

Bruker AVANCE III HD 400 MHz Spectrometer Operation

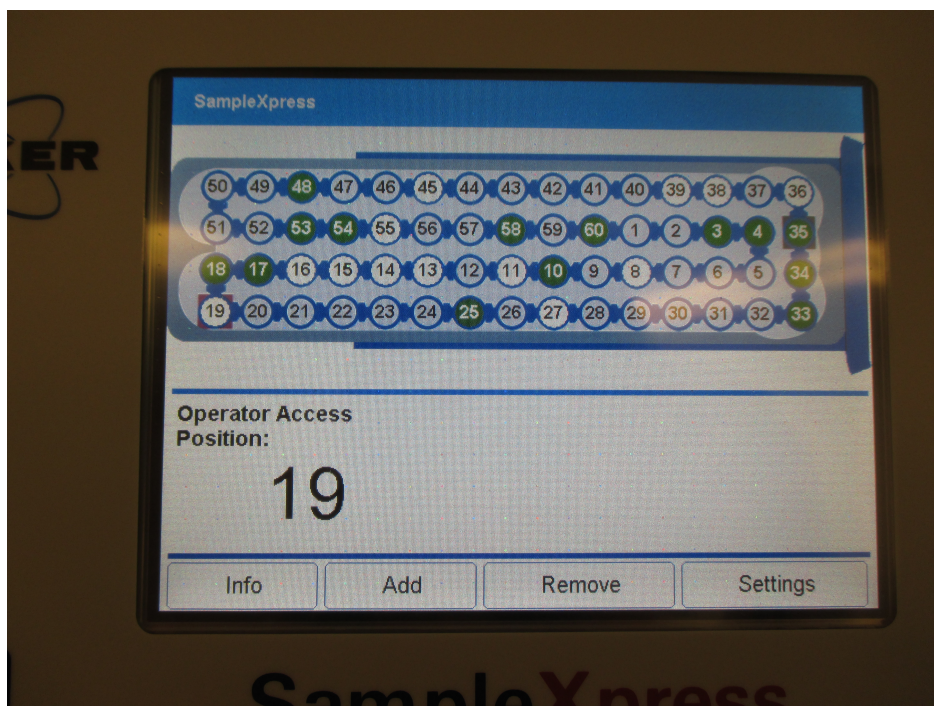
The spectrometer is located in Room 312 of Physical Sciences I. It is equipped with a SampleXpress sample changer for full automation. To utilize the instrument, obtain training and an account from the NMR Facility staff. Sections below describe how to work with the sample changer and IconNMR software to obtain basic one-dimensional spectra. Many pulse sequences are available for one- and two-dimensional spectra. If you do not see a sequence that you need in the pull-down menu when selecting the experiment, request that the staff update your account to permit use of that sequence.

NMR Sample Preparation Concerns

The system accepts 5 mm diameter NMR tubes. DO NOT use any NMR sample tube that is broken (such as with a jagged edge on top), chipped, cracked, scratched, or warped. DO use high quality tubes rated for at least 400 MHz. DO NOT dry NMR tubes in an oven for more than 30 minutes at 125 °C, to avoid warping. Kimble Chase tube cleaning and handling procedures are recommended. The ideal volume of the liquid sample is 0.6 – 0.7 ml; a range of concentrations are possible but something in the mg/ml range is probably reasonable. (Higher concentrations may be needed for detecting a less sensitive nucleus such as ^{13}C at natural abundance.) Remember to put deuterated solvent in your sample, which is required for ^2H locking, a procedure that compensates for drift of the large magnetic field and so allows for narrow lines and good resolution.

SampleXpress Sample Changer Operation

To insert and remove samples from the sample changer, use its interface, depicted below.



Holder positions in the picture on the interface are color coded according to the activity:

Gray means a holder is empty and available for use,

White means the holder is occupied,

Green means the experiments on an occupied holder are complete,

Pink means the sample is about to be placed in the magnet,

Blue means the sample is in the magnet and experiments on that sample are in progress,

An Orange square around White means the position is selected for adding or removing a sample.

Use the “Add” button at the bottom of the interface to add a sample. This brings up another menu screen in which you can choose “Go to free”. The chain will rotate to the next free position and display its number, at which point the sample may be placed in the sample changer at the opening in the cover. If you have several samples and prefer to put them in adjacent positions, choose “In sequence from position” instead of “Go to free”. Select an empty gray position for adding the sample. The chain will rotate in ascending order from this position, to allow adding a series of samples. Select “Back” to exit from the sample addition screens and return to the main menu. If you do nothing, the interface will automatically go back to the main menu in about 30 seconds. Select “Remove” to remove a sample, then “Go to position” on the next menu screen. On the position selection screen, press the number of the sample you need to remove. When the chain has rotated to that position, the screen will display a message requesting you to remove the sample. After removing the sample, the position selection screen will be displayed again, so that you can select another position to remove, or select “Back” to end sample removal.

IconNMR Automation

Introduction:

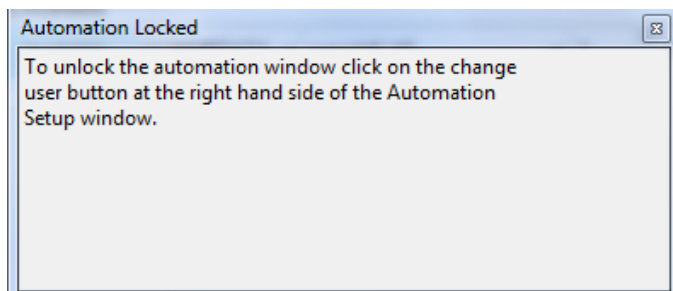
The 400 MHz Bruker Avance III spectrometer provides the IconNMR Automation interface for easy operation. Experiments can be set up in a queue that in normal operation runs constantly. Tuning, locking, shimming, data acquisition, and saving of data are all done automatically for experiments submitted to the queue.

Archiving of data includes automatic writing to a shared disk (Dept Shares, see below for connection instructions), which you can access from any computer. In the Automation mode, on-spectrometer processing will not be allowed and the normal procedure will be to process data offline on another computer. To facilitate offline processing, two computers are set up in Room 312, a PC and a Mac. On both of these computers, both Bruker TopSpin and MNova processing software may be run for data processing.

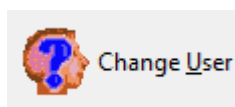
Logging ON and OFF:

You will be given a username (your O-key Account Username) and password (which you may choose) when you are allowed to operate the instrument. (To see what your O-key Account Username is, log in with your O-key email address and password at <https://app.it.okstate.edu/okey/>.)

When you arrive to log in, you should see on the spectrometer computer screen the following window, indicating that IconNMR Automation is locked:



Whether or not you see this window, log in by clicking on the **Change User** button:



Select your login name from the list and click the OK button. Another window will come up in which you type your password.

You must log in to your account to submit samples and collect spectra so that your data will be saved in your directory on the shared drive; if you do not, you will not be able to access your data for processing. Also, accounting is done by consideration of who submitted and ran each experiment. Do NOT submit your experiments while another user is logged in, or the other user will be charged for them.

When you are finished submitting experiments, click on the **Change User** button, and in the change user window click on the **Lock IconNMR** button to log out.

Queue and holders:

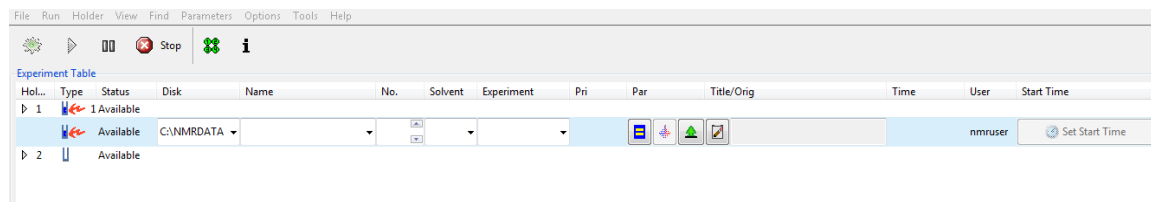
The IconNMR Automation interface has a list of holders on the top part. Just above this is the following set of buttons:



Normally, the green Start button icon should appear inactive (faded, as in above picture) and the red Stop button icon should be active (full color). In this case, the queue is running. If the opposite is true, and the queue is not running, you can start the queue, allowing experiments to be acquired, by clicking on the Start button.



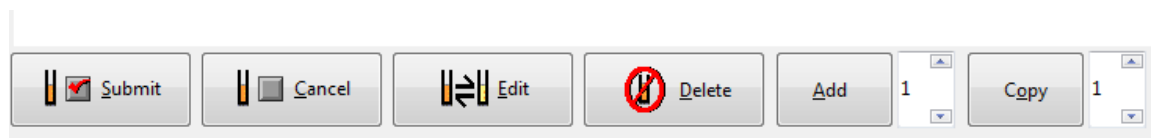
Holders are equivalent to samples; after experiments for one holder are complete, the sample changer ejects the sample and inserts a new sample. Our current setup has sixty holders corresponding to the 60 sample positions on the changer. Using these, you may set up a series of spectra to take on several samples as needed.



In the Status field, holders are labeled as follows:

Status	Meaning
Available	Holder is ready to be used
Running	Spectra are currently being acquired on the sample in this holder
Finished	Acquisition is complete on this sample
Failed	Abnormal situation in which data collection has been stopped

After a holder moves to “Finished” status, it can be returned to “Available” for reuse by highlighting the holder and clicking on the Delete button below the holder list (picture below). Any user can delete finished experiments from a holder, even if another user submitted the experiments. Data has already been saved, and this action does not delete the acquired data. A “Finished” holder may correspond to a position in the sample changer that is still holding a sample, or the position may be empty. Thus, if you place a sample in the sample changer, and find that holder in IconNMR is “Finished”, simply convert it to “Available” before setting up your experiments. *Everyone is requested to remove their samples from the sample changer promptly after acquisition is done, so in most cases users will not need to remove others’ samples from the sample changer.*



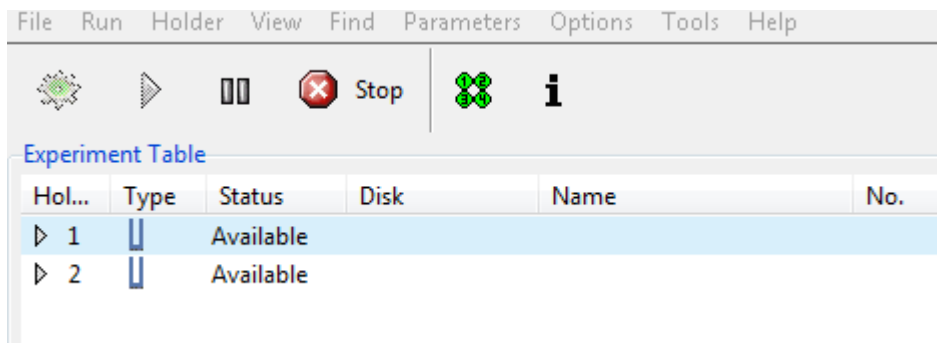
These comments will make more sense after going through the step-by-step instructions below.

