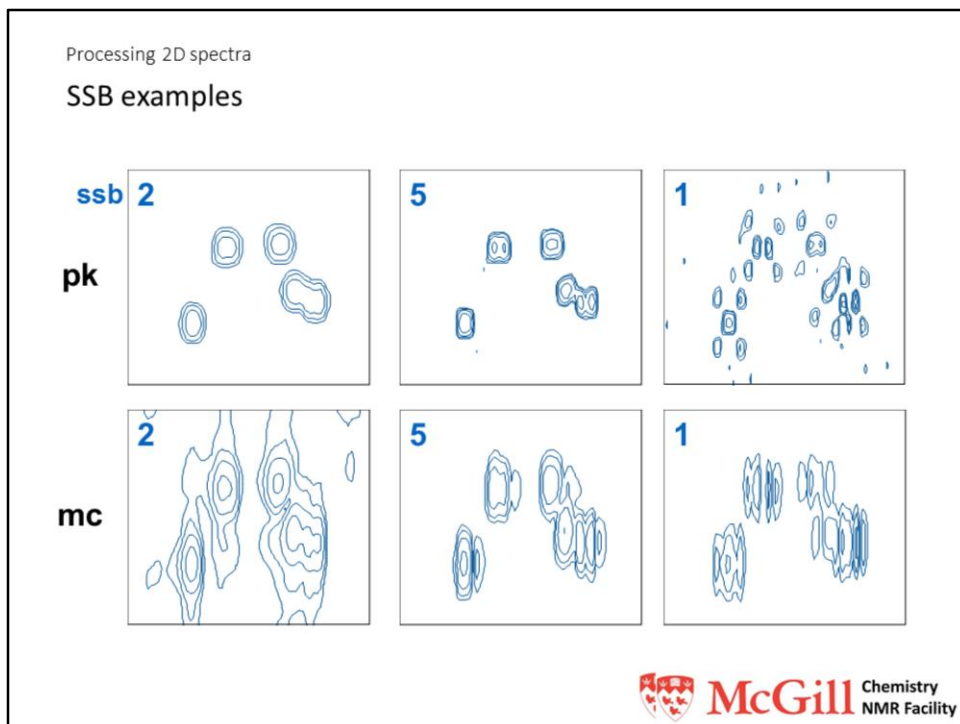
The difference between the two examples above is the result of the intrinsic lineshape difference between a phased spectrum (normal absorption Lorentzian lineshape) and the much broader magnitude-mode lineshape (a mixture of the absorption and dispersion lineshapes). If you want to illustrate this just 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.
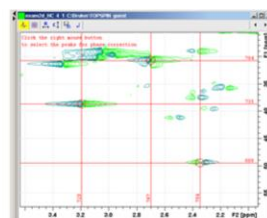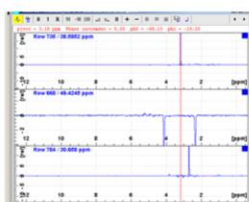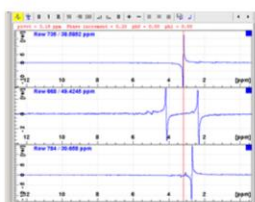
Phasing a 2D experiment is very similar to a 1D, except that there are 2 dimensions: both f1 (vertical) and f2 (horizontal) can be phase corrected.

