

## Introduction to the TOPSPIN program

These notes include a general introduction to the TOPSPIN program, and a step-by-step guide to the acquisition and processing of 1D spectra. TOPSPIN runs on the Windows PC platform and you must use the “*nmr-user*” domain account to login to the spectrometer. When logged in use the TOPSPIN icon on the desktop to start the program.

### The TOPSPIN program

#### 1. Interacting with the program

Interaction with the NMR program is either *via* keyboard commands or via choices made by selecting pull-down menus and icons with the mouse. In addition, the mouse (which has three buttons referred to as left, middle and right in the following descriptions) is used to interact with the spectrum directly. The use of the menus saves you having to remember many commands and allows you to browse through the options available, although the keyboard input may be faster when you are familiar with the system. Figure 1 shows the basic TOPSPIN display which you will always get when you run the program. Other windows also appear at various stages displaying other features such as the lock display or variable temperature controls, and other information such as parameter tables or peak-pick list etc.

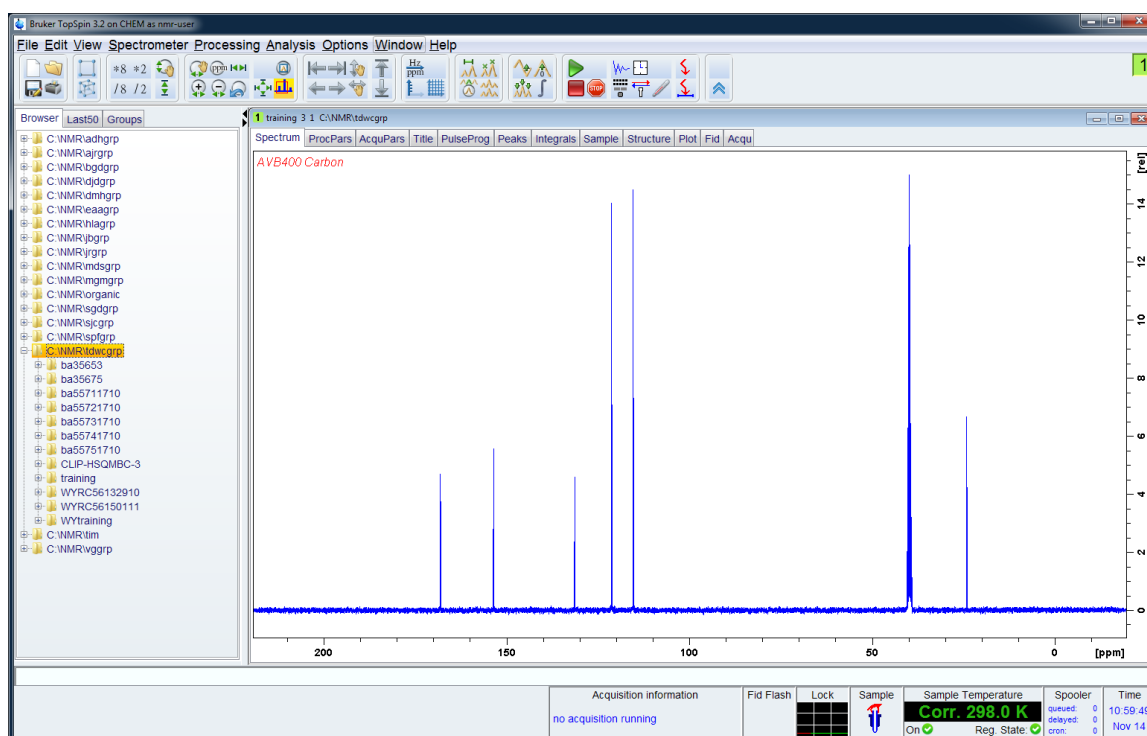


Figure 1: The main TOPSPIN display window.

The main areas of the display include:

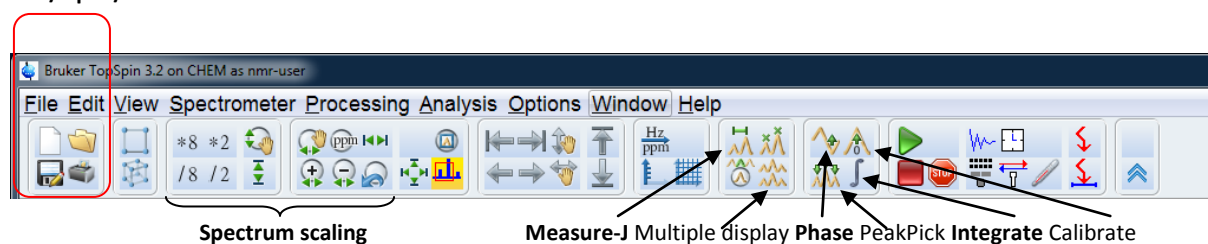
- **Pull-down menus:**



Most common commands can be found within these menus but for most operations it is usually easier to type in commands directly when you are familiar with the software operation.