Step-by-step procedure for acquiring spectra in IconNMR with the sample changer:

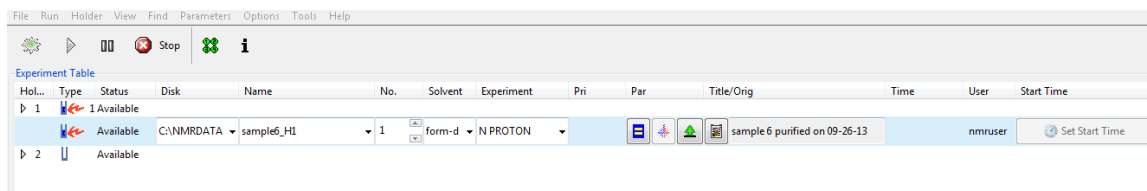
1. Login to IconNMR on the spectrometer computer.
2. Insert your sample into the spinner turbine, and use the gauge to determine appropriate sample depth. This is very important, as it makes certain that your sample tube will not damage the probe.
3. Approach the sample changer interface screen, and press “Add”, then “Go to free”. The sample changer rack will rotate around to put a free holder position in the corner where samples can be inserted. Place your turbine with sample in the

sample changer and note the number of the holder displayed on the screen. Press “Back” after sample addition to return to the main menu.

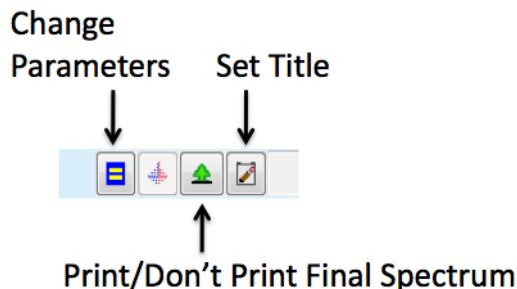
- Write in the notebook next to the spectrometer computer the date, sample holder number, your name, and a brief description of your sample.
- Select the holder in Icon NMR with the same number as you used in the sample changer, by highlighting it. (If your holder says “Finished”, highlight it and click on the **Delete** button, then highlight the “Available” holder.)



- Click on the **Add** button in IconNMR (picture above). Fill in the fields *Name*, *No*, *Solvent*, *Experiment*, and *Title/Orig*. Name is the file name for saving the data; it is best to include in this at least some indication of the sample identity and type of spectrum. Number (No) is a designation always used in the Bruker system and allows multiple experiments to be run with the same Name but different Number value. If you devise a system (and stick to it and remember it!) in which a particular number corresponds to a spectrum type (1 = ^1H 1D, 2 = ^{13}C 1D, etc.), this might be useful for saving a series of different spectra for one sample. Solvent can be chosen from a large list when you click on the down arrow to the right of the field. Likewise for experiment, which allows you to choose the type of spectrum. Title is a description of your sample, experiment, or conditions as you like and may include spaces. Click the **Set Title** button to get your text loaded into the Title/Orig field.



- Before submitting an experiment, a few more choices can be made using the set of buttons on the holder line.



Click on the blue/yellow box button to change a few parameters, such as the number of scans to take (ns) or pulse delay (d1). After parameters are changed, the parameter icon changes to a yellow exclamation point on red background.

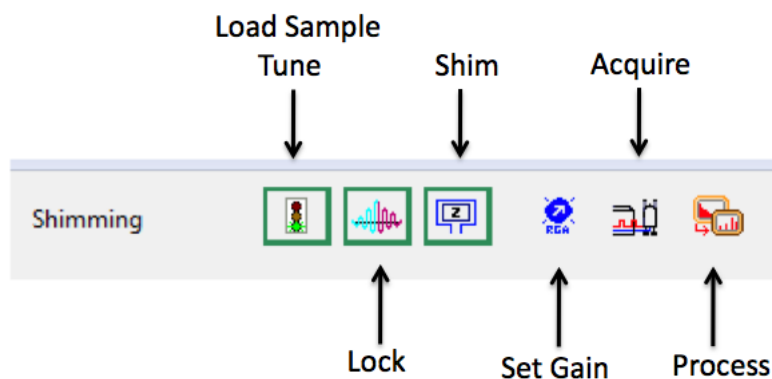


Click on the green tree icon to toggle between printing (picture of a printer with paper, sort of diagonal?), and not printing (green tree – save the trees by using less paper!) If you are not sure about changing parameters, just use the parameters that are automatically set up. However, you can get the Time the experiment will take to acquire (in the field after Title/Orig) if you change one of the parameters, such as number of scans. If you do not change any parameters, the time will show up after the experiment is submitted (see item 6 below).

5. Click the **Add** button to set up further experiments for this sample if needed.
6. To submit an experiment to the queue for acquisition, click on the **Submit** button while that experiment/holder is highlighted. To submit the entire series of experiments set up for one holder at once, highlight the first row for the holder, which represents the entire holder, and click the **Submit** button.
7. Note that if you want to replicate an experimental setup for additional samples (such as running proton 1D spectra in the same way for several samples), the **Copy** button copies the experiments in one holder to the other holder. Remember to change the Name and Title appropriately before running the new experiment(s).
8. When you are finished submitting samples and leaving the spectrometer to run independently, log out by clicking on the **Change User** button and then the **Lock IconNMR** button.
9. Remember to come back and retrieve your samples from the sample changer after data acquisition is complete. To remove a sample from the changer, select “Remove” on the sample changer screen, then “Go to position” on the next menu screen. On the position selection screen, press the number of the sample you need to remove. Press “Back” to return to the main menu after removing samples.

Watching progress of experiments:

1. Examine activity icons in the upper right of the IconNMR interface, and the status reports on the lower half of the screen to see that your experiments are proceeding normally. The activity icons will be surrounded with a blinking dark green border as each task is performed:



In the unlikely event of a mishap in the automation, an X instead of check may appear for one of the automation activities in the experiment list on the lower part of the screen. Be alert to this, as it may indicate your data is bad. For example, in the case of the picture below shimming failed (note the X under the Shim heading, and red text), the shims were very bad and lines in the proton spectrum were overly broad. Problems are unlikely, but contact the NMR facility staff for assistance if you encounter one.

Preceding Experiments														
#	Date	Holder	Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User	Disk
2	2013-10-24 11:53:07	2	10242013_chol_LH_c	10	PROTON	✓	✓	✓	✓	✓	✓	✓	dongtac	C:\NMRDATA\TA\dongtac
1	2013-10-24 11:30:57	1	10242013_chol_LH	10	PROTON	✓	✓	✓	✓	X	✓	✓	dongtac	C:\NMRDATA\TA\dongtac

- When acquisition for an experiment is complete, "Finished" will appear in its status field. When a sample is removed from the sample holder, the holder in IconNMR will not automatically become "Available." Highlight it and click on the **Delete** button to return it to "Available."

Connection to the shared drive to access NMR data:

Below are the instructions for mapping our shared drive on Windows and MacOS, so that you can access it on your own computer. (When you log in to the Mac or PC in room 312 with your O-key email and password, your directory on the shared drive should be an icon on the desktop. If it is not, follow these procedures.)

You will need to know your O-key Account Username (see how to find this above under logging on).

Mapping the drive on Windows:

- Right-click on the My Computer icon (Windows XP) or Computer (Windows VISTA/Win7)
- Click on "Map Network Drive..."
- Click on the down arrow next to the Drive text box.
- Select a free drive letter such as "R:"
- In the path field, type the path you wish to map to (PS312 NMR Users):

\\cas-fs01.cas.okstate.edu\Dept Shares\chemistry

You may be asked to login; if so, use your O-key Account Username (not email address) prefaced by "OSU" and use your O-key password.

The "Reconnect at logon" box should be checked ONLY if you wish to have this folder mapped EVERY time you log into this computer. This folder will only be mapped when you login with your respective login and password, and will not be mapped for anyone else who logs in with their own O-key credentials.

6. Click Finish.

Mapping the drive on Macintosh:

1. Click on the Go menu
2. Click on "Connect to Server..."
3. In the path field, type the path you wish to map to:
smb://CAS-FS01.cas.okstate.edu/Dept Shares/chemistry/PS312_NMR_Users/
4. Login with your O-key Account Username and password
5. You will have to navigate the directory structure (Dept Shares -> Chemistry -> PS312_NMR_Users -> <your name>).
6. You can create an alias (File -> Make Alias) and move it to the Dock for your convenience. When you click on the alias you will be asked to authenticate.

Note: If you are attempting to access this shared drive from off campus, you MUST go through the University's VPN first, (<http://osuvpn.okstate.edu>), then map or connect to the drive.

Offline data processing:

The offline data processing computer in Rm 312 has both Mnova and Bruker TopSpin programs. You may also put MNova on your own computer using the campus license.

To obtain MNova for a PC:

The software can be downloaded from Mestrelab, but there is a registration file that needs to be run on Windows machines to "link" it to the CAS license server.

Mnova Download Link

<http://mestrelab.com/software/mnova-suite/download/>

qNMR Download link

(Mnova qNMR must be downloaded additionally and dragged and dropped inside the Mnova Program.)

<http://mestrelab.com/downloads/qnmr/win/Mnova-qNMR-1.1.0.1159.zip>.

And finally, the registration file that links it to the license server can be run from here:

<\\cas-licensing.cas.okstate.edu\Mnova Client License Repository\MNOVA LICENSE REGISTRATION.reg>

To obtain MNova for a Mac:

1. Download and install the Mnova software using the vendor prompts.

Mnova Download Link

<http://mestrelab.com/software/mnova-suite/download/>


qNMR Download link

(Mnova qNMR must be downloaded additionally and dragged and dropped inside the Mnova Program.)

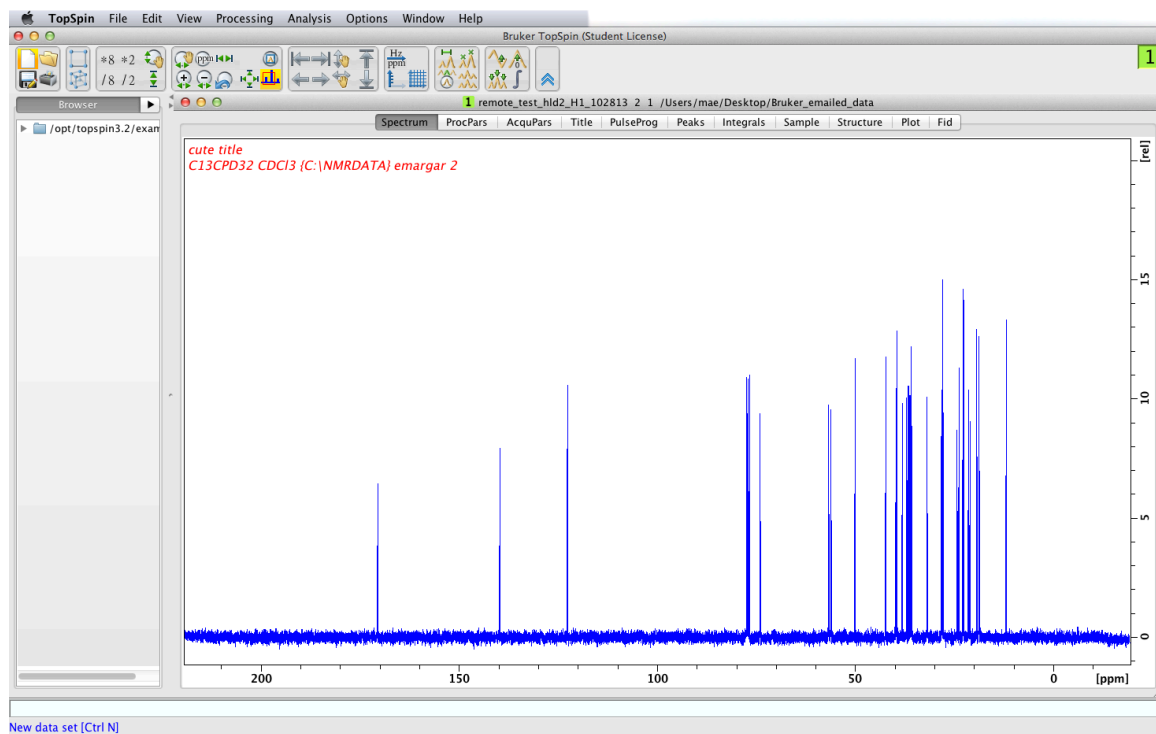
<http://mestrelab.com/downloads/qnmr/win/Mnova-qNMR-1.1.0.1159.zip>.

