

NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

1D NMR Processing and Analysis with TopSpin 4.0

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The purpose of this document is to help researchers become proficient in basic 1D processing and analysis of NMR data using the academic edition of TopSpin 4. For most of the procedures described here, Bruker provides both automatic as well as manual approaches. This document will attempt to describe both in a concise and straight-forward fashion. Please note that the pictures contained within were extracted from the Windows version of TopSpin.

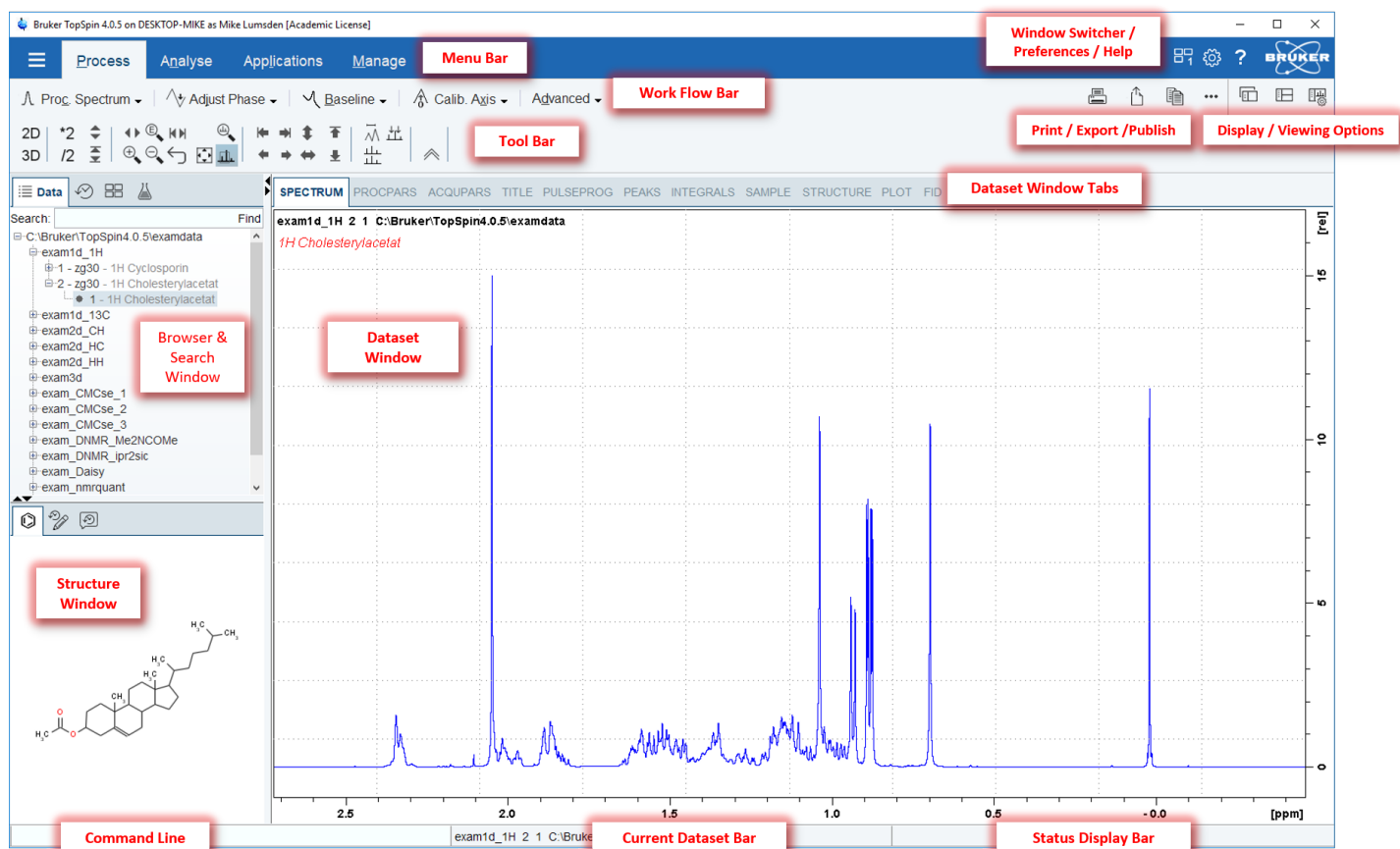
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Section 1: Opening NMR Data & Customizing the Display

1.1 The TopSpin Display

Before beginning, it is important to familiarize yourself with the basic TopSpin 4 layout. The figure below illustrates the various important areas of the interface. These designations and the associated terminology will be used throughout this document and so the reader is advised to become familiar with this figure before reading ahead.

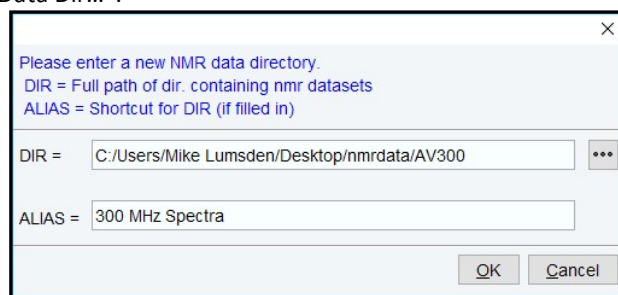


1.2 Opening NMR Data

Once you have a local copy of your NMR data, the first task is of course to load the data you wish to work with into a TopSpin data window. There are several ways to accomplish this but one of the most convenient is to use the Browser & Search Window. After performing a default installation, note that the browser window shows a directory <TS HOME>\examdata. You may delete this if you wish by right-clicking on it and selecting "Remove Selected Data Dirs...".

On my desktop I have a folder called nmrdata and below that two folders entitled AV300 and AV500. To add these to the browser, I would proceed as follows:

- right-click in an empty area of the browser and choose "Add New Data Dir..."
- In the window that opens you specify the directory to add and optionally an alias as shown in the figure. After clicking OK, the new directory will be available.
- Click the + sign to expand and see the available datasets. Clicking the + sign on any dataset to show the available experiment numbers and drilling into an experiment number you will see the available processed data numbers (usually only "1").
- To open you can either double click on the processed data number or drag it into the dataset window to open.



- If you want to open the data in a new window (leaving existing open data windows untouched), right-click on it and choose “Display in New Window”.
- If you have a dataset open and wish to load a different experiment number from the same dataset, type in the command line “re <expno>” where expno is the desired experiment number.

