



Agilent VnmrJ 4.2 Spectroscopy

User Guide

Notices

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This guide is valid for 4.2 revision of the Agilent VnmrJ software, until superseded.

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5301 Stevens Creek Boulevard
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C Liquids Spectra

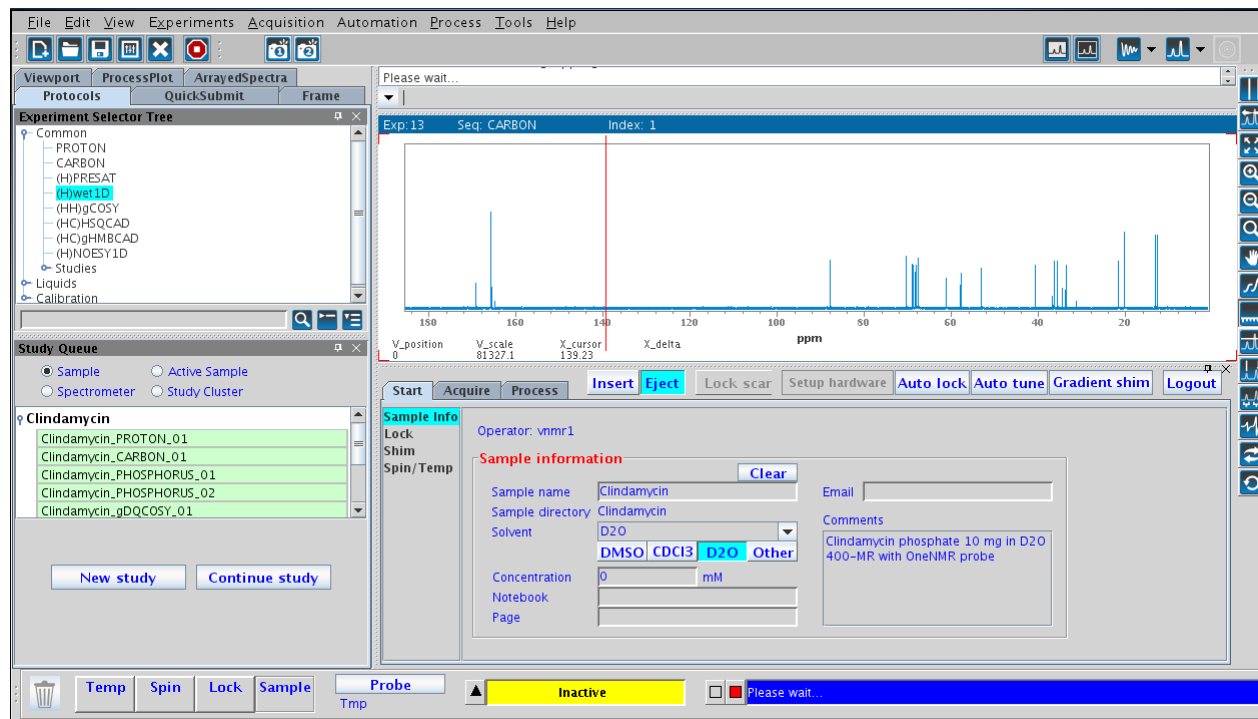
Ethylindanone	530
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1 Running Liquids NMR Experiments

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This chapter describes the use of VnmrJ to run liquids NMR experiments with the VnmrJ spectroscopy interface. The tasks involved in running a liquids NMR experiment generally follow the VnmrJ interface layout, moving from left to right over the interface, from the vertical panels to the Start, Acquire, and Process tabs. The VnmrJ interface is described in the *Agilent VnmrJ Familiarization Guide*.



NMR Experiment Tasks

The following table lists the required tasks and the related section in this manual. The VnmrJ panels and pages listed are typical, but may vary depending upon the choice of experiments.

Task	For more information
Prepare for an experiment	See “Prepare for an experiment” on page 18
Select an experiment	See “Selecting an Experiment” on page 44
Set up an experiment	See Chapter 3 , “Experiment Setup”
Acquire NMR data	See Chapter 6 , “Data Acquisition”
Process the data	See “Process the data” on page 20
Display the data	See Chapter 8 , “Displaying FIDs and Spectra”
Print the data	See Chapter 9 , “Printing, Plotting, and Data Output”
Save the data	See “Saving NMR Data (Optional)” on page 23

Prepare for an experiment

Perform the following tasks before beginning an experiment:

- Start VnmrJ.
- Prepare the sample and position the sample tube in a turbine.
- Load the probe file, if changing the probe.
- Install the probe, tune, and calibrate if necessary.

See [Chapter 2](#), “Preparing for an Experiment”.

Select an experiment

Select an experiment from the Experiment Selector or Experiment Selector Tree in the Protocols vertical panel, from the **Experiments** menu, or drag-and-drop a protocol from the Locator. See [“Selecting an Experiment”](#) on page 44.

Set up an experiment

The VnmrJ experimental setup and the functions available under the **Start** tab are described in [Chapter 3](#), “Experiment Setup”.

Set up the experiment using the pages in the **Start** tab.

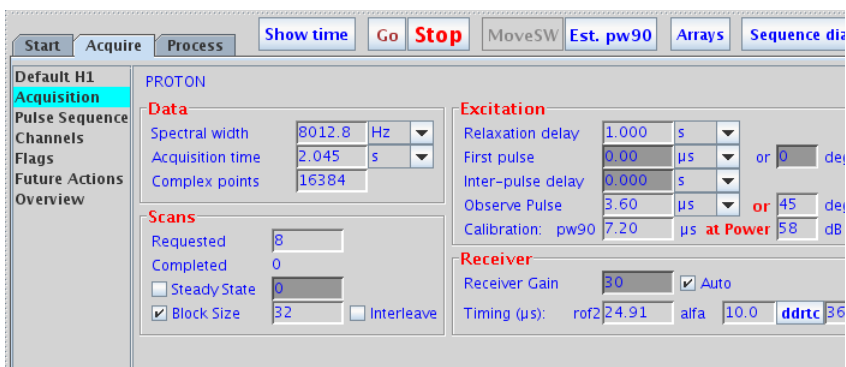
The screenshot shows the 'Sample Info' page in the software. At the top, there are tabs for 'Start', 'Acquire', 'Process', 'Insert', 'Eject', 'Lock scan', 'Setup hardware', 'Auto lock', 'Auto tune', and 'Gr'. Below these, a sidebar on the left lists 'Sample Info', 'Lock', 'Shim', and 'Spin/Temp'. The main area is titled 'Sample information' and contains the following fields:

- Operator: vnmr1
- Sample name: Clindamycin
- Sample directory: Clindamycin
- Solvent: D2O (with buttons for DMSO, CDCl3, D2O, and Other)
- Concentration: 0 mM
- Notebook: [empty field]
- Page: [empty field]
- Email: [empty field]
- Comments: Clindamycin phosphate 10 mg in D2O 400-MR with OneNMR probe

- 1 Select the **Sample Info** page.
 - a Fill in the information for the sample, select a **Solvent**, and enter the comments.
 - b Enter a name in the **Sample name** field to name the sample.
 - c Define the sample, if desired, by filling in the optional **Email**, **Concentration**, **Notebook**, **Page**, and **Comment** fields.
 - d Insert the sample.
- 2 Regulate spinning and temperature on the **Spin/Temp** page.
- 3 Find Z0 and adjust the lock using the **Shim** and **Lock** pages.
- 4 Shim the system to adjust the field homogeneity using the controls provided on the **Shim** page.

Acquire a spectrum

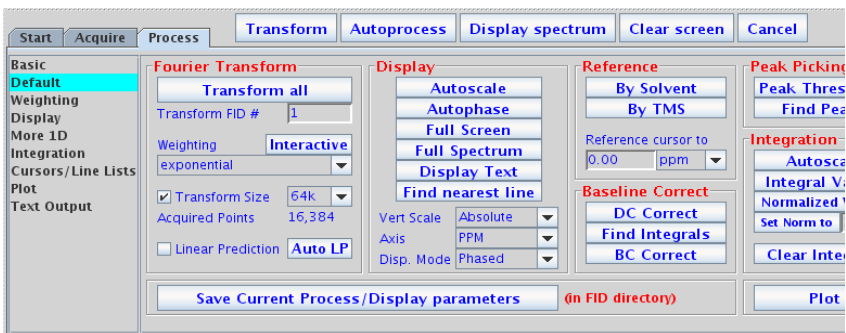
VnmrJ NMR data acquisition and the functions provided under the **Acquire** tab are described in Chapter 6, “Data Acquisition”. Set acquisition and acquire data using the pages in the **Acquire** tab.



- 1 Set up experimental parameters and post acquisition actions.
- 2 Click the **Acquire** button to acquire NMR data.

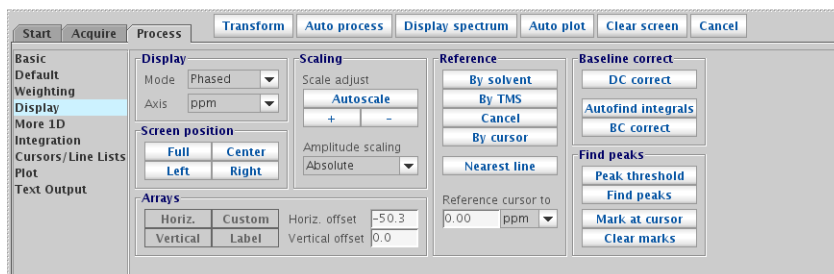
Process the data

VnmrJ NMR data processing and the functions accessed by clicking on the **Process** tab are described in Chapter 7, “Processing Data”.



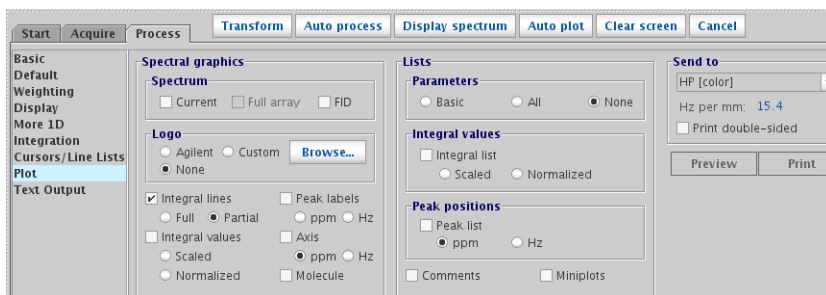
Display the data

VnmrJ data display is described in Chapter 8, “Displaying FIDs and Spectra”. Click the **Process** tab and select the **Display** page and the **graphic control** buttons to manipulate the display of the data.



Print or plot the data

VnmrJ data display is described in [Chapter 9](#), “Printing, Plotting, and Data Output”. Use the **Plot** page in the **Process** panel to create a print or plot.



Saving NMR Data (Optional)

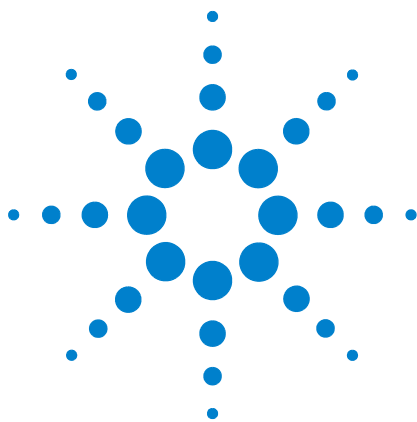
If the data is acquired and the Automatic FID save feature in the **Future Actions** page under the **Acquire** tab is not selected, use the **Save As** window, the **Auto Save** option, or the **Future Actions** page to save the data.

Method	Description
Future Actions page	Click the Acquire tab, Future Actions tab, and Save FID Now button
Main VnmrJ menu options	<p>Click File. Select either Save as or Auto Save. Selecting Auto Save saves the data as specified in Edit/Preferences. Use the Edit/Preferences window to customize where and under what name data is saved (see the <i>VnmrJ Administration User Guide</i>). VnmrJ default data saving templates include DATE (producing filenames such as 20040921_01.fid), and seqfil (for example, HMQC_01.fid).</p>

Stopping an Experiment

There are four ways to stop an experiment:

- Click the **Stop** button.
- Click **Acquisition** in the main menu, then **Abort Acquisition**.
- Click the **Stop** button in the **Action** bar (when either the **Start** or the **Acquire** panel is selected).
- Enter aa on the command line.



2 Preparing for an Experiment

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This chapter describes how to prepare for an experiment by preparing the sample, ejecting and inserting the sample, loading a probe file, and tuning probes on systems with ProTune or on standard systems.



Starting VnmrJ

- 1** Log in to the workstation.
- 2** Double-click the VnmrJ icon.

Preparing the Sample

Sample preparation and positioning in the turbine affects the efficiency of the auto shimming methods. Variations in bulk magnetic susceptibility at air-to-glass, glass-to-solvent, and solvent-to-air contact points can greatly degrade the field homogeneity from sample to sample. The time spent on shimming, or even the need to shim, is largely dependent upon the care in controlling the effects of these contact points.

Selecting a solvent

Most samples are dissolved in a deuterated solvent that does not react or degrade the sample. The instrument can be run unlocked if the sample must be run using a solvent that is not deuterated.

Setting the sample height

Experimentation and calculation show that the length of the liquid column must be at least three times the length of the observe coil window to minimize end effects (for a 5-mm tube in a 5-mm probe). A typical sample length is 5 cm (for 5 and 10-mm probes; it is a bit less for 3-mm sample tubes). Solvent volumes of 0.6 ml in a 5-mm tube and 3.1 ml in a 10-mm tube are adequate for removing the end effects. See the manual provided with the probe in use for specific sample height and volume specifications.

Reduction of sample volume to attain higher concentration usually fails (special plugs for low volume samples are available and will help with line shape) because the increased signal is found around the base of the NMR resonance, not within the narrow portion of the signal. In fact, a well-shimmed 0.4 ml sample will be lower in sensitivity than the same solution diluted to 0.6 ml and also shimmed well. The questionable gain in sensitivity is further degraded by the longer time it will take to shim the system. Small variations of sample height, which would be insignificant in a 0.6 to 0.8 ml sample, can be dominant when the sample is only 0.4 ml in volume.

For best results and reduced shimming times, samples should be prepared to each have the same liquid height as much as possible. Above 0.7 ml, there is little sensitivity to sample length, as long as the bottom of the tube is

positioned properly. Prepare every sample up to the same height and obtain shim values using samples of that height.

Table 1 Sample tube depths

Volume	Length mm	Depth(Range)
700 μ L	50 mm	68 mm (65–69 mm)
600 μ L	42 mm	65 mm (63–67 mm)
500 μ L	36 mm	62 mm (60–64 mm)
400 μ L	28 mm	59 mm (58–62 mm)

Table 1 shows the depths at which to place samples, for samples of the indicated size (for Wilmad 528 or 535 tubes with no restricting plugs). The depth is the distance in mm from the bottom of the turbine to the bottom of the sample tube.

Sample position using the depth gauge

Set the sample position to a repeatable position. Use the sample depth gauge provided, shown in Figure 1. (Use location 0 on the sample tray if an SMS sample changer is present.)

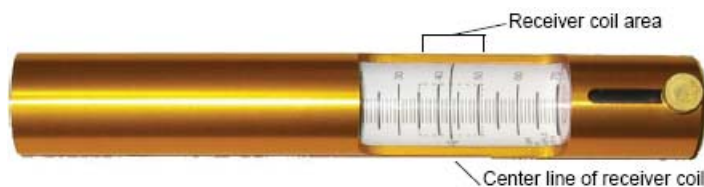


Figure 1 Sample depth gauge

- 1 Insert the turbine into the top of the sample depth gauge.
- 2 Insert the NMR sample tube into the turbine. Gently push the sample tube down until it touches the moveable bottom of the sample depth gauge.
- 3 Loosen the knob on the sample depth gauge.
- 4 Raise the bottom of the gauge, along with the sample tube and turbine, until the sample volume is centered on the centering mark (CL, between 35 and 51 mm) in the back of the gauge.
- 5 Tighten the knob.

- 6 Remove the sample tube and turbine from the depth gauge.
- 7 Gently pull up on the sample tube in the turbine, replace the turbine/sample tube into the depth gauge, and gently push down on the sample tube until it touches the repositioned bottom of the depth gauge.

Sample tubes

Buy the best quality NMR sample tubes and clean the outside of each tube with a solvent such as isopropyl alcohol, followed by a careful wiping with a wiper tissue before placing the tube in the probe. Fingerprints left on the sample turbine (in particular) will get transferred into the spinner housing and eventually create difficulties in spinning samples.

Sample changes and probe tuning

Probe tuning is required when there is a significant change in the polarity of the solvent. Changing from a non-polar organic solvent to a more polar organic solvent or aqueous solvent generally requires retuning the probe. Changes in the ionic strength of the solution (for example, low salt to high salt) also require retuning of the probe.

Ejecting and Inserting the Sample

The spectrometer is equipped with hardware and software to provide computer control of sample ejection, insertion, spinning, locking, and shimming. This section covers computer-controlled sample ejection and insertion.

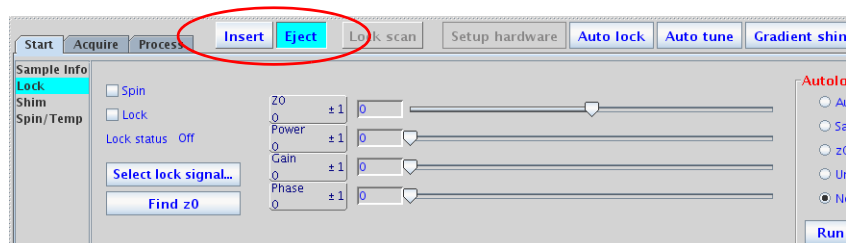


Figure 2 Insert and Eject buttons on the Action Bar of the Start tab

Manual control of ejection and insertion is also provided on each of these systems to enable you to withdraw samples if necessary. But we strongly recommend that you use computer control for maximum reproducibility and safety.

Ejecting a sample

Always eject first (even if no sample is in the magnet) to start airflow to carry the sample. The ejected air is turned on. Under computer control, the sample, if present, rises back to the top of the upper barrel. Remove the sample and replace it with another sample.

Using the start tab

The **Insert** and **Eject** buttons are on the Action Bar of the Start tab.

- 1 Click the **Start** tab.
- 2 On the Action Bar, click the **Eject** button.

Manual ejection

VNMRS or 400-MR:

- 1 Flip up the switch at the top of the upper barrel. Flip the switch up to eject, and down to insert.

Inserting a sample

The sample tube gradually lowers down the upper barrel

under computer control. With the current upper barrel, which can hold 3-, 4-, 5-, and 10-mm tubes, the turbine makes initial contact and alignment before the sample tube encounters any close tolerance.

Using the start tab

- 1 Perform a sample ejection (even if no sample is in the magnet) to start airflow to carry the sample. (See [“Ejecting a sample”](#) on page 30.)
- 2 Insert the sample by placing it in the top of the upper barrel.
- 3 Click the **Start** tab.
- 4 On the Action Bar, click **Insert**.

Manual insertion

VNMRS or 400MR:

Flip down the switch at the top of the upper barrel. (Flip the switch down to insert, and up to eject)

Loading a Probe File

Probe files can be created at any time and are typically created during system or probe installation. Procedures for creating probe files and probe calibration files are provided in the *VnmrJ Administration* manual. For more information on Using Probe ID, see “[Calibrating the Probe](#)” on page 84.

- 1 Click the **Probe** button on the **Hardware** bar (bottom left corner of the VnmrJ interface).

The probe selection window appears.

- 2 Click on the pull-down menu at the top of the **Current probe** section in the popup window and select the desired probe file.
- 3 Click **Close** to dismiss the window.

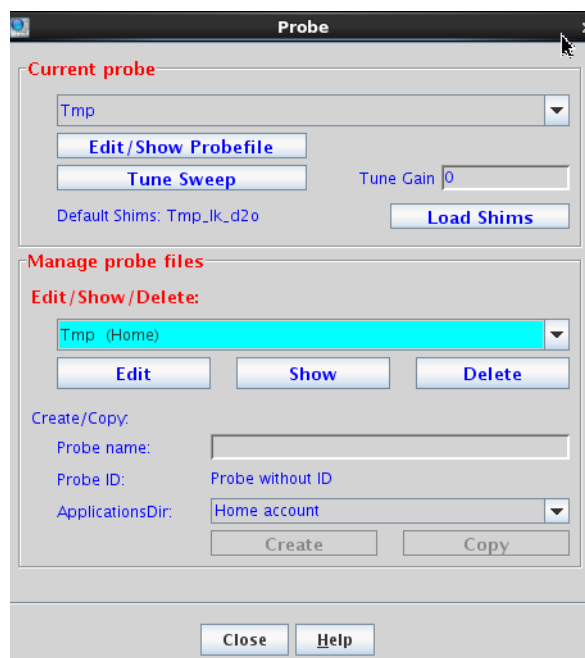


Figure 3 Loading a probe file

Tuning Probes on Systems with ProTune

This section applies to Agilent NMR systems with ProTune Accessory or OPTIMa enabled.

Configuring for operation with automated sample handlers

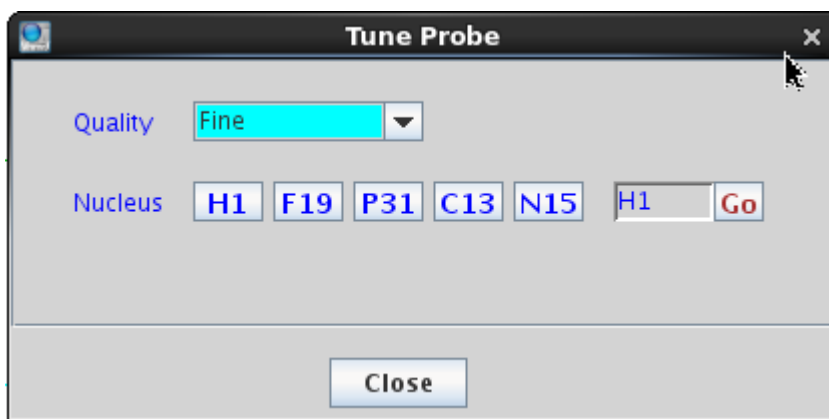
- Applies to systems equipped with ProTune.
 - The system must be properly configured and ProTune-calibrated. See the *VnmrJ Administration Guide* for configuring the software and calibrating ProTune.
- 1 Log in as the account administrator and start VnmrJ.
 - 2 Use the **Tuning** check box on the **Start** panel.
 - 3 Gradient shimming will start after each auto tune event if both auto tune and gradient shimming are selected.

The tune parameters (`wtune`, `tunemethod`) are specified in the probe file.

Running ProTune on systems without automated sample handlers

- 1 On the main menu bar, click **Tools > Probe Tuning > Auto Tune Probe**.

The Tune Probe window appears.



- 2 To select a nucleus not in the above list, or to select a different criterion:
Select a criteria from the drop-down menu next to Quality:
Coarse – within 5 percent of optimum pw

Medium – within 2 percent of optimum p_w

Fine – within 0.5 percent of optimum p_w

- 3 Select the next nucleus and repeat. Continue to the next step when all desired tuning is completed.
- 4 Click **Close** to exit the Tune Probe module.

Remote tuning from the ProTune window

The ProTune interface window (shown in [Figure 4](#) on page 35) can also be used to tune the probe. Functions and features of ProTune are listed in [Table 2](#) on page 35.

- 1 Start ProTune by entering the following in the VnmrJ command line:

```
protune('calibrate')
```

The calibration files for the probe shown on the hardware bar of the VnmrJ interface are loaded. See [Figure 4](#) on page 35.

- 2 Make sure that the motor and sweep communication read OK before starting manual tuning after the **ProTune** window appears.

[Figure 4](#) on page 35 shows the **ProTune** window.

- 3 Enter the frequency (MHz) in the Tune Probe field.
- 4 Click **Tune Probe**.

The software reads the appropriate channel file and starts tuning.

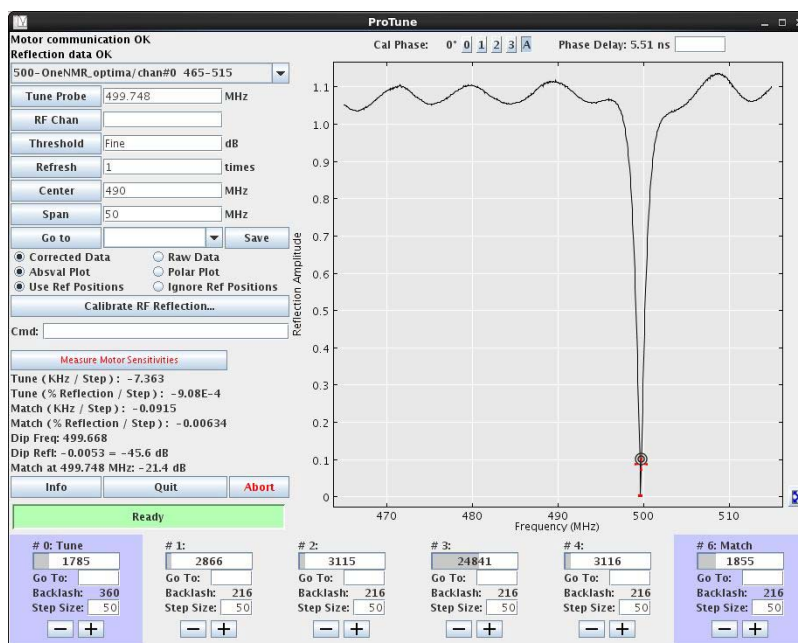


Figure 4 ProTune window

- 5 Do the following to tune manually:
 - a Select the appropriate frequency range/channel from the drop-down list.
 - b Click the appropriate **plus or minus** buttons to adjust the tune and match.
 - c Enter a frequency in the **Center** box and click **Enter** to update the reflection window to center the window.
 - d Enter a frequency width in the **Span** entry box and click **Enter** to update the reflection window to set the window span.
- 6 Close the **ProTune** window – acquisition will stop automatically.

Table 2 ProTune features and functions

Feature	Button, label, or message	Description
Diagnostic and tuning	Motor Communication OK, Sweep Communication OK	Status of Ethernet communications between the module and the workstation
	Abort Command	Stops current command
	Drop-down list	Probe name and channel number

2 Preparing for an Experiment

Table 2 ProTune features and functions (continued)

Feature	Button, label, or message	Description
	Corrected Data/Raw Data	Toggles window to display corrected or raw data
	Tune Probe (MHz)	Tunes frequency
	Threshold (dB)	Criteria for successful tuning
	Refresh (times)	The number of times the plot should update with new data
	Center (MHz)	Sets center value of the sweep range
	Span (MHz)	Sets span value of the sweep range
	Absval Plot/Polar Plot	Toggles between polar plot (imaginary and real reflection) and absolute value plot (reflection v. frequency).
	Cmd	Executes the ProTune command in the field.
Settings	Tune/Match Backlash	Difference between the number of steps traveled from the tune frequency and the number of steps traveled back to the tune frequency.
	Tune/Match KHz/Step Size	Number of KHz the dip moves in 1 step
	Tune/Match Reflection/Step	Minimum dip movement in one step with no regard to frequency. Positive values are inside the circle of origin, and negative values are outside the circle of origin.
	Dip Frequency	Frequency the dip occurs
	Dip Reflection	Reflection value at the dip
	Match at Freq MHz	Reflection at the desired frequency
Controls and output	Tune	Tune motor control
	Match	Match motor control
		Displays the graph in full scale view.

Tuning Probes on Standard Systems

Typically, probes are tuned using the Tune Interface shown in [Figure 5](#) on page 38.

Selecting correct quarter-wavelength

When a large change is made in the frequency of the observed nucleus on broadband stems, such as switching from ^{13}C to ^{15}N , an additional change is made in the quarter-wavelength cable—a coiled cable located on the system, as follows:

- Attached to the preamplifier housing for VNMR spectrometers
- Attached to the inner face of the magnet console interface unit as part of the observe circuitry on other systems

The quarter-wavelength cable is *not* changed for each nucleus, but only for broad ranges of frequencies (for example, 40 to 80 MHz), it usually changes, covering a factor of two (an octave) in frequency. An incorrect cable does not typically affect signal-to-noise, but may have a profound effect on the 90° pulse length.

Note that ProTune probes have a universal quarter-wavelength cable that spans the entire range of X nuclei. When this cable is used, it never needs to be changed (at least when the probe is connected to ProTune).

Tuning using Mtune

The Mtune routine runs in the graphics canvas and uses VnmrJ panels. Start **Mtune** as follows:

- 1 Click **Tools > Probe Tuning > Manually Tune Probe** on the main menu.
or enter `mtune` on the command line.
- 2 Select the center frequency of the nucleus to tune.
For example, set H1 on channel 1.
- 3 Select **Start Probe Tune** on the **Mtune** panel, see [Figure 5](#) on page 38.

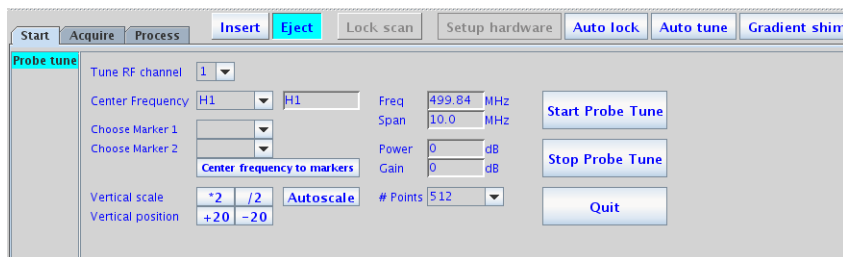


Figure 5 Manual tuning panel

- 4 Optional: Choose an appropriate tuning Power (may be channel dependent)—typical values are between 0 and 10 dB.
- 5 Optional: Set the tuning Gain to 50dB (a typical gain).
- 6 Set a Frequency Span that shows the tuning dip in full size:

A typical Frequency Span for ^1H is 10 MHz.

The Frequency Span for lower frequencies (like ^{15}N) may be 10 MHz, or less if desired. Wider spans may be desired when tuning manually over a wide frequency range.

- 7 Adjust any of the parameters as needed.
- 8 Set one or two markers.

Up to two frequency markers are selectable. This is useful for tuning ^1H and ^{19}F at the same time, for example with double-tuned coils like in the ATB or 4-nucleus probe families (Figure 5 on page 38).

- a Click the **Center frequency to markers** button to set the tuning frequency in between the two cursors.
- b Set the Span to a sweep width that covers both dips.

A span of 50 MHz is typical for showing both ^1H and ^{19}F at 400 MHz, as shown in Figure 5 on page 38.

Maximize the graphics canvas horizontally to have the best screen resolution.

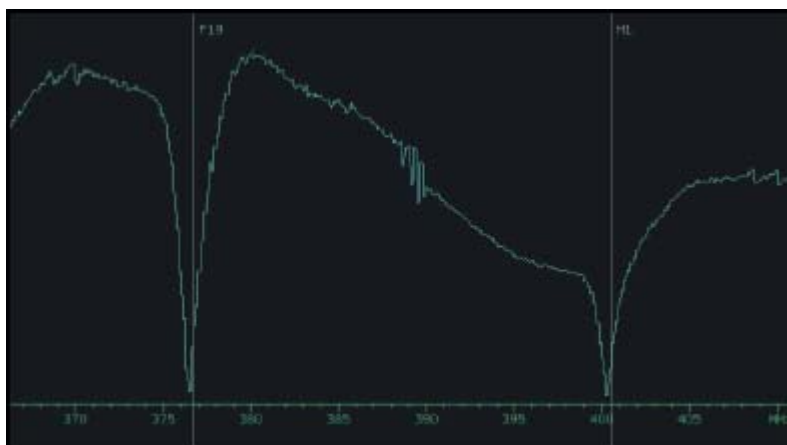


Figure 6 Frequency span display showing both ^{19}F and ^1H tune dips

- 9 Click the **Autoscale** button to fit the tuning dip vertically into the graphics canvas or scale.
- 10 Shift the display manually using the **Vertical scale/ Vertical Position** controls.
- 11 Keep the default number of acquired points, or change the number of points by selecting a new value from the **# Points** drop-down menu.

Increasing the number of points provides better resolution at large spectral widths. Higher resolutions will have smaller update rates.

- 12 Tune the other channels as follows:
 - Switch tuning off by selecting **Stop Probe Tune**.
 - Select a different channel and nucleus.
 - Switch tuning back on by selecting **Start Probe Tune**.
- 13 Repeat Step 1 through Step 13 for each channel.
- 14 Exit **Mtune** after tuning is done:

Click **Stop Probe Tune**, then click **Quit** to return to the previous NMR experiment.

Tuning using TUNE INTERFACE remote

Using the interface remote

The TUNE INTERFACE remote is attached to the RF front-end and can be extended to the magnet while tuning the probe.



Figure 7 TUNE INTERFACE remote

- The TUNE INTERFACE display, a rectangular liquid-crystal display that shows a numerical value two ways—as a digital readout and as an analog representation along the oval surrounding the digital readout at the top of the screen.
- Two single-digit readouts labeled CHAN and ATTN are below the display. The CHAN readout can be set to 0 for OFF or to the channel being tuned (1, 2, 3, etc.), and the ATTN readout is the amount of attenuation (analogous to the TUNE LEVEL knob on older systems). The attenuation is selected in units of 10 dB. The maximum attenuation is 79 dB, which is selected by a setting of 8. (A readout value of 9 is functionally identical to a value of 8.) The buttons above and below each readout allow you to change the value of the readout.

Tuning a probe

Tuning a probe using the TUNE INTERFACE remote involves the following steps:

- 1 Set up the spectrometer to observe the nucleus of interest.

Often, the system is already set to the correct nucleus; if not, proceed as if to set up an experiment.

- 2 Set the nucleus for each channel.

The TUNE INTERFACE remote will not work after either powering on or resetting the acquisition console until the tune frequencies are set.

- 3 Type `su`.

When a `su` is executed, the console frequency is set for each channel that is defined for the experiment. This frequency is also used for tuning (in this mode). The table below shows the relationships between the channel selected and the associated parameters:

Channel 1	tn	Sfrq	Tof
Channel 2	dn	Dfrq	Dof
Channel 3	dn2	dfrq2	dof2
Channel 4	dn3	dfrq3	dof3

See the *Command and Parameter Reference* for descriptions of these parameters.

- 4 Press the **CHAN** buttons until the readout is the number of the RF channel to tune. Start with channel 1.

This turns on the tuning function for the channel. The TUNE INTERFACE display should show a number, and the red indicator light should not flash. (If the light flashes, check the connector to the cable for an improper connection.)

- 5 Press the **ATTEN** buttons until the readout is 6, 7, or 8.
- 6 Optional: Insert the appropriate sticks into the probe if necessary. See the probe installation manual to choose the sticks that are needed to tune to the desired nucleus.
- 7 Tune the probe. As the probe gets closer to being tuned, the number on the TUNE INTERFACE display will decrease.
- 8 Press the **ATTEN** button until the readout is 8, to increase the tuning level sensitivity. Continue tuning until the number displayed on the TUNE INTERFACE display is at a minimum. (The minimum will be around 50-60 for VNMRS-generation console.)
- 9 Disconnect the tuning function by pressing the **CHAN** buttons until the readout is 0. (During normal operation, CHAN must be set to 0 or acquisition is not allowed.)
- 10 Repeat the steps above for each channel on the system.

For further information about probe installation and tuning, see the probe installation manual that was shipped with your probe.

2 Preparing for an Experiment



3 Experiment Setup

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This chapter sequentially describes how to set up experiments by selecting an experiment, spinning the sample, setting the sample temperature, handling spin and error temperature, working with lock and shim, optimizing lock, adjusting field homogeneity, selecting shims to optimize, shimming on the lock signal manually, shimming PFG systems, and calibrating the probe.



Selecting an Experiment

This section describes using the Protocol vertical tab, the menu bar, the Locator, and the Browser to choose and load an experiment. After an experiment is selected, VnmrJ reads and loads the standard parameters, reads the probe file, then loads the probe calibrations.

Protocols vertical tab

- 1 In the left vertical tabs, select **Protocols**.
- 2 Under either the Experiment Selector Tree or the Experiment Selector, double-click an experiment to open in the active viewport.

NOTE

If a study is not selected, the experiment parameters will go into the active viewport, rather than into the Study Queue.

Main menu selections

Experiment

- 1 Click **Experiments** in the menu bar.
- 2 Click an experiment in the list to bring it into the active viewport, see the **Experiments** menu.

The list of experiments contains some submenus.

File

- 1 Click **File**.
- 2 Select **Open**.
- 3 Navigate to the directory containing the experiment or data set.
- 4 Click an experiment or data set from the Browser to a viewport.
- 5 Click **Open** to bring it into the active viewport.

Locator

- 1 Click **Tools**.
- 2 Select **Locator**.
- 3 Click and drag an experiment from the locator to a viewport or double-click the experiment to bring it into the active viewport.

Browser

- 1 Click **Tools**.
- 2 Select **Browser**.
- 3 Navigate to the directory containing the experiment or data set.
- 4 Click and drag an experiment or data set from the Browser to a viewport.

NOTE

Loading a data set does not set parameter values from the current probe file. So, the settings may be incorrect for any new acquisition.

Spinning the Sample

When the **Spin/Temp** page under the **Start** tab is selected, the Liquids Spinner panel or the MAS Spinner panel, depending on the spectrometer hardware, is displayed.

Liquids spinner

To configure the software so that the **Liquids Spinner** panel is displayed, select a probe file in which the parameter `Probespintype` has been set to **liquids**. Optionally, set `spintype='liquids'` to show the **Liquids Spinner** panel.

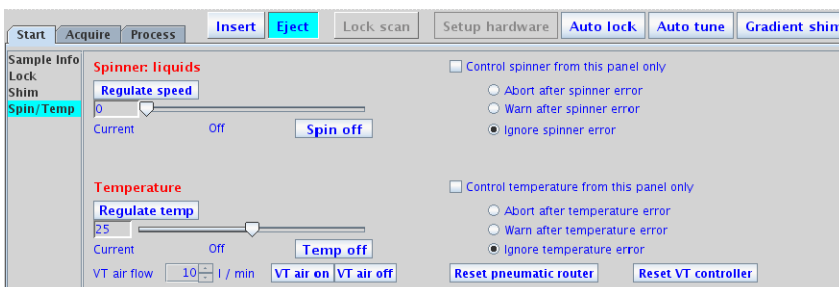
Adjust the spin rate using the **Input** window or the **Acquisition** window. Typical spin rates are 20 for 5-mm tubes and 15 for 10-mm tubes.

The last entered spin rate is used to regulate sample spinning when a new sample is inserted. The actual spin rate is indicated in three ways:

- The **Spin chart** display button on the hardware bar displays a history of the sample spin rate.
- The **Acquisition Status** window shows the actual rate as well as a spin regulation indication.
- The remote status unit signals the spin rate using the spin light status:
 - off – the sample is not spinning.
 - blinking – the sample is spinning but not at the requested rate.
 - on steady – the spin rate is being regulated at the requested rate.

Using the start tab

The **Spin/Temp** page is under the **Start** tab.



- 1 Click the **Start** tab. Select the **Spin/Temp** page.
The controls for the spinning speed consist of an entry field, a slider bar, and a button for disabling or enabling spinning.
- 2 Adjust the spinning speed with any of these methods:
 - Enter a spin rate in the text entry field.
 - Drag the slide control. The value changes proportionally as the mouse moves.
 - Click in the slider bar to move the slider by one increment.
- 3 Optional:
 - Specify preferences for the control of spinner and temperature by selecting the **Control spinner from this page only** and **Control temperature from this page only** check boxes. This forces all spinner and temperature changes to happen from this panel (rather than the command line or some other window). This is a recommended option, and prevents unintended changes in temperature or the spin rate.
- 4 Specify error handling for spinner and temperature by selecting one of the three options under each of the spinner and temperature sections.

MAS spinner

To configure the software so that an **MAS Spinner** panel is displayed, select a probe file in which the parameter `Probespintype` has been set to 'mas'. Optionally, set `spintype='mas'` to show the **MAS Spinner** control panel. (For the Nanoprobe, set `spintype='tach'` instead.)

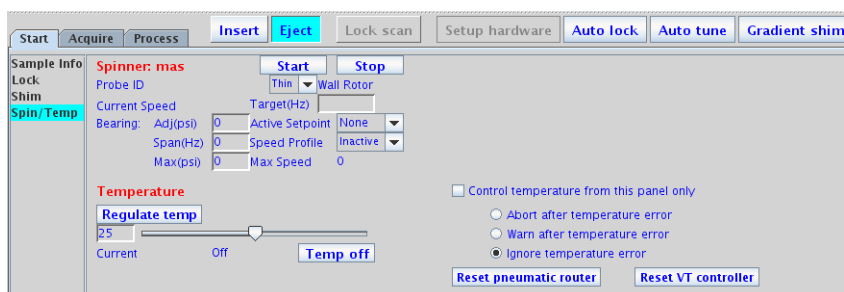
The **MAS Speed Controller** controls the flow of the bearing gas according to a linear algorithm based on the speed of the spinning rotor. The bearing pressure profile starts from the

final pressure at start up and increases linearly to the maximum bearing pressure in proportion to the speed of the rotor. The speed controller already has bearing profiles for all solids spinning modules stored in nonvolatile memory. Use the bearing control items to customize these settings. Changes made to the bearing profile remain in effect until the power is cycled or the probe is changed. To make permanent changes to the bearing profile, use the TIP interface.

WARNING

Thinwall rotors can shatter at high speeds, causing injury and damage. If a thinwall rotor is used, be sure that Thin is selected for Rotor Wall.

The **MAS Spinner** panel (which controls the **MAS Speed Controller**) is accessed from the **Spin/Temp** page under the **Start** tab.



Setting `spintype='mas'` gives the **MAS Spinner** control panel described here. Setting `spintype='liquids'` gives the liquids spinner panel.

Start Button	Click the Start button to start the rotor spinning at the target set into the active setpoint.
Stop Button	Click the Stop button to initiate the speed controller stop cycle, which slows down the rotor in a controlled manner until it is stopped. The Stop button is intended to provide a normal stop and is not an emergency stop.
Target (Hz)	<p>The Target Speed field is used to set the spinning speed stored in the active setpoint. Any changes made to the setpoints remain in effect until the power is cycled or the probe is changed. Setting the target will not start the rotor if it is not spinning, it just sets the value in the setpoint.</p> <ul style="list-style-type: none"> -- Only the currently active setpoint can be changed. -- If neither setpoint is active, any change made to the value in the Target Speed field activates setpoint one and sets it to that value. -- If the rotor is spinning and the active setpoint is changed, the rotor's spinning speed changes as soon as the return key is pressed.

Probe ID	The probe ID is detected automatically by the solids speed controller when the tachometer cable is plugged into the probe. Early solids probes (7.5 mm) had no designated ID type. So, an unplugged cable or a cable plugged into an unknown probe may cause a 7.5 mm probe type to be displayed. Although the probe type is detected immediately by the speed controller when the probe is connected, the probe ID in the panel is updated about once per minute. It is also displayed in the hardware bar.
Current Speed	This item displays the current speed of the rotor. This value is also displayed in the hardware bar.
Wall Rotor	Displays rotor wall thickness. Setting the Rotor Wall to Thin causes the Max Speed to be reduced to protect the rotor from shattering. The value defaults to Thin after the probe is changed or the controller is reset. If the current rotor is a standard wall rotor, change the setting to Std to get the allowable rotor speed for the probe.
Bearing Adjust (psi)	The value in the Adjust field shifts the bearing profile up or down, up to ± 80 psi. The bearing adjustment profile can be a negative number, meaning that the operating profile is lower than the factory setting.
Bearing Span (Hz)	The value in the Span field determines when the controller stops following the bearing profile. When the speed of the rotor is within Span Hz, the speed controller freezes the bearing value and does not change it until the spinning speed error exceeds this value. The factory-defined setting for Span is 100 Hz for all spinning modules. When adjusting the bearing profile, set Span to 0, meaning that the bearing pressure is continuously controlled. Then, when finished entering values in the Adjust and Maximum fields, enter a new value in the Span field.
Bearing Max (psi)	The value in the Maximum field sets the maximum bearing pressure in psi. This value controls the upper cutoff point in the bearing profile. The maximum bearing pressure is limited to 80 psi.
Active Setpoint	Two setpoints are available. The one selected is used to control the speed and is the one that is set when the target is changed. Changing the setpoint while the rotor is spinning immediately changes the target to the value for that setpoint. Selecting None stops the control algorithm and leaves the bearing air and drive air unchanged, which is sometimes referred to as coasting.
Speed Profile	Select Active to activate the speed profile feature, which causes the speed controller to constantly compare the drive pressure required to spin the rotor against its preprogrammed estimate of the required pressure for the current speed. If the pressure required to spin the rotor falls outside this range, the speed controller assumes that the rotor is vibrating. If the speed controller cannot stabilize the rotor, it brings the rotor to a stop.
Strip Chart	Clicking on the Spin button in the hardware bar will bring up a strip chart of the spinning speed with time.

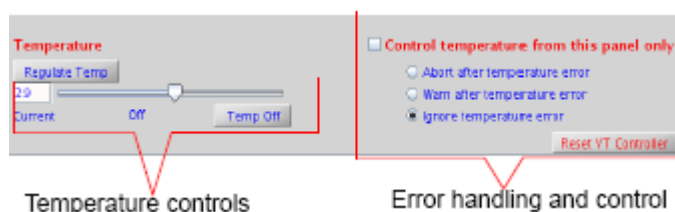
The following safety measures have been implemented for the high-speed spinner probes (for example, MAS) to prevent rotor and stator damage:

- The air flow selected from the spinner window is ramped to the new value at a safe rate.
- Air flow is shut off if the spinner speed drops to zero, and the spin setting is nonzero to prevent spinner runaway if the tachometer fails.
- Air flow is shut off if, for any reason, the spinning speed cannot be reached to prevent continued attempts to spin a crashed rotor.

Setting the Sample Temperature

Set the temperature for VT control and regulate the temperature by clicking on **Spin/Temp** page.

See [Appendix A](#), “Status Codes” , for more information on using the Variable Temperature module. The following steps describe a typical operation sequence:



- 1 Open the **Spin/Temp** page under the **Start** folder.
- 2 Set the desired temperature by entering a value or using the slider, and click **Regulate Temp**.
- 3 Set up the acquisition for the experiment as usual, using the **Acquire** folder.
- 4 Click the **Temp** button on the hardware bar to display the temperature display chart.
- 5 Start temperature control with the **Setup Hardware** button on the Start folder, or with the **Acquire** button on the Acquire folder. These commands act as follows:
 - **Setup Hardware**
The temperature control and acquisition hardware controls are set and the sample temperature changed to the desired temperature. The experiment is not started when the desired temperature is reached. Wait for the delay time, *vtwait*, (seconds), then push the **Acquire** button to begin data acquisition.
 - **Acquire**
The same actions occur as in Setup Hardware, except that after reaching the desired temperature, the system waits until the temperature stabilizes, or until the delay time, *vtwait*, has elapsed (whichever is shorter). Then the pulse sequence and data acquisition begins. The spectrometer waits until the temperature has stabilized, or it waits the delay time, *vtwait*, (whichever is shorter) every time the temperature is changed under program control.

Selection of the VT-gas routing occurs after clicking on either **Setup Hardware** or **Acquire**. The VT controller begins to control the gas temperature in the probe at the requested temperature. The temperature readout will begin to change, and the VT indicator light will begin flashing. At this time, if the requested temperature is below ambient, either add coolant liquid to the coolant bucket or reset any FTS device to a temperature 10° C or more below the desired operating temperature.

The VT indicator light stays on steadily after the probe temperature reaches the requested temperature (it may initially overshoot). A sample to be studied at non-ambient temperature can now be transferred into the probe. The VT readout is the temperature of the cooling/heating gas as measured just below the RF coil, and this may be slightly different from the true sample temperature. The exact temperature of the sample is correctly determined by a calibration curve that must be constructed for each probe, and must include flow rate and equilibration time.

CAUTION

Do not use aromatic, ketone (including acetone), or chlorinated solvents in the coolant bucket. Such coolant media attack the standard polystyrene bucket. Another type of container must be used (not supplied by Agilent).

CAUTION

Operating the system with the coolant bucket filled with liquid nitrogen and with the requested temperature greater than the value of VT cutoff (v_{tc}) results in the condensation of liquid nitrogen inside the exchanger coil tube. If the exchanger coil is then warmed above -210 C or if nitrogen gas is passed through the coil (when temperature is less than VT cutoff), then liquid nitrogen will be forced through the transfer line and into the probe. This will cause a sudden pressure surge in the transfer lines and probe as the liquid nitrogen boils, and it can blow the flexible connector apart. If the liquid nitrogen reaches the glass components of the probe and sample tube, the glass will probably break. Instrument damage can be avoided by following these precautions:

- Do not immerse the exchanger coil in liquid nitrogen when no nitrogen gas is flowing through the coil.
- Do not stop the VT nitrogen gas flow while the exchanger is immersed in liquid nitrogen.
- Arrayed VT experiments that have a temperature range from above VT cutoff to below VT cutoff should be set up starting at the lowest temperature and ending at the highest temperature. When the experiment passes the VT cutoff crossover, remove the liquid nitrogen coolant.
- To avoid water in the exchanger when the low temperature experiment is complete, warm up the exchanger by removing it from the liquid nitrogen and maintain a flow of dry nitrogen until room temperature is reached.

WARNING

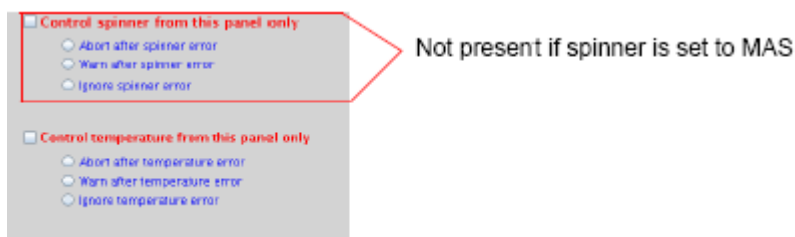
Sealed samples containing volatile materials can rupture when heated, resulting in potential injury, exposure, and equipment damage. Before running sealed samples at elevated temperatures, heat the samples in an oven at a temperature higher than the highest temperature expected during the experiment. If the tube ruptures while in the probe, the glass components and insert coil will probably be destroyed.

WARNING

Sealed samples containing materials with boiling points at or below room temperature can rupture as the sample warms, causing potential injury, exposure, and equipment damage. Equilibrate the probe to a temperature below the sample boiling point before the sample is placed into the probe.

Handling Spin and Temperature Error

Use the **Spin/Temp** page of the **Start** tab to select spin and temperature error handling. The provided choices specify the action to be taken if a spinner or temperature failure occurs. Also use the **Spin/Temp** page to specify whether spinning and temperature can be controlled on panels other than the **Start** tab.



- **Control spinner/temperature from this panel only** – prevents the spinner and temperature parameters from being overwritten when old datasets are recalled, but it also locks out control of the temperature from the command line or other panels. Most users prefer to have this option selected.
- **Ignore spinner/temperature error** – stops any system checking so that acquisition continues regardless of the spin speed or temperature.
- **Warn after spinner/temperature error** – makes the system check the spin speed and temperature. A warning message is added to the log file if the spin speed or temperature is set to a particular value and the spin speed or temperature goes out of regulation. However, acquisition is not stopped.
- **Abort after spinner/temperature error** – makes the system check the spin speed and temperature. Acquisition is halted if spin speed or temperature is set to a particular value and the spin speed or temperature goes out of regulation.

Working with the Lock and Shim Pages

Mouse control of buttons and sliders

To change the increment

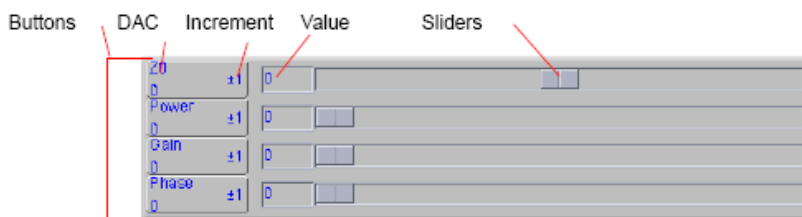
- 1 Middle-click the increment button until the desired value is displayed. The defaults are 1, 10, and 100.
- 2 To change to a customized increment value, shift/middle-click the button, enter a new value, and press **Return**.

To change the DAC value

- 1 Enter a new value in the input window and press **Return**.
- 2 Alternatively, Shift/left-click the **DAC** button itself. Enter a new value and press **Return**.