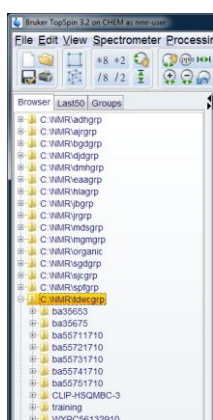
- **Icon panels:** Some useful functions are indicated below and explained in the sections which follow.

### New/Open/Print



- **Spectral Window:** This area is used to display FIDs and spectra.

- **Browser window:** This emulates the windows explorer menu and allows ready access to acquired datasets.



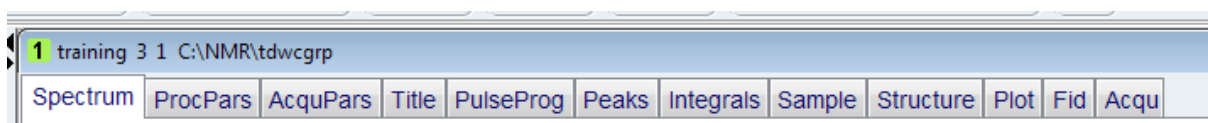
Data is held in group folders and may be transferred into the open spectrum window by a “drag & drop” process. Alternatively, right-click the mouse in the browser panel and select **Display in New Window** to open the data set in a separate window within TOPSPIN. The top right-hand corner of the TOPSPIN display shows small coloured squares, each indicating a separate window in TOPSPIN (data set or display). Move between windows by selecting different squares; multiple windows may be displayed using the *Window* → *Arrange ...* menu options.

- **Command line.** This sits at the bottom of the window and displays all keyboard command entries. Most commands found in the menus may be entered here also. Below this often appears a help line explaining the action of the icon the mouse currently sits on.

- **Identifier.** This text shows the data set currently in use. The syntax for this is described below under Naming Data Sets, but the example below indicates we are looking at a data set called *training*, looking at experiment acquisition 3 and processed version 1 and that the data is stored in the C:\NMR folder and belongs to user group *tdwgrp*.



This Main Display is used for interacting with *processed* data. Within this there are various tabs that take you to display tables for **Parameters**, a **Title** window, **Peak lists**, **Integral list**, and so on, plus the main interactive **Plot** tab. Help is available on-line also in the form of tutorials and complete manuals and is accessed from the Help menu.



## 2. Data acquisition

**2.1. Defining data sets** All data generated by the spectrometer are automatically saved to disk and stored in your group directory. Therefore it is always necessary to define your group and a name for your data set before doing anything else; *this should always be the first thing you do when you start a session on the spectrometer*. There is a

very real chance of you overwriting a previous data set if you fail to do this. Any given data set is identified by five parameters. They are:

- a) The sample name (NAME): this can be any name and should be used to identify the sample e.g. camphor, tdwc-sample99 etc. The use of your initials first is strongly recommended as this makes finding your data easier in the future.
- b) The experiment number (EXPNO): Identifies the "raw" acquired data. This means you may run a number of experiments under the same sample name, each identified by a unique sample number.
- c) The processing number (PROCNO): This identifies the processed version of the above raw data. This allows you to process the raw data in a number of ways and to keep each processed data under a unique number. For example, you may wish to transform an FID with and without resolution enhancement; you are able to store the two results with different PROCNO and compare them. Note however that if you process a raw data set with PROCNO set to 1 and then decide to process it differently, say with a window function, you must change the PROCNO first if you do not wish to overwrite the originally processed data.
- d) The data folder (DIR): this should always be your group data folder and is most easily defined by loading a previously acquired data set from your group folder before defining the new data set details.
- e) The user (USER): this will be your group log-on name

An example of the way data sets are identified may be:

NAME	EXPNO	PROCNO	DIR	USER
camphor	1	1	C:\NMR tdwcgroup	
camphor	2	1	C:\NMR tdwcgroup	
camphor	2	2	C:\NMR tdwcgroup	

These values are those in the identifier shown in Figure 1.

To define your current data set, use the command **new** (or **edc**) and complete the table entries. It is not necessary to complete the solvent, experiment or title entries at this stage.

## 2.2. Reading and editing parameters

### 2.2.1 Parameter groups

Parameters are stored in separate categories for acquisition & processing and may be loaded simultaneously with the **rpar** command. This will display a table of the available parameter sets through which you may scroll and select your desired parameters. Choose the appropriate parameter set (e.g. h1acq...) for your experiment and probe. Note that a different probe will use different parameters and the list can be restricted to the probe in use by the command **rpar \*xyz** where xyz represents the three-letter code of the probe in use. Select your parameter set and then Copy all... **Always ask if you are unsure which parameter set you should be using.**

The parameters can be viewed and edited with the commands **eda** (edit acquisition; **AcquPars** tab) and **edp** (edit processing; **ProcPars** tab). At this point it is necessary to point out the two different parameter classes; "Set parameters" are those you enter for use during acquisition, processing etc. and can be viewed in the tabbed views. "Status parameters" actually relate to the data already acquired or processed and can be seen by selecting the **S** icon in the parameter windows. For example, suppose you were to set initial parameters to acquire 64 scans, but you halted acquisition after only 32 scans. The **AcquPars** table will still indicate 64 scans, as would the parameter ns (number of scans) although this is clearly incorrect for the acquired data. However, the Status table would show ns as being 32, the actual number of scans for the data set. Similar considerations would apply for processing parameters.