2. Connect to the license server using "Finder - Go – Connect to Server"
 3. Enter "[SMB://cas-licensing.cas.okstate.edu/Mnova Client License Repository/](http://cas-licensing.cas.okstate.edu/Mnova_Client_License_Repository/)
 4. When prompted, enter your OKEY username and password.
 5. Launch MestReNova
 6. At the Registration Wizard, choose install. Follow the prompts and select the two .lic files found in the connected file share above.
 7. Finally, edit the plist file which is located at: /Users/<your user name>/Library/Preferences/com.mestrelab.MestReNova.plist (On some systems, this may be a hidden folder)
- You can use any mac preferences editor to do it. Basically, you will need to add a new item with the name License.SharedRepository and the corresponding path of the license.
- `"/cas-licensing.cas.okstate.edu/Mnova Client License Repository/"`
`<http://mestrelab.com/images/stories/resources/installing/installing-license-server-5.jpg>`
- You can use for example use this free editor:
<http://www.nightproductions.net/prefsetter.html>

Processing with Bruker TopSpin

The TopSpin interface (a user-friendly GUI) is similar in some ways to the VNMRJ interface. Various buttons and menus allow you to take actions, but a command line for typing in text commands is also available (light blue line near the bottom). There is also a browser for locating data on the left of the TopSpin interface, but you may find it just as easy to browse by opening a file chooser as you would in any program (**File -> Open** on the Mac or  on the Mac or PC). The Browser window can be shown or hidden with **View -> Browser Panel On/Off** or **Control-D**. Here we will cover only the simplest methods to get processing tasks done using the buttons and menus in the GUI. These instructions are brief and do not cover all possible options in data processing. Typed commands will not be given here, but can be seen next to the selection for each menu item.

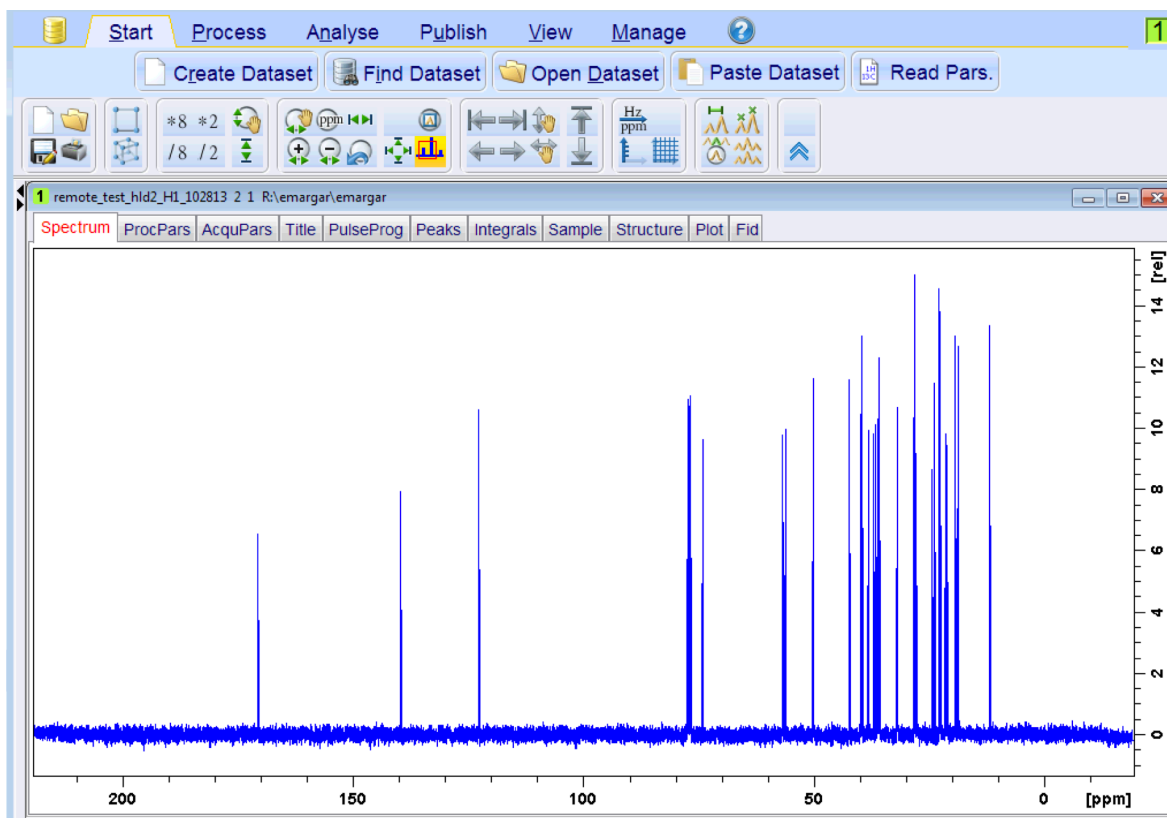
Below is the appearance of the TopSpin interface on the Mac:



The screenshot shows the top portion of a software window. At the very top is a menu bar with options: File, Edit, View, Windows, Help, and a dropdown arrow. Below the menu bar are several toolbars. The first toolbar contains icons for file operations (new, open, save, print) and a folder icon. The second toolbar has icons for molecular structures (a square with four dots, a cube, a ball-and-stick model). The third toolbar includes mathematical symbols (*8 *2, /8 /2), a hand cursor, and zoom controls (vertical double arrows). The fourth toolbar features a circular arrow, a ppm scale indicator, a plus-minus sign, a magnifying glass over a spectrum plot, and a zoom-in button. The fifth toolbar contains horizontal and vertical double-headed arrows, a hand cursor, and a zoom-out button. The sixth toolbar displays frequency units (Hz, ppm), a grid pattern, and a zoom-in button. The seventh toolbar shows three different spectral plots (bar chart, line graph, and another bar chart) and a zoom-in button. Finally, there is a large blue double-upward arrow button on the far right.

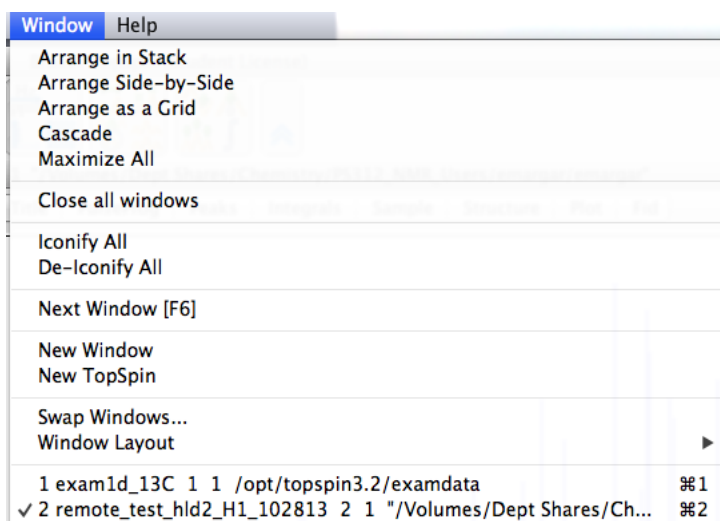


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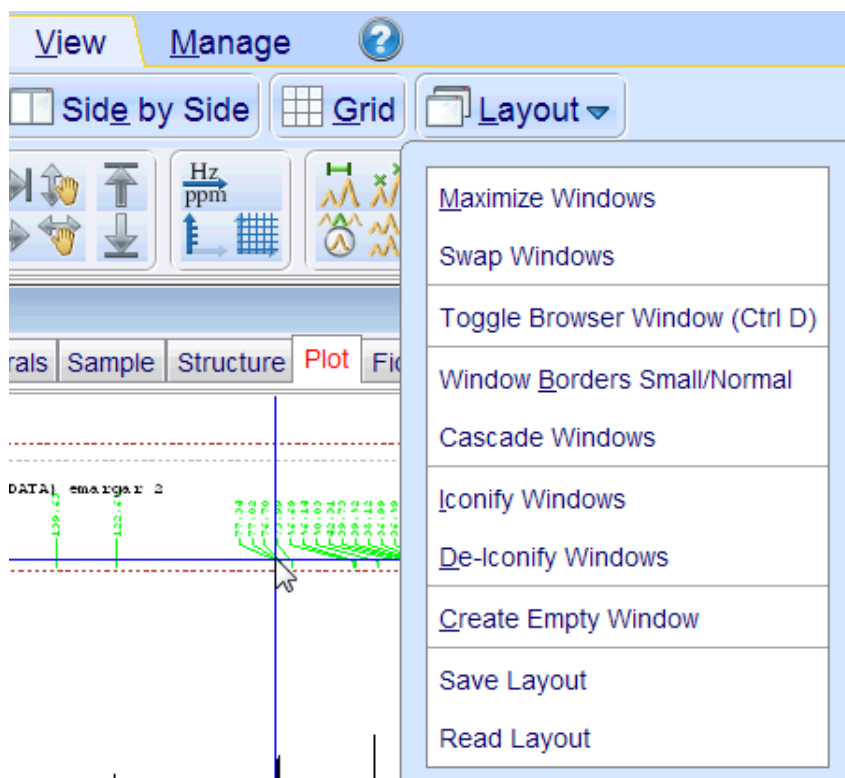


Managing data windows:

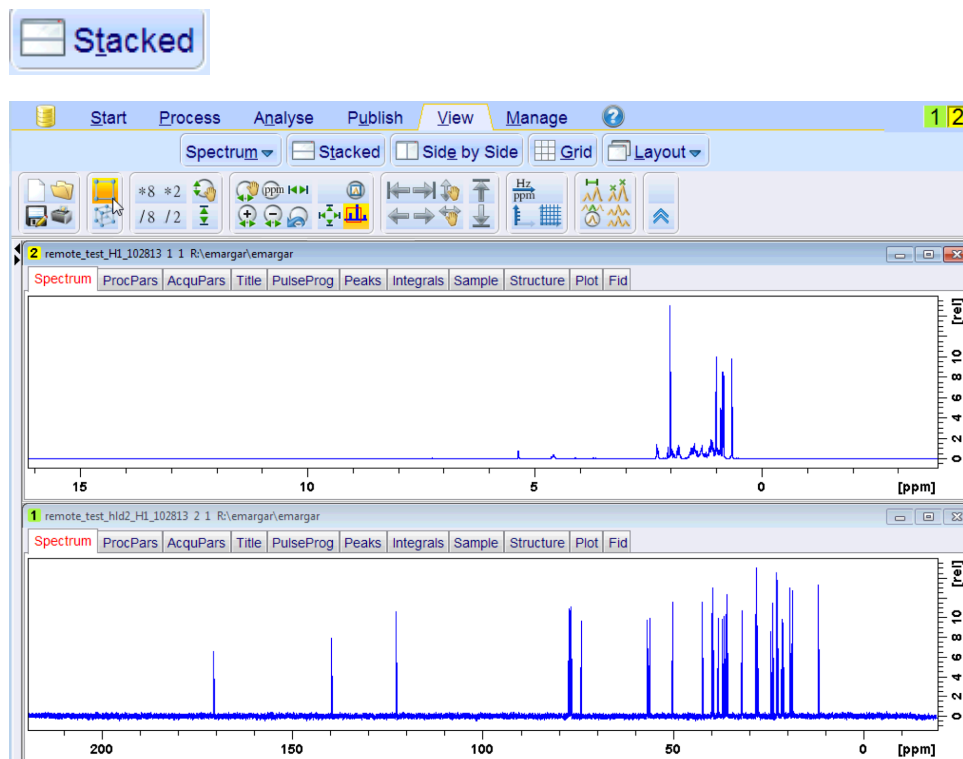
When you start TopSpin and open some data, a new data window will automatically be created. Notice the data window in each picture above, with its border and buttons for closing it or changing its size (on the top right for the PC, top left for the Mac). You may have several data windows, and may display them one at a time or simultaneously. This feature can be handy for examining more than one data set, or simultaneously viewing different sections of a single data set. A new data window can be created with the **Window -> New Window** menu choice on the Mac:



Or with the **View -> Layout -> Create Empty Window** choice on the PC:



To display more than one window at once, select an option in the **Window** (Mac) or **View** (PC) menu, such as **Arrange in Stack**:



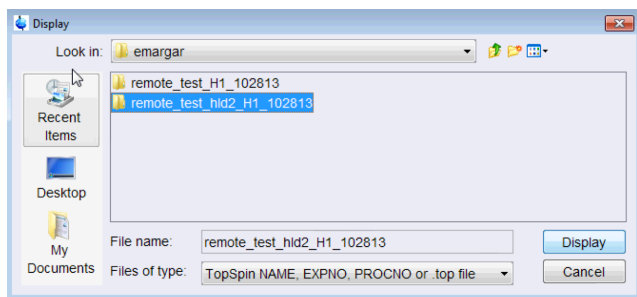
To make a window the active window, click on it to highlight its border. The active window will be the window that data is read into (see below). When only one window is displayed, it is the active window.

Opening data files:



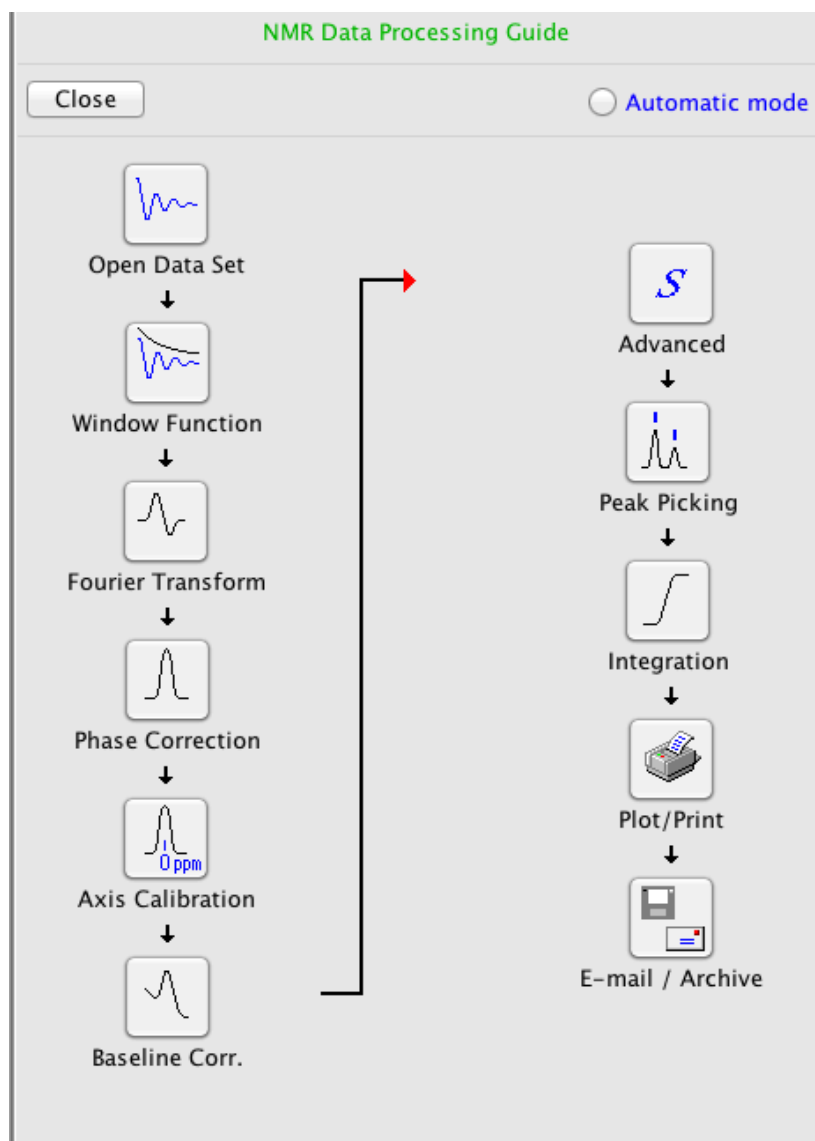
Click on the open file button/icon:

A window will appear that allows you to locate the desired data file; click the **Display** button to open the highlighted file in the active window.



Data Processing Guide:



This feature appears to be available in the Mac version of TopSpin only. Bring up the Data Processing Guide window by selecting **Processing -> Data Processing Guide**. This allows the various functions outlined below required for 1D data processing to be invoked sequentially by a series of buttons. Start at the upper left and proceed down the left column then to the top of the right column, following the arrows. Select each desired function in turn. This guide can be used in either automatic or interactive mode. If the automatic mode is selected (by highlighting the circle), when each function button is selected, the action is taken automatically without any user input. In the interactive mode (automatic mode not selected) as each button is selected the user is able to interactively complete the task. Note that if you select interactive mode in the Data Processing Guide, you will need to learn the interactive processing procedures in the sections below.



Examining the spectrum:

Use the buttons at the top of the interface, below the menus, to resize the spectrum and move around in it. Vertical scale is controlled by this button cluster, or by the *middle mouse button*:



General horizontal zooming in and back out to the full spectrum are easily done with the buttons  (click and drag with the left mouse button to select the zoom region) and , respectively.

Shifting the spectrum up and down or side-to-side are accomplished with this button cluster:

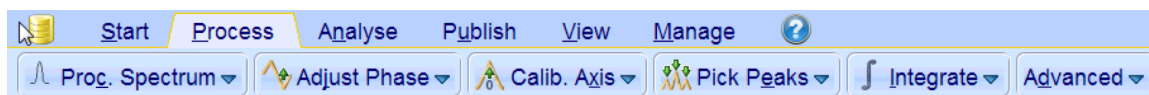


Another useful button toggles the axis between Hz and ppm:

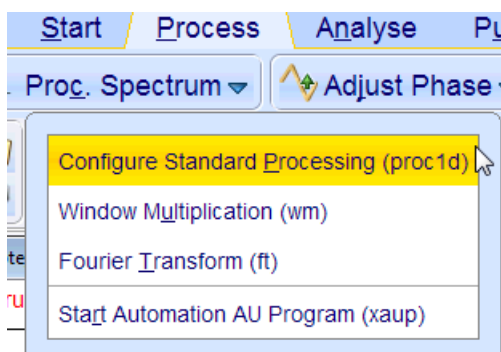


For these and other buttons not depicted, place the cursor over the button for an explanation of its function.

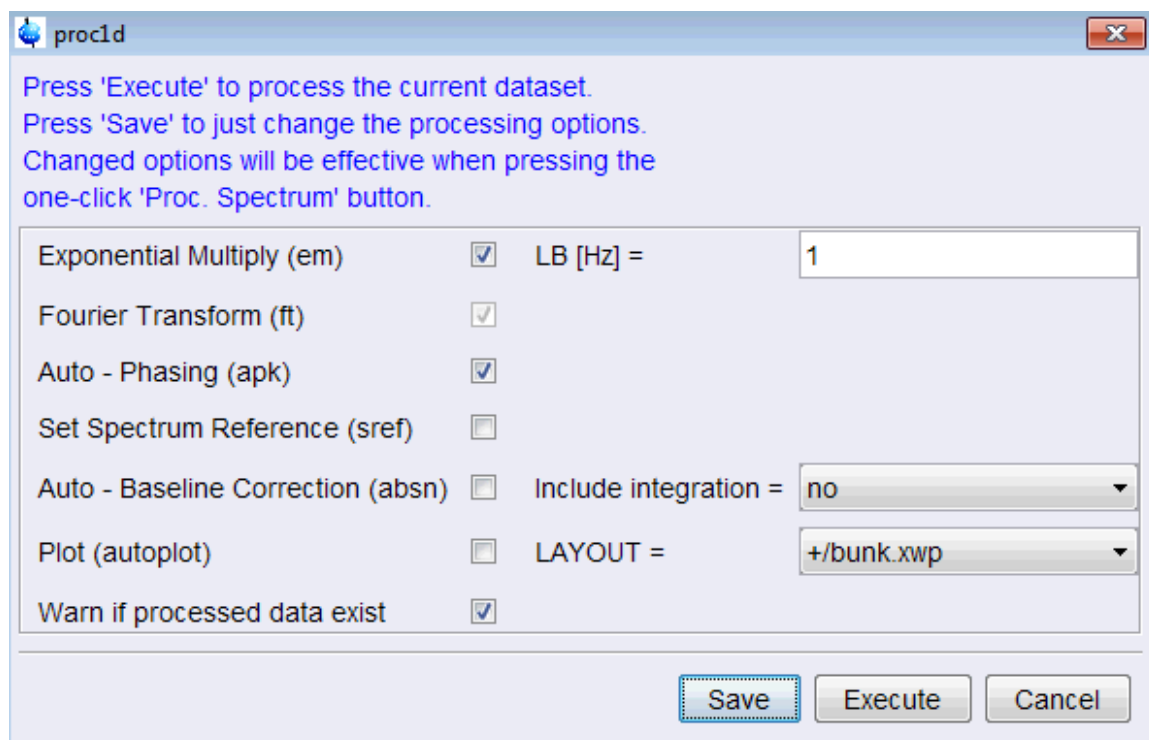
*Window function multiplication, Fourier transformation and baseline correction **on the PC**:*



The **Process** -> **Proc. Spectrum** tab may be clicked to transform the spectrum; however, data will be transformed automatically when it is read in. To adjust the parameters used, such as exponential window function line broadening or baseline correction, right-click on the down arrow next to **Proc. Spectrum** to get the **Configure Standard Processing** choice.