Lock buttons and controls

The **Lock** and **Shim** buttons (z0, Lock Power, Lock Gain, Lock Phase) provide on-the-fly adjustment. The slider values can be moved with the mouse or entered directly.



Shim buttons and controls

Lock DAC buttons

Lock meter

Highest lock lever

Lock receiver gain

Shim DAC buttons

Shim file name

Shim DAC

DAC value

DAC increment

Z1	1091	±1	X1	191	±10	X3	-1057	±100	Z3X	-9721	±1	ZX3	8878	±1
Z2	-93	±5	Y1	1599	±10	Y3	5559	±100	Z3Y	-1027	±1	ZY3	19498	±1
Z3	1358	±10	XZ	-2332	±100	XZ2	9862	±100	Z2X2Y2	963	±1	Z4X	27927	±1
Z4	1902	±10	YZ	9099	±100	YZ2	-3029	±100	Z2XY	7138	±1	Z4Y	17513	±1
Z5	-5852	±10	XY	-1986	±100	ZXY	9795	±100						
Z6	2918	±1	Z7	0	±1	X2Y2	-766	±100	ZX2Y2	-7179	±100			

Working with Proshim

Use the Proshim window to build or edit Proshim methods. Select **Tools > Standard Calibration Experiments > Shim Editor**. The window is divided into the following areas:

Current method, Method editor, Manage shim method files and FID-shim parameters.

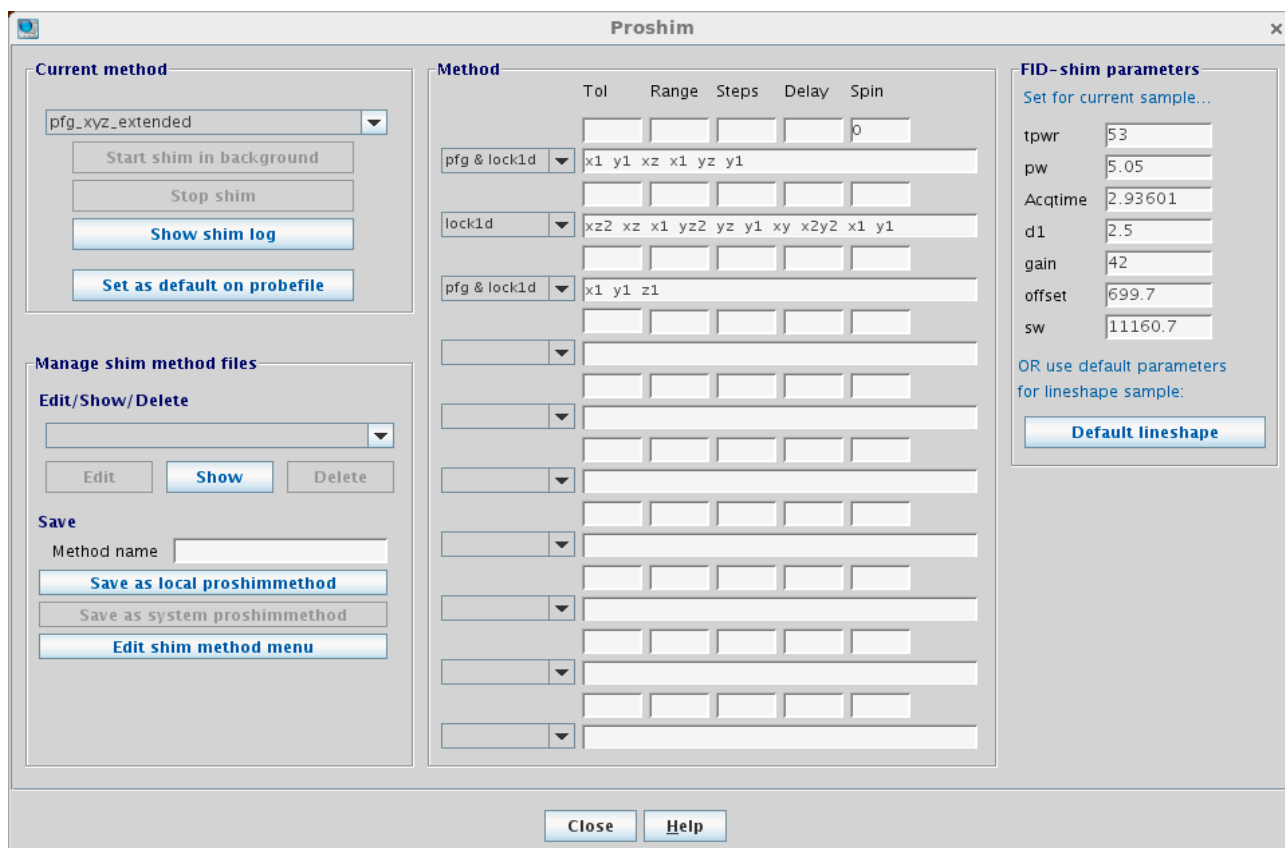
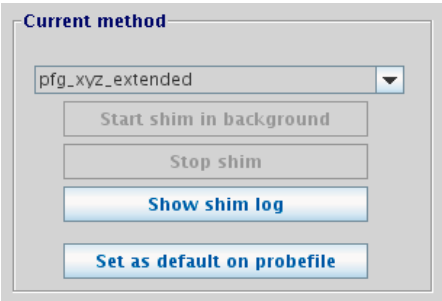


Figure 8 Proshim window

Current method

Table 3 Proshim —Current method

Item	Description
Current method	
drop-down list of Proshim methods	Select a method to start, edit, or delete. The list displays Proshim methods that are available for the current experiment, including user-created Proshim methods. The selected Proshim method is displayed in the Method group.
Start shim in background	Click to start the Proshim method displayed in the above drop-down list. The method will run in a background VnmrJ session.
Stop shim	Click to stop the Proshim method currently running in background.
Show shim log	Click to open a terminal window that displays the shim log.
Set as default on probefile:	Sets the current method as the default method on the probefile.

Method editor

Use the Proshim Method editor to:

- display the method selected in the Current Method group.
- edit the method selected in the Current Method group.
- build a new method.

NOTE

Use [“Manage shim method files”](#) to save any edits to a shim method.

Table 4 Proshim —Method editor

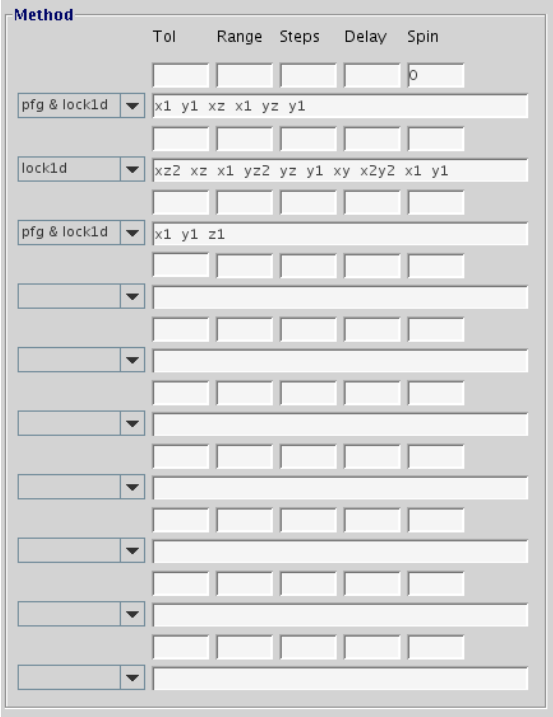
Item	Description
<p>Method editor</p> 	
<p>Method column</p>	<p>Click to display a drop-down list of shim optimization types:</p> <ul style="list-style-type: none"> • lock—optimizes the lock shim variables as a group. • lock1d—optimizes the lock shim variables independently in listed order. • fid—optimizes the FID shim variables as a group. • fid1d—optimizes the FID shim variables independently in listed order. • Pfg – optimizes the shims using a z-gradient shim method. • Gxyz – optimizes the shims using a 3D gradient shim method.
<p>Tolerance column</p>	<p>The default value is based on the optimization type for the shim method and appears blank. Enter a new value to change the tolerance. This sets the distance between the maximum and minimum value in each shim dimension for simplex shimming. Values are in DAC units.</p>

Table 4 Proshim —Method editor

Item	Description
Range column	The default value is based on the optimization type for the shim method and appears blank. Enter a new value to change the range. This is the initial step size for optimization. Values are in DAC units.
Steps column	The default value is based on the optimization type for the shim method and appears blank. Enter a new value to change the steps. Values are in DAC units.
Delay column	The default value is based on the optimization type for the shim method and appears blank. Enter a new value to change the delay. This is the delay between changes in shim value during optimization.
Spin column	The default value is based on the optimization type for the shim method and appears blank. Enter a new value to turn the spinner on or off.

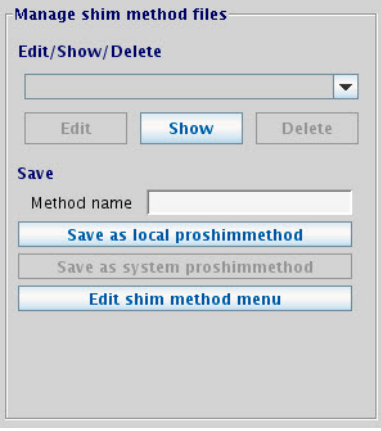
Manage shim method files

After making edits to the shim method and Start Shim is run, the edited Shim is available to be saved.

Use Manage shim method files to

- save the edited shim method as a local or system shim method
- display a shim method
- edit a shim method
- delete a shim method

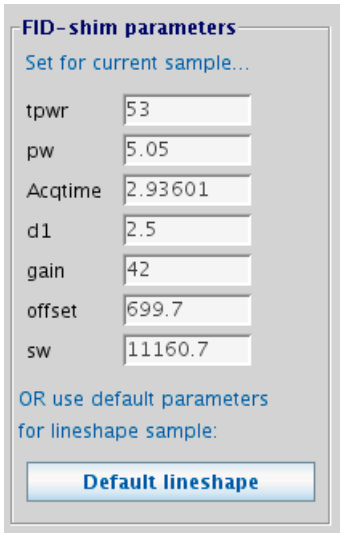
Table 5 Proshim —Manage shim method files

Item	Description
Manage shim method files	
Edit/Show/Delete	Click the drop-down list to display the list of Proshim methods associated with the current experiment.
	Edit—Click to edit the selected shim method.
	Show—Click to display the selected shim method.
	Delete—Click to delete the selected shim method.
Save	<p>After making edits to a shim method, save the edited method as a local or system shim method.</p> <p>Method Name—Enter a name for the edited shim method.</p> <p>Local shim methods are saved to the <code>/vnmrsys/proshimmethods</code> directory.</p> <p>System shim methods are saved to the <code>/vnmr/proshimmethods</code> directory.</p>
Edit shim method menu	Opens the “Shim Menu Editor” popup. This popup is used to add methods to the shim menu on the sample info page.

FID-shim parameters

This group in the Proshim window lets you specify acquisition parameters to be used for FID shimming

Table 6 Proshim — FID-shim parameters

Item	Description
FID-shim parameters	
Set for current sample	Allows the user to specify acquisition parameters to be used for FID shimming.
Default lineshape	Loads parameters appropriate to FID shimming on the chloroform in acetone lineshape standard sample.

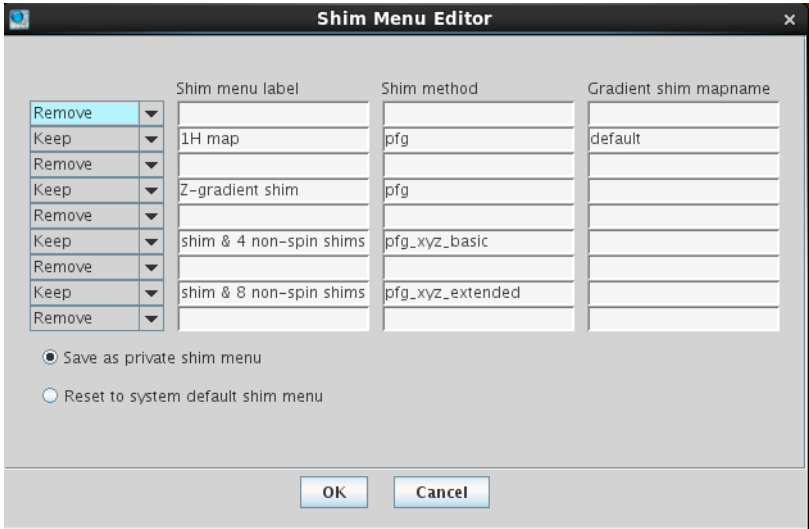
Shim Menu Editor

The Shim Menu Editor popup is used to associate a gradient shim map with a Proshim method, if needed. It is also used to place a label for the Proshim method into the Select shim method menu on the sample info page when submitting a Study to acquisition.

To open the Shim Menu Editor,

- 1 Select **Tools > Standard Calibration Experiments > Shim Editor**.
- 2 In the Manage shim methods files group, select **Edit shim method menu**.

Table 7 Shim Menu Editor—opened from the Proshim window

Item	Description
Shim Menu Editor	
Shim menu label	This is the label that will appear in the shim method pull-down when submitting a Study to acquisition.
Shim method	The name of the Proshim method that the user wants to add to the shim method pull-down.
Gradient shim map	The shim map that is to be used when gradient shimming is included in a shim method.

Proshim in Automation

Once a Proshim method has been created it can be used to shim a sample in automation.

- 1 Create a New Study and add the desired experiments to the Study Queue.
- 2 On the Sample Info page, use the Select shim map pull-down to choose a Proshim method
- 3 The specified Proshim method will now be used when the study is submitted to acquisition.

Using Proshim with Study Clone

After a Proshim method has been assigned to a study, a Study Clone can be created which will include the Proshim customization.

NOTE

For more information on the Study Clone utility, see the *VnmrJ Automation User Guide*, "Customizing VnmrJ Actions for Each User Account".

Scheduling Shim Maintenance

Use the scheduler to create a shim maintenance schedule:
Tools > Standard Calibration Experiments > Shim Scheduler

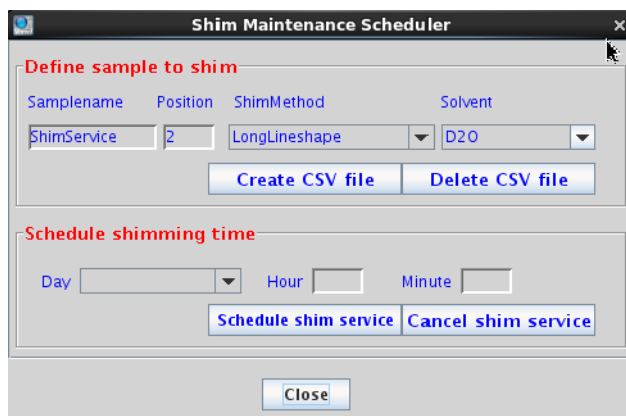


Figure 9 Shim maintenance scheduler

Table 8 Shim Maintenance Scheduler

Item	Description
Define sample to shim	
Sample Name	Enter sample name for the shimming study.
Position	Enter the location for the lineshape sample.
Shim Method	Select a Proshim method: long lineshape, fast lineshape, fidshim lineshape, fidshim lineshape fast, lineshape no shim.
Solvent	Select a solvent from the drop-down list. Solvents are typically acetone-d6.
Create CSV File	Click to save Shim.csv to vnmrsys/Spreadsheets. Click Schedule Shim Service to schedule the shim service. Shimming can be scheduled for more times by setting another day and time and clicking Schedule Shim Service. It is not necessary to re-create the Shim.csv simply to schedule. Once it is placed in the csvlib directory, it is ready to use as desired.
Delete CSV File	Click to delete Shim.csv from vnmrsys/Spreadsheets.
Schedule shimming time	
Day	Select the day for shim service.

3 Experiment Setup

Table 8 Shim Maintenance Scheduler

Item	Description	
Hour	Enter the desired hour for shim service in a 24-hour format.	
Minute	Select the minute for shim service.	
Schedule Shim Service	Click to schedule the shim service. Shimming can be scheduled for more times by setting another day and time and clicking Schedule Shim Service. It is not necessary to re-create the Shim.csv simply to schedule. Once it is placed in the csvlib directory, it is ready to use as desired.	
Cancel Shim Service	Click to cancel all scheduled shim services.	
Help	Click to display help content.	

Optimizing Lock

Under computer control, the lock system maintains a constant field at the sample as the static field generated by the superconducting magnet drifts slowly with time or changes due to external interference. Locking makes the resonance field of the deuterium in the deuterated solvent coincide with the lock frequency.

The lock level can be viewed by clicking on the **Lock** button on the hardware bar.

The entire lock optimization process can be skipped if optimum lock parameters are already known for a particular solvent and probe combination. Values for these parameters are stored in the probe file and are set automatically.

It is important to obtain an optimal lock signal if automatic shimming is to be used. Manual adjustment often is done to achieve the maximum lock amplitude. This can result in a partly saturating condition, and a true non-saturating power is usually 6 to 10 dB lower. The response of the lock level is governed by the T_1 of the deuterated lock solvent as well as the magnet-determined or chemical exchange-determined T_2^* of the solvent. This can vary widely, from about 6 seconds for acetone- d_6 to about 1.5 seconds for $CDCl_3$ and lower for more viscous solvents. To allow a reliable, repeatable selection of lock power, automatic optimization may be used.

Finding Z0 and establishing lock

Find Z0 and establish the lock either manually or by using Auto lock (through the **Sample Info** page of the **Start** tab).

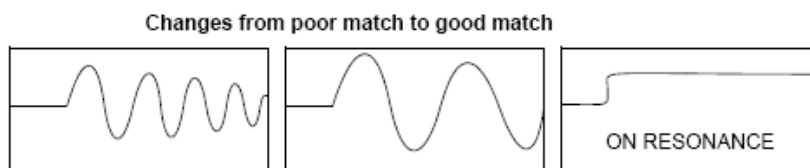
The screenshot shows the 'Sample Info' page in the software. The 'Operator' is vnmr1. The 'Sample information' section includes a 'Clear' button and the following fields:

- Sample name: Clindamycin
- Sample directory: Clindamycin
- Solvent: D2O (selected from a dropdown menu with options D2O, DMSO, CDCl3, D2O, Other)
- Concentration: 0 mM
- Notebook: (empty)
- Page: (empty)
- Email: (empty)
- Comments: Clindamycin phosphate 10 mg in D2O 400-MR with OneNMR probe

Manual method

Establish lock using manual locking on the **Lock** page. The line that crosses the spectral window represents how close

the deuterium resonance field is to the lock frequency. When the two are matched, the line should be flat (with perhaps some noise, depending on the lock gain and lock power). The greater the number of sine waves in the line, the poorer the match.

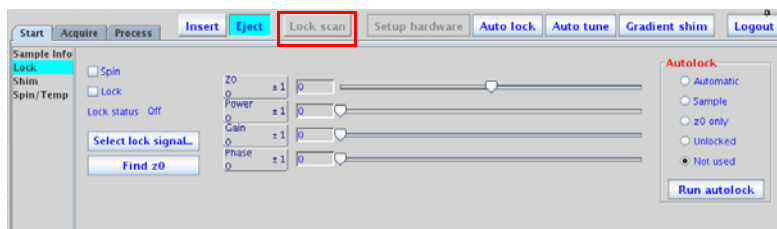


- 1 Make sure a sample is inserted and seated properly. Spinning is optional.
- 2 Click the **Lock** page in the **Start** tab.
- 3 Select Spin On or Spin Off by selecting or clearing the **Spin** box.
- 4 Click **Lock Scan** to open the lock display.
- 5 Clear the **Lock** check box. (The lock should not be *on* and regulated when adjusting Z0.)
- 6 Find Z0 by clicking on and dragging the Z0 slider bar until the lock signal is on resonance.
- 7 Adjust the lock power, gain, and phase by clicking on and dragging the slider bars, or by clicking on the buttons.

The actual value needed for lockpower and lockgain depends upon the concentration of the deuterated solvent, the nature of the deuterated solvent—the number of deuterium atoms per molecule—and the relaxation time of the deuterium. At this point, do not be too concerned about optimizing power and gain; just look for a sine wave.

- 8 Click the **±10** or **±100** button for Z0 until some discernible wave appears if no sine wave (perhaps just noise) is seen.
- 9 Reduce the lock power if the concentration of the lock solvent is high (>50%).
- 10 Reduce the lock power if the signal oscillates (goes down and then back up) and it is difficult to establish lock. The deuterium nuclei become “saturated” if the lock power is too high. Acetone is more easily saturated than most solvents.

- 11 Adjust Z0 until the signal changes from a sine wave to an essentially flat line. The line may start to move up on the screen as the lock condition is approached if the solvent is concentrated.
- 12 Select the **Lock** check box.
- 13 Click **Lock Scan** again to close the lock display.



AutoLock with probe file

This requires a probe file with proper probe calibrations. See the *VnmrJ Installation and Administration* user guide.

- 1 Click **Find Z0**. This button can be found the **Lock** page of the **Start** tab.

AutoLock

- 1 Click the **Sample Info** page of the **Start** tab.
- 2 Select Spin On or Spin Off by selecting or clearing the **Spin** box.

Select an option under the **Autolock** section.

- 3 Click the **Autolock** button – the spectrometer will find Z0 and make all specified adjustments.
- 4 Choose **Find Z0** or **Run autolock**.

Lock power, gain, and phase

Lock power, gain, and phase are set by the lock parameters—lockpower, lockgain, and lockphase—when using autolock. The parameters set the following limits and step sizes:

- lock power is 0 to 68 dB, step size of 1 dB (68 is full power)
- lock gain is 0 to 48 dB, step size of 1 dB (48 is full gain)
- lock phase is 0 to 360 degrees, step size of 1 degrees.

The Z0 field position parameter z0 holds the current value of the Z0 parameter. The limits of z0 are: -2047 to +2047, in steps of 1, if the parameter shimset is set to 1, 2, or 10, or; -32767 to +32767 if shimset is set to another value.

Lock control methods

Navigate to the **Start > Lock parameter** panel to access the following lock methods and controls.

Leaving lock in the current state

Set Autolock to **Not Used**.

When Autolock is set to **Not Used**, the inserted sample will lock only if the sample has the same solvent as the previous sample, and if the values of Z0, lock power, and lock gain have not been re-adjusted.

Running an experiment unlocked

Set Autolock to **Unlocked**.

Lock is deactivated at the start of acquisition.

Simple Autolock

Set Autolock to **Sample**.

The system searches for the lock signal and, if necessary, optimizes lock power and gain (but not phase), whenever an acquisition is initiated with go, ga, au or with any macro or menu button using the go, ga, or au if Autolock is set to Sample at the beginning of each experiment (each initiation of an acquisition).

Find Z0

Find Z0 acquires a ^2H spectrum, finds the offset of the tallest peak, and uses that information to set the value of Z0. It does not adjust lock power, lock gain, or lock phase. This option is selected whenever **Autolock** is set to **Every sample** or **Every expt**. However, if the lock is not captured by this method, the system starts using the Autolock Optimization options listed in (cross-ref) [“Optimizing Autolock”](#) .

Optimizing Autolock

Optimizing Autolock uses a sophisticated software algorithm to search the field over the full range of Z0 (as opposed to hardware simple Autolock), captures lock, and automatically adjusts lock power and gain (but not lock phase).

- **Lock Find Resonance** is set to **Every Sample**.
- The same process as **Simple** occurs, but only if the sample has just been changed under computer control and acquisition is started (when manually ejecting or inserting a sample, the software cannot keep track of the action and **Every Sample** has no effect).
- z0 is inactive and when an autolock operation is started, autolock searches for the lock signal by changing the lock frequency.

Spectrometer frequencies are computed from the lock frequency. So, if the lock frequency changes as a result of an Autolock operation, frequencies for that acquisition are off by the amount of that change. Switching from chloroform to acetone requires a change in the lock frequency of about 5 ppm, which is then reflected in the acquired spectrum.

Full optimization

Full optimization is the most complete optimization of lock parameters. A fuzzy logic autolock algorithm automates the parameter control process in order to find the exact resonance and the optimum parameters (phase, power, gain) automatically and quickly with high reliability. Fuzzy rules are used in the program to find the exact resonance frequency and for adjusting power and phase. The fuzzy rules are implemented at different stages of the autolock process. First, the software finds the resonance. If the exact resonance cannot be found, phase and power are adjusted and the software looks for the exact resonance again. The software then optimizes the lock power to avoid saturation, optimizes the lock phase, and optimizes the lock gain to about half-range.

RF frequencies, decoupler status, and temperature are also set during full optimization.

Adjusting Field Homogeneity

See also [Chapter 4](#), “Gradient Shimming”, if the system is equipped with gradient shimming capabilities.

Shim coils produce small magnetic fields that are used to compensate for inhomogeneities in the static field. In shimming, the current in the shim coils is adjusted to make the magnetic field as homogeneous as possible.

Computer-controlled digital-to-analog converters (DACs) regulate the room-temperature shim coil currents. Users should plan to adjust the shims every time a new sample is introduced into the magnet or a probe is changed. See [Appendix B](#), “Shimming Basics” for more information on shimming.

Loading shim values

- 1 Click the **Shim** page in the **Start** panel.
- 2 Click the **Read shims from pars** button.

This is the equivalent of the command line instructions:
`load='y' su.` Shim values stored in the current experiment are loaded (this may not be suitable).

Loading a shim file

Load a shim set from the Locator to the shim buttons area of the **Shim** page as follows:

- 1 Click the **Locator Statements** button (magnifying glass icon).
- 2 Select **Sort Shimsets**. Shim sets can also be sorted by probe or filename.
- 3 Select a shim set and drag-and-drop it onto the graphics canvas or shim buttons area of the **Shim** page.

Saving a shim file

Save the shim values to a file as follows:

- 1 Enter a file name in the field next to the **Save Shims** button, and press **Return**.
- 2 Click the **Save Shims** button.

Shim gradients

The shims coils are printed coils wrapped around a

cylindrical form. The probe slides into the resulting shim tube. A coil (or sum of coils) whose field is aligned along the axis of the magnet is called a Z axial shim gradient (Z1, Z2, Z3, etc.). Coils whose fields are aligned along the other two orthogonal axes are called X and Y radial shim gradients (X1, XY, X2Y2, Y1, YZ, etc.). The field-offset coil Z0 (“zee-zero”) alters the total magnetic field.

Each shim gradient is controlled by its own parameter; for example, the X1 shim gradient is controlled by a parameter named x1.

Depending on the value of the shimset parameter, shim values range from -2047 to +2047 or from -32767 to +32767, with a value of zero producing no current.

Shimming on the Lock Signal Manually

Monitor the intensity of the lock signal while adjusting the shim settings. Each shim setting controls the current through shim coils that control magnetic field gradients in different directions. The Z direction must be parallel to the vertical direction of the probe, and it is for this reason that the height of the sample in the NMR tube affects the Z shim settings rather dramatically.

Routine shimming

- 1 Load the shim settings that have been most recently established for the probe in use as a starting point if the shim settings are way off the mark (for example, if the temperature has changed).
- 2 Click **Setup Hardware**.
- 3 Make sure that:
 - the probe has a sample
 - it is spinning at the correct speed
 - the system is locked onto the deuterium resonance from the lock solvent
- 4 Check that the lock signal is not saturated. The signal is saturated if changing the lock power by 6 units (6 dB) does not change the lock level by a factor of two. Adjust the lock gain as necessary.
- 5 Open the **Shim** page.

Try a change of +10 or -10 in the setting for Z1. If the lock level goes up with one of these, continue in that direction until the level is maximized (it no longer increases, but instead begins to fall).
- 6 Change the setting for Z2C or Z2 by +10 or -10 and continue in that direction until the level is maximized.
- 7 Adjust Z1 for maximized lock level, then adjust Z2 for the same. Continue this iterative process until the lock level goes no higher. If the lock level increases to 100, decrease lock gain and then continue to adjust Z1 and Z2. Lock power can be adjusted as needed.

The routine adjustment is sufficient in most cases. Critical experiments, in some cases, do require adjustment of higher order Z shims and the non-spin shims.

The following procedure is suggested for a second level of shimming:

- 1 After Z1 and Z2 have been adjusted for maximum lock signal, write down the lock level, adjust Z3 in one direction, by +10, and then repeat the optimization of Z1 and Z2 (iteratively) until the lock signal is at a maximum. Note this level of the lock signal. Continue changing Z3 in the same direction if the lock signal is higher than it was initially. Every change in Z3 must be followed by optimization of Z1 and Z2 until the lock level is at a maximum.
- 2 Repeat step 1 with Z4. That is, change Z4 in one direction, then optimize Z1 and Z2. If the lock level does not go up, change Z4 in the opposite direction and optimize Z1 and Z2. Continue until the highest possible lock level is obtained.
- 3 Repeat steps 1 and 2 iteratively until the highest possible lock level is obtained.
- 4 Turn the spinner off and go through the non-spin shims, one at a time, maximizing the lock level for each one. Then return and go through each again. Continue through all until the lock level is as high as possible. If lock is lost, increase the lock gain.
- 5 Turn the spinner on and optimize Z1 and Z2 as described above, return to the non-spins (turn the spinner off) and re-optimize these. Continue until the highest lock level is obtained.

Insert the lineshape sample (CHCl₃ in deuterioacetone for ¹H, and dioxane in deuterobenzene for ¹³C) for an ultimate check and examine the lineshape to make certain that the homogeneity is close to the original specs, especially for the lineshape at 0.55% and 0.11% of the total peak height. Also examine the height of the spinning sidebands. See the *Probe Installation* manual that was shipped with your probe.

Setting low-order (routine) shims

The following procedure describes how to set the low-order, or routine, shims. Resetting Z0 and lock phase is normal when making very large changes in the room temperature shims. With this procedure, concentrate on improving the symmetry of the main resonance as well as the half-height resonance and lineshape.

- 1 Adjust the lock level to about 80 (if possible).

Maximize lock level with Z1.

Maximize lock level with Z1 and Z2. Do this by making a change in Z2 followed by maximizing with Z1 again. Continue to iterate in this manner until there are no further increases in the lock level.

- 2 Acquire the spectrum.

Resonance lines are symmetric if the sample is properly shimmed.

Resonances that are asymmetric or unusually broad at the base require added attention; see [Table 35](#) on page 385 in [Chapter 14](#), “Data Analysis”, for which shims to adjust. Adjusting Z4 or the non-spins is not required for most routine samples.

- 3 Adjust Z3 by interactively shimming Z1 and Z3 in the manner described in step 3 for Z1 and Z2. Changes in Z3 may affect Z2. So, after shimming Z3, maximize Z1 and Z2 again.

Removing spinning sidebands (non-routine)

Use this procedure to reduce or eliminate spinning sidebands that are not within specification.

- 1 Write down the lock level, set SPIN to **OFF**, and write down the lock level.
- 2 Adjust lock to about 80 if possible.
- 3 Maximize lock level with X.

4 Maximize lock level with Y

5 Maximize lock level with X and Y.

Do this by making a change in Y followed by maximizing with X again. Continue to iterate in this manner until there are no further increases in the lock level.

6 Maximize lock level with X and ZX.

Do this by making a change in ZX followed by maximizing with X again. Continue to iterate in this manner until there are no further increases in the lock level.

7 Maximize lock level with Y and ZY.

Do this by making a change in ZY followed by maximizing with Y again. Continue to iterate in this manner until there are no further increases in the lock level.

8 Repeat step 3 above.

9 Maximize lock level with XY and ZXY (ZXY not available on 13 or 14 channel shim systems).

10 Repeat step 3 through step 5.

11 Set SPIN to **ON** and acquire a spectrum.

If the sample is properly shimmed, the lines should be symmetric.

12 See [Table 52](#) on page 517 and the previous sections for which shims to adjust if the lines are not symmetric or are unusually broad at the base. For most routine samples, adjusting Z4 or the non-spins is not required.

13 Adjust Z3 by interactively shimming Z1 and Z3 in the manner described in step 3 in the previous procedure ([“Setting low-order \(routine\) shims”](#) on page 79) for Z1 and Z2.

Changes in Z3 may affect Z2. So, after shimming Z3, maximize Z1 and Z2 again.

Setting the high-order axial shims (non-routine)

See “[Shim Interactions](#)” on page 511, for information about shim interactions.

- 1 Look at which side of the peak has asymmetry to determine how to adjust Z4 – low field to the left and high field to the right.
- 2 Use [Figure 148](#) on page 513, in [Appendix B](#), “Shimming Basics” , to determine which direction to move Z4. A large asymmetry implies that Z4 is far off. Change Z4 by a considerable amount to try to push the asymmetry to the other side of the peak. This provides two important pieces of information:
 - Confirms that Z4 is the problem if the asymmetry moves.
 - Indicates what the actual value of Z4 should be when Z4 is changed. Because the values that caused it to be on either side of the peak are known, the correct value must be between the two extremes.
- 3 Set Z4 to the value that produces neither a high-field nor low-field asymmetry.

Z4 affects all the shims below it. So, repeat the procedure “[Setting low-order \(routine\) shims](#)” on page 79.

- 4 Maximize the lock level with Z5.
- 5 Repeat step 3 and step 4 until no further increase is obtained.

Setting high-order radial shims (non-routine)

Note that Z2X, Z2Y, ZX2–ZY2, Z3X, Z3Y, and Z5 are not available on 13-channel shim systems.

- 1 Set SPIN to **OFF** and write down the new lock level.
- 2 Set the lock level to about 80.
- 3 Maximize the lock level by shimming Z2X against ZX.
- 4 Maximize the lock level by shimming Z2Y against ZY.
- 5 Repeat the procedure “[Removing spinning sidebands \(non-routine\)](#)” on page 79.
- 6 Maximize the lock level by shimming ZXY against XY.
- 7 Maximize the lock level by shimming ZX2–ZY2 against (X2–Y2).

- 8 Set SPIN to **ON** and adjust the lock level to 80.
- 9 Maximize the lock level by shimming Z1, Z2, Z4, and then Z1, Z2, Z3.
- 10 Repeat step 1.
- 11 Maximize the lock level by shimming X3 against Y3.
- 12 Maximize the lock level by shimming Z3X against Z3Y if available.

See the installation data for your magnet for approximate Z3X and Z3Y values.

- 13 Look at the spectrum and decide where to concentrate your effort:
 - For a broad base, adjust Z4 and Z5.
 - For spinning sidebands, adjust the proper order radial shims.

The contribution of Z3 to the breadth of the base becomes evident (as does the contribution from the high-order radial shims) as Z4 and Z5 are optimized. Several cycles of shimming are required.

Local maxima are encountered from time to time that can cause problems. A local maxima is indicated if a high-order shim continues to increase and eventually reaches the maximum output of the shim supply, without having reached the optimal lock level.

Carefully re-examine the lower-order shims by making large excursions (systematically), beginning with the lowest-order shim and working up. This is a particularly difficult issue when dealing with the high-order radial shims such as X3, Y3, Z3X, and Z3Y, because their perturbation of the lock level is small relative to the change in the shim current.

The effects of X3, Y3, Z3X, and Z3Y on the spectrum are significant in experiments such as water suppression, but can go unnoticed or may not be important in some routine 1D spectra where large solvent peaks are not encountered.

Shimming PFG Systems

These procedures apply to the Performa I, Performa II, Performa IV, and Performa XYZ PFG systems. Once in operation, leave the amplifier powered on to allow the amplifier to reach a long-term equilibrium.

Performa I and Performa II

- 1 Open the System settings window (**Edit > System settings**).
- 2 Next to the Gradient amplifier label, set X, Y, and Z to off. Click **OK**.
- 3 Click the **Setup Hardware** button. This button is available when the **Start** tab is open.
- 4 Verify a drop in the lock level from the small dc zero current from the amplifier.
- 5 Shim the system to the desired level.
- 6 Open the **System settings** window and set Gradient amplifier Z to on. Click **OK**. The shimming changes from the small dc offset current.
- 7 Click **Setup Hardware**.
- 8 Adjust Z1 to restore the homogeneity. The lock level should have identical stability on the meter.

This two-stage approach is not strictly necessary, but it does separate any problems that might arise.

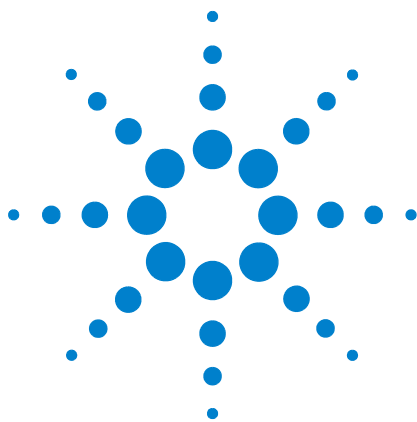
Performa XYZ

- 1 Prepare the amplifier by moving the switch from **STANDBY** position to **ON**.
- 2 Open the **System Settings** window (**Edit > System settings**) and set **Gradient amplifier X, Y, and Z** to on. Click **OK**.
- 3 Click the **Setup Hardware** button. This button is available when the **Start** tab is open.

The yellow RUN light should turn on.

Calibrating the Probe

Probe calibrations are stored in the probe file. Use these procedures and the standard calibration samples to update and maintain the calibration file for the probe. See the *VnmrJ NRM Systems Calibration User Guide* for detailed instructions on automatic and manual probe calibration procedures.



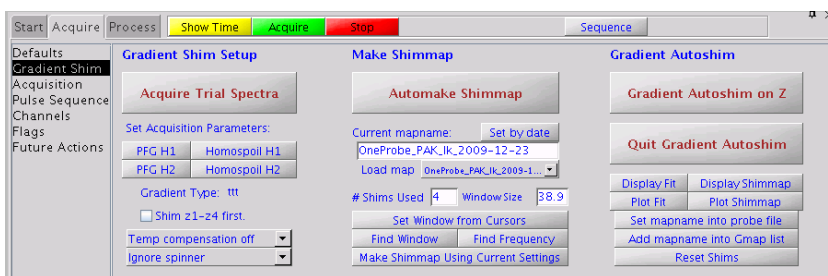
4 Gradient Shimming

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Introduction to Gradient Shimming

Gradient autoshimming provides rapid, automatic adjustment of axial room-temperature shims. It is a reliable way to set high-order shims, which shorten the time needed for shimming. A typical gradient autoshimming time is only a few minutes, and all steps are done with a few clicks of a mouse button.



The default gradient autoshimming is designed to use the axial gradients. It is typically done by using a PFG amplifier and probe, although it can also be done by using the homospoil gradient (Z1 room temperature shim coil). The homospoil method requires a bit more calibration to use. See [“Homospoil gradient type”](#) on page 88 for more details on how to set up the homospoil gradient.

Gradient autoshimming methods support shimming on a wide variety of samples with different volumes and solvents. Gradient autoshimming can be performed on either the 1H or 2H nuclei. For aqueous samples, water protons provide sufficient signal for shimming. For deuterated solvents, gradient shimming can be performed if there is sufficient deuterium signal. Deuterium gradient shimming is feasible on most samples where the lock solvent is a single, strong resonance, which includes the majority of solvents of interest for routine NMR use.

PFG gradient autoshimming is available on systems that are configured with a PFG accessory, whereas homospoil gradient shimming is available on all systems, with or without PFG.

Deuterium Gradient Shimming

Deuterium gradient shimming is feasible for most deuterated solvents for which the lock solvent has a single, strong deuterium resonance with sufficient signal.

Automated deuterium gradient shimming automatically holds the lock at its current value and switches the transmitter cable to pulse the lock coil when an experiment is run with `tn='lk'`.

The system administrator must make a shimmap on deuterium before deuterium gradient shimming can be used. Follow the procedure [“Mapping Shims and Gradient Shimming”](#) on page 91, using the deuterium signal for all steps. The transmitter power (tpwr) should be kept low to avoid probe arcing, with a 90° pulse greater than about 200 μ s.

Homospoil Gradient Shimming

Homospoil gradient type

VnmrJ allows homospoil (room temperature Z1 shim coil) as a general gradient type. It does not require the use of a pulsed-field-gradient module and thus is available on systems without PFG.

When homospoil is switched on in a pulse sequence, the shim current is set to maximum for a given period of time.

- To use homospoil as a quick homogeneity spoil in a pulse sequence, use `hsdelay`. This is the traditional homospoil method, and is usually done at the beginning of a relaxation recovery delay (for example, `hsdelay(d1)`). The parameter `gradtype` is ignored. See the *User Programming* manual for details of how to use `hsdelay`.
- To use homospoil as a general gradient type in a pulse sequence, in other words, for gradient shimming during automation or gradient shimming in general:

Click either **Homospoil H1** or **Homospoil H2** button on the **Gradient Shim** page. This sets the parameter `tmpgradtype` to `'nnh'`.

The value of `gradtype` is ignored if the parameter `tmpgradtype` exists. The parameter `pfgon` is ignored for homospoil gradients, since a separate gradient amplifier is not needed. Homospoil is then triggered by gradient statements such as `rgradient('z',gzlv11)`. If the value of `gzlv11` is non-zero, homospoil is switched on. If the value of `gzlv11` is zero, homospoil is switched off. Only one sign and strength of gradient current is available during a pulse sequence and is set by hardware.

Homospoil gradients may be switched on only for a limited period of time, usually 20 ms. This time limit is determined by hardware in spectrometer systems (see [Table 9](#) for system configurations). Check your pulse sequences to ensure this time limit is not exceeded.

Table 9 Homospoil control

Shim Supply	Homospoil Time Limit
Agilent 14	20 ms/200 ms
Agilent 18 to 40	20 ms/200 ms

The behavior of homospoil gradients is quite different from that of a pulsed field gradient. The gradient strength is much weaker than the traditional PFG, and the recovery time is much longer because of eddy currents. The strength and recovery of the gradient depends on the shim coils and system hardware. Typically, these gradients are suitable only for profile-type experiments and unsuitable for gradient coherence-selection experiments such as GCOSY and GNOESY. For most gradient experiments, pulsed field gradients are preferred, if they are available.

Homospoil gradient shimming for ^1H or ^2H

Follow the procedure in [“Mapping Shims and Gradient Shimming”](#) on page 91. Use **Find z0** before gradient shimming to use homospoil deuterium gradient shimming with different solvents.

Configuring Gradients and Hardware Control

- 1 Confirm that PFG or homospoil gradients are installed on your system. See the previous sections in this chapter. A PFG probe is required.
- 2 Confirm that the gradients are active by checking that `tmpgradtype`, `gradtype`, and `pfgon` are set appropriately for your system. Use `config` to change `gradtype` if necessary. Use System Settings to set `pfgon` if necessary.

Mapping Shims and Gradient Shimming

The shims must be mapped before autoshimming is used. Mapping the shims is necessary when a new probe is installed, but can be repeated at any time.

Spinning the sample during gradient shimming can cause motion artifacts. In most applications, using 5 mm or smaller NMR tubes, spinning is not recommended. Spinning larger 8 mm NMR can produce better results than not spinning the sample. See “[Spinning During Gradient Shimming](#)” on page 108, for instructions on spinning during gradient shimming.

Recommended samples for gradient shimming

The following samples are recommended for gradient shimming.

^1H shimming	10% to 90% H_2O in D_2O
^2H shimming	doped 1% H_2O in 99% D_2O or autotest sample

Manually calibrating the 90 degree pulse for ^1H and ^2H

These values are calibrated during the automatic probe calibration routines. Listed below is a way to re-calibrate them manually.

- 1 Insert a sample and find lock.
- 2 Disable sample changer control (`loc='n'`).
- 3 Adjust lock power, lock gain, and lock phase. Make coarse shim adjustments on Z1, Z2, X1, and Y1.
- 4 Select the **Proton** protocol.
- 5 Click the **Acquire** tab.
- 6 Select the **Channels** page.

- 7 Do one of the following procedures.
 - Lock (2H) 90° pulse calibration:
 - a Set the Observe Nucleus to lk.
 - b Set 90 Degree Pwr to 42 (to avoid arcing).
 - c On the **Acquisition** page, set the Observe Pulse to 200.
 - d Continue to [step 8](#).
 - Proton (1H) 90° pulse calibration:
 - a Verify that the observe nucleus is set to H1.
 - b Continue to [step 8](#).
- 8 Click the **Acquire** button or enter ga and wait for acquisition to finish.
- 9 Click the **Process** tab and select the **Cursors/Line List** page.
- 10 Click the **Transform** button.
- 11 Place the cursor near the peak and click the **Place on Nearest Line** button.
- 12 Click the **Move Transmitter** button.
- 13 Select the **Acquire** tab, then the **Acquisitions** page, and click the **Arrays** button.
- 14 Enter pw for the **Param Name**, **Array** size 20.
- 15 Set the following:

¹ H shimming	² H shimming
First value =4	First value =100
Increment =4	Increment =100

- 16 Select **Acquisition > Acquire** and **WFT**, (or enter ga) and wait for acquisition to complete.
- 17 On the **Acquisition** page set pw90 to the value of pulse width corresponding to first maximum.
- 18 Click **Arrays** and click **UnArray** in the **Array** window.
- 19 Set the observe pulse to the pw90 value.
- 20 Enter pw90 and tpwr in the probe file, if desired.

NOTE

The strong signal may cause an ADC overflow for 1H operation. If so, set gain = 0.

Mapping the Shims

- 1 Stop sample spinning.
- 2 Disable sample changer control (`loc='n'`).
- 3 Adjust lock power, lock gain, and lock phase. Make coarse shim adjustments on Z1, Z2, X1, and Y1.
- 4 Click **Tools**.
- 5 Select **Standard Calibration Experiments**.
- 6 Select **Set up Gradient Shimming**.

Standard parameters are retrieved from `gmapz.par` the first time **Set Up Gradient Shimming** is clicked, or if a shimmap was previously made, parameters are retrieved from the current shimmap.

- 7 Click the **Acquire** tab.



- 8 Select the **Gradient Shim** page.
- 9 Click one of the following buttons under Set Acquisition Parameters to retrieve the parameters from the probe file (if available) and set up gradient shimming acquisition parameters:

PFG H1	Homospoil H1
PFG H2	Homospoil H2

Selecting Homospoil gradient shimming parameters requires enabling of homospoil gradients (see [“Homospoil Gradient Shimming”](#) on page 88).

Solvent	Scans	Relaxation delay, sec.	Gain
deuteriochloroform	8-32	2	36 (18 Mercury)
dms0-d6	4-16	2	28 (10 Mercury)
D ₂ O	1-4	2	24 (6 Mercury)
deuterobenzene	1-4	2	24 (6 Mercury)
deuteroacetone	1-4	6-12	24 (6 Mercury)

Actual parameters might vary, depending on solvent concentration, probe, and system hardware.

- 10 Set `pw` as follows:
 - For PFG, set `pw` to the **90-degree pulse** or less.
 - For homospoil, set `pw` to the **90-degree pulse** and `p1` to **180-degree pulse**.
- 11 Click the **Gradient Shim** page.
- 12 Click **Acquire Trial Spectra** on the **Gradient Shim** page to test the parameters.

Two top-hat profile spectra should appear if the parameters are correctly set. If these spectra do not appear, check the following:

- Gradients are active (`pfgon` is set correctly).
 - Acquisition parameters, `pw`, `tpwr`, `nt`, and `gain` are correct.
 - Adjust parameters to see good signal-to-noise with no ADC overflow.
- 13 Enter a map name for the shimmap in the **Current mapname** field (any string valid for a file name). Or click the **Set by date** button.
 - 14 Click **Automake Shimmap** on the **Gradient Shim** page.
 - 15 The number of shims used to make the map is controlled by the `gsize` parameter (which defaults to 4).
 - 16 If the mapname already exists, a prompt appears to make a new mapname and to overwrite the current mapname.
 - 17 After acquisition is finished, click **Set mapname into probe file** button on the **Gradient Shim** page.

Starting gradient shimming

Click **Gradient Autoshim on Z** on the **Gradient Shim** page to start shimming as a system administrator. This button starts gradient shimming using current parameters and displays the curve fit and shim adjustments for each iteration.

When doing gradient shimming manually, the software shims “gssize” number of shims, even if the map was made with more shims. This is in contrast to gradient shimming during automation, in which case the software shims will have as many gradients as were used to make the map, and the value of gssize is ignored.

Quitting gradient shimming

Click the **Quit Gradient Autoshim** button to quit gradient shimming and exit the Gradient Shimming Setup panel. This also retrieves the previous parameter set and data, including any data processing done on the previous data set.

Gradient shim commands and parameters

The following commands and parameters are useful for performing special functions or can be set manually. See the *VnmrJ Command and Parameter Reference* for full descriptions.

Commands	Description
<code>gmapshim<('files' 'quit') ></code>	Run gradient autoshimming, quit
<code>gmapsys*</code>	Enter Gradient Shimming setup panels, make shimmap
<code>gmapz<(mapname) ></code>	Get parameters/files for gmapz pulse sequence
<code>* gmapsys<'shimmap'<,'auto' 'manual' 'overwrite' mapname></code>	

Parameters	Description
<code>d2</code>	Incremented delay for 1st indirectly detected dimension.
<code>d3</code>	Incremented delay for 2nd indirectly detected dimension; arrayed to two values
<code>gradtype*</code>	Gradients for x, y, and z axes.
<code>gzlvl {DAC value}</code>	Pulsed field gradient strength
<code>gssize {integer, 1 to 6}</code>	Number of z-axis shims used by gradient shimming
<code>gzwin {0 to 100}</code>	Percentage of spectral window used by gradient shimming

Parameters	Description
p1	First pulse width—If > 0, it is used between the gradient pulses as a 180 refocusing pulse, and the gradients have the same sign
pfgon{'nny' if on}	PFG amplifiers on/off control
pw	Pulse width; it can be 90° if p1=0
solvent	Lock solvent
vtcomplvl	Variable temperature compensation for gradient shimming
gmapspin	Enable or disable spinning during gradient shimming
gmap_z1z4	Gradient shim initially on z1-z4
* gradtype {3-char string from 'c', 'd', 'n', 'w', 'l', 'p', 'q', 's', 't', 'u', 'h'}	

How making a shimmap works

An experiment with the shims arrayed is run to map the shims and processed to make the shimmap. See [Figure 10](#).

Coarse shims are used if present. The parameters and data for the shimmap are stored in the file `userdir + '/gshimlib/shimmaps/' + mapname + '.fid'`. These parameters are retrieved the next time gradient shimming parameters are retrieved.

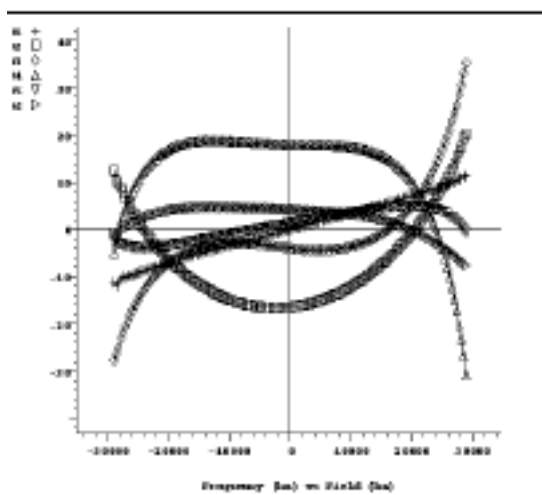


Figure 10 Shimmap plot of Z1 through Z6

How automated shimming works

The shims must be mapped before gradient automated shimming is used, see “[Mapping the Shims](#)” on page 93, for details. When gradient shimming is run from the **Gradient Shim** page, the curve fit plot is displayed for each iteration. The plot shows the raw data as #1 and the curve fit as #2. See [Figure 11](#).

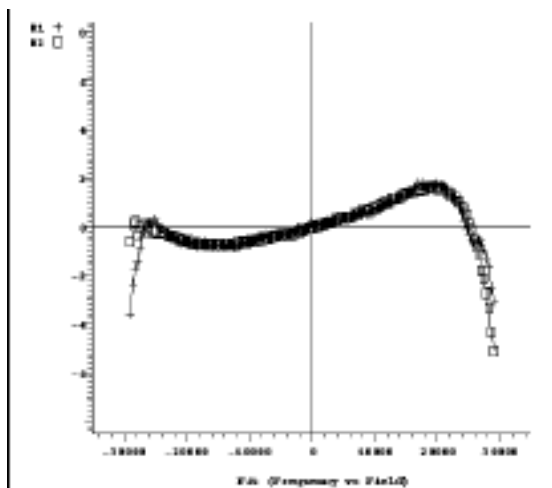


Figure 11 Curve fit plot

Shim adjustments for each iteration are also displayed in the **Text Output** window and have converged when the rms error number is less than 1.0. Gradient shimming continues until convergence or until a maximum of 5 iterations is reached. See [Figure 12](#).

mapname 5mm_Triax_01					
shimset 4 gsize 6					
					rms err 1.892
Shim	Offset	Old	New	Diff	Error
z1	800	-9405	-9269	-136	48
z2	800	-3118	-3104	-14	13
z3	3200	-4356	-4321	-35	37
z4	-3200	4049	4895	-836	104
z5	-3200	13443	14537	-1094	322
z6	3200	-15619	-12568	-3051	467
z7	3200	0	0	0	0
z8	3200	0	0	0	0

Figure 12 Display of shim adjustments for each iteration

If a shim goes out of range, the shim is set to maximum and shimming continues with the remaining shims. If convergence is then reached, shimming is tried once more with all Z shims and is continued, unless a shim goes out of range again.

