

Bruker Topspin NMR Training Manual

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User-guide for USC's Bruker NMR Spectrometers. (Avance III-HD 300, Avance III-HD 400A and Avance III-HD 400B)

I: Overview of operation:

All three of these systems have sample changers and usually run fully automated using a program called **IconNMR**. Data collection is determined by your choice of experiment(s) for each sample you submit. While the parameters from the default experiments are usually valid for general survey spectra, parameters can be customized for more specific results. Please consult with the NMR staff for instructions related to parameter choices.

Processing is done with **Topspin** and is covered later in this manual.

Details for the steps in this outline are in following sections.

Automated NMR Instrument Operation Steps:

1. Sign into the logbook!
2. Clean the outside of your NMR tube
3. Properly gauge sample in a spinner. (page 2)
4. Check for **IconNMR** or start it if necessary (page 3)
5. Click on the **Change User** button and log onto your group account (page 4)
6. Find next open position in sample changer
7. Place spinner with sample into holder and note number
8. Enter sample details in correct sample holder line in **IconNMR** automation window (page 6-8)
9. Select holder line and click the Submit button
10. Hit the Start button if necessary (page 9)
11. Wait for system to run experiment
12. Perform any manual processing plotting, saving etc. (pages 15-24)
13. Log out of **IconNMR** by clicking the **Change User** button
14. Remove sample **AND spinner** from sample changer.
15. Sign out of logbook.

II: Properly Gauging the Sample in the Spinner

Make sure your hands are clean and dry. Also make sure the outside of your NMR tube is also clean and dry. Wipe it off with a Chem-Wipe before you begin. Isopropanol is also available to clean the tube if necessary.

No Gloves are allowed in the NMR Lab. Gloves might be protecting you, but they have been exposed to chemicals and are contaminating everything you touch. This exposes others to the hazards of your lab as you handle doorknobs, pens, spinners, mice and keyboards. The outside of your NMR tubes and any other materials you carry into the NMR lab should all be clean and free of any hazardous materials. Tubes should be tightly capped.

Insert the NMR Tube Into a Spinner

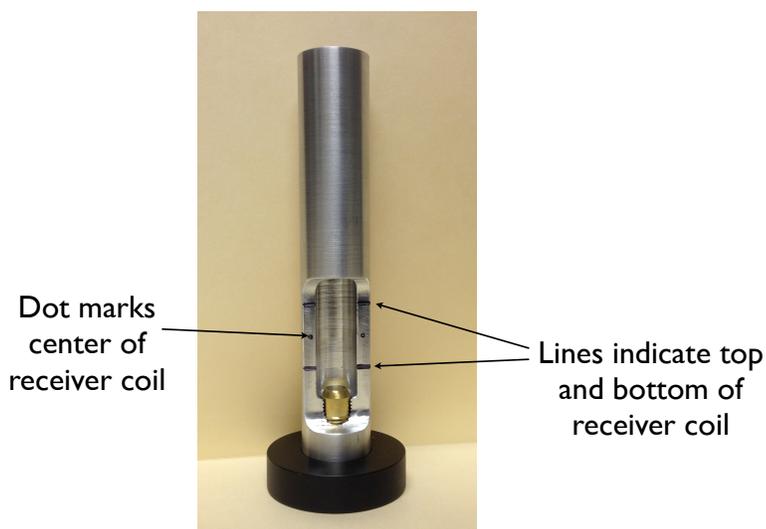
Hold the spinner at the top at the black and white band. You should try not to touch the lower part of the spinner. Push the tube through the spinner from the large (top) end. It should slide quite freely but remain where it is placed when pressure is removed. Do not force a tube that seems too tight. The O-ring in the spinner grips the tube. Push the tube so that only about four cm extends out the bottom of the spinner.



Adjust the Spinner Position

Insert the spinner with the sample into the depth gauge. Slowly push the sample down until it hits the bottom stop. The sample is properly gauged for samples with heights of 40 mm or more.

If you need to use less than 0.5 ml of solvent (< 40 mm height) for any reason, you must center the liquid volume in the area between the two lines on the gauge. This centers the sample in the receiver coil.



**NEVER PLACE AN EMPTY SPINNER
INTO THE MAGNET OR INTO THE
SAMPLE CHANGER**

III: Starting and logging into *IconNMR*:

You should find the system with *IconNMR* running. If for some reason it is not, you can start it by either two ways.

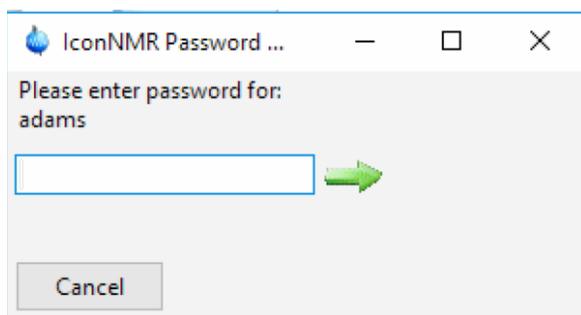
1. Type *icon* on the command line and hit the return key.
2. Choose from the Menu: **Acquire -> Options -> IconNMR Automation**

The window at the right should open. Click on the **Automation** button to continue.

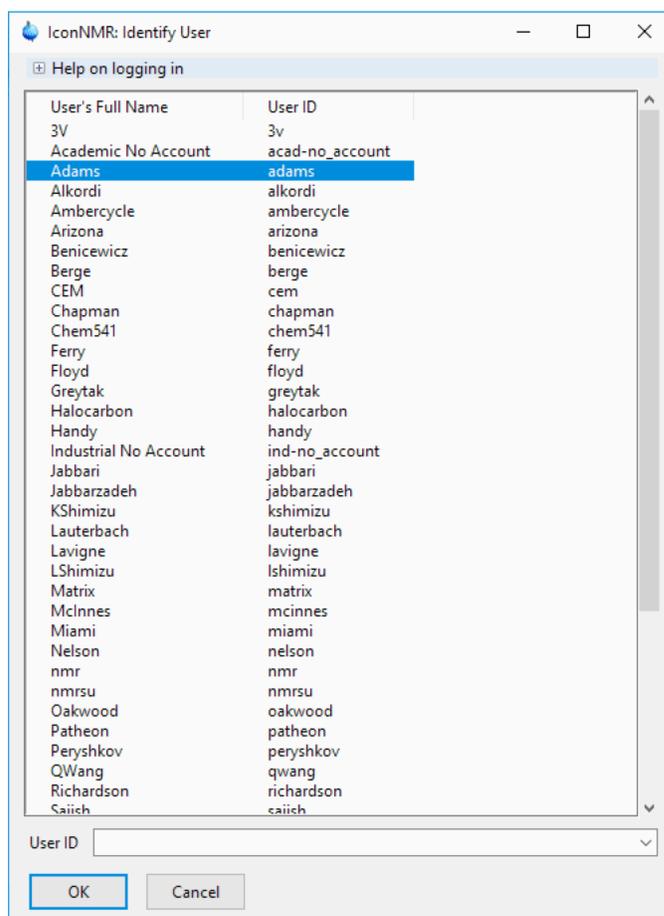


This should open the user identity window. Select **your group name (or accounting unit)** as the user ID and click the **OK** button.