1.3 Manipulating Your Spectrum with the Toolbar

The Topspin Toolbar allows you to manipulate your spectrum in many ways. For example, you can control the intensity of your peaks, zoom into an interesting spectral region, shift your spectrum horizontally, or reset the view to the complete spectrum with default scaling. The figure to the right shows the various sections of the toolbar. A few additional tips & points:

- double-headed arrows (for example \updownarrow) are for an interactive manipulation of the display by clicking on the arrow, holding and dragging.
- : Perform an exact zoom by specifying the limits
- : Reset zoom, vertical intensity or both
- : Toggles the interactive zoom method: off and interactive zoom only selects a horizontal region (vertical intensity and baseline position remain the same); on and you draw a box to select your region.



Intensity Scaling

Can also be done by scrolling with the mouse

Chemical Shift Axis Expansions

Can also be done by clicking on one end in the spectrum and dragging to the other end of the desired expansion region

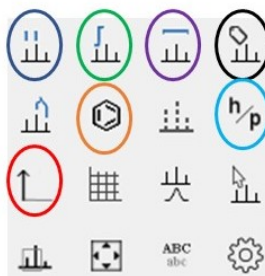
Move Spectrum Up & Down / Left & Right

Can also be done by hovering directly over the horizontal and vertical axes in the dataset window

1.4 Spectrum Display Properties

The spectrum display properties window contains many useful toggles that you can use to customize what information gets displayed within your spectrum window. To open the window, click the right-most icon below the Bruker logo. A picture of the various options is included here along with some brief color-coded descriptions:

- Toggle Display of Peak Picking
- Toggle Display of Integral Trails
- Toggle Display of the Molecular Structure
- Toggle the Y-Axis Display & Units

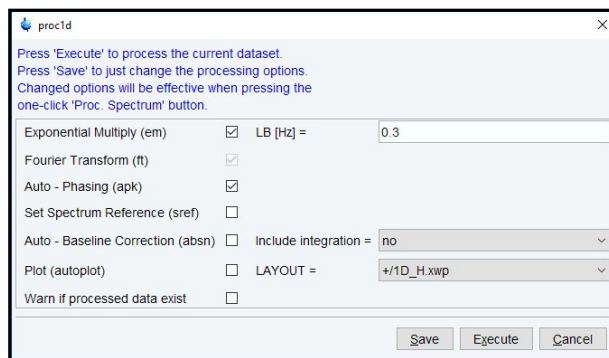


- Toggle Display of Integral Ranges
- Toggle Display of the Title
- Toggle Units Between Hz / ppm

Section 2: 1D NMR Processing

2.1 Fourier Transformation (FT)

The first step in processing raw NMR data is performing a Fourier transform (FT) to convert the time domain data (FID) to the frequency domain (spectrum). This is typically done with the command “ef” which performs an exponential multiplication of the FID (controlled by LB in Hz) which is automatically followed by a FT. Prior to “ef”, you should check the value of SI which controls zero-filling. At minimum, SI should be TD/2 (no zero-filling). However, a value equal to TD is usually more appropriate (zero-fill once) and sometimes even larger values are used. The default values of LB are often appropriate but you are free to change the value prior to issuing the ef command. Use larger values to improve the S/N in your spectrum at a cost of slightly broader peaks. If you are trying to resolve closely spaced peaks in a spectrum and have good S/N, use smaller values of LB or skip it entirely by using the command “ft”.