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_3 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:
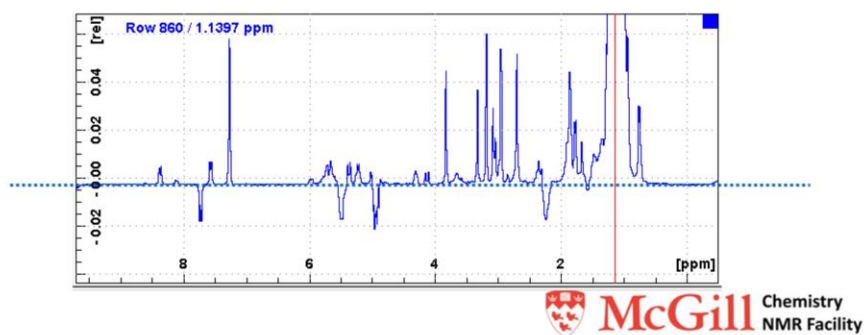
When phased correctly, the bulk of the baseline should usually be flat (even if not at zero) or at least a smooth continuous curve. You might see deviations at the extreme edges of the spectrum due to digital filters.
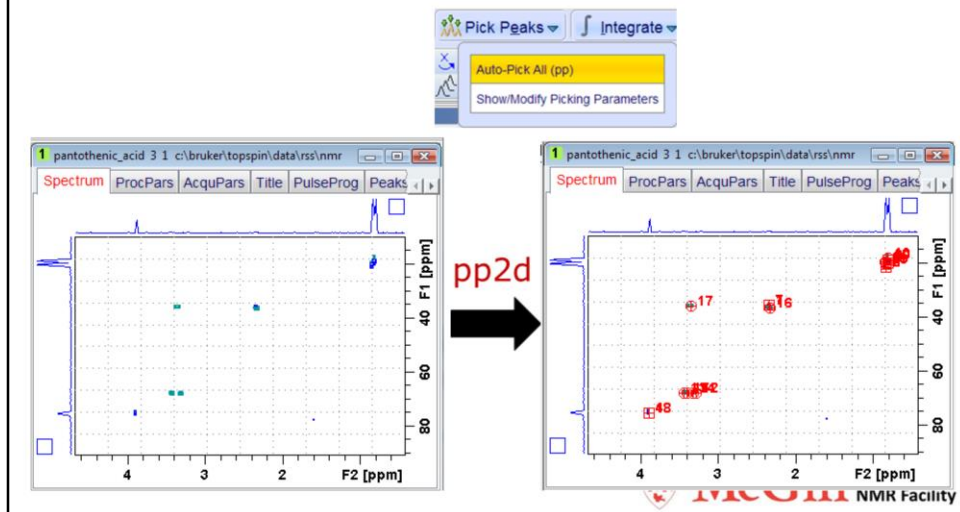
Referencing 2D spectra

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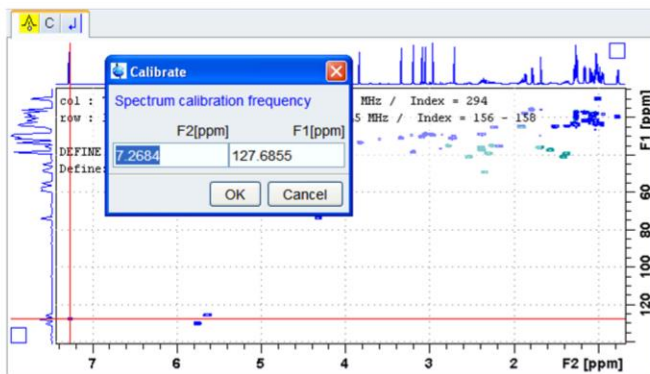
The pp2d dialogue box lets you set the threshold based on the contour levels. Set the contours so you can see (only) the peaks of interest and save them before starting pp2d. Then, under Minimum intensity / Set to, choose "Lowest contour level."

The command int2d allows for integration of the peaks chosen with the pp2d command.
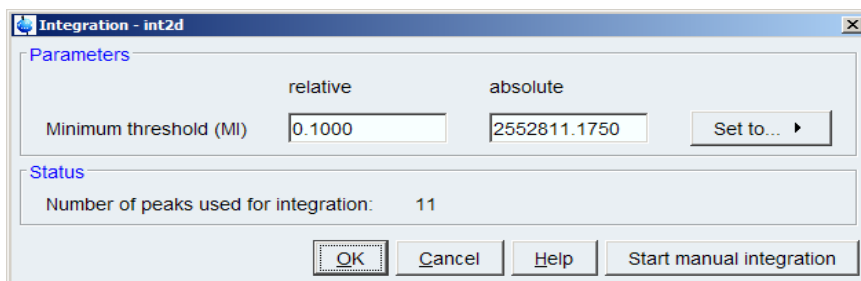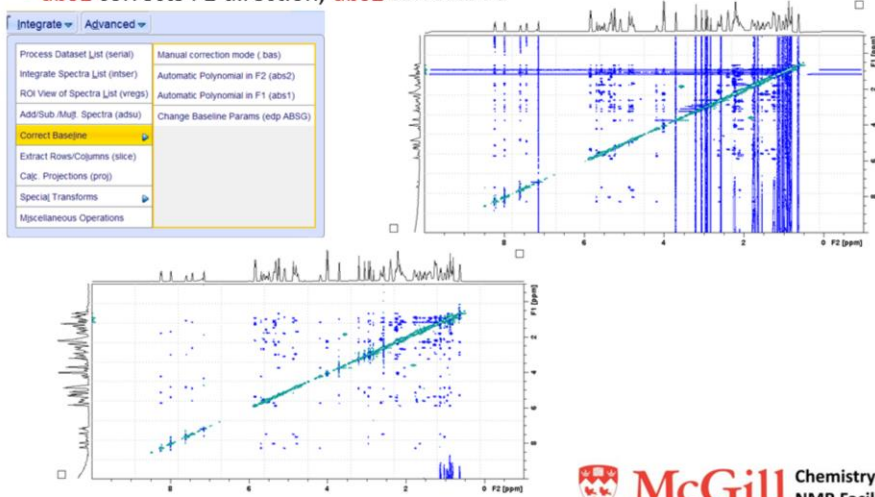
NOESY/ROESY are usually the worst spectra for needed baseline corrections, especially in t1, because of t1 noise.