This will open the password dialog box. The default password in the initial code for your group that you also use for saving datasets (see staff for details):



Click on the **green arrow** and an empty automation window should open and the system is ready to run samples.



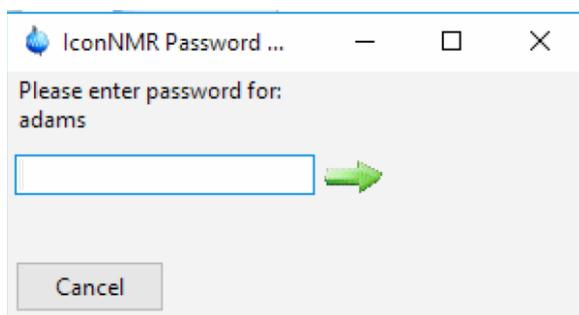
IV: Logging into IconNMR:

The NMR Facility uses the information collected with the automation software for billing. Therefore, it is important to correctly log into **IconNMR** and make sure you log out when you are done. If you find the system logged into a (different) account you should click on the **Changer User** button that is on the lower right of the automation window

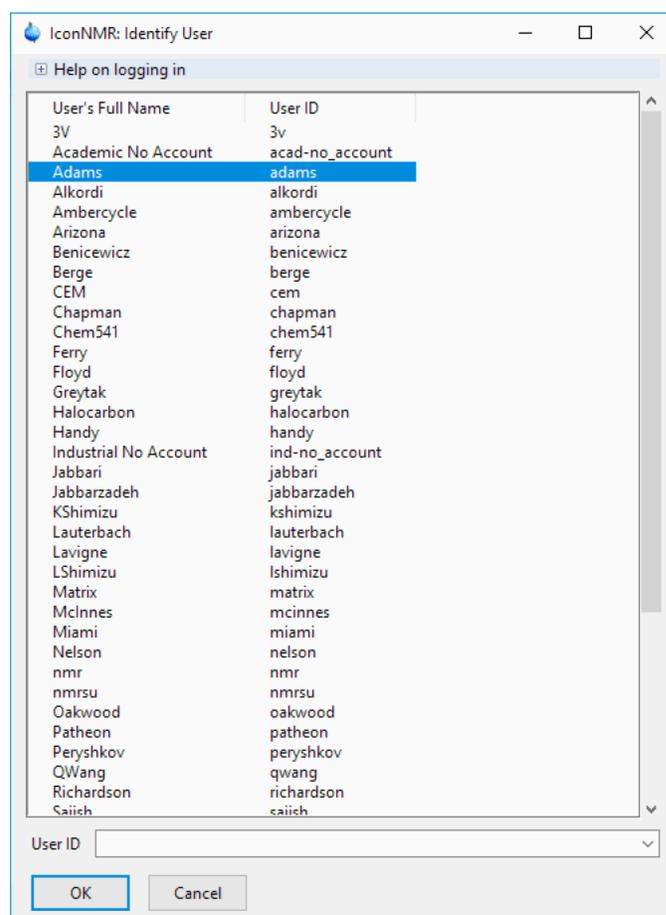


This should open the user identity window. Select **your group name (or accounting unit)** as the user ID and click the **OK** button.

This will open the password dialog box. The default password in the initial code for your group that you should also use for saving datasets (see staff for details):



Click on the **green arrow** and an empty automation window should open and the system is ready to run samples.



The system should automatically log your account off after 10 mins. However, to avoid confusion and allow other groups running samples under your professor's account make sure you log out of IconNMR each time you run by clicking on the **Change User** button.

V: Acquiring Data with *ICONMR* Automation window

Automated data collection is performed with software called *IconNMR*. It is Bruker's easy to use interface that is actually fairly powerful. On instruments with a sample changer the software will insert and eject the samples as they are run.

IconNMR Automation Window:

The automation window is organized as a table where the columns are specific entries that are needed to run samples. The numbered rows represent samples holders.

The screenshot shows the IconNMR Automation window with the following data:

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Pri	Par	Title/Orig	Time	User
1		Red	E:\Data	auto5_140107	5	DMSO	dimetf N proton_0-12			Final Product	00:01:17	auto
2		Green	E:\Data	auto5_140107	6	DMSO	dimetf N proton_0-10			Mixture A	00:01:17	auto
3		Yellow	E:\Data	auto5_140107	7	DMSO	dimetf N proton_0-12			Diene	00:01:17	auto
4		Yellow	E:\Data	auto5_140107	8	CDCl3	chlorof N proton_0-12			Reaction 103	00:01:17	auto
5		Black	E:\Data	auto5_140107	9							auto

#	Date	Holder	Name	No.	Experiment	Load	ATM	Lock	Shim	Acq	Proc	User	Disk	Title/Orig	Remarks
3	2014-01-07 13:11:51	2	auto5_140107	6	proton_0-10	✓						auto	E:\Data\data\auto\nmr	Mixture A	
2	2014-01-07 13:08:52	1	auto5_140107	5	proton_0-12	✓	✓	✓	✓	✓	✓	auto	E:\Data\data\auto\nmr	Final Product	xauw: Compiling AU program 'user/proc default calibration done
1	2014-01-07 13:01:30	1	auto5_140107	1	proton_0-12	✓	✓	✓	✓	✓	✓	auto	E:\Data\data\auto\nmr	Final Product 1	Automation was stopped

The status column shows the progress of the queued samples in a run. With a sample changer it is possible to have many samples in the queue.



Red indicates the experiment(s) for this sample have been completed normally.

Green indicates this sample/experiment is currently running.

Yellow shows samples and experiments that are waiting to be run, i.e., queued.