### 2.2.2 Important acquisition parameters

Here we shall run through the most significant parameters for *routine acquisition of a proton spectrum*.

Data acquisition is *always* performed by running a specified "pulse program". Therefore it is necessary to specify parameters for the spectral region you wish to study, and for the pulses, powers and delays used by the pulse program.

**Pulprog:** The pulse program you wish to run. Basic acquisition will use the "zg30" or "zg60" programs, which are simple pulse acquire programs using a 30° or 60° observation pulse respectively.

**Nucleus:** The nucleus to be observed (1H).

**D1:** The relaxation delay between scans.

**P1:** The 90° pulse width.

Note that delays, pulse lengths and powers are actually held within arrays in these tables and can be seen by clicking on the appropriate Edit buttons, which will present a table of all delays, powers or whatever.

**PL1:** The power attenuation of the transmitter pulse, defined in either absolute Watts (W) or as a power attenuation relative to 1W output (dB). The output power is fully controllable and should be set to a high power for pulsing. This value also effects the value of P1. **Do not change this unless you are absolutely sure you know what you are doing!**

**SFO1:** The transmitter frequency in MHz. This can be set more conveniently by setting the frequency offset (O1 in Hertz or O1P in ppm); this frequency is that at the centre of your spectral window.

**SW:** This specifies the total spectral width in ppm. **SWH** is the equivalent parameter in Hertz. Typically 12 ppm for <sup>1</sup>H.

**TD:** The Time Domain data size (i.e. the number of data points in the FID). Typically 32k points.

**NS:** The number of scans to be acquired.

**DS:** The number of dummy scans before data is collected. Typically 2-4 to obtain a steady-state.

**RG:** The Receiver Gain. Set as described below in 2.5.

Most of these parameters will be set correctly in the standard parameter sets and it is not usually necessary to alter them. A useful way of checking the necessary parameters have been set is to use the **ased** command (the pulse icon within the **AcquPars** tabbed view), which will present a reduced table of the parameter values needed for the set pulse program. You may edit any of the values in this table if necessary but only do this if you know what you are doing. Having set the basic acquisition parameters, we must now lock and shim the sample.

### 2.3. Locking and shimming

It is first necessary to recall the appropriate shim set by entering **rsh**. A table will be presented from which you should select the appropriate shim set.

To lock onto a chloroform sample, for example,, simply enter **lock cdcl3** (alternatively just enter **lock** and you will be presented with a list of solvents to choose from; select one and the lock procedure will continue) and wait until you see the lock signal settle about 2/3 up the screen. (To see the lock display you must use **lockdisp** command). The lock power, gain and phase should be set correctly automatically.

Shimming (magnetic field optimisation) most conveniently uses automated gradient shim procedures which may be executed with **tshim**. Manual sample shimming makes use of software interactions via the mouse (called with the **bsmsdisp** command; see Figure 2 figure below), and will not be discussed at length here other than to note as a minimum you will always need to optimise Z and Z<sup>2</sup> and also X and Y for non-spinning samples (the default in most cases).

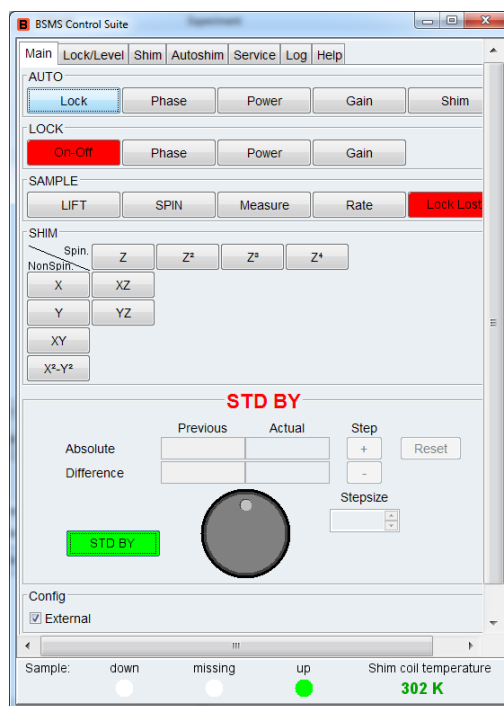


Figure 2: The *bsmsdisp* lock and shimming interactive panel

#### 2.4. Tuning the probe

Prior to acquiring data the probe must be tuned and matched to optimise its performance at the frequency of interest (analogous to tuning a radio to get the best reception). You must set the acquisition parameters and insert your sample before attempting to tune the probe. The tuning process is nowadays automated and is executed with the *atma* command (automatic tune and match). After a short while, you see a display on the screen that looks something like Figure 3 which will go when the process is complete. For experiments involving more than one nucleus in the parameter file, all nuclei will undergo this process after *atma* is issued and you must wait for this to complete before proceeding.