Setting projections

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.
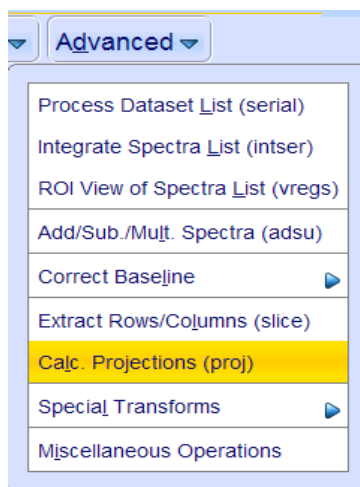
Another option is to right-click on a spectrum listed in the data browser and choose Display as 2D Projection
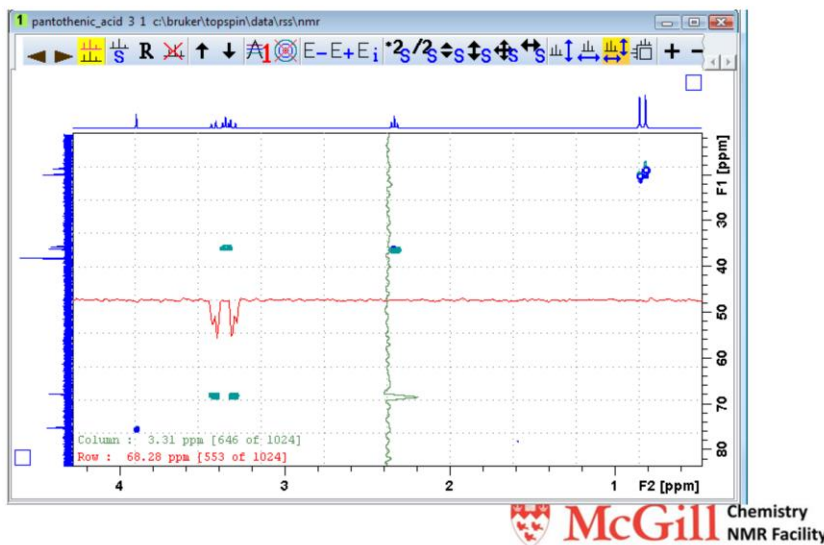
To view individual rows and/or columns, go into the multiple display mode by clicking on the icon ⋀⋀ The scan rows/columns buttons within multiple display mode allow you to look at individual rows/columns of a 2D spectrum. If you want to do more with a row/column, like compare it in multiple display 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

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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)

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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_1-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_1 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.

Generating a printout quickly: prnt or the Plot tab
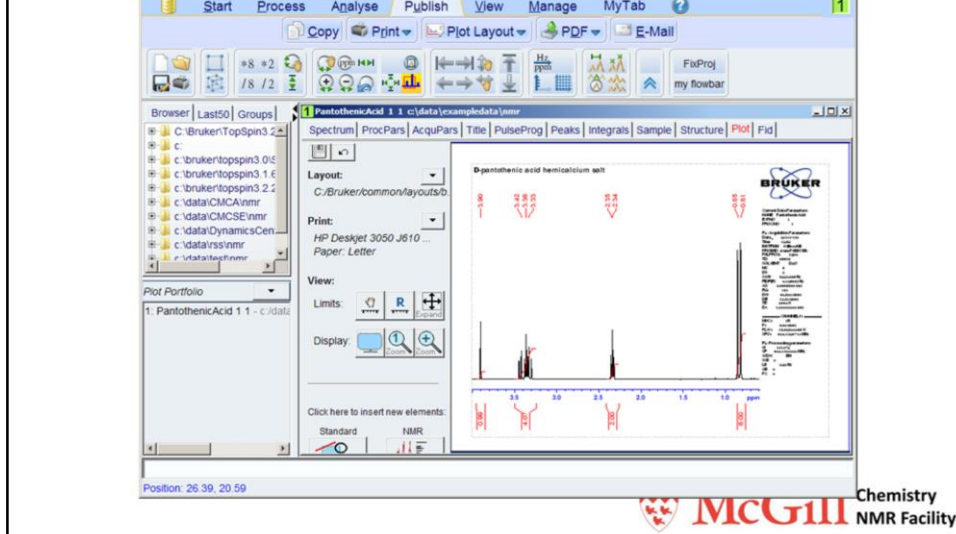
There are several ways to create a printed or PDF output of a spectrum.