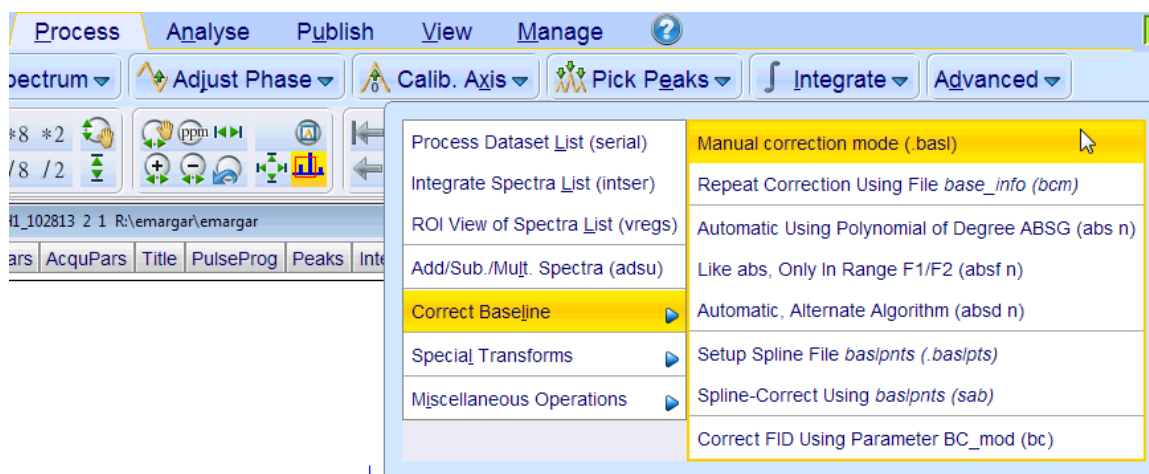


This brings up the proc1d window, in which you can change some settings:

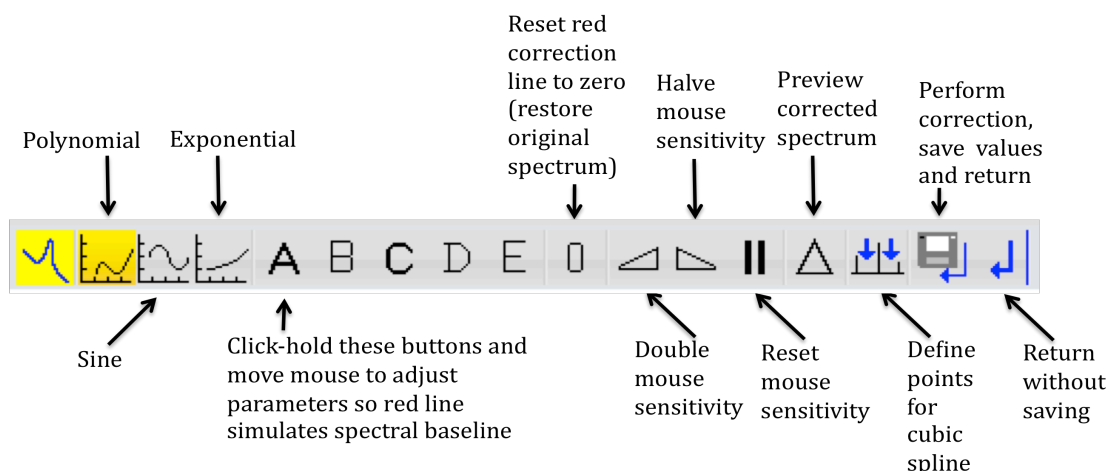


Window Multiplication in the above menu acts as shown below for the Mac.

Interactive baseline correction can be entered in the PC version of TopSpin through **Process -> Advanced -> Correct Baseline -> Manual correction mode**. This will only be needed in unusual cases when the baseline is distorted.



The series of buttons in baseline correction mode is defined below:



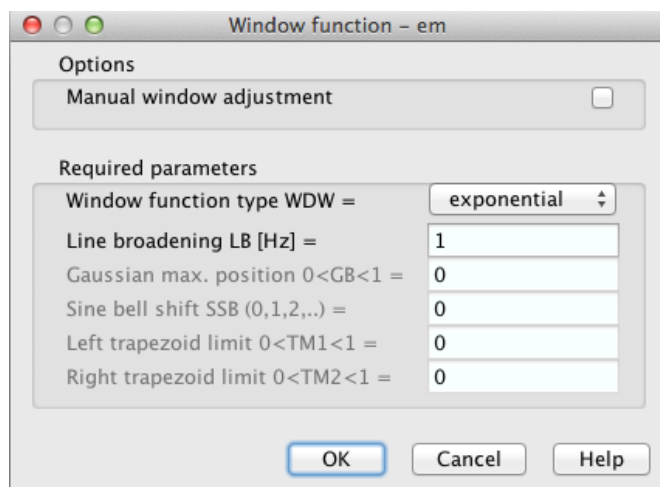
Three types of functions depicted on the left end of the bar can be chosen as possible simulators of the baseline curve. Above, the polynomial function is selected (since it has a golden background). It allows adjustment of parameters A through E, according to the function

$$f(x) = A + B*x + C*x^2 + D*x^3 + E*x^4$$

To formulate a baseline correction curve by hand, manipulate the parameters until the red line matches the baseline of the experimental spectrum, then click the perform correction, save and return button.

Window function multiplication, Fourier transformation and baseline correction on the Mac:

Clicking on the **Processing -> Window Multiplication** menu choice will open a dialog box, shown below, in which the window function and its parameters can be entered. Select the window function type from the menu of choices by left-clicking on the button. Parameters become activated and enterable when a function that uses them is selected. Below, an exponential function with 1 Hz line broadening parameter is selected. Clicking the OK button applies the chosen window function.



Clicking on the **Processing** -> **Fourier Transform** menu choice will open a dialog box in which the Fourier transform and linear prediction parameters can be entered. The size of the real spectrum (number of points) can also be entered in this window, in the case that zero filling is required. Clicking the OK button performs the Fourier transform.

Fourier transform - ft

Options

☒ Standard Fourier transform
☐ Advanced Fourier transform

Required parameters

Size of real spectrum SI [pnts] = 32768
of fid data points to be used TDeff = 0
Index of first output point of strip transform STSR = 0
Total # of output points of strip transform STSI = 0

Fid linear prediction (LP) mode ME_mod = No LP
of LP coefficients NCOEF = 0
of fid data points contributing to backward LP LPBIN = 0
of fid data points to be predicted TDOFF = 0

Reverse spectrum REVERSE = No
Weighting factor for first fid point FCOR = 0.5
Apply 5th order phase correction (A*X only) PKNL = Yes

OK Cancel Help

Baseline correction - abs

Options

☐ Correct baseline manually
☒ Auto-correct baseline using polynomial
☐ Auto-correct spectral range ABSF1..ABSF2 only
☐ Auto-correct baseline, alternate algorithm
☐ Define baseline points for cubic spline correction
☐ Correct baseline using cubic spline
☐ Correct baseline using base_info file
☐ Correct baseline of the FID

Required parameters

Degree of polynomial ABSG (0..5) = 5
Left limit for correction region ABSF1 [ppm] = 240
Right limit for correction region ABSF2 [ppm] = -10
Number of averaging points = 1
Baseline points file defining cubic spline = baslpnts
Baseline info file stored by manual correction = base_info
Fid baseline mode BC_mod = quad

OK Cancel Help

Clicking on the **Processing** -> **Baseline Correction** menu choice will open a dialog box, shown above, in which the baseline correction method and its parameters can be entered. Automatic polynomial correction, as shown, is easy, because it involves only setting the

degree of the polynomial. Clicking the OK button performs the baseline correction when an automatic choice is selected. Selecting “Correct baseline manually”, “OK” enters interactive baseline correction, with the button menu the same as shown above for the PC.

Phasing of 1D spectra:

Manual phasing:

Click on the window with the data to be phased (highlighting the bar at the top).



Click on the phase adjust button on the top toolbar or select **Processing -> Phase Correction -> Manual phasing** (Mac), or select **Process -> Adjust Phase** (PC).

Select the pivot point by clicking at the desired location with the right mouse button and selecting “Set Pivot Point”. This pivot point is most conveniently placed near the rightmost peaks of the spectrum but not on top of a peak.

Adjust the zero order phase by clicking, holding, and moving with the left mouse button on the **0** in the phasing toolbar (shown below). Correct the right side of the spectrum so the rightmost peaks are phased positive, absorptive.



Similarly, adjust the first order phase, correcting the left side of the spectrum, by clicking, holding, and moving with the left mouse button on the **1** in the phasing toolbar.

To get finer or courser control of the phase changes, use the buttons to increase or

decrease mouse sensitivity:



Moving the middle mouse button on the spectrum background will increase or decrease the vertical scale of the spectrum. Increasing the vertical scale will be essential to setting the phase parameters carefully.

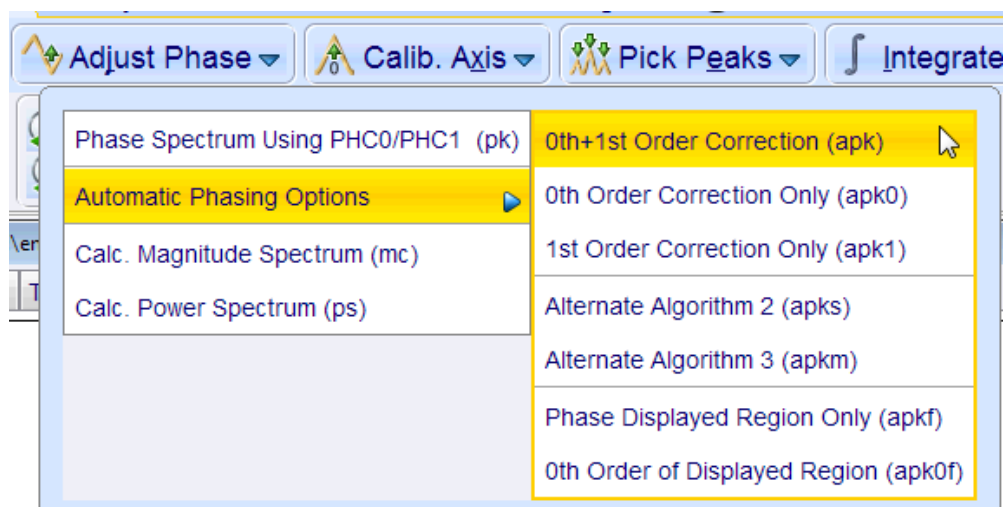
Click on the save, execute, return button to execute the phasing, save the phases, and

leave the phase correction mode.

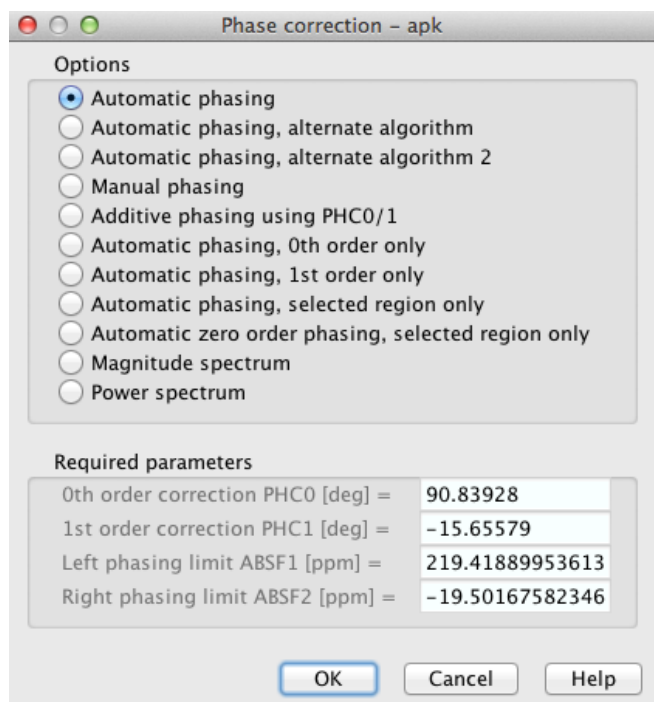


Automatic phasing:

On the PC, select **Process -> Adjust Phase -> Automatic Phasing Options -> 0th + 1st Order Correction**. 0th order correction alone may be used for a single peak or if the peaks are not spread out much across the spectrum.