No lights indicate that the experiment has not been submitted

Lights that are all **black** indicate there was an error with the run

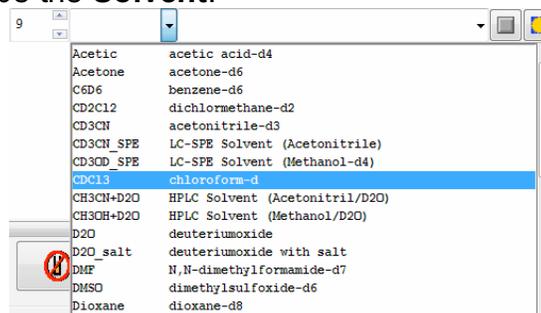
Entering a new sample

Double clicking an unused holder line will activate the line for entry:

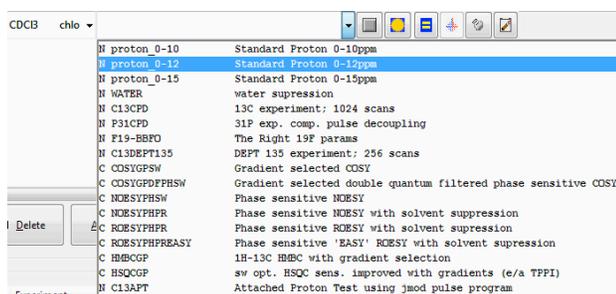


The first three columns, **Disk**, **Name** and **Number** will self populate and cannot be changed. The first empty column should be the **Solvent**.

Clicking in the **Solvent** field brings up a list of recognized solvents. Choose the one that is correct for your sample and click the left mouse button again to make the selection. Use the scroll wheel or scroll bar if necessary.



The next field is the **Experiment**. Again, click in the blank area, scroll to your required experiment and click on the option to select. (options on the spectrometer will look different than those shown in this manual).



The next five entries are a series of buttons. For experiments using complete default parameters they can be ignored.



The first one (left to right) is a check "box". This can be marked to give this sample/experiment priority and will be run next. You should not abuse this to jump ahead of samples entered by other users.

The yellow sun signifies that the experiment will be placed into the normal or daytime queue. This icon can be toggle to a moon icon. This sets the experiment to be run in the predefined evening time slot i.e., experiment will not start before 7:00PM.



Clicking the equal sign button opens a panel that allows adjustment of selected parameters. New values can simply be typed into each box. Access to full parameters can be achieved by selecting “**Edit all Acquisition Parameters**” or “**Edit Processing Parameters**” found under the **Parameter** menu. Both of these options switch you over to the main **Topspin** window. Look for the small **Return to IconNMR** button to resume entering the sample information.

D1	1 [sec]	"Relaxation Delay"
NS	16	Number of scans
SW	19.9915 [ppm]	Spectral width
O1P	6.175 [ppm]	Spectrum Center (PPM)

OK

The faint blue and red icon allows a user to change the way the system locks and shims the sample. This function is inactive and the button is safe to ignore.

The last icon controls the printing of the collected spectrum. In the default state (the green *tree* icon) the system will not print the results of the experiment upon completion. You can manually plot after you make any changes to the processed results. Click this icon and toggle it to the “printer” icon and printed output is automatically generated.



The last field to be completed is the “Title”. Clicking on the field will open a box where you can enter details of your sample. Multiple lines are allowed, but try to keep it to two or they can be cut off on the plot. Click **Set Title** when done. **Set & Copy Title** is used for entering a title across multiple experiments on a single holder.

Title or Comment can be entered here

Set Title Set & Copy Title

The “**Time**” column gives an estimate of how long each experiment will take to run. After all the required information is entered into the table, the experiment can be submitted by highlighting the holder line and then clicking the **Submit** button.



Other buttons below the experiment table include **Cancel**, **Edit** and **Delete**. A holder row must be selected/highlighted for these buttons to work.

The **Add** button allows multiple experiments to be entered into one sample holder. This allows queuing of many experiments for the same sample. For instance you can set up to run a proton, carbon and COSY spectrum. Hitting **Submit** will run them in the order entered.

Copy will take the selected holder and copy the entered values into the next unused holder. The experiment number should automatically increment. This is a good way to

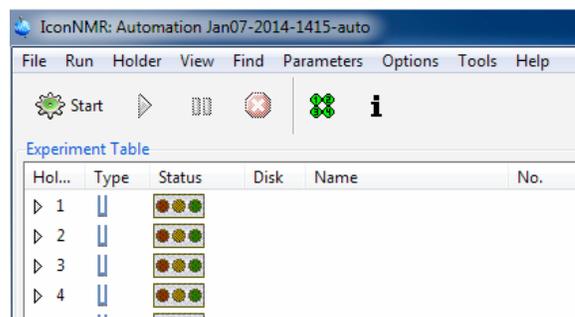
set up multiple samples or to save time when setting up a new run. Select the newly created holder information and click edit to make any changes.

At the bottom of the **IconNMR** automation window is a list of experiments that have been completed. Double clicking on an entry will open that dataset in **Topspin**. The Remarks will indicate if there were any errors.

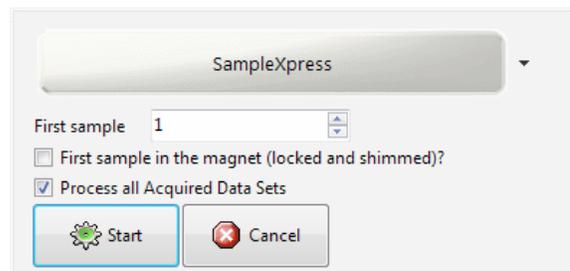
Preceding Experiments															
#	Date	Holder	Name	No.	Experiment	Load	ATM	Lock	Shim	Acq	Proc	User	Disk	Title/Orig	Remarks
5	2014-01-07 13:17:42	4	auto5_140107	8	proton_0-12	✓	✓	✓	✓	✓	✓	auto	E:\Data\data\auto\nmr	Reaction 103	sref: reference peak not found default ca
4	2014-01-07 13:14:47	3	auto5_140107	7	proton_0-12	✓		✓	✓	✓	✓	auto	E:\Data\data\auto\nmr	Diene	sref: reference peak not found default ca
3	2014-01-07 13:11:51	2	auto5_140107	6	proton_0-10	✓		✓	✓	✓	✓	auto	E:\Data\data\auto\nmr	Mixture A	sref: reference peak not found default ca

Starting and Stopping the Automation

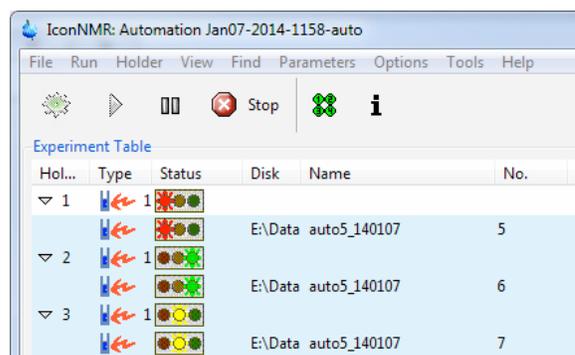
If **IconNMR** had been just launched or if the automation was stopped, you will have to click on the green **Start** button on the top left of the automation window.