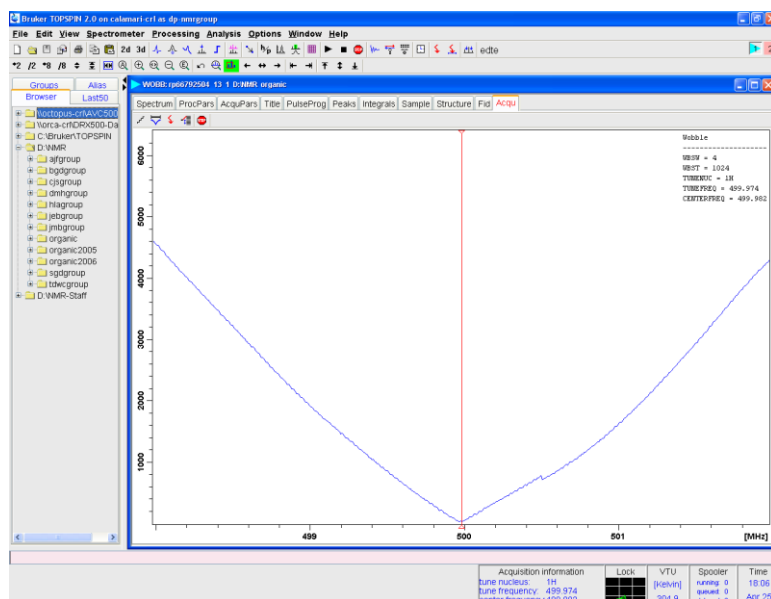


Figure 3: The *atma* tuning response of a well tuned and matched probe.

## 2.5. Acquiring data

It is now necessary to set the receiver gain (signal amplification factor) , and this is most conveniently achieved by using the automated sequence. Enter **rga** and wait a few minutes until you get the **rga finished** message at the bottom of the screen. To start data acquisition enter **zg**; the acquisition status box will display the target number of scans, the actual number of scans to date and the time remaining to the end of the experiment..

To stop the acquisition before reaching the specified number of scans, enter **halt**, which will write your data to disk at the end of the current scan and allow processing. Entering **stop** or clicking the STOP icon, will terminate acquisition immediately but will not save your data; it will be lost so be very careful with this command! If you wish to process and examine the data already acquired and you wish to leave the acquisition running e.g. to check the signal-to-noise ratio, you must force the data onto disk from the computer memory, where it is held during the acquisition. Use the transfer command for this: **tr**. You may then process the data in the Main Display, as described below. Once you have data on disk, you may now process it and interact with the result in the Main Display.

## 3. Data processing

The parameters that define the processing of data may be examined on the **ProcPars** tab (or **edp**) which will present them in a table. Many of these will be set automatically by the software, which is able to determine the type of processing required for the type of data acquired. One thing to notice here is the fact that the size of the spectrum (SI) should normally be set to one half the size of the FID (TD).

### 3.1 From FID to spectrum

#### 3.1.1 Fourier transformation

To produce the basic spectrum from the raw data you must Fourier transform them by entering **ft** . The spectrum will appear on the screen and can be manipulated as described below. The spectrum is saved with the processing number defined with **edc** and the raw FID remains on disk also, unchanged.

#### 3.1.2 Window functions

Often, you may wish to apply a suitable window function to your data before the transform, such as for sensitivity or resolution enhancement. Sensitivity enhancement is controlled by the parameter **lb** which defines the line-broadening required in Hertz. To carry out the weighting you should enter **em** (exponential multiplication) and then you may perform **ft**. A more convenient way is to use **ef** which performs both exponential multiplication and Fourier transformation. For resolution enhancement, you must define **lb** again (this time as a negative number as you require line narrowing) and **gb** which defines the point at which the window function reaches its maximum value (must be between 0 and 1). Similarly, **gm** will perform the weighting and **gf** will perform both weighting and transform.

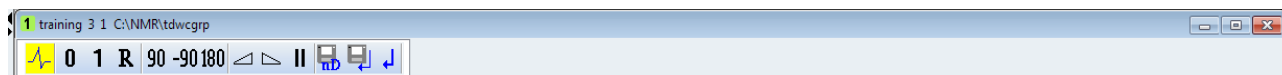
### 3.2. Manipulating the spectrum

Basic spectrum manipulation is performed with the icon buttons illustrated above. In addition to the icons, the spectrum display region may be expanded with the mouse buttons alone by dragging across the spectrum with the left mouse button selected and in TOPSPIN version 2 or higher the wheel mouse button may be used for vertical scaling. Clicking on the right button in the Spectrum window reveals a number of options; the **Display properties** option allows one to turn on/off integral display, peak picking etc on the spectrum.