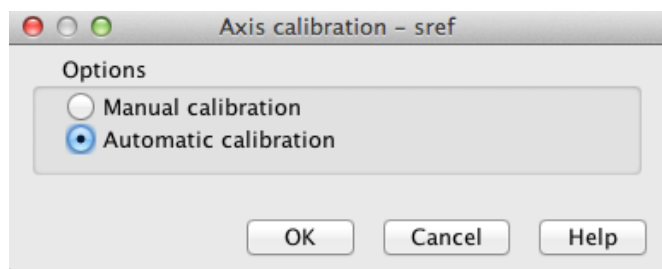


On the Mac, choose **Automatic phasing** from the window after selecting **Processing -> Phase Correction**.



Referencing:

Clicking on the **Analysis -> Axis Calibration** menu choice on the Mac will open a window that allows interactive or automatic setting of the reference:

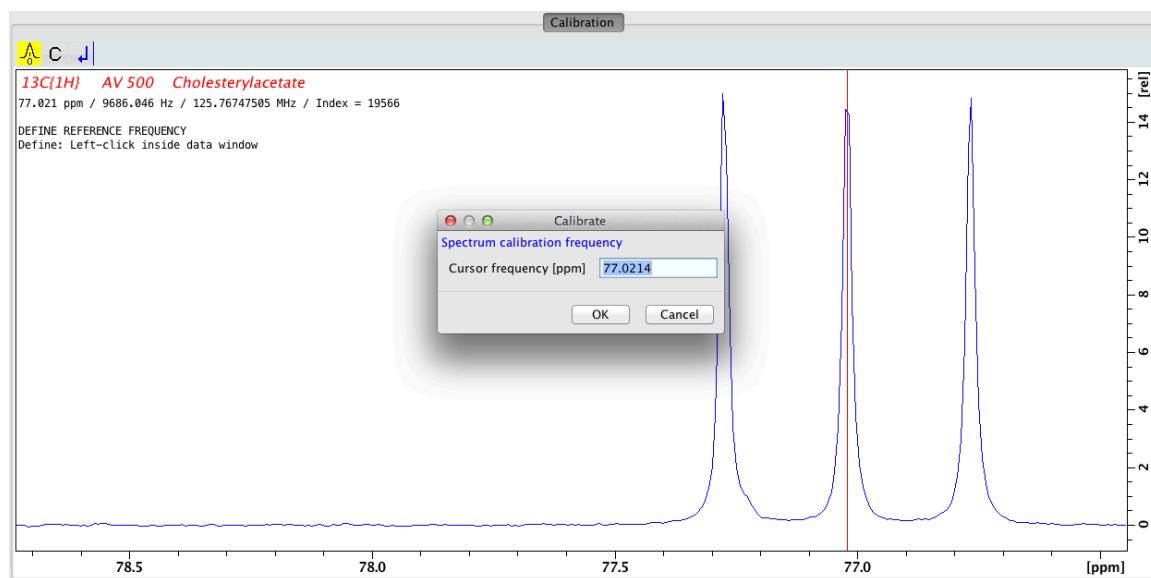


If you request “Automatic calibration”, TopSpin uses a TMS signal as the reference.

Interactive referencing allows the user to set any peak to a particular ppm value, which will be required when TMS is not present in the sample. To enter interactive referencing (axis calibration), select “Manual calibration”, rather than “Automatic calibration”.

On the PC, select **Process -> Calib. Axis** for manual calibration, or select the down arrow on this tab for automatic calibration to TMS.

The data window goes into calibration mode as indicated by a yellow copy of the calibration icon in the upper left corner.

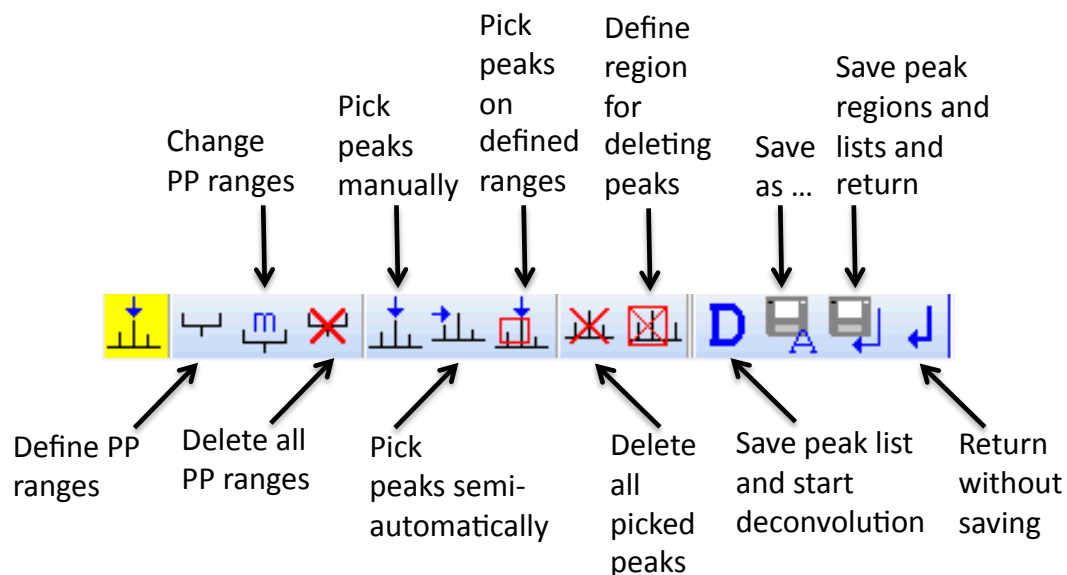


Position the red cursor at the top of the desired reference peak and left-click at that position to bring up a dialog box in which the required frequency in ppm can be entered. After clicking on OK in the dialog box, TopSpin automatically exits calibration mode.

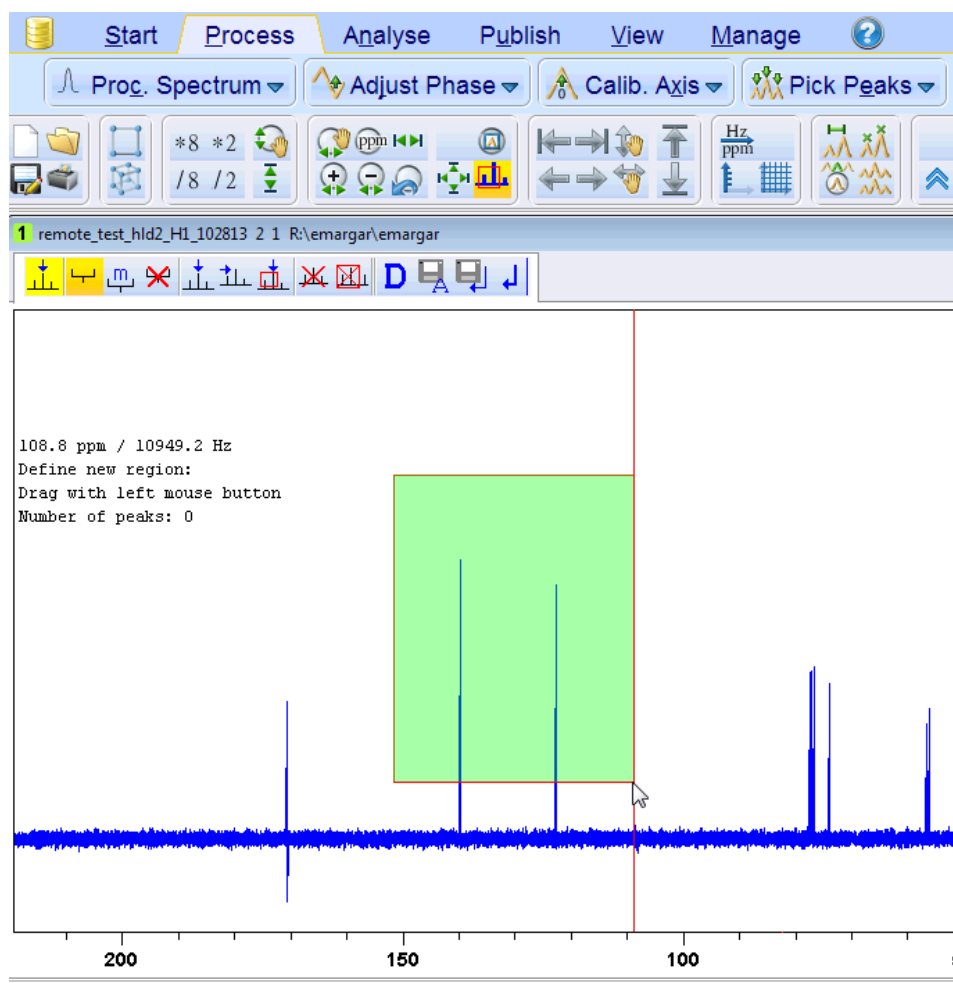
Peak Picking:

To enter interactive peak picking mode, select **Analysis -> Peak Picking -> “Define regions/..”** on the Mac or **Process -> Pick Peaks** on the PC.

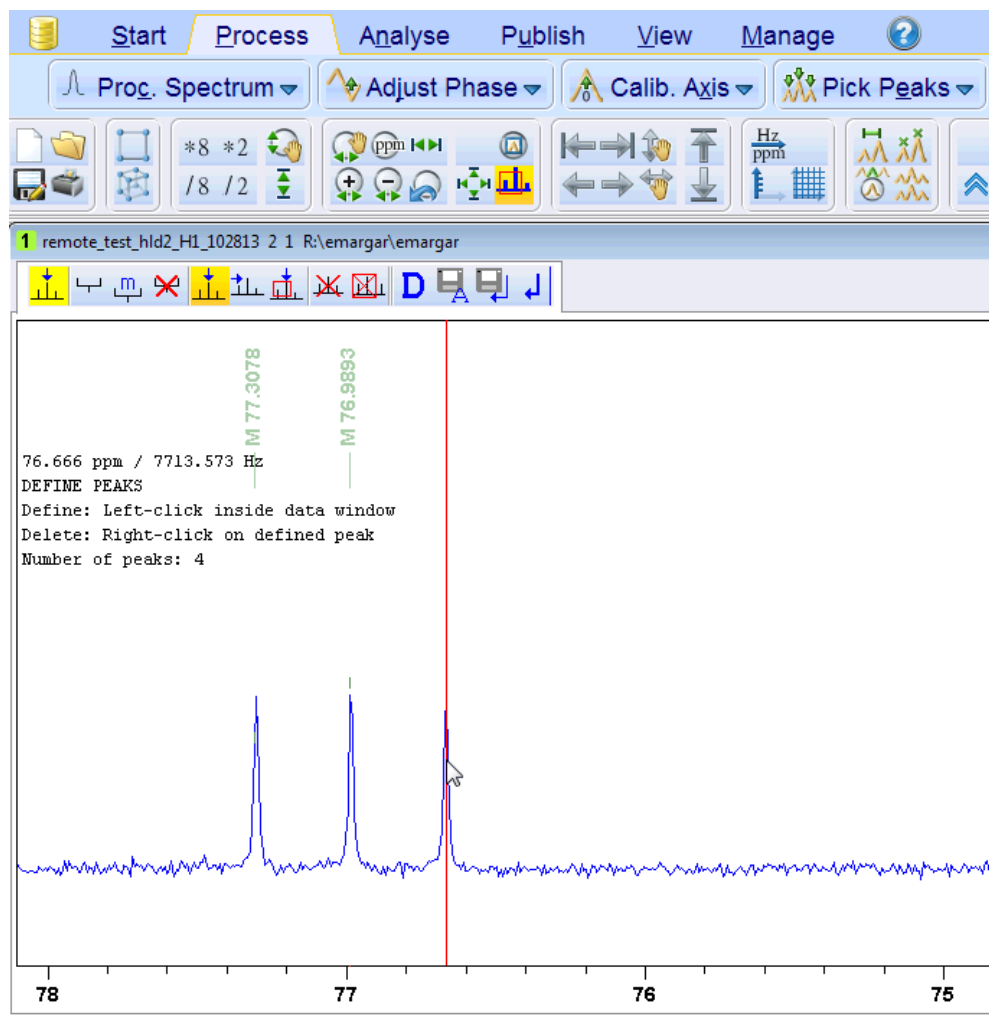
The series of buttons in peak picking mode is defined below:



In the mode of defining peak picking ranges, click and drag with the left mouse button to select a region as shown below:



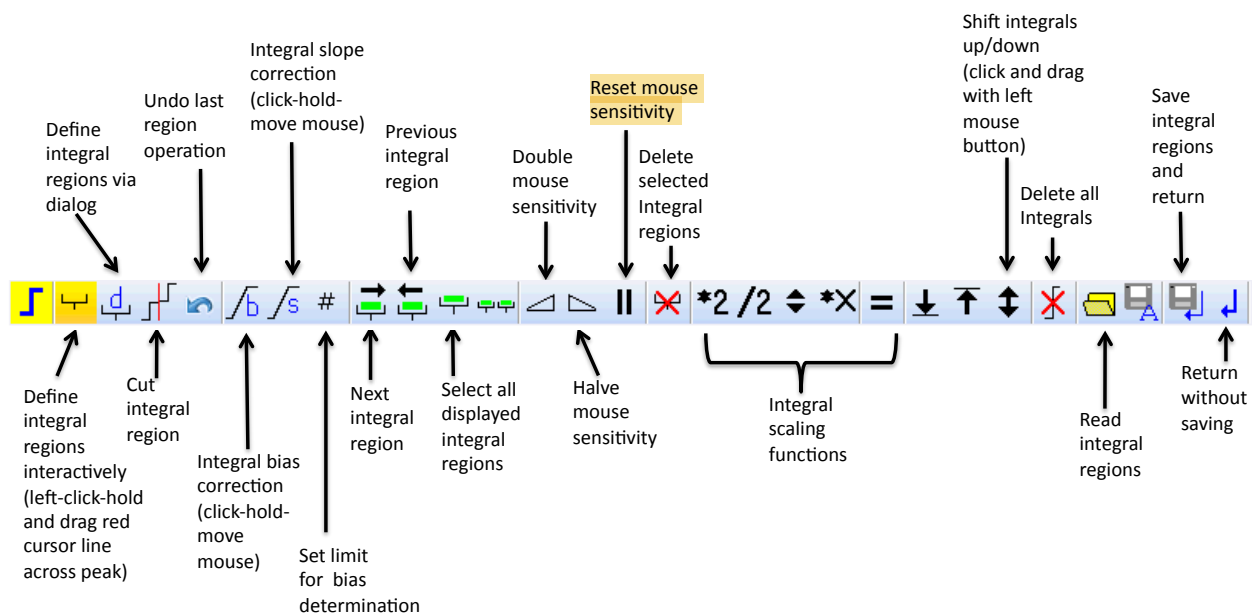
In the mode of picking peaks manually, click with the left mouse button on top of each individual peak as shown below:



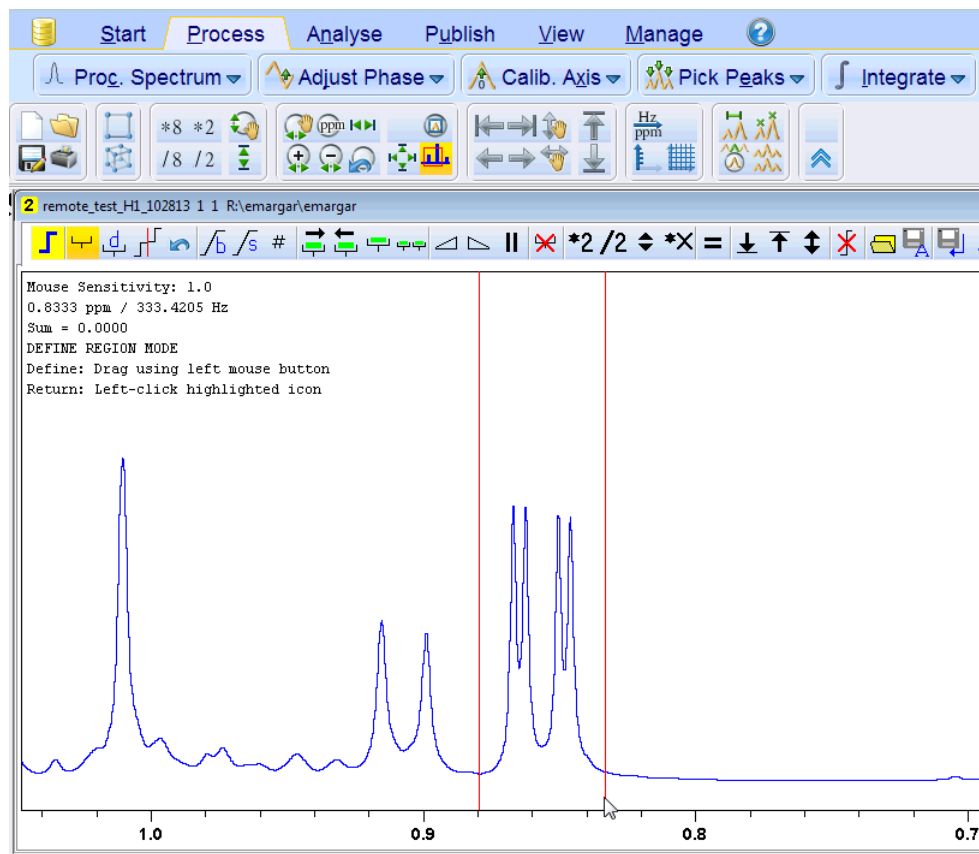
How to integrate peaks in a 1D proton spectrum:

To enter interactive integration mode, select **Analysis -> Integration -> “Define integral regions manually”** on the Mac or **Process -> Integrate** on the PC.

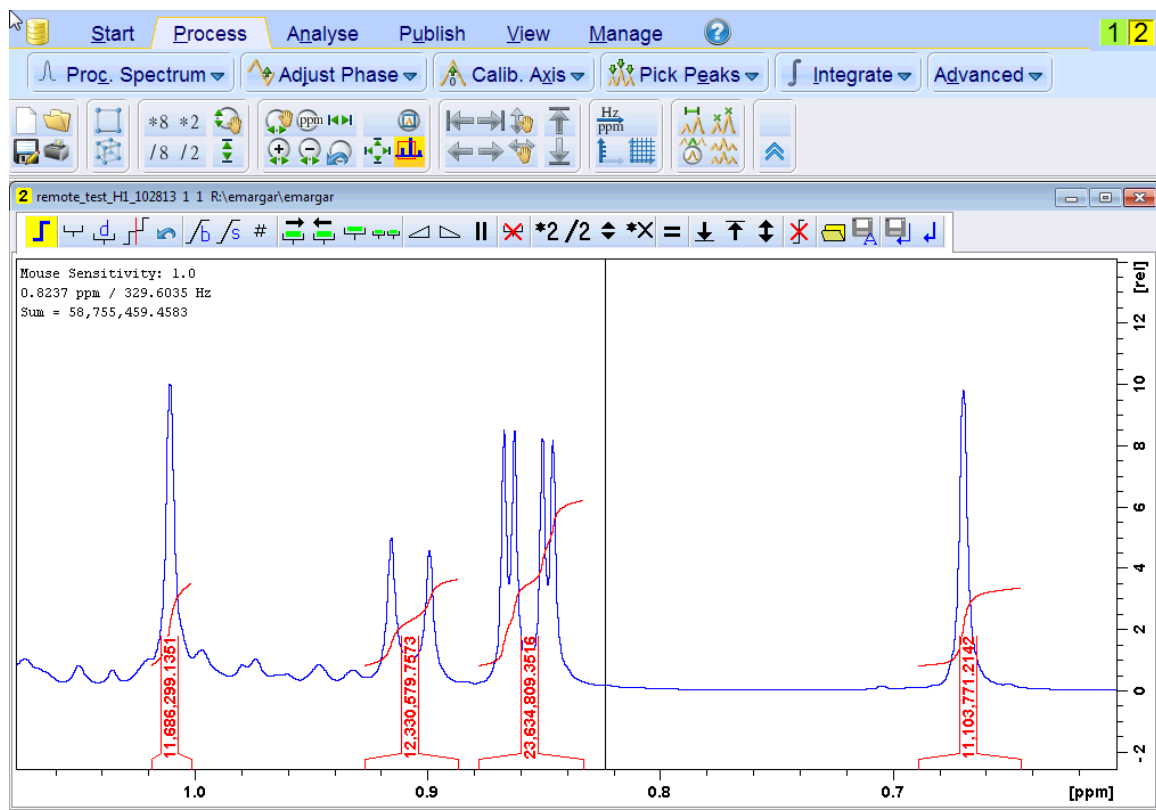
The series of buttons in integration mode is defined below:



Employing the button highlighted in gold above (Define integral regions), allows regions to be integrated to be selected by left-clicking and dragging across the peak:



The picture below shows how the integrals are reported after this manual selection of regions:



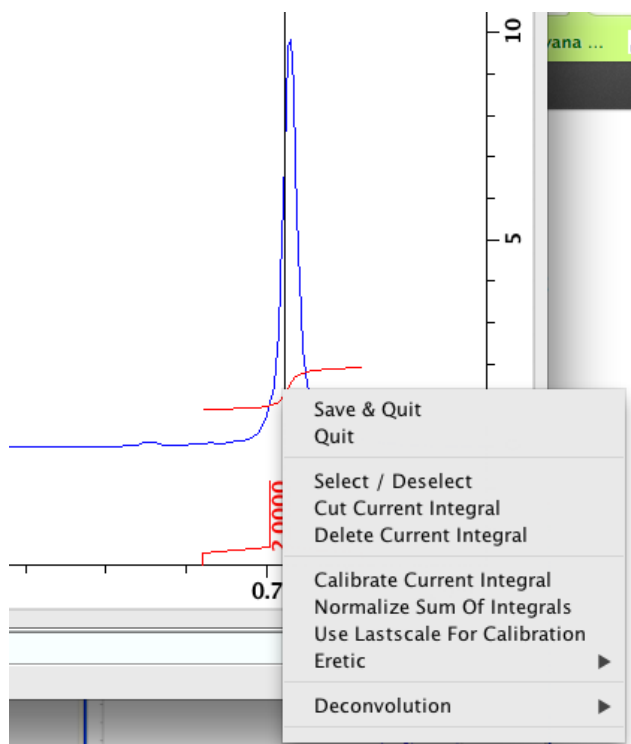
To delete a selected integral, in case of a mistake, select it with one of the green buttons.



