Vanderbilt Small Molecule NMR Center

Instructions for Using TOPSPIN

DRX-300: Setting up ^{1}H, ^{13}C (^{1}H decoupled), and ^{31}P NMR Experiments

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1 Getting started

1.1 Sample Preparation

Make sure your sample has a **minimum volume of 0.5ml.** Samples with less volume will be more difficult to shim and may require large changes in Z^3 and Z^4 in order to see a "good" lineshape. **Please remember to use a NMR tube holder for transporting tubes to the NMR Vault**. They are available in the Chemistry Stockroom.

1.2 Logging in to Spectrometer

Log into the PC using the username and password that was assigned to you by the NMR Staff

Once you're logged in, double click on the TOPSPIN icon to launch the program:

1.2.1 Find your data

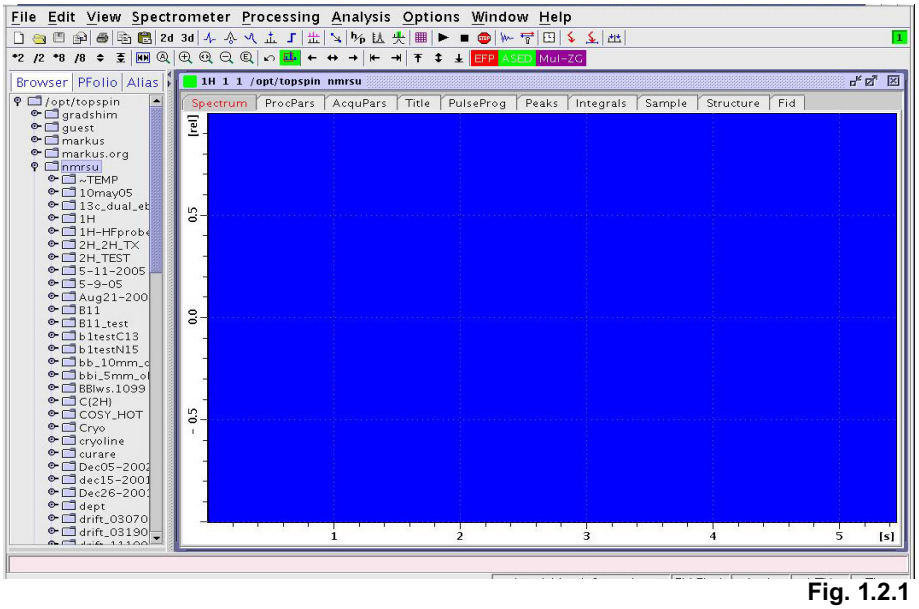


Fig. 1.2.1

All data is located in **/opt/topspin/data (Fig. 1.2.1).**

Simply locate your username on the left-hand side and click on the icon to view the sub-directories.

To select a file, click on the file and drag into the active window on the right.

If you have multiple experiments in that directory the following window should appear (**Fig. 1.2.2**):

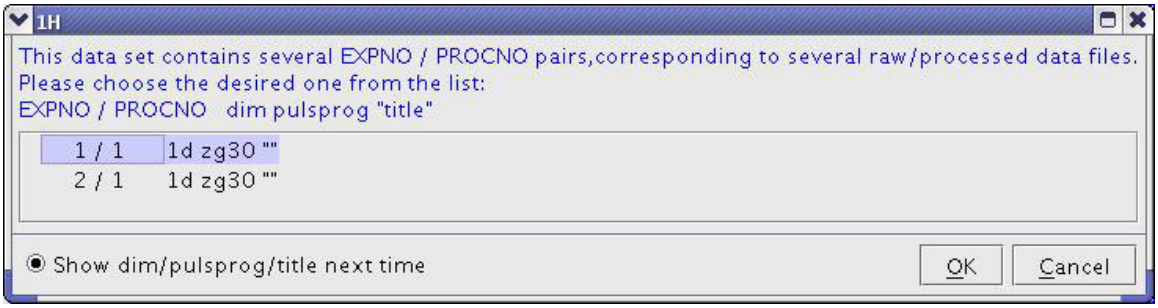


Fig. 1.2.2

2 Acquisition

2.1 Creating a New File

There are 2 Ways To Open the Window That Creates New Files

1) type "**edc**"
2) Go under "**File**" than "**New**"

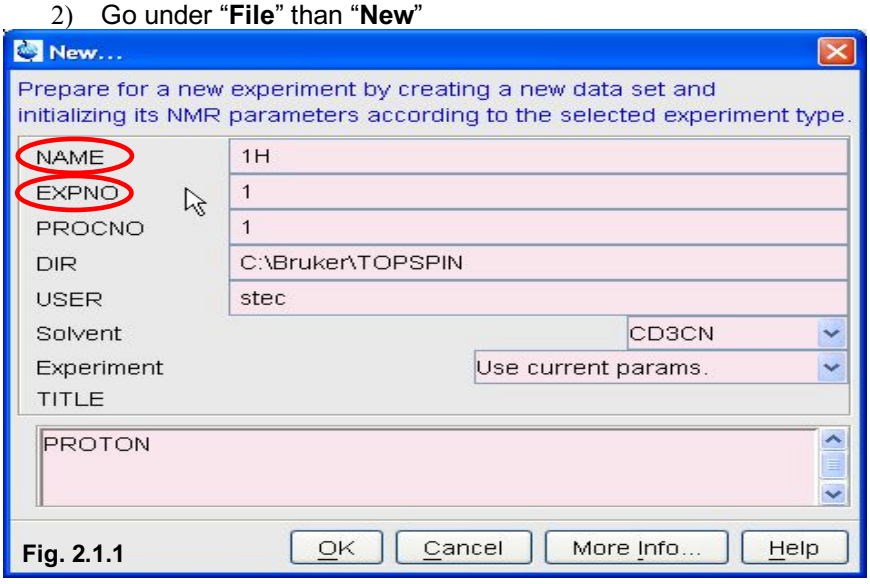


Fig. 2.1.1

A **NEW** file can be created by either changing the Name OR by keeping the same Name and incrementing the EXPNO # (**Fig. 2.1.1**).

If the name is changed use up to a maximum of 13 characters (alphabetical or numeric). Do not use any special characters such as (!@#$%&?).

Also, **DO NOT** include any spaces since this will create problems when the data is transferred.

Since data files are overwritten once a new experiment is started it is very important to create a new file before a new acquisition is started!!

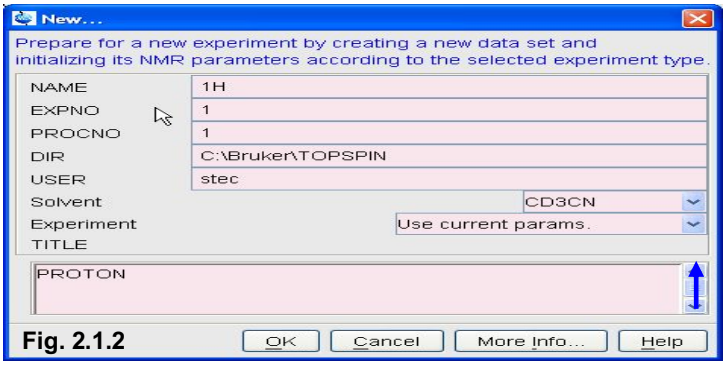


Fig. 2.1.2

The type of experiment can be selected by clicking on the icon across from "**Experiment**" (**Fig. 2.1.2**)

For **^{1}H** experiments select **AA_PROTON.MV**
For **^{13}C** experiments select **A_C13CPD.MV**
For **Coupled ^{31}P** experiments select **A_P31.MV**
For **Decoupled ^{31}P** experiments select **A_P31CPD.MV**

Finally, Type "gpro" to read in the current power levels

OR

the correct power levels can be recalled by clicking on the appropriate Icon (Fig 2.1.3):