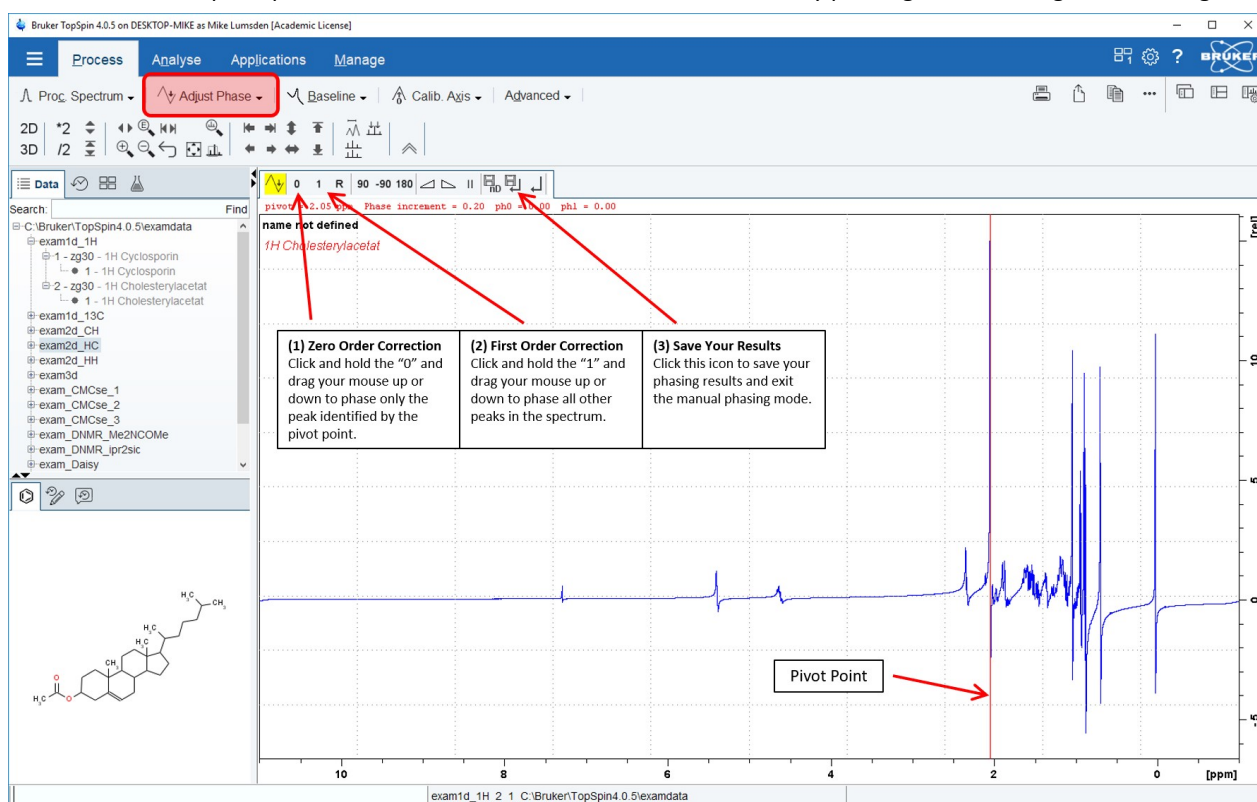
Use an Automatic “One-Click” Processing

- Click “Process” in the Menu Bar to open the Processing Flow bar.
- Click the left-most button in the Flow bar - “Proc. Spectrum.”
- You can control what this automatic processing does to some extent by selecting the down arrow beside “Proc. Spectrum” and choosing “Configure Standard Processing”. The figure on the right shows the available options. You can configure:
 - The amount of line-broadening to apply (0.3 Hz for ^1H and 1 Hz for ^{13}C are typical numbers).
 - Whether or not to perform automatic phase correction, baseline correction and/or referencing steps.
 - The auto baseline correction can optionally be combined with an integration by choosing Yes in the “Include Integration” field.
- If you wish to save your changes so that they apply to all future one-click processing, click Save.
- Click Execute to perform the processing.


2.2 Phasing

If you used one-click data processing, your spectrum may have been phased automatically. In most cases, the result of this automatic phasing is adequate. However, there may be times where you want to improve the result. To manually phase, click on “Adjust Phase” in the processing flow bar. By default, the tallest peak is chosen as the reference and its position is shown by a red vertical line known as the “pivot point”. The interface and instructions for manually phasing 1D data are given in the figure.

A few



additional tips & points about manual phasing:

- To change the position of the pivot point, hover your mouse over the desired peak, right click, and choose “Set Pivot Point”.
- **R** : Reset the 0 and 1st order phase correction
-  : Increase or decrease the mouse sensitivity when adjusting the phase

Automatic 1D Phasing from the Command Line

- The “**apk**” command will perform an automatic phase correction using both a zero and first order correction.
- In some cases, only a zero-order correction should be necessary leaving the first-order correction at zero. This can be done using the “**apk0**” command.

2.3 Baseline Correction

Baselines from present day spectrometers are impressive and rarely require a rigorous manual baseline correction. Automatic routines are available to flatten any residual curvature and work very well. Only a few tips & tricks are provided here:

- The automatic procedure can be called from the command line via “**abs**”. This will automatically fit your baseline to a polynomial of degree given by the processing parameter ABSG (usually 5). Note that the abs procedure may be executed as part of the one-click processing.
- Bundled with the abs command is an automatic integration of your spectrum. I find this to rarely be of any value and so a more useful command might be “**abs n**” – the n specifies no integration.
- It is also possible to flatten a region of your spectrum, ignoring the rest. To do this, set the parameters ABSF1 and ABSF2 to represent the region in ppm you are interested in (ABSF1 > ABSF2). Then issue the command “**absf n**”.

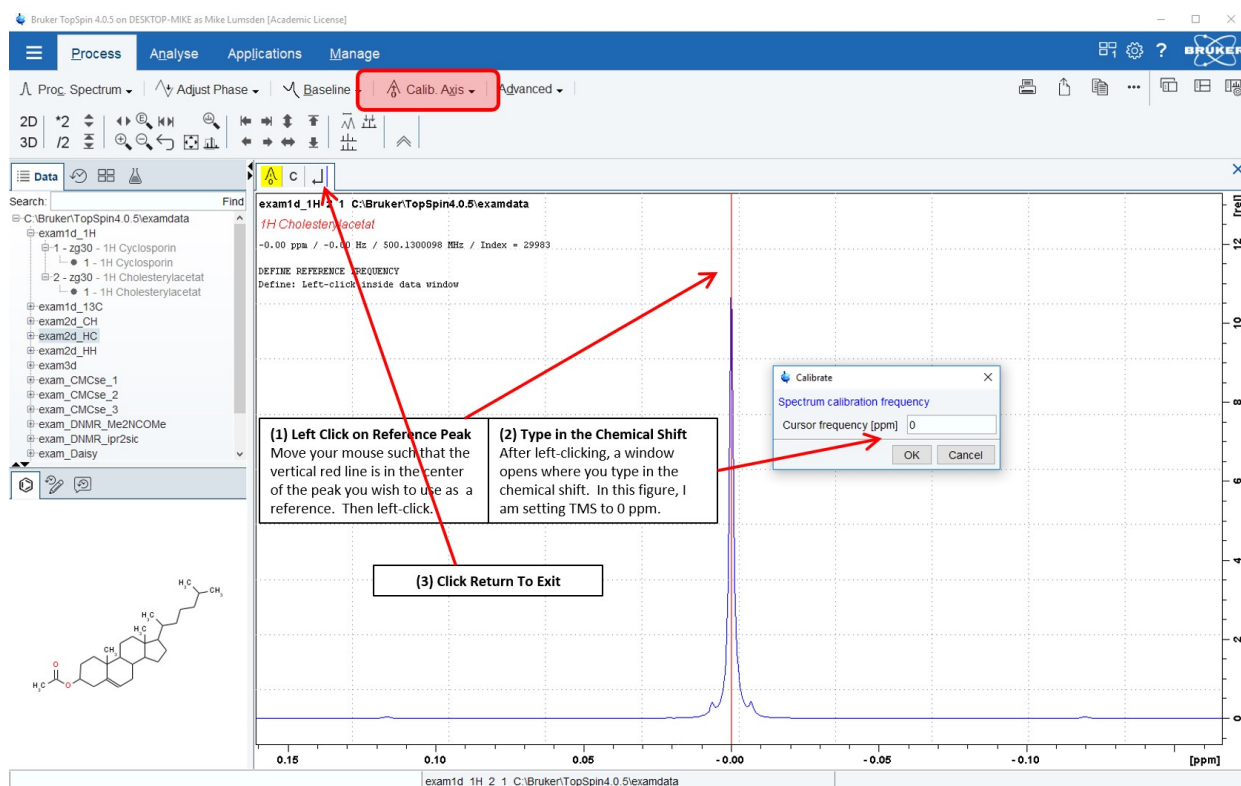
NOTE

Since version 4.0.6, a new processing command “**apbk**” is available which performs both baseline and phase correction for problematic X-nuclei spectra (^{13}C , ^{19}F and ^{31}P). This algorithm appears more robust than issuing separate apk and abs commands for these nuclei. In addition, “**apbk**” can be called with the following options:

- -po → phase only
- -bo → baseline only
- -n → do not write integral regions
- -f → force the algorithm to run for nuclei other than C/F/P.