This brings up the panel to the right. Clicking the **Start** button on this panel will initiate the experiment for the sample in the lowest holder number.

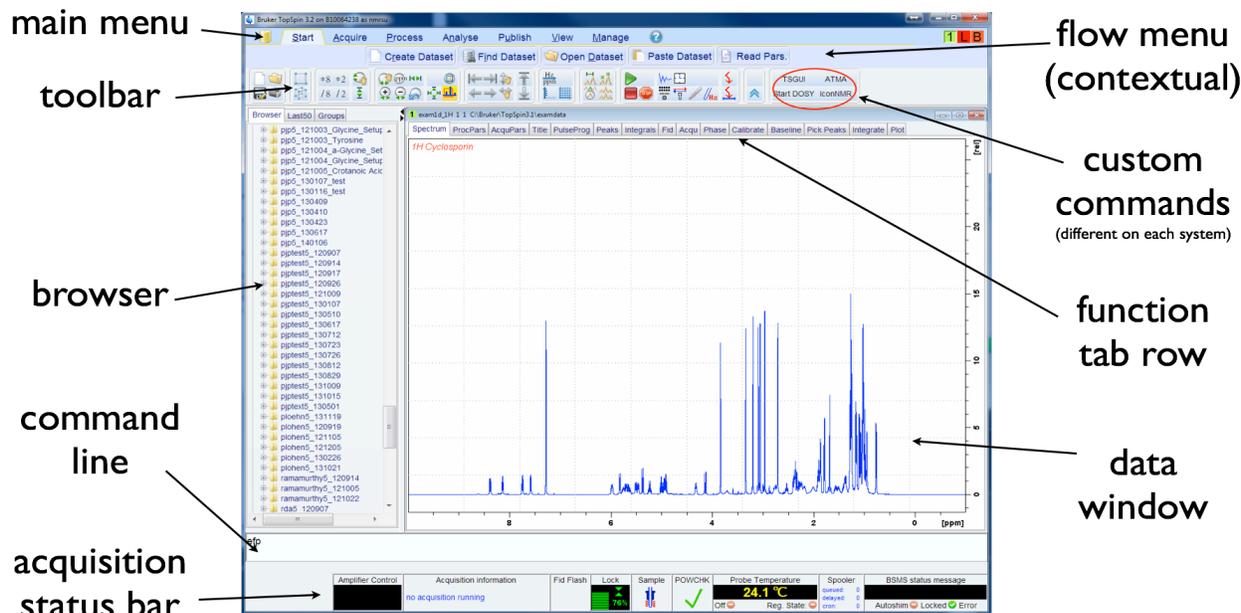


When automation is running the **Start** button is greyed out and the red **Stop** button is visible at the top of the automation window. Use the **Stop** button to immediately stop the queue of experiments (data that is being collected will be lost). The Pause button || will allow the current experiment to finish before stopping the queue.



VI: Using Topspin

Topspin is the program that is used to collect, process, save and plot NMR results. The **Topspin** main window consists of a data window, a file browser, toolbar and a menu-bar. The data window has a row of tabs that allows different displays and several processing functions. Note that the browser can be inactive [hit **Ctrl+d**] or displayed as a separate window.



Note that the menus and toolbars depend on the data dimensionality. The descriptions below hold for 1D data.

How to Use the Menu bar

The menu bar contains the following menus:

- **File:**  Perform data/file-handling tasks
- **Start:** Create or open an existing dataset
- **Process:** Data processing commands
- **Analyze:** Data analysis commands
- **Publish:** Output generation
- **View:** Set view options and display multiple data windows
- **Manage:** Set various options, preferences and configurations
- **Help:**  Access various information, indices, manuals etc.

Experienced users will usually work with keyboard commands rather than menu commands. Note that the main keyboard commands are displayed in square brackets [] behind the corresponding menu entries. Furthermore, right-clicking any menu entry will show the corresponding command.

How to Use the Toolbar (1D data)

The toolbar contains buttons for data handling, switching to interactive modes, display settings, and starting acquisition. They are grouped into several related subsections.



Buttons for file management:

-  Create a new dataset [**Ctrl+n, new**]
-  Open a dataset [**Ctrl+o, open**]
-  Save the current dataset [**Ctrl+s, sav**] use the **wr** command for fewer options
-  Print the current dataset [**Ctrl+p, print**] use the **plot** command for more options.
-  Switch to the last 2D dataset [**.2d**]
-  Switch to the last 3D dataset [**.3d**]

Buttons for vertical scaling (intensity):

-  Increase the intensity by a factor of 8 [***8**]
-  Increase the intensity by a factor of 2 [***2**]
-  Decrease the intensity by a factor of 8 [**/8**]
-  Decrease the intensity by a factor of 2 [**/2**]
-  Change the intensity smoothly (hold left mouse button, drag up or down)
-  Reset the intensity to tallest peak (baseline positions remains unchanged) [**.vr**]

Note that vertical scaling can also be changed with the mouse wheel.

Buttons for horizontal scaling (zooming):

-  Zoom in/out smoothly
-  Exact zoom via dialog box [**.zx**]
-  Reset zooming (horizontal scaling) to full spectrum [**.hr**]
-  Toggle interactive zoom mode. When switched off, interactive zooming only selects a horizontal region; baseline position and intensity scaling remain the same. When switched on, interactive zooming draws a box selecting the corresponding area.

-  Zoom in to the center (spectrum) or left edge (FID) of the displayed region, increasing the horizontal scaling. [*.zi*]
-  Zoom out from the center (spectrum) or left edge (FID) of the displayed region, decreasing horizontal scaling) [*.zo*]
-  Undo last zoom [*.z1*]
-  Display the entire spectrum (baseline position and intensity scaling are adjusted if necessary) [*.a11*]
-  Retain horizontal and vertical scaling when modifying dataset or changing to different dataset. Global button for all data windows [**.keep**]

Buttons for horizontal shifting

-  Shift to the extreme left edge of the spectrum [*.s10*]
-  Shift to the extreme right edge of the spectrum [*.sr0*]
-  Shift to the left, half of the displayed region [*.s1*]
-  Shift to the right, half of the displayed region [*.sr*]
-  Smoothly shift to the left or to the right

Buttons for vertical shifting

-  Smoothly shift the spectrum baseline up or down.
-  Shift the spectrum baseline to the middle of the data field [*.su*]
-  Shift the spectrum baseline to the bottom of the data field [*.sd*]