### 3.3. Phase correction

This may be performed automatically or manually. Automatic correction is executed with the command **apk** and further small adjustments may be carried out with manual interaction

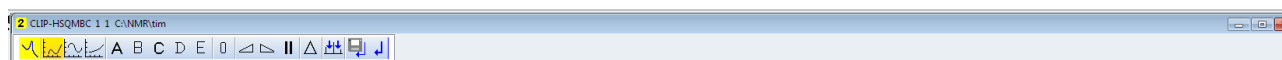
To enter the correction routine, click on the *phase* icon and a new icon panel will appear within the spectrum window.




By default the biggest peak is selected as the reference point for phasing as shown by the vertical line but may be redefined by placing the cursor on an appropriate peak at one edge of the spectrum, right clicking and selecting **Set Pivot Point**. Now place the cursor over the **0** icon, hold down the left mouse button and drag to correct the phase of the reference peak then repeat the process for the **1** icon and correct all other peaks in the spectrum. Click on the Save and Return icon (the disk and arrow symbol) to store the new phase data. Spectra may be processed identically at a later date with *pk* or *fp* (ft and pk) to give the correctly phased data.

### 3.4 Baseline Correction

Again, this may be performed automatically or manually. The automatic polynomial correction is executed with *abs* and is sufficient for most cases (note this is the same command as for performing automatic integration; to execute a baseline correction without regenerating a new integral list use *abs n*). The manual mode is entered via the *bas* command; select **Correct baseline manually** from the options.



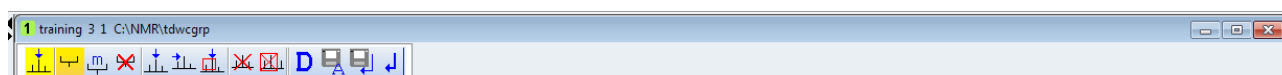
The default mode is a five function polynomial (A to E in the buttons). A line appears that represents the baseline zero and the object is to get this line to match exactly the spectrum baseline by adjusting the coefficients via click and drag on each. This begins with A (the linear offset) and goes through to E, with each causing curvature of the line at frequencies further to the right hand side of the spectrum. When a suitable fit has been found, click on  to see the result; further corrections in real time can be made at this point. Return from the difference mode with the *save and return* (disk) icon.

### 3.5 Calibration/referencing

This allows you define the chemical shift for any reference peak in the spectrum. Click on the *calibrate* icon and move the cursor over the spectrum. Place the cursor on the top of the reference peak and select with the left mouse button. Type in the desired reference in the window that appears (the units are as those chosen for the axis).

### 3.6 Peak Picking

Before attempting peak picking, you may wish to correct the spectrum baseline if it shows significant distortion in the regions of interest, as above. Automatic peak-picking may be executed with the *pps* command; the peak list may be viewed in the **Peaks** tab. If this proves inadequate the manual mode provides greater flexibility. Enter this mode with the *peak-pick* icon and select the icon shown in green (shaded) below (in general in TOPSPIN, a gold icon indicates this mode is selected or is turned on). You can now drag a shaded box over the peaks of interest, excluding those that are too small or too large as desired, and everything within the box is added to the peak list. You may draw multiple boxes to include all the required peaks, then use the save and return (disk) icon.

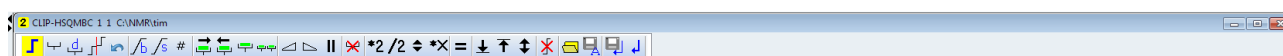


### 3.7 Integration

Before attempting integration it is necessary to have a well phased spectrum and any necessary baseline correction must have been completed (see above). As with phase correction, integration may be performed automatically or manually.

To automatically integrate a spectrum, enter the command **abs**; this procedure will also carry out a polynomial baseline correction on the data before selecting integral regions, so you will not usually need to do baseline correction yourself. The integrals are written to a file named 'intrng' and may be viewed in the **Integrals** tab).

To enter the manual integration routine, click on the *integrate* icon and a new icon panel will appear. To generate new integrals, it is necessary to define the appropriate regions. Select the first icon and drag over each of the peak regions to be integrated. Place the cursor over a integral region and use the right mouse button to calibrate the peak intensity or delete the region. Use the save and return (disk) icon to store the new integral regions.



### 3.8 Other features

#### 3.8.1 Multiple display

It is often useful to overlay spectra for comparison and TOPSPIN is able to overlay multiple spectra, both 1D and 2D (Figure 4). Enter this mode with the *multiple display* icon and a new panel appears in the Browser window that will contain the list of the displayed spectra. Add data to the current spectrum simply by dragging & dropping into the spectrum window the required processed data set. The displayed spectra may be scaled or moved (or removed from the display) independently by selecting a spectrum *via* clicking directly on this and using the scaling buttons on the icon bar within the spectrum window.