2.4 Calibration (Referencing)

When spectra are acquired locked on a deuterated solvent, chemical shift values for all nuclei are already accurate. Bruker uses the chemical shift of the lock solvent as well as the deuterium Larmor frequency to calculate the absolute deuterium frequency



corresponding to 0 ppm. The frequency corresponding to 0 ppm for any other nucleus can then be easily calculated. Although very robust, there are times when a manual calibration might be necessary (mixed solvent systems, running spectra unlocked, etc.). To manually calibrate your spectrum, click on “Calib. Axis” in the processing flow bar. The interface and instructions for manually calibrating the spectrum are given in the included figure.






Automatic Calibration from the Command Line

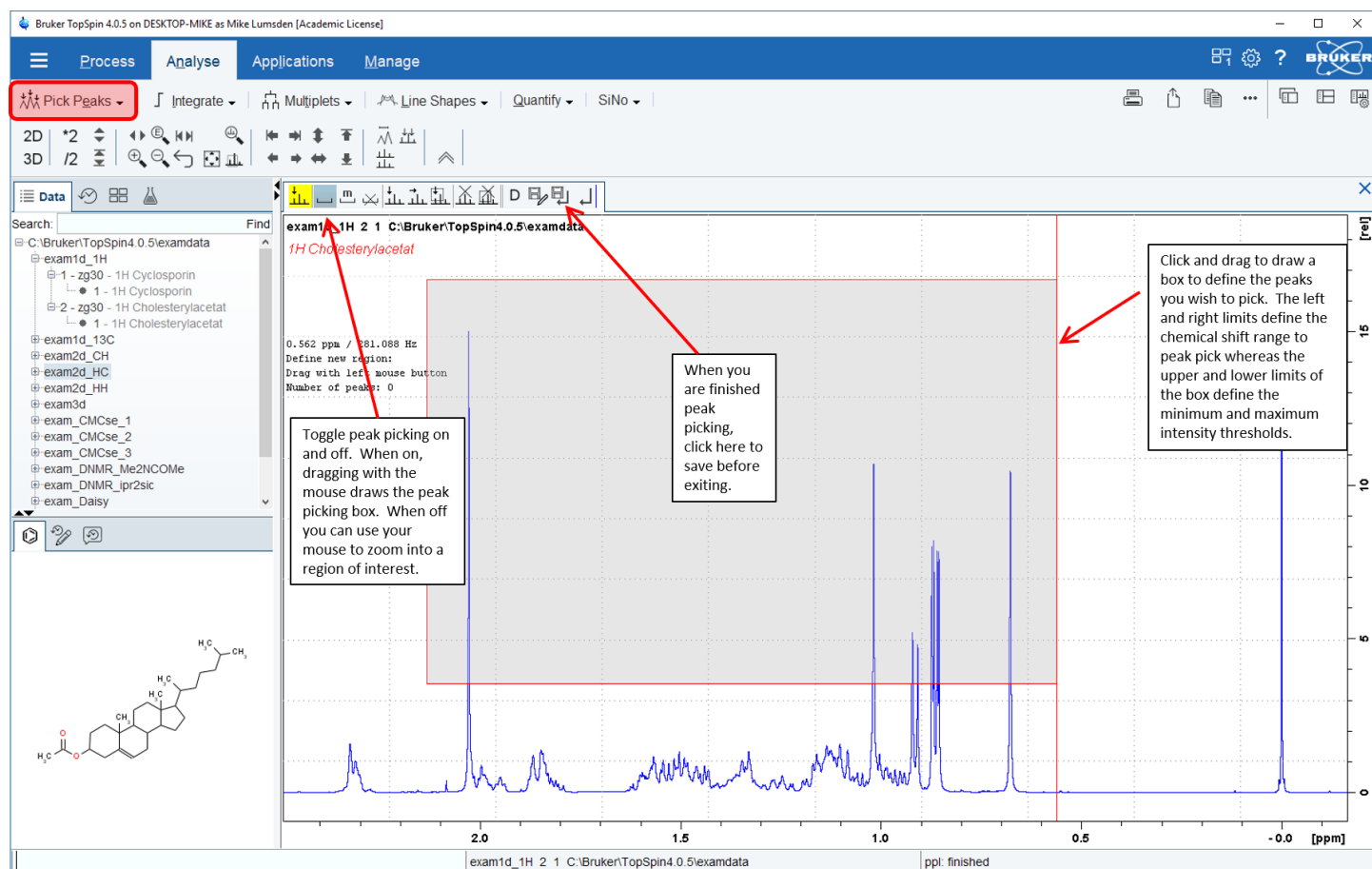
- If your NMR sample contains TMS, you can use the “**sref**” command to automatically set this signal to 0 ppm. This can also be executed as part of the one-click processing. If you have other silyl groups in your sample, use this command with caution as the wrong signal could be positioned at zero!

Section 3: 1D NMR Analysis

3.1 Peak Picking

To perform manual peak picking, select the Analyse menu bar item to open the Analysis flowbar. Select “Pick Peaks” from the flow bar. The interface and the instructions for manually peak picking are given in the figure on the next page. A few additional tips & points:

- : Modify existing peak picking regions.
- : Delete all existing peak picking regions.
- : Pick peaks manually. Left click on a peak to pick it; right click over a peak already picked to delete it.
- : Move your mouse slightly to the left of the peak to pick and left click (a red line should be positioned over the desired peak). Then right-click and select “Add Peak to List”.
- : Delete all peak picking
- After saving and exiting the peak picking routine, you may wish to have the chemical shifts displayed directly in the spectrum window. To do this, right-click anywhere in the window and select “Spectra Display Preferences”. In the window that opens, make sure the option “Peak labels” is selected and then click Apply. In that same window, you can control the number of digits in the labels by clicking Change beside “Number of digits for integral / peak labels”.
- To print a list of peaks, select the “PEAKS” tab in the dataset tab bar. Right click in the peak table area and choose “Print...”