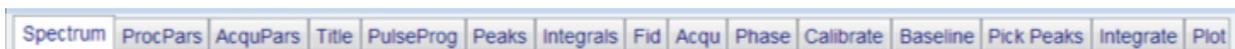
Buttons for display options:

-  Toggle between Hz and ppm axis units
-  Switch the y-axis display between abs/rel/off
-  Switch the overview spectrum on/off
-  Toggle grid between fixed/axis/off
-  Enter distance measurement mode
-  Setup pulse program frequency list [*.freqlist*]
-  Toggle display of spectrum overview [*.ov*]
-  Enter multiple display mode [**.md**]

Buttons for Acquisition functions:

	Start Acquisition [zg]
	Halt Acquisition (saves data) [halt]
	Emergency Stop Acquisition (discards data!!!) [stop]
	Show Acquisition window (i.e., Acqu Tab) [acqu]
	Estimate experiment time [exptf]
	Open BSMS control panel (manual locking and shimming) [bsmsdisp]
	Open Lock display [lockdisp]
	Open Variable Temperature control [vtudisp]
	Open MAS control panel [masdisp] (NOT AVAILABLE ON ALL SYSTEMS)
	Set RF frequency with cursor, [seto123]
	Set SW to current display region (also changes center freq. i.e. SFO1) [setsw]

The Topspin Data Window Tab Row



At the top of the data window is a row of tabs that help display or perform certain processing functions. There are both commands and menus that activate these functions, but the tabs make them more accessible. The tabs displayed are highly customizable and not all may be shown or they may not be in this order. Here a list of some of the more useful tabs and what they do.

- **Spectrum** Display the spectrum in an interactive mode
- **ProcPars** Display the processing parameters
- **AcquPars** Display the acquisition parameters
- **Title** Open title editor (appears on plots)
- **PulseProg** Open pulse sequence display and editor
- **Peaks** Display list of peaks (peak picking results in a table)
- **Integrals** Display list of integrals (integral results in a table)
- **Fid** Display the time domain results i.e., FID
- **Acqu** Show data collection in real time (Either FID or spectrum)
- **Phase** Enter manual phase correction function
- **Calibrate** Enter manual chemical shift reference function
- **Baseline** Enter baseline correction function
- **Pick Peaks** Enter into peak picking function (manual or automatic)
- **Integrate** Enter into manual integration function
- **Plot** Open the plot editor

Command Line Usage, How to Put the Focus in the Command Line

In order to enter a command on the command line, the focus must be there. Note that, for example, selecting a dataset from the browser, puts the focus in the browser. To put the focus on the command line:

Hit the **Esc** key or

Click inside the command line

How to Retrieve Previously Entered Commands

All commands that have been entered on the command line since TOPSPIN was started are stored and can be retrieved. To do that:

Hit the **↑ Up-Arrow** key on the keyboard

By hitting this key repeatedly, you can go back as far as you want in retrieving previously entered commands. After that you can go forward to more recently entered commands as follows:

Hit the **↓ Down-Arrow** key on the keyboard

How to Change Previously Entered Commands

1. Hit the **← Left-Arrow** or **→ Right-Arrow** key to move the cursor
2. Add characters or hit the **Backspace** key to remove characters
3. Mark characters and use **Backspace** or **Delete** to delete them, **Ctrl+c** to copy them, or **Ctrl+v** to paste them.

In combination with the arrow-up/down keys, you can edit previously entered commands.

How to Enter a Series of Commands

If you want to execute a series of commands on a dataset, you can enter the commands on the command line separated by semicolons, e.g.:

em; ft; apk

If you intend to use the series regularly, you can store it in a macro as follows:

right-click in the command line and choose **Save as macro**. Use your initials to start the name.

VII: Command Mode Options

This section lists some of the basic functions available in *Topspin* command mode. It is not an attempt to present a complete description of *Topspin* and every command available. If you wish to explore more of the capabilities of *Topspin*, refer to the manuals. These manuals are located by the spectrometer workstation. All typed commands are entered into the *Topspin* command input window that is on the bottom of the screen.

Data Collection

Acquisition parameters can be found on the “*AcquPars*” tab in the data window. The “pulse icon”  will show only parameters that are relevant to the current experiment.

zg deletes any current data and starts a new collection. The data is **NOT** automatically processed after data collection.

Data Processing

eft does an exponentially weighted Fourier transform of the data using the *line broadening* defined by the **lb** parameter.

ft does an un-weighted Fourier transform of the data. **em** can be used before **ft** to give a *line broadened* spectrum (same result as **eft**).

efp does a weighted Fourier transform of the data and applies the **last** phase correction to the data.

Data Displaying and Manipulation

The “*Fid*” tab displays the fid

The “*Spectrum*” tab displays the transformed data and enters an interactive mode. In this mode the mouse will move a vertical cursor line across the spectrum. You can hold the left mouse button down and drag the cursor to horizontally zoom the data. Release the left button when you reach the limit you want to expand. This works in either direction.

The right mouse button will bring up a context sensitive menu.

The **exactzoom** panel can be used for finer control of the displayed frequency span of the spectrum. Access the exact zoom panel by clicking on the  menu button [or typing **.zx**] and enter the left and right limits of the spectrum region you require. The displayed chemical shift range is passed onto the Plotting routine and should be adjusted *prior* to manual plotting.

The scroll wheel on the mouse can be used to change the vertical scale of the spectrum. Alternately the vertical scaling buttons can be used. However, vertical scaling information is **not** passed onto the **Plotting** tab.

VIII: Phasing a Spectrum

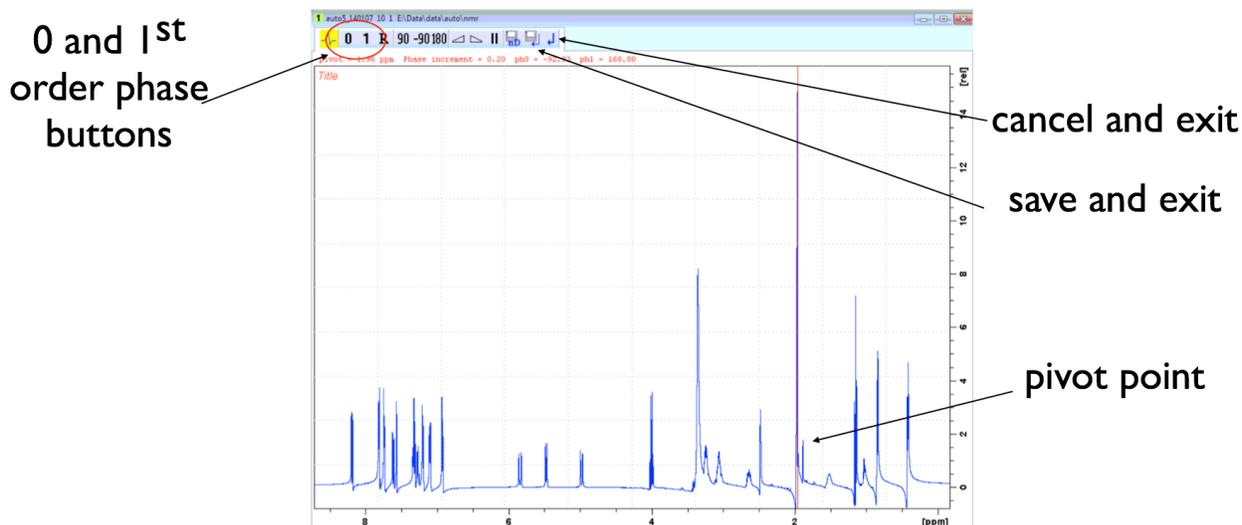
Automatically Phasing a Spectrum – *apk* command