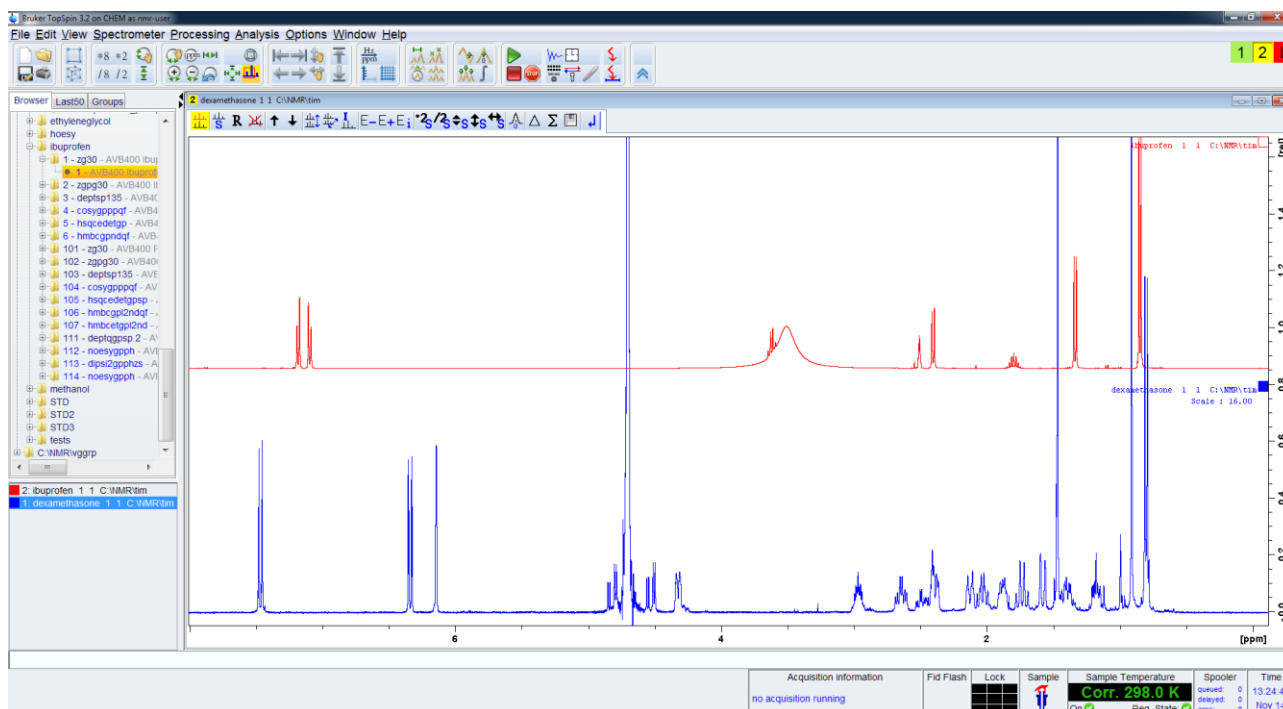


Figure 4: The multiple display window.



### 3.8.2 Measuring $J$ values

The values of coupling constants made be measured with the distance measurement tool. First expand the spectrum to show the multiplet to be analysed and then activate the *measurement tool* icon (it will turn green). Use the left button to fix the reference point on a multiple line and the drag to another line. Repeat this process for all splitting to be measured and then right-click to exit this mode (or move the cursor out of the spectrum window).

### 3.8.3 Sample information

As an alternative to the title window the **Sample** tab allows you to record pertinent information about the sample under study and may be a useful way to cross-reference the NMR data with laboratory book information. It is possible to add new named text boxes of your choice with the **+** icon should you wish.

### 3.8.4 Molecular structures

The **Structure** tab allows a chemical structure to be stored with the NMR data set. This can be drawn with the Bruker tools within the window or it may be imported as a mol file or pdb file for example. This facility is more appropriate when using TOPSPIN as a processing tool on local workstations rather than on the NMR spectrometers themselves.

## 4. Plotting

There exist two possible routes to plot from TOPSPIN:

- 1) Use the interactive plot editor in the **Plot** tab, or
- 2) plot directly the spectrum as on screen .

Method 1 provides most flexibility and highest quality whereas method 2 (print active window) is simple for routine output. Use the printer icon to reveal the printer options; those making use of **Plot** require layouts to be defined that position the various graphics items on the page, a list of which appear in the LAYOUT pull-down list in the dialogue box in the left-hand panel. The **Plot** editor is a fully interactive plot editor which allows you to position multiple plots, text, arrows, labels etc. on the page, has a similar mode of operation to graphical drawing programs and is now the recommended route for all plotting. Images may also be exported as graphics files according to the file extension used (eg .png, .pdf, .ps, .jpeg) and is again most appropriate for off-line data processing. The images below illustrate the main option panels that allow editing of the plot areas according to type (spectrum, title, parameters etc)- each area is activated by clicking within the area and is marked by green resize markers- see Figure 6.