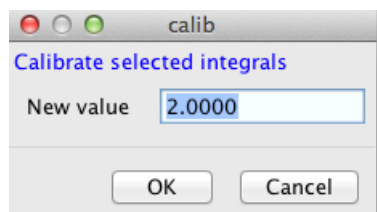
This will highlight the integral with a yellow/green color. Next, select the “delete selected integral regions” button to remove the integral:



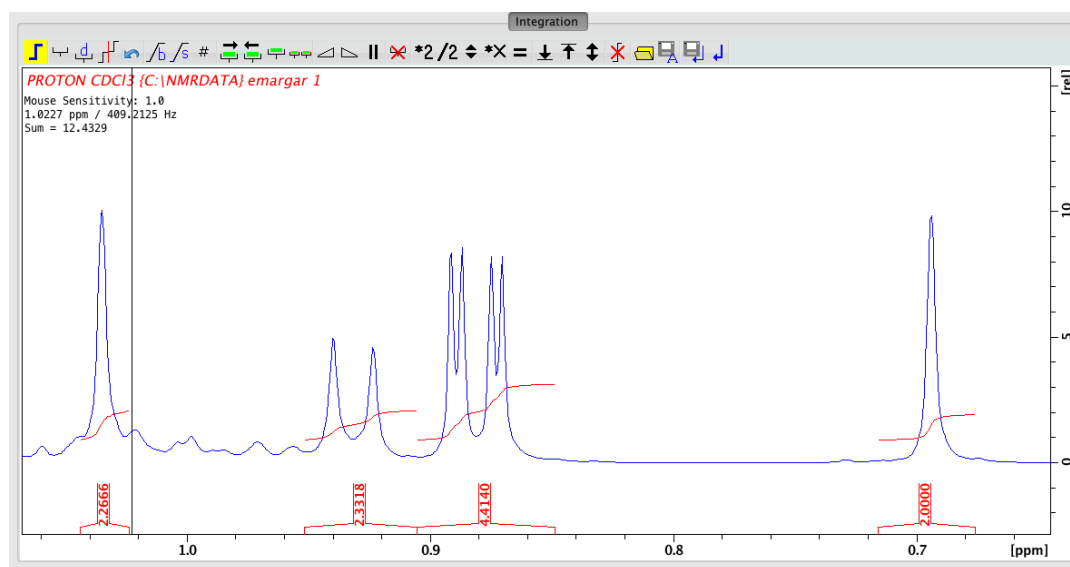
The numbers are large, so you may want to change the scale to make them reasonable numbers equivalent to number of protons, such as 1,2,3, etc. In the following example, the rightmost integral is set to a value of 2.0. To do this, right-click on the integral and select “Calibrate Current Integral” from the menu.



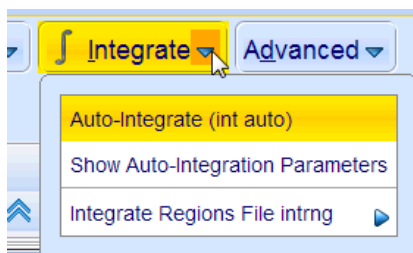
Next, enter the value (corresponding to the assumed number of protons).




The result is as follows, with all integrals reported relative to the calibrated one:



It can be easier some times to let TopSpin automatically select the integral regions, by selecting **Analysis -> Integration -> “Auto-find regions...”** on the Mac or **Process -> Integrate -> Auto-Integrate** (right-click on down arrow) on the PC.



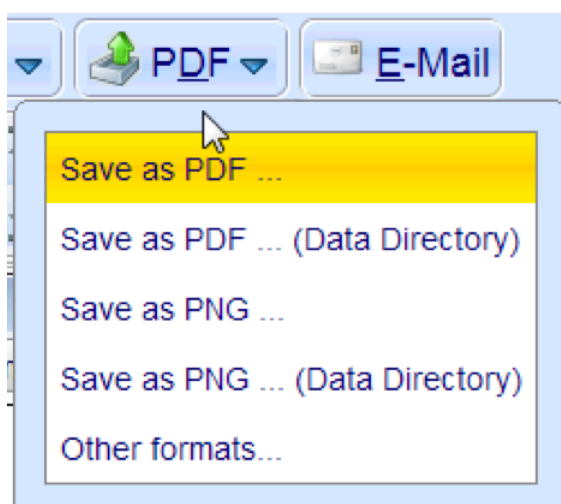
However, the integral regions may not be selected as you would like, so that the cut integral button may be needed , as well as select and delete buttons as described above.

The integral list may be copied and pasted into Excel by going to the **Integrals** tab in the spectral window, highlighting all the rows in the table, copying (**command-c** or **Publish -> Copy** on the PC), and opening Excel and pasting with **command-v**.

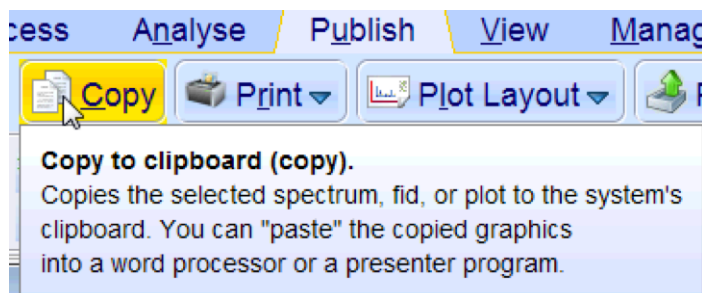
Spectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Struc
△ Object	Integral [abs]	Integral [rel]	Peaks	v(F1) [ppm]				
Integral 1	163197067.06	11686299.13...	0	1.0101				
Integral 2	172194330.16	12330579.75...	2	0.9072				
Integral 3	330055864.75	23634809.35...	0	0.8558				
Integral 4	155062169.34	11103771.21...	0	0.6670				

Plotting and Saving Pictures of Spectra:

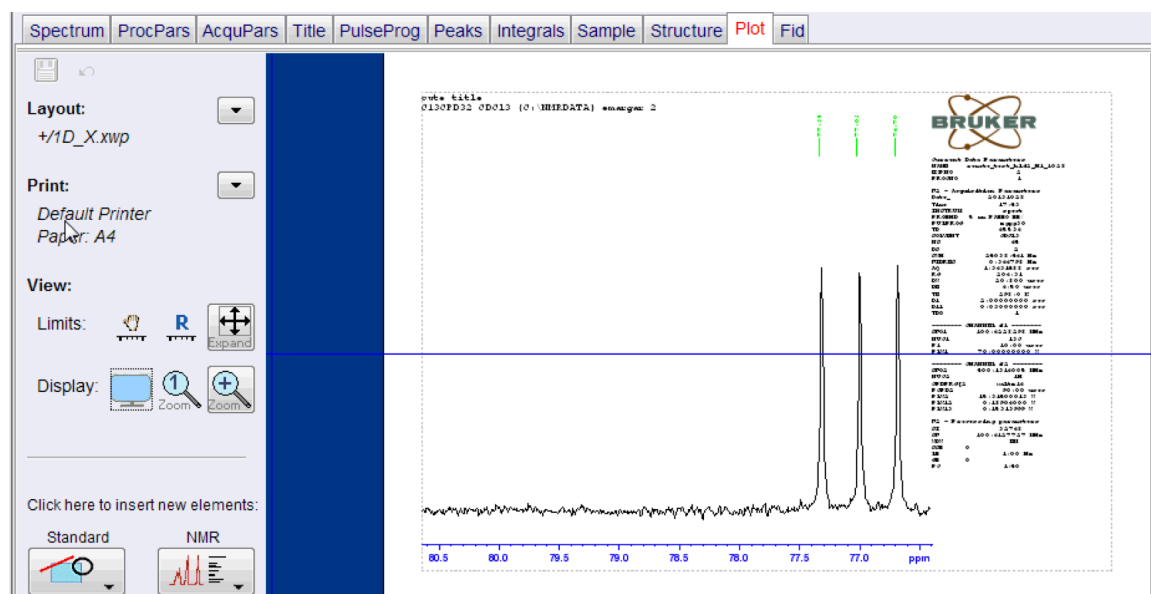
The spectrum depicted in the active window can be written out in PDF or PNG (or several other formats) using the **File -> Export** menu choice on the Mac or the **Publish -> PDF** tab on the PC.



It is also possible to copy a plotted spectrum using the **Publish -> Copy** button on the PC, for pasting into another file such as a Word or PowerPoint document.

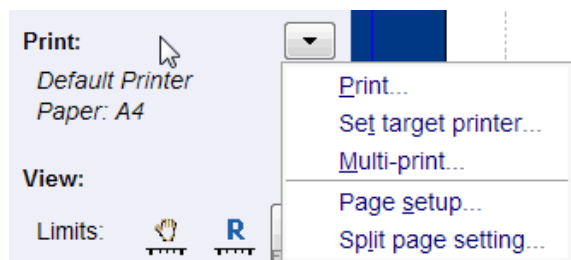


Plots may be setup and made in the Plot mode, selected by clicking on the **Plot** tab at the top of the spectral window (or also by selecting **Publish -> Plot Layout** on the PC).

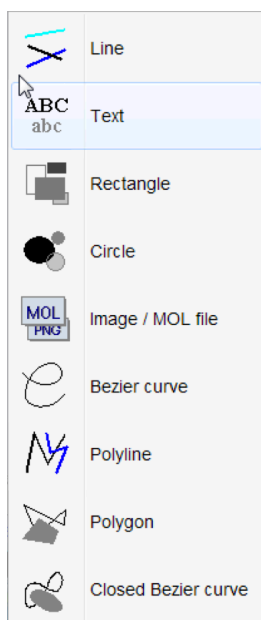


Addition of text, lines and shapes, pictures (including *mol* files with chemical structure pictures) is possible through the **Standard** menu. Just click and drag in the location on the plot where the item is to be added. Double clicking on an item highlights it and allows its dimensions and position to be entered.

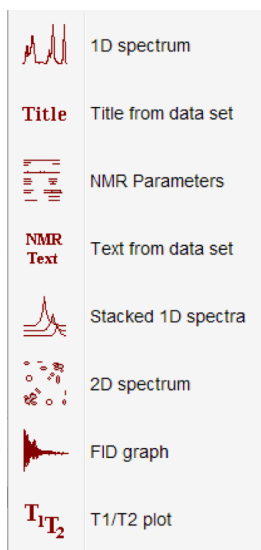
Printing to paper can be accomplished by selecting **Print** from the menu next to:



The **Standard** menu in the **Plot** tab:



The **NMR** menu in the **Plot** tab:



Choose a provided standard layout for the plot with **Layout:** -> **Open**, or by selecting **New** under **Layout:** a new layout can be constructed by adding elements from the **Standard** and **NMR** menus. Saving a new layout (**Layout:** -> **Save**) seems to work on the PC, but causes TopSpin to hang up on the Mac (?). There is a lot that can be done, but it looks like easy control over the fonts and other details of the plot is not available (?). Therefore, users may find this is handy for notebook printouts and internal reports, but may want to use MNova for preparing figures for publication.