Under the "AcquPars" window

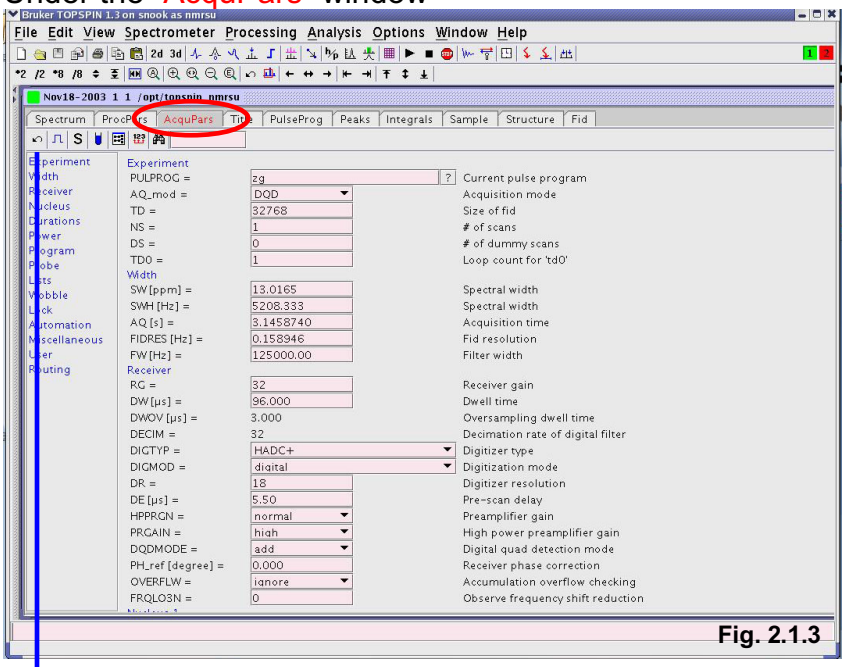
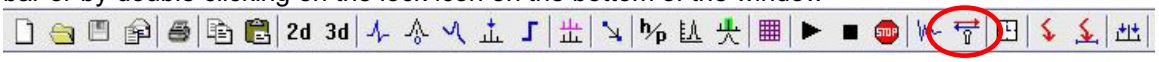


Fig. 2.1.3

click on the following icon:

2.2 Inserting and Locking Sample

Next, open up the Lock Window by either typing in "**lockdisp**" or selecting the "**Lock**" icon found on the top bar or by double clicking on the lock icon on the bottom of the window



Once TOPSPIN is opened, the sample can be placed inside the magnet.

1) You might want to check the temperature (type edte)
2) Make sure the **Lock, Autoshim and Spin** are **OFF** before ejecting the sample from the magnet!! Anything on the BSMS keypad that is illuminated green is on. To turn off the lock or spin, push the appropriate keypad button
3) **Remove the bore cap**
4) **Eject sample** (press lift on/off button, top left hand corner of keypad)
5) **Clean Sample and Place in depth gauge**
6) **Lower Sample** (by pressing the lift on/off button)

(type) "lock" or click on the "LOCK" button

Fig. 2.2.1

When the solvent list displays, select the proper solvent by clicking on it. (**Fig 2.2.1**) Computer will automatically adjust Field & auto-Lock

2.3 Sample Spinning

Since the main probe on the 300 MHz spectrometer is a direct direction probe it is recommended to spin the sample for increased resolution. Sample spinning is started by depressing the "**SPIN ON/OFF**" button located on the **BSMS** keypad. The sample is spinning when the green light is stabilized.

2.4 Shimming

Once the sample is spinning and you have locked on to the appropriate solvent, the next step is to shim the sample. The recommended method for sample shimming uses pulse field gradient shim routine which is fully automated.

2.4.1 Gradient Shimming with Gradshim

Before you start the gradient shimming program it is VERY important to return the shims to their default value. This is done by typing the following:

Type "**rsh chloshim.600**"

Once the default shims values are recalled, the gradient shimming program is started by typing "**gradshim**" or click on the "**SHIM**" button

Make sure your username is spelled correctly in the "**USER**" box, "**Shimming Method**" is set to "**1D2H**" and "**FILENAME**" is "**deflt1d2h**" (**Fig. 2.4.1**).

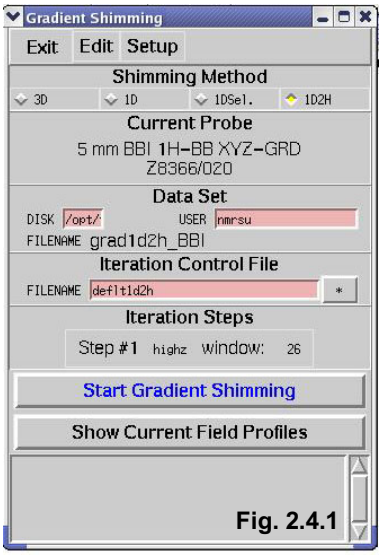


Fig. 2.4.1

When the automatic gradient shimming is completed the following box should appear (see below, **Fig 2.4.2**).

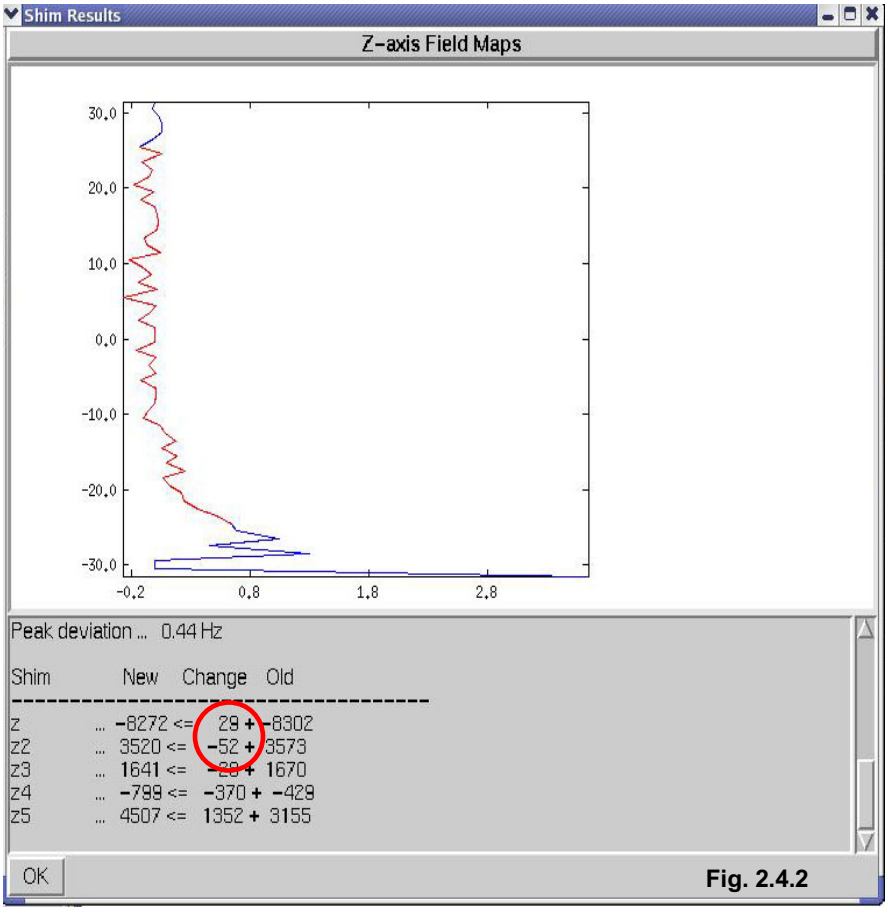
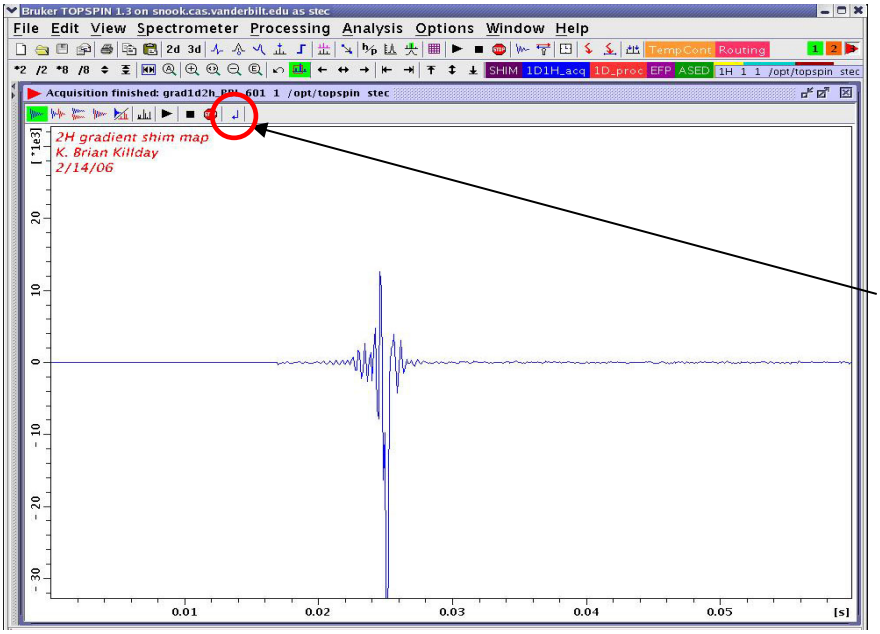