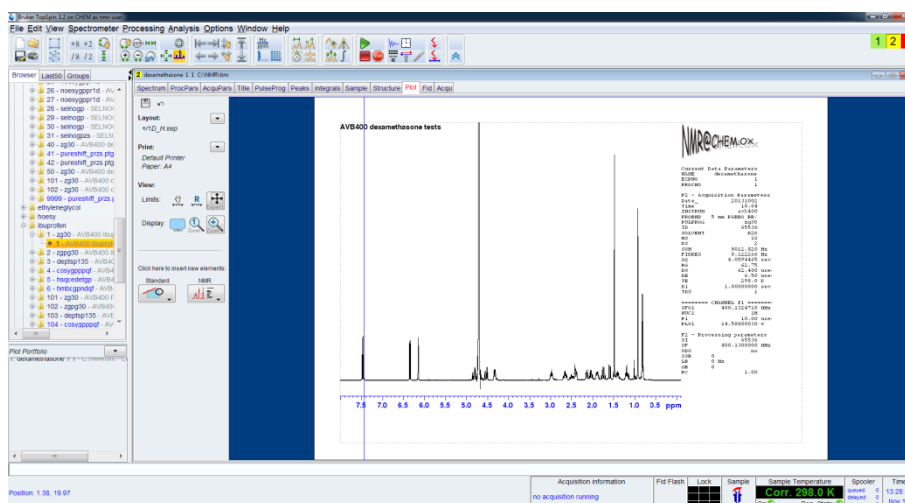


Figure 5: The main **Plot** tab showing Layout and Print options

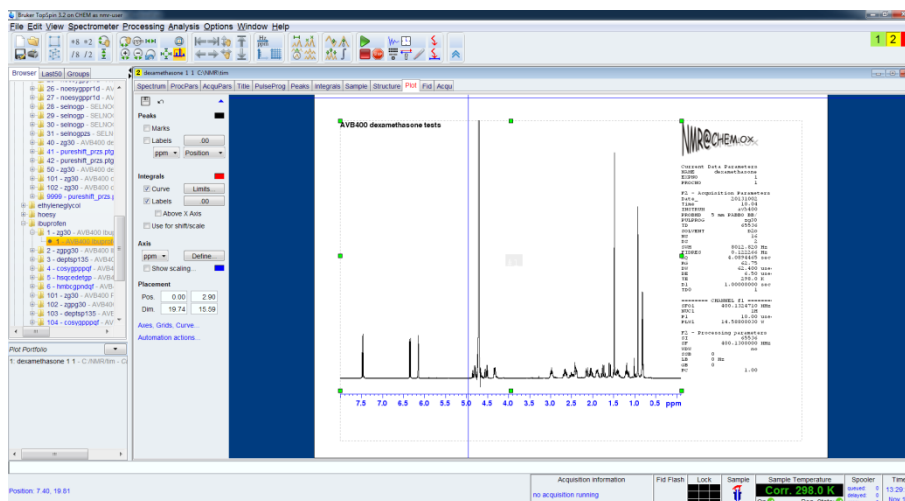


Figure 6: The *Plot* tab showing Peak Pick, Integral and Axis options

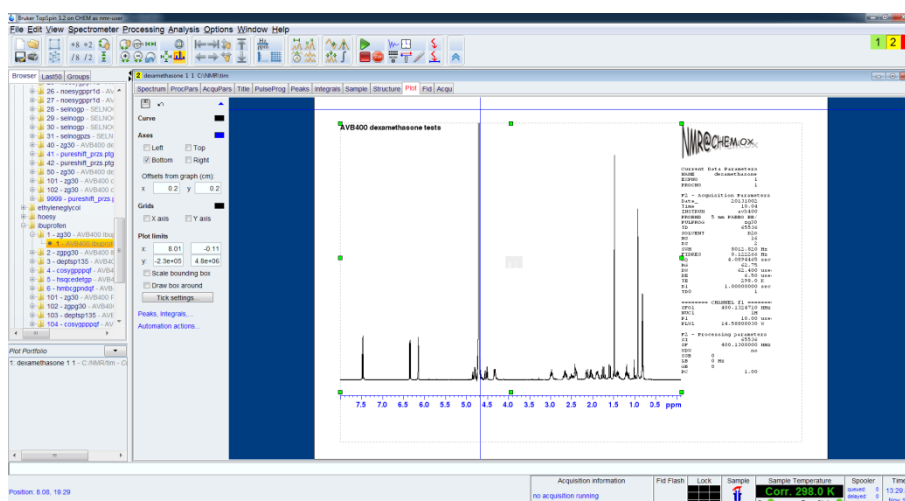


Figure 7: The main *Plot* tab showing Plot limit definition and other display options