Shimmap Display, Loading, and Sharing

Displaying the shimmap

After the shims are mapped, display the shimmap by clicking the **Display Shimmap** button on the **Gradient Shim** page (under the **Acquire** tab).

The shimmap display is a multicolored plot of the shimmap, with Z1 as #1 and Z2 as #2, etc. See [Figure 13](#).

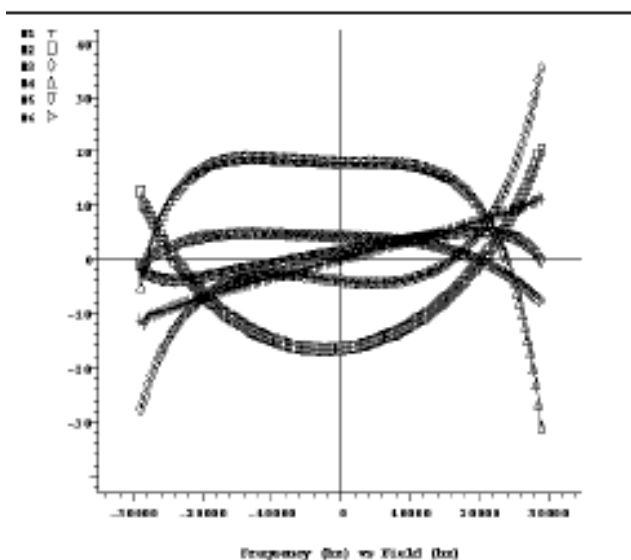


Figure 13 Shimmap plot

The shimmap is specific to the probe used, and can also be dependent on sample volume for small volumes. The shimmap shows the actual field dependence of the shims, except for a dc offset added for display purposes. Good signal-to-noise in the shimmap is needed for the shimming to work well. Poor signal-to-noise might result in incorrectly set shims.

Loading a shimmap

Do the following to change shimmaps (as a system administrator):

- 1 Click the **Acquire** tab.
- 2 Select the **Gradient Shim** page.
- 3 Select a previously created shimmap from the choices in the **Load Map** menu.

This loads parameters and loads the shimmap files `gshim.list` and `gshim.bas` from `gshimlib/shimmaps/mapname.fid` into `gshimlib/data`.

Sharing a shimmap

The system administrator can copy a shimmap file from `vnmrSYS/gshimlib/shimmaps` into the directory `/vnmr/gshimlib/shimmaps` so that the file is accessible to all users.

Do the following steps to copy files:

- 1 Log in as `vnmr1`.
- 2 Open a terminal window.
- 3 Enter `cd ~/vnmrSYS/gshimlib/shimmaps` and find the maps to copy.
- 4 Enter `cd /vnmr`.
- 5 If `gshimlib` does not exist, enter `mkdir gshimlib`.
- 6 Enter `cd gshimlib`.
- 7 Enter `cd shimmaps`.
- 8 Enter the following for each map in `vnmrSYS/gshimlib/shimmaps`:

```
cp -r ~/vnmrSYS/gshimlib/shimmaps/mapname.fid.
```

Remember the final dot at the end of the command and the substitution of a name for *mapname*.

- 9 Do the following for each user account:
 - a Log in to the user's operating system account.
 - b Start VnmrJ.
 - c Click **Tools**.
 - d Select **Standard Calibration Experiments**.
 - e Select **Set up Gradient Shimming**.
 - f Select a file from the **Load Map** menu on the **Gradient Shim** page.

Shimmap files and parameter sets

The parameters and shimmap files saved under a mapname are retrieved when that mapname is retrieved. When reinserting a probe, reload the shimmap for that probe. If the correctness of the shimmap is in doubt, make a new shimmap, which typically takes only a few minutes. The last parameters and files used are automatically retrieved the first time `gmapsys` is entered. If `gmapsys` is entered again, the parameters are not retrieved. Gradient shimming uses the current parameters after the pulse sequence is loaded (`seqfil='gmapz'`).

Gradient Shimming for the General User

The general user can run gradient shimming from outside `gmapsys` from any experiment. Any one of the following methods is recommended for routine use:

- In the menu system, click **Acquisition> Do Gradient Shimming**.
- Parameters are retrieved from the current mapname, which is displayed at the start of shimming, and the spinner is automatically turned off. The curve fit and shim adjustments are not displayed. The previous parameter set and data are retrieved when shimming is finished. This button only functions after a shimmap is made.
- Click the **Start** tab, on the **Standard** or **Lock** pages, then click the **Gradient Shim** button.
- Enter `gmapshim`. This performs the same action as clicking on **Gradient Autoshim on Z**.
- Use `wshim='g'` within parameter sets.
- When running in the Study Queue, select the **Shim** check box before submitting the experiment.

Use one of the following methods to stop gradient shimming before it is completed:

- Under the **Acquire** tab, select the **Gradient Shim** page and click the **Quit Gradient Autoshim** button. Quitting aborts the experiment and retrieves the previous parameter set and data.
- Abort the acquisition with `aa` and click **Cancel Cmd**. Then enter `gmapshim('quit')` to retrieve previous data set and parameters.

Deuterium Gradient Shimming Procedure for Lineshape

Use this procedure for optimizing both the spinning and non-spinning shims.

Setting up

- 1 Insert the appropriate lineshape sample (chloroform in acetone-d6) and find lock.
- 2 Turn off spinning and disable sample changer control.
- 3 Click the **Start** tab.
- 4 Select the **Lock** page.
- 5 Adjust lock power, lock gain, and lock phase as necessary.
- 6 Do quick shimming on z1, z2, x1, y1 (use z1c, z2c, if present).

Making the ShimMap

- 1 Use 90° pulse for `tn='lk'`.
- 2 Click **Tools**.
- 3 Select **Standard Calibration Experiments**.
- 4 Select **Set up Gradient Shimming**.
- 5 Click the main menu bar.
- 6 Standard parameters are retrieved from `gmapz.par` the first time **Set Up Gradient Shimming** is clicked, or if a shimmap was previously made, parameters are retrieved from the current shimmap.
- 7 Click the **Acquire** tab.
- 8 Select the **Gradient Shim** page.

Click one of either the **PFG H2** or **Homospoil H2** buttons under **Set Acquisition Parameters** to retrieve the parameters from the probe file (if available) and set up gradient shimming acquisition parameters:

Selecting Homospoil gradient shimming parameters requires that homospoil gradients are enabled

- PFG –set **Observe Pulse** to one half the 90° pulse found in step 2 and set **Relaxation Delay** to 6.
- Homospoil H2 – set **Observe Pulse** to the 90° pulse and **Relaxation Delay** to 6.

- 9 Click **Acquire Trial Spectrum** and wait for acquisition to be completed. A two profile spectra is displayed.
- 10 Click **Automake Shimmap** to map the shims.
- 11 Under Current mapname, click the **Set by date** button or, enter a file name.
- 12 Wait for the acquisition to be completed and the message, “shimmap done!” to be displayed.

Starting Z gradient shimming

- 1 Click **Gradient Autoshim** on Z.
- 2 Wait for the acquisitions to be completed, and the message, “Gradient Autoshimming on Z done! N iterations” to be displayed.
- 3 Click the **Start** tab and select the **Lock** page.
- 4 Click the **Lock Scan** button and adjust lock phase.

Optimizing non-spinning shims

- 1 Shim on the lock level only on low-order nonspins (x1, y1, xz, yz, and so on).
- 2 Do not shim on z's (z1, z2, and so on.).
- 3 Repeat Starting Z Gradient Shimming Starting Z Gradient Shimming.

Evaluating homogeneity

- 1 Measure proton lineshape.
- 2 Turn on spinner if appropriate.
- 3 Click the **Lock Scan** button to make fine shim adjustment.
- 4 Shim on all shims as necessary.

Calibrating $gzwin$

The parameter $gzwin$ is the percentage of the spectral window used in calculating the field maps. $gzwin$ should be adjusted only when making a new shimmap. If this parameter is not calibrated correctly, excess noise data at the edge of the shimmaps appears, which corresponds to the region in the profile spectrum where the signal goes to zero. It is normal to have a few noise data points at the edge of the shimmap, but if it is more than a few data points (greater than 25% of the window), $gzwin$ may be incorrectly calibrated. This can occur if there is low signal-to-noise or if $gzwin$ has not previously been calibrated for the current parameter set. If the gain is too high, “wings” will appear on the sides of the spectra and may result in incorrectly calibrated $gzwin$. This can also occur if there are multiple chemical shifts for the nucleus chosen for gradient shimming in the presence of a weak gradient.

Automatic calibration of $gzwin$

Click the **Automake Shimmap** button or do the following:

- 1 Click the **Find $gzwin$** button on the **Gradient Shim** page. This calibrates $gzwin$ and sets tof to center the window used for calculation.
- 2 Click **Make Shimmap Using Current Settings** button. This makes the shimmap with the current values of $gzwin$, tof and other parameters.
- 3 Optional: Click through each step to verify correct calibration of $gzwin$. The box cursors at the end of step 1 should be at either edge of the profile.

Manual calibration of gzwin

Manual calibration of `gzwin` can be used to avoid noise spikes in the spectrum, or other artifacts. To manually calibrate `gzwin`, do the following:

- 1 Click the **Acquire Trial Spectra** button on the **Gradient Shim** page. Wait until the experiment is done.
- 2 Display a spectrum using the graphics control buttons. Set the box cursors near the edges of the profile.
- 3 Click **Set Window from Cursors**.
- 4 Click **Make Shimmap Using Current Settings**.

The parameter `gzwin` should be adjusted only when making a new shimmap. The calibrated value of `gzwin` is saved when the new shimmap is saved at the end of the mapping experiment. The same value of `gzwin` must be used in shimming as in making a shimmap, and should not be adjusted when shimming.

Varying the Number of Shims

The maximum number of shims available for gradient shimming is determined by the:

- shim hardware
- number of shims (up to the limit set by the hardware) used when the shimmap was created

Changing the number of shims used for gradient shimming

- 1 Map the shims, see “[Mapping the Shims](#)” on page 93, or load an existing shimmap.
- 2 Enter a number in **# Shims Used** entry box on the **Gradient Shim** page. The number must be less than or equal to the number of shims used to make the shimmap.
- 3 Click the **Gradient Autoshim** on Z button.
- 4 All the shims specified in the **# Shims Used** entry box are used to optimize the field homogeneity.

Selecting optimization of Z1 through Z4 shims first

Optimization of Z1 through Z4 shims before optimizing all the shims applies only to systems with room temperature shims Z5 and higher order. Gradient shimming takes longer and goes through more iterations, but this may avoid the problem on some systems where a high-order shim (for example, Z5, Z6) goes out of range because it contains impurities from lower-order shims. Select this optimization as follows:

- 1 Load or make a shimmap of more than four shims.
- 2 Click the **Shim z1-z4** first check box.
- 3 Set the shims specified in the **# Shims Used** entry box to a value greater than 4.

Shimming starts with Z1–Z4 optimization and then proceeds with all shims specified by **# Shims Used**. The check box may be set at any time before or after shimming from the **Gradient Shim** page. In order to use this parameter in user autoshimming, set it before making a shimmap, or in the corresponding parameter set in `gshimlib/shimmaps`.

Variable Temperature Gradient Compensation

Temperature gradients or convection currents may be compensated for with the addition of a 180° pulse in the pulse sequence prior to data acquisition. Sample viscosity, effective T₂, and signal-to-noise affect the quality of the gradient shimming. Variable Temperature gradient compensation is compatible with spinning during gradient shimming. Enable variable temperature gradient shimming as follows:

- 1 Select one of the following from the **Temperature Compensation menu** on the **Gradient Shim** page.
 - **Temp compensation off** – no compensation.
 - **Temp compensation on** – systems with homospoil or PFG.
 - **Temp compensation plus** – systems with pulse field gradients; this option adds additional dephasing gradients.
- 2 Set **p1** to 180° pulse.
- 3 Map the shims; see [“Mapping the Shims”](#) on page 93.
- 4 Start gradient shimming; see [“Starting gradient shimming”](#) on page 95.

Spinning During Gradient Shimming

Spinning during gradient shimming is useful for sample sizes 8 mm and larger. Spinning the sample requires synchronization of the pulses and delays with the period of the rotor. Sample viscosity, effective T₂, and signal-to-noise affect the quality of the gradient shimming. Spinning during gradient shimming is compatible with temperature gradient compensation.

Set up for spinning during gradient shimming as follows:

- 1 Select **Synch with spinner** from the menu options under **Gradient Shim Setup** on the **Gradient Shim** page.
- 2 Set the spinner speed either from the command line or from the panel as follows:
 - a Click the **Start** tab.
 - b Select the **Sample Info** page.
 - c Enter the spinning speed in the box next to the **SPIN** button, press **Enter**, and activate the spin setting by placing a check in the box next to the Spin Speed field.
 - d Click the **Acquire** tab.
 - e Select the **Gradient Shim** page.
- 3 Select a nucleus and gradient option by clicking on one of the following Set Acquisition Parameter buttons:

PFG H1	Homospoil H1
PFG H2	Homospoil H2
- 4 Map the shims; see [“Mapping the Shims”](#) on page 93.
- 5 Verify that the spinner is on.

NOTE

The map must be made with the sample spinning at the speed set in Step 2.

- 6 Start gradient shimming. See [“Starting gradient shimming”](#) on page 95.
- 7 Verify that the spinner is on.

NOTE

Gradient shimming must be run with the sample spinning at the speed set in Step 2. The shims will not be set correctly if the sample is not spinning at the same speed that was used when the shimmap was created.

Suggestions for Improving Results

Calibrate the 90° pulse and adjust t_{pwr} , p_w , and gain to optimize signal-to-noise. Reduce gain if ADC overflow occurs, which may appear as wings on the profile. Optimal signal-to-noise is the most important criteria for gradient shimming.

Stimulated echoes may result for solvents with long T_1 and appear as excess noise, a beat pattern in the spectrum, or as secondary echoes in the FID (use d_f to observe this). Do one or more of the following:

- set d_1 to 3 to 5 times T_1
- use a smaller flip angle for p_w

The phase encode delay d_3 is arrayed to two values, the first of which is zero. The second value can be increased for better signal-to-noise in the phase maps, up to about the point where the amplitude of the second profile is half that of the first (about $2/3 T_2$ without radiation damping).

Radiation damping can be severe in water (1H). However, longer d_3 values increase the phase excursion, and can make it difficult to shim large shim corrections (especially Z_1).

Typical 1H values are 5 to 30 ms, and typical 2H values are 30 to 200 ms. If the shims are far off when making a shimmap, the second value of d_3 might be too small. If this problem occurs, decrease the second value of d_3 temporarily to one-half to one-quarter its value.

When reinstalling a probe, make sure it is in the same vertical position in the magnet barrel as when the shimmap was made. If vertical position of the probe is uncertain, make a new shimmap, which typically takes only a few minutes.

Alternate between z-axis gradient shimming and shimming the low-order x- and y-axis shims by other methods (for example, on lock level). The z-axis shims account for the majority of sample volume changes (changes in height), and the x- and y-shims are relatively insensitive to change in height. Evaluate shimming for a particular application, since the ideal lineshape may vary with the application.

The high-order shims can sometimes be set off-scale during shimming. This may occur if the sample is short, if the sample is improperly seated in the probe, or if the high-order shims are weak or other effects. In such cases, the off-scale shim is set to maximum, and shimming

continues with lower-order shims. Superior results can be obtained in some cases by varying the number of shims used, see “Varying the Number of Shims” on page 106. On a short sample, it also can be useful to remap the shims.

Some shim systems may need additional time when running the shim-mapping experiment to allow the shims to settle. The added time is especially noticeable on some systems for Z4. To account for added time, lengthen the `d1` delay or add dummy scans in between each array element (for example, `ss=-2`). Decreasing the amount a shim is offset also allows the shim to settle more quickly. Enter `gmapsys('vi')` to edit the values in the Offset column, and then enter `gmapsys('shimmap','manual')` on the command line to map the shims with user-defined offsets. A new mapname may also be set using `gmapsys('vi')`.

Coarse shims are used on systems on which they are available. To use fine shims on these systems, enter `gmapsys('vi')` to edit the entries in the shim column (for example, change `z1c` to `z1`), and then enter `gmapsys('shimmap','manual')` to map the shims.

The water protons provide sufficient signal for shimming for samples in H_2O . Deuterium gradient shimming is strongly recommended for samples other than water if there is sufficient deuterium signal. Proton gradient shimming can be made to work in samples other than water if there is sufficient proton signal and the signal is well-resolved (does not overlap with other strong resonances). Gradient shimming can also be done on a water sample of equal solution height of the sample of interest, and then the sample of interest can be inserted.

For further information, see the entries for `gmapshim`, `gmapsys`, and `gmapz` in the *Command and Parameter Reference*.

Gradient Shimming Using Selective Excitation

Gradient shimming is predicated on the correlation between the resonant frequency of a given molecule and its physical location within the sample. When the spectrum used for gradient shimming is not dominated by a single, large resonance, this relation is no longer valid. To overcome this limitation, a frequency-selective shaped pulse can be used in the place of the normal broadband pulse to excite only one signal from the sample.

To create a z-gradient shim map using selective excitation:

- 1 Select the **Shaped Pulses** check box in the Gradient Shim panel. This will enable the “Define band” button (Figure 14).

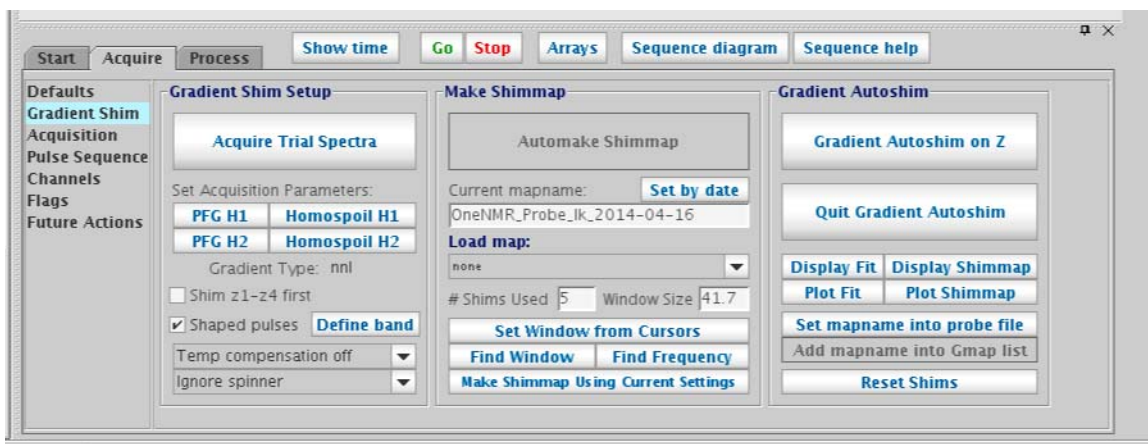


Figure 14 Gradient Shim panel with Define band button enabled

- 2 Click the **Define band** button. This will acquire a spectrum, display the result in the graphics canvas, and present the Gradient Shimming Band Selection pop-up (Figure 15).

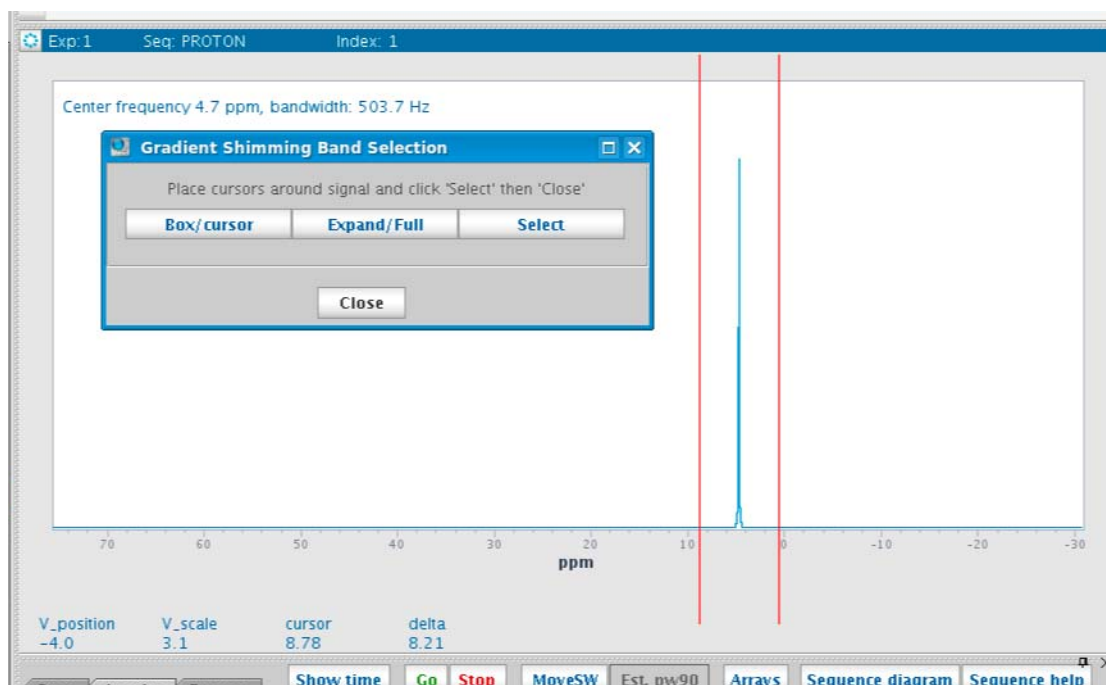


Figure 15 Define band result showing Gradient Shimming Band Selection pop-up

- 3 Place the cursors around the peak to be used for selective gradient shimming and click the **Select** button. This will capture the frequency and bandwidth required for the selective excitation pulse.
- 4 Click **Close**.
- 5 The shaped pulse will be calculated automatically and loaded into the current parameter set. The graphics canvas will be updated to display the pulse sequence using the selective pulse (Figure 16).

4 Gradient Shimming

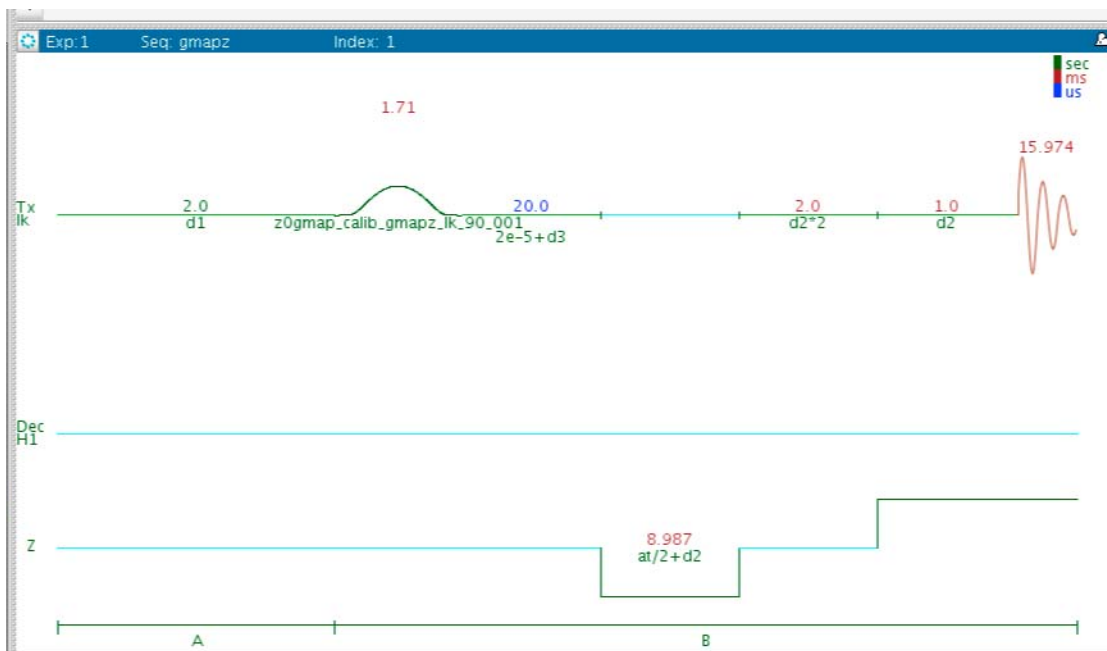


Figure 16 Pulse sequence using the selective pulse

- 6 Once the selective pulse has been created, a gradient map can be recorded using only the signal selected by the shaped pulse. The procedure is identical to that used for a normal shim map once the pulse parameters have been optimized. See “[Mapping the Shims](#)” on page 93, starting at [step 12](#).

Gradient Shimming Pulse Sequence and Processing

The basis of gradient shimming is differential phase accumulation from field inhomogeneities during an arrayed delay. The phase is spatially encoded by a pulsed field gradient.

The gradient shimming pulse sequence in Figure 17 is shown with $p_1=0$, in which case p_w can be set to a small flip angle. If $p_1>0$, the pulse field gradients are both set to the same sign, and p_1 should be set to 180° and p_w to 90° , so that RF inhomogeneities are refocused. $p_1=0$ is usually sufficient for most cases.

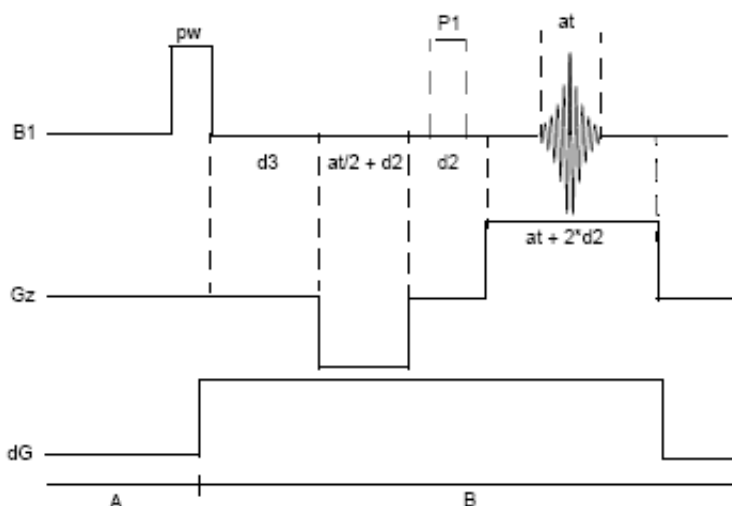


Figure 17 Gradient shimming pulse sequence

Phase accumulation from all gradients present is as follows:

$$\phi = z G_z (-at/2 + t) + dG (d_3 + at/2 + 3*d_2 + t)$$

where t is the time during acquisition at , G_z is the z -axis pulsed field gradient strength, and dG is the sum of the shim gradient fields, shown as being on during relevant times in the pulse sequence.

The effect of the shim gradients dG can be isolated by arraying d_3 and taking the difference in the phases:

$$\Delta\phi = \phi_2 - \phi_1 = dG * (d_3 [2] - d_3 [1])$$

For example, at a particular point, $\Delta\phi$ can be $2\pi * 100 \text{ Hz} * 10 \text{ ms}$, or 2π radians. Thus, a pair of profiles with different d_3 values can be used to calculate the B_0 field along z .

The effect of any one shim gradient can be isolated by arraying the shim value, represented by dG , and taking the difference in the phase differences:

$$\begin{aligned}\Delta(\Delta\phi) &= \Delta\phi_2 - \Delta\phi_1 = dG_2 * (d_3 [2] - d_3 [1]) - dG_1 * (d_3 [2] - d_3 [1]) \\ &= (dG_2 - dG_1) * (d_3 [2] - d_3 [1])\end{aligned}$$

Therefore, two pairs of profiles can be used to map out the effect of a shim. By arraying all the shim values, a set of phase difference maps or shim field maps can be constructed for a given shim set. Shimming can then be performed by constructing a background field map for the starting shim values ($\Delta\phi$) and fitting the result to the shimfield maps. The calculations are quite fast. So, the entire shimming process is usually limited by the data acquisition time, typically taking only a few minutes.

In practice, the phase is calculated from $\phi = \arctan(x, y)$ from the real and imaginary values at each point in the spectrum, and $\Delta\phi$ is calculated from the difference in the phases of a pair of spectra with d_3 arrayed. [Figure 18](#) shows an example of mapping the z_1 shim.



Figure 18 Mapping the z_1 shim

Gradient Shimming References

- P. C. M. Van Zijl, et al. *J. Magn. Reson.* 1994, 111 (Series A), 203–207.
- S. Sukumar, et al. *J. Magn. Reson.* 1997, 125 (Series A), 159–162.
- H. Barjat, et al., *J. Magn. Reson.* 1997, 125 (Series A), 197–201.
- C. L. Evans, et al., *J. Magn. Reson.* 2002, 154 (Series A), 325–328.

4 Gradient Shimming



5 Study Queue and Studies

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A study is an ordered list of experiments that can be performed on any given sample. When data is collected using the Study Queue, information regarding that sample including raw data, processed data, and plots are linked together and automatically collected into a Sample Directory. These links allow customized information from one experiment (that is, calibrated pulse widths, optimized sweep widths, solvent suppression conditions, etc.) to be automatically incorporated into subsequent acquisitions, and the automatic addition of high-resolution reference spectra to 2D plots. The location and structure of each Sample Directory is controlled by choices made in the User Preferences panel under the Templates tab.



Introduction to Studies

A study can be created, modified, extended, and saved using different tools described in this chapter. It is important to note that these tools can be applied with or without the use of a robot sample changer.

The sequence of steps for data acquisition using studies is initiated by clicking the **New Study** button on the lower portion of the **Study Queue** vertical panel. This places the software into Submit mode, where tools are available to build, customize, and submit a study. Review mode is used for all other tasks, such as manual data collection, processing and plotting.

While the Study Queue is used to collect data, it also serves other functions. The Study Queue presents a graphical representation of the status of all experiments submitted to acquisition in the current automation run. Experiment nodes that have completed are displayed as green, the active experiment is blue and pending experiments that have been submitted and not yet begun are displayed in yellow. Double-clicking on a completed node loads the associated data and processes that data in the current workspace.

The Study Queue can display an overview of all the studies submitted to automation by choosing **View> Spectrometer** from the top of the **Study Queue** window. Lists of nodes under the headings of Active Study, Completed Studies, Studies in Progress, Errored Studies, Priority Studies, and Studies in Queue are displayed, as well as the estimated completion time for each active and pending experiment node.

The various options and choices available for using the Study Queue will be discussed in this chapter.

Study Clusters

There are several practical scenarios where you would want to treat a set of FIDs (from different studies) as a single group. For example, different synthetic compounds for a given project over a period of time; quality assurance scenario where the same compound is analyzed from manufactured batches over a period of time; data from different arms of a biological studies or same arm of different biological studies. In these cases, group such “related” FIDs into a cluster for post-acquisition processing. The StudyCluster utility in VnmrJ offers such a tool. While this is a generic tool, it is used extensively in CRAFT when multiple (related) FIDs are analyzed. This section describes the functionality of this tool as well as a guide to create a cluster.

The study cluster tools are accessed from the main menu using **MainMenuTools > Study Clusters**.

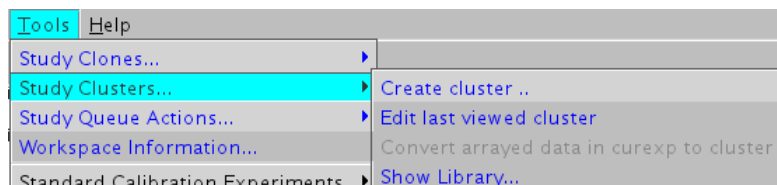


Figure 19 Study Clusters submenu

To create a study cluster

- 1 In the menu bar, click **MainMenuTools > Study Clusters > Create Cluster**.

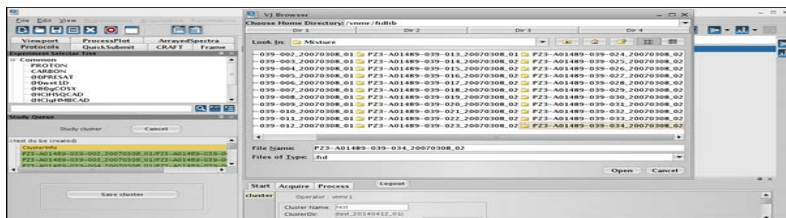


Figure 20 Create cluster submenu

- 2 Select one of the options to start the process of creating a new cluster.
 - a Select **New cluster** to put the Study Queue into “cluster” mode which allows you to add FIDs to the cluster list.
 - b The **Add data from current workspace option** does the same thing as **New cluster** but in addition adds the FID from the current workspace to the cluster list (as a convenience).
 - c **Add study from current workspace** is same as (b) but all the FIDs from the study in the current workspace will be added to the cluster list.
 - d **Add data from all viewports** is also the same as (b) but all the FIDs from all the currently active viewports will be added to the list.
- 3 The Study Queue will now be in cluster mode (ready to add any FID from the browser or locator).

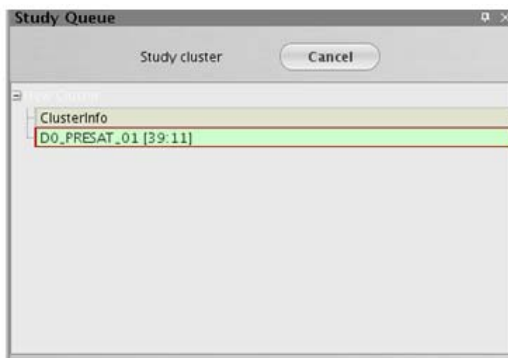


Figure 21 Study Queue in cluster mode

- 4 In the VnmrJ browser, navigate to studies of interest and double click the FID to be added to the Study Queue. This builds the Study Queue.
 - Alternately, you can drag and drop a study from the browser to the Study Queue area to add all the FIDs in that study to the Study Queue.
 - Another alternative is to keep a text file with the list of full pathname of the FIDs to be added to Study Queue in userdir/clusterlib. Double click this text file (this file must be in an appdir/clusterlib to be recognized as a list to be added to the Study Queue) to add all the FIDs in that list to the Study Queue.

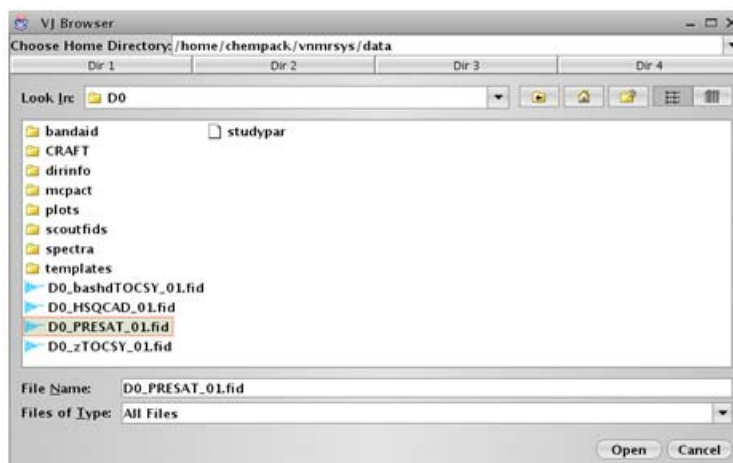


Figure 22 VJ Browser

- 5 Once the Study Queue is constructed with the FIDs of interest, type a Cluster Name for the cluster in the panel.

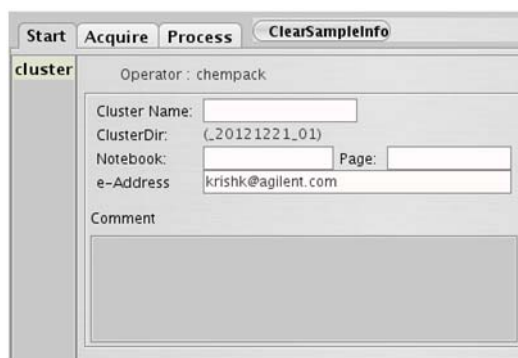


Figure 23 Cluster panel

- 6 Click **Save Cluster**. This will save this cluster in `userdir/clusterlib` with a directory name as dictated by the `ClusterName` and the sample directory template.

To edit the last viewed cluster

- In the menu bar, click **Study Clusters > Edit last viewed cluster**.

This utility behaves exactly like the new cluster utility. The Study Queue will be put in a cluster mode and all the FIDs in that cluster will be added to the Study Queue. You add/delete FIDs to this list and resave the cluster.

To show the cluster library

- In the menu bar, click **Study Clusters > Show Library...**

This utility will show a popup with access to all clusters in all available and valid appdirs. You can choose a cluster of interest and display that in the Study Queue area for review.

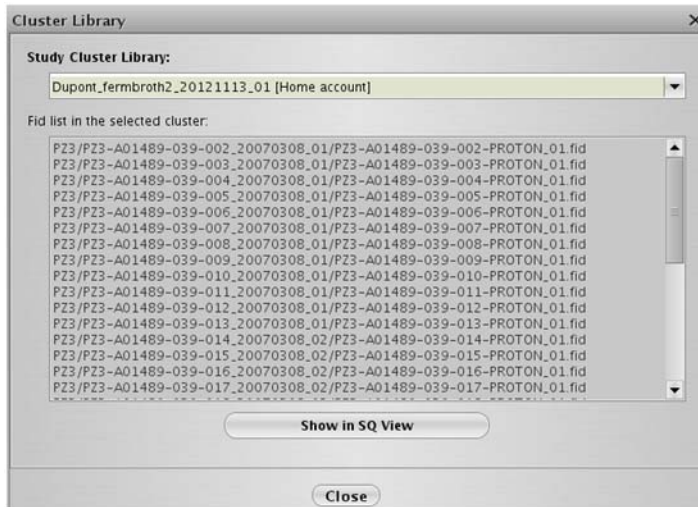


Figure 24 Cluster Library popup

To define cluster attributes

When a cluster is created, the tool recognizes if all the FIDs in the cluster are of same 1D type (the comparisons are done for `seqfil/tn/np/sw`). If they are, an arrayed spectrum that has all the FIDs as an array will be created in the cluster directory. This is a convenient feature for quick

visualization of the entire set (and a required feature for CRAFT cluster analysis – meaning all FIDs in a cluster must have the same key acquisition parameters). Once such an array is created, it can be recalled in current workspace. If the arrayed data in current workspace is such a cluster array, the “Define cluster attribute” tool becomes available.

- In the main menu, click **MainMenuTools > Study Clusters > Define cluster attribute**

Selecting this tool opens a popup where attributes for individual FIDs within that cluster be defined.

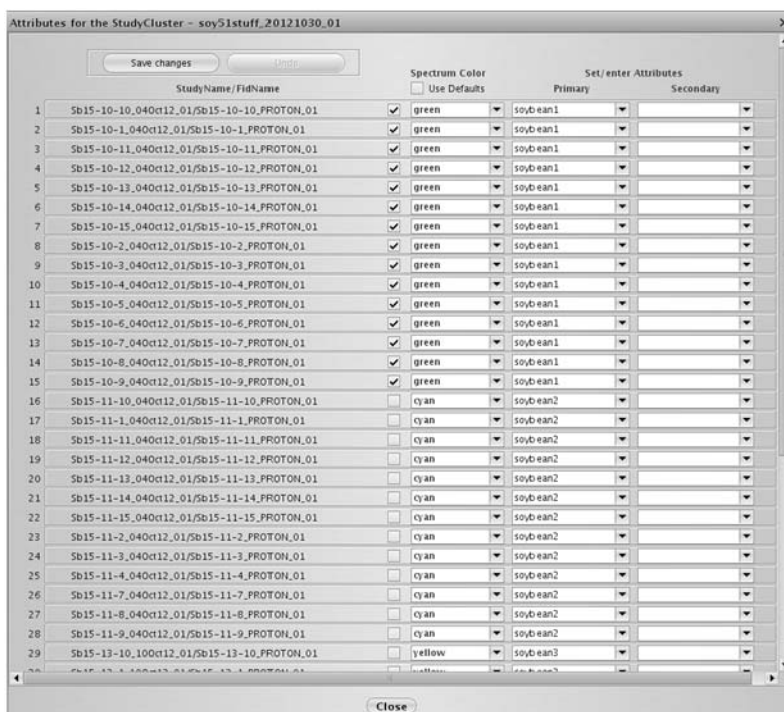


Figure 25 Attributes popup

For each StudyName/FidName, up to three attributes can be defined or assigned:

Color – the color for display of the trace while reviewing via interactive dssi mode

Primary attribute – for example, vehicle, drug1, drug2

Secondary attribute – for example, a secondary subgroup within the primary subgroup, such as 10mg/kg, 20mg/kg and 100mg/kg within drug1

5 Study Queue and Studies

These attributes are saved with the cluster and are conveniently used by CRAFT. For example, to assign green color to spectrum 1 thru 12, select check boxes for FIDs 1 and 12 – the intermediate FIDs will be automatically selected, select green from the color menu and save.

Operations Using a Robot Sample Changer

The **Study Queue** window changes to reflect movement between the two modes of software operation: **Review** mode and **Submit** mode.

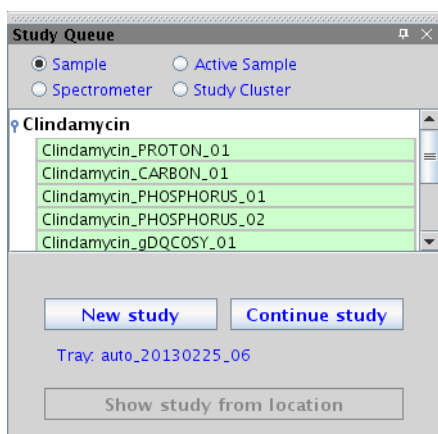


Figure 26 Study Queue in Review mode

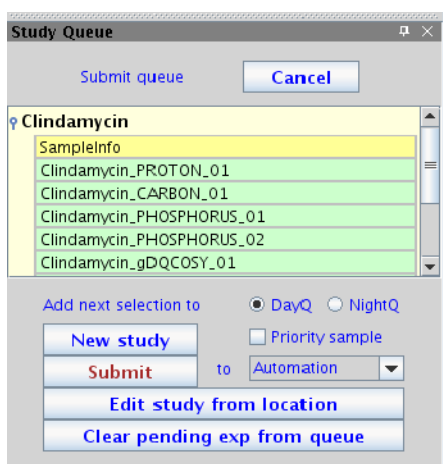


Figure 27 Study Queue in Submit mode

Study Queue controls-Review mode

In Review mode, the **Study Queue** window is used to display information about the current automation run.

You can select the following Study Queue view using four view selections:

- Sample
- Spectrometer
- Active Sample
- Study Cluster

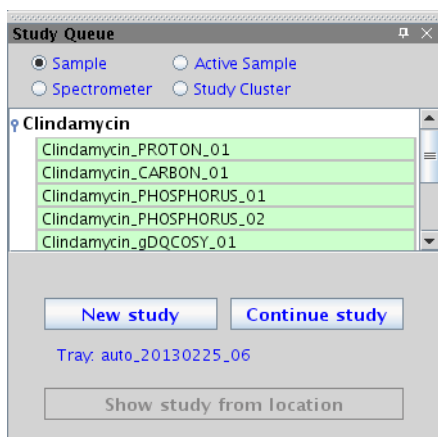


Figure 28 Study Queue in Review mode

Study Queue—Sample View

The Sample view displays the study linked to the data in the current workspace. See [Figure 26](#).

If the **ScoutFids** check box is selected in **Edit > Preferences > SQview** all prescan FIDs will be displayed in the Study Queue.

Study Queue—Spectrometer View

The Spectrometer view displays all studies in the current automation run.

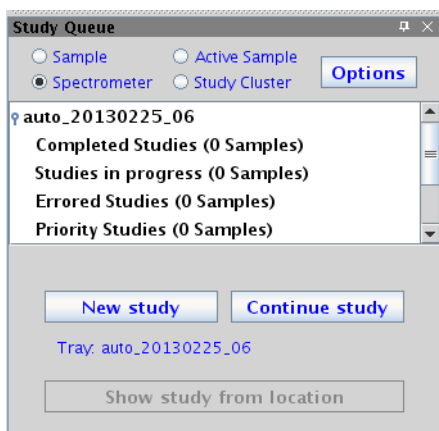


Figure 29 Study Queue-Spectrometer view

Click **Options** to configure and update display settings on the Spectrometer View Preference window, see [Figure 30](#).

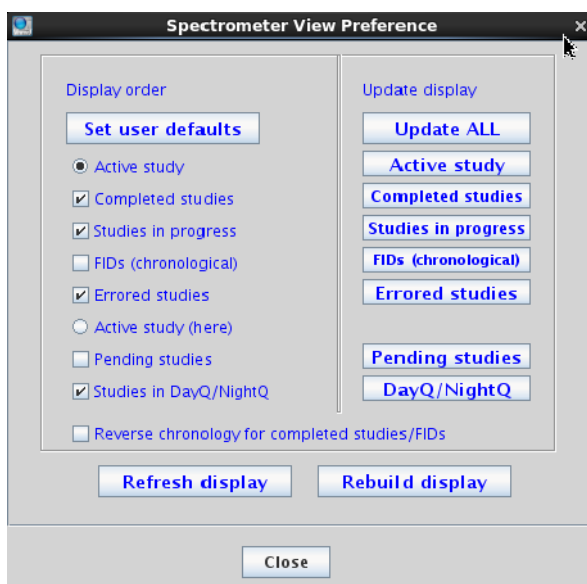


Figure 30 Spectrometer view options

Study Queue—Active Sample

The Active Sample view displays the currently acquiring study.

Study Queue—New Study

The **New Study** button initializes a new study and moves the software to **Submit** mode. The **Edit Study** button allows the

user to modify the current study displayed in the Study Queue. The **Show Study from Location** button loads a study from the tray display.

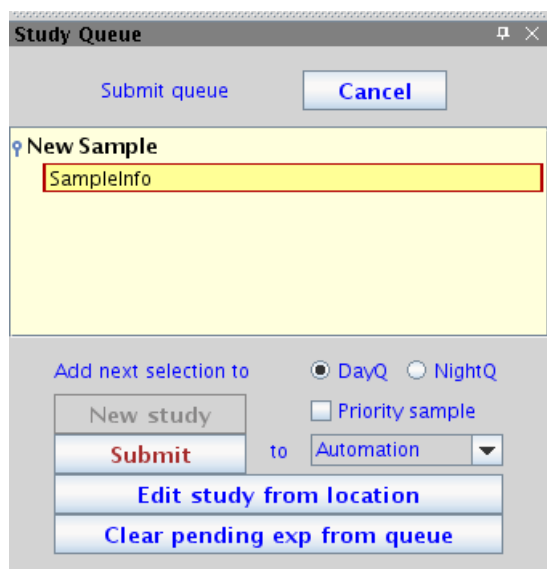


Figure 31 Study Queue–Submit mode new study

Study Queue controls-Submit mode

In Submit mode, the **Study Queue** window is used to build a study and submit it to acquisition.

The **Cancel** button abandons any changes made to the current study and returns the software to Review mode. Selecting the **DayQ** and **NightQ** buttons determines when an experiment will be run. The schedule for when the DayQ and NightQ begin is set by the account administrator through the Preferences panel, see Automation Tab.

The **Priority sample** check box allows a sample to be submitted ahead of all other samples in the current automation run. This feature is controlled by the system administrator on an operator-by-operator basis.

The **New Study** button creates a new study.

The **Submit** button submits the current study to acquisition. The associated pull-down menu determines how the acquisition is to be performed:

- **Automation**–submits the study to the Spectrometer Queue.
- **Foreground exp**–submits the study to acquisition in the current workspace.

- **Background**—submits the study to a background copy of VnmrJ.

The Edit Study from Location button loads a study into the Study Queue from the tray display in preparation for modification of that study.

The Clear Pending Exp from Queue button deletes all pending experiments from the current Study Queue.

Using the Study Queue-full automation

To collect a series of spectra in a study queue:

- 1 Place the sample in a spinner turbine and adjust its position using the sample depth gauge. Place the sample/spinner into the robot sample rack and note its position.
- 2 Click **New Study** in the Study Queue vertical panel. This initializes the Study and moves the software into "Submit mode."
- 3 Click on the buttons in the **Experiment Selector** to add the desired experiments to the Study Queue. Alternatively, double-click an experiment in the Experiment Selector Tree to add it to the Study Queue. The order of the experiments in the Study Queue can be changed using a click-and-drag action. Dragging an experiment node to the trash can deletes it from the queue.
- 4 Experimental parameters for an individual experiment can be customized by double-clicking on the experiment node in the study queue. (This is dependent upon the rights and privileges permitted for each user by the System Administrator.) The parameter set is loaded into the current workspace where any desired changes can be made using the horizontal panels under the **Acquisition** tab. Once the desired changes are complete, click **Save**.
- 5 Fill in the information associated with the sample, such as sample name, solvent, email address, comments about the sample, and so on. The sample name parameter is typically required as part of the automatic data saving routine, and the solvent information is used to determine where the lock must be set to in order to get correct referencing.
- 6 Select the check boxes to control automatic printing, locking, shimming, and tuning, as appropriate.

- 7 Select the location in the tray display that corresponds to your sample. Once selected, the location will show a flashing highlight ring.
- 8 Click **Submit**. Depending on the settings in the preferences panel, the system remains in "submit mode" with the current queue still loaded in the **Study Queue** window. The same queue can be submitted to a new sample location by editing the sample information, clicking the appropriate location in the tray, and clicking **Submit** again.
- 9 Click **Cancel** to return the interface to Review mode.

Day Queue, Night Queue, and Priority Queue

The account administrator can choose to restrict the time available for any given sample, (See Automation Tab). Depending on preferences chosen by the account administrator, the Study Queue either moves any experiment that exceeds the time limit into the Night Queue or will not allow further additions to the study. The user has the ability to explicitly direct an experiment to occur at night by selecting the "NightQ" radio button in the **Study Queue** window before adding an experiment to the study.

If allowed by the account administrator, users can select the **Priority sample** check box before submitting a Study Queue to acquisition. The priority sample will be inserted into the magnet as soon as the current sample is completed, as compared to being added to the Spectrometer Queue in the order that the studies were created.

Editing experiments in a pending study

Until acquisition has begun on a queued experiment, that experiment can be modified or deleted from the study.

- 1 Load the study to be edited into the Study Queue by right-clicking the location in the tray display.
- 2 Select **Edit Study** from the options displayed after right-clicking on the location in the tray display.
- 3 Double-click the experiment node to be edited in the Study Queue.
- 4 Click **Save** after changing the parameters.
- 5 To delete a node, drag the node to the trash can icon.
- 6 Select the location of the sample from the tray display, and click **Submit**.

- 7 Click **Cancel** to return the interface to Review mode.

Extending an existing study

After a study has been created, it is possible to add experiments to the study. Study status (pending, active, completed) does not affect adding experiments to the study. More than one instrument can be used if the VnmrJ study does not need to be collected on the same instrument that the study will continue on. After a Study is loaded and ready for edit, the workflow is identical to the creation of a new Study.

- 1 Load the study to be edited into the Study Queue using one of the following methods:
 - a Right-click the location in the tray display and select **Edit Study**.
 - b Recall a data set from the study of interest using **File> Open>** (select the file or FID) and select **Edit Study**.
 - c Recall a previous Automation file using the Tray Archives tool (**Automation > Tray Archives**) and select the appropriate location in the tray display, and select **Edit Study**.
- 2 Add experiments to the queue from the Experiment Selector.
- 3 Select the location of the sample in the tray display and click **Submit**.
- 4 Click **Cancel** to return the interface to Review mode.

Deleting a sample from the queue

To delete a sample from the Study Queue:

- 1 Right-click on the location in the tray display and select **Delete Study**.

Operations Without a Robot Sample Changer

This section describes using the Study Queue without a robot sample changer installed.