Fig. 2.4.2

The shim map on the left represents a map for a sample that was shimmed properly. Notice the change in the "Z" shim is less than 80. Any changed in "Z" less than 80 does not need extra shimming. I

Change in "Z" of +/- 80 or higher requires an additional shim!!

If the lock level becomes too high on the display and goes out of view, decrease **"lock gain"** on the BSMS keypad until the signal is observable on the lock display.



Once gradient shimming is completed it is very important to make sure that the proper experiment is selected for acquisition.

This is done by closing the gradient shim by simply clicking on the circled icon (Fig. 2.4.3).

Fig. 2.4.3

2.5 Acquiring a Spectrum

After maximizing the lock level, take one scan and check the lineshape. Before you take a scan you want to set the receiver gain. The command to set the receiver gain is:

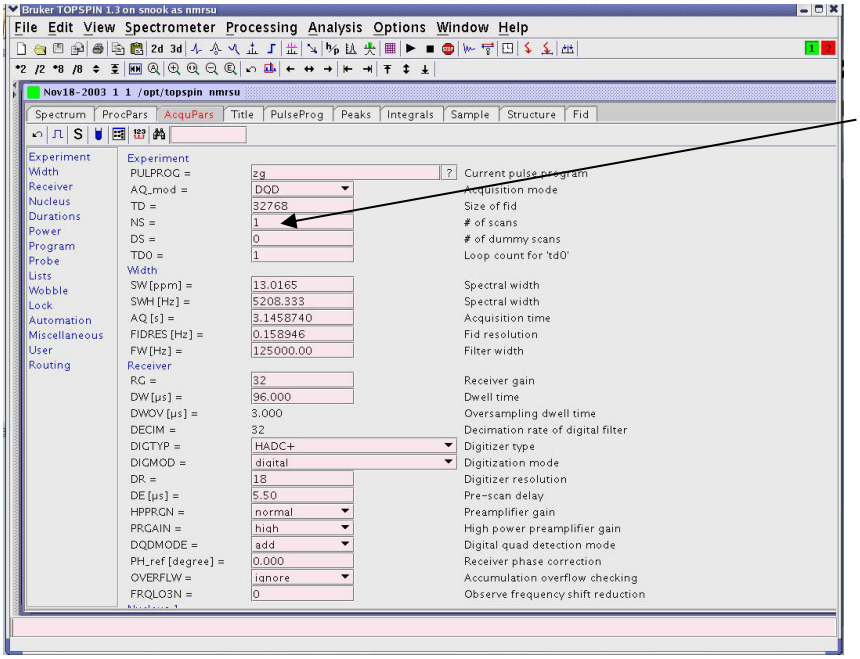
(Type) **"rga"**

Wait for the instrument to message that is has completed setting the receiver gain. Once it is done setting the gain you are ready to take a scan. The command to start an experiment is:

(Type) **"zg"** or click on the  icon located on the top-right of the TOPSPIN window

Type "**ns**" to set the number of scans or set the number of scans under the "AcquPars" window. The recommended number of scans is 16.

After setting the number of scans start the acquisition as described above.



You might want ot check the shimming and parameter settings by acquiring a single scan (NS=1) experiment followed by a fourier transformation and phase correction as described below

Fig. 2.5.1

3 Processing Spectrum

3.1 Fourier Transformation

(Type) **"efp"** or click on the **"EFP"** button
Keep in mind, that this uses the LB parameter (LB for 1H ~ 0.3 Hz, 13C ~ 2Hz).

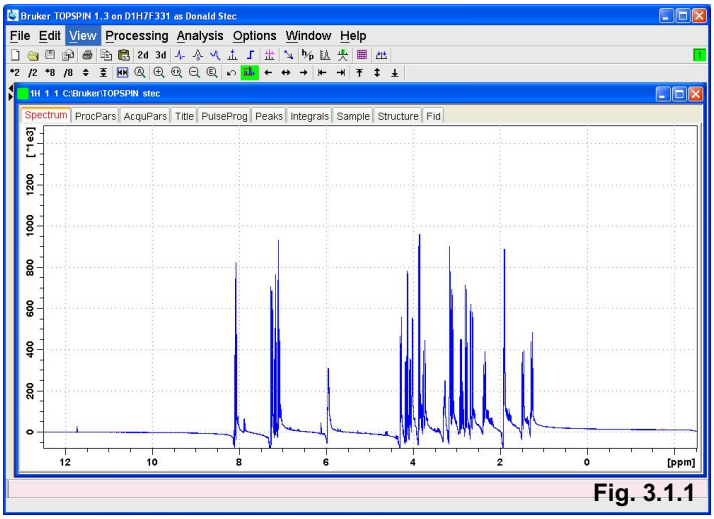


Fig. 3.1.1

- use these buttons to increase the scale so the baseline becomes easier to view
- use the “up” and “down” buttons to bring the spectrum to the center of the screen and to the bottom
- use these buttons to expand or contract your spectrum
- use this button to go back to the full display for your spectrum

3.2 Phase Correction

Click on the “Phase” icon

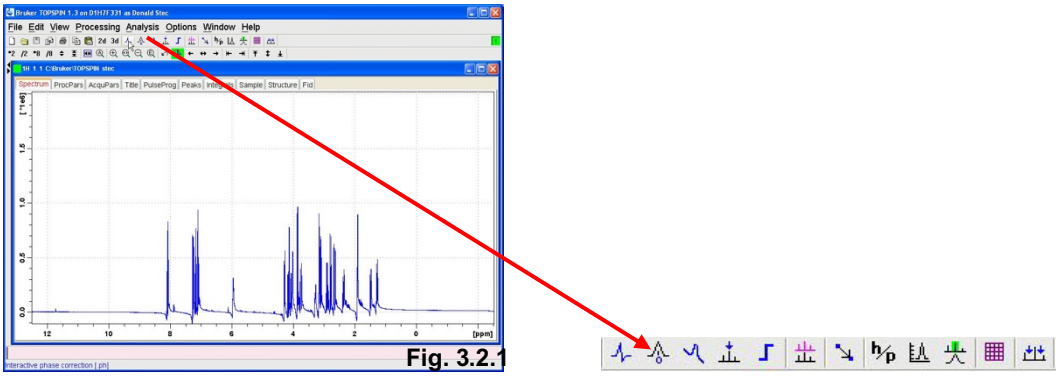


Fig. 3.2.1

This should open the following window:

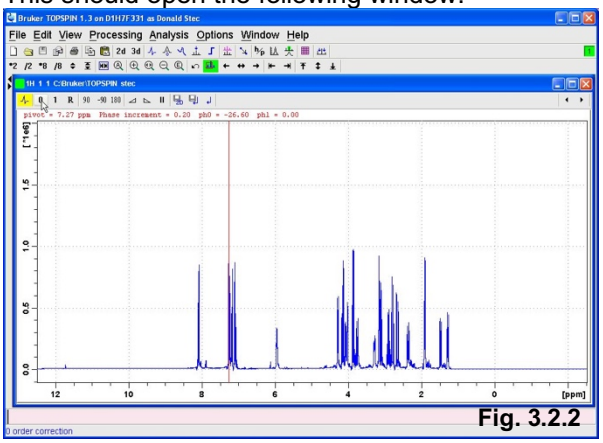


Fig. 3.2.2

To phase the spectrum, click and hold in on the “0” button for zero order phasing and the “1” button for 1st order phasing.

The “R” button resets the phase back to its original value while the 90, -90 and 180 buttons apply a 90, -90, and 180 degree phase automatically. Once the appropriate phase is applied click on the icon in the red box to save.

Check the solvent peak and other narrow peaks to make sure the lineshape is narrow and symmetric. This requires expanding the spectrum display.

3.3 Expand Spectrum

Click mouse 1 on either side of the spectrum where you would like to expand and hold down while dragging the mouse (**Fig 3.3.1**). A second cursor should appear. The expansion will finish once you release mouse 1 (**Fig 3.3.2**).

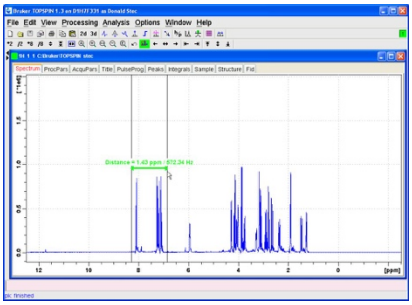


Fig. 3.3.1

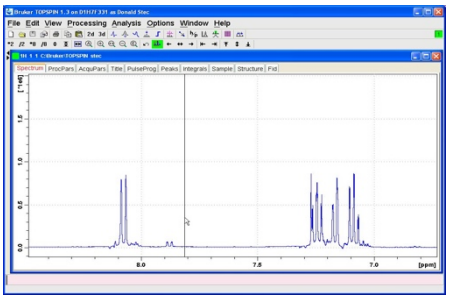


Fig. 3.3.2

3.4 Referencing

Now you can set the reference. The reference is usually set to a peak who’s position you are certain of such as a solvent peak or TMS. The command sref does a rough calibration of the spectrum.

To set the reference click on the **Calibrate** button:

Selecting the calibrate button will open the following window (**Fig. 3.4.1**)

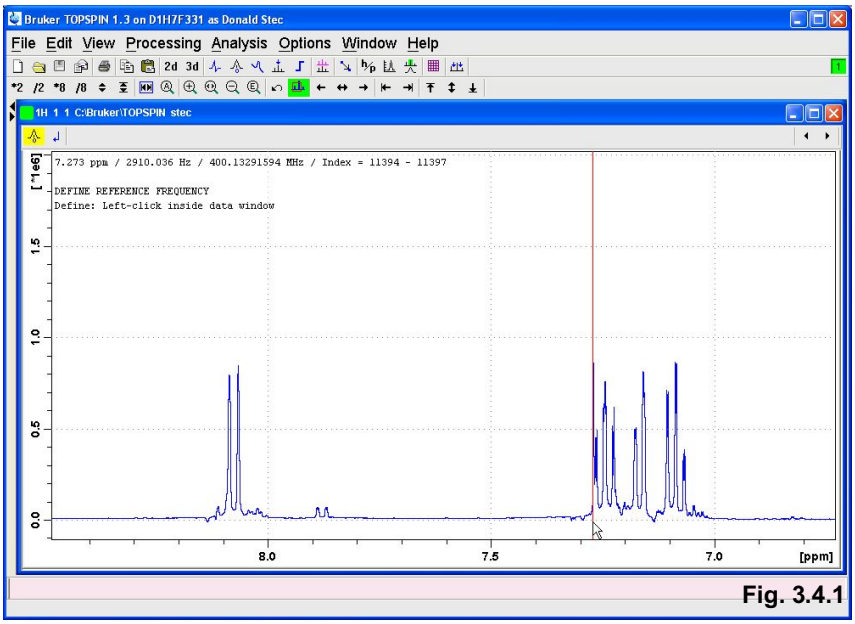
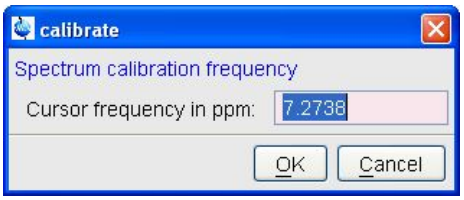


Fig. 3.4.1

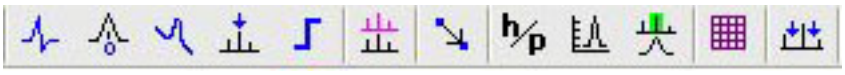
With the red cursor in the center of the peak you would like to use as a reference click with mouse 1 and the following window should appear



Simply type the correct value into the box and select “OK”

Now that you have set all the parameters, it’s time to collect a spectrum with multiple scans. If you click on the “**1D1H_ACQ**” Icon it will automatically acquire, process and integrate a 1H NMR spectrum with 16 scans.

3.5 Baseline Correction



Select the baseline correction icon and the following window will open (**Fig. 3.5.1**):

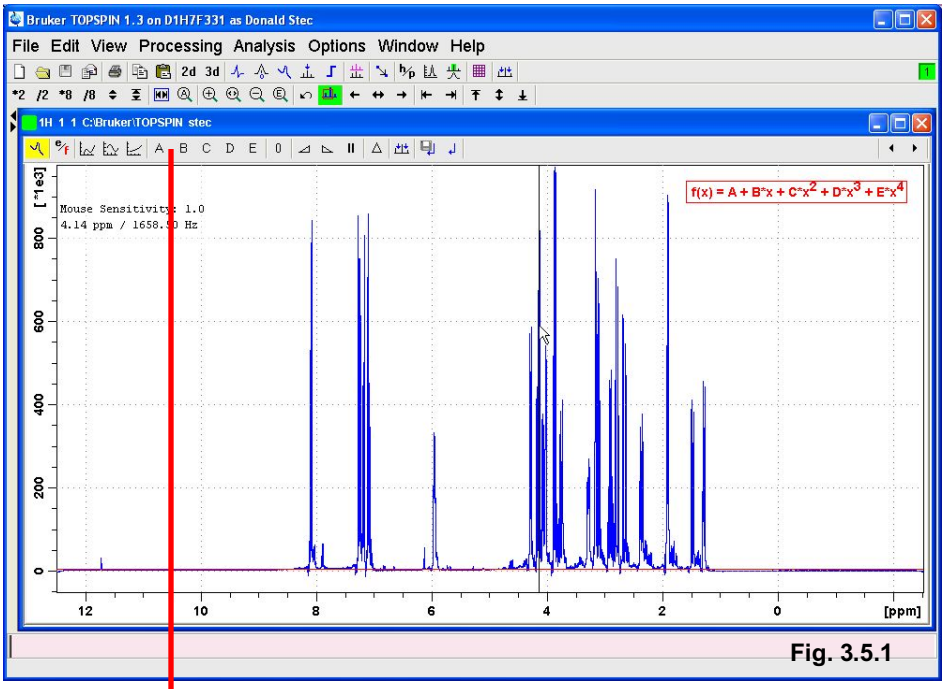


Fig. 3.5.1

- Selects the proper function (polynomial, sine, exponential). The default is polynomial
- Use these buttons to compensate of the offset (A) and to make sure the fit is running through the center of the spectral baseline (B,C,D,E)
- Once corrections are made select this button to SAVE and RETURN