Automatic phasing is done as part of an experiment in *IconNMR*; Specifically, automatic phasing is done when *Topspin* processes automatically collected data. Alternatively, you can enter the *apk* command in the *Topspin* command line after the spectrum is displayed.

Manually Phasing a Spectrum

This procedure describes how to manually phase a spectrum.

1. Display the spectrum by clicking on the **Spectrum** tab and adjust the intensity and chemical shift range. The vertical scale should be high enough to see small phase errors in the baseline of the peaks.
2. Click on the **Phase** tab to start the interactive phase function.



3. Locate the red vertical “line” that the software places over the most intense peak. This is the “**Pivot point**” of the phase correction. (This can be changed by pointing the mouse cursor on a different peak and then right click to bring up the context sensitive menu.
4. Place the mouse cursor onto the 0-order phase correction button **0**, then click and **hold** the left mouse button
5. Move the mouse up or down to adjust the phase of the spectrum. Focus your attention on JUST THE PEAK AT THE PIVOT POINT. This phase correction makes changes that are constant across the whole frequency range but it should be easier to judge its effect on just the one (tallest) peak. Release the button when the adjustment is complete.

6. Place the mouse cursor onto the 1st-order phase correction button , then click and **hold** the left mouse button
7. Move the mouse up or down to adjust the phase of the spectrum. Focus your attention on peaks that are FAR AWAY FROM THE PIVOT POINT. This phase correction makes changes that increase as the frequency moves away from the pivot point. It is impossible to judge its effect on the pivot point. Release the button when the adjustment is complete. If successful, all peaks should be correctly phased. If not repeat the procedure.
8. To accept the phase correction and exit the interactive phasing mode, click on the save and return icon . If you want to cancel your changes, i.e., exit without saving, click on the return button . Either option brings you back to the **Spectrum** tab

You can read about the other options of the interactive phase correction in the full manual or ask the NMR staff for further assistance.

IX: Chemical Shift Referencing (i.e., Calibrate)

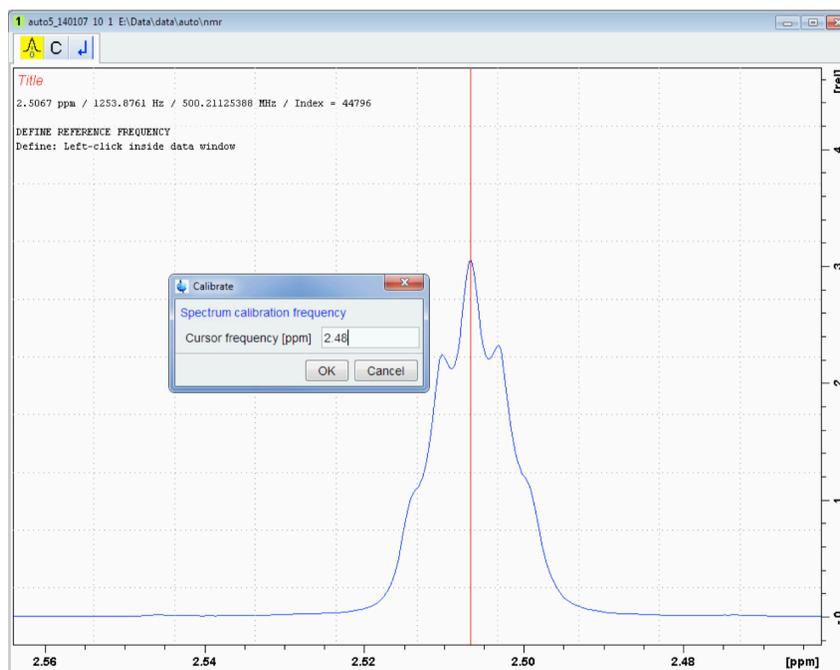
Automatic

The **sref** command (used by the automation) can be used for automatic referencing. If TMS is present in the sample the **sref** will automatically assign this peak to zero ppm. However, this command can erroneously assign other peaks that fall near TMS to the “zero” point (i.e., silicon grease). If there are no peaks near 0 ppm a quick referencing based on the lock frequency is performed. This gives a reasonable but not totally accurate chemical shift reference.

Manual:

Traditionally, TMS is added to a sample and its resonance is used, but **Topspin** also allows you to reference a spectrum with any known line. Typically the residual resonance from the solvent is used. (see table of solvents for their chemical shift values)

Before you reference a spectrum you should expand the region of the line you want to reference (i.e., the residual solvent peak). Clicking the **Calibrate** tab opens the manual calibration function. To reference the spectrum, move the vertical cursor with the mouse to the exact center of the line you want to reference. Click the left mouse button to open a dialog box where you can enter the value you want to assign to the chosen peak. Click **OK** and you will be switched back to the **Spectrum** tab. Peak picking and integration should be performed after you reference the spectrum.



X: Peak Picking.

Generating the list of spectrum frequencies is performed by the **pp** command. Peaks shown with **pp** will be included on plots that include peak values. Typing **pp** brings up the following dialog box. The settings in this example are a good starting point for creating an automated listing. Changes to these values can either give more or less peaks.

It is always safer to choose “**Auto-Pick peaks on full spectrum**” than “**Auto-Pick peaks on displayed spectrum region.**”

The “**Intensity of the reference peak**” (CY) is always the *largest* peak in the spectrum. Here, we are giving it a *relative* value of 15 cm.

The *relative* “**Minimum intensity**” (MI) sets the threshold for peaks to be included in the peak picking. A smaller number will usually include more peaks in the list.