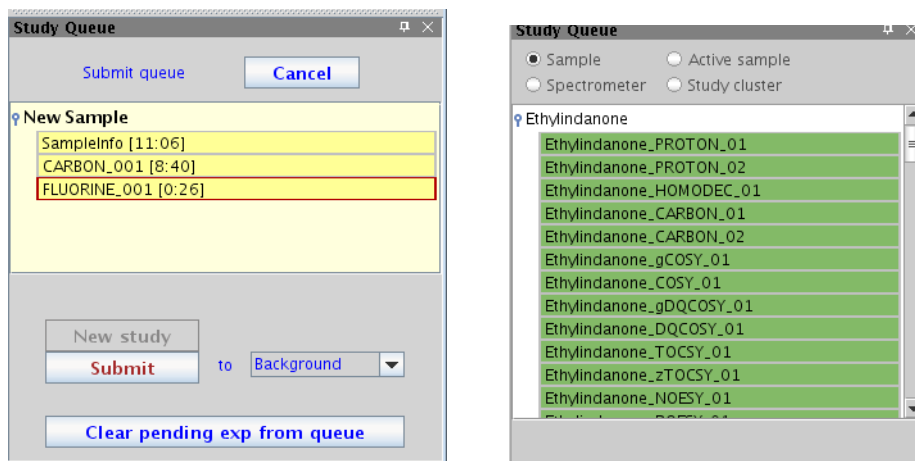


Figure 32 Study Queue—Submit mode (left) and Review mode (right)

Study Queue Controls-Review mode

When in **Review** mode the **Study Queue** window is used to display information about the current study.

The selections at the top of the panel determine what information is displayed:

- **Sample**—shows the study linked to the data in the current workspace.
 - If the **ScoutFids** check box is selected all prescan FIDs will be displayed in the Study Queue.
- **Spectrometer**—shows all studies in the current automation run.
 - Options - the Spectrometer View Preference popup will display to configure and update the Spectrometer display.
- **Active Sample**—shows the study that is currently running.
- **Study Cluster**—shows the study cluster, if defined.

The **New Study** button initializes a new study and moves the software to **Submit** mode. The **Continue Study** button modifies a completed study currently displayed in the Study Queue.

Study Queue Controls-Submit mode

In Submit mode, the **Study Queue** window is used to build a study and submit it to acquisition.

Table 10 Submit mode table

Button	Description
Cancel	Abandons any changes made to the current study and returns the software to Review mode.
New Study	Creates a new study.
Submit	Submits the current study to acquisition. The associated pull-down menu determines how the acquisition is to be performed.
Foreground exp	Submits the study to acquisition in the current workspace.
Background	Submits the study to a background copy of VnmrJ.
Clear Pending Exp from Queue	Deletes all pending experiments from the current Study Queue.

Using the Study Queue-full automation

To collect a series of spectra on a sample using the Study Queue:

- 1 Place the sample in a spinner turbine and adjust its position using the sample depth gauge. Insert the sample/spinner into the magnet.
- 2 Click **New Study** in the Study Queue vertical panel. This initializes the Study and moves the software into "submit mode."
- 3 Click on the buttons in the Experiment Selector to add the desired experiments to the Study Queue. The order of the experiments in the Study Queue can be changed using a click-and-drag action. Dragging an experiment node to the trash can deletes it from the queue.
- 4 Experimental parameters for an individual experiment can be customized by double-clicking on the experiment node in the study queue. (This is dependent upon the rights and privileges permitted for each user by the System Administrator.) The parameter set is loaded into the current workspace where any desired changes can be

made using the horizontal panels under the "Acquisition" tab. Once the desired changes are complete, click **Save**.

- 5 Fill in the information associated with the sample, such as sample name, solvent, email address, comments about the sample, etc. The sample name parameter is typically required as part of the automatic data saving routine, and the solvent information is used to set the spectral window.
- 6 Select the check boxes to control automatic printing, locking, shimming, and tuning, as appropriate.
- 7 Click **Submit**.
- 8 The software will submit the Study Queue to acquisition and return to Review mode.

Creating a Study Queue from data acquired manually

A common workflow in the research laboratory is to collect multiple proton spectra on a sample as the user optimizes conditions, such as shimming or solvent suppression, for that sample. Once the experimental conditions are deemed correct, a series of spectra can be acquired. The Study Queue supports this common workflow.

- 1 Acquire a PROTON spectrum (any experiment could be used but PROTON is most common) in foreground using manual acquisition. See *Chapter 1, Spectroscopy User Guide*.
- 2 Use the **File> Autosave** function to save the FID using the data save template established in User Preferences (**Edit > User Preferences**). This action creates the directory structure required by the Study Queue.
- 3 In the **Study Queue** window, select **View> Sample** to refresh the Study Queue display.
- 4 Click **Continue Study** to open the Study and move the software into "submit mode."
- 5 Click on the buttons in the Experiment Selector to add the desired experiments to the Study Queue. The experiments are created based on the optimizations done in the PROTON experiment, retaining optimized parameters such as sweep width, solvent suppression, and so on.
- 6 Once the Study has been built and customized as desired, click **Submit**.

Editing experiments in an active study

Until acquisition has begun on a queued experiment, that experiment can be modified or deleted from the active study.

- 1 Load the active study into the **Study Queue** window by choosing **View> Sample**.
- 2 Click **Edit Study**.
- 3 Double - click the experiment node to be edited, or simply drag the node to the trash can to delete it.
- 4 After changing the parameters as desired, click **Save**.
- 5 Click **Submit**.

Extending a completed study

Once a Study has been created, it is possible to add more experiments to that Study whether the study is active or completed. The Study does not have to have been collected on any particular instrument. Studies can be continued at any time on any spectrometer under VnmrJ. The workflow is identical to the creation of a new study once the study to be edited is loaded.

- 1 Load the study to be edited into the **Study Queue** window:
 - By choosing **View> Sample** in the **Study Queue** window when the current workspace holds a data set from the study of interest.
 - By recalling a data set from the study of interest using **File> Open** and then selecting **Tools> Study Queue Actions> Refresh Study Queue**.
- 2 Once the desired Study has been loaded into the Study Queue, click **Continue Study**.
- 3 Experiments can now be added to the queue from the Experiment Selector, Experiment Selector Tree, or Experiment menu, and customized as described above.
- 4 Click **Submit**.

Data Acquisition Using a Spreadsheet

Sample entry and acquisition can be controlled by using the `csv2cpQ` macro to import acquisition parameters and variables from a "comma separated values," or csv file. A csv file can be created by most common text editing or spreadsheet applications.

The macro requires at least one argument, this being the path name to the csv file (both relative and absolute path names are supported). The macro also supports up to 2 keyword arguments with valid keywords being `auto` (submit to an automation run - this is the default value), `acq` (submit directly to acquisition), and `enter` (submit to an autofile); and `print` or `noprint` to control the printing of the submission information. For example:

```
csv2cpQ('/home/vnmr1/my_spreadsheet')
csv2cpQ('my_spreadsheet', 'acq', 'noprint')
```

The first line of the csv file is treated as a header and contains the keywords for all variables or parameters to be imported from the spreadsheet. The order of the entries in the header is arbitrary, and any element in the header that is not a VnmrJ parameter or variable is ignored. Each entry is enclosed in quotation marks and must be separated by a comma.

To allow some flexibility in the creation of the `csv2cpQ` header, a file named `csv2cpQ_synonym` is located in the `/vnmr/adm/walkupadm` directory. This file contains a list of terms that can be used as synonyms for a parameter or keyword in the csv file header. For instance, this file aliases both "1H" and "H1" to mean "PROTON" when used to enter an experiment protocol in the csv. The `csv2cpQ_synonym` file can be modified by the system administrator to accommodate site-specific needs.

Once the header has been constructed as desired, each subsequent line is used to fill in a value for each of the entries in the header. Any value that is left blank (i.e., two commas without a white space between them) is ignored.

As an example, a file containing the following entries would submit three sample for analysis.

```
"SAMPLE", "DAY", "NIGHT", "operator", "samplename", "solvent", "c  
omments"  
1, "PROTON", "CARBON", "chemist_1", "ibuprofen", "DMSO", "refere  
nce #32"  
2, "PROTON", , "chemist_2", "aspirin", "methanol-d4", "Commercia  
l sample"  
3, , "PROTON  
gHSQCAD", "chemist_3", "acetaminophen", "meod", "synthetic"
```

The first sample is located in tray location 1, and this line submits a PROTON experiment to the day queue and a CARBON experiment to the night queue for that location. For sample #2, a PROTON experiment would be submitted to the day queue from location 2, while PROTON and gHSQCAD would be submitted to the night queue for sample #3.

Study Mimic

Study Mimic simulates submission of experiments to acquisition using the Study Queue by inserting saved data and parameters instead of newly acquired data. These sample runs can be used for testing new experiments, checking parameters, or training purposes. Study Mimic can be set up from a console or workstation computer.

Using Study Mimic

- 1 Launch Study Mimic from **Tools > Study Queue > Actions > Mimic Study Acquisition**. The Study Queue–Submit button is replaced by the Mimic Study Acquisition button.

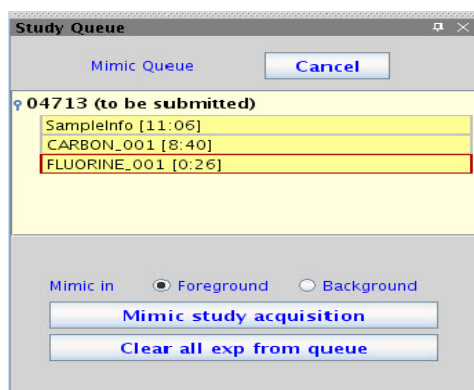


Figure 33 Mimic Queue

- 2 Add sample information on the **Start tab > Sample Info** Parameter panel.
- 3 Add experiments to the Study Queue from the Experiment Selector vertical panel.
- 4 Modify parameters for each experiment in the Acquire–Parameter panel.
- 5 After the queue is built, select whether to run the simulations in **Foreground** or **Background** acquisition in the Mimic Queue.
- 6 Select whether to use data from an archived study or whether to save parameters only and click **OK**.

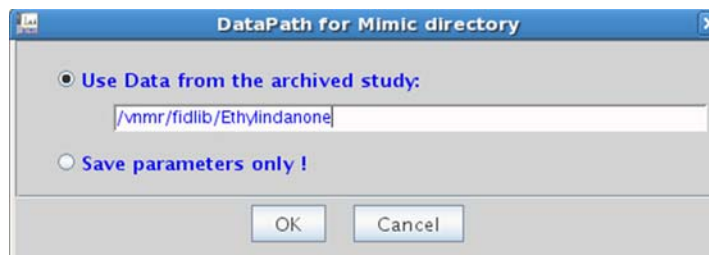


Figure 34 DataPath for Mimic directory

The Study Queue will sequentially run all appropriate experimental macros, load parameters, calculate pulses, and report any errors in the command line.

- 7 Upon completion, a **DONE** message will appear in the Graphics Canvas.

If the user has selected to save data, the chosen FIDs will be available in the Study Queue.

Study Mimic-multiple submissions

- 1 To configure a system to run Study Mimic by default, go to **Edit > Preferences > UserPrefs**.

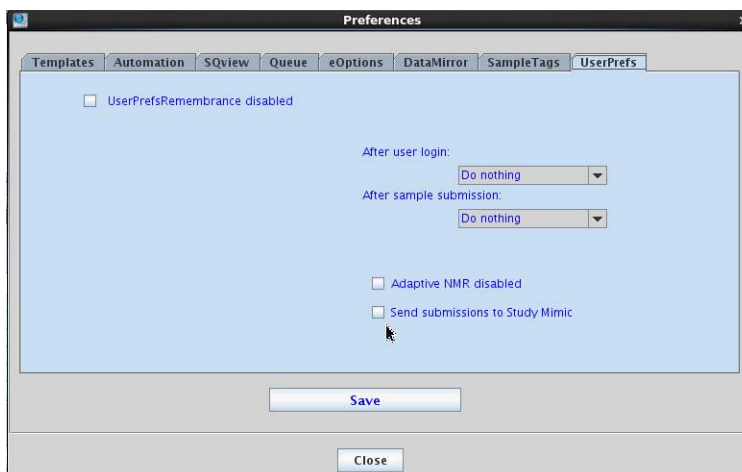
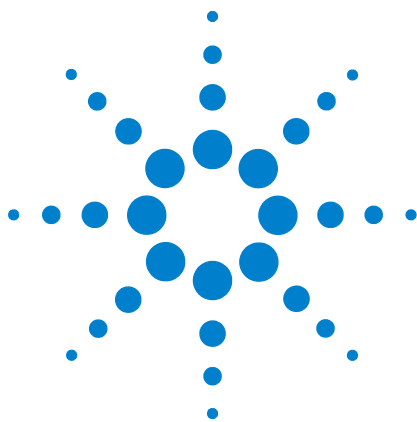


Figure 35 Multiple submissions for Study Mimic

- 2 Select the **Send Submission to Study Mimic** check box.
- 3 Click **Save**.
- 4 Click **Close**.

The Study Mimic mode will now be the default mode.

5 Study Queue and Studies



6 Data Acquisition

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This chapter describes how to select the experiment, set the parameters, and acquire data. Two aspects to selecting parameters are the frequency-related aspect—setting the position and size of the spectral window, and the pulse-sequence-related aspect—power levels and pulse widths.



Acquiring a Spectrum

VnmrJ reads the probe file and sets up the experiment according to the type of probe in use.

Start an acquisition from the **Acquisition** menu, or use the pages under the **Acquire** tab.

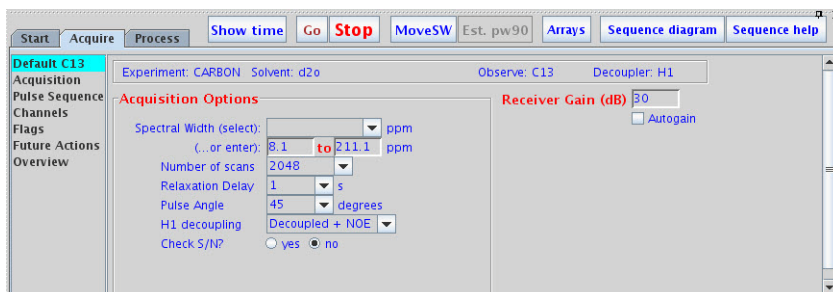


Figure 36 Acquire tab for Carbon experiment

- 1 Accept the default settings or set acquisition parameters (by clicking on the different pages in the **Acquire** tab).
- 2 Click the **Go** button to start the acquisition.
- 3 Click the **Stop** button to stop the acquisition.

Acquisition Settings

Acquisition and post acquisition actions

Use the **Flags** page of the **Acquire** tab to determine the data acquisition precision (32-bit double precision is normal), what actions to take during acquisition if the spinner or VT fails to remain in regulation, how often to save the FID during the acquisition (block size), and other acquisition-related actions.

Preacquisition delay

Usually set to 0.5 (seconds) to allow the hardware to set up at the beginning of the experiment. This parameter can also be varied for kinetics experiments.

Delays $rof1$, $rof2$ and α

$rof1$ is normally fixed as 10 μ s. After the final pulse in each pulse sequence, the receiver is gated off for $rof2$ μ s before the acquisition begins. α and $rof2$ are important where the flatness of the baseline is of concern.

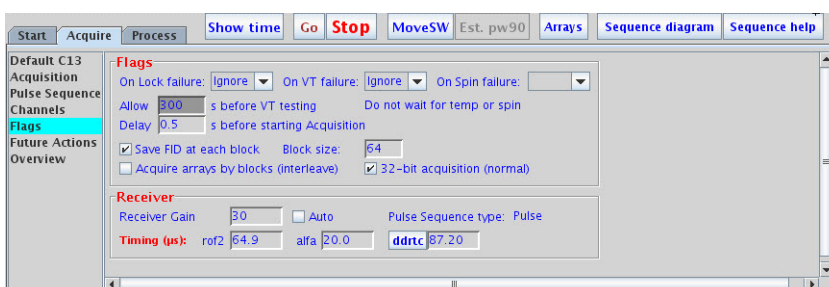


Figure 37 Flags page in the Acquire tab

Use the **Future Actions** page to specify actions that are to occur automatically after acquisition finishes. For example, Save FIDs or set automatic FID saving.

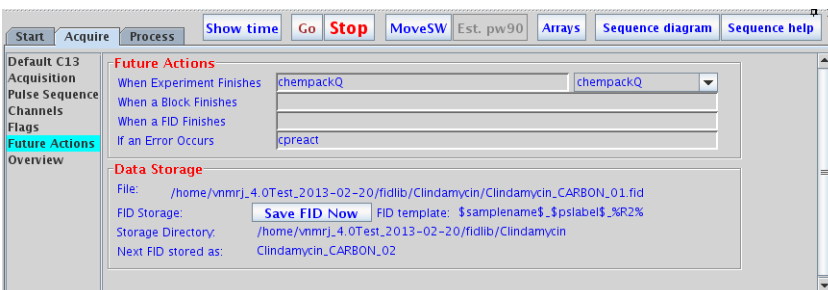


Figure 38 Future Actions page in the Acquire tab

Nucleus-specific frequency settings

Use the **Acquire/Defaults** page to adjust nucleus-specific parameters.

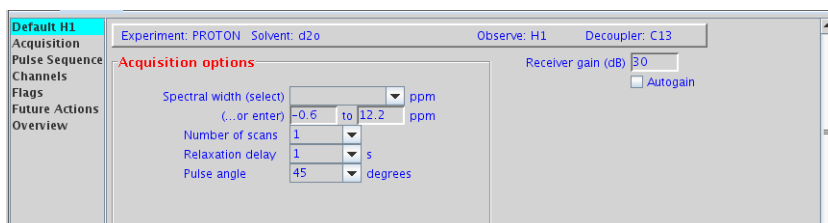


Figure 39 Default page for Proton experiment

Transmitter and decoupler positioning

Use the **Channels** page to set transmitter and decoupler values.

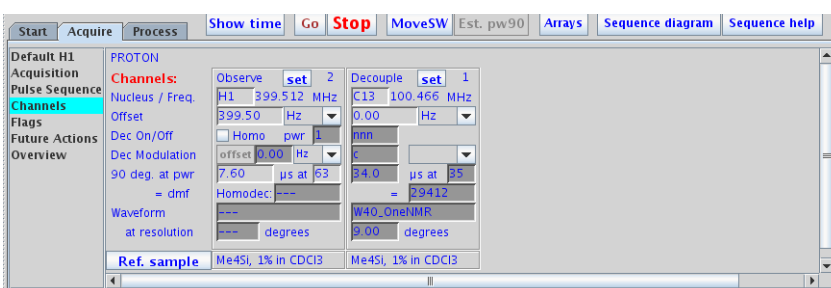


Figure 40 Channels page for Proton experiment

The **Move transmitter** button in the **Process / Cursors** page allows you to move the observe transmitter offset so that the current cursor position becomes the center of the spectrum. This method maintains the current referencing. If you wish to specify the transmitter frequency directly, rather than using the cursor position, you can enter a value in the **Offset** field in the **Acquire / Channels** page.

Spectral window

Set the **Spectral** window size in the Spectral width field in the **Acquire/Acquisition** page.

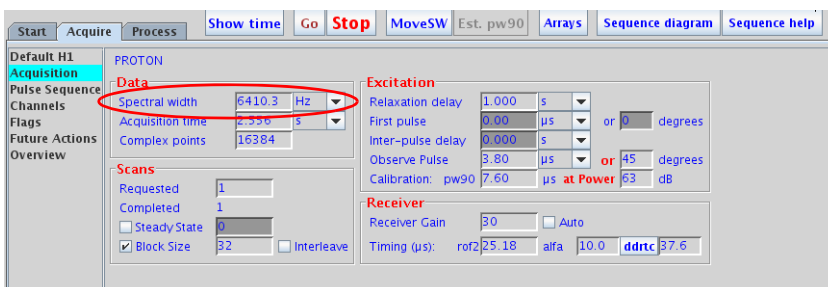


Figure 41 Acquisition page showing spectral width setting

Pulse sequence settings (standard two-pulse)



Figure 42 Setting pulse sequence values

Use the **Acquisition** and **Pulse Sequence** pages to set the values for the pulse sequence.

Complex points

Generally calculated automatically when the spectral width or acquisition time is changed. VnmrJ calculates a new acquisition time spectral width value if a value is entered in the Complex points field.

Acquisition time

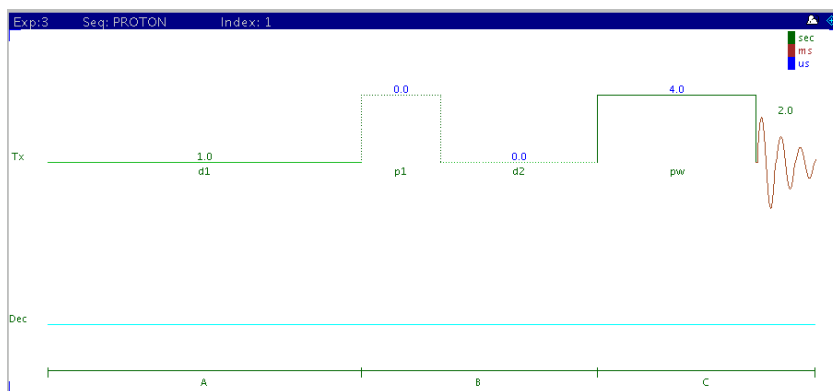
The length of time during which each FID is acquired. Acquisition time values that would use an "unallowed" number of data points are readjusted automatically.

Steady-State	The number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data. In a multi-FID experiment, if Steady-State is a positive value, the steady-state pulses are applied at the start of the first FID only. Whereas, if Steady-State is a negative value, the steady-state pulses are applied at the start of every FID.
Scans requested	The number of repetitions or scans performed to make up the experiment—the number of transients acquired. Set Scans Requested to a very large number, (for example, 1e9) to set up an indefinite acquisition. The Scans Completed field changes during the course of an experiment to reflect the number of completed transients.
Relaxation delay, First pulse, Inter-pulse delay	First pulse ($p1$) and Inter-pulse delay ($d2$) are zero for “normal” 1D NMR. The Relaxation delay ($d1$, used to allow recovery of magnetization back to equilibrium) may be zero as well, reducing the total pulse sequence to a pulse of the time entered in the Observe Pulse field (pw), followed by the Acquisition time (at).
Homospoil	Homospoil is a process by which the homogeneity is temporarily made very bad (“spoiled”) to cause any transverse magnetizations present at that time to decay rapidly to zero.
Receiver Gain	Low gain in multiline, high-dynamic range samples can cause a number of problems, including intermodulation distortions, lower sensitivity, and extra lines in the spectrum. Too high a gain, on the other hand, can cause ADC overload and consequent baseline distortion. Autogain sets the gain optimally for a wide variety of samples. $gain=60$ represents the highest possible actual receiver gain and $gain=0$ the lowest. The functional step size is 2. $gain='n'$ activates Autogain, in which the gain is automatically adjusted at the start of acquisition. Setting $gain='y'$ turns this off.
Calibration: pw90	This field displays the length of the 90° pulse, in μs . This value is determined when the probe is installed, calibrated and tested as described in the probe installation manual and will usually need to be changed for a new sample.

Pulse Sequences

Display the pulse sequence

- 1 Click the **Acquire** tab.
- 2 In the Action Bar, click the **Sequence Diagram** button to display the pulse sequence in the graphics canvas.



Standard two-pulse parameters

Most experiments will be acquired using a pulse sequence known as the standard two-pulse, or S2PUL. Figure 43 shows a two-pulse sequence and the associated labels from the **Acquire** pages.

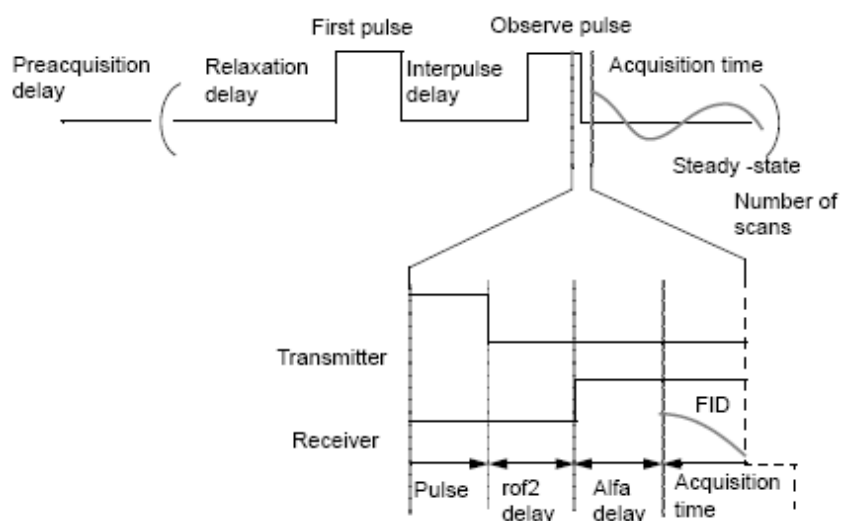


Figure 43 Acquisition parameters for standard two-pulse sequence

A preacquisition delay (pad) is usually set to 0.5 seconds at the beginning of the experiment.

Following the preacquisition delay are these:

- 1 Relaxation delay (d1)
- 2 First pulse (p1)
- 3 Inter-pulse delay (d2)
- 4 Observe pulse (pw)

Dead times `rof2` (with receiver off) and `alfa` (with receiver on) are associated with the observe pulse. The complex data points are acquired during the acquisition time.

This process is repeated `Steady-State` number of times plus the `Requested Scans` field. Data is actually acquired only during the `Requested Scans` scans and not during the initial `Steady-State` scans.

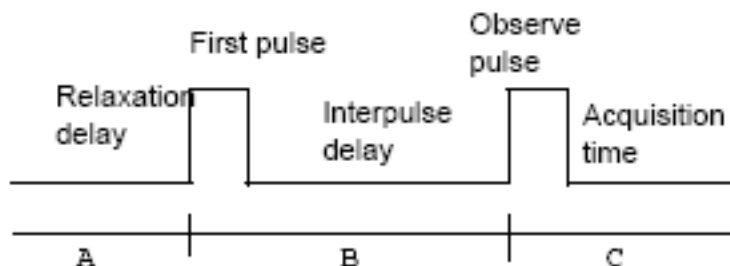
The receiver is off during the pulse sequence and on only during `Acquisition time`. The amplifier can be unblanked at any time, but no longer than 10 ms. Blanking and unblanking are implicitly done around pulses.

After the final pulse in each pulse sequence, the receiver is gated off for $\text{rof2 } \mu\text{s}$ before the receiver is turned on and is followed by the `alfa` delay ahead of the start of data acquisition. If “pulse breakthrough” effects are seen (spike in the beginning of the FID), increasing `rof2` can reduce or eliminate the problem.

The status concept

Every pulse sequence can be divided logically into “periods” of time. The standard two-pulse sequence, for example, can be divided as shown below. This sequence has three logical periods, referred to in the diagram as A, B, and C. These periods are used in controlling the decoupler “status” (as well as the “homospoil” status, discussed later in this chapter).

Logical Periods A, B, C in Standard Two-Pulse Sequence



The decoupler fields in the **Channels** page use the following letters:

- N no, or an off status
- Y yes, or an on status

For example, to have the decoupler be ON during period A, ON during period B, and OFF during period C, describe the desired decoupler status as `yyn`. Setting the decoupler to `yyn` will produce a coupled spectrum with NOE (in the heteronuclear case). Setting it instead to `nny` would give us an experiment with the decoupler only ON during period C, the acquisition time, which (in the heteronuclear case) would be a decoupled spectrum without NOE.

Observe transmitter and decoupling settings

Transmitter power levels are set through attenuators, which are, in turn, controlled through fields in the **Channels** page.

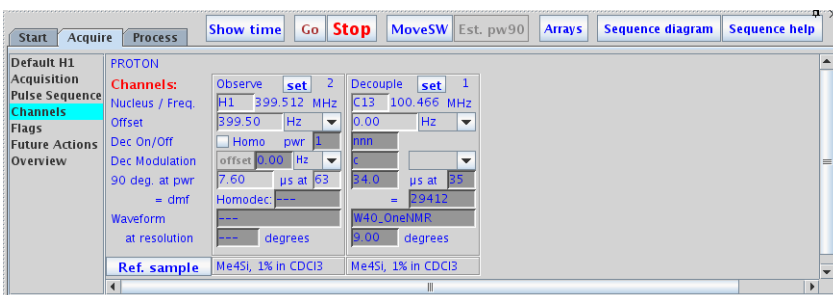


Figure 44 Channels page

The observe transmitter power, which is under computer control, is set in the Observe power field.

The Decoupler ON/OFF field determines the decoupler output:

- **y, ynn, yyn**, etc., turns the first decoupler ON.
- **n** or **nnn** turns the first decoupler OFF.

The transmitter fine power level is controlled by the t_{pwr} (or t_{pwr}) parameter (if present). The attenuation is linear and spans 60 dB. If no fine attenuator is present, the value offsets the coarse power, simulating fine power.

The Homo selection box sets the homonuclear decoupling control for the observe channel. Selecting Homo specifies that the receiver is gated, which is done by controlling the observe L.O. (local oscillator) line. The first RF, amplifier, and preamplifier are gated only if decoupling is on. If the decoupling is off, no gating of these signals takes place. When Homo is selected, Modulation is typically set to **c** for continuous wave (CW) modulation.

WARNING

Decoupler power greater than 2 watts in a switchable probe will damage the probe. Always carefully calibrate high-power decoupling to avoid exceeding 2 watts of power. The maximum value for $dpwr$ on many systems has been set to 49, corresponding to approximately 2 watts of power. Before using $dpwr=49$ for continuous decoupling, ensure safe operation by measuring the output power. This safety maximum may be adjusted in the config program.

The decoupler power is set in the Decouple Pwr field. This field accepts values from --16 (lowest power) to 63 (highest power). However, the output power should be measured to make sure that no more than 2 watts is applied to switchable probes. This safety maximum, which limits the value that can be entered, can be adjusted in the System Settings and System Configuration windows. The decoupler power for the second, third, and fourth decoupler channels, respectively, also have safety maximums.

Decoupler modes

Several other efficient decoupling schemes are available from the **Modulation** pull-down menu, including GARP, MLEV-16, and XY32 decoupling. See the description of `dmm` in the *Command and Parameter Reference* for other modulation modes available.

Although modulation normally has just a single “state” in the standard two-pulse sequence, multiple states are possible. For example, 'ccw' gives single-frequency decoupling during the first part of the pulse sequence and WALTZ-16 decoupling during acquisition.

Set `dmm` to 'p' to select programmable decoupling. To specify the decoupling sequence during any period of waveform generator programmable decoupling, use the `dseq` parameter for the first decoupler, `dseq2` for the second decoupler, and `dseq3` for the third decoupler. The parameters `dres`, `dres2`, `dres3`, and `dres4` control the tip-angle resolution used within a programmable decoupling sequence on the first, second, third, and fourth decouplers, respectively. See the manual *User Programming* for further information on pulse control of programmable decoupling (that is, waveform generators).

The following values are typical for decoupling:

- Homonuclear decoupling:

<code>homo='y'</code>	Homonuclear decoupling on
<code>pwr=5-15</code>	Decoupler power level range

- Heteronuclear decoupling:

<code>dm='y'</code>	Decoupler mode on
<code>dmm='w'</code>	WALTZ-16 decoupling
<code>dpwr=40</code>	Decoupler power level
<code>dmf=10000</code>	Decoupler modulation frequency

Parameter Arrays

- 1 Click **Acquisition** on the main menu.
- 2 Select **Parameter Arrays** to open the **Array Parameter** window and set arrayed parameters. Parameter arrays are explained in detail in Multi-FID (Arrayed) Spectra.

This window can be used both with in or outside of a study.

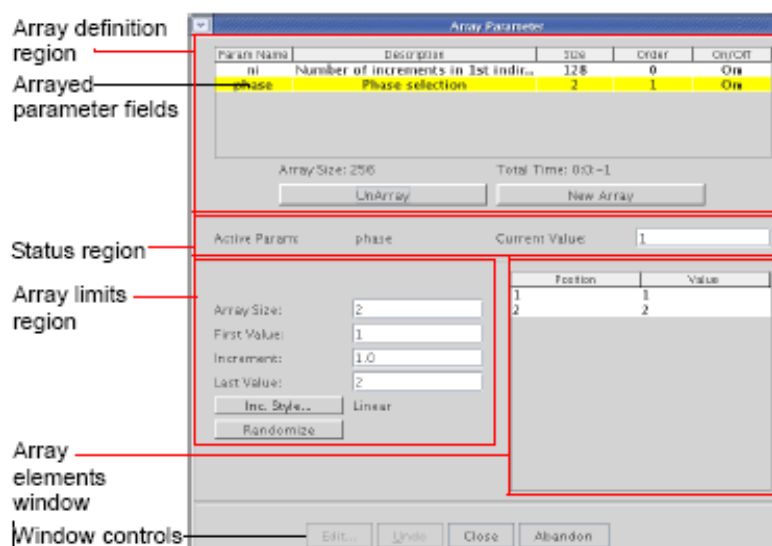


Figure 45 Window regions

Array definition

Table 11 Arrayed parameter field columns

Param name	Defines name of arrayed parameter
Description	Displays the text description of the array
Size	Displays the number of steps or increments in the array
Order	Displays the precedence for running the array – double-click in the field and enter the array order. Arrays with sequential numbers create a full matrix (array A x Array B) and each array can be a different size. Arrays with the same order number (and the same size) create a diagonal array.
On/Off	Uses array or does not use array on this parameter

Table 12 Fields and buttons

Array Size field	Shows size of selected array
Total Time field	Shows estimated time to complete the array
UnArray button	Removes selected parameter from the list of arrayed parameters
NewArray button	Adds new row to the list of arrayed parameters
(Status Region)	Shows active parameter during acquisition and current value
Array limits	<p>Array Size field – enter the size of the array and press Return</p> <p>First Value – enter the starting value of the array and press Return</p> <p>Increment – enter the array increment and press Return</p> <p>Last Value – enter the ending value of the array and press Return</p> <p>Inc. Style ... button – click and select linear or exponential</p> <p>Randomize button – click to create a random array</p>
Array elements	Change the value of any individual array element by double clicking on the value, entering a new value, and pressing ENTER .
Window buttons	<p>Edit—Not active</p> <p>Undo—Undoes the change; restores the change (if clicked again)</p> <p>Close—Closes the window (and saves changes)</p> <p>Abandon—Closes the window and makes no changes</p>


The top panel in the window is a table of currently arrayed parameters. The Parameter Name, Description, array Size, array Order and On/Off status are displayed. Add parameters with the **New Array** button and remove parameters with **Unarray**. Turn on or off an arrayed parameter by double-clicking on **On/Off**. Edit the array order (except for implicit arrays n_1 , n_2 , etc.) to enable nested or parallel (diagonal) arrays. Only parameters of the same array size can be parallel, that is, have the same array order.

Highlight the array parameter table one row at a time with single clicks. The values of the highlighted array parameter (Active Param) are displayed in a table in the middle panel, along with editable entries for Array Size, First Value, Increment, and Last Value. Edit the parameter values. There are also buttons for increment style (Inc. Style, which allow you to chose linear or exponential), and randomizing (Randomize) the order of array values. Specific values can also be entered manually for every element in the list of values. String variables can also be arrayed and modified.

The Current Value of the parameter is displayed above the array parameter values. Select this value from the arrayed values by double-clicking on the position number in the list of values or change it manually. When the parameter is unarrayed or turned off, it is set to the current value.

Abandon restores the original state of the window (the state it was in when it opened) and closes the window. **Close** keeps the changes and closes the window.

Stopping and Resuming Acquisition

Click either the **Stop** icon  or the **Stop** button in the Action Bar of the Acquire parameter panel to stop an acquisition experiment that has been submitted for acquisition.

If the experiment is waiting for execution, no data is saved when it is stopped. If the experiment is active, the data is retained when it is stopped.

An acquisition generally continues to completion unless stopped early. Several situations can stop the acquisition early. The system may detect an error, it may detect an overflow, or the operator may stop the system with an aa or an sa command.

Stop an acquisition (because sufficient signal-to-noise has been obtained or because the experiment has proved useless) by selecting **Acquisition > Abort Acquisition**. The acquisition is aborted immediately. All current FID data is discarded and the experiment is interpreted as an error. Any data collected from an earlier block size transfer or earlier FIDs are retained. All defined Future Actions processing occurs followed by any queued experiments.

Automatic Processing

Set up automatic processing on the **Future Actions** page.

Block-size allows users to examine data while an experiment is in progress. By setting the parameter `bs` to a non-zero value (for example, `bs=64`), the spectrometer is instructed to send the accumulated data to the host computer at the end of every 64 transients. The host computer stores the data in an appropriate disk file (overwriting earlier data). An updated version of the experiment in progress is available for viewing by the user. Weighting and transforming the data processes the current FID as of the last block size transients and displays the resulting spectrum on the screen.

Use the **Future Actions** page (the **When a Block Finishes** field) to make the process automatic. Enter `wft` in the **When a Block Finishes** field and click **Acquire**. Data is transferred to the host computer after each `bs` number of transients is completed, the macro `wft` is run, and the data is displayed in the active VnmrJ viewport. Any command or macro can be set to occur automatically using the **When a Block Finishes** field.

When a Block Finishes processing can be started after acquisition is started. Enter a `wbs` command, (for example, `wbs('wft')`) on the VnmrJ command line. The `wbs` processing may also be disabled by entering `wbs('stop')`. Setting `bs='n'` before starting the acquisition disables this block-size storage. For long experiments, it is not a good idea to set `bs='n'`. If `bs='n'`, data is stored on disk only at the end of the experiment, and if the experiment is aborted prior to termination, data will be lost.

There are other times when automatic processing is desirable:

- Automatically transform the FID upon completion of data acquisition.
Set the `wnt` (for “when number of transients”) parameter, (for example, `wnt='wft'`). This is automatically performed by the `ga` command.
- Automatically process data from an experiment that acquires data sets after all FIDs are collected. A 2D experiment is an example of such an experiment.
Set the `wexp` (for “when experiment”) parameter, (for example, `wexp='wft2da'`).

- Take corrective action in the event of an acquisition error. Set the `werr` (when error) parameter, (for example, `werr='react'`).

Acquisition Status Window

Click the **black triangle** next to the status display to open the **Acquisition status** window. The display contains fields for acquisition status information. Fields are displayed based upon the hardware configuration of the system or the parameters set on the system.

Table 13 Fields in the acquisition status window

Field	Description
Status	Present status of acquisition. The values displayed should be self-explanatory (for example, "Shimming") with two exceptions: "Active" means that the acquisition computer started but the console is not active yet, and "Inactive" means that <code>acqstat</code> cannot communicate with the acquisition computer or that the acquisition computer is not executing.
Queued	Number of experiments queued by multiple go commands
Exp	Number of the active experiment (for example, <code>exp1</code> , <code>exp2</code> , <code>exp3</code>)
Fid	Number of the FID being acquired if in an arrayed experiment
Ct	Number of completed transients
Sample	Sample number in magnet if in automation mode
Lock	Lock status: Off, Regulated, Not Regulated (NotReg)
Complete	Estimated time when experiment will complete
Vt	Variable temperature unit status: Off, Regulated, or Not Regulated (if VT is set as present and <code>vttype=2</code>)
Stored	Last time data was transferred to disk



7 Processing Data

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Introduction to Data Processing

To turn data into a spectrum after acquisition, data must be Fourier Transformed. Use the **Process** tab to access other data-processing options: adjusting the weighting function, zero filling, linear prediction, phasing, and referencing.

Processing and plotting options can be accessed on the **Process/Basic** page.

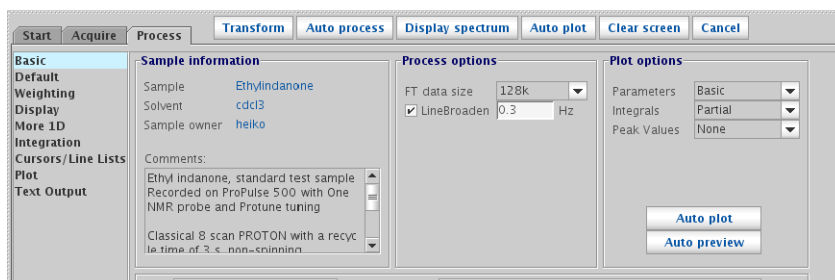


Figure 46 Process/Basic page

Other data processing options can be accessed on the **Process/Default** page.

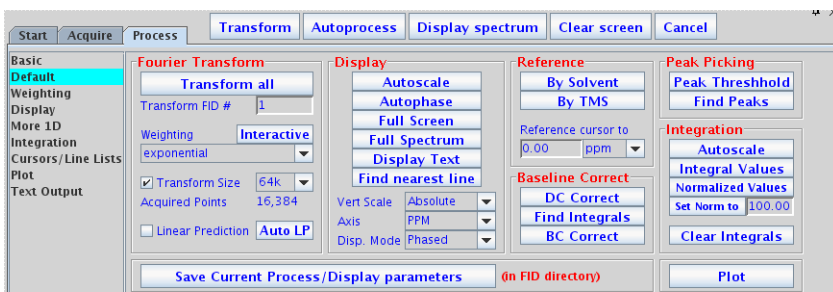


Figure 47 Process/Default page

Weighting Function

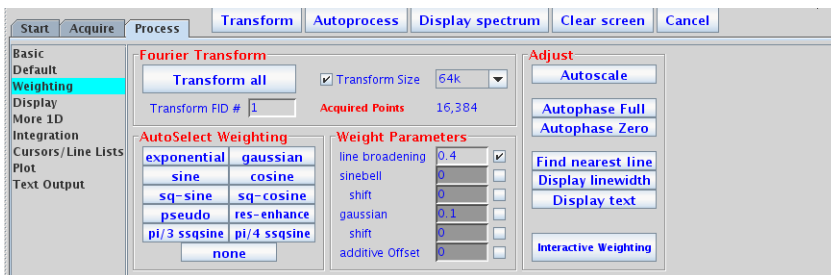


Figure 48 Process/Weighting page

The **Process/Weighting** page contains a full set of weighting-function action buttons. These automatically set the Weight parameters in the following way:

- **exponential** – A positive value gives the desired line broadening in Hz. This is also called a decaying exponential function. A negative value gives a resolution enhancement function. The parameter name is lb.
- **gaussian** – Time constant, in seconds, that defines a Gaussian function of the form $\exp(-(t/gf)^2)$.
- **shift** – shifts the center of the Gaussian function $\exp(-((t-gfs)/gf)^2)$.
- **sine** – A positive value, in seconds, applies a sinebell of the form $\sin(t*p/(2*sb))$. A negative value applies a squared sinebell function of the form $\sin^2(t*p/(2*sb))$.
- **shift** – a sinebell shift constant, in seconds. It allows shifting the origin of the sinebell function according to the formula $\sin((t-sbs)*p/(2*sb))$. Again, the square of this function is applied if sb is negative.
- **additive Offset** – An additive weighting constant that adds the constant awc to each value of the weighting function. It is applied after the sinebell and exponential function but before the Gaussian function.

As part of the Transform process, all weighting functions are set and applied simultaneously. Deselect the associated check box to remove a particular weighting function from use.

The effects of combining sinebell, exponential, and Gaussian weighting can be complicated and should only be used after experimenting with the individual parameters. The use of either Gaussian apodization (which leads to Gaussian line shapes) or line broadening (values greater than 0 lead to Lorentzian lineshapes) is especially critical for deconvolution.

Other line shapes cannot be handled by the deconvolution program, but may be appropriate for 1D resolution enhancement or for absolute-value 2D experiments. Weighting functions (other than exponential) can alter the relative areas of the resonances within a spectrum, and so they should be used with great care if quantitative results are required.

The **res-enhance** button sets defaults of a equal to 0.1 and b equal to 0.3 into the formulas $lb = -0.318 / (a * sw)$, and $gf = b * sw$, thereby calculating “reasonable” values for the resolution enhancement parameters lb and gf . The arguments a and b can also be selected by the user.

Several macros exist that set weighting parameters to give certain window functions. These include `gaussian`, `pi3ssbsq`, `pi4ssbsq`, `sqcosin`, and `sq sinebell`.

The parameter `wfile` is available for handling user-written weighting functions; see the manual *VnmrJ User Programming* for details.

Interactive Weighting

Click the **Interactive Weighting** button on the Process panel to start interactive weighting.

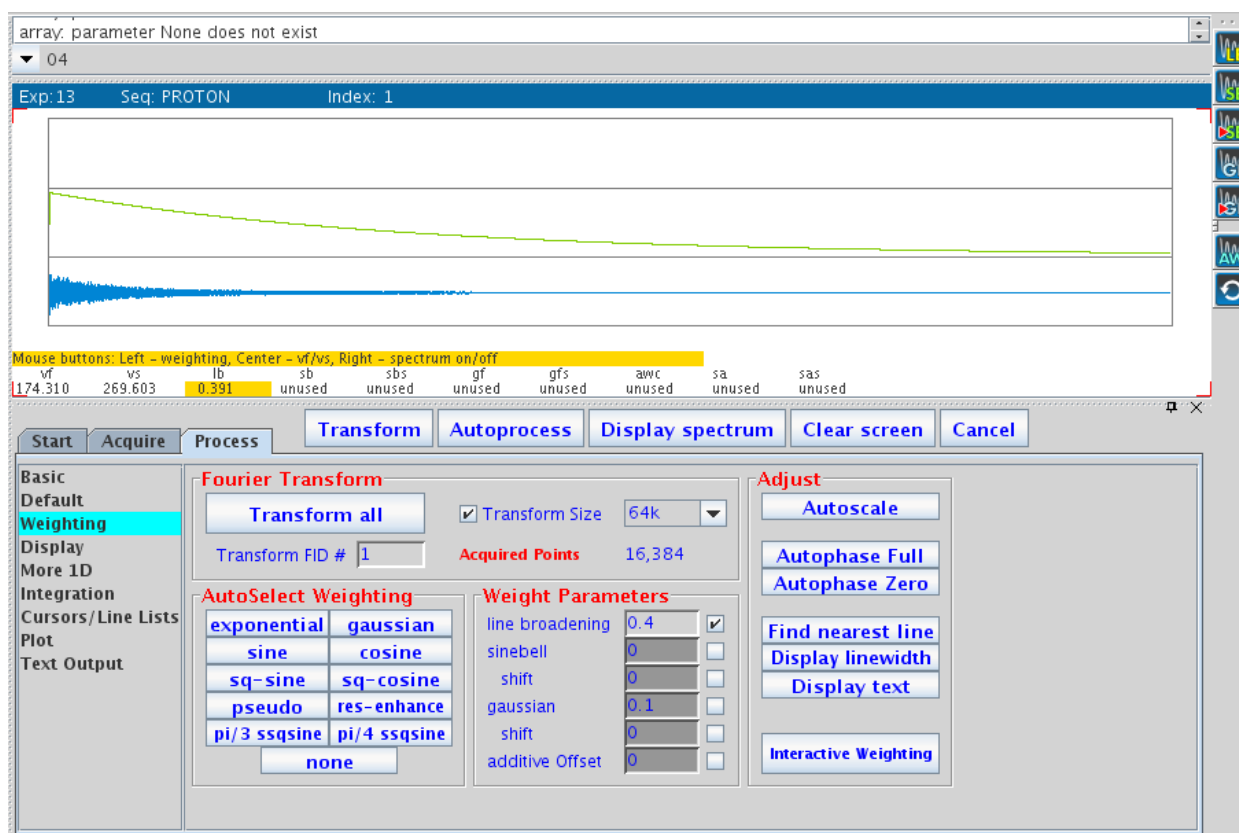









Figure 49 The graphics display for interactive weighting

Buttons next to the Interactive Weighting display provide access to the following functions:

Check box	Icon	Function
Line broadening		Selects line broadening or exponential weighting. A negative value gives resolution enhancement.
Sinebell		Selects the sinebell constant. A negative value gives squared sinebell. Change sign by clicking outside the box at the left.
Shifted Sinebell		Selects the sinebell shift constant (if sinebell is active).
Gaussian		Selects the Gaussian time constant.
Shifted Gaussian		Selects the Gaussian shift constant (if Gaussian is active).
Additive weighting		Selects the additive weighting constant.
Return		Returns to the previous menu.

Currently active weighting parameters can be changed by moving the cursor to the appropriate field in the weighting function box and pressing the left mouse button. New values for weighting parameters can also be typed in. Note that all other parameters, unless set to “not used”, are also used to calculate the weighting function.

Use the center mouse button within the FID box to adjust FID intensity (parameter v_f). Use the center mouse button within the spectrum box to adjust spectrum intensity (v_s). Use the right mouse button to turn the display of the transformed spectrum off and on. (It will be off by default whenever $f_n > 25000$.)


Fourier Transformation

The **Transform all** button will Fourier transform one or more FIDs. It uses the Transform Size parameters, and weighting functions are simultaneously applied whenever any weighting options are selected. Other options listed on the **More 1D** page are also simultaneously applied (such as **Linear Prediction**, **Solvent Subtraction**, **FID Shift**, and **FID phase rotation**). The data processing options **Baseline Correct** and **Drift Correct** are only applied as separate discrete actions that are used after the Fourier Transform.

The Transform Size field is the number of points to be Fourier transformed (f_n), and the number must be a power of two; typical numbers are 16384, 32768, or 65536 (listed as 16K, 32K, and 64K, where K is a multiplier of 1024). The most common entry for Transform Size is Default. This value specifies that however many data points (n_p) were acquired, the first power of two greater than or equal to n_p will be used as f_n . If f_n is greater than n_p , or if f_n is 'n' and n_p is not a power of two, the remaining points in the transform are filled in with values of zero (*zero-filling*). (If n_p is <10% bigger than f_n , the software will round down instead of up.) Thus, there is no explicit zero-filling command. This process is an implicit one governed by the relationship between f_n and n_p . The number of complex data points is $f_n/2$.

Phasing

Phasing spectra may be considered part of either data processing or data display. Performing a complex Fourier transformation produces two sets of data, referred to as the *cosine* and *sine* transforms, or the *real* and *imaginary* data sets, respectively. The absorption spectrum (peaks “in-phase”) and the dispersion spectrum (peaks “out-of-phase”) generally do not coincide with either the real or the imaginary channels, but must instead be produced from a linear combination of the two spectra.

Phasing can be adjusted using **Phase button**  for interactive phasing or using the **Autophase functions** on the **Process/Default** page.

Phase parameters

The process of phasing a spectrum requires the determination of an angle θ that can be used to “mix” these two data sets to produce one data set, according to the formula:

$$\text{Absorption spectrum} = \text{real} * \cos\theta + \text{imaginary} * \sin\theta \quad [\text{Eq. 1}]$$

The process is complicated by the fact that the phase angle θ is a function of frequency:

$$\theta = r_p + (w-w_0) / sw * l_p \quad [\text{Eq. 2}]$$

where l_p (left or first-order phase) and r_p (right or zero-order phase) are constants that must be determined.

The following is clear about the terms in Equation 2:

- r_p is *frequency independent*. Changes in r_p affect all peaks in the spectrum equally.
- l_p is *frequency dependent*. Changes in l_p affect peaks with a differing amount as a function of frequency.

There are several ways in which l_p and r_p can be adjusted:

- Like any parameter, they can be recalled with a particular parameter set. They can also be entered directly (for example, $l_p=150$).

- Fully automatic phasing is also provided with the `aph` and `aph0` commands. The `aph` command optimizes both the frequency-dependent (`lp`) and the frequency-independent (`rp`) parameters, and is independent of the starting point. The `aph0` command adjusts only `rp`. The `aphx` macro optimizes parameters and arguments for the `aph` command. `aphx` first performs an `aph` then calculates a theoretical value for `lp`. If `lp` set by the `aph` is different from the calculated value by 10 percent, the calculated value is used and an `aph0` is performed.

The command `phase` (`phase_change`) changes the phase of all peaks in the spectrum by adding `phase_change` to the current value of `rp`, and can remove any excess in `rp` more than 360°.

Autophase algorithm

The automatic phasing algorithms `aph` and `aph0` have the following features:

- Weighting parameters do not affect the algorithms.
- Spectra with very low signal-to-noise can be phased.
- In vivo spectra can be phased, which is very difficult for most autophasing algorithms.
- Spectra with inverted lines can be phased. Such spectra include APT or DEPT data or selectively inverted lines obtained with shaped pulses. This type of phasing is difficult for traditional autophasing algorithms.

The autophasing algorithm uses many rules that are used in a manual phasing procedure. First, it finds the peak areas. Then, it estimates the correct phase for each peak. An initial guess of the first-order phasing parameter `lp` is made based upon the estimated phases of two “normal” peaks. The peaks are categorized into three classes: normal, inverted, and bad. The peaks in the normal and inverted group will be used to find the optimal values for the phasing parameters `lp` and `rp`. A final check is made to determine whether autophasing was successful or unsuccessful.