3.6 Integration

Click on the "**Integrate**" icon

The following window should appear (**Fig. 3.6.1**):

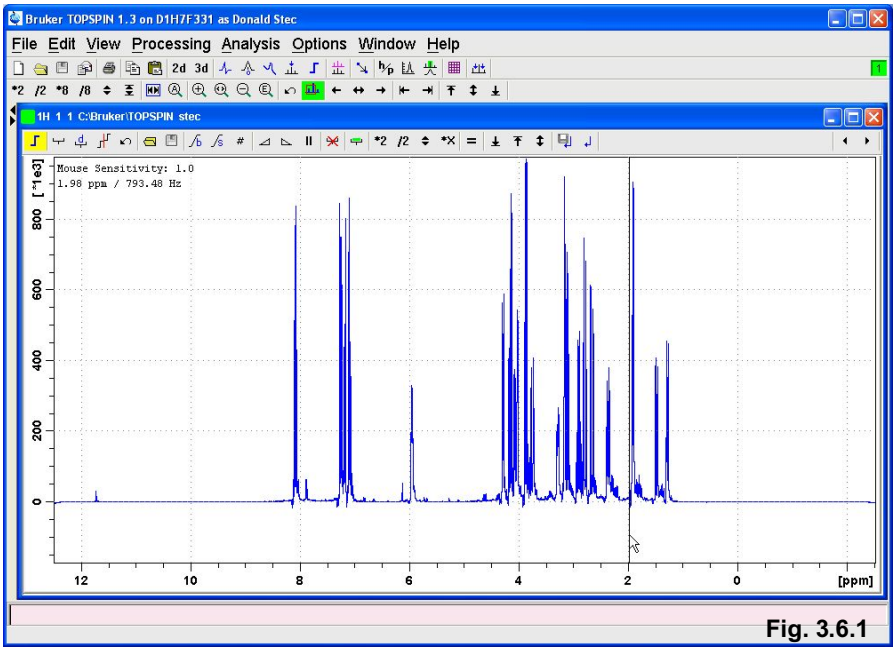


Fig. 3.6.1

Before you integrate, expand around the area of interest following the directions for expansion listed above.

Click on this icon to activate the integral mode and the following window will appear (**Fig. 3.6.2**) (once the icon is "active" it will change color to green)

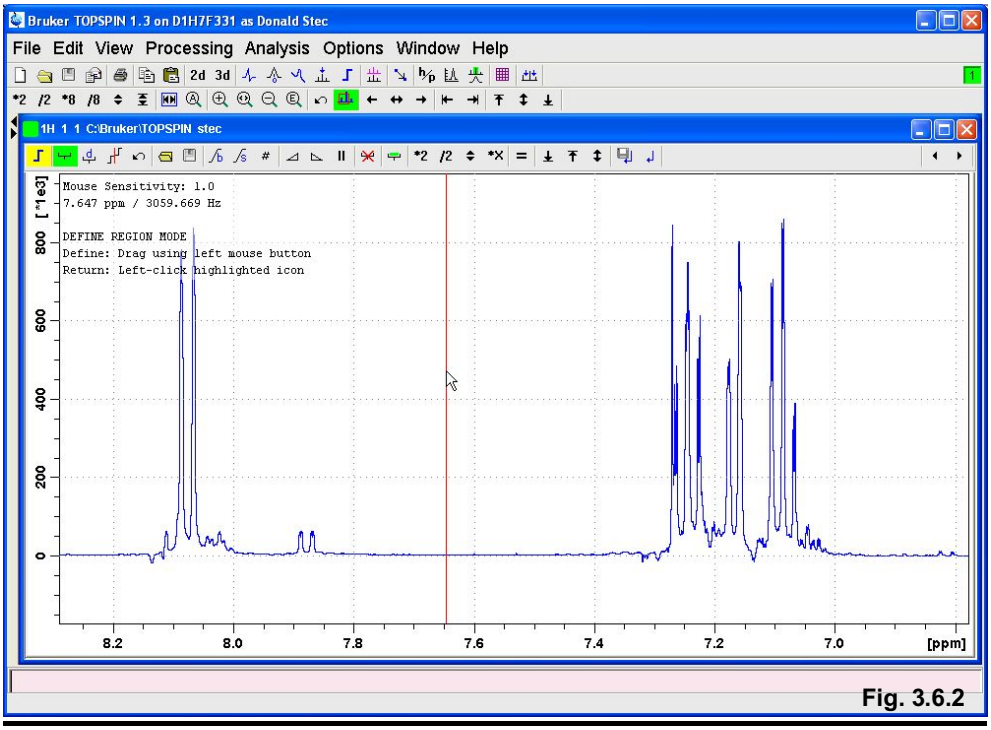


Fig. 3.6.2

To integrate, click on one side of the peak of interest using the left mouse and hold down. A second cursor will appear. Release the mouse when the cursor area covers the peak you would like to integrate (**Fig. 3.6.3**)

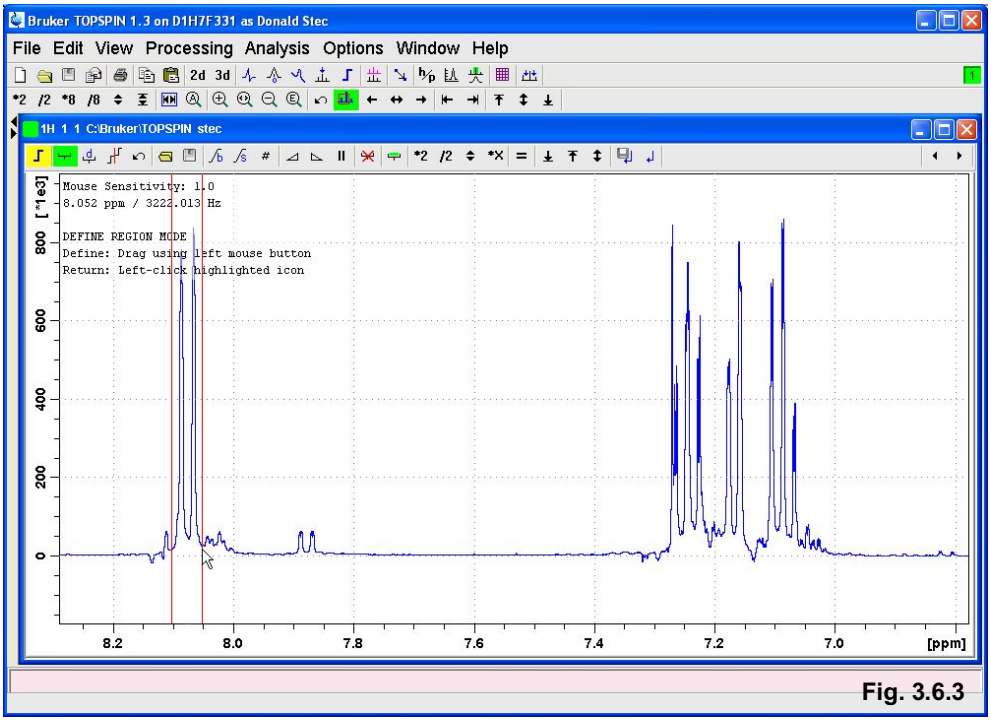


Fig. 3.6.3

Once an integral is set, a value will appear. To calibrate the integral to a reference number, click on the right mouse between the integral limits and select “Calibrate” in the window that appears. (**Fig. 3.6.4**) A new box will appear where you can modify the value. You can also delete the current integral using the same window