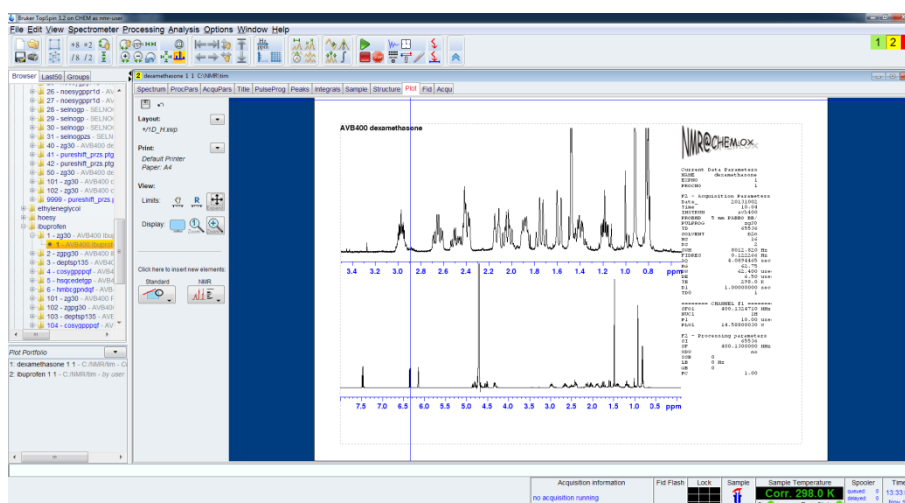


Figure 8: The main *Plot* tab showing multiple display options. The panel on the bottom left shows the selected data sets for multiple display options which may be populated via drag and drop of data from the browser.

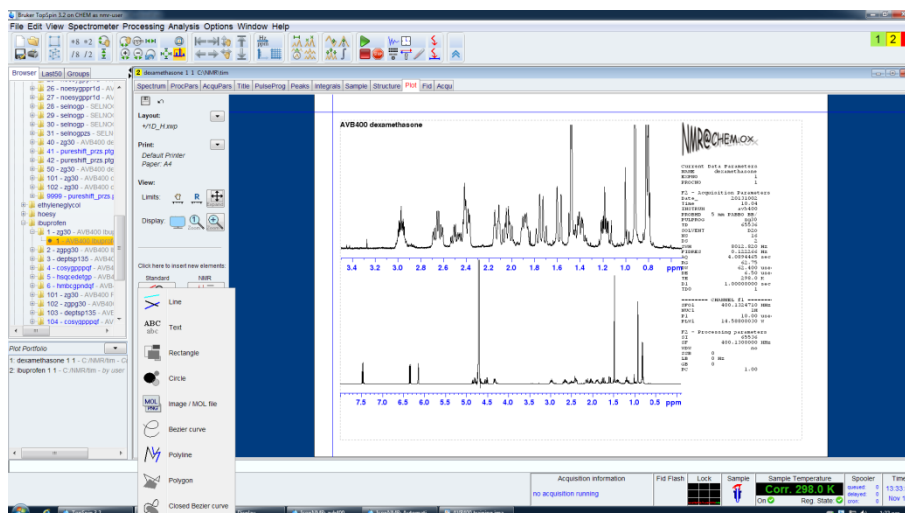


Figure 9: The main *Plot* tab showing drawing and labeling options

### Summary

This section serves as a short summary of the steps to follow to obtain a basic spectrum and may be useful when you have had some experience on the instrument.

1. Log-on to the system (if not already logged-in) by entering the *nmr-user* domain account and password.
2. Place your sample in the magnet ensuring you have set the sample tube in the turbine using the depth gauge. When using the robot use the command *sx n* to place a sample in the magnet (where *n* is the holder location) and *sx ej* to remove the sample. **ONLY INTERACT WITH THE SAMPLE WHEN THE ROBOT DOME COLOUR IS GREEN.**
3. Define your data set name with *new* (or *edc*).
4. Read in the appropriate parameter set with *rpar*.
5. Check the acquisition parameters with the **AcquPars** tab (or *eda*) if necessary.
6. Recall a shim set with *rsh*.
7. **Lock** onto your solvent with *lock "solventname"*.
8. **Shim** using *tshim* or manually as appropriate.
9. **Tune** the probe by use of the *atma* commands and let all channels complete.
10. Set the receiver gain with the *rga* command and wait for it to complete.
11. Acquire data with the *zg* command.
12. After the acquisition has completed or after you have used the *halt* command or the *tr* command you may process the FID. Use *ft* or *ef* to produce the spectrum.
13. You are now in the Main Display and may manipulate your spectrum as necessary.
14. Plot your spectrum directly or use the **Plot** tab to set the display as it will appear on paper and plot from there.
15. Turn off sample spinning (if used) and eject (*sx ej*) and remove your sample.