Algorithms are complicated but fairly “intelligent.” The key point of an algorithm is to use a set of fuzzy rules to estimate the correct phase for each peak. The use of these rules makes an algorithm less sensitive to the signal-to-noise ratio, the weighting parameters, and the baseline quality. Fuzzy logic also makes it possible to do the classifications on the peaks.

The command `aphb` autophases Bruker data. See the *Command and Parameter Reference* for more information about this command.

Spectrum display

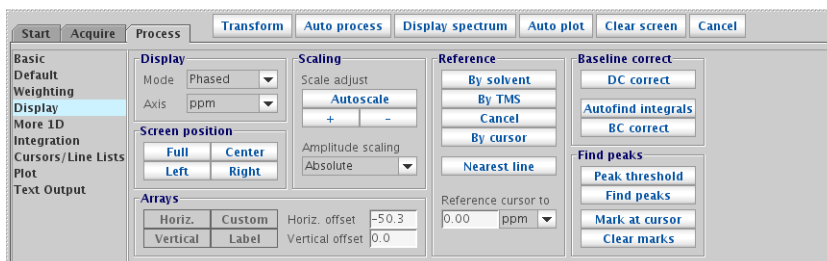


Figure 50 Process/Display page

The displayed spectrum is calculated in one of the following four *mutually exclusive* modes. The first three can be selected by menu selection in the **Process/Display** page.

- The *phase-sensitive mode* (**Phased**) is selected by the command `ph`. In this mode, the displayed spectrum is calculated using the phase parameters `lp` and `rp`. This is the most commonly used mode.
- The *absolute-value mode* (**Absvalue**) is selected by the command `av`. In this mode, the displayed spectrum is calculated according to the equation

$$\text{absorption spectrum}(\omega) = (\text{real}^2(\omega) + \text{imaginary}^2(\omega))^{1/2}$$

- The *power mode* (**Power**) is selected by the command `pwr`. In this mode, the displayed spectrum is the square of the displayed spectrum calculated in the absolute value mode.
- The *phase-angle mode* is selected by the command `pa`. In this mode, each point in the displayed spectrum is the arc-tangent of the phase angle of the real and imaginary point.

Once a spectrum is displayed using the interactive display command `ds`, the spectrum can be interactively phased by selecting the **Phase** button from the graphical tool bar. When the spectrum is in the Phase mode, the integral and cursors are not displayed.

Advanced Data Processing

This section covers the functions available on the **More 1D** page: advanced data processing, including linear prediction, FID shifting, FID phase rotation, and frequency shifting.

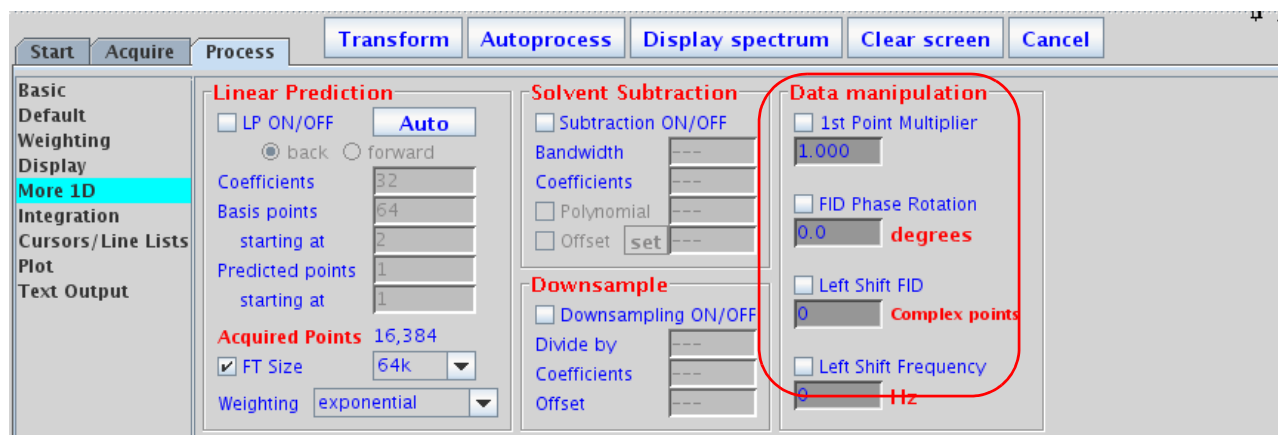


Figure 51 Data manipulation options in the More 1D page

FID manipulation

Check Box	Function
1st Pt Multiplier	Allows correction of the first point of the FID if it is distorted. See the <code>fpmult</code> parameter in the <i>VnmrJ Command and Parameter Reference</i> .
FID Phase Rotation	The parameter <code>phfid</code> is a zero-order FID phasing constant. If <code>phfid</code> is set to a value other than 'n', the FID is phase-rotated by <code>phfid</code> degrees before weighting or Fourier transformation is performed.
Left Shift FID	The parameter <code>lsfid</code> is a constant used in left-shifting the FID. If <code>lsfid</code> is set to a value other than 'n', the FID is left-shifted by <code>lsfid</code> complex points before weighting or Fourier transformation is performed. The value for <code>lsfid</code> must lie between 0 and $n_p/2$. The <code>tmove</code> macro provides a graphical method of setting the parameter <code>lsfid</code> —position the left cursor at the place that should be the start of the FID, then enter <code>tmove</code> to adjust the parameter <code>lsfid</code> .
Left Shift Frequency	Sets the frequency shift of spectral data, in Hz. See <code>lsfrq</code> in the <i>VnmrJ Command and Parameter Reference</i> . Sets a frequency shift of spectral data, in Hz, with a negative value resulting in peaks being shifted upfield (to the right) and a positive value in peaks being shifted downfield (to the left). <code>lsfrq</code> operates in the time domain on complex FID data, and so the desired value must be entered prior to Fourier transformation.

Data processing methods

All data processed in VnmrJ are processed by using the Fourier transform, but there are three variations, which are governed by the `proc` parameter:

- Two orthogonal (real and imaginary, or x and y) data points are sampled at the same time and form a single complex data point in the FID. Such data are processed using a normal complex Fourier transformation, using `proc='ft'`.
- Some spectrometers (Bruker Instruments) acquire pseudo-quadrature data by sampling two orthogonal data points sequentially, rather than simultaneously. Such data must be processed using a real Fourier transformation, with `proc='rft'`.
- For complex data only, it is possible to include “linear prediction,” as part of the data processing. `proc='lp'` is used to trigger this operation.

Linear prediction

Use the **Linear Prediction** group in the **More 1D** page to control linear prediction and to adjust its parameters.

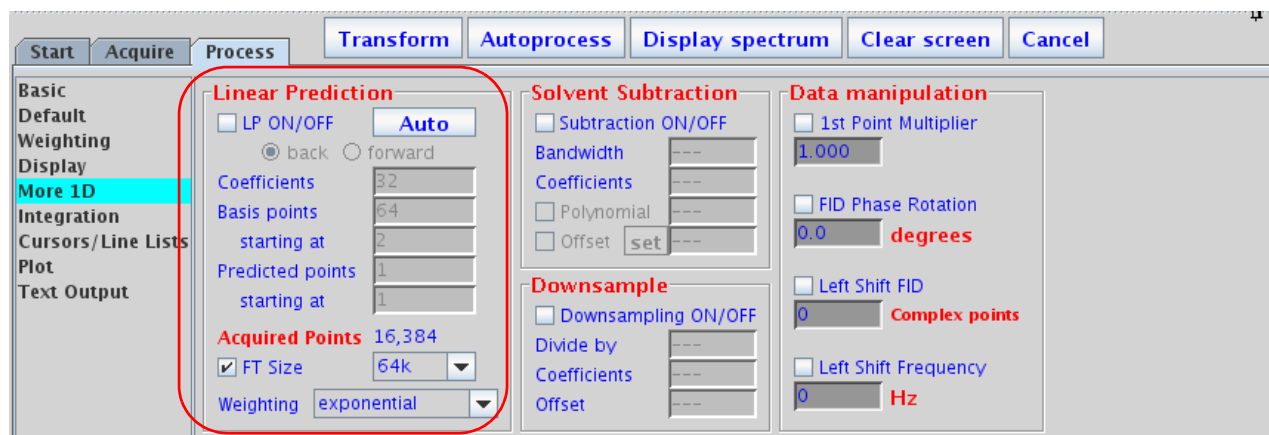


Figure 52 Linear prediction options in the More 1D page

Linear Prediction in VnmrJ

Linear prediction is incorporated into the Fourier transform routine, so that you do not normally see the “improved” FID, but merely the resulting spectrum (which results from Fourier transforming the linear predicted FID). This is

accomplished by selecting the **LP ON/OFF** check box in the Linear Prediction panel and clicking on the **Transform** button.

Enter `ft('nofft')` to suppress display of the linear-predicted spectrum and perform all the steps of the Fourier transform routine except the actual Fourier transformation. Real points of the FID are displayed by setting `lp=0 rp=0`, or display the imaginary points by setting `lp=0 rp=90`.

Linear prediction involves solving a series of equations for appropriate coefficients based on the actual FID. It involves quite a number of parameters and can be somewhat tricky to optimize (if not optimized properly, or if the data are not amenable, the analysis may simply fail, just like any least-squares fit process may fail to converge).

Linear prediction can be run in an iterative fashion by arranging the LP parameters—first extending backward, then forward, and backward again for more complex problems.

Why Use Linear Prediction?

Raw time-domain data acquired during a pulsed NMR experiment can have two flaws:

- Early points in the FID may be distorted due to a host of hardware characteristics, such as preamplifier saturation and probe ringing. Even on a perfect spectrometer, these distortions cannot always be avoided.
- The acquisition time of each FID may have been too short to allow for full decay of the signal, leading to distortion in the Fourier transformed spectrum.

Both types of distortions can be solved using *linear prediction*. This uses the “good” part of the FID to analyze for the frequencies that are present in the signal, and then uses that information to extend the FID either in a reverse direction (to “fix” the first few “bad” points) or in a forward direction (to eliminate truncation problems, or even single “bad” points). Following this process, the “new, improved” FID is then Fourier transformed in the usual way.

See H. Barkhuijsen, R. de Beer, W.M.M.J. Bovee, and D. van Ormondt, *J. Magn. Reson.*, 61, 465-481 (1985) for more information on the algorithm implemented in the software, and on linear prediction in general.

Solvent subtraction filtering

Numerous solvent suppression pulse sequences exist that reduce the signal from a large solvent peak to a level where the desired resonances can be observed. Often, however, experimental solvent suppression does not entirely eliminate an unwanted solvent peak. Digital filtering of the data can further suppress or eliminate a solvent peak.

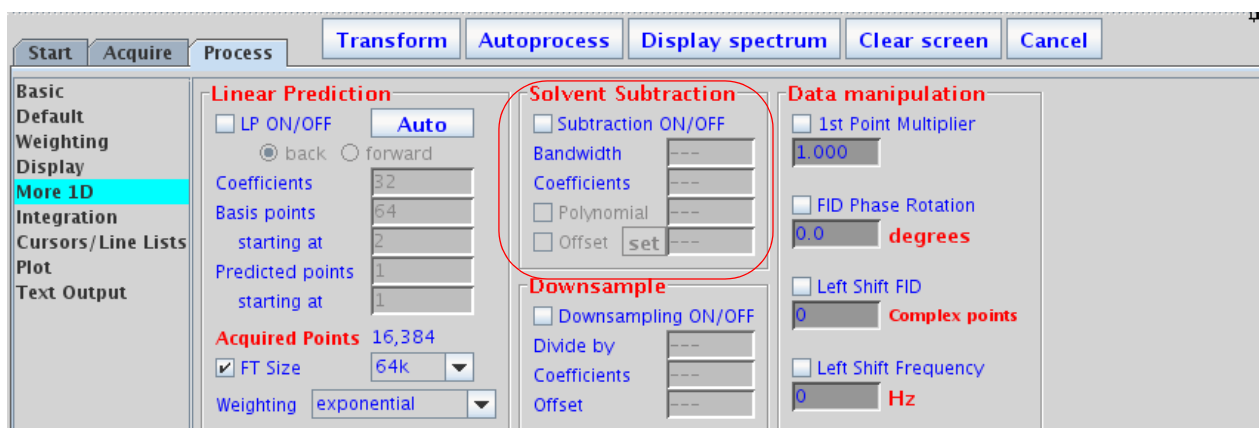


Figure 53 Solvent Subtraction options in the More 1D page

VnmrJ incorporates two algorithms for solvent subtraction by digital filtering:

- **bandwidth** – Sets the value of `ssfilter` to specify the full bandwidth of the low-pass filter applied to the original FID to yield a filtered FID. Its default value is 100 Hz.
- **coefs.** – Sets the value of `ssntaps` to specify the number of taps (coefficients) used for the digital filter. The default value is 121, but the value can range from 1 to $n_p/4$. The more taps in a filter, the flatter the passband response and the steeper the transition from passband to stopband, giving a more rectangular filter. An increased number of taps also increases the computational time dramatically (so that it might become noticeable). The default is suitable for low-frequency suppression option. A value between 3 and 21 works better for the zero-frequency suppression option.

- polynomial – Sets the value of `ssorder` to determine the polynomial used to create a low-pass filter applied to the FID acquired with the solvent on resonance. The resulting FID is subtracted from the original FID to remove the on-resonance frequencies. Transforming the resulting FID produces a solvent-subtracted spectrum. Another name for this is zero-frequency suppression.
- offset – Sets the value of `sslsfrq`, which adjusts the location of the solvent-suppressed region of the spectrum. Setting `sslsfrq` to a non-zero value shifts the solvent-suppressed region by `sslsfrq` Hz. Setting `sslsfrq` to 'n' (the default value) solvent suppresses a region centered about the transmitter frequency. The parameters may be arrayed to achieve multiple-frequency suppression.

The quality of zero-frequency suppression diminishes rapidly as the solvent peak moves off the exact center of the digital filter. Adjust `sslsfrq` to move the center of the filter to within ± 0.2 Hz of the solvent peak for optimal solvent suppression.

Downsample

User-controlled downsampling is not routinely needed on data acquired on newer consoles, but it can be used on virtually all NMR data if desired.

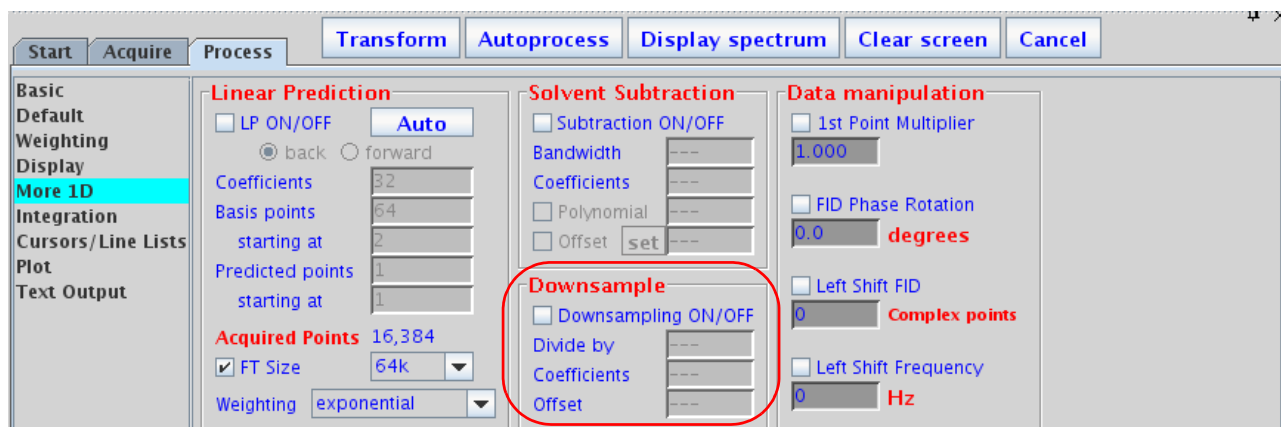


Figure 54 Downsample options on the More 1D page

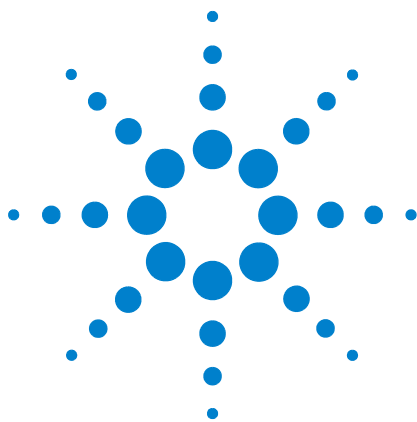
- `divide by` – Sets the value of the downsampling factor that will be applied after digital filtering. The spectral width of the data set after digital filtering and downsampling is `sw` divided by `downsamp`, where `sw` is the acquired spectral width.
- `coefs` – Sets the value of `dscoef` to specify the number of coefficients used in the digital filter. This parameter is automatically adjusted by VnmrJ to give filter cutoffs that are the same value of `downsamp` by using $dscoef * downsamp / 2$ coefficients in the digital filter. VnmrJ always rounds $dscoef * downsamp / 2$ to an odd number. The default is 61.
- `offset` – Sets the value of a bandpass filter, in Hz, that is not centered about the transmitter frequency. A positive value selects a region upfield from the transmitter frequency; a negative value selects a downfield region.

Interleave FIDs

The `ilfid` command converts a multiple FID element into a single FID by interleaving the FIDs. When invoked in an experiment of `nf` FIDs, each of `np` points, `ilfid` sorts the data into a single FID of $np * nf$ points that can then be transformed. The interleaving takes the first complex point of each of the `nf` FIDs and places them in sequential order in the new FID. It then takes the second complex point from each of the `nf` FIDs and appends them sequentially to the new FID. This operation is repeated for all complex points. Although `ilfid` adjusts `np` and `nf`, it does not alter other parameters such as `sw`. See the *VnmrJ Command and Parameter Reference* for further information on `ilfid`, including an example.

CAUTION

Because `ilfid` alters the data irrevocably, it is strongly recommended to save the FID before using `ilfid`.



8 Displaying FIDs and Spectra


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Displaying a FID or 1D Spectrum

Click the **Display FID** graphics control button to display a FID. Click the **1D Spectrum** graphics control button to display a 1D spectrum.

FID display

An FID is available for displaying upon completion of the acquisition of acquisition block size. Clicking the **FID icon**  displays a FID and enables interactive manipulation of the FID display.

The FID display graphics buttons change to show that multiple FIDs can be viewed. [Figure 55](#) shows a typical display with a FID and two vertical cursors (box mode).

The FID is also phase-rotated (zero-order only) by the number of degrees specified in the FID Phase Rotation field on the **Linear Prediction** page.

1D spectrum display

After data is transformed, a spectrum becomes available for display and plotting.

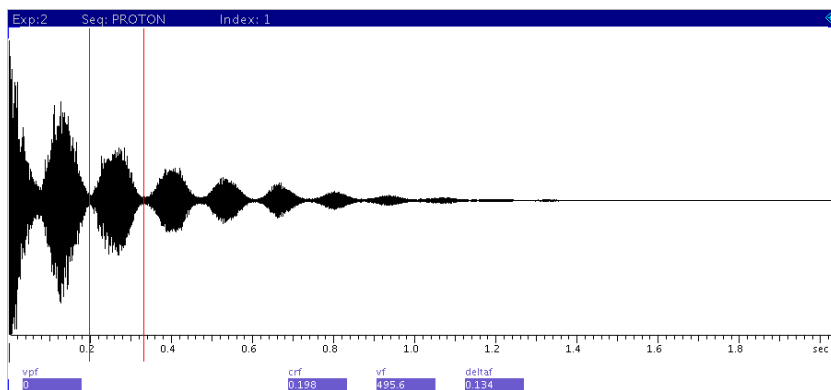


Figure 55 Interactive FID display

The normal spectrum display enables interactive manipulation of a single 1D spectrum. A spectrum is displayed by clicking on the **1D Spectrum graphics control icon**



or by transforming a data set.

A spectrum displays in the graphics window similar to Figure 56.

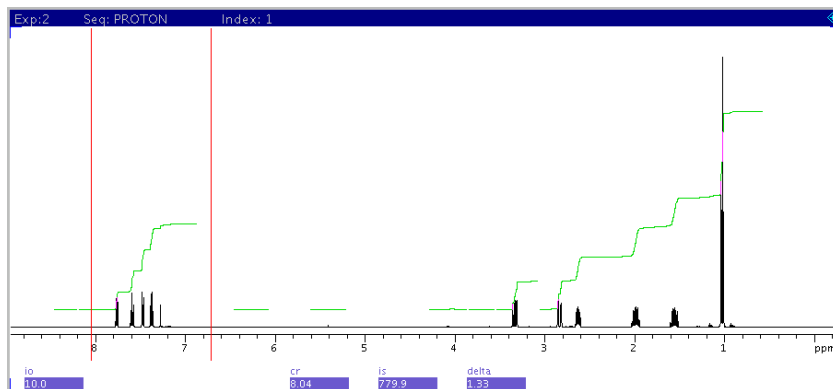


Figure 56 Interactive spectrum display

Display Tools

VnmrJ provides interactive tools for creating individualized displays of NMR data.

Interactive display tools

These tools are described below:

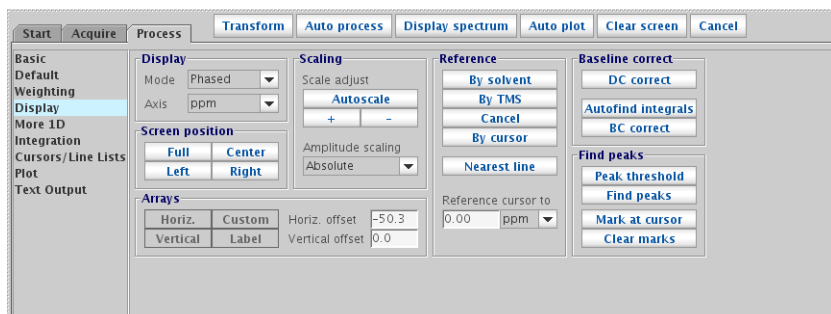
Mouse buttons	The mouse buttons correspond to the display parameters shown on the lower right part of the graphics window. The display parameter changes as different graphics control functions are selected. Typically, the left button controls the left cursor position, the middle button controls vertical scaling, and the right button controls the right cursor or delta between the two cursors.
Graphics control buttons	The graphics control bar next to the graphics canvas provides graphics control buttons for annotation, cursors, zooming, scales, grab and move, threshold, phasing, and refresh. Different functions appear for FID or spectrum display.
Display page	The Display page on the Process tab provides appropriate display parameters, including display mode, axis, and amplitude scaling.
Display menu	The Display menu provides tools for displaying multiple spectra, plotting, and creating insets.

A typical use of these tools might be to expand a region on a spectrum:

- 1 Display the spectrum – click the spectrum icon on the system toolbar.
- 2 Select the region to expand – left click the spectrum to place the cursor on the left boundary of the region of interest, and right-click to designate the right boundary. Use the left mouse button to drag the left cursor and right button to drag the right cursor until the desired region is between the cursors.
- 3 Expand the region – click the magnifying glass icon on the graphics control bar.

Display parameters

FID and spectral display is governed by parameters on the **Display** page of the **Process** tab.



Display Mode

The Display Mode parameters set the display mode along the directly or indirectly detected dimension.

Phased	Each real point in the displayed spectrum is calculated from a linear combination of real and imaginary points comprising each respective complex data point.
Absvalue	(Absolute value mode) Each real point in the displayed spectrum is calculated as a square root of the sum of squares of the real and imaginary points comprising each respective complex data point.
Power	Each real point in the displayed spectrum is calculated as a sum of squares of real and imaginary points comprising each respective complex data point.

Axis

The Axis parameters set the labeling of plot scales, peak frequencies, etc. Typically, FID display is in seconds, and spectrum display is in PPM, Hz, or kHz.

Amplitude scaling

The amplitude scaling, or vertical scale, parameters set the scale intensities for the display:

Normalized	The largest peak in the spectrum is automatically found, then the display is normalized to make the peak vertical scale on the plot in millimeters.
Absolute	The appearance on the display screen is used as a guide to adjust the vertical scale to produce the desired height. This mode enables comparing intensity from one experiment to another, a necessity for <i>all</i> arrayed experiments.

For vertical scaling, full scale on the screen represents full scale on the plotter. This relationship is used to adjust the vertical scale in the Absolute display mode, because vertical scale is not the height of the largest peak. Use the Normalized amplitude scaling mode when the largest peak is to be off-scale.

An exception to the general rule of plotting is provided by the *wysiwyg* parameter. This parameter is set in the **Edit > System settings** window, on the **Display/Plot** tab: *Set display from plotter aspect ratio (wysiwyg)*.

Checked	Scales the image to the current plotter setting (wysiwyg).
Unchecked	Scales the image to the full window, which is easier to view. This option scales the window but does not change the ratio of the image.

Screen position

The screen position parameters set the horizontal position of the display on the screen and the plotter. Clicking one of the buttons updates the display:

Full	Display or plot on the entire screen or page.
Center	Display or plot in the center of the screen or page.
Left	Display or plot in the left half of the screen or page.
Right	Display or plot in the right portion of the screen or page.

Controlling cursors and vertical scale

Click the mouse buttons in the graphics display window to position cursors and adjust the FID or spectral vertical scale and position.

Left cursor	Click the left mouse button to position the cursor and update the value displayed for the <code>crf</code> or <code>cr</code> parameter (<code>crf</code> for a FID or <code>cr</code> for a spectrum).
Right cursor (box)	Click the right mouse button to display and position a second cursor to the right of the original cursor. The value of the parameter <code>deltaf</code> for a FID or <code>delta</code> for a spectra changes with the position of the right cursor and is the difference in seconds or Hz between the two cursors.
Two cursors	If both cursors are displayed, the left mouse button moves both cursors simultaneously, leaving the distance between them (<code>deltaf</code> or <code>delta</code>) unchanged.
Vertical scale	Click the middle mouse button to adjust the vertical scale of the FID (<code>vf</code> parameter) or spectrum (<code>vs</code> parameter).
Vertical position	Adjust the vertical position of the FID by clicking and holding the middle mouse button near the left edge of the graphics display and sliding the FID or spectrum up or down. The value of <code>vpf</code> or <code>vp</code> (or <code>vpfi</code> if the imaginary channel) is will change.

Display limits

The screen position buttons (**Full**, **Enter**, **Left**, **Right**) on the **Display** page place the display and plot in the desired portion of the page.

The `wysiwyg` parameter is useful for scaling the image to a full window instead of the same size as the plot. This parameter is set in the **Edit > System** settings window, on the **Display/Plot** tab: Set *display from plotter aspect ratio* (`wysiwyg`).

Checked	Scales the image to the current plotter setting (<code>wysiwyg</code>).
Unchecked	Scales the image to the full window, which is easier to view. This option scales the window but does not change the ratio of the image.

Display parameters files

The parameters that control the appearance of the spectrum can be saved in temporary display parameters files, which allow the user to easily jump between different displays of

one dataset. There are eight display-parameter files available for each experiment (workspace) in each user account. Each display-parameter file saves the parameters that describe the appearance of the spectrum like `sp`, `wp`, `sp2`, `wp2`, `sc`, `wc`, `sp2`, `wc2`, `vs`, `is`, `vs2d`, `th`, and the integral reset regions. This file does not store or alter phasing, referencing, or baseline-correction information. The files are created with the typed command `s1`, `s2`, `s3`, up to `s8`, and recalled with the typed commands `r1`, `r2`, `r3`, up to `r8`. The typed commands `fr1` through `fr8` ("full recall 1") also recall the integral reset regions, which are not recalled with the simpler `r1` style command.

These display-parameter files can be copied to other experiments by using the typed command `md (m,n)` (where `m` is the experiment with the files and `n` is the experiment to which you want to copy the files). This copy function can also be accomplished by using the **Edit / Move Display** parameters menu. This capability is quite useful for looking at exactly the same expansion of multiple NOESYs run with different mixing times, or looking at exactly the same expansion region in multiple NOESY, TOCSY, ROESY, and COSY datasets.

The display-parameter files remain in the local experiment until the experiment is either deleted or the files removed. The display-parameter files are not useful for experiments run with different spectral widths. For example, if an `s1` was saved on a proton spectrum, if a carbon spectrum is later brought into this same experiment, the `r1` will give a non-useful expansion.

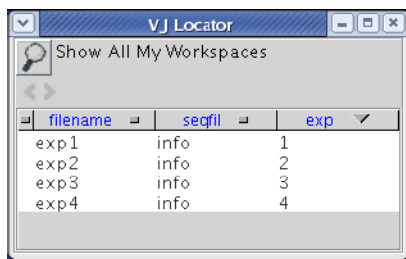


Figure 57 VJ Locator

- 1 Click **Tools** from the main menu.
- 2 Click **Locator...**

Click the magnifying glass icon to open a menu of searches. Selecting a search changes the search sentence (next to the magnifying glass icon). The results of the search are

displayed in the list. Those items in the white part of the list satisfy the search sentence. Those in the gray part do not. Three attributes are displayed for each item that is found by the search. The attributes correspond to the three columns in the list. Clicking the attribute name at the top of the list opens a menu of attribute choices.

Click an item in the Locator list to select that item. The selected item can be dragged to the graphic area or the parameter panel area to cause the appropriate action.

For example, dragging a data set to the graphic canvas retrieves that data set into the current workspace (experiment). Dragging a workspace to the graphic canvas causes that workspace (experiment) to be joined with the graphic area. Double-clicking an item performs the same action as dragging the item to the graphics canvas.

Save Current Process / Display Parameters

In the **Basic** and **Default** pages of the **Process** tab, there is a button labeled **Save Current Process/Display Parameters**. Click this button to save a parameter set in the dataset with all the current processing and display information, in a manner that is suitable for GLP requirements. This action stores parameters such as phasing, integral regions (resets points and isadj), and the display information that would be stored by the `s1` and `r1` commands (described above). (It does not store information about referencing, symmetrization, baseline correction, or drift correction.) The next time any **Process** button is clicked, the last-stored set of display parameters will be used to display the resulting spectrum.

Spectrum Annotations

Buttons for creating annotations are located on the Graphics Toolbar. They are available for "ds", "dss", and "dconi" display modes. The information in this section describes how you create or modify annotations on a spectrum. For a complete list of the annotation buttons and their functions, see the *Agilent VnmrJ Familiarization Guide*.

The position of an annotation object is defined by frequencies (in PPM). Optionally, you can also define an annotation by chart position (in mm). See [“Select and modify annotations”](#) on page 189 for details.

To create an annotation

- 1 Display the spectrum (click the spectrum icon on the system toolbar).
- 2 On the Graphics toolbar, click the icon for the annotation you want to add. This starts the create mode.
- 3 Move the mouse to the spectrum and click or click and drag to create the desired annotation.
- 4 When you complete the annotation (click or release the mouse after dragging) you are returned to select mode.

To create a text annotation

- When you select the Text annotation, a Property Editor popup appears where you define the text annotation. After you define the text properties, click **Close** to add the annotation.


To create multiple annotations of the same type

- After you select an annotation icon on the toolbar, hold down the **Ctrl** key and click on the spectrum. Annotations will be added each time you click until you release the **Ctrl** key.

To create a polygon or polyline

- 1 Select the polygon or polyline icon on the graphics toolbar to start creation mode.
- 2 Using the left mouse button, click multiple points on the spectrum to define the shape, except for the last point.

- 3 Click the right mouse button to add the last point and exit annotation creation mode.


Once you have created the polygon or polyline, you can do the following when in select mode ():

- Move the mouse over the annotation to select it, click a point on the polygon or polyline, and drag it to a new location.
- To add a point to a polygon or polyline, select the line where a point will be added, then hold down the **Ctrl** key and double-click.
- To delete a point from a polygon or polyline, select the point, then hold down the Ctrl key and double-click the point.


Select and modify annotations

You can move, resize, and change the properties of a selected annotation.


To select an annotation

- 1 Click  to enter select mode.
- 2 Move the mouse cursor over the annotation until it is highlighted.


To move an annotation

- 1 Click  to enter select mode.
- 2 Move the mouse cursor over the annotation until the annotation is highlighted, then click and drag the annotation to the new location.
- 3 Release the mouse button at the desired location.

To resize an annotation

- 1 Click  to enter select mode.
- 2 To resize (non-text annotation), move the cursor over the annotation until a “handle” appears, then click and drag the handle to the desired size.
- 3 Release the mouse button to accept the new size.

To change the properties of an annotation

- 1 Click  to enter select mode.
- 2 Move the mouse cursor over the annotation until the annotation is highlighted, then right-click.
- 3 In the menu that appears (Figure 59 on page 192) select **Properties**.

The Property Editor popup appears with options for the selected type of annotation. An example of the Property Editor for a point is shown in Figure 58

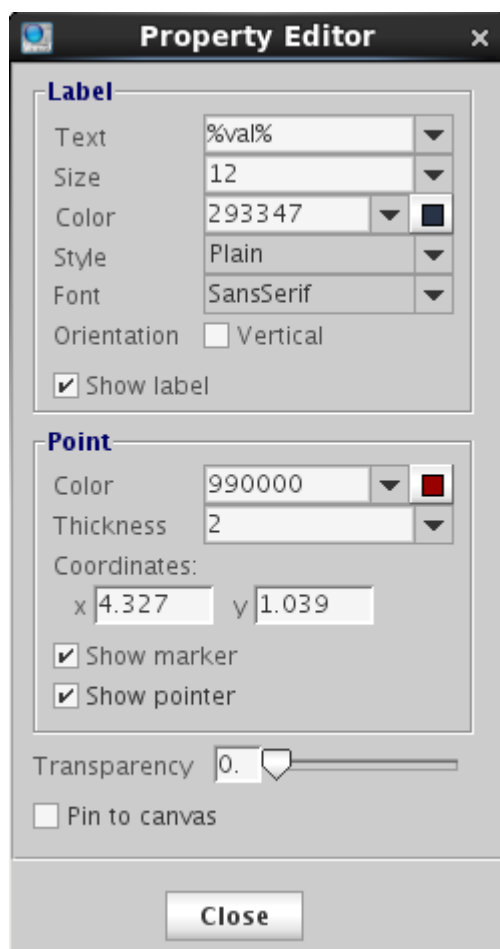


Figure 58 Property Editor popup for a “point”

The default colors, fonts, line thickness of annotation objects are defined in the Annotation tab of the Styles and Themes dialog (**Edit > Display Options > Display > Annotations**). Changes

made in the Property Editor for an annotation object temporarily override the properties defined by the display options.

Labels

A label may contain a keyword between two % symbols. Valid keywords are %val%, %ppm%, %hz%, %amp%, %vol%. The keyword will be substituted by the appropriate value in the annotation. Only one keyword is allowed in a label.

A label may also contain parameter substitutions. A valid parameter name is placed between two \$ signs, and will be replaced by its value when displayed. The parameter value can be real or string.

Transparency


The transparency of an annotation object is defined by a number ranging from 0 to 100, with 0 being totally opaque and 100 being totally transparent.

Pin to canvas


This option toggles whether the annotation is associated with a position in the spectrum, or a position on the screen. If this option is turned off, the annotation will retain its position relative to the spectrum as the spectrum is zoomed and scaled.

To reset the properties of an annotation to the default, select **Reset Properties**.

To show or hide annotations

Click  to show or hide annotations. Hidden annotations are saved in curexp+' /datdir/annos'.

To delete an annotation

- 1 Click  to enter select mode.
- 2 Right-click an annotation. A menu appears where you can select to delete the selected annotation or all annotations (Figure 59 on page 192).

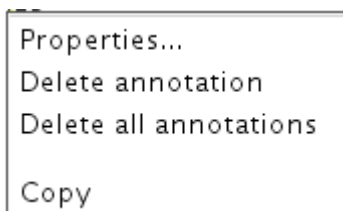


Figure 59 Menu when you right-click an annotation

To copy and paste annotations

To copy and paste an annotation, hover over an annotation to highlight it, then right-click and select **Copy** from the menu. Move the mouse to a desired location, right-click and select **Paste**.

To save or load annotations

Annotations can be saved to a file and loaded back later.

- 1 Right-click in the graphics canvas.
- 2 In the menu that appears, click **Save Annotations**.
- 3 In the Save Annotations dialog, specify the directory and file name. By default, the directory is `$HOME/vnmrsys/Annotations`, and the file is `annoNN.txt`, where NN are auto-generated numbers.
- 4 Click **OK**.

To load a saved annotations file

- 1 Right-click in the graphics canvas.
- 2 In the menu that appears, click **Load Annotations**.
- 3 In the Load Annotations dialog, select the annotations file to load from the drop-down menu.
- 4 Click **OK** to load the saved annotations into the graphics canvas.

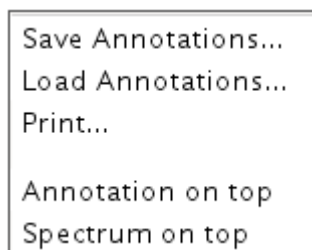


Figure 60 Menu when you right-click in graphics canvas

To print an annotated spectrum

To print the annotated spectrum, right-click in the graphics canvas and then select **Print**. A preview will be displayed in Adobe Reader and can be printed from the File menu.


To move annotation in front of spectrum

By default, the annotation is placed on top (in front) of the spectrum. To change this, click **Spectrum on top**. To return the annotation to the top, click **Annotation on top**.



Figure 61 Annotation on top (left) - Spectrum on top (right)

Phasing

The **Phase** button  starts the interactive phasing mode. Any integral and cursors that are displayed along with the spectrum are removed. The width of the update region is set by the *Spectrum updating during phasing (0-100)* field in **Edit > System settings > Display/Plot** tab, which sets the percentage of the screen display to be updated.

FID phasing

The **Phase** button activates the interactive phasing mode:

- 1 Position the mouse arrow on a FID region of interest, about halfway vertically up the screen, and click the left mouse button.

A horizontal cursor intersects at the mouse arrow, and two vertical cursors are placed on either side of the mouse arrow. A small region of the FID is displayed in a different color if a color display is present. Only this spectral region is interactively updated.

- 2 Move the mouse above or below the horizontal cursor, but within the two vertical cursors. Click the left or right button to adjust the FID phase parameter `phfid`.

Click the mouse above the horizontal cursor to increase `phfid`. Click below the horizontal cursor to decrease `phfid`. Place the mouse arrow right on the horizontal cursor and click the left button to restore the initial phase.

- 3 To exit the interactive phasing mode, make another selection from the menu. Select the **Cursor** or **Box** button if no other choice is desirable.

Spectrum phasing

- 1 Position the mouse arrow on a spectral region of interest toward the right side of the spectrum, about halfway vertically up the screen, and click the left mouse button.

A horizontal cursor will intersect at the mouse arrow. Two vertical cursors will be placed on either side of the mouse arrow. A region of the spectrum will be displayed in a different color if a color display is present, and only this spectral region will be interactively updated (for the case of less than 100% updating).

- 2 Move the mouse above or below the horizontal cursor, but within the two vertical cursors. Click the left or right button to adjust the zero-order or frequency-independent phase parameter r_p .
 - Click above the horizontal cursor to increase r_p (cause a clockwise rotation of the peaks).
 - Click below the horizontal cursor to decrease r_p (and cause a counter-clockwise rotation).
 - Place the arrow on the horizontal cursor and click the left button to restore the initial phase.

The left and right buttons of the mouse differ only in their sensitivity. Full scale (top to bottom of the screen) corresponds to approximately 180° for the left button, and 20° for the right button. The left button is a “coarser” adjustment, and the right button is a “finer” adjustment.

- 3 Move the mouse arrow to another region of the spectrum, near the left edge of the display, outside the vertical cursors, and click the left mouse button again.

The frequency-independent phase-correction made so far is first applied to the entire spectrum. A new horizontal cursor is displayed at the mouse arrow, and two new vertical cursors are displayed on either side of the mouse arrows. The mouse now controls the first-order or frequency-dependent phase parameter l_p .

- 4 Click the left or right button above or below the horizontal cursor to increase or decrease l_p so that the phase at the center of the previous region bracketed by the vertical cursors is held constant.

This process eliminates or substantially reduces the necessity to iteratively adjust the two parameters r_p and l_p . As with the zero-order correction, the left button acts as a “coarse” adjust, and the right button as a “fine.”

Define a new update region by clicking the mouse outside the two vertical cursors.

Subsequent first-order phase changes cause the zero-order phase to be adjusted so that the phase angle at the center of the previous region bracketed by the vertical cursors remains constant. Click the **Phase** button again if to return to the zero-order phase correction.

Adjust the vertical scale and apply the latest phase correction by clicking the middle mouse button at the top of a peak that is on scale. This leaves the vertical scale unaffected but recalculates the phase of the entire spectrum. Clicking the center button above or below the peak raises or lowers the vertical scale.

- 5 Exit the interactive phasing mode by clicking another graphics control button.

Line Tools

Find nearest line and line resolution

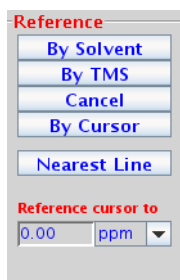
- 1 Place a cursor near the line of interest.
- 2 Select the **Process** page and click the **Find nearest line** button. The cursor moves to the nearest line and displays its height and frequency (in Hz and ppm) in the message window.
- 3 Click **Display** linewidth to display the resolution of a line, as well as the limiting digital resolution of the spectrum. The resolution is determined by a width at half-height algorithm and not by least-squares.

Display line list

- 1 Click the **Threshold** graphics control button and use the middle mouse button to vertically position the yellow threshold line.
- 2 Select the **Line List** page and click the **Display Line List** button. This process displays line frequencies and intensities that are above a threshold.

Spectral Referencing

Frequency referencing is set on the **Display** page.



Button	Description
By Solvent	Reference the spectrum for selected solvent.
By TMS	Reference the spectrum to a TMS line. In the case of other signals (for example, from silicon grease) immediately to the left of the TMS line (even if they are higher than the reference line), <code>tmsref</code> tries avoiding those signals by taking the line furthest to the right in that area, as long as it is at least 10% of the main Si-CH ₃ signal. Large signals within 0.6 ppm for ¹ H (or 6 ppm for ¹³ C) to the right of TMS might lead to mis-referencing.
Cancel	Clears the reference line by removing any spectral referencing present, and turns off referencing.
By Cursor	References the spectrum based on the current cursor position. To reference the spectrum based on a line position in the spectrum, first use the Find nearest line button on the Process page, then click By Cursor .

Table 14 Parameters used in spectral referencing

Reference line (frl)	The distance, in Hz, of the reference line from the right edge of the spectral window. This line is the spectral position used to set the referencing. It can be the signal of a frequency standard (such as TMS), or any line (such as a solvent signal) with a known chemical shift (in ppm), or a position in the spectrum where such a line is expected to appear.
-----------------------------	--

Table 14 Parameters used in spectral referencing (continued)

Reference position (rfp)	The difference between the reference line and the reference frequency (zero position of the scale) in Hz. Referencing a spectrum using the signal of a frequency standard, such as TMS, use reference position is 0. The distance of the reference frequency from the right edge of the spectrum is <i>reference line reference position</i> .
Spectrometer frequency	The absolute frequency, in MHz, of the center of the spectrum (the transmitter position). Use the <code>spcfreq</code> command in order to see the accurate value of the spectrometer frequency (<code>sfreq</code> parameter).
Reference frequency	The frequency, in MHz, of the frequency standard, that is, the zero position of the frequency scale, and the divider (unit) for the calculation of ppm scales (<code>reffreq</code>).

The **By Solvent** and **By TMS** buttons assume that the system is locked (and that the lock solvent is defined in `/vnmr/solvents`). Ensure that the field offset has been adjusted so that the lock frequency is on resonance with a sample of similar susceptibility if the experiment is to run unlocked and these buttons are used to set the field offset. Adjust the field offset is adjusted using the following procedure:

- 1 Insert a sample with deuterated solvent.
- 2 Adjust `z0` (or `lkoF`) in `acqi` so that the lock frequency is on resonance.
- 3 Switch off the lock.
- 4 Insert the non-deuterated sample.

The accuracy of the solvent and **TMS** referencing buttons is mostly limited by the accuracy of the chemical shift of the lock resonance line, which may depend on the concentration and the chemical properties (acidity/basicity) of the components in the sample. But they should normally be accurate enough to find an actual reference line close to its predicted position.

Estimate the position of the reference frequency in spectra from unlocked samples, provided the spectrometer is first locked on a sample with similar susceptibility, then the lock is disengaged and the field offset adjusted such that the lock signal is on-resonance. Now, acquire a spectra without lock and calculate their (estimated) referencing using `setref`,

8 Displaying FIDs and Spectra

provided the solvent parameter is set to the solvent that was last locked on.

Working with Viewports and Inset Frames


All VnmrJ graphics are displayed in frame(s). The viewport has a default frame that occupies the entire viewport graphics area. An inset frame initially shares the same workspace and data as the original frame and is manipulated in the same way as the default frame.

Creating an inset frame



An inset frame has the full capability of the default frame. The only difference is that the default always exists, while an inset frame can be created and removed. Create an inset frame within the default viewport frame as follows:




Figure 62 Creating an inset frame

- 1 Click the **Frame** vertical panel.
- 2 Select the inset mode tool. 
- 3 Place the cursor at the low field (left) side of the region to be expanded as shown in [Figure 62](#) frame 1a.
- 4 Hold the left mouse button down and drag the inset window to the high field (right) side of the region.
- 5 Drag the cursor down to set the height of the inset frame as shown in [Figure 62](#) frame 1b.
- 6 Release the mouse button to create the inset frame, see [Figure 62](#) frame 2.


Zooming in on a region within an inset frame

- 1 Select the default mode tool .
- 2 Click inside the frame to make the frame active.
- 3 The frame has a yellow border when it is active and white border when it is inactive (these are the default colors of inactive and active frames).
- 4 Select the zoom mode tool .
- 5 Place the cursor at the low field (left) side of the region to be expanded as shown in [Figure 62](#) frame 3a.
- 6 Hold the left mouse button down and drag the inset window to the high field (right) side of the region, [Figure 62](#) frame 3b.
- 7 The region selected is indicated by a transparent gray rectangle.
- 8 Release the mouse button and the selected region expands to fill the inset box, [Figure 62](#), frame 4.

Resizing an inset frame

- 1 Select the default mode tool .
- 2 Click inside the frame to make the frame active. An active frame has a yellow border.
- 3 Move the mouse cursor to a corner of the inset frame. The cursor changes from a single-headed arrow to a double-headed arrow.
- 4 Hold down the left mouse button and grab the corner of the frame.
- 5 Drag the corner to resize the frame.
- 6 Release the mouse button when the frame is at the desired size.

Moving an inset frame

- 1 Select the default mode tool .
- 2 Click inside the frame to make the frame active. An active frame has a yellow border.
- 3 Move the mouse cursor to an edge of the inset frame. The cursor changes from a single-headed arrow to a four-headed arrow.
- 4 Hold down the left mouse button and grab the edge of the frame.
- 5 Drag the frame to the new position.
- 6 Release the mouse button when the frame is at the desired position.

Stacked 1D Display

Stacked display using the main menu display

- 1 Click **Display** on the main menu.
- 2 Select a display mode from the drop-down menu:
 - Display Multiple Spectra Horizontally
 - Display Multiple Spectra w/ Labels
 - Display Multiple Spectra Vertically
 - Increase vertical Separation by 20%
 - Decrease vertical Separation by 20%
 - Create an Inset of the current Display
 - Save Current Display Parameters
 - Plot Current Display before Making Inset
 - Make Inset
 - Plot Inset and Return Original Display

Stacked display using the display page

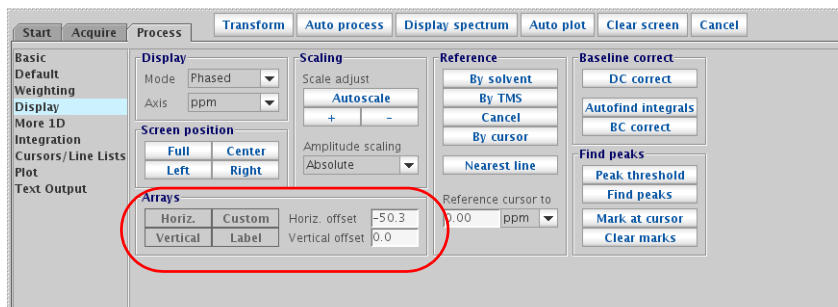










Figure 63 Options for controlling the display of arrayed data








- 1 Click the **Process** tab.
- 2 Select the **Display** page.

- 3 Click a **Display Arrays** button:
 - **Horizontal** – Display arrayed spectra horizontally and divide available display width into equal portions.
 - **Vertical** – Display arrayed spectra stacked vertically with each spectrum displayed using the full width of the screen.
 - **Label** – Add a label to the spectra.
 - **Custom** – Use a custom Label.
- 4 Enter values for the Display offsets
 - **Horizontal offset** – Enter a value in mm for the separation between spectra.
 - **Vertical offset** – Enter a value in mm for the separation between spectra.

Stacked spectra display using the graphics tools

Icon	Function
	Display the first arrayed spectrum and display 1D graphics toolbar with the following icons at the top (or left side if the bar is horizontal).
	Display the next spectrum.
	Display the previous spectrum.
	Display arrayed spectra stacked vertically with each spectrum displayed using the full width of the screen.
	Display arrayed spectra horizontally and divide available display width into equal portions.
	Hide or show axis under the spectra.
	Label the spectra.
	Return to previous graphics display tool.

Stacked FID Display Using the Graphics Tools

Icon	Function
	Display the first arrayed FID and display 1D FID graphics tool bar with the following icons at the top (or left side if the bar is horizontal).
	Display the next FID.
	Display the previous FID.
	Display arrayed FIDs stacked vertically with each spectrum displayed using the full width of the screen.
	Display arrayed FIDs horizontally and divide available display width into equal portions.
	Label the FIDs.
	Return to previous graphics display tool.

Aligning and Stacking Spectra

Requirements for aligning and stacking spectra

Spectra can be a mixture of 1D and 2D data sets, all 2D data sets, or all 1 D data sets provided these requirements are met:

- All selected viewports need to use a common scale.
- Data in the viewports may have different nuclei, different spectrum width, or different spectral regions. The common scale is determined based on data in all selected viewports and determines whether alignment or stacking is possible. Overlaid and stacked spectra are drawn based on the common scales.
- Alignment is enabled if more than one axis in more than one viewport has the same axis (H1, C13 etc.).
- Stacking is enabled when data in all viewports have the same axis/axes.

Setting up stacked aligned spectra

- 1 Select the **Viewport** tab from the vertical tabs panel.
- 2 Load each data set into a different viewport and process the data. Data must meet the requirements mentioned in [“Requirements for aligning and stacking spectra”](#) .
- 3 Select viewports containing spectra to overlay by placing a check in the check box under **select**.
- 4 Click the **Overlay Viewports** button to overlay all selected viewports.

The **Stack Spectrum** button, [Figure 64](#), is displayed below the **Overlay Viewports** button if all spectra have the same dimension (all 1D or all 2D) and all axis/axes (nuclei) match. Stacked spectra are aligned and each spectrum is shifted along x and y. The shift between spectra is specified by x and y offset in the entry fields below the **Stack Spectrum** button. Spectral axes are also synchronized to enable zoom and pan of the spectrum without losing the alignment.

8 Displaying FIDs and Spectra

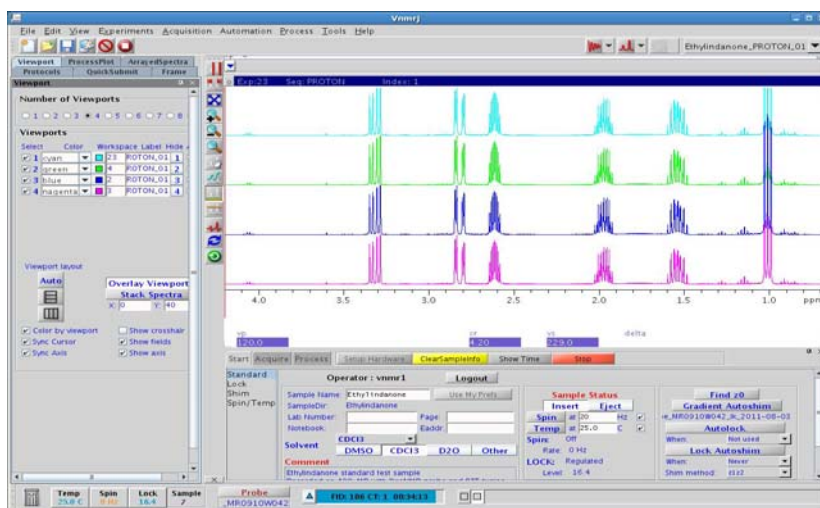


Figure 64 1D stacked spectra

The **Align Spectra** button, [Figure 65](#), is available if it is a mix of 1D and 2D spectra when multiple spectra are overlaid. All 2D spectra must have matching axes. All 1D data must match one of the 2D axes. 1D spectra are aligned and displayed at the margins of the 2D spectrum. 1D spectrum will be rotated if necessary to align with the 2D spectrum. Zoom and pan are synchronized when the spectra are aligned.