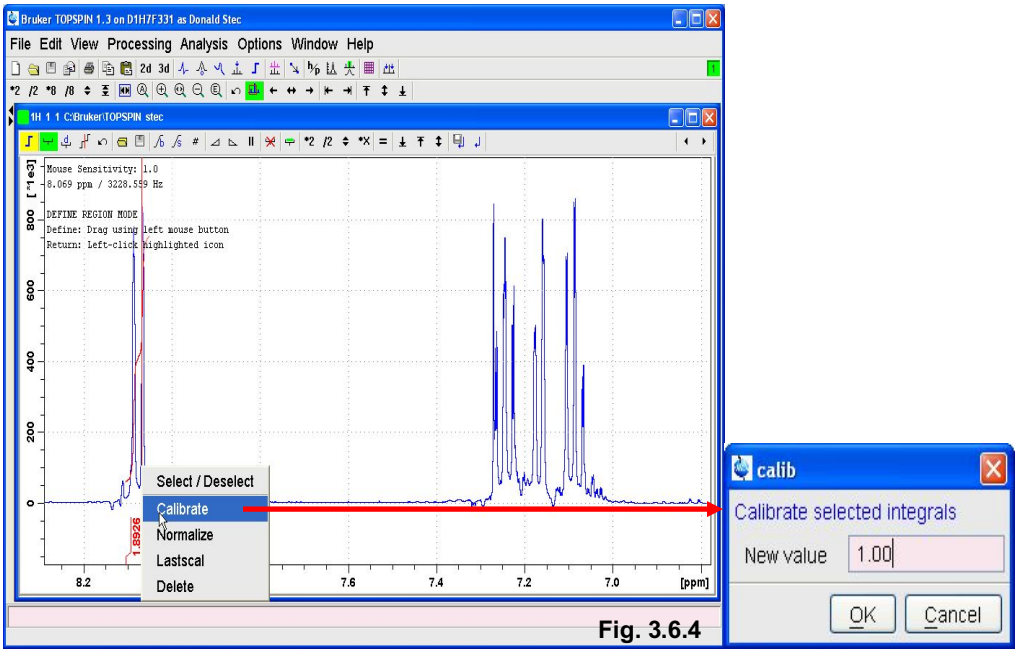


Fig. 3.6.4

- Use these keys to adjust the basis and slope of the integral
- Use these keys to define and delete integral regions
- Use these keys to manipulate vertical scale of integrals
- **USE THIS KEY TO SAVE INTEGRALS**

Once integrals are saved, they may disappear from your active window. To make your integrals appear in your active window, right click on the mouse and select "**DISPLAY PROPERTIES**". The following window will appear (**Fig. 3.6.5**):

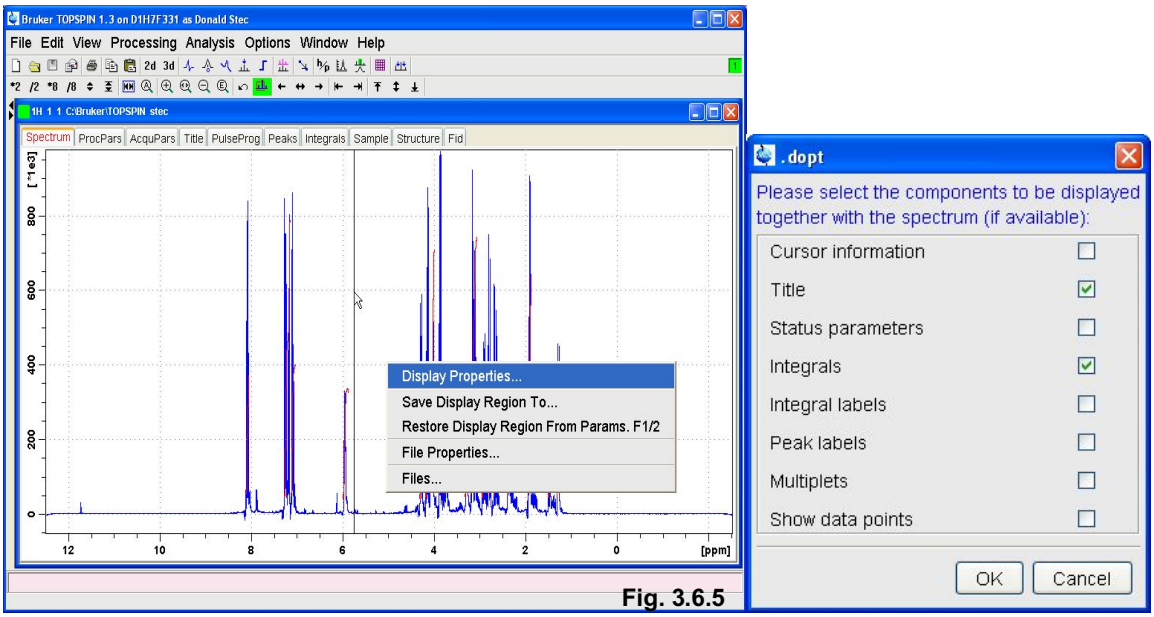
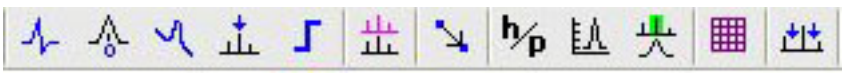


Fig. 3.6.5

Simply click on the box for the parameter you would like to appear on your active window.

3.7 Peak Picking

Click on the "**Peak Pick**" icon



The following window should appear (**Fig. 3.7.1**):

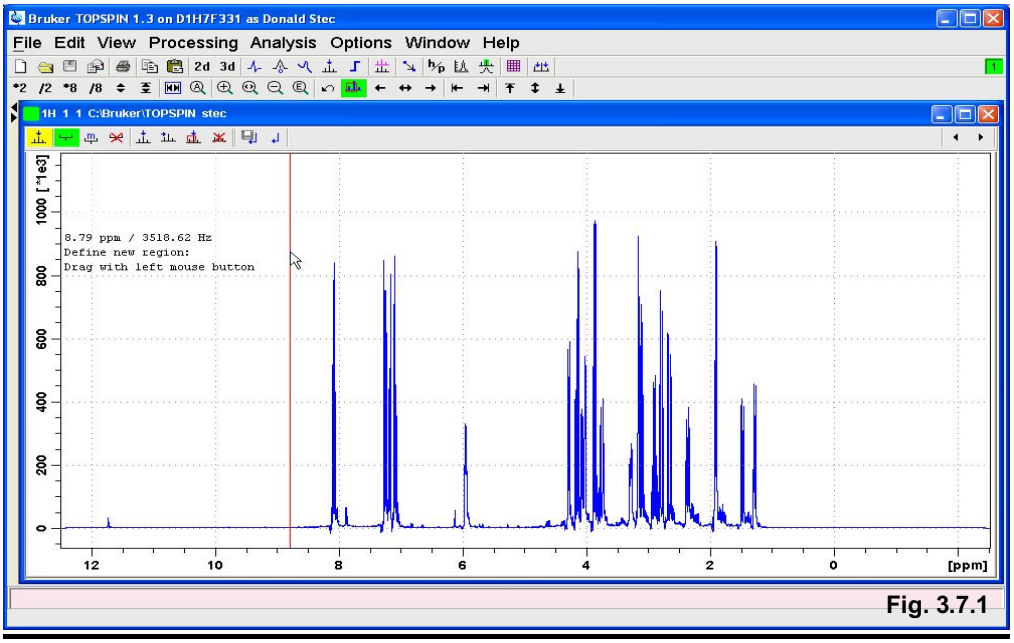


Fig. 3.7.1

To pick peaks, click on the left mouse and drag. A green box should appear as in the following (**Fig. 3.7.2**):