“**Maximum intensity**” (MAXI) is only relevant when it is less than the reference intensity (CY).

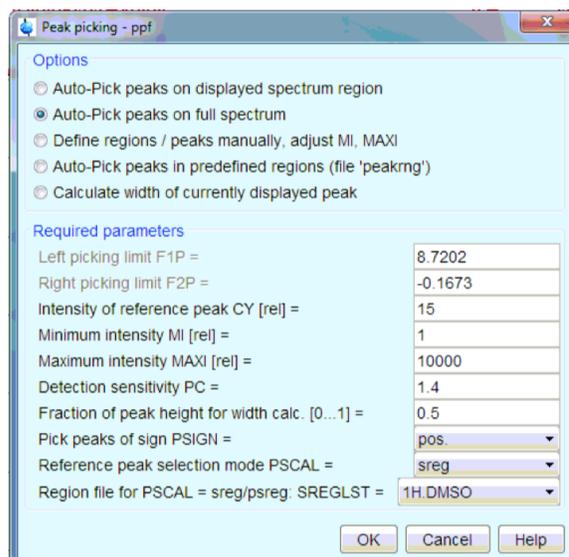
The “**Detection sensitivity**” (PC) sets how aggressive shoulders on larger peaks or partially overlapped lines are recognized as peaks to include in the picking. A smaller number will produce more peaks (usually in baselines of larger signals) while a larger number will force only the main peak to be reported.

Other options on the panel allow more manual interaction with the spectrum. See the NMR staff for help or refer to the full manual for more information.

Printing Peak list

A listing of the peak picking can be viewed by clicking on the **Peaks** tab. When this screen is shown, clicking the print button at the top tool bar will produce a printout. However, to get a **much** cleaner listing use the command (script):

pp_print



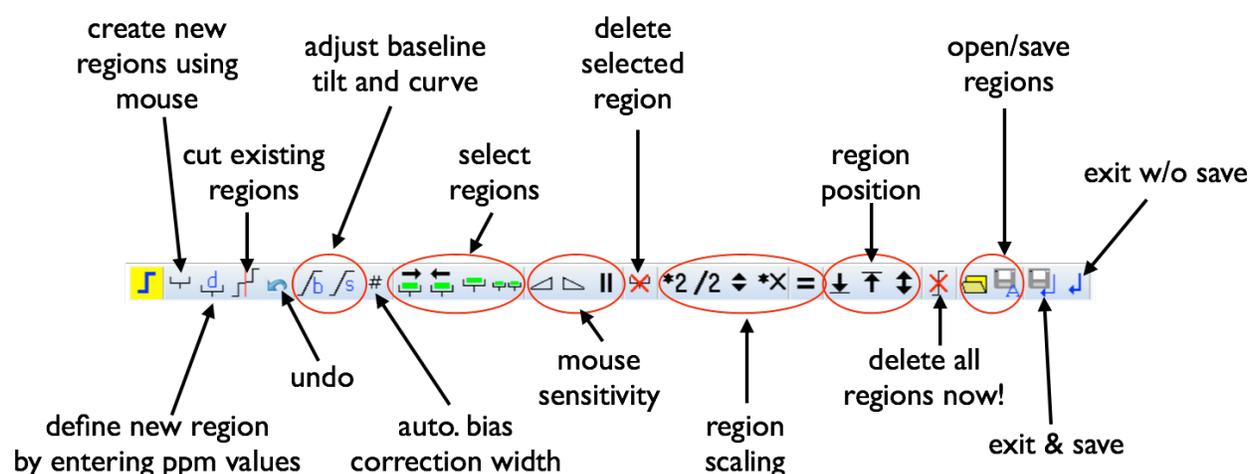
XI: Integration

Automatic Integral Resets

The **abs** command automatically creates integral regions around “major” peaks and performs a baseline correction. This is what the automation executes when the data is processed. For many spectra this is all you will need.

Manual Integration

The **Integrate** tab opens up the interactive integration function. From this routine you can create new integral regions, delete existing regions and cut existing regions into smaller ones. Most of the functions are accessed from the button bar that opens at the top of the data window:



The spectrum can still be manipulated with either the main button bar or the mouse but the vertical cursor action changes depending on which sub function is active.

Create Integral Regions

1. Click on the “**Create new region**” button  to activate edit mode. While active the icon will turn yellow (clicking it again toggles it off).
2. Using the mouse, move the vertical cursor to the point in the spectrum where you want an integral region to start.
3. Click and **hold** down the left mouse button.
4. Drag the vertical cursor to the point you want the region to end.
5. Release the mouse button. An integral curve for the region you just defined should now be shown.
6. Repeat (steps 2-5) for all desired regions
7. When done make sure you save your edits and exit the function by clicking the  button

Delete existing integral regions

To delete all integral regions click on the  button

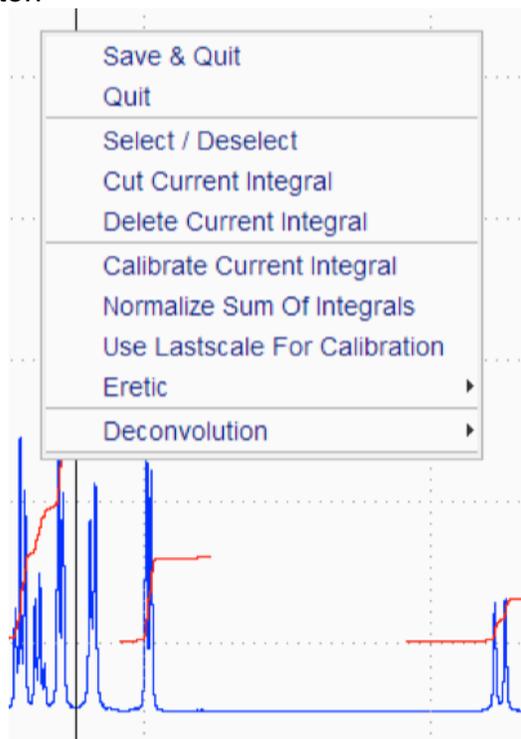
To delete a single region:

1. Position vertical cursor over region
2. Click the right mouse button to bring up the context sensitive menu
3. Choose **“Delete Current Integral”**

Breaking Existing Integral Regions

To break an existing region into two:

1. Position vertical cursor at point you want to cut
2. Click the right mouse button to bring up the context sensitive menu
3. Choose **“Cut Current Integral”**



If there are multiple regions that need to be cut into smaller regions:

1. Click on the **“Cut Existing Region”** button  (icon will turn yellow when active)
2. Position vertical cursor at point where you want to cut region
3. Click the left mouse button and the region will cut into two
4. Repeat (steps 2-3) for all desired cuts.
5. Click on the **“Cut Existing Region”** button  to deactivate

Integral Region Calibration and Normalization

To assign a numeric value to specific integral region:

1. Position vertical cursor over integral region you want to define
2. Click the right mouse button to bring up the context sensitive menu
3. Choose **“Calibrate Current Integral”**
4. Enter value into box that is displayed and click OK.