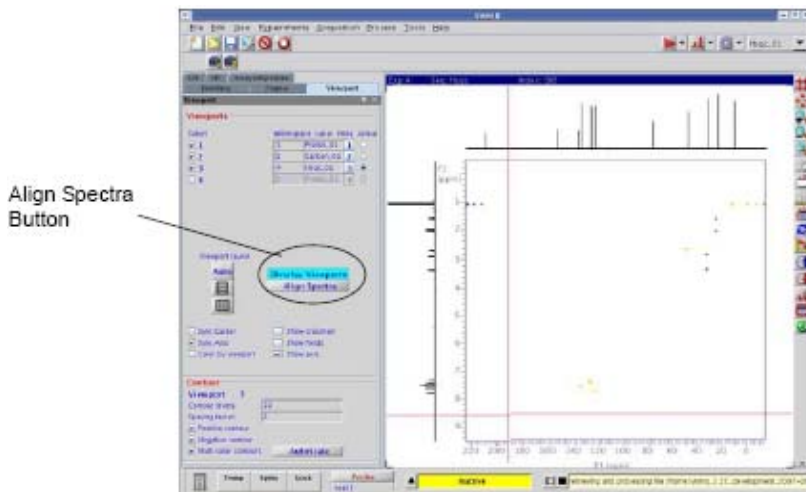


Figure 65 2D spectra with overlaid 1D's

The **Stacked Spectrum** button for 2Ds, **Figure 66**, is displayed below Overlay Viewport if all 2D spectra axes and nuclei match. Spectral axes are synchronized to enable zoom and pan of the spectra without losing alignment.

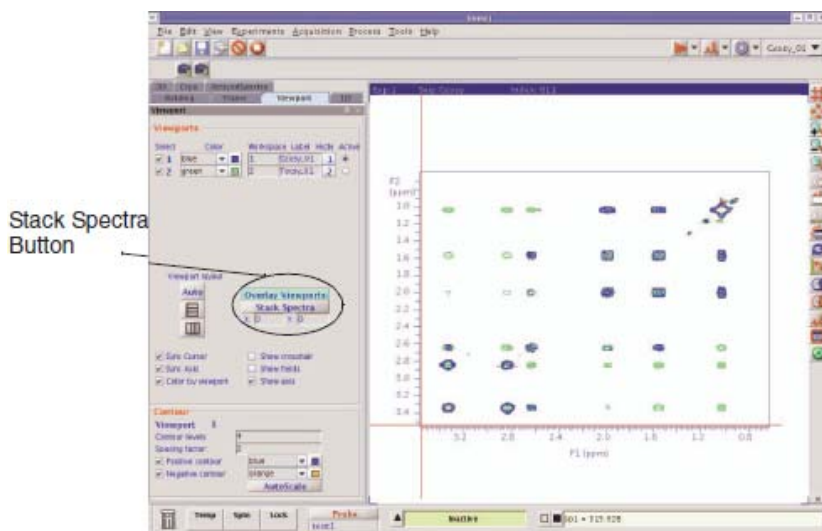


Figure 66 Stacked overlaid 2D spectra

Integration

This section describes methods and tools for displaying and plotting integrals.

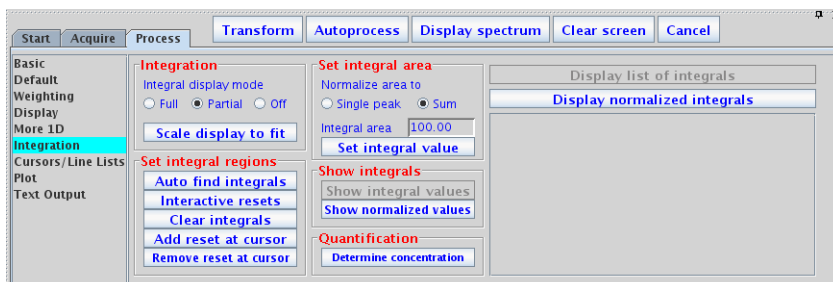


Figure 67 Integration page in the Process tab

Interactive zero- and first-order baseline correction mode

The **Integral Lvl/Tlt** button activates interactive zero and first order baseline correction mode. The zero order correction is represented by the `lvl` parameter; the first order correction is represented by the `tlt` parameter. If no integral is displayed when the **Integral Lvl/Tlt** button is activated, the integral is automatically displayed.

- 1 Left-click an integral region of interest, about halfway vertically up the screen.

A horizontal cursor intersects at the mouse arrow. Two vertical cursors are placed on either side of the mouse arrow.
- 2 Right or left-click above or below the horizontal cursor, but within the two vertical cursors, to adjust the zero-order baseline correction parameter `lvl`.
 - Clicking above the horizontal cursor increases `lvl`.
 - Clicking below the horizontal cursor decreases `lvl`.
 - Clicking on the horizontal cursor restores the initial baseline correction value.
- 3 Left-click another region of the spectrum, outside the vertical cursors.

A new horizontal cursor is displayed at the mouse arrow, two new vertical cursors are displayed on either side of the mouse arrow, and a single vertical cursor is displayed in the

middle of the region where $lv1$ was being updated. The mouse now controls the first-order baseline correction parameter $t1t$.

- 4 Right or left-click above or below the horizontal cursor to increase or decrease $t1t$, and change $lv1$ so that the total drift correction at the single vertical cursor in the middle of the previous region is held constant.

This process eliminates or substantially reduces the necessity to iteratively adjust the two parameters $lv1$ and $t1t$. As with the zero-order correction, placing the mouse arrow right on the horizontal cursor and clicking the mouse button will restore the initial baseline correction values.

Each time the mouse is clicked outside the two vertical cursors, new vertical and horizontal cursors are displayed.

The left and right mouse buttons both adjust the baseline correction parameters and differ only in their sensitivity. The left button causes changes a factor of eight times larger than the right button, making the left button a “coarse” adjust and the right button a “fine” adjust. The overall sensitivity of these adjustments can also be controlled by the parameter $lv1t1t$. This parameter is a multiplier, with a default value of 1.0, for the size of the changes. To make larger changes, make $lv1t1t$ larger than 1.0. To have finer control, set $lv1t1t$ to be between 0.0 and 1.0.

The middle mouse button adjusts the integral scale (parameter is) or the integral offset (parameter io), exactly as whenever an integral is displayed.

- 5 Exit the interactive baseline correction mode by clicking on another graphics control button.

Displaying integrals step-by-step

The following methods provide an opportunity to compare procedures. Before starting each procedure, be sure to obtain a typical spectrum.

- 1 Load a data file into the active viewport using the file browser or the Locator.
- 2 Transform the data if necessary.
- 3 Click the **Process** tab.
- 4 Select the **Integration** page.

- 5 Click an **Integration Display Mode** radio button: **Full**, **Partial**, or **Off**.
 - **Full** shows integrals over the entire spectrum, including the noise.
 - **Partial** shows even integrals regions and hides all the odd integral regions.
 - **Off** turns off the integral display.
- 6 Click **Auto Find Integrals** to automatically set the integral resets and display the data as set by the **Integration Display Mode** radio button.
- 7 Click the **Scale display to fit** button to automatically scale the display.
- 8 Set the integral area.
- 9 Enter a value in the Integral Area field.
- 10 Click one of the following radio buttons under the **Normalize Area to:** page region.
 - **Single Peak** – selects the region or peak under the cursor as the reference and sets the single peak integral to the value in the Integral Area field when the **Set Integral Value** button is clicked.
 - **Sum** – sets the entire integral to the value in the Integral Area field. Do not click the **Set Integral Value** button. This button sets the single peak reference.
- 11 Display the integral results as follows:
 - **Single Peak** – both the **Show Integral Values** and **Show Normalized Value** buttons are active.
 - **Integral Values**
Click **Show Integral Values** to display the values of the integral regions on the screen below the spectrum.
Click **Display Lists of Integrals** to list the display regions and the value of the integral over each region.
 - **Normalized Integral Values**
Click **Show Normalized Values** to display the values of the integral regions normalized to the reference region on the screen below the spectrum.
Click **Display Normalized Integrals** to list the display regions and the value of the integral over each region normalized to the reference region.

Manual method

- 1 Load a data file into the active viewport using the file browser or the Locator.
- 2 Transform the data if necessary.
- 3 Click the **Process** tab.
- 4 Select the **Integration** page.
- 5 Click **Clear Integrals**.

Any currently defined integral reset points are cleared.

- 6 Set up the integral resets from left to right (down field to up field).
 - a Click the **Interactive Resets** button.
 - b Place the cursor to the left of the first integral region.
 - c Click the left mouse button.
 - d Move the cursor to the right of the first integral region.
 - e Click the left mouse button.
 - f Repeat Step b through Step e until all the required integral regions are defined.
- 7 Click **Scale display to fit** button to automatically scale the display.
- 8 Set the integral area:
 - a Enter a value in the Integral Area field.
 - b Click one of the following radio buttons under the **Normalize Area to:** page region.
 - **Single Peak** – selects the region or peak under the cursor as the reference and sets the single peak integral to the value in the Integral Area field when the **Set Integral Value** button is clicked.
 - **Sum** – sets the entire integral to the value in the Integral Area field.
Do not click the **Set Integral Value** button. This button sets the single peak reference.

9 Display the integral results as follows:

- **Single Peak** – both the **Show Integral Values** and **Show Normalized Value** buttons are active.

- **Integral Values**

Click **Show Integral Values** to display the values of the integral regions on the screen below the spectrum.

Click **Display Lists of Integrals** to list the display regions and the value of the integral over each region.

- **Normalized Integral Values**

Click **Show Normalized Values** to display the values of the integral regions normalized to the reference region on the screen below the spectrum.

Click **Display Normalized Integrals** to list the display regions and the value of the integral over each region normalized to the reference region.

Command line equivalents for VnmrJ interface driven integration

Use the parameter page editor to view the commands on the current parameter page.

- 1 Click **Edit** on the main menu.
- 2 Select **Parameter Pages**.
- 3 Place the mouse cursor on a button or entry field.
- 4 Double-click (left mouse button).
- 5 Read the associated command next to the field Vnmr Command.

Baseline correction

Most operations performed on spectra assume a quality baseline. Line lists, integrations, resolution measurements, 2D volume integrations, etc., all measure intensities from “zero” and do not perform any baseline adjustments. If the baseline in your spectrum is not flat perform a baseline correction operation before performing further data reduction. Two types of baseline correction are provided, linear and non-linear, and are available using the buttons on the **Display** page.

Baseline correction commands

Using the beginning and end of the displayed spectrum to define a straight line to be used for baseline correction, the `dc` command turns on a linear baseline correction. `dc` calculates a zero-order baseline correction parameter `lv1` and a first-order baseline correction parameter `tl1t`. The `cdc` command turns off this correction. The results of the `dc` or `cdc` command are stored in the `dcb` parameter, which can be queried (`dcb?`) to determine whether drift correction is active. If active, `dcb=''`; if inactive, `dcb='cdc'`.

The `bc` command performs a 1D or 2D baseline correction. The 1D baseline correction uses spline or second to twentieth order polynomial fitting of predefined baseline regions. `bc` defines every other integral, that is, those integrals that disappear in partial integral mode (`intmod='partial'`) as baseline and attempts to correct these points to zero. A variety of parameters can be used to control the effect of the `bc` command.

For more information about the `bc` command, see the entry for `bc` in the *Command and Parameter Reference*.

Integral reset points commands

The `z` command (or the equivalent button or icon) resets the integral to zero at the point marked by the displayed cursor. `z(reset1,reset2,...)` allows the input of the reset points as part of a command, instead of using the position of the cursor. Reset points do not have to be entered in order. The resets are stored as frequencies and will not change if the parameter `fn` is changed. The command `cz` (or the equivalent button) removes all such integral resets. `cz(reset1,reset2,...)` clears specific integral resets.

The `liamp` parameter stores the integral amplitudes at the integral resets points, and the `lifrq` parameter stores the frequencies of integral reset points, for a list of integrals. To display the values of `liamp`, enter `display('liamp')` with a **Text Output** page selected. Frequencies are stored in Hz and are not adjusted by the reference parameters `rfl` and `rfp`.

Integral regions commands

The region command divides a spectrum into regions containing peaks. A variety of parameters can be used to control the effect of the region command. For more information, see the *Command and Parameter Reference*.

Integral display and plotting commands

Display and plotting of the integral trace is independent of the values of the integrals. The height of the trace is controlled by the parameter `is` and can be interactively adjusted with the `ds` command. The macro `isadj(height)` (or the equivalent button) adjusts the integral height so that largest integral fits the paper or is height mm tall if an argument is provided, for example, `isadj(100)`.

The command `dli` (or the equivalent button) displays a list of integral values at the integral reset points. The frequency units of the reset points are defined by the parameter `axis`. The reset points are stored as Hz and are not referenced to `rfl` and `rfp`. The amplitudes are stored as actual values, they are not scaled. The integral values are scaled by the parameters `ins` and `insref` and the Fourier number. Typically, `ins` is set to the number of nuclei in a given region. For example, if a region represented a single methyl group, the following procedure would scale the integral values of that region:

- 1 Set `ins=3`.
- 2 Set `insref` to the Fourier-number-scaled-values of that integral.
- 3 Enter `dli`. The integral value of that region is displayed as 3 and all other integral values are accordingly scaled.

Integral value scaling can be interactively set with the `ds` command. The `setint` macro can also be used to adjust integral value scaling. The `setint` macro sets the value of an integral and scales integral values in conjunction with the command `dli`. Normalized integral values can also be selected. In this case, `ins` represents the total number of nuclei. The individual integral values will be scaled so that their sum is equal to `ins`. The normalized mode may be selected by setting `insref` to “not used.” The integral is scaled by `ins` and `insref`.

Two commands are closely related to `dli`:

- `nli` is equivalent to `dli` except that no screen display is produced.
- `dlni` normalizes the values from `dli` using the integral normalization scale parameter `ins` and then displays the list.

The `dpir` command displays numerical integral values below the appropriate spectral regions, using the integral blanking mode in which only every other integral is plotted. The

command `dpirn` shows the normalized integral values in an analogous fashion.

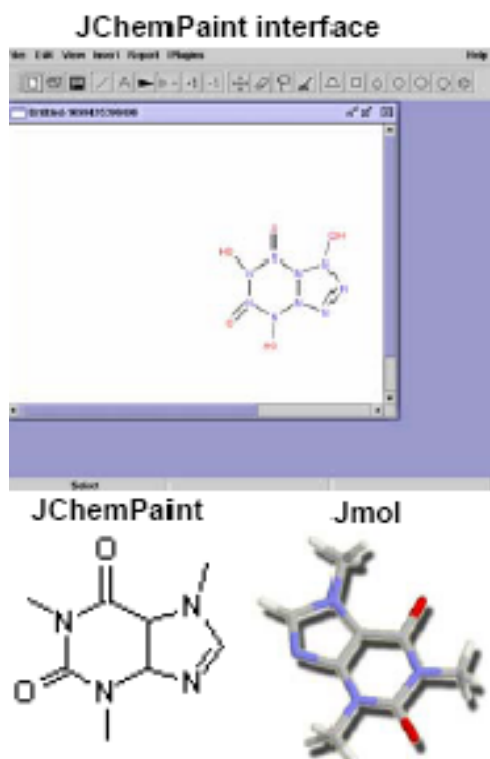
The `pir` command plots digital integral values below the spectrum, using the integral blanking mode in which only every other integral is plotted. The command `pirn` plots the normalized integral values in an analogous fashion.

Molecular Display and Editing (JChemPaint and Jmol)

Tools for editing, viewing, and printing molecular structures are installed as options with VnmrJ. These tools are derived from *JChemPaint* and *Jmol*.

JChemPaint and Jmol are open source software packages available from <http://sourceforge.net/>.

JChemPaint is a graphical editor for 2D molecular structures. Jmol is a visualization and analysis tool for 3D structures.



Running JChemPaint

- 1 Click **Tools**.
- 2 Select **Molecular Structures**.
- 3 Select **JChemPaint** menu.

See <http://jchempaint.sourceforge.net> for documentation.

File Formats

JChemPaint can edit, save, and export the file formats listed here.

Format	Action
MDL MOL	edit, save
SMILES	edit, save
IUPAC Chemical Identifier	edit
MDL SDF Molfile	edit
Chemical Markup Language	edit
Scalable Vector Graphics	save
CDK source code fragment	save
BMP	save
JPEG	save
PNG	save
TIFF	save
Gaussian Input	export

- 1 Save files as MDL MOL (*.mol) in one of the mollib directories: /vnmr/mollib or ~username/vnmrsys/mollib.
- 2 Import an existing mol file into VnmrJ by copying it into the mollib directory.
- 3 Click **Tools** on the main menu.
- 4 Select **Open**, browse to the file, and drag it to the VnmrJ graphics screen.

Molecular structures

Molecular structures are displayed and manipulated in the VnmrJ graphics window. View as many graphics as wanted. The graphics are displayed in the current experiment, and they are saved per experiment.

Use the following steps to display a molecular structure in the VnmrJ graphics window:

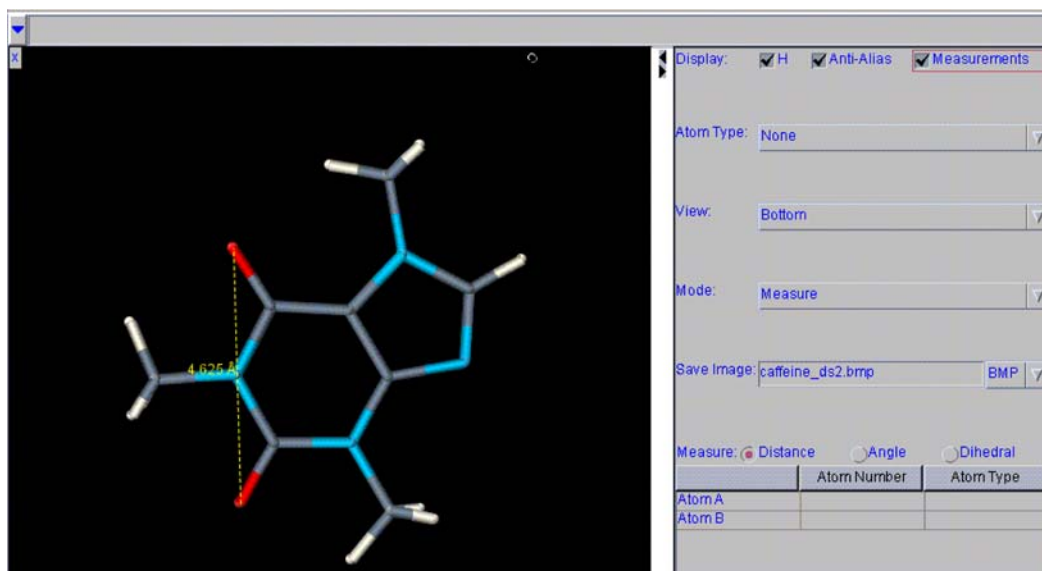
- 1 Click **Tools** on the main menu.
- 2 Select **Browser**, browse to the mollib directory:
`/vnmr/mollib` or `~username/vnmrsys/mollib`
- 3 Select the appropriate files and drag them to the VnmrJ graphics window:
 - Molecule file -- select a file from the mollib directory that ends in the mol extension.
 - Graphic file -- open the icons directory and select a **TIFF, GIF, JPEG, or PNG** file.

After a molecular structure is displayed, use the mouse to move, resize, delete, or view the corresponding 3D version with Jmol.

To:	Do:
Select	Double-click the molecule.
Move	Select the molecule and drag with the left mouse button.
Resize	Select the molecule and drag with the middle mouse button.
Delete	Select the molecule and drag to the trash can.
View a molecule with Jmol	Select the molecule and click the right mouse button. This only works with MOL files. See Jmol Interface in <i>VnmrJ Jmol Interface in VnmrJ</i> .

Jmol interface in VnmrJ

VnmrJ provides some Jmol tools to view a molecule.



Menu	Description
Display	H– Displays hydrogen atoms. Anti-Alias – Turns on anti-aliasing and smooths the graphics display. Measurements – Displays measurements.
Atom Type	Displays the atoms with atomic symbols, atom types, atom numbers, or nothing.
View	Views the molecule from front, top, bottom, right, or left.
Mode	Rotate – rotates the image. Zoom – zooms in/out. Translate – moves the image. Select – selects the atoms. Measure– measures distance, angle, or dihedral. See “Measuring a molecule” on page 222.
Save Image	Saves the molecule image as BMP, JPEG, PPM, PNG, or PDF. The image is saved in the directory <code>~username/vnmrsys/mollib/icons</code> with the name entered in the field. See “Saving a molecule image” on page 222.

Measuring a molecule

- 1 Select the measure mode: distance, angle, dihedral.
- 2 Click the appropriate atoms to create the measurement:
 - distance – click two atoms.
 - angle – click three atoms
 - dihedral – click four atoms
- 3 Display the measurement by selecting the **Measurement** display option.

Saving a molecule image


- 1 Select the file format for the image: **BMP, JPG, PPM, PNG, or PDF.**
- 2 Enter a name for the image and add a file extension that corresponds to the file format chosen in step 1.
- 3 Press **Enter**.

The file is saved in the directory
~username/vnmrsys/mollib/icons/.

Jmol display options

- Change the foreground color of the molecule window: enter the following command on the VnmrJ command line:

```
vnmrjcmd('mol', 'foreground', 'color')
```

where *color* is a color name or a hex value. The foreground color by default is set to the most visible color according to the background color.
- Change the font of the labels on 3D molecule graphics: use the **Edit > Display Options** window and change the font of Plain Text.
- Click  to exit Jmol.

Full-Featured Jmol

Select **Tools > Jmol** to view a molecule with the full-featured Jmol software package,

See <http://jmol.sourceforge.net/> for Jmol documentation.

Licenses for JChemPaint and Jmol

The licenses for JChemPaint and Jmol are included on the VnmrJ CD in the licenses directory.

8 Displaying FIDs and Spectra



9 Printing, Plotting, and Data Output

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This chapter describes how users can print and plot data.



Printing Graphics

After processing, the 1D or 2D spectrum is displayed in the graphics canvas so that the scale, expansion, and threshold can be adjusted.

To print the spectrum:

- 1 Click **File** from the menu.
- 2 Select **Print Screen**.

The pop-up in [Figure 67](#) appears.

- 3 Select the **name** of the printer to print to it.
- 4 Select the print area, either **Viewports** or **VnmrJ Window**. Viewport will capture the contents of the Viewport, while VnmrJ Window will capture the entire VnmrJ window.
- 5 Choose the **number of copies** to print.
- 6 Click the **Print** or the **Preview** button.

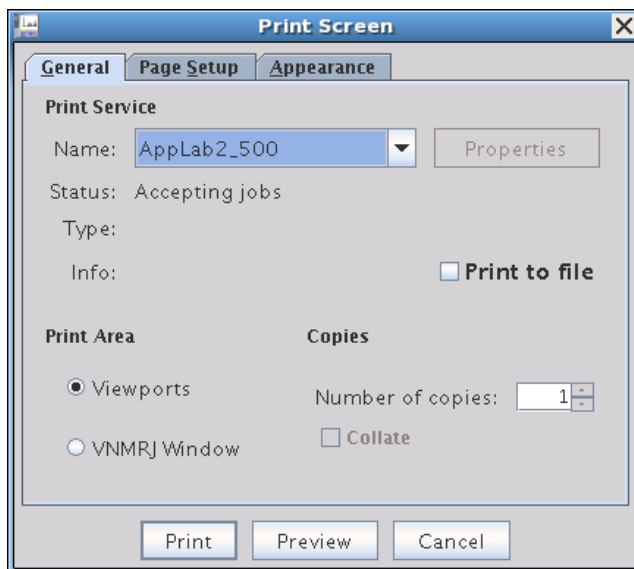


Figure 67 General tab of the Print Screen pop-up

Printing to a file

Select the **Print to file** check box. [Figure 68](#) shows the pop-up that appears when the **Print to file** check box is selected and the **Print** button is clicked.

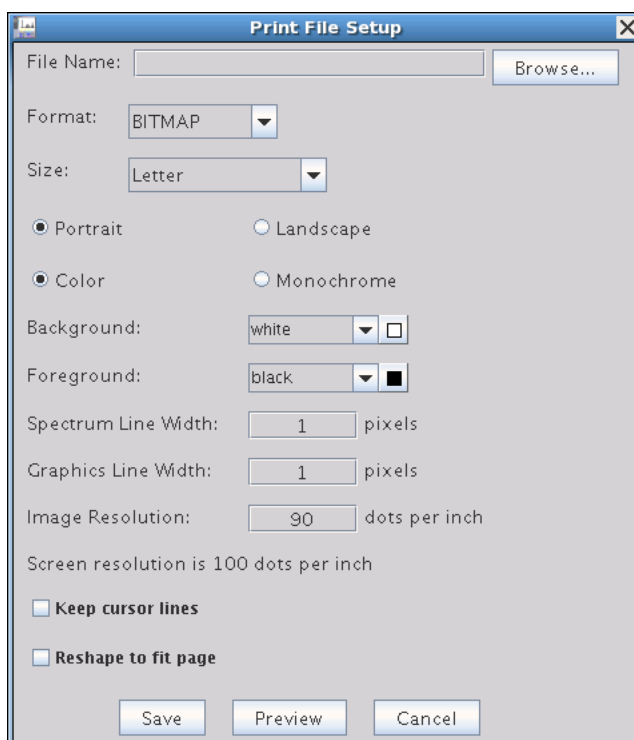


Figure 68 The Print to File pop-up

- **File Name:** name of the file to be saved.
- **Browse...:** allows one to browse to a folder to set the location where the file will be saved.
- **Format:** drop-down menu that offers a choice of various outputs.
- **Size:** drop-down menu that offers choice for paper size.
- **Orientation selection:** radio-button to set Portrait or Landscape.
- **Graphics color:** radio-button to select Color or Monochrome output.
- **Background and Foreground color selection:** Pull-down menus for various color choices.
- **Spectrum Line Width:** spectrum line width in pixels.
- **Graphics Line Width:** graphics line width in pixels.
- **Image Resolution:** image resolution in dots per inch.

- **Screen resolution:** the current screen resolution in dots per inch.
- **Keep cursor lines:** check box to include cursor lines as they appear in the graphics area.
- **Reshape graphics to fit paper:** check box to resize graphics to fit paper size.
- **Save:** saves output to File Name.
- **Preview:** starts Adobe Acrobat Reader and displays (without saving) the output.
- **Cancel:** closes Print to File pop-up (without saving).

The **Page Setup** and **Appearance** tabs (Figure 69 and Figure 70) allow for settings similar to the **Print File Setup** to be applied to the printed output. Additionally, entries are available for paper source, margins, and print quality (Draft, Normal, and High) while finer control of image resolution and preview is unavailable.

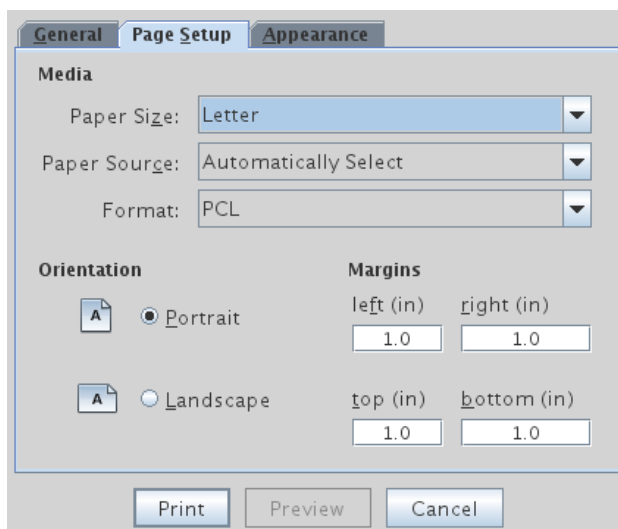


Figure 69 The Page Setup tab of the Print Screen pop-up

- **Paper Source:** drop-down menu choices for tray a printer will use.
- **Margins:** margins on the sides of the page in inches (in).

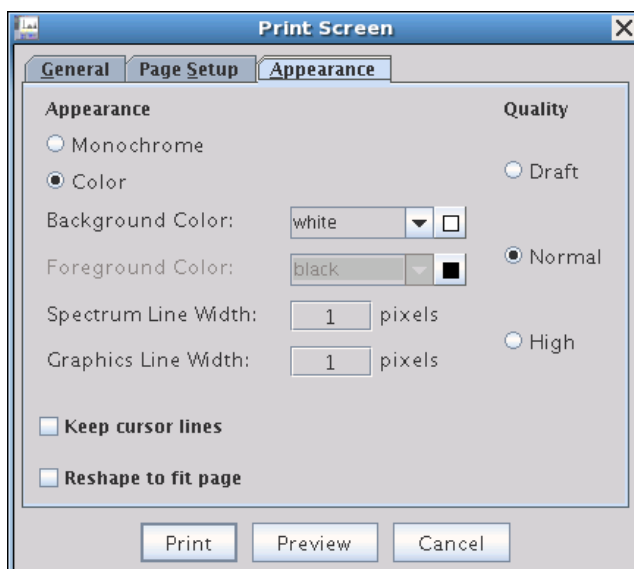


Figure 70 The Appearance Setup tab of the Print Screen pop-up

- **Quality:** a choice of Draft, Normal, and High.

Plotting

Setting up and submitting a plot can be done from the vertical and horizontal panels and from the command line.

An example of a 2-page plot output is shown in [Figure 71](#) and [Figure 72](#), with various options shown. You can customize the plot to include the information most important to you.

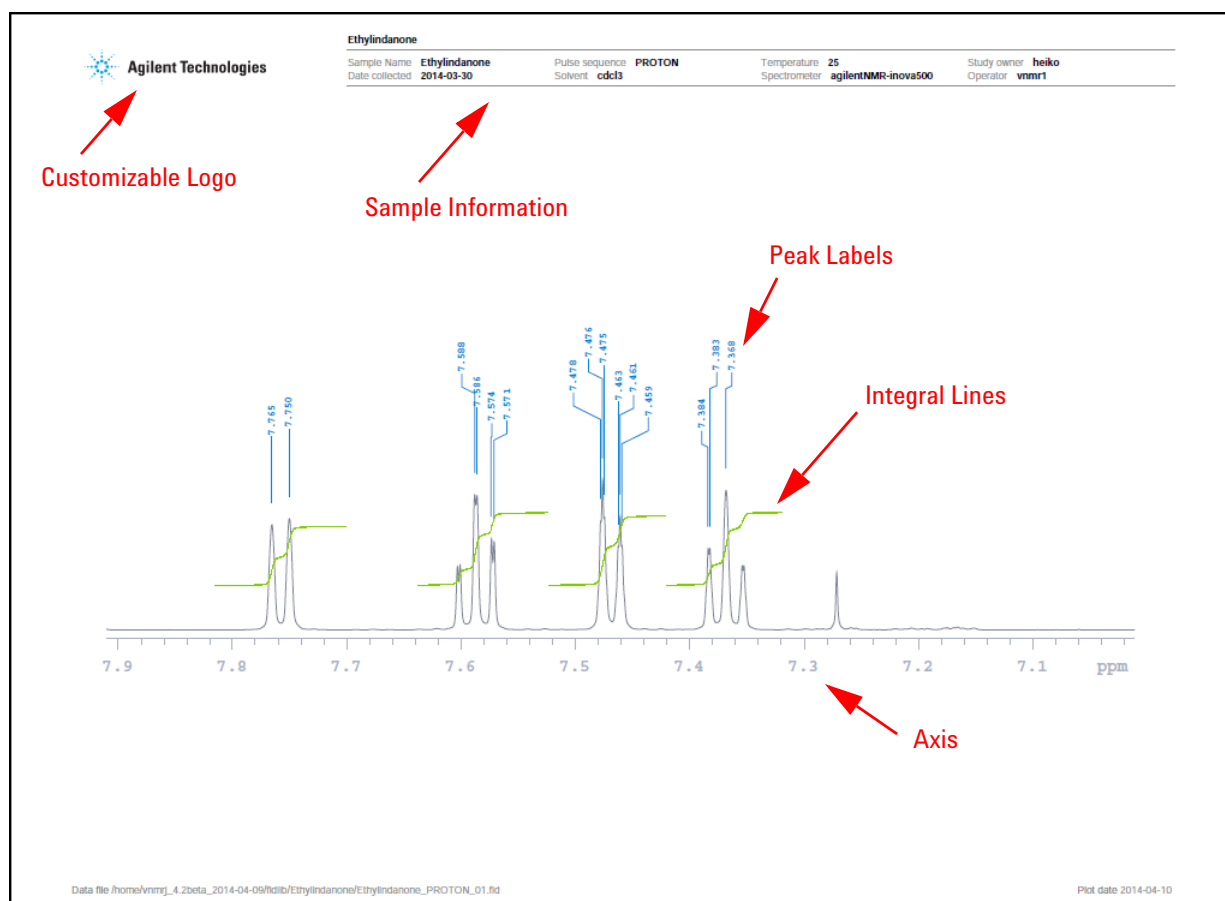


Figure 71 Example of a 1D spectrum plot output with options identified - page 1

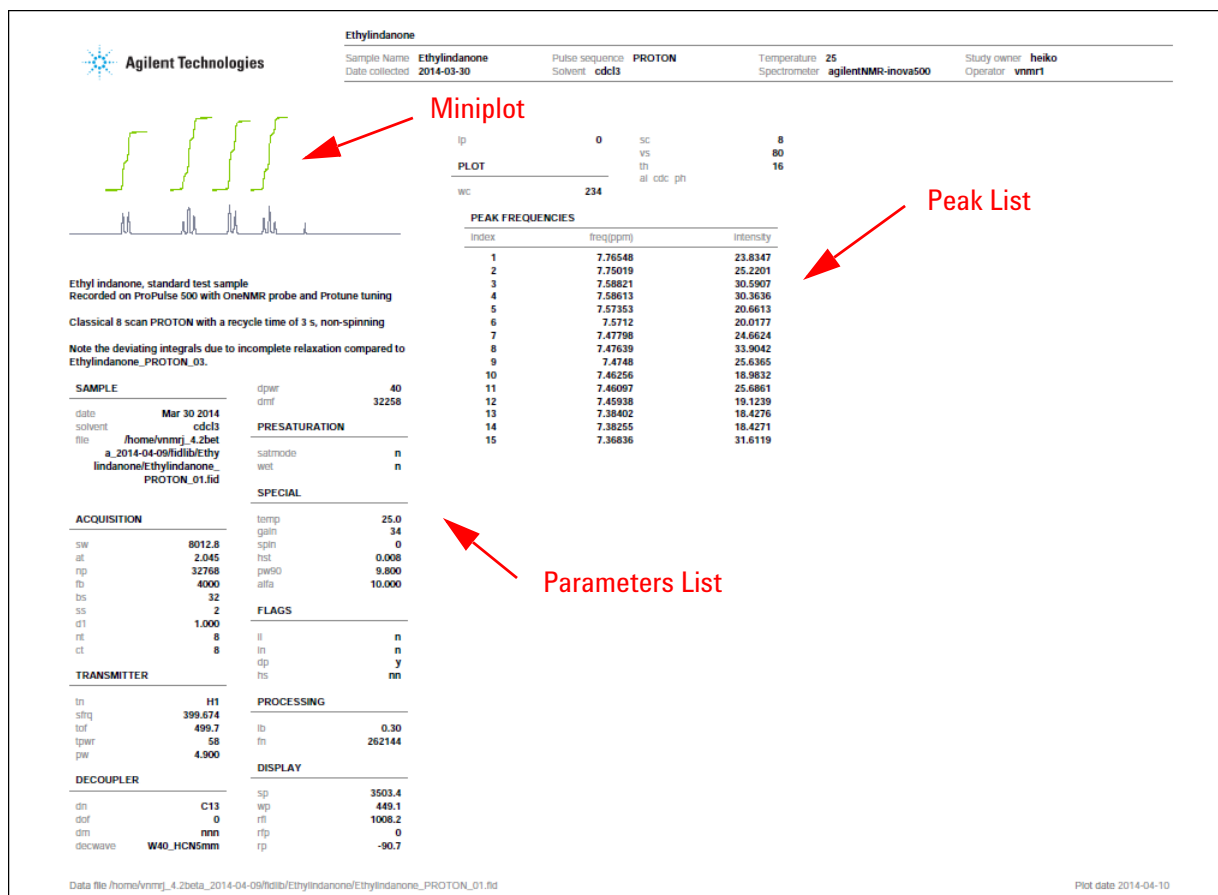


Figure 72 Example of a 1D spectrum plot output with options identified - page 2

Plotting from the ProcessPlot vertical panel

The **ProcessPlot** vertical panel (Figure 73 on page 232) contains four buttons in the **Plot** group.

- **Auto Plot Page** sends the plot to the selected printer. (This is the same function as the **Auto Plot** button in the Process > Basic parameter panel – executes the plot macro; then the resetplotter macro.)
- **Auto Plot Preview** button opens the plot for preview in Adobe Reader. This is the same function as the **Auto Preview** button in the Process > Basic parameter panel – executes the macro pageview('auto') to generate the output.

- **Print Screen** button does the same function as **File > Print Screen** command, which opens the Print Screen window, where you can setup and preview the current viewports or VnmrJ window.
- **More plotting – parameter pages** button opens the **Plot** parameter panel, where more plotting options are available. See “[Plotting from the Plot parameter panel](#)” on page 233.

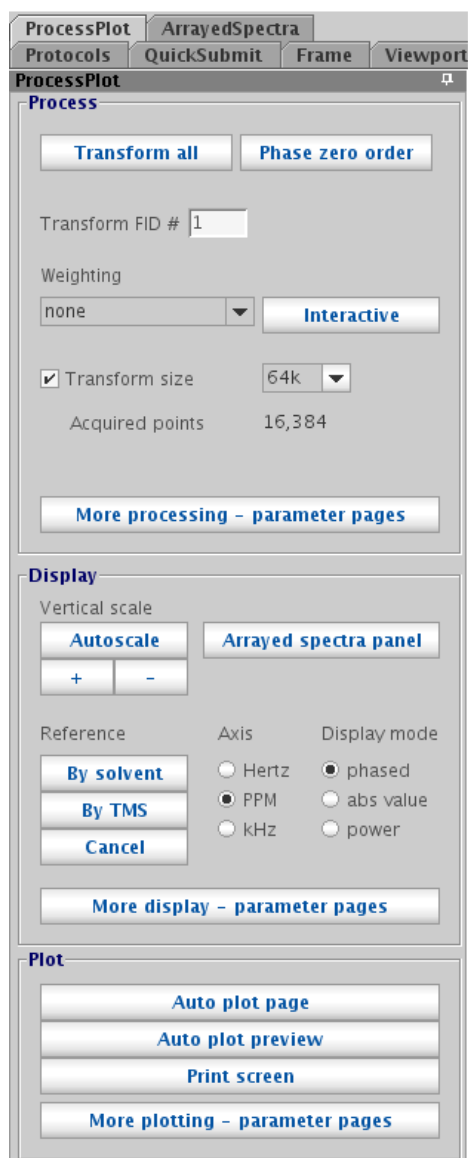


Figure 73 The ProcessPlot vertical panel - 1D

Plotting from the Plot parameter panel

The **Plot** parameter page is accessed from the **Process** tab after the spectrum or FID is displayed. Plot options selected on the **Plot** and **Basic** parameter pages (Figure 75 on page 234 and Figure 78 on page 236) are added to a temporary plot file, and the **Print** button submits the plot file to the selected plotter.

The **Preview** button starts Adobe Reader (Figure 76) and displays the output of the plot file. Some of the menu options in Adobe Reader are available for use. The **Print** button executes "doplotoption page" which prints the manually chosen plot options and the **Preview** button executes "doplotoption pageview" which generates a preview of manual printing options. The Plot View pop-up (Figure 77) appears during a preview, which allows the saving of the view to a pdf format file, sending the output to the default plotter, a file, and to one or more e-mail addresses.

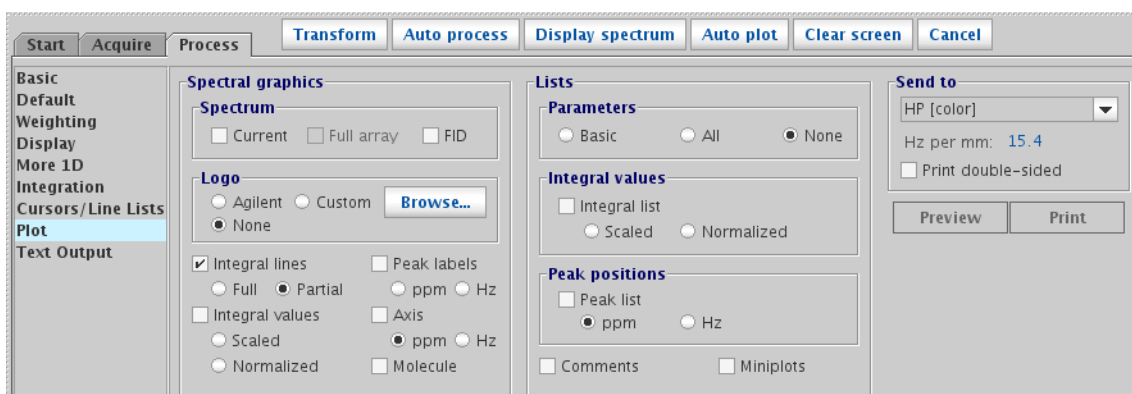


Figure 74 The Plot parameter page for a 1D data set

The Plot parameter page for 1D data contains options for setting up the 1D plot. For a description of the options contained in this panel, see the *Agilent VnmrJ 4 Familiarization Guide*.

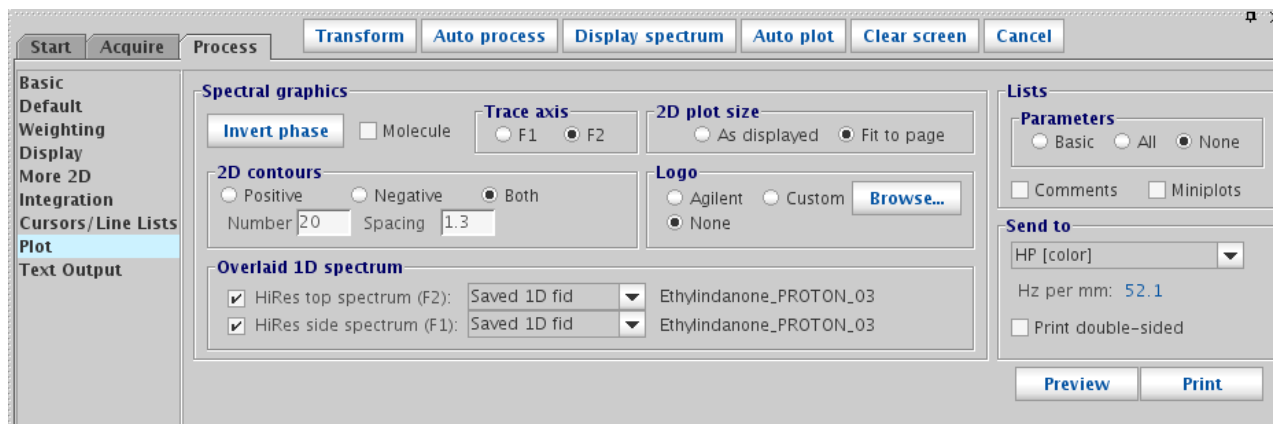


Figure 75 The Plot parameter page for a 2D data set

The **Plot** parameter page for a 2D data set enables you to set options for plotting the 2D data for descriptions of each option shown, see the *Agilent VnmrJ 4 Familiarization Guide*.

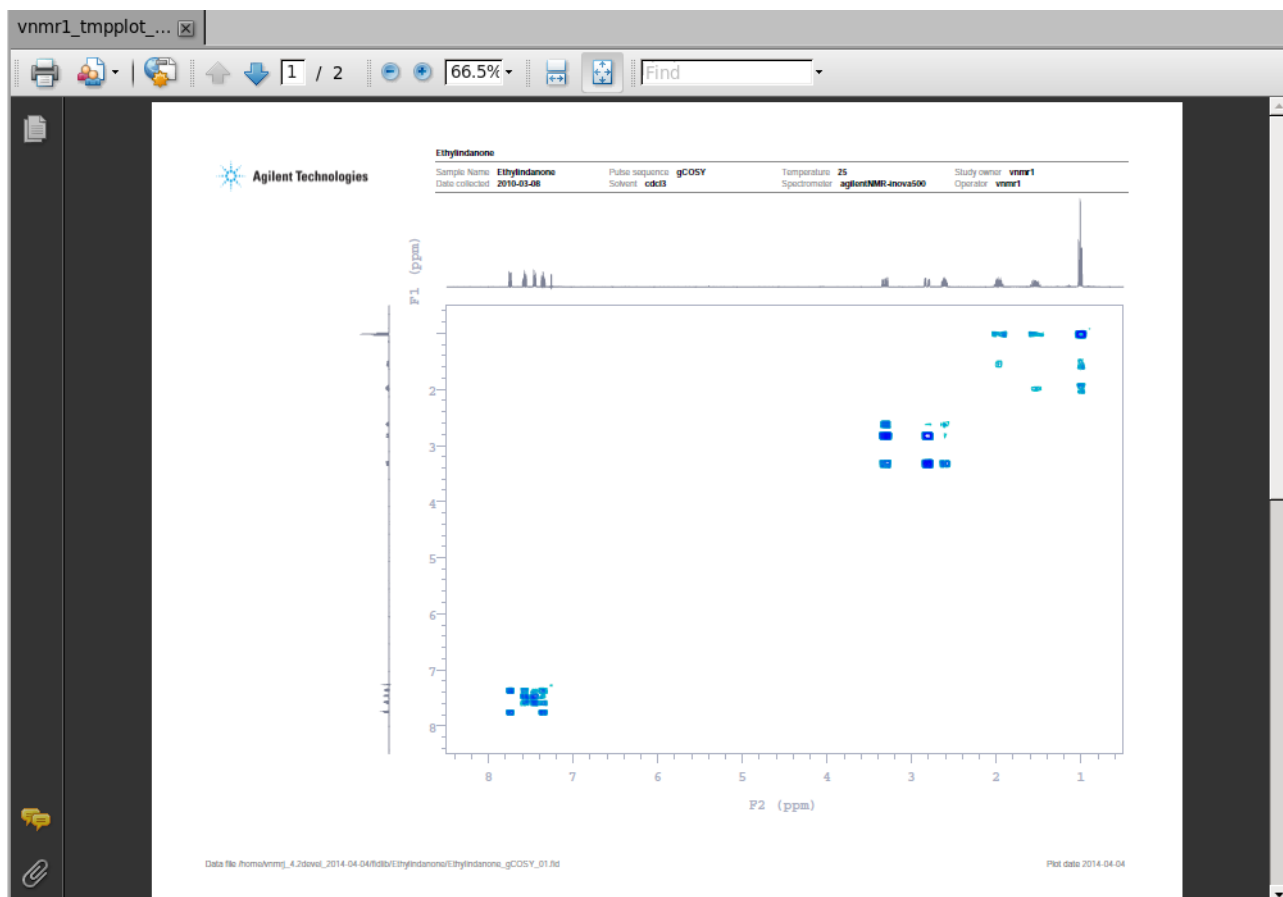


Figure 76 Preview of a 2D plot file initiated from clicking the Preview button

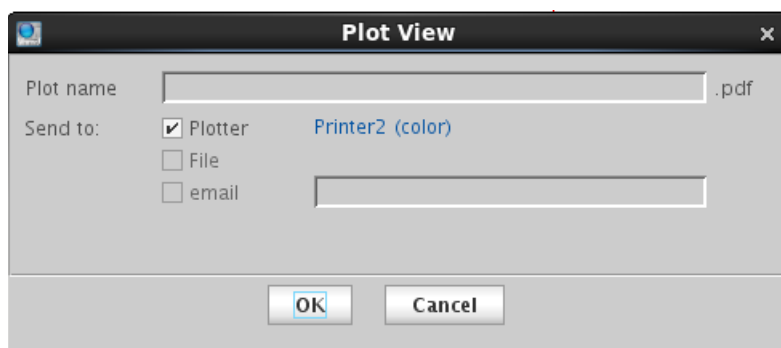


Figure 77 The Plot View pop-up

The **Basic** parameter page (Figure 78) contains **Auto Plot** and **Auto Preview** buttons; both have the same function as the **Print** and **Preview** buttons found on the **Plot** parameter page.



Figure 78 The Basic parameter page for a 1D data set

Using the Plot Designer

NOTE

Due to new advanced annotation functions added to VnmrJ, the Plot Designer function has been deprecated. The Plot Designer functions are still available by typing `jdesign` in the command line.

Plot designer provides the following tools:

- Interactive plot composition fine-tuning of the layout on the screen prior to plotting.
- Label spectra with text in various fonts and draw lines, boxes, and arrows.
- Save customer plot layouts and templates for reuse.
- Export plots for further annotation and incorporation into reports and publications.

System requirements

Plot Designer is a Java-based application. The Java Runtime Environment (JRE) provides an environment in which Java applications run. Any required updates are available from the update area of the Sun Microsystems Web site at <http://www.sun.com>.

Using plot designer

Select a viewport and process the data set for plotting.

Start the Plot Designer program as follows:

- 1 In the command line, type `jdesign` then press Enter.

The **Plot Designer** window opens. See [Figure 79](#).

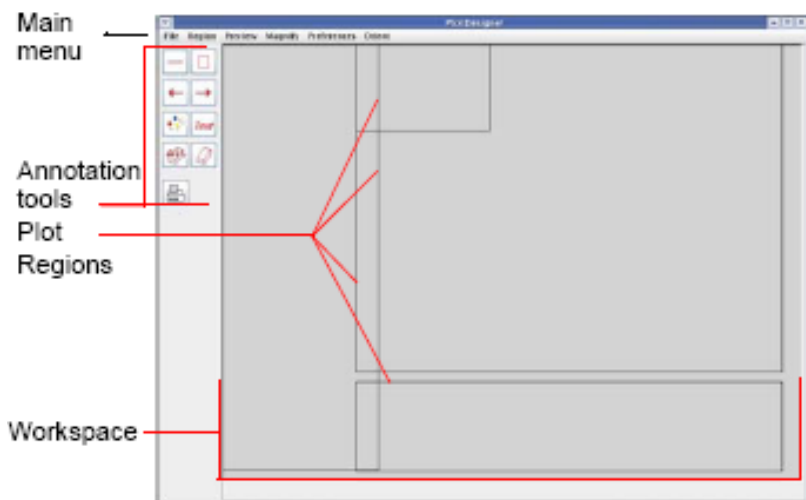


Figure 79 Plot Designer with current default template

- 2 Load a template:
 - a Click **File**.
 - b Click **Templates**.
 - c Select from the following standard templates or any custom user-created template:

deptB	dicom.default	chemParray	chemP1d
basic2D	oneD	Whitewash	ChemP2d

- d Select **Use this template as default** to keep this as the template that loads each time Plot Designer is started. The name of the default template is shown on the message line above the **Plot Templates** window control buttons.
 - e Click **Open**.
- 3 Select **Preview** from the **Plot Designer** menu.
- 4 Select **All**.

The data from the active viewport is imported into the various regions of the template based upon the commands associated with each region.

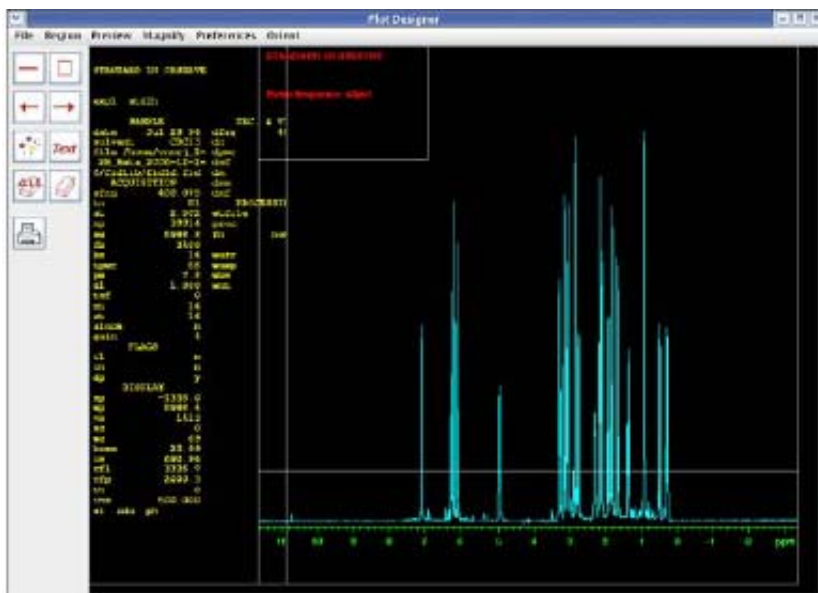


Figure 80 Default oneD template with imported data

Creating a customized template

Create a template from scratch or base a customized template on one of the supplied templates.