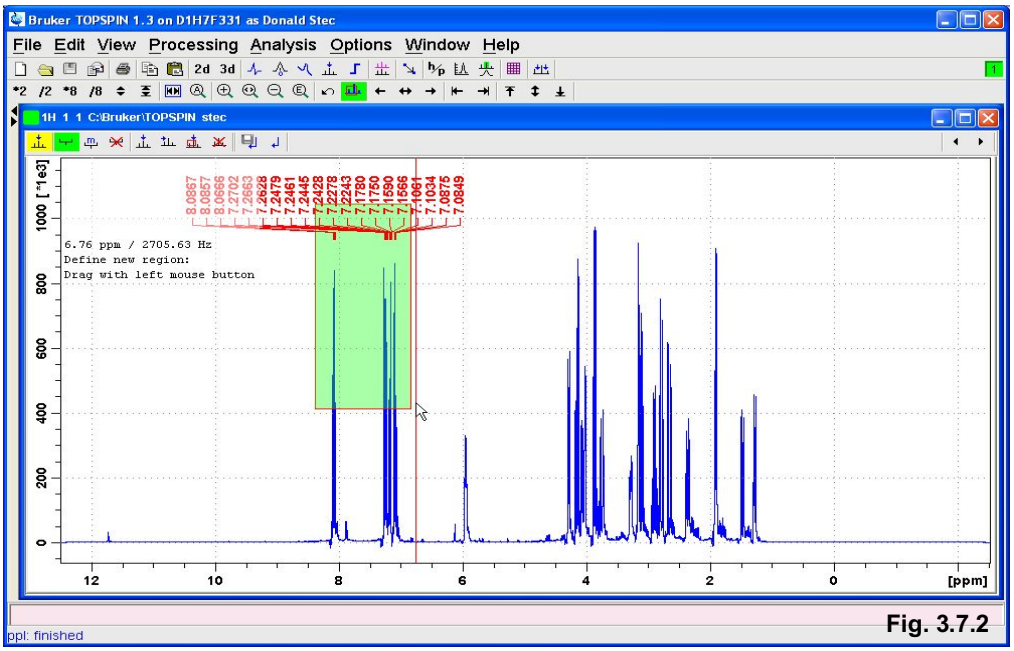


Fig. 3.7.2

Once you release the mouse the peaks should appear. Please remember, the lower the box is towards the baseline, the MORE peaks will be selected. To delete peaks, right click and select "**Delete All Regions**" (**Fig. 3.7.3**)

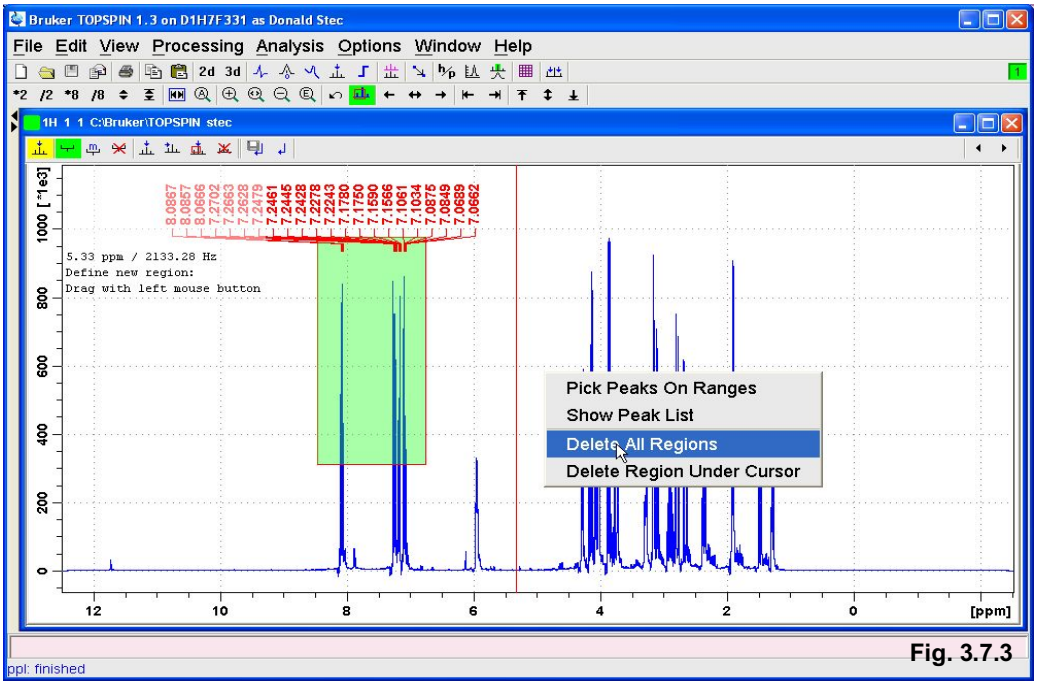


Fig. 3.7.3

- Use this icon to delete all peaks
- Use this icon to manually pick peaks
- USE THIS ICON TO SAVE PEAKS

To make your peaks appear in your active window, right click on the mouse and select "**DISPLAY PROPERTIES**" and make sure you select the "**Peak Labels**" box.

3.8 Setting the Title

Before printing you can set the title for the spectrum by selecting the "Title" icon. The following window should appear (**Fig. 3.8.1**):

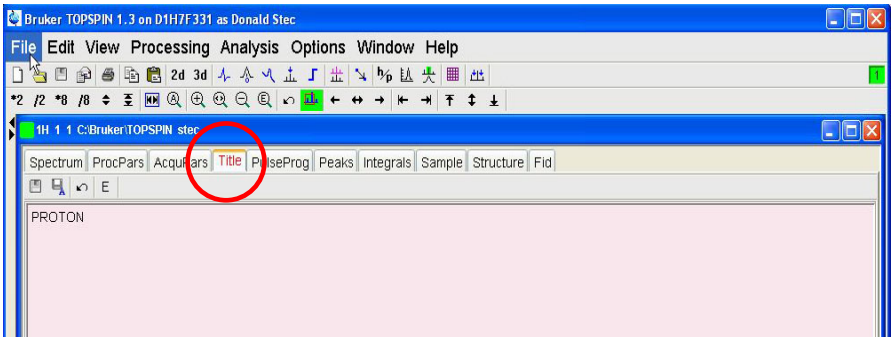


Fig. 3.8.1

To set the title, just click in the pink box and type the desired title. There are no character or space limitations.

3.9 Plotting

In version 1.3 of TOPSPIN, plotting is done using the Plot-editor.

To open up the plot editor click on the "**PLOT**" button

or

select "**File**" on the top left side of the window and than choose "**Print**" (**Fig. 3.9.1**)

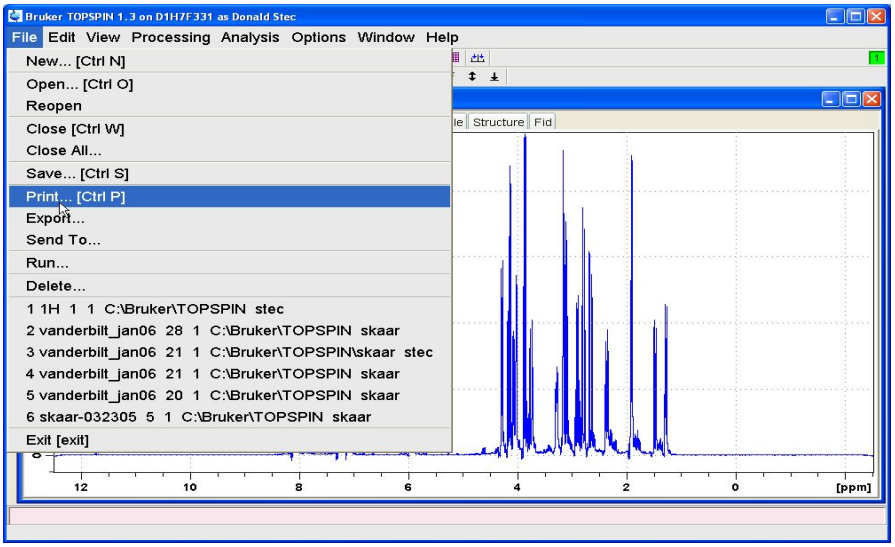


Fig. 3.9.1

Once you select "**Print**", the following box will appear (**Fig. 3.9.2**):