Automatic Peak Picking from the Command Line

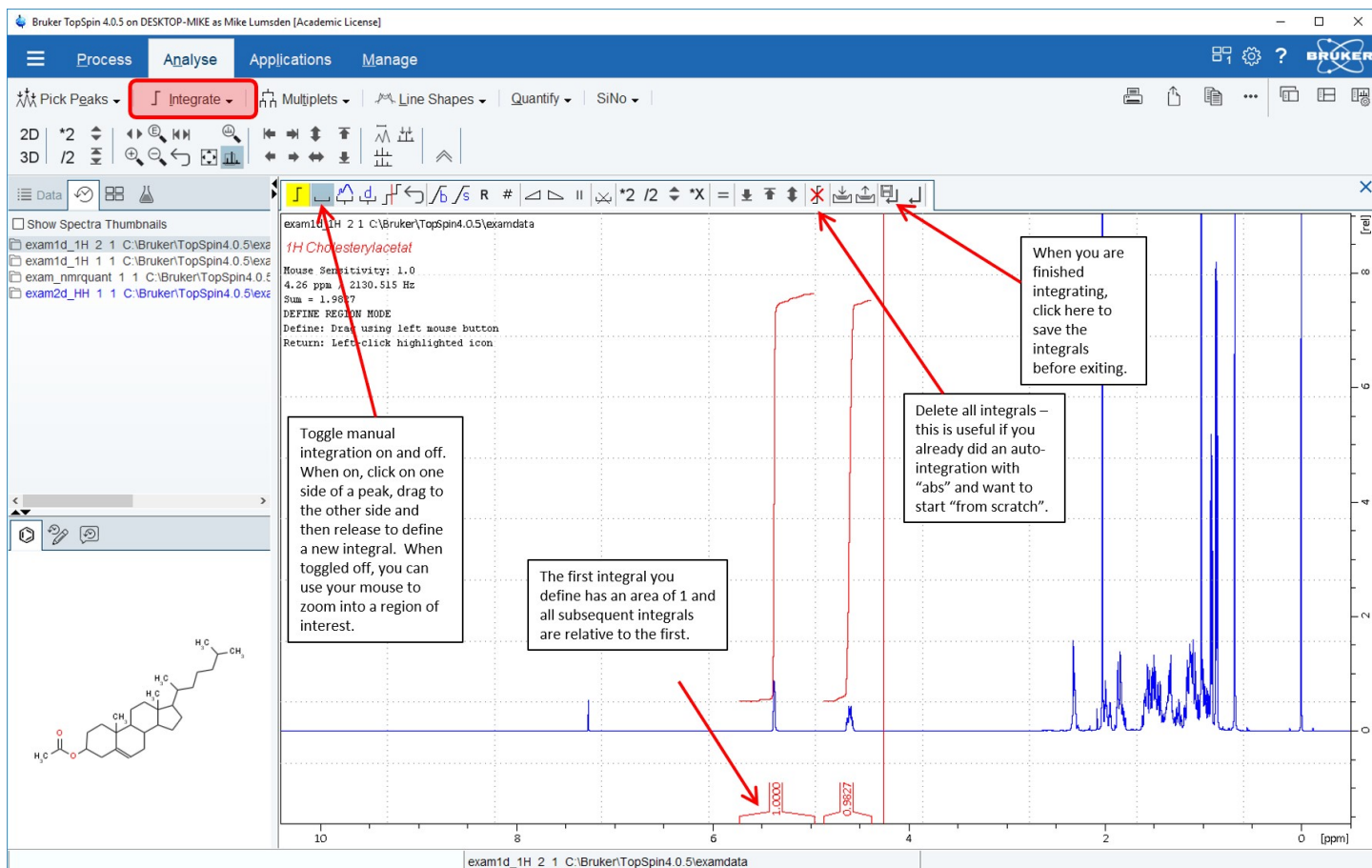
- The commands “ppf” and “pps” are for automatic peak picking. The former will peak pick the entire spectrum whereas the latter will only pick the region of the spectrum displayed in the data window.
- The main parameters controlling the algorithm are MAXI, MI, and PC. MAXI specifies the maximum intensity to pick, MI the minimum or the threshold intensity, and PC is a sensitivity parameter (smaller values lead to a more sensitive peak picking). One use of PC is to detect a shoulder on a larger peak by making the parameter smaller.
- PSIGN parameter: normally set to “pos.” meaning only positive signals are picked. However, for edited experiments like DEPT135 you would set this to “both” such that negative and positive peaks are picked.

- If you perform an automatic peak picking and then need to heavily modify the result, this is most conveniently done by entering the peak picking manual mode.
- You may view the peak picking list at any time by selecting the “PEAKS” tab.

3.2 Manual Integration

To perform a manual integration, select the Analyse menu bar item to open the Analysis flowbar. Select “Integrate” from the flow bar. Note that it is crucial to have a well-phased spectrum and a flat baseline for accurate integrals. The interface and the instructions for manually integrating are given in the figure on the next page. A few additional tips & points:

- : Define an integral region exactly via a dialogue window rather than using your mouse.
- : Delete selected integral regions.
- : auto-scale toggle: click on and all integrals get scaled to the same height and the scaling factor used to do this is also displayed; click again and this auto-scaling is turned off.
- Calibrating an integral: for example, suppose you have an integrated signal in your proton spectrum that you know is from a single methoxy-group. To calibrate this integral to three, move your mouse over the integral and right-click. In the context menu that appears, choose “Calibrate Current Integral” and then type in 3 for the new value. This integral gets set to 3 and all others will be calculated relative to this calibration.
- When you save and exit the integration routine, you may not see your integrals in the spectrum window. If you want them displayed, right-click and select “Spectra Display Preferences”. Turn on integrals/integral labels as desired.



****NOTE****


Since version 4.0.6, integrals can be selected via a simple mouse-click in the integrals region rather than needing to right-click and choosing Select. Multiple integrals can be selected by holding down the Shift key while performing the mouse click.