To prepare to customize a template:

- 1 Start Plot Designer.
- 2 Click **File**.
- 3 Select **Create a Plot Design**.
- 4 Load a template or use the default template.
- 5 Do any or all of the following:
 - Click a region to delete. See [“Deleting a region”](#) on page 240.
 - Delete all the regions. See [“Clearing all regions from the workspace permanently”](#) on page 240.
 - Add a new region. See [“Adding a region”](#) on page 241.
 - Edit an existing region: double on a region, click **Region** on main menu, select **Edit**, and enter the plotting command for the content of the selected region. See [“Editing plotting commands in a new or existing plot region”](#) on page 241
 - Add text and graphics elements: see [“Adding and editing text and graphics elements”](#) on page 242.

Deleting a region

- 1 Double-click a region.
- 2 Click **Region-Delete**.

Restoring a single deleted region

Click **Region-Undelete**.

Clearing all regions from the workspace permanently

Click **Delete All**: no undelete. Regions removed with Delete All cannot be restored with Undelete.

Adding a region

- 1 Click **Region** on main menu.
- 2 Select **New** (mouse cursor changes to a cross hair).
- 3 Draw the new region on the screen.

Editing plotting commands in a new or existing plot region

- 1 Double-click a region to make it active. Active regions have red borders with control handles.
- 2 Enter new plotting commands or edit existing plotting commands in the region editor window. Any plotting command currently support plotting command is allowed.

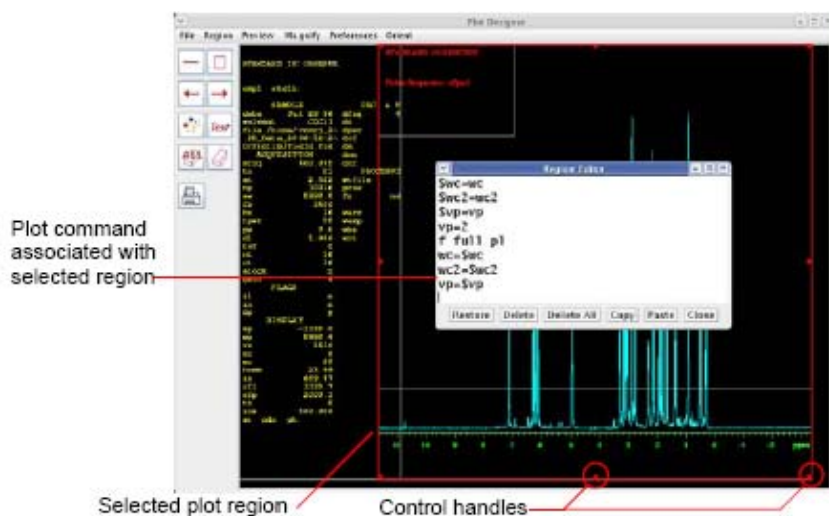


Figure 81 Editing a plot region commands

The **Region Editor** window control buttons as listed in [Table 15](#) are found.

Table 15 Region editor buttons

Button	Function
Restore	Applies the original template to a region. Restores template to its original design if it was opened and changes were made to it, using this button.
Delete	Removes text. This option is not similar to Copy. Deleted text is not stored in a buffer. Do not use Delete to cut and paste text.
Delete all	Clears all text from the input area.
Copy	Duplicates text.

Table 15 Region editor buttons (continued)

Button	Function
Paste	Inserts copied text in the input area.
Close	Exits the Region Editor.

Resizing and moving plot regions and objects


Move an object or region by double-clicking on it and dragging the mouse across the workspace. The arrow keys can be used to move objects.

Resize a region by double-clicking on it, grabbing a control handle (see [Figure 81](#)) on the border, and dragging it to the new size.

Adding and editing text and graphics elements

Change the size and color of objects in a region with the **Item Preferences** window, shown in [Figure 82](#).

**Figure 82** Item preferences window

Click **Region-Preferences** to open this window or click the **Item Preferences** icon , described in “[Plot designer tools](#)” on page 245.


Changing line width

Change the width of a line by doing the following procedure:

- 1 Highlight the line or region by double-clicking on it.
- 2 Enter a new width in the Line Width field.
- 3 Click **Apply** to change the line.
- 4 Click anywhere in the workspace to deselect the line.


Changing fonts

Plot Designer has three font families: Sans Serif, Monospace, and Serif. Fonts can be Plain, Bold, or Italic. Change the family, style, and size of a font as follows:

- 1 Highlight the text or region.
- 2 Click the **Item Preferences** tool  to open the **Item Preferences** window.
- 3 Choose a family, style, and enter a size in the Font field.
- 4 Click **Apply** to change the text.

Changing colors

Change the color of a line by doing the following:


- 1 Highlight the line or region.
- 2 Click the **color** button  to open a pop-up menu showing a range of colors.

Move the tuning slider either left or right to change a color, or change a color by clicking on the left or right arrows in the Red, Green, and Blue fields. The values in the Color (RGB) field automatically change as the slider moves.


- 3 Click **Apply** when the required colors are selected.
- 4 Place the cursor anywhere in the workspace and click once to apply the color change.

Adding text

Do the following to add text into your design:

- 1 Click the text input tool  to open the text input window.
- 2 Type text in the field at the top of the window.
- 3 Customize the text by clicking on the desired options and entering a font size in the indicated field.
- 4 Click **Put** and drag the cursor into the workspace, then click once to paste in the text.

Use the following procedure to copy and paste text that is already on the workspace and change the font styles:

- 1 Highlight the text.
- 2 Click the text input tool  to open the **Text Input** window shown in [Figure 82](#).
- 3 Select a Font family and Font style, and enter a Font size.
- 4 Click **Put** to paste the text in the workspace.

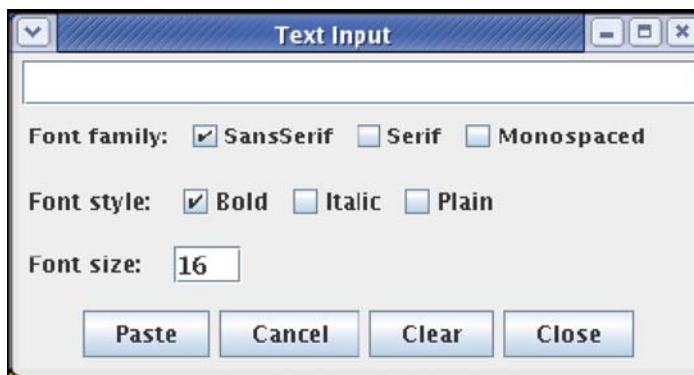


Figure 83 Text input window

Changing font color

Repeat the procedure given in [“Changing colors”](#) on page 243 to change font colors.

Saving a custom template

Save the custom a template as follows:

- 1 Click **File**.
- 2 Select **Templates** to open the **Plot Templates** window.
- 3 Enter a name in the **Template** field.

Optional: Select the box next to **Use this template as default** to make the file the default template. The default template is automatically loaded when Plot Designer is started.

- 4 Click **Save** to store the template in `$vnmruser/templates/plot` directory.

A warning is displayed if the saved template overwrites a current template.

- 5 Click **Cancel** to not replace the file.
- 6 Quit the **Plot Templates** window by clicking on **Close**.

Plot designer tools

The Plot designer tools are listed in [Table 16](#). Press and hold down the left mouse button and drag the cursor in the workspace to use a drawing tool.

Table 16 Plot designer tools








Icon	Function	Description
	Line Drawing	Draws a line
	Box	Draws a box
	Arrows	Draws an arrowhead and places it at the origin of the line. Draws an arrowhead and places it at the point of the line
	Item Preferences	Sets the color and size of lines and fonts. Select an object to edit by double-clicking on it. See “Adding and editing text and graphics elements” on page 242 for a description of its properties.
	Text Input	Adds text into the design and controls the size and appearance of the text. See “Adding text” on page 244.

Table 16 Plot designer tools (continued)

Icon	Function	Description
	Erasers	The ALL eraser removes all objects The eraser tool removes selected objects. See also “Adding and editing text and graphics elements” on page 242, “Adding a region” on page 241, and “Deleting a region” on page 240.
	Print	Prints a file

Changing an aspect or property of plot designer

- 1 Click **Preferences** from the main menu.
- 2 Select **Set Up** to open the Workspace Preferences panel.



- 3 Click the corresponding button to open a pull-down menu.
- 4 Select a color preference.
- 5 Click **Apply** to execute the changes.
- 6 Click **Close** to exit the window.

Table 17 Workspace preference controls

Control	Function
Background	Changes the background color of the window
Border Color	Changes the color of the border surrounding the workspace
Highlight Color	Color of an object after double-clicking on an object to indicates that it is selected
Grid Color	Changes the color of the grid
Plotter	Selects a black and white or color plotter
Border	Shows (on) and hides (off) region borders
Grid	Shows (on) and hides (off) grid in the workspace
Snap	The center of the border of an object snaps to the grid when an object is created or moved if snap is turned ON . Turn Snap OFF to disable this feature.
Snap Spacing	Controls the amount of space on the grid to which an object snaps. Spacing is in inches, centimeters, or points.

Changing the shape of the plot designer window

Plot Designer can be viewed in two orientations- Landscape or Portrait (which is the default orientation). Change the shape of the **Plot Designer** window in the **Orientation** menu.

Changing the size of the plot designer window

Increase or decrease the size of the **Plot Designer** window by clicking on the sizes listed in the **Magnify** menu.

Saving a Plot File

Do the following procedure to save a plot:

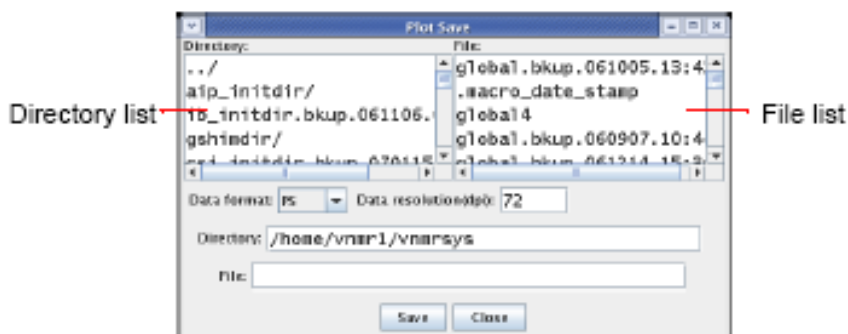


Figure 84 Plot save window

- 1 Click **File** in the **Main Menu**, then **Save Data** to open the **Plot Save** window shown in [Figure 84](#).
- 2 Scroll down the list of directories and choose a directory or enter a path for the file in the Directory field.
- 3 Select a **Data format** for your file and enter a **Data resolution**. [Table 18](#) lists the formats that are available.

Table 18 Output formats supported by plot designer

Format	Description
AVS	AVS X image file
BMP	Microsoft Windows bitmap image file
EPS	Adobe Encapsulated PostScript file
FAX	Group 3 FAX
FITS	Flexible Image Transport System
GIF	Compuserve Graphics Interchange Format (version 89a)
GIF87	Compuserve Graphics Interchange Format (version 87a)
JPEG	Compressed format from Joint Photographic Experts Group
MIFF	Magick image file format
PCD	Photo CD
PCX	ZSoft IBM PC Paintbrush file
PDF	Portable Document Format
PICT	Apple Macintosh QuickDraw/PICT file
PGM	Portable gray map
PNG	Portable Network Graphics
PS	Adobe PostScript file
PS2	Adobe Level II PostScript file
SGI	Irix RGB image file
SUN	Sun Rasterfile
TGA	Truevision Targa image file
TIFF	Tagged Image File Format
VIFF	Khoros Visualization image file
XBM	X11 bitmap file
XPM	X11 pixmap file
XWD	X Window System window dump image file

- 4 Label your file by entering a name in the File field.
- 5 Click **Close** to exit the window.

Printing a plot

Click the print tool.




Exiting plot designer

Click **File-Quit**.

Any design in the window when Plot Designer is closed is automatically opened in the workspace the next time the program is started.

Color Printing and Plotting

Printer and Plotter color output is defined using the **Styles and Themes** pop-up, which provides access to the VnmrJ interface, display, and plot colors. To open the Styles and Themes pop-up, click the Display Options icon  or select **Display Options from the Edit menu**.

Setting colors

View the current settings or define new color settings as follows:

- 1 Click **Edit**.
- 2 Select **Display options**.

OR, click the Display Options icon .

The **Style and Themes** pop-up opens. See [Figure 85](#).

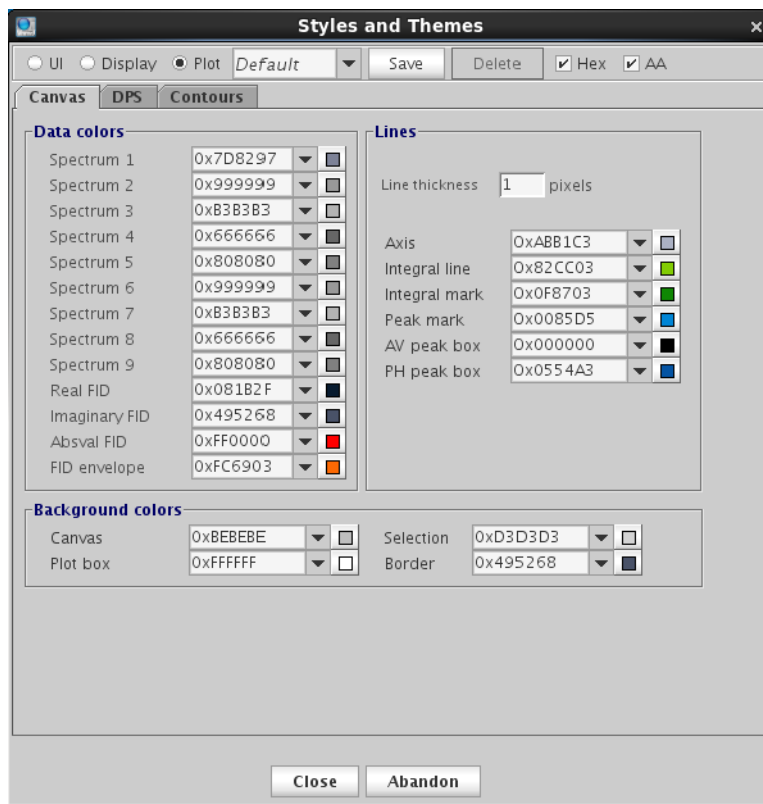


Figure 85 Styles and Themes Plot tab- 1D and 2D

- 3 Select **Plot** to set the colors and options for plotting data (Canvas), pulse sequence (DPS), and contours.
- 4 In the drop-down menu, select a theme (additional themes only appear if they were previously saved).
- 5 Select a color or keep the current color for each display or function shown.
- 6 Click the **Contours** tab to select colors for each contour level.

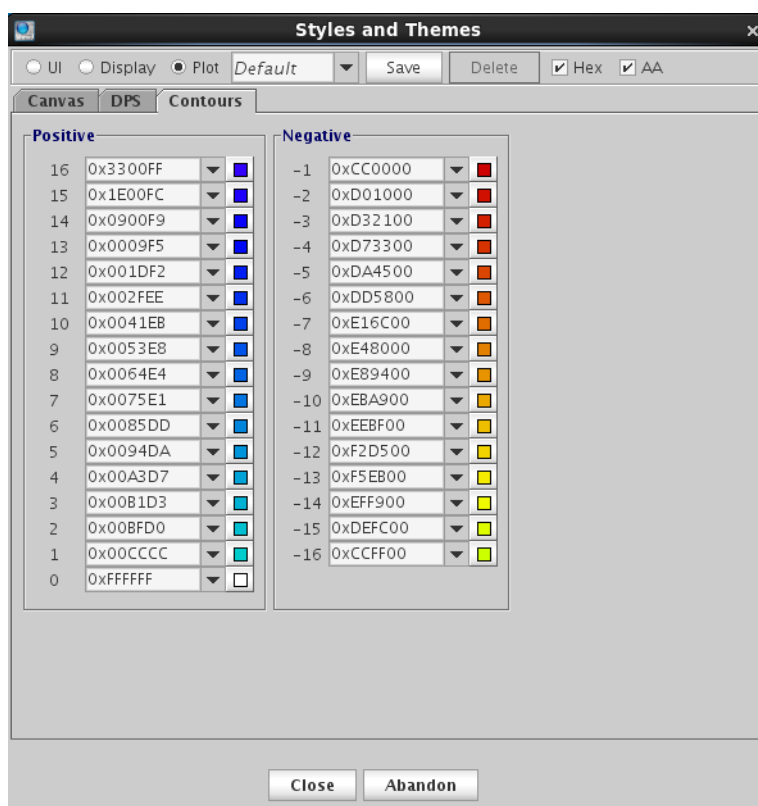


Figure 86 Contours tab for selection of contour colors

- 7 Click **Save** to save the color selections to the selected theme.
OR, highlight the name in the drop-down menu next to the **Save** button and type a name. Then click **Save** to save the current color theme file using a new name.

Loading a color theme

To retrieve a color theme:

- 1 Select a theme file from the drop-down menu.

Changing or renaming a color theme

To change the colors in a file:

- 1 Select a theme from the drop-down menu.
- 2 Follow the procedure in [“Setting colors”](#) on page 250.
- 3 Click **Save** to save the theme.

To change the name of a color theme:

- 1 Select a theme from the drop-down menu.
- 2 Highlight the theme name, and then type a new name.
- 3 Click **Save** to save the file.
- 4 Optional: To delete the theme with the old name, see [“Removing a color theme”](#) on page 252.

Removing a color theme

To remove a color theme from the list:

- 1 Select a color theme from the drop-down menu.
- 2 Click **Delete**.

The deleted file is removed from the drop-down menu.

NOTE

You cannot delete the default theme.

Sending a Plot Via Email

After setting the plot options in the **Plot** parameter page, click **Preview**.

- 1 Enter a **Plot name** in the Plot View pop-up.
- 2 Select **File**.
- 3 Select **email**.
- 4 Type valid email addresses in the email entry field.
- 5 Click **OK**.

There are advanced macros that can be issued from the command line (`eplot`, `epage`, `espec`, `fplot`, `fpage`, `efid`, `esampledire`). These can be used to send the output to an email address. See the *VnmrJ Command and Parameter Reference Guide* for details on the usage of the macros.

Advanced Printing Commands

Printing from within VnmrJ is initiated with the `printon` command. Once the `printon` command is issued, all further output that normally appears in the text output window is redirected to a temporary file and no further output will appear in the text output window. When the `printoff` command is issued, the temporary file is sent to a printer, the file is purged from the system, and future text output is resumed to the text output window. This output includes the following:

- Parameter listings from `dg`, `dgl`, `da`, etc.
- Line listings from `dll`.
- Integral listings from `dli`.
- System configuration parameters generated by `config ('display')`.
- Text files using the `text` command.
- Results of calculations from `h2cal`, `adept`, `t1`, `t2`, etc.
- Any other information that some program or macro may write to the text window.

For descriptions of commands and macros in VnmrJ, see the *Agilent VnmrJ Command and Parameter Reference Guide*.

This output is saved in a temporary file in the VnmrJ subdirectory `tmp`. The VnmrJ parameter printer determines the printer to which the output is directed. When the `printoff` command is issued, VnmrJ executes a UNIX script called `vnmrprint` that sends the temporary file to the printer using standard UNIX printing utilities. This script is supplied with the name of the temporary file to be printed, the name of the printer (corresponding to a `printcap` entry), and the type of printer (corresponding to a `devicetable` entry).

NOTE

The `devicetable` information is used to distinguish PostScript printers. The `vnmrprint` script allows users to customize.

The macro `ptext(file)` prints out the text file given as an argument. For example, the command `ptext('/vnmr/psglib/DEPT.c')` prints the text file `DEPT.c`.

Print jobs for the currently active printer in VnmrJ are held in a print queue. The `showprintq` macro displays the current

print jobs in the print queue. The `killprint` macro will stop a print job and remove it from the print queue. Unless the user executing this macro is `root` (superuser), only that the user's print job is deleted from the print queue.

Table 19 Printer-Associated commands and parameters

Commands	
<code>killprint</code>	Stop print job and remove from print queue
<code>printoff<('clear' file)></code>	Stop sending text to printer and start print operation
<code>printon</code>	Direct text output to printer
<code>p_{text} (file)</code>	Print out a text file
<code>showplotter</code>	Display currently defined plotters and printers
<code>showprintq</code>	Display print jobs in print queue
<code>vnmrprint*</code>	Print text files (UNIX)
* <code>vnmrprint printfile <printcap> <printer_type <clear file>></code>	
Parameter	
<code>printer {string}</code>	Printer device

Advanced Plotting Commands

This section describes advanced plotting commands available in VnmrJ. For details on individual commands and macros, see the *Agilent VnmrJ Command and Parameter Reference Guide*.

Spectral plotting

The `pl` command plots the currently displayed region of the currently active spectrum, or spectrum plus integral (or the region which would be displayed if there were a spectral display on the screen). `pl('int')` plots the integral only. `pl('pen2')` plots the spectrum using pen number 2 of a multi-pen plotter.

The `pscale` command plots a scale under a spectrum. The syntax is:

```
pscale<(<axis><,vertical_start><,plot_start><,pen>>>
```

If the letter `p`, `h`, `k`, etc. is supplied as an optional argument for `axis` that is used instead of the current value of the parameter `axis`, the optional argument `vertical_start` defines the vertical position where the scale is drawn (the default is 5 mm below the current value of the parameter `vp`). The second optional argument `plot_start` is interpreted as a modified start of plot. The `pen` option defines the pen number to be used.

The `ppf` command plots peak frequencies in units specified by the `axis` parameter above the peaks, selecting only those peaks greater than `th` high. `ppf('noll')` plots peak frequencies using the last previous line listing while `ppf('pos')` plots only positive peaks. Other arguments for noise suppression (`noise_mult`) and label positioning work the same as the `dpr` command.

The `p11` command produces a columnar line list on a plotter, similar to what would appear on a printer. The output is automatically formatted into multiple columns, depending on the number of lines. The syntax is `p11<(x,y,minimum_y)>`. The arguments `x` and `y` are the `x` and `y` position of the upper left of the line list, and `minimum_y` is the minimum `y` at which to reset back to the top.

The `plh` command plots a proton spectrum based on parameters `pltmod` and `intmod`:

```
pltmod='off' sets no plotting.
```


`pltmod='fixed'` takes `sp` and `wp` as is.

`pltmod='full'` adjusts `sp` and `wp` to plot the full spectrum.

`pltmod='variable'` adjusts `sp` and `wp` to plot only the region of interest.

`intmod='off'` gives no integral.

`intmod='partial'` gives a series of integrals over each region.

`intmod='full'` gives a single integral over the entire spectrum.

Given a spectrum divided into regions by the `region` command or by the cursors in the `ds` program, the macro `aexppl<(expansion_factor)>` automatically plots each region at the horizontal scale requested (in Hz/ mm). The default scale is 2 Hz/ mm.

Several generic plotting macros, such as `plot` and `plot1d`, are available that call specialized plotting macros, depending on the user definition or other wise on the type of data in the experiment. For details, see the *Agilent VnmrJ Command and Parameter Reference Guide*.

Display limits

Because of the use of different plotters with different dimensions, the parameters `sc`, `wc`, `sc2`, and `wc2` need to be set differently to position plots and displays in the same relative position on the page. The `full`, `center`, `left`, and `right` commands do nothing more than modify `sc`, `wc`, `sc2`, and `wc2` to place the display and plot in the desired portion of the screen and page. The `f` command is used to set the `sp` and `wp` parameters to display a full spectrum. The `zoom(width)` macro adjusts the display limits to the width specified, in Hz, setting the limits to $\pm \text{width}/2$. Also available is the `split` macro, which repositions the left-hand cursor halfway between its original position and the position of the left cursor.

A scaling factor helpful for 1D plotting is the `hzmm` parameter, which contains the quotient of `wp` divided by `wc`.

The `wysiwyg` parameter is useful for scaling the image to a full window instead of the same size as the plot. Setting `wysiwyg='n'` sets a full display and `wysiwyg='y'` sets a plot display (the default).

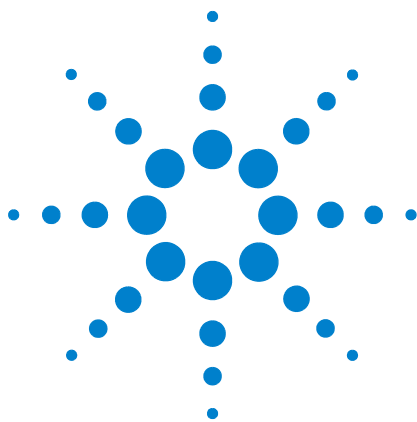
Table 20 Plotting-Associated commands

Commands	Descriptions
aexppl<(expansion_factor)>	Automatic plot of spectral expansion
ap<(template)>	Print out all parameters
bpa:\$sc2_minimum	Plot boxed parameters
hpa	Plot parameters on special preprinted chart paper
killplot	Stop plot job and remove from plot queue
page<(num_pages<,'clear' file)>>	Submit plot and change plotter page
pap<(template,>x,<y><size>>	Plot out "all" parameters
pir	Plot integral amplitudes below spectrum
pirn	Plot normalized integral amplitudes below
pl*	Plot spectra
plc<(pltmod)>	Plot carbon spectrum
plh<(pltmod)>	Plot proton spectrum
pll<(x,y,minimum_y)>	Plot a line list
plp<(pltmod)>	Plot phosphorus spectrum
plot	Automatically plot spectra
plot1d	Plotting macro for simple (non-arrayed) 1D spectra
ppf*	Plot peak frequencies over spectrum
pscale*	Plot scale below spectrum or FID
pltext*	Plot text file
ppa<(x<,y>>	Plot parameter list in "English"
setpen<(maxpen,max_num_pen)>	Set maximum number of HP pens
setplotdev<:plotter_type>	Return characteristics of a named plotter
showplotq	Display plot jobs in plot queue
showplotter	Display currently defined plotters and printers
vnmrplot<file>	Plot files (UNIX)
*pl<(start,finish,step<,'int'><,'all'><options>>	
*pltext<(file<,>x,<y,<width>>>> <:\$x_rext,\$y_rext,\$y_increment>	
*ppf<(noll'><,'pos'><,>noise_mult<,>'leader'><,>length>>	
*pscale<(fid'><,>axis<,>yertical_start<,>plot_start<,>pen>>	

Plotting-Associated parameters

Parameters	Descriptions
ap { string}	"All" parameters display control
maxpen { 1 to number of pens}	Maximum number of pens to use
plotter { string}	Plotter device
wcmax { number, in mm}	Maximum width of chart
wc2max { number, in mm}	Maximum width of chart in second direction
x0 { number, in mm}	X -zero position of HP plotter or Postscript device
y0 { number, in mm}	Y -zero position of HP plotter or Postscript device
hzmm { number}	Scaling factor for plots
pltmod*	Plotter display mode
sp { number, in Hz}	Start of plot
wp { number, in Hz}	Width of plot
* pltmod { ' off' , ' fixed' , ' full' , ' standard' , ' user' , ' variable' }	

9 Printing, Plotting, and Data Output



10 Advanced 1D NMR


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This chapter describes how to work with 1D NMR liquids experiments.



Working with Experiments

In VnmrJ, NMR experiments are contained in Workspaces (also called experiments). Use the Locator to navigate to different workspaces.

- 1 Open up the Locator from within the **Tools** menu.
- 2 View the experiments in the Locator by clicking on the **Locator Statements** icon () and selecting **Sort Workspaces**.
- 3 Open (connect to or join) an experiment by dragging it to the graphics window.
- 4 Create a new experiment (in sequential numerical order) by clicking on **File > New Workspace**.
- 5 Delete an experiment by dragging the experiment from the Locator to the trash can icon.

Enter `explib.` to view the experiments on a system in the **Process / Text Output** page. The output will show the library of the currently available experiment files (`exp1, exp2, ..., exp9999`).

Although multiple experiments can be created, only one experiment at a time is allowed to be currently active in the foreground for manipulation. Simultaneously, background processing or acquisition can occur in other experiments.

To use the menu system to copy selected bits of information between Experiments:

- 1 Click **Edit**.
- 2 Select one of the following options:
 - **Move Parameters**
 - **Move FID**
 - **Move Text**
 - **Move Display parameters**
 - **Move Integral resets**
- 3 Fill in the information requested in the pop-up window.
- 4 Join the new experiment if desired.

These same functions can be accomplished on the command line by using the `mp`, `mf`, and `md` commands to move (copy) files between Experiments.

- `mp (<n, >m)` moves parameters from Experiment `n` to Experiment `m`, for example, `mp (4, 5)`. If `n` is omitted, parameters are moved from the currently active Experiment to Experiment `m`.
- `mf (<n, >m)` moves the last acquired FID and the associated parameters.
- `md (<n, >m)` moves only those “saved display” parameters associated with the commands `s1` through `s9`.

Multi-FID (Arrayed) Spectra

Many NMR experiments acquire a series of FIDs, related to each other through the variation of one or more parameters. For example, suppose it is necessary to run a series of spectra at four different temperatures: 30 °C, 50 °C, 70 °C, and 90 °C. Instead of acquiring four separate sets of data, it is possible to create an array in which the `temp` parameter is given four successively different values. These four subexperiments are now all treated as a single experiment. Entering `go` begins successive acquisition of all four experiments. One advantage of acquiring data like this – as an "array" – is that they can all be transformed, displayed, phased, referenced, plotted, and saved with single commands. Using arrays to acquire data has always been a very powerful feature of the Agilent software.

Arrayed parameters

Use the **Array Parameter** window (called from the **Acquisition/Parameter Arrays** menu; see “[Parameter Arrays](#)” on page 155) to create an arrayed experiment. Alternatively, you can use the command line to create arrays by using either the `array` macro, or by entering the parameter and its desired arrayed values (separated by commas; that is, `temp=30,50,70,90` or `pw=5,10,15,20,25`). Alphanumeric parameters can also be arrayed (that is, use `dm='n','y'` to perform two experiments in which the decoupler is off in the first experiment and on in the second experiment).

Not all parameters can be arrayed. Non-arrayable acquisition parameters include display parameters, most processing parameters, and any parameter that changes the number of data points to be acquired, such as `np`, `sw`, `dp`, and `at`.

To display the values of the currently arrayed parameter, either open the **Array Parameter** window or type `da`. The typed `da` command displays all values of arrayed parameters if entered without an argument. If one or more parameters are listed as an argument, `da` displays only the specified parameters.

Multiple arrays

Two or more parameters can be arrayed in an experiment. For example, an experiment to perform a series of decoupling experiments using an array of decoupler power levels and an array of decoupler frequencies might be set up with `dpwr=17,20,23` and `dof=295,345,507,1245`. In this example, twelve experiments are performed (for example, three different values of decoupler power `dpwr` are used for each of the four different values of the decoupler offset `dof`).

Setting array order and precedence

Whenever an array of one or more parameters is set up, the parameter array becomes important. This parameter tells the system the name of the parameter or parameters that are arrayed and the order and precedence in which the arraying is to take place. The **Array Parameters** window, see [“Parameter Arrays”](#) on page 155, may be used for setting all of the parameters associated with setting up the array.

The string parameter array can have one of several forms:

`array= ''` means no parameter is arrayed (this value is two single quotation marks with no space between, not a double quotation mark).

`array='x'` means parameter x is arrayed.

`array='y,x'` means parameters x and y are arrayed, with x taking precedence. The order of the experiments is $x_1y_1, x_2y_1, \dots, x_ny_1, x_1y_2, x_2y_2, \dots, x_my_2, \dots, x_my_n$, with a total of $m \times n$ experiments being performed. The order of precedence behaves the way an odometer would; right to left.

`array='x,y'` means parameters x and y are arrayed, with y taking precedence. The order of the experiments is $x_1y_1, x_1y_2, \dots, x_1y_n, x_2y_1, x_2y_2, \dots, x_2y_n, \dots, x_my_n$, with a total of $m \times n$ experiments being performed.

`array='(x,y)'` means parameters x and y are jointly arrayed (“diagonallized”). The number of elements of the parameters x and y must be identical, and the order of experiments is $x_1y_1, x_2y_2, \dots, x_ny_n$, with n experiments being performed.

Entering one or more arrayed parameters automatically sets up an array. It is necessary to enter the array directly only if the order or precedence needs to be changed.

All of these changes can also be accomplished within the **Array Parameter** window.

Interactively arraying parameters

Separate from the array *parameter* is the array *macro*. Entering the array macro without an argument starts it in the interactive mode and it prompts for the following information in this order:

- The name of the parameter to be arrayed.
- The number of values of the parameter.
- The starting value.
- The step size (the difference between values in the array).

An arrayed parameter is set up using the information provided. The restrictions with this macro are that only numeric parameters can be arrayed and all values of the array must satisfy the limits of the parameter.

Entering an array with a parameter name as an argument, (for example, `array('pw')`) still starts an interactive mode, but the program only asks for the remaining three items of information. Alternatively, an array bypasses the interactive mode completely if it is started with all four arguments (in the proper order: parameter name, number of steps, starting value, and step size). For example, entering `array('tof',5,1000,-50)` sets the `tof` parameter to have 5 elements with the values in the order 1000, 950, 900, 850, 800.

Resetting an array

Once an array is created, it is possible to change the value of a single element of the array by typing, for example, `pw[2]=11.3`, where the 2 enclosed in brackets indicates which element of the array to modify (array elements are counted starting at 1). This can also be done within the **Array Parameter** window.

Set a single value for the parameter (for example, `pw=10`) to reset an arrayed parameter to a single value. The array parameter is automatically modified to reflect this change.

Array limitations

Regular multiple arrays can include up to 20 parameters, each of which can be a simple parameter or a diagonal array

(a set of parameters), which can include up to 10 parameters. The total number of elements of all arrays is essentially unlimited ($2^{32}-1$).

Acquiring data

Once any parameter is arrayed, acquisition generates not just one, but an entire array of spectra. These spectra can then be examined either individually or as a group, as described below.

Autogain cannot be used in an arrayed experiment. Either use `gain='y'`, which sets the gain to the previously determined value, or set the gain equal to a fixed value. The `gain` parameter itself cannot be arrayed.

Arrayed acquisitions can be interleaved, in which, a part of each experiment is done in turn rather than starting and finishing each experiment sequentially. The `interleave` function is controlled by the parameters `il` and `bs`.

- `il='y'`

Experiments are interleaved: `bs` transients are performed for each member of the array, followed by `bs` more transients for each member of the array, which continues until `nt` transients are collected for each member of the array. Thus, `il` is relevant only if `bs` (block size) is less than `nt` (number of transients).

- `il='n'`

All `nt` transients are acquired for the first experiment in the array, then all `nt` transients for the second experiment, continuing until `nt` transients have been acquired for each member of the array. The `bs` parameter is still active, but it is not involved in interleaving when `il='n'`.

Processing



The **Transform**, **Transform all**, and **Process** buttons reprocess all elements of the array. The typed commands `ft` or `wft` can also be used to transform all of the spectra. Both commands take the same arguments and options:

- 'acq' does not transform elements that have already been transformed.
- 'nods' prevents an automatic spectral display (same as `ds` command).
- 'zero' zeroes the imaginary channel of the FID before Fourier transform.

Phasing can be done on any spectrum. Only one set of phase correction parameters exists, so all spectra have the same phase at any one time (although the phase can of course be changed when examining different spectra).

Display and plotting

The Vertical Panel “Arrayed Spectrum” can be used for displaying arrayed data. The command `ds(index)` or the

 and  icons, interactively display the requested spectrum from the array. The index argument can have one, two, or three numbers, each separated by commas, depending on the size of the spectral array.

Note that spectra are always scaled according to the number of completed transients `ct`; if `nt` is arrayed (`nt=1,2,4,8`), each spectrum is scaled by its *own* `ct`.

Other spectra display commands are not interactive like the `dc` command: `dss`, `dssn`, `dssa`, `dssan`, `dssh`, `dsshn`, and `dssl`. They display stacked spectra in which each spectrum is offset with respect to the previous spectrum. The order of stacking can be left to right, right to left, top to bottom, or bottom to top, depending on whether the horizontal offset (`ho`) and vertical offset (`vo`) parameters are positive or negative. Some of these commands set `ho` and `vo` automatically.

The spectra display commands function as follows:

- `dss` displays stacked spectra using the current values of `ho` and `vo` to set the order of stacking.
- `dssn` displays stacked spectra the same as `dss`, but the graphics window is not erased before starting the display. This allows composite displays of many spectra to be created.
- `dssa` displays stacked spectra automatically (that is, `vo` and `ho` are automatically adjusted to fill the screen in a lower left to upper right presentation).
- `dssan` displays stacked spectra automatically the same as `dssa`, but the graphics window is not erased before starting the display.
- `dssh` displays stacked spectra horizontally (that is, `vo` is set to zero, and `ho` is adjusted to fill the screen from left to right).
- `dsshn` displays spectra horizontally the same as `dssh`, but the graphics window is not erased before starting the display.
- `dssl` displays a label for each element in a set of stacked spectra. The label is an integer value starting with 1 and extending up to the number of spectra in the display. (Note that it always starts numbering with 1, regardless of which spectra are displayed.)

The command `p1` plots stacked spectra with the same format as displayed by `dss`.

The argument syntax `<(start,finish<,step>)><,options>` is used by the `dss` command, the variants of `dss`, and by the `p1` command. The arguments are the following:

- `start` is the index number of a particular trace to be viewed when displaying arrayed 1D spectra or 2D spectra. It is also the index of the first spectrum when displaying multiple spectra.
- `finish` is the index of the last spectra when displaying multiple spectra. Because the parameter `arraydim` is automatically set to the total number of spectra, if `finish` is set to `arraydim`, all remaining spectra in the array will be shown.
- `step` is the increment for the spectral index when displaying multiple spectra. The default step is 1.

Options can be any of the following:

- `'all'` is a keyword to display all of the spectra.

- 'int' is a keyword to display only the integral, independently of the value of the parameter `intmod`.
- 'top' or 'side' are keywords that cause the spectrum to be displayed either above or at the left edge, respectively, of a contour plot. This assumes that the parameters `sc`, `wc`, `sc2`, and `wc2` are those used to position the contour plot. This option does not apply to `dssa`, `dssan`, `dssh`, or `dsshn`.
- 'dodc' is a keyword for all spectra to be drift corrected independently.
- 'red', 'green', 'blue', 'cyan', 'magenta', 'yellow', 'black', and 'white' are keywords that select a color. This option does not apply to `dssa`, `dssan`, `dssh`, `dsshn`, or `pl`.
- 'pen1', 'pen2', 'pen3', etc. specify a pen number on a plotter. This option does not apply to `dss` or any of its variants.

Pulse width calibration step-by-step (using an array)

Note, for illustration, how the following steps perform a pulse-width calibration using arrays:

- 1 Set up parameters and obtain a normal spectrum of any sample. For best results, one or more intense signals should appear near the center of the spectrum.
- 2 Enter 5 for the value of `pw` (for example, `pw=5`) or use some other small value.
- 3 Enter 1 for the value of `nt` (for example, `nt=1`).
- 4 Obtain a spectrum and phase it properly. Set `d1` to $5 * T_1$ (or set it to a long value like `d1=10` if you do not know the T_1).
- 5 Make an array of `pw` from 5 to 30 in steps of 5 or use some other set of suitable values for the `pw` array.
- 6 Select **Absolute Intensity** and acquire. For example, enter `pw=5,10,15,20,25,30 ai go`. (Absolute intensity mode is also set by Process / Display / Amplitude Scaling / Absolute.
- 7 Transform and display the stacked data after the experiment finishes acquisition (for example, `wft dssh`).
- 8 Find the experiment where the signal goes through its 360° (or 180°) null. Enter `da` to remind yourself of the values of the `pw` array.

- 9 Enter pw equal to one-quarter of pw360 (or, less desirably, to one-half of pw180) to reset the array.

T1 and T2 Analysis

A common form of arrayed experiment is the "inversion-recovery T_1 " experiment. In this experiment the nuclei are allowed to relax to equilibrium (d_1) then inverted with a 180° pulse (p_1), given a variable time to return to equilibrium (d_2), and finally given a monitoring 90° pulse (p_w) to measure their peak height as a function of d_2 . Under most circumstances, the behavior of the peak heights as a function of d_2 will be exponential and this exponential time constant is the T_1 .

Setting up the experiment

The standard two-pulse sequence is used to perform the T_1 experiment. The experiment can be set up by using any one of the following three methods.

- 1 Entering appropriate values for p_1 , p_w , d_1 , and an array of values for d_2 .
- 2 Using the `dot1` macro.

`dot1` sets up all parameters to perform a T_1 experiment, including d_1 , p_w , p_1 , nt , and an array of d_2 values, based on information entered. The three arguments that can be input are the minimum expected T_1 , the maximum expected T_1 , and the total time in hours the experiment should take. If no arguments are provided, `dot1` prompts the user for the information.

- 3 Click the **T1_measure** protocol button, or use the menu as follows:
 - a Click **Experiment**.
 - b Select **H1 Relax. Measurements**.
 - c Select **T1 Measurements**.
 - d Fill in the information on the **Sample Info panel** of the **Start tab**.
 - e Adjust parameters on the **Acquire / Defaults** page.

The parameter `pw90` must contain a correctly calibrated 90-degree pulse width because `dot1` uses this information.

Processing the data

Once the data is acquired and transformed, use one of the following two methods to set up the data for analysis.

- 1 Go to the **Process / T₁ Analysis** panel, and follow the directions listed there.
- 2 Display the last spectrum of the T₁ arrayed dataset.
- 3 Phase this spectrum properly.
- 4 Select a threshold and adjust the threshold line position.
- 5 Enter `dpf`, or `d11`, or click the appropriate button to display a line list and locate lines for the system.
- 6 Enter `fp` to "find peaks" (measure the peak height of each peak in an array of spectra). If optional line indexes are supplied to `fp` as arguments (for example, `fp(1,3)`), only the peak heights of the corresponding lines are measured.
- 7 Type `t1` to run the T₁ analysis.

The `npoint` parameter (if defined and set "on") determines the range of data points over which the `fp` command searches for a maximum for each peak.

Analyzing the data

T₁ and T₂ analysis is performed by the `t1` and `t2` macros, respectively. `t1` and `t2` measure relaxation times for all lines in the line listing and display an extended listing of observed and predicted peak intensities. `t1s` and `t2s` perform the same calculation as `t1` and `t2`, but produce a shorter output, showing only a summary of the measured relaxation times.

The **Process / T₁ Analysis / Display All Fits** button, or the command `expl`, displays the exponential/polynomial curves resulting from T₁ and T₂, or kinetic analysis. Optional input of line numbers as arguments allows you to display only selected lines. Similarly, the command `pexpl` will plot the same curves.

The macro `autoscale` returns the command `expl` to autoscaling, in which scale limits (set by `scalelimits`) are determined that will display all the data in the `expl` input file. The macro `scalelimits` causes the command `expl` to use typed-in scale limits. If no arguments are given, `scalelimits` asks for the desired limits. The limits are retained as long as an `expl` display is retained.

Enter `dels(index1<, index2>...)` to delete spectral data from the `t1` or `t2` analysis (or from `t1s` or `t2s`). This command deletes data, for the spectra selected by the indexes, from the output file `fp.out` that is used by the `t1` or `t2` analysis. The deleted data can be restored by rerunning `fp`.

T₁ data workup: step-by-step

The following procedures accomplish a manual T₁ analysis:

- 1** Enter `rt('/vnmr/fidlib/T1_measure_01.fid')`.
- 2** Enter `wft dssh full ds(arraydim) aph`.
- 3** Use the left mouse button to set the threshold.
- 4** Enter `dll fp t1 center expl`.

Kinetics

The arraying capability of the VnmrJ software provides for the acquisition of data for the study of kinetics.

Setting up the experiment

Typically, the best procedure is to array the preacquisition delay parameter `pad`. For example, if `pad=0,3600,3600,3600,3600`, the system acquires the first spectrum immediately (`pad[1]=0`), waits 3600 seconds (`pad[2]=3600`), acquires the second spectrum, waits another 3600 seconds, etc. Because 3600 seconds is 1 hour, this inserts a wait of one hour between acquisitions. After all the spectra have been obtained, they are processed much like T_1 or T_2 data.

Processing the data

If the signal decreases exponentially with time, the output is matched to the equation:

$$I=A1*EXP(-T/TAU)+A3$$

The analysis is done by the macro `kind` or by macro `kinds` if a short output table is desired.

If the signal increases exponentially with time, the output is matched to the equation:

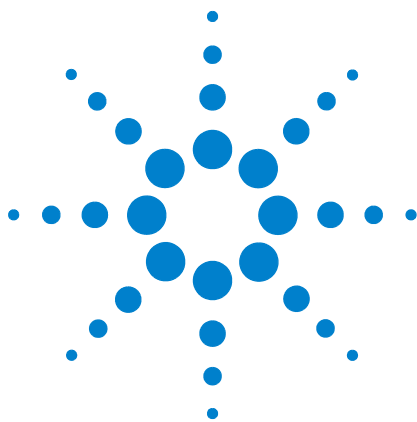
$$I=-A1*EXP(-T/TAU)+A3-A1$$

with analysis done by the macro `kini`, or by the macro `kinis` for a short output table.

Kinetics step-by-step

The following steps are typical in processing a kinetics experiment using the command line:

- 1 Enter `wft dssh full ds aph`.
- 2 Click the **Threshold** icon in the **Graphics Control** menu. Use the left mouse button to set the threshold.
- 3 Enter `dll fp`.
- 4 Enter `kind`, `kini`, `kinds`, or `kinis`, as appropriate.
- 5 If desired, adjust `sc`, `wc`, `sc2`, and `wc2` by entering `center` or `full`.
- 6 Enter `expl`.



11 Multidimensional NMR

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2D NMR data are similar to arrayed 1D data in many respects. A series of FIDs are acquired in 2D experiments as a function of time (using one of the time variables in the pulse sequence) and transformed into a series of spectra. 2D experiments do not explicitly specify the values of the time array. Instead, the timing is specified by two parameters, $sw1$ and ni , that respectively describe the "2D" spectral width (to be discussed shortly) and the "number of increments" (which determines the number of elements in the array, and the number of different experiments).



Hadamard Spectroscopy

Hadamard spectroscopy is a technique for acquiring multidimensional data sets using a small number of transients and reconstructing the nD spectrum using a Hadamard transform based on selective excitation of a predetermined set of frequencies using Hadamard encoding. A waveform generator is required due to the complexity of the shaped pulses. On the 400MR and VnmrS consoles, a waveform generator is included on the RF board of each channel.

Acquiring the data

A list of selectively excited frequencies is created from a 1D spectrum or other means. A series of shaped pulses is created from the frequency list using a Hadamard matrix to selectively excite or invert the signals of interest. The matrix size is greater than the number of frequencies in the list. A typical Hadamard matrix is shown in [Figure 87](#).

+	+	+	+	+	+	+	+
+	+	+	+	-	-	-	-
+	+	-	-	+	+	-	-
+	+	-	-	-	-	+	+
+	-	+	-	+	-	+	-
+	-	+	-	-	+	-	+
+	-	-	+	+	-	-	+
+	-	-	+	-	+	+	-

Figure 87 Hadamard Matrix

The first shaped pulse selectively excites all of the frequencies in the 8 x 8 matrix above. A second shaped pulse selectively excites the first four frequencies and inverts the second four frequencies. Frequencies 1, 2, 5, and 6 are selectively excited, and frequencies 3, 4, 7, and 8 are inverted by the third shaped pulse. The process is repeated until each row of the Hadamard matrix has been acquired.

Processing the data

Hadamard processing in the indirect dimension is done by summing, adding, or subtracting the acquired data increments in combinations according to the Hadamard matrix elements. Each sum gives a trace corresponding to a frequency in the list and is placed at the appropriate

frequency in the indirect dimension. Areas between the frequencies in the list are zero-filled. The data sets acquired in the direct dimension are Fourier transformed to produce a 2D spectrum.

The trace for the first frequency in the list is usually found in the second row of the Hadamard matrix. The second row in an 8 x 8 Hadamard matrix is created by adding the first four acquired elements and subtracting the second four acquired elements. The process is repeated until all traces for the selected frequencies are complete. A Hadamard matrix is created that is greater in size than the number of frequencies and contains more Hadamard sums than are needed.

2D and higher dimensional experiments using the Hadamard method are run and processed in a significantly shorter time than the same experiment acquired using hypercomplex or TPPI methods. Current software supports only 2D versions of Hadamard spectroscopy.

Implementing the method

A list of frequencies used for 2D Hadamard spectroscopy is contained in the parameter `htfrq1`. The Hadamard matrix size is set to a value that is both greater than the number of frequencies contained in the parameter `htfrq1` and a power of two multiplied by 1, 12, 20, or 28. All shaped pulses are created in the pulse sequence using `Pbox`.

Hadamard data is processed using `ft2d` (or one of its variations) with `proc1='ht'`. The zero-filling size in the indirect dimension is set by `fn1`, which may be used to adjust the separation of the frequencies of interest.

Setting up a homonuclear 2D hadamard sequence

The currently available homonuclear 2D Hadamard sequences are TOCSY, COSY, DQ-COSY, NOESY, and ROESY. These experiments are accessible by either selecting the experiment from the pull-down menu **Experiments > Hadamard Experiments > Use current parameters, Load new parameter set** or **AutoHT** or from the **Experiment Selector**. Alternatively, they can be loaded by entering their macro name on the command line (`tocsyHT`, `gcosyHT`, `dqcosyHT`, `noesyHT`, and `roesyHT` respectively) from the Locator.

- 1 Acquire a 1 D spectrum (^1H or other nucleus).
- 2 Process and phase the spectrum.

- 3 Select **Experiments > Hadamard Experiments > Setup Hadamard encoding** to open the Edit Hadamard Encoding Frequencies window. Alternatively, enter `editht` on the command line.
- 4 Enter a line width in the HT linewidth, lb [Hz] entry box in Hz. Typically, a linewidth of 20 – 25 Hz is used for ^1H spectra. Use 25 Hz for samples with large germinal couplings. Use 1 – 5 Hz for ^{13}C spectra.
- 5 Adjust the threshold to select the peaks of interest.
- 6 Press the **Create Line List** button and edit the line list as needed in the Line List Box. It is also possible to use the cursor to selectively add or remove entries in the line list with the Select HT Frequencies buttons.
- 7 Click **Set list into parameters** button to save the frequency list in the current workspace.
- 8 Click **Close**.
- 9 Load the Hadamard homonuclear 2D experiment.

Either use the **Experiments** menu, Use current parameters, or enter the macro on the command line.

- 10 The Defaults panel can be used to customize basic parameters while the Pulse Sequence panel is used for advanced parameters.

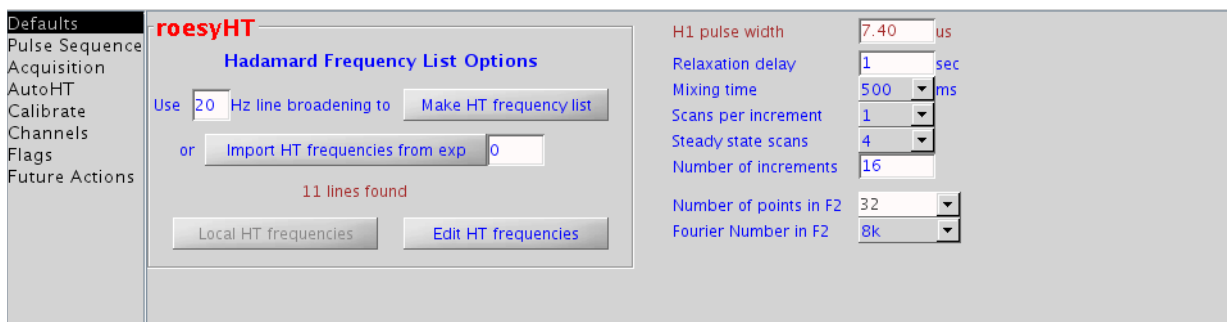


Figure 88 roesyHT experiment – default panel

Note the menu option for Mixing time (which is displayed only for tocsyHT, noesyHT and roesyHT). The Hadamard Frequency List Options section supplements the Edit Hadamard Encoding Frequencies window.