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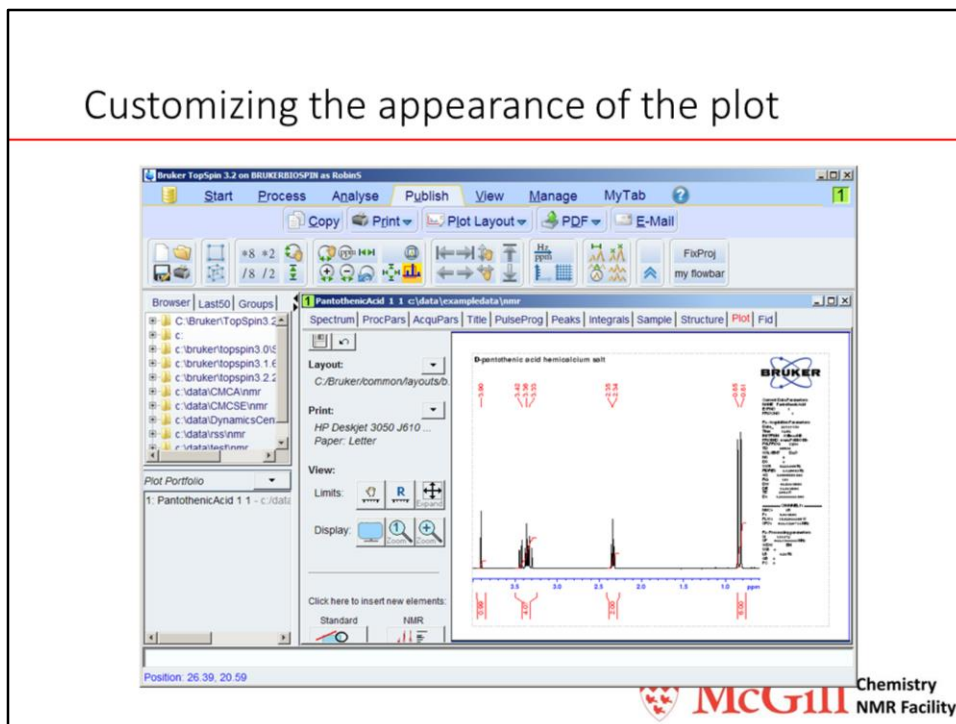
The plot tab can be used in two ways: to customize a view of the current spectrum, or to create a more generally useful plot arrangement, known as a layout.

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, 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).

Modifying objects already on the plot

- Clicking on an item brings up options for modifying it (in the left panel)
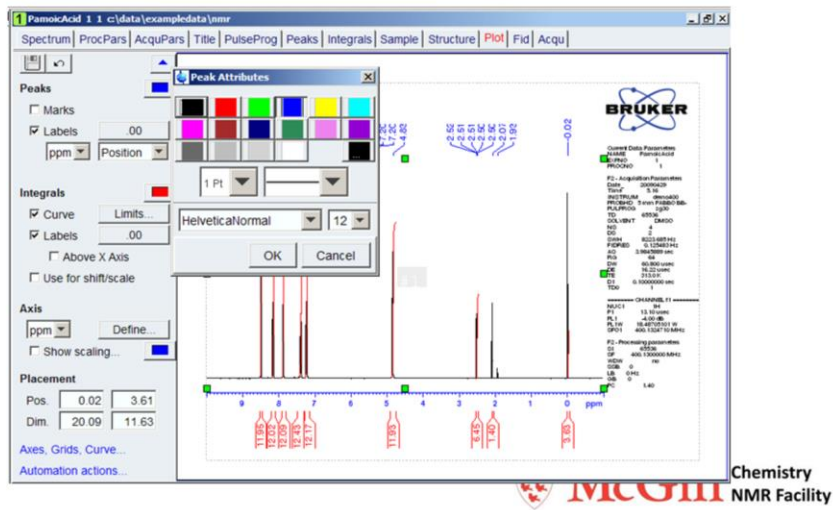
In this window, the spectrum is selected, so the options in the left panel are those appropriate for modifying the appearance of the integrals, the peaks, the axis, etc. The meaning of the buttons is:

Undo

Save

Return to main panel (to add items and adjust printer options, etc.)