If you want to have all the integral regions add up to a specific number (i.e., 100%) right click over any region and select **Normalize Sum of Integrals**. Enter the value into box that is displayed and click OK.

XII: Plotting Spectra

Plotting results with *Topspin* can be a very complex procedure. This manual is going to show the simplest way to use our pre-defined layouts to get output with the options you want. However, each of these layouts can be modified to give you a large variety of plot options. See the NMR Staff to discuss these options and learn more about how to use them.

Typing *plot* at the command line is equivalent to clicking on the **Plot** tab. The plot function shows a preview of what the printed output will look like. There is a panel at the left where you can choose a different layout. These layouts give options to include peak values and integrals (see next page for the options). A default layout is defined in the parameter file and is set for the common choices for the experiment type and nucleus of the spectrum. Proton defaults to include the integral curve and values. ^{13}C spectra have a default layout that includes the chemical shift values on peaks (in ppm).

choose layout

Adjust the vertical scaling of the spectrum using either the toolbar buttons or the mouse scroll wheel. Chemical shift range selection is easier done before you enter the plot

function. When you have the desired output click on the printer icon  on the main toolbar and click **OK** in the dialog box that opens (printer and other options should be correct).

Available Plot Layouts

1D_H.xwp	Spectrum with integral curves and values (Default for ^1H)
1D_X.xwp	Spectrum with shift peak values (Default for ^{13}C)
1D_BB.xwp	Same as 1D_X (Default for ^{31}P , ^{11}B etc.)
1D_Peaks+Integrals.xwp	Spectrum with integral curves and peak values
2D_homo.xwp	2D layout for COSY spectrum
2D_inv.xwp	2D layout for hetero-nuclei experiments, i.e., HSQC, HETCOR etc.
2D_2proj.xwp	2D layout for hetero-nuclei experiments with projections on both axes
ppfile.xwp	Layout for generation of Peak Pick Lists

Making Changes to Spectrum Options in the Default Layout

Click in the spectrum area to bring up panels that have options for the spectrum printout. The three different panels can be accessed by the lower blue choices:

exit back to main panel

toggle to these panels

Most of the choices are self-explanatory. The Automation Actions panel has information used for plots generated by *IconNMR* and can be ignored. Custom plot layouts can be saved (Save as option from main panel/ Layout list) for future use. Always start the layout name with your initials. Never overwrite the default layouts. See NMR Staff for assistance with creating custom plots.

XIII: Saving and Retrieving Data:

Saving a data set:

Datasets acquired using **IconNMR** are saved in a temporary file labeled with the date and numbered subfolders that indicates the order in which they were run. These files will be periodically deleted (monthly). If you want to have your data archived you must save the data (using the **wr** command) using a different file name. **We require you to use our standard file-naming scheme. All data files that do not comply with these rules will be deleted with no warning!**

The structure of the file name is:

abc4_yymmdd[_x]

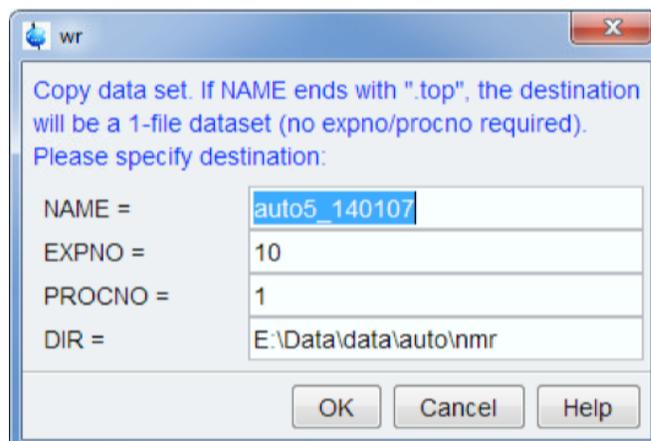
Where:

- abc* represents the initials of your major professor (**lower case only**)
- 4* signifies that the data was collected on the 400 MHz system (use "3" for the 300)
- yy* is the last two digits of the year
- mm* is the number of the month (January = 01 etc.)
- dd* is the day
- _x* are optional letters/numbers for additional information to distinguish between multiple datasets run on the same day. Use only letters, numbers, dashes(-) or underscores(_). Any other character is NOT ALLOWED.

Please note that the underscore is important and single digit months and days should be preceded with a zero. Also note the limit for the whole file name is 32 characters. Files with longer file names will be archived with a truncated label.

To save the data set:

1. Make sure the dataset is open in the data window
2. Enter **wr** in the command line
3. The dialog box at the right should open. (Note the date code should be correct)
4. Change "auto" to your **major professors initials** (see staff if you are uncertain on correct initials)
5. Add any additional information to the end of the name (remember the "_")



6. The **EXPNO** can be left as shown or it can be reset to “1” (or any other number). Multiple datasets can be added to the same file (i.e., folder) **NAME** but each must be given a unique **EXPNO**.
7. Click OK

The **wr** command will not allow you to overwrite an existing dataset.

Retrieving a data set:

If you recently ran the sample with **IconNMR**, double-click on the line for the dataset in the *lower* panel (marked **Preceding Experiments**).

There are several ways of opening a dataset that was **saved** earlier.

Using the Folder button  on the top tool bar:

1. Click on the folder button  at the top toolbar: a browser box should open
2. If necessary navigate to the correct folder where the data is stored: (E:\data\data\auto\nmr).
3. Click on the folder name
4. Click the **Display** button at the bottom of the browser box.
5. If more than one dataset was saved in that folder, another dialog box will open. Select the dataset you want and click the **Open** button.

Using the Menus:

1. Click on the File icon  at the top menu-bar and choose **Open**: a browser box should open
2. If necessary navigate to the correct folder where the data is stored: (E:\data\data\auto\nmr).
3. Click on the folder name
4. Click the **Display** button at the bottom of the browser box.
5. If more than one dataset was saved in that folder, another dialog box will open. Select the dataset you want and click the **Open** button.

Using the Browser:

1. Navigate to the correct folder where the data is stored: (E:\data\data\auto\nmr).
2. Right-Click on the folder name
3. Choose **Display** from the context sensitive menu
4. If more than one dataset was saved in that folder, another dialog box will open. Select the dataset you want and click the **Open** button.