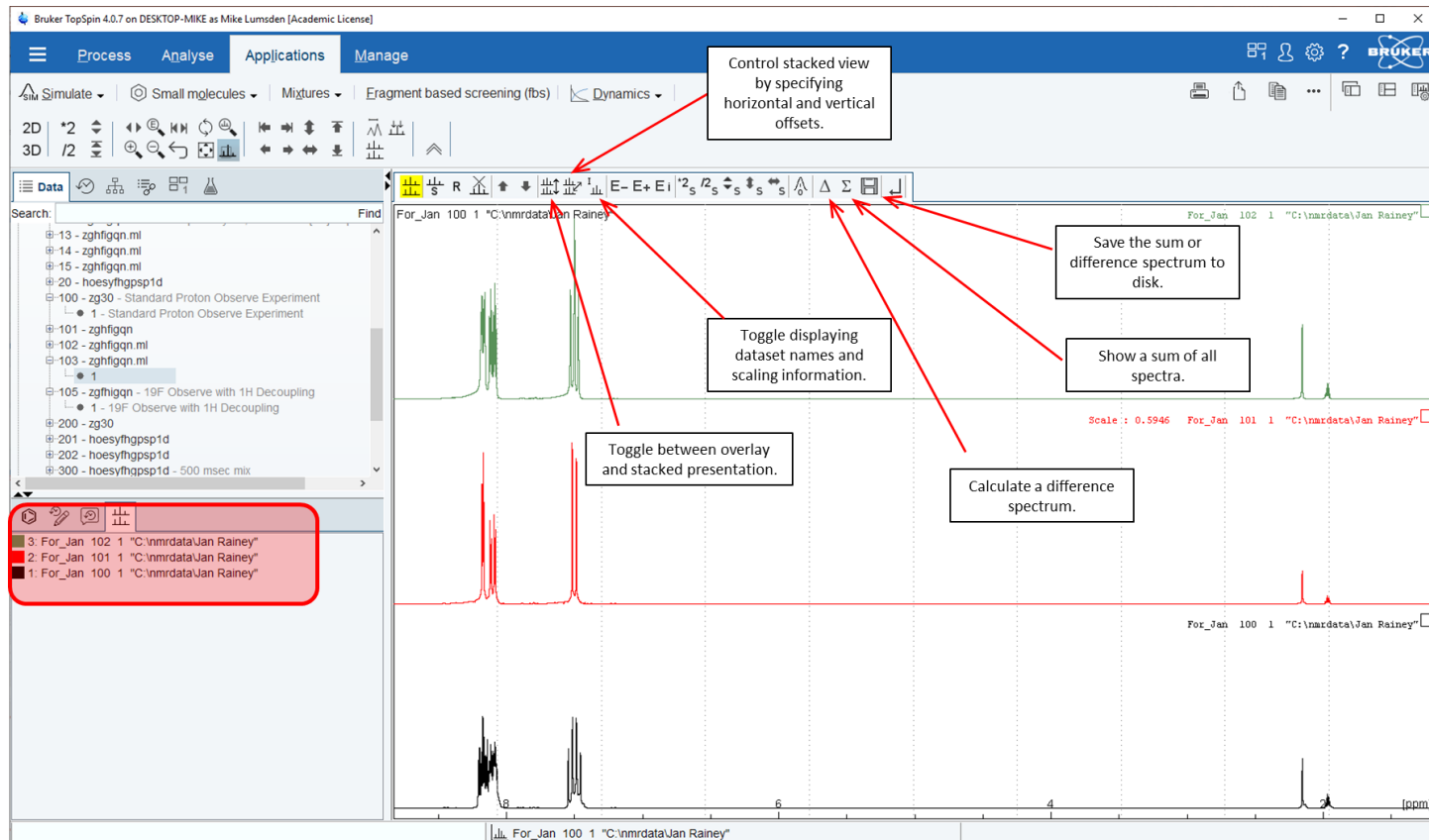
Automatic Integration from the Command Line

- As mentioned in the baseline correction section, an automatic integration comes “bundled” with the “abs” command.



- The main parameters that control this algorithm are as follows:
 - ABSL: sensitivity parameter relative to the noise level (like peak picking PC parameter). Make ABSL larger for less sensitivity. Range is 0 to 100 (default 3).
 - ISEN: another sensitivity parameter but in this case relative to the largest integral and as such, controls the discarding of small integrals. Small integrals are rejected if they are ISEN times smaller than the largest integral in the entire spectrum. Default value is 128.
 - AZFW: peaks further apart than AZFW ppm are treated independently; those less than AZFW ppm apart are considered overlapping and combined into a single integral. This can be useful if you have a multiplet where the individual resonances are being integrated individually as they are further apart than AZFW. Increase the value of AZFW (in ppm) to a value larger than the peak separation and they will be combined.
 - AZFE: an integral extension factor which extends integral regions on both sides by AZFE ppm. If the extension causes adjacent regions to overlap, the center of the overlap is used as the limit of the two regions.

3.3 Multiple Display Mode

It is often useful to overlay two (or more) spectra in order to facilitate comparisons. TopSpin offers an interface called multiple display mode for this purpose. Enter multiple display mode by typing “.md” in the command line or selecting the toolbar icon . Data can be added to multiple display mode simply by dragging the processed dataset(s) directly from the dataset browser. The interface as well as some instructions are provided in the next figure. Note that when you launch multiple display mode, the left-side bottom panel automatically shows a dataset list (indicated by the red rectangle). This list corresponds to the experiments and their order of display within the multiple display window.




Some hints concerning multiple display mode:




- Reordering the spectra: suppose you wish to position the green spectrum in the above figure in the middle and the red one at the top. To do this, select the green spectrum in the dataset list and click the down arrow  in the multiple display toolbar. Alternatively, you could select the middle spectrum and choose the up arrow.
- Deleting Spectra from the Display: to remove a spectrum from the display, select it in the dataset list and use . Note that multiple spectra can be selected and deleted simultaneously by holding down the SHIFT key before clicking.
- Expansions & Offsets: There are numerous icons in the main topspin toolbar to perform scaling, horizontal or vertical expansions and offsets. Using these while in multiple display mode will affect all the displayed spectra equivalently. Note

that similar icons appear within the multiple display mode toolbar, but they have “S” included in the icon. This is because the operation only applies to the Selected spectrum. For example, to vertically expand only the bottom spectrum in the display, select it first in the dataset list and then use either the 2_S to scale up by 2 or \updownarrow_S to scale interactively.

Section 4: NMR Data Output

4.1 Print Active Window

For routine tasks, it is normally easiest to simply print the active TopSpin spectrum window. The idea is you customize the spectrum to show the region you want plotted as well as the information you want included such as peak picking, integrals, etc. (using the spectral display preferences ()). Even finer control of the display (color schemes, fonts, line thickness, etc.) can be achieved via a right-click in the data window and selecting “Spectra Display Preferences”. Once everything looks the way you want, generate the output using the icons near the upper right corner of the TopSpin window:

- Hardcopy: click on the printer icon (), choose the correct printer, and clicking Print. Note that parameters like orientation, print quality, margins, etc. are also accessible from this window.
- Electronic Copy: to export your data as a file, click on the export active data icon (). In the window that opens, you specify the location, filename and format (i.e. pdf, jpg, tiff, bmp, etc).
- Copy & Paste: you can also easily copy the display to the clipboard and paste it into a program like PowerPoint using the “Copy to Clipboard” icon ().