Do / do not display peak marks or labels

Format text and line style of peak marks and labels

Format peak labels (decimal places, Hz / ppm, position of label)

Do / do not display integral curve or labels

Format text and line style of integral curve and labels

Change units of chemical shift axis

Format text and line style of chemical shift axis

Move spectrum on the page

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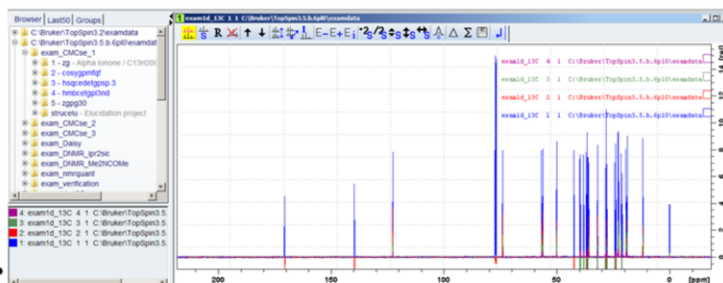
Use the multiple display mode in the Spectrum tab (the main view of the spectrum), not the plot editor tab. Use the standard techniques for viewing spectra (dragging and dropping from data browser or typing re <#>, for example) to load up many spectra.

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.
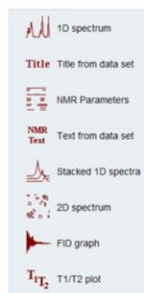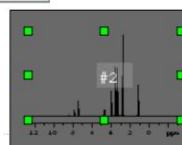
The number displayed over an element when it is selected shows which number spectrum in the plot portfolio list is being plotted. It can be difficult to see, so here a grey box was plotted behind the spectrum so that the highlighted number appears clearly.
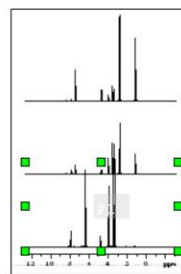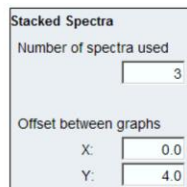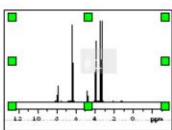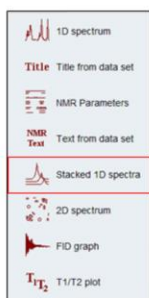
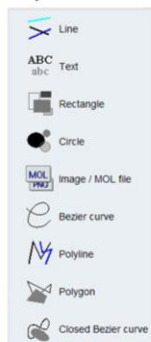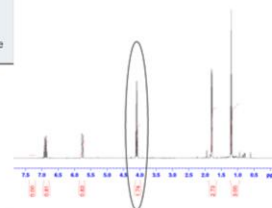The green box only defines the size of the initial spectrum. The spectrum which appears here (#1 in this example) is taken from the position in the Plot Portfolio; if you wanted to plot spectra 2-5, for example, you would drag spectrum 2 into the stacked spectra region and then plot 4 stacked spectra.
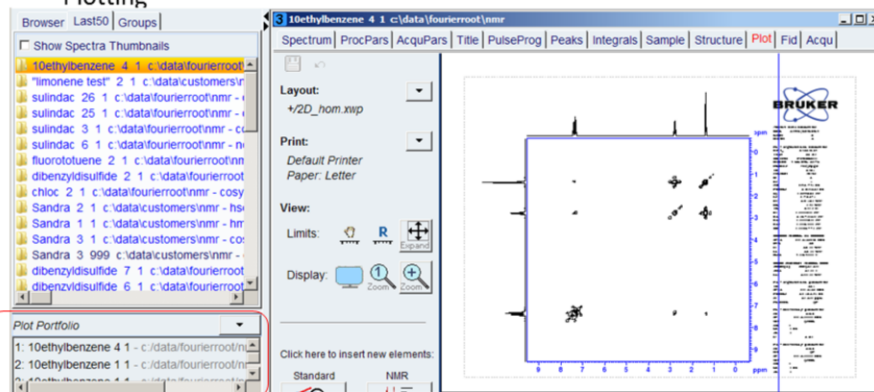
In earlier versions of the plot editor, objects were added by selecting them, clicking on the page to place them, and then editing them.

Plotting 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.

The standalone plot editor available in TopSpin 1 and 2 contains all the same functionality as the plot tab of TopSpin 3, but most settings are contained under Edit and 1D/2D Edit or under Attributes. Datasets must be set under the Data button.