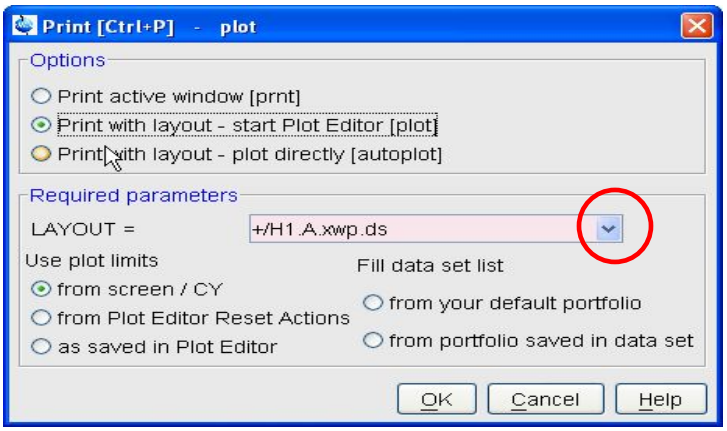


Fig. 3.9.2

Make sure "**Print with layout-start Plot Editor [plot]**" is selected and a Layout is selected as well. To select a layout, click on the arrow button. Once the proper layout is chosen the Plot-editor should open.

4 Finishing Up

1) Lock = Off Spin = Off Eject Sample (make sure bore cap is removed before ejecting)
Autoshim = off (if used)

2) Put standard sample back inside the magnet

3) Put the bore cap back in place!!!!

4) Exit Topspin program and log-out of instrument (right click on desktop)

If Problems Occur Please Contact Don or Markus

4.1 Copying Data onto a Flash Drive

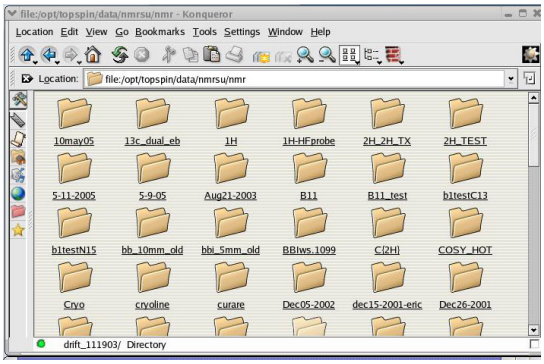
1) Open up data window by clicking on your home icon 

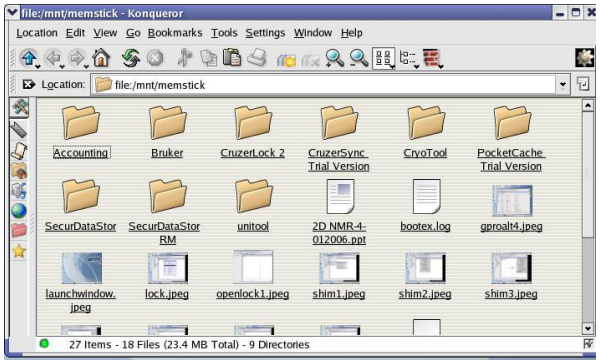
located on the Desktop.

2) All data is located in the following path**:/opt/topspin/data/*your_username*/nmr**

3) Open up data stick window by clicking on icon 

4) To transfer data:
Simply drag a file from your data directory to the USB Stick directory





5) Once you drag the file over the following message will appear:

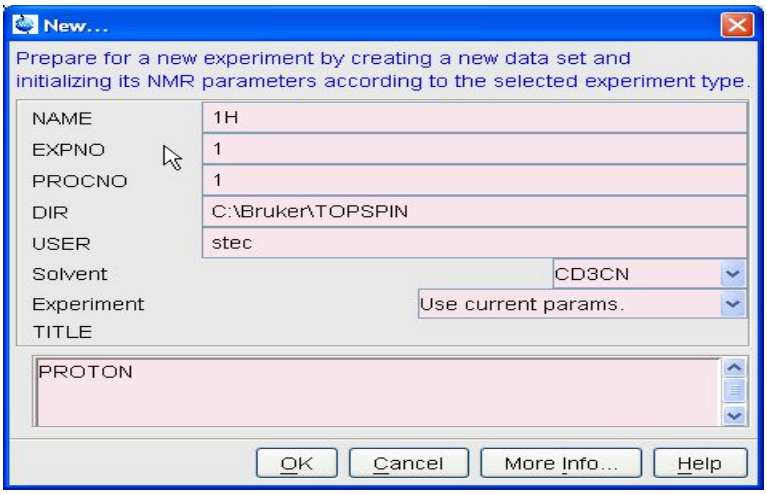
Select “Copy Here” and file should be saved on USB Stick

6) Remove USB Stick properly:
   a. Right click on USB drive icon
   b. Select the “unmount” option

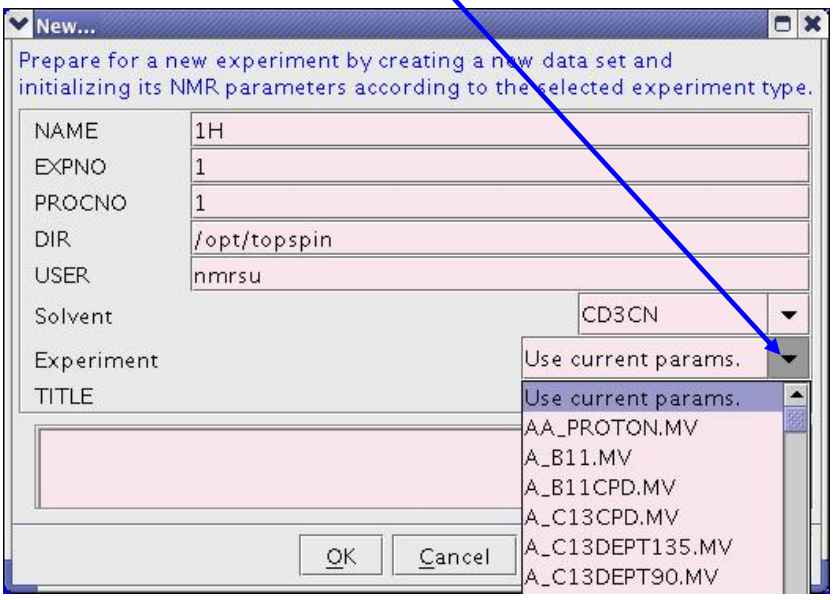
   c. Remove USB stick

5 Acquiring ^{13}C, ^{19}F & ^{31}P NMR Spectrum

Create a new file as described above:



Under "Experiment" click on the  icon and select the following:



FOR ^{13}C: A_C13CDP.MV
A_C13DEPT135.MV
A_C13DEPT90.MV

FOR ^{31}P: A_P31.MV (no ^{1}H decoupling)
A_P31CPD.MV (with ^{1}H decoupling)

FOR ^{19}F: A_F19.MV (no ^{1}H decoupling)
A_F19CPD.MV (with ^{1}H decoupling)

Set **ns for 13C**

128 scans = 6 mins 512= 24 min 2000= 1.5 hrs 20000 = 15 hrs.

ZG or  starts the experiment

tr **(**lets you look at the data before it's finished)
wait for the message "checklockshift: finished" then type

efp (phase as needed)

halt to stop acquisition (do not use "stop", this will not save the FID)

efp to Fourier Transform and display the final spectrum