4.2 AutoPlot

Higher-quality figures can be produced using layouts. Layouts are files that specify the objects to be included in your figure (spectra, parameters, titles, etc.) as well as the exact positioning and attributes of each object. Commonly, layout files serve as a coarse template which get modified as needed via the TopSpin plot editor. However, if a layout file pre-exists that can be used “as-is”, one can plot the figure directly (bypassing the plot editor) by issuing the “**autoplot**” command. To ensure the proper layout file is being used (or to change it), simply type “layout” in the TopSpin command line. Alternatively, the LAYOUT parameter can be accessed via the PROCARS tab in the Automation block of parameters.

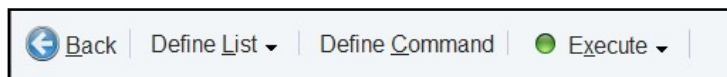
Maximum flexibility and figure quality are provided using layouts in combination with the TopSpin plot editor. Researchers are advised to consult the document “TopSpin 4 Plot Editor for details.

Section 5: Serial Processing

Many different types of NMR studies involve the acquisition of multiple NMR spectra. Often these spectra are acquired in an identical fashion and need to be processed in an identical fashion. It can be very tedious and laborious to process large numbers of spectra manually and identically. Fortunately, TopSpin provides auto-processing tools for exactly this purpose.

5.1 Processing a List of Experiments

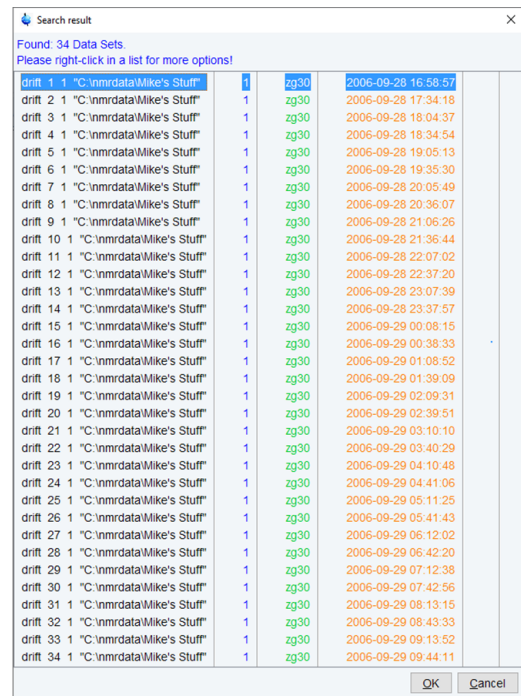
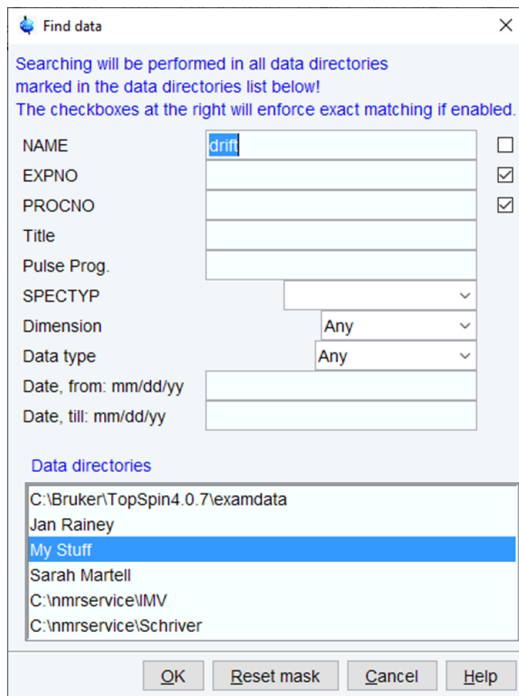
Serial processing of NMR data can be launched by typing the command “**serial**” in the command line. This will open a new flowbar as shown in the accompanying figure. The process involves three steps:



1. Define List: Specify the dataset names you want to include in the serial processing.
2. Define Command: Specify the processing commands you want TopSpin to perform on each dataset in your list
3. Execute: start the serial processing

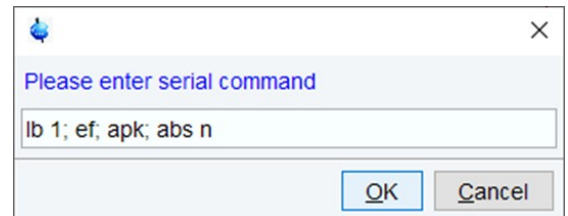
5.1.1 Define List

Serial processing requires a dataset list. If one already exists, click on the Define List button to specify the list name or to browse for it. If you are building a new list, click the drop-down arrow and select “Build Dataset List Using Find”. This will open a window allowing you to specify your search criteria as shown in the next figure. For example, at the bottom you can specify which data directories to search. In addition, you can filter according to a date range. Experiment names can be specified in the NAME field. Once your criteria have been specified, click OK which will show you the list of experiments that match your search criteria. From here, select the experiments you want to include in the serial processing and then click OK. Hold down the CTRL key to select individual experiments or the shift key for a range of experiments.



5.1.2 Define Command

The next step is to click on the Define Command button which will open a window to specify the individual processing commands you wish to apply. Although multiple commands could be applied individually, it is more time efficient to specify them serially. The figure shows a serial request for setting LB to 1 Hz, exponential multiplication and FT, automatic phase correction, and an automatic baseline correction without integration. Note that individual commands must be separated by a semi-colon.



Another approach would be to specify the processing commands in a TopSpin macro (use the “**edmac**” command). For example, I could create a macro called “myproc” which contains the same commands specified serially above. In this case, in the serial command field instead of typing out the individual commands one would call the macro using “xmac myproc”.

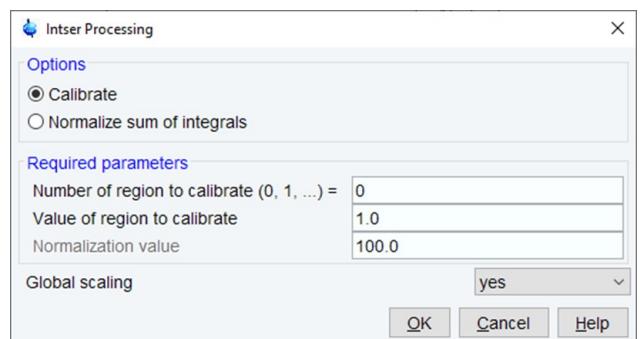
5.1.3 Execute

The final step is to click on the Execute button. A window will open showing a summary of the processing commands and your data set list. Click ok to start the serial processing. Note that by default the processing is done in the foreground. If you wish to continue to work in TopSpin during the serial processing, click the drop-down arrow beside Execute and select Execute in background. When finished, a window opens specifying Serial Processing Finished.

5.2 Integrating a List of Experiments

In an analogous fashion, a list of NMR experiments can be integrated serially. The procedure is closely related to serial processing with one major difference; the first experiment in the list must be integrated manually before starting (using the methods described in Section 3.2). When finished manually defining your integral regions, ensure you save them before exiting.

Next, start the serial integration by typing “**intser**” in the command line. This opens a flowbar which allows you to define an experiment list, define parameters, as well as execute the serial integration. The procedure for defining experiment lists is identical to that described in the previous section and will not be repeated here. Clicking on Define Parameters opens a window like that shown in the figure. The following options are possible:



- Calibrate: allows you to specify the numerical value of one integral; all other integrals will be relative to this one. When calibrate is chosen, in the required parameters section you must specify which integral region to calibrate as well as the

value for this region. Regions are numbered 0, 1, 2... and are always ordered starting at the high frequency end (left side) and moving towards the lower frequency end (right side).

- Normalize sum of the integrals: this sets the sum of all the integrals to a desired value which is specified in the "Normalization Value" field within the required parameters section.
- Global Scaling: set to either yes or no. When set to yes, all integrals in all spectra get scaled relative to the calibrated or normalized values in the reference spectrum (the first spectrum). When set to no, each spectrum in the list is individually calibrated or normalized depending upon your selection.


When the parameters are set, click Execute to start. The serial integration happens in the background. Once complete, an information window opens showing the integration results, which can easily be imported into a spreadsheet program for further processing/plotting.

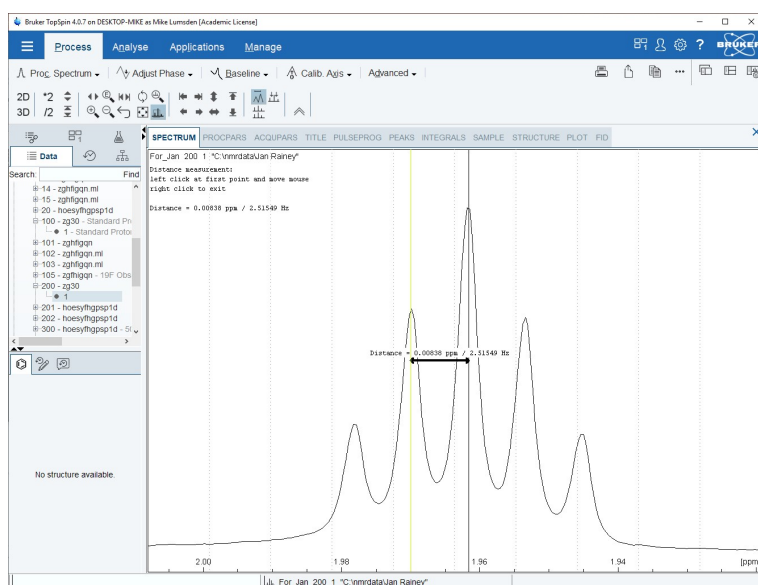
Section 6: Miscellaneous

This final section will be reserved for several options or measurements that you may find useful when analyzing your 1D NMR spectra.

6.1 Measuring Coupling Constants

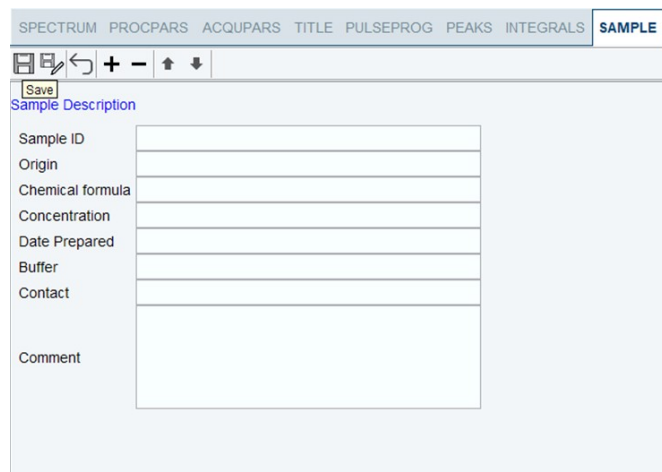
Coupling constants (J-values) are most easily obtained using the distance measurement tool. To measure a J-value, proceed as follows:

- Expand the spectrum such that the multiplet you are interested in occupies the display
- In the upper toolbar, click on the distance measurement tool  (it stays shaded)
- Position the vertical marker to correspond with the center of one of the peaks of the multiplet and then left click and hold.
- While holding, drag the mouse to position the second marker such that it corresponds with the center point of another line of the multiplet. The distance (J) is displayed on the screen in both ppm and Hz.
- Repeat for any additional couplings you are interested in. To exit, right-click in the spectral window or simply move the mouse anywhere outside of the window.



6.2 Sample Description Tab

The Sample tab allows you to expand on title information and optionally record additional details about your sample. The default interface is shown in the figure, but it is important to note that the format can be completely customized. Individual items can be selected for deletion using the minus-sign or re-ordered using the up-down arrows. New fields can be added using the plus-sign.



The screenshot shows the "SAMPLE" tab in the Bruker TopSpin software. The "Sample Description" section contains a form with the following fields: Sample ID, Origin, Chemical formula, Concentration, Date Prepared, Buffer, Contact, and Comment. Each field has a corresponding input box. The "Contact" field has a larger, multi-line input area. The form is designed for entering detailed information about the sample being analyzed.