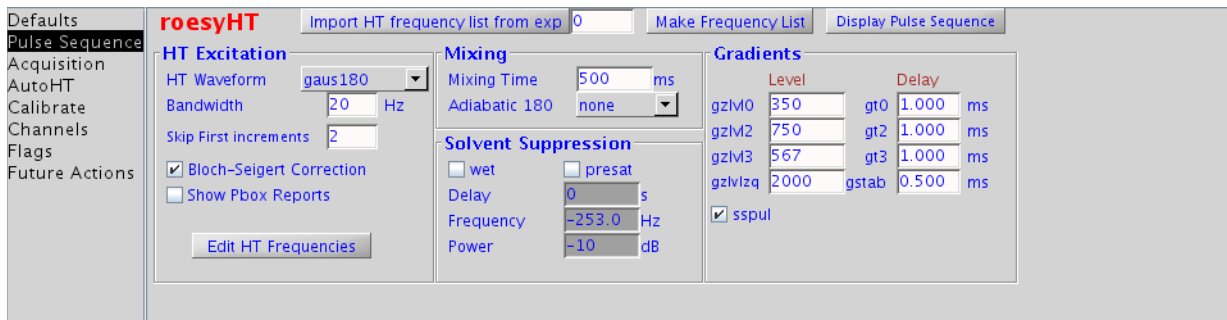


Figure 89 roesyHT experiment – pulse sequence panel

The parameters for controlling Hadamard Transform Excitation, Solvent Suppression, Mixing time, and Gradients are provided. Note that the buttons for **Import** and **Edit HT Frequencies** can also be found on this panel.

- 11 Start acquisition.
- 12 Process using the **Full 2D Transform** button on the **Default Process** page or enter `wft2da` on the command line.

Setting up a heteronuclear 2D Hadamard sequence

The currently available heteronuclear 2D Hadamard sequences are HSQC, HSQC-TOCSY, HMBC, and HETCOR. These experiments are accessible by either selecting the experiment from the pull down menu **Experiments > Hadamard Experiments > Use current parameters, Load new parameter set or AutoHT** or from the **Experiment Selector**. Alternatively, they can be loaded by entering their macro name on the command line (`hsqcHT`, `hsqctocsyHT`, `hmbcHT`, and `hetcorHT` respectively) from the Locator.

- 1 Acquire a 1D spectrum (^{13}C or other nucleus).
- 2 Process and phase the spectrum.
- 3 Select **Experiments > Hadamard Experiments > Setup Hadamard** encoding to open the Edit Hadamard Encoding Frequencies window. Alternatively, enter `editht` on the command line.
- 4 Enter a line width in the HT linewidth, lb [Hz] entry box in Hz. Typically, a linewidth of 1 – 5 Hz for ^{13}C spectra.
- 5 Adjust the threshold to select the peaks of interest.
- 6 Press the **Create Line List** button and edit the line list as needed in the Line List Box. It is also possible to use the

cursor to selectively add or remove entries in the line list with the **Select HT Frequencies** buttons.

- 7 Click **Set list into parameters** button to save the frequency list in the current workspace.
- 8 Click **Close**.
- 9 Acquire a 1D ¹H spectrum and adjust the spectral width and decoupling as desired.
- 10 Load the Hadamard heteronuclear 2D experiment.

Either use the **Experiments** menu, Use current parameters, or enter the macro on the command line.

The **Defaults** panel can be used to customize basic parameters while the **Pulse Sequence** panel is used for advanced parameters.

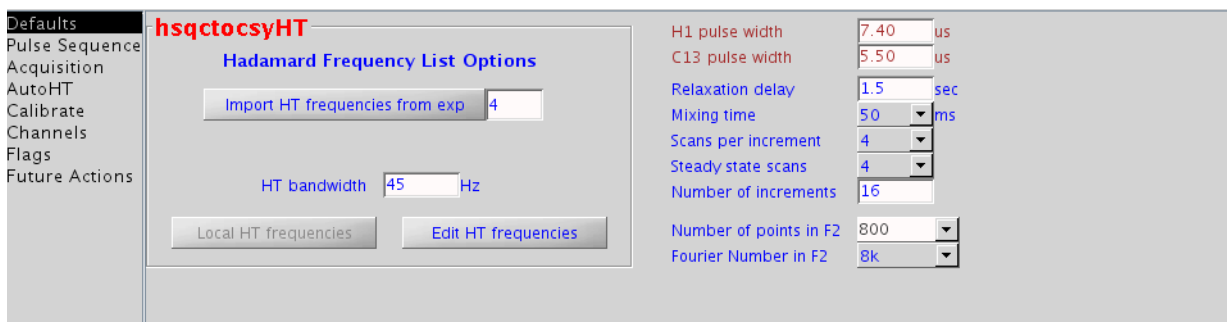


Figure 90 hsqctocsyHT experiment – default panel

Note that the menu option for Mixing time (which is displayed only for the hsqctocsyHT experiment) and the addition of the C13 pulse width. The Hadamard Frequency List Options section supplements the Edit Hadamard Encoding Frequencies window.

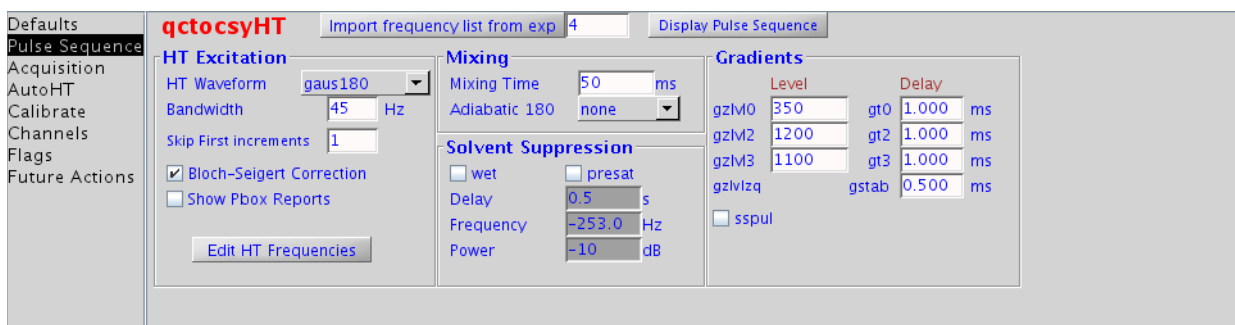


Figure 91 hsqctocsyHT experiment – pulse sequence panel

Parameters for controlling Hadamard Transform Excitation, Solvent Suppression, Mixing time, and Gradients are provided. Note that the buttons for **Import** and **Edit HT Frequencies** can also be found on this panel.

Figure 92 hmbcHT experiment – pulse sequence panel

Parameters for controlling Hadamard Encoding, Decoupling, Solvent Suppression, J-couplings, and Gradients are provided.

Figure 93 hsqcHT experiment – pulse sequence panel

- 11 Start acquisition.
- 12 Process using the **Full 2D Transform** button on the **Default Process** page or enter `wft2da` on the command line.

Editing or creating hadamard frequencies

- 1 Select **Experiments > Hadamard Experiments > Setup Hadamard encoding** to open the **Edit Hadamard Encoding Frequencies** window or enter `editht` on the VnmrJ command line to open the **Edit HT Freq window**.
- 2 Edit the Hadamard frequencies from a 1D spectrum using the editable text entry window under Line List. See [Figure 94](#).
- 3 Click **Display** to show the Hadamard frequencies.
- 4 Create or edit a Hadamard frequency list using the **Edit Hadamard Encoding Frequencies** window buttons.

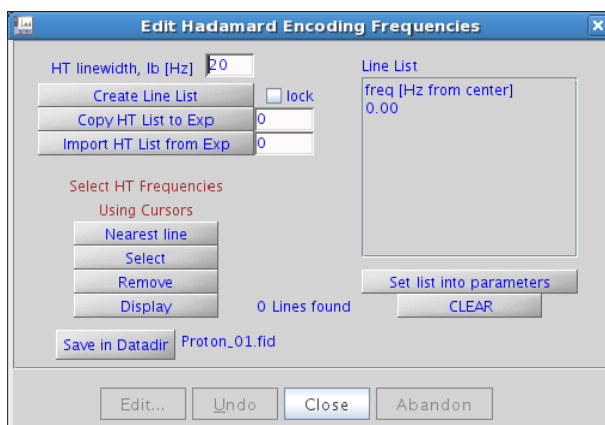
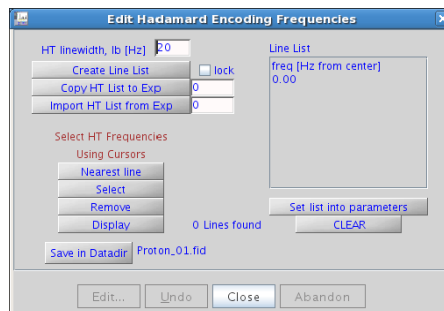


Figure 94 Spectrum and edit HT freq window

Table 21 Edit hadamard encoding frequencies window buttons

Button	Function
Create Line List	Processes the spectrum using the line broadening specified in the field next to Min line width [Hz] to smooth out multiplet structures. Peak thresholds are adjusted automatically and a line list is created.
Nearest line	Places the cursor on the nearest line



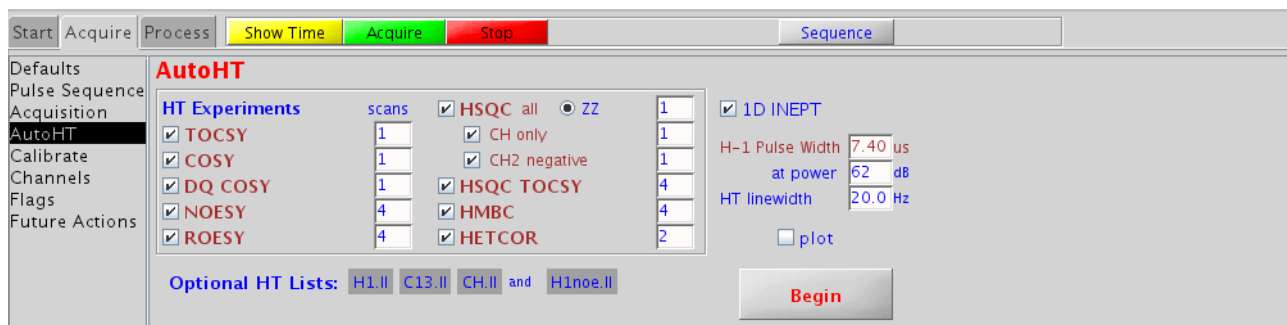
The line list is set into the parameter `htfreq1`. Only frequencies separated by the minimum line width are used.

Table 21 Edit hadamard encoding frequencies window buttons (continued)

Button	Function
Select	Adds the current cursor position to the line list. The cursor must be more than the minimum line width from an existing frequency in the line list.
Remove	Removes the line nearest the cursor position from the line list
Display	Displays the frequency list. If a 1D spectrum is displayed, show the frequencies using δ_{ppf} in units set by the axis parameter
CLEAR	Clears all frequencies from the frequency list
Save in Datadir	Saves the current frequency list as a Hadamard line list for the current nucleus (t_n). It saves the frequency list, band width, current nucleus, spectral width, and frequency offset in a persistence file. The frequencies and other parameters are loaded from the persistence file when loading a Hadamard experiment.
Line List	Displayed in the editable text entry window under Line List. The first column contains the Hadamard frequency list (parameter $htfrq1$). The second column of numbers, if present, contains the bandwidth, in Hz, for each frequency.
Set list into parameters	Sets the changes from the Line List text entry window into the parameters $htfrq1$ and $htbw1$
Lock	Locks the current line list so the list cannot be changed unless it is unlocked first.
Copy HT list to Exp	Copies a line list file from the current line list into a workspace (Exp). The file name: <code>/export/home/vnmr1/vnmrsys/exp2/ht11.htfrq1</code> has the same format as the Line List in the text entry window.
Import HT list from Exp	Copies a line list file from a workspace (Exp) into the current line list, and sets the line list into the parameters. The file name: <code>/export/home/vnmr1/vnmrsys/exp2/ht11.htfrq1</code> has the same format as the Line List in the text entry window.

autoHT

The autoHT panel allows a queue of Hadamard experiments to be run sequentially. The only customization allowed for the queue from this panel is the number of scans for each experiment.



To set up autoHT experiments:

- 1 Create a working directory.
- 2 Record 1D H-1 and C-13 experiments and save in the working directory as H1.fid and C-13.fid.

Optional CH.fid file contains only the CH region (for example INEPT or DEPT).

- 3 Create H1.ll, C13.ll and optional CH.ll and H1noe.ll Hadamard line lists and save in the working directory. All frequency lists in autoHT series are optional and ensure more reliable results.
- 4 From the **Hadamard Experiments** menu, choose **autoHT**.
- 5 In the **autoHT** page, select the required experiments, set the working directory.
- 6 Check the H1 pulse length and click **Begin**.

Running Hadamard experiments from the study queue

The Study Queue allows a queue of Hadamard experiments to be run in the order of the Study Queue. They can be customized individually, and can be mixed with other protocols within the queue. If a 1D experiment has a Hadamard line list already saved with it.

- 1 Acquire a Proton spectrum using the Study Queue.
- 2 Display the Proton spectrum.
- 3 Select **Experiments/Hadamard Experiments/Setup Hadamard encoding** to open the **Edit Hadamard Encoding Frequencies** window. Alternatively, enter `editht` on the command line.
- 4 Enter a line width in the HT linewidth, lb [Hz] entry box in Hz. Typically, a linewidth of 20–25 Hz is used for 1H spectra.
- 5 Adjust the threshold to select the peaks of interest.
- 6 Click the **Create Line List** button and edit the line list as needed in the **Line List Box**. It is also possible to use the cursor to selectively add or remove entries in the line list with the **Select HT Frequencies** buttons.
- 7 Click the **Set list into parameters** button to save the frequency list in the current workspace.
- 8 Click **Save** in Datadir.

If a heteronuclear Hadamard experiment will be run, such as a ^1H - ^{13}C hsqc, then a Carbon spectrum will also need to be acquired, repeating steps 1-8 for the Carbon spectrum.

- 9 Click **Continue Study**.
- 10 Click the **Hadamard** tab in the **Experiment Selector** then select the homonuclear hadamard experiment. The experiment will be added to the **Study Queue**.
- 11 The Experiment node can be customized, more experiments can be added to the queue or to start the queue click the **Submit** button.

Commands, macros, and parameters

For more information on the following commands, macros, and parameters used in Hadamard Spectroscopy, see the *VnmrJ Command and Parameter Reference* manual.

Command/Macro	Description
d11	Display listed line frequencies and intensities (C)
editht	Edit a Hadamard frequency list (M)
ft1d	Fourier transform along f_2 dimension (C)
ft1da	Fourier transform phase-sensitive data (M)
ft2d	Fourier transform 2D data (C)
ft2da	Fourier transform phase-sensitive data (M)
getht	Retrieve or save a Hadamard frequency list file (M)
n11	Fine line frequencies and intensities (C)
selexHT	Set up the selexHT experiment (M)
sethtfrq1	Set a Hadamard frequency list from a line list ((M)
gcosyHT	Set up the gcosy experiment (M)
dqcosyHT	Set up the dqcosyHT experiment (M)
tocsyHT	Set up the tocsyHT experiment (M)
noesyHT	Set up the noesyHT experiment (M)
roesyHT	Set up the roesyHT experiment (M)
hsqcHT	Set up the hsqcHT experiment (M)
hsqctocsyHT	Set up the hsqctocsyHT experiment (M)
hmbcHT	Set up the hmbcHT experiment (M)

11 Multidimensional NMR

Command/Macro	Description
hetcorHT	Set up the hetcorHT experiment (M)
wft1d	Weight and Fourier transform f_2 for 2D data (C)
wft1da	Weight and Fourier transform phase-sensitive data (M)
wft1dac	Combine arrayed 2D FID matrices (M)
wft2d	Weight and Fourier transform 2D data (C)
wft2da	Weight and Fourier transform phase-sensitive data (M)

Required Parameters	Description
htfrq1	Hadamard frequency list in n_i (P)
n_i	Number of increments in 1st indirectly detected dimension (P)
htofs1	Hadamard offset in n_i (P)
procl	Type of processing on n_i interferogram (P)
fn1	Fourier number in 1st indirectly detected dimension (P)

Optional Parameters	Description
htbw1	Hadamard pulse excitation bandwidth in n_i (P)
pxrep	Flag to set the level of Pbox reports (P)
pxbss	Bloch-Siegert shift correction during Pbox pulse generation (P)
htbitrev	Hadamard bit reversal flag (P)
htss1	Stepsize for Hadamard waveforms in n_i (P)
htcall	RF calibration flag for Hadamard waveforms in n_i (P)
htpwr1	Power level for RF calibration of Hadamard waveforms in n_i (P)
nimax	Maximum limit of n_i (P)

Reference

E. Kupce and R. Freeman, "Two-dimensional Hadamard spectroscopy", *J. Magn. Reson.* (2003), 162:300-310.

Real-Time 2D

Real-time 2D allows the user to perform 2D actions while the experiment is still in progress. A full 2D transform on the data can be performed when eight or more increments are completed.

Experiments such as heteronuclear chemical shift correlation and homonuclear 2D-J experiments require only a few increments to resolve the resonances of interest. Other experiments require more increments. The experiment can be stopped at any time if there is sufficient data to solve the problem.

Interferograms

Interferograms are produced by transposing a matrix created from a series of spectra in the acquisition dimension. The peaks' heights in a 1D arrayed experiment, like an inversion-recovery T_1 experiment, behave exponentially as a function of time. In a 2D experiment, the peaks' heights oscillate, and these oscillations contain the information of interest in a 2D-experiment. The decaying oscillations are called interferograms, and they resemble a free induction decay (FID).

Each interferogram in the 2D dataset contains a series of points that represent the peak height at a particular frequency in the original spectrum as a function of time. Many of the interferograms contain only noise because, at that point, the original spectrum contained only noise. Interferograms that correspond to the peaks in the original spectrum contain useful information.

Evolution time, or t_1 , is the time that is varied in a 2D experiment and is the first of two evolution time periods in the 2D experiment. The t_1 evolution time is controlled by the parameter d_2 . This time is normally calculated automatically from the number of increments (the parameter n_1) and $1/sw_1$. The value of n_1 determines if a 2D experiment will be run. The initial value of d_2 (at the start of the experiment) is usually set to zero but can be set to any value.

A d_2 array does not appear in the display da (for example, d_2 is "implicitly" arrayed). Only the first value of d_2 appears as a parameter value in the display dg . A minimum of eight increments is required before the 2D transform can be

performed, but a more typical range is 32 to 512.

Detection time, or t_2 , is the time during which the signal is detected and is the second of the two evolution time periods. After transformation of the signals detected during the time t_2 , the "normal" spectrum appears along the F_2 axis. The second transform reveals information about the frequencies of oscillations during the t_1 time period and places them along the F_1 axis. (By convention, when an axis is still in the time domain, we label that axis with "t", whereas after the FT, when the axis shows frequency, we use the "F".)

Parameters that refer to the F_1 axis in a 2D experiment are identified by the number 1 (for example, sw_1 , lb_1 , fn_1), whereas the normal 1D parameters control F_2 .

The process of "transformation, transposition to interferograms, and second transformation" is often reduced to a single command, button, or menu choice. Most buttons that start the acquisition of a 2D experiment also perform all the necessary processing when the experiment is done.

Phase-Sensitive 2D NMR

Phase-sensitive 2D NMR acquisition and processing offers better sensitivity, resolution, and the ability to display and plot phased data (as opposed to absolute-value data). There are four kinds of experiments in which a user might want to examine phase-sensitive data:

- Experiments in which two different experiments are performed for each value of t_1 , typically using different phase cycles or gradients, producing a full complex data set for the second transformation. This method is called, in the case of phase cycling, (popularized by States, Haberkorn, and Ruben, *J. Magn. Reson.* 1982, 48, 286), the *hypercomplex method*.
- Experiments in which the phase of the excitation pulse is updated as a function of t_1 (TPPI or Time Proportional Phase Incrementation (see Marion and Wuthrich, *Biochem. Biophys. Res. Commun.* 1983, 113, 967), which produces real data along the t_1 axis.
- A 2D experiment in which the data, processed in a suitable way, is expected to appear in absorption mode in both directions, that is, Heteronuclear 2D-J.

- A 2D experiment in which the data is not expected to appear in absorption mode in both directions, but in which it is nonetheless desirable to observe the data in a phase-sensitive presentation.

Complex transforms are usually performed along t_1 . This is the ideal situation for the hypercomplex method. TPPI data can be processed along t_1 with either a complex FT or a real FT, depending upon the method of data collection.

The recommended hypercomplex method requires two data tables (TPPI method requires one). TPPI requires sampling at twice the frequency along t_1 to create one data table twice the data size per data table as the hypercomplex method to produce the same real resolution. In terms of data storage requirements and execution time, the experiments are equivalent. The TPPI method is no longer routinely supplied or supported.

2D Experiment Set Up

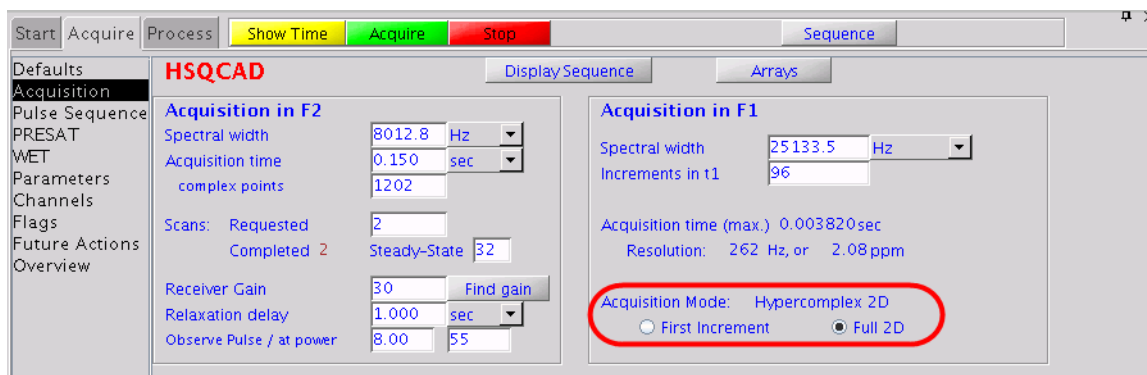
- 1 Drag-and-drop or double-click the desired experiment, using the **Experiment Selector**.
- 2 Use the **Experiments** menu, which offers more options for customization.
- 3 Use the Locator to list the available 2D experiments:
 - a Click the **Locator Statements** button (magnifying glass icon), and select **Sort Protocols** for experiments.
 - b Set the columns to name, apptype, and seqfil.
 - c Modify the Locator Statement to show hetero2d (heteronuclear 2D) or homo2d (homonuclear 2D).

Data Acquisition: Arrayed 2D

2D experiments have one implicitly “arrayed” parameter, *d2* and can have explicitly arrayed parameters like 1D experiments. Explicitly arrayed parameters have nothing to do with phase-sensitive 2D, but are used in experiments such as a series of 2D-NOE experiments using different mixing times. This feature opens up a variety of experiments, including addition/subtraction of two or more 2D experiments.

Hypercomplex method

The hypercomplex method of phase-sensitive 2D NMR requires two data tables. The pulse sequence must generate a sequence of pulses or pulse phases suitable for generating the two component experiments of the hypercomplex method.



Any parameter may be used for this purpose. VnmrJ uses the parameter *phase* for many pulse sequences, which takes on values of 0, 1, or 2:

- A value of *phase*=0 produces a phase cycle suitable for a non-phase-sensitive 2D experiment.
- Running an array of experiments with *phase*=1, 2 produces two experiments suitable for the hypercomplex method.

TPPI Method

The TPPI method of phase-sensitive 2D NMR requires one data table. The data is processed along t_1 with a complex Fourier transform by setting `proc1` (which sets the type of data processing to be performed on the t_1 interferogram) to `'ft'`. This manner of implementing TPPI leads to a doubling of the f_1 frequency axis.

When an arrayed 2D experiment is run in this manner, there is in reality a double array: d_2 (the evolution time) and phase. The order of these arrays is such that the phase array is cycled the most rapidly. Observe the order of these experiments; for example:

Method	Evolutiontime	Phase
States-Haberhorn	$d_2=0$	phase=1
	$d_2=0$	phase=2
	$d_2=1/sw_1$	phase=1
	$d_2=1/sw_1$	phase=2
TPPI (non-arrayed)	$d_2=0$	phase=3
	$d_2=1/sw_1$	phase=3
	$d_2=2/sw_1$	phase=3
	$d_2=3/sw_1$	phase=3

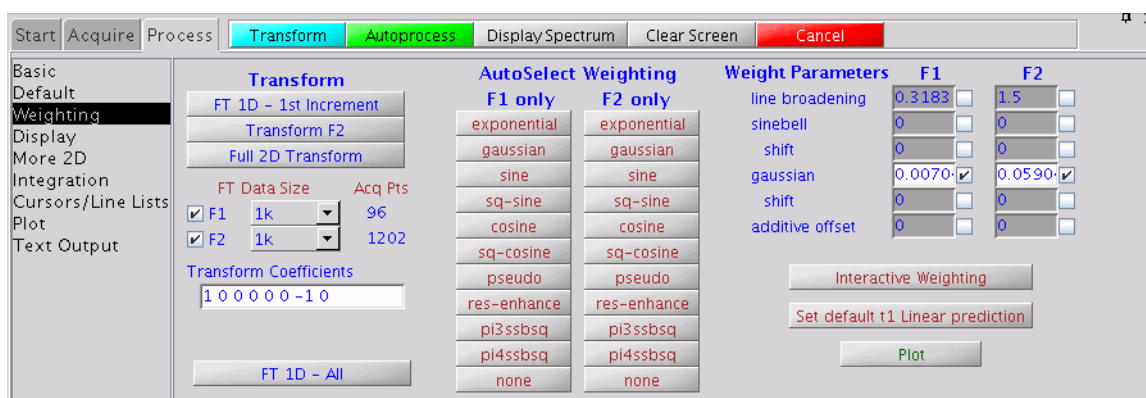
Not all pulse sequences have the TPPI method incorporated. The acquisition status window displays a count of the current FID and the number of completed transients (`ct`) in that FID. The current FID number is the total count of completed FIDs to this point, including all arrays. As the phase parameter is cycling the most rapidly, and since, typically, phase is an array of two values, the current FID number is typically twice the number of the current increment. For example, when the counter reads FID 54, this means that 27 FIDs of the first type of experiment have been completed, 26 of the second type, and the system is working on the 27th experiment of the second type.

Weighting

This section describes weighting functions for processing 2D experiments.

2D weighting parameters

The 2D weighting parameters used for processing the t_1 domain (the interferogram) or first indirectly detected dimension (n_1) are set on the **Process** page in the **Process** panel and are analogous to weighting parameters for 1D experiments.



Non-phase-sensitive (absolute-value and power) 2D experiment

“Pseudo-echo,” sinebell, or sinebell-squared weighting is typically used to attenuate long dispersion tails. This weighting is often responsible for a significant loss in sensitivity in absolute-value 2D experiments.

Weighting	AutoSelect weighting button
pseudo-echo	Pseudo
sinebell	Sine
sinebell-squared	sq-sine

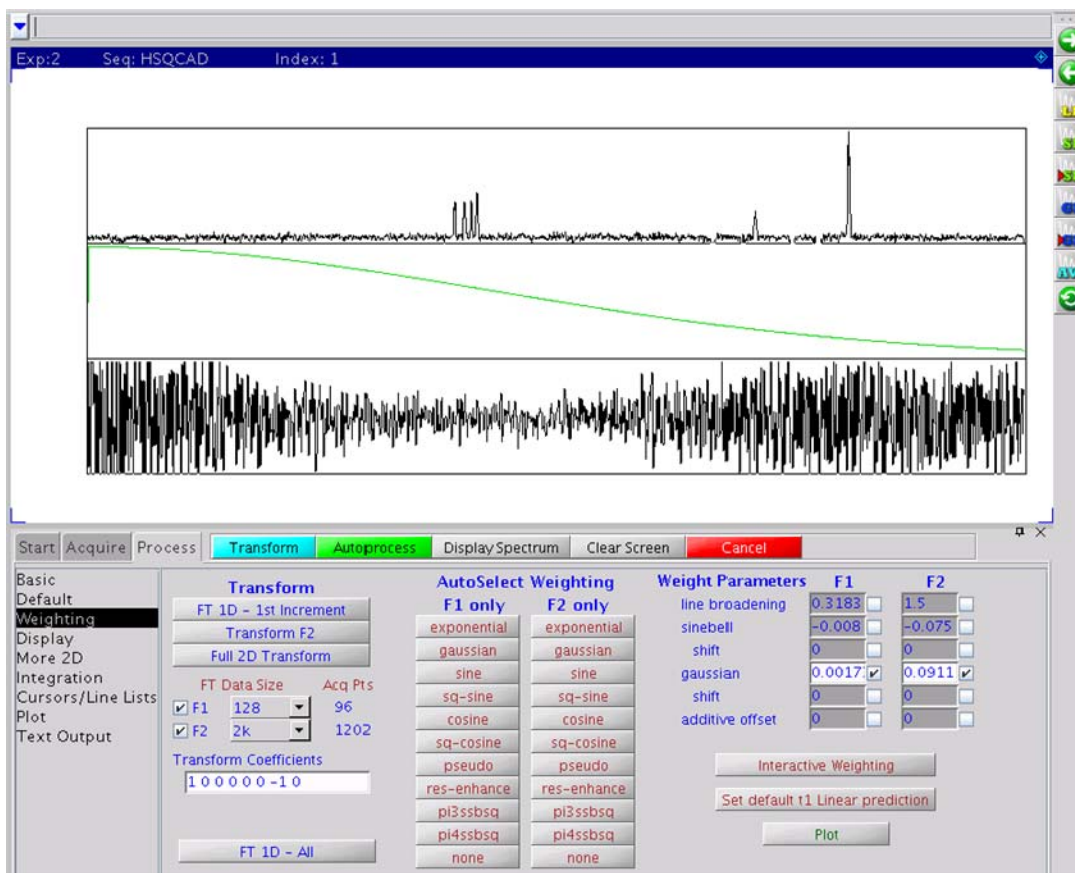
Phase-sensitive 2D experiments

The key to using weighting functions is to use them to ensure that the weighted FID or interferogram decays to zero by the end to avoid “truncation wiggles.” The Gaussian function is ideally suited for this, typical values might be $gf=0.6*at$, $gf1=0.6*ni/sw1$ ($=0.6*at1$), which are entered








in the Weight Parameters fields. Resolution enhancement (using negative line broadening) may be helpful in cases of spectral overlap, but can also be dangerous, since the “wiggles” that it can produce around the sides of peaks show up as peaks of opposite sign in the 2D plot, which complicates the analysis of the spectrum.

Interactive weighting

The **Interactive Weighting** button that shows up in several pages of the **Process** tab (or the `wti` command) allows interactive setting of the weighting parameters for any selected dimension (either the t_2 FIDs or the t_1 interferograms, for example, both the n_1 and n_2 dimension). The currently active element or trace is used in adjusting the weighting parameters.



The following graphics toolbar buttons control these parameters used with interactive weighting:

Check Box	Icon	Function
Line broadening		Selects line broadening or exponential weighting. A negative value gives resolution enhancement.
Sinebell		Selects the sinebell constant. A negative value gives squared sinebell.
Shifted Sinebell		Selects the sinebell shift constant (if sinebell is active)
Gaussian		Selects the Gaussian time constant
Shifted Gaussian		Selects the Gaussian shift constant (if Gaussian is active)
Additive weighting		Selects the additive weighting constant
Return		Returns to the previous menu

The values displayed in the graphics display window correspond to the values displayed in the Weight Parameters fields on the **Process** page. Clicking a graphics control button toggles the weighting function on and off.

Enter values in the fields next to the Weight Parameters and select the box to activate the parameter. Press **Return** to enter the value.

On the graphics screen, the left mouse button changes the selected parameters. The right mouse button turns the spectrum on and off. (This produces a faster response to changes in the weighting function, which was useful years ago on slower computers.)

- 1 `pmode=''` is both `ph` and `ph1` must be executed before performing 2D FT.
- `pmode='partial'` is only `ph` must be executed before performing 2D FT.
- `pmode='full'` is a command must be executed before 2D FT.

Baseline and Drift Correction

Use the **Linear Prediction** and **Display** pages in the **Process** tab to correct baseline and drift.

Calculating the preacquisition delay

Unless the first-order phase in the directly-detected dimension is approximately zero (which it should normally be on modern spectrometers), the non-zero l_p value will affect both the spectral drift correction (dc) offset and the curvature of the spectrum during 2D data processing. Delay values are shown on the **Acquire / Flags** page. The ideal first-order phase correction should be close to zero. If the probe file is calibrated properly, this will occur automatically. If the probe files are not properly calibrated, do one of the following.

- 1 Enter `setrc` to calculate a new value for the `ddrtc` delay so that l_p is rendered approximately 0 after acquisition. This is done before acquisition, and does not require an existing spectrum.
- 2 Enter `setlp0` to calculate a new value for the `ddrtc` delay so that l_p is rendered approximately 0 after acquisition. This macro requires an existing spectrum, properly phased (it looks at l_p), and it must be followed by a reacquisition of the spectrum.

On older spectrometers, do one of the following:

- 1 Obtain a trial spectrum and phase it to pure absorption. This spectrum provides the current pre-acquisition delay and first-order phase values. Using these values, the `calfa` macro will calculate proper values for the pre-acquisition delays (like `alfa`) such that when the spectrum is re-acquired, l_p should now be approximately 0 when the spectrum is properly rephased. This macro requires an existing spectrum, properly phased (it looks at l_p), and it must be followed by a reacquisition of the spectrum.
- 2 Enter `setlp0` to calculate a new value for the pre-acquisition delay (`alfa`) so that l_p is rendered approximately 0 when the spectrum is reacquired. This macro requires an existing spectrum, properly phased (it looks at l_p), and it must be followed by a reacquisition of the spectrum.

Setting the receiver gating time

Set the receiver gating time (typically `rof2`) to a value appropriate for the current probe and observe nucleus. On DDR systems (that is, VNMR5), the appropriate gating time for a cold probe is approximately 25 μsec to prevent ringing, and the appropriate gating time for a room temperature probe is approximately 25 μsec . On older spectrometers, the appropriate gating time for a cold probe is approximately 20 μsec , and the appropriate gating time for a room temperature probe is approximately 4 μsec .

First point multipliers

The fields under 1st Pt Multiplier (on the **Linear Prediction** page) multiply the first point of each FID by the F2 value and the first point of each interferogram (for the indirectly-detected dimension) by the F1 value. The 1st Pt Multiplier compensates for first point distortion in analog receivers (see Otting, Widmer, Wagner and Wuthrich, *J. Magn. Reson.* 1986, 66, 187). The default `fpmult` value on older spectrometers is 1.0, except if the processing involves backward extension of the time-domain data with linear prediction, in which case, the value is then 0.5. On newer spectrometers, the default `fpmult` value should always be 0.5. The default `fpmult1` value is 0.5.

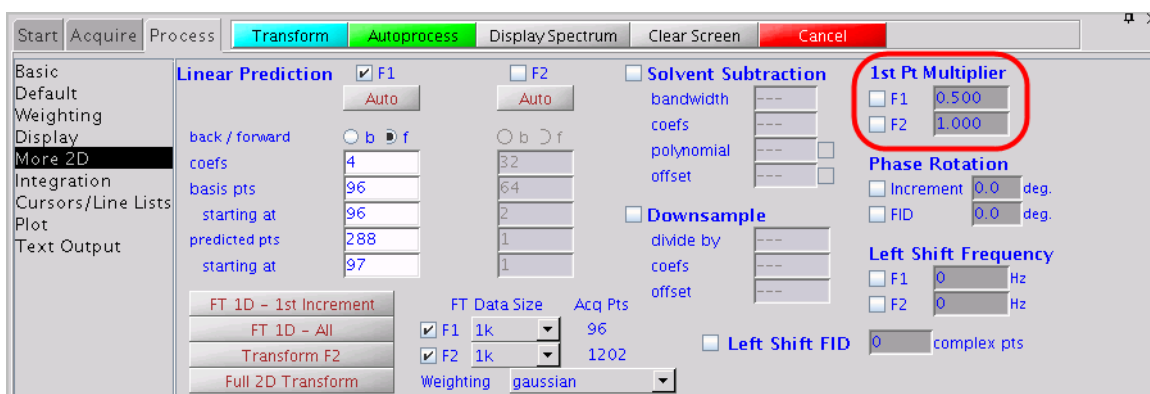
The effect of using the F2 value in 1st Pt Multiplier is to perform a linear baseline correction on all F_2 data, reducing negative-going ridges along F_2 in phase-sensitive 2D data. This correction is not needed in experiments such as COSY, where the FID starts at zero and grows. It is also not needed in absolute-value mode presentation, if pseudo-echo or sinebell processing is used, because the processing function goes to zero at $t_2=0$, forcing all FIDs to start at zero amplitude.

Typically, no correction for 1st Pt Multiplier is necessary. Automatic empirical setting of `fpmult` is available through the macro `cfpmult`, which is used on a first increment spectrum. Clearing the check boxes next to F1 and F2 disables the first-point multiplier feature. This is the usual value for sinebell or pseudo-echo processing.

The best empirical value of the 1st Pt Multiplier can also be determined manually. It can be determined manually during or after the acquisition of the 2D data by using the **FT 1D -1st Increment** button in the **Process** pages.

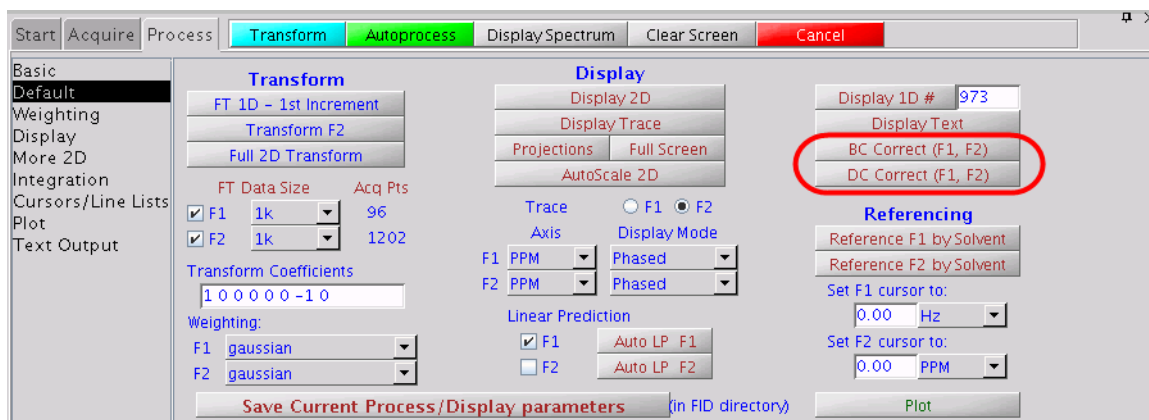
- 1 Enter `dc` or use the **DC Correct** button with a properly phased first increment spectrum on the screen.
- 2 Position the mouse at the right edge of spectrum baseline (to keep track of the ideal baseline position).
- 3 Enter `cdc` and observe the new position of the baseline. It typically drops.
 - Baseline goes negative: set the F2 1st Pt Multiplier to greater than 1.0 (try 1.5) and click **FT 1D - 1st Increment** on the **Process / Default or Process / Display** pages.
 - Baseline rises but does not return to the position indicated by the mouse arrow: increase the value of F2 1st Pt Multiplier and click **FT 1D - 1st Increment** again. If in doing so the baseline rises above the ideal level, reduce the value and try again.

Usually, only a few tries are required before the proper value of F2 1st Pt Multiplier is found.



Spectral drift correction

Drift corrections can be performed on the 2D datasets as well. This can sometimes help remove ridges from the processed data. The **DC Correct (F1, F2)** button on the **Process / Default** page performs drift corrections along both axes, and should only be performed after the 2D transform. The drift corrections can also be called via the command line using `dc2d('f2')` and `dc2d('f1')`, in which case, they can be using either during or after the 2D transform, as long as the indicated axis has been transformed.



Baseline correction

Baseline Correction is related to the 1st Pt Multiplier and Drift Correction tools, which are used to remove ridges from the processed data. Access baseline correction on a 2D dataset by clicking on the **BC Correct (F1, F2)** button on the **Process / Default** page. It performs baseline corrections along both axes, and should only be performed after the 2D transform. Baseline correction in 2D processing uses the spline or second to twentieth order polynomial fitting of predefined baseline regions. These regions are set up prior to the use of `bc` by setting integral resets so that integrals appear only over regions of the spectrum with signals present. These can be set after clicking **FT 1D - 1st Increment**. The quality of the baseline correction can be assessed by `bc(1)`. The baseline corrections can also be called via the command line using `bc2d('f2')` and `bc2d('f1')`, in which case they can be used either during or after the 2D transform, as long as the indicated axis has been transformed.

In setting baseline regions near the ends of the spectrum, the actions of the `bc` and `dc` operations are similar to the 1st Pt Multiplier, because they all represent a simple spectral drift correction.

Processing Phase-Sensitive 2D and 3D Data

The complete 2D transformation can be performed after 2D data has been acquired by using one of the **Transform** buttons on the **Process** pages. A typical example would be the **Full 2D Transform** button on the **Process / Default** page.

A series of complex FIDs, obtained as a function of t_1 , are transformed to become a series of spectra. Each spectrum contains a real and imaginary part. Each spectrum is then phase-rotated, according to the phase correction determined from an individual spectrum. This produces a series of spectra, each consisting of an absorptive and a dispersive part, formed as linear combinations of the original real and imaginary parts. Complex interferograms then form out of corresponding points along the frequency axis from each of the spectra and this is transformed to produce the final 2D spectrum.

The real and imaginary part of the interferograms can be formed from any linear combination of the real and imaginary parts of one or more spectral sets after the first Fourier transformation. Refer to these coefficients below according to the following scheme: RR1 is the coefficient used to multiply the real part (first R) of spectra in set 1 (the 1) before it is added to the real part (second R) of the interferogram. IR2 thus represents the contribution from the imaginary part of spectra in set 2 to the real part of the interferogram, and so on.

Another set of complex interferograms are formed from these two sets of F_2 spectra for some experiments. This set of interferograms is 90° out-of-phase in F_2 to the previous set and can be constructed without any additional coefficients.

Different experiments require different coefficients. Some, such as heteronuclear 2D-J experiments, consist of only one FID and spectral set, and hence need a total of four coefficients to be processed correctly. Others, including hypercomplex 2D experiments, consist of two original data sets and need a total of eight coefficients to allow it to be processed correctly. Other experiments are possible with three or even more data sets, requiring in each case, four times as many coefficients as the number of data sets (see the macro `wft2dac`).

$4n$ coefficients must be supplied if there are n data sets to be transformed, as in typical phase-sensitive experiments. The first

$2n$ coefficients are the contributions to the real part of the interferogram, alternating between real and imaginary parts of the successive data sets. The next $2n$ coefficients are the contributions to the imaginary part of the interferogram, in the same order.

Thus, using the definition that the first letter refers to the source data set, the second letter refers to the interferogram, and the number identifies the source data set, we have the cases shown in the table.

Data Sets	Coefficient Order
1	RR1, IR1, RI1, II1.
2	RR1, IR1, RR2, IR2, RI1, II1, RI2, II2.
3	RR1, IR1, RR2, IR2, RR3, IR3, RI1, II1, RI2, II2, RI3, II3.
...	...

The coefficients are generally 1, 0, or -1 , but other coefficients are acceptable. Any *real* coefficient can be used, and as many coefficients can be non-zero as is desired. Up to 32 coefficients can be supplied, which at four per data set, allow the addition, subtraction, etc., of eight 2D data sets (that is, eight different phase cycles). See the macro `wft2dac` for more information.

Processing programs

A number of processing programs are available:

- `ft1d(coefficients)` performs only the first Fourier transformation along the F_2 dimension (without weighting) and matrix transposition, allowing the display of interferograms with the `wti`, `dcon`, and `dconi` commands.
- `wft1d(coefficients)` functions the same as `ft1d` except weighting is included.
- `ft2d(<option,>coefficients)>` performs a complete transformation in 2D, without weighting, after 2D data has been acquired. If the first Fourier transformation has already been done using `ft1d`, `wft1d`, `ft1da`, or `wft1da`, then `ft2d` performs only the second (t_1) transform. 'ptype' or 'ntype' can be used as the first argument to select P-type or N-type peak selection. The coefficients arguments are discussed below.

- `wft2d(<option,>coefficients)>` performs the same as `ft2d`, except weighting is included. To perform a normal 2D transform on the n-th element in an arrayed 2D experiment, type `wft2d(n)`.
- `ft2da('bc',polynomial_order)>` runs complete phase-sensitive Fourier transform after the 2D FID data has been acquired. 'bc' is a keyword to perform a baseline correction on the F_2 spectra prior to the Fourier transform along F_1 . The `polynomial_order` is the order of the polynomial used in the baseline correction.
- `wft2da('bc',polynomial_order)>` functions the same as `ft2da`, except weighting is included.
- `ft1da` functions the same as `ft2da`, except a Fourier transform along F_1 is omitted.
- `wft1da` functions the same as `ft1da`, except weighting is included.

You can reduce the processing time for some 2D data sets by selectively transforming the t_1 interferograms. The command `ft2d('f2sel')` allows only preselected F_2 regions to be transformed along t_1 ; the t_1 interferograms in the non-selected F_2 regions are zeroed but not transformed. The same mechanism that is used to select baseline regions for baseline correction (bc) is used to select the F_2 regions that are to be transformed along t_1 . Partition the integral of the spectrum into several regions. The even numbered F_2 regions, for example, 2, 4, etc., will be transformed along t_1 ; the odd numbered ones will not be transformed along t_1 .

Unreliable peak heights can be caused by Fourier transformation of truncated time-domain data. To obtain properly defined signals, take one of the following steps:

- Collect data until the signal has decayed to zero in the time domain.
- Transform the data with zero-filling (`fn>=2*np`, `fn1>=4*ni`).

Taking one of these steps is particularly important in 2D spectra with antiphase or dispersive signals, where underdigitization can lead to signal cancellation.

Common coefficients for wft2d processing

To enter process coefficients, use the **Transform Coefficients** field on the **Process/Default** page. Typically, the coefficients are already set in the 2D parameter sets (4 coefficients for absolute value mode and 8 coefficients for phase-sensitive).

A magnitude-mode transform, in which the real part of the interferogram is formed from the real part of the spectra and the imaginary part of the interferogram is formed from the imaginary part, would require $1, 0, 0, 1$. Changing the sign of the imaginary part of the interferogram serves to change the effective direction of the F_1 frequency axis, as is required for data in which N-type peaks are detected. This can be done with $1, 0, 0, -1$.

In some experiments, including heteronuclear 2D-J, the basic data are purely amplitude modulated, with a starting amplitude of +1. After the first transformation and phasing operation are complete, the dispersion part of each spectrum serves only to produce a phase-twist in the final spectrum without contributing any information. Setting the imaginary part of the second transform to zero produces a pure absorption display in both domains: $1, 0, 0, 0$.

In the hypercomplex method of acquiring pure absorption 2D data, we have two complete sets of spectra and must therefore provide eight coefficients to specify the composition of the interferograms. A typical execution of the method described by States, Haberkorn, and Ruben, assuming that the first spectrum of the first data block has been phased for absorption, requires $1, 0, 0, 0, 0, 0, 1, 0$ to produce pure absorption spectra.

Other manipulations of two data blocks are formatted similarly. A magnitude-mode 2D experiment that is the sum of the two different experiments can be constructed by $1, 0, 1, 0, 0, 1, 0, 1$. For a COSY experiment, this would produce the P-type experiment. Subtracting data block two from block one, which for a COSY experiment gives the N-type COSY, would be accomplished by $1, 0, -1, 0, 0, 1, 0, -1$. Thus, two different absolute-value 2D experiments, (P-type and N-type) and a phase-sensitive 2D experiment, can be produced from the *same* data set, without acquiring the data again.

Different combinations of data sets with appropriate phase cycling might allow selection of various quantum orders in a single experiment. Note that since the coefficients may be different from one, it is possible essentially to phase shift each experiment *separately* (phase shift the receiver) *after* the experiment is done. For TPPI data with phase=3, only one data set is collected, and the imaginary part of the second transform is set to zero: $1, 0, 0, 0$.

The parameter `f1coef` is used to store the above coefficients as text strings and is displayed under the label *Transform Coefficients*.

2D solvent subtraction filtering

2D solvent subtraction is set up on the **Process / Linear Prediction** page. In a 2D transform, solvent subtraction is invoked on t_2 FIDs. The parameters `ssfilter` and `ssorder` select the processing option as follows:

- The `zfs` (zero-frequency suppression) option is selected if both bandwidth (`ssfilter`) and polynomial (`ssorder`) are set to a value.
- The `lfs` (low-frequency suppression) option is selected if bandwidth is set to a value and polynomial is not selected.
- The solvent subtraction (both the `zfs` and `lfs` options) is turned off if the bandwidth is blank.

Left shift FID, left shift frequency, phase rotation

Use the **Process / Linear Prediction** page to adjust the Left Shift FID, Left Shift Frequency, and the Phase Rotation.

Check **Left Shift FID** to left-shift the FID by the entered number of complex (or hypercomplex) points before the weighting and Fourier transformation are performed. The parameter name is `lsfid`; the value must be between 0 and `np` minus 1.

Enter a negative value for Left Shift Frequency to shift the peaks upfield (to the right) or a positive value to shift the peaks downfield (to the left). The Left Shift Frequency (`lsfrq`) operates only on the F_2 data. A related parameter (`lsfrq1`) performs a similar function in F_1 .

Apply a phase-rotation to the interferogram by checking the appropriate box and entering a value in degrees (zero-order phase rotation). This causes zero-order phase rotation before weighting and Fourier transformation are performed.

2D processing of 3D data

Acquisition and full processing of 3D data requires the parameters `ni2` and `sw2` (`d3` is the delay increment in the `ni2` dimension). 2D processing of “slices” of the 3D data matrix is accomplished using the following commands:

<code>ft2d('ni2')</code>	Transforms non-arrayed 2D data that have been collected with <code>ni2</code> and <code>sw2</code> (instead of <code>ni</code> and <code>sw1</code>). The <code>addpar('3d')</code> macro creates the necessary processing parameters for the <code>ft2d('ni2')</code> operation (<code>par3d</code> functions the same as <code>addpar('3rf')</code>).
<code>ft2d('ni',#)</code>	Selectively transforms selected <code>np-ni</code> 2D plane within a non-arrayed 3D data set, <code>#</code> is an integer that can range from 1 to <code>ni2</code> .
<code>ft2d('ni2',#)</code>	Selectively transforms selected <code>np-ni2</code> 2D plane within a non-arrayed 3D data set, <code>#</code> is an integer that can range from 1 to <code>ni</code> .
<code>wft2d</code>	Acts the same as <code>ft2d</code> , but applies weighting before transformation.

The format of the arguments to `ft2d` changes if an arrayed 3D data set is selectively processed. For example, `ft2d('ni',#1,#2)` performs a 2D transform along `np` and `ni` of the `#2`-th `ni2` increment and the `#1`-th element within the explicit array. This yields a 2D `np-ni` frequency plane. `#1` ranges from 1 to `ni2`; `#2` ranges from 1 to `[arraydim/(ni*ni2)]`.

Arrayed 3D data sets can also be subjected to 2D processing to yield 2D absorptive spectra. If the States-Haberhorn method is used along both F_1 (`ni2` dimension) and F_2 (`ni` dimension), there will generally be four spectra per (`ni,ni2`) 3D element. In this case, the command `ft2d('ni2',#1,<16 coefficients>)` performs a 2D transform along `np` and `ni2` of the `#1`-th `ni` increment using the ensuing 16 coefficients to construct the 2D `t1`-interferogram from appropriate combinations of the four spectra per (`ni,ni2`) 3D element. Use the `proc2` parameter to specify the type of data processing to be performed on the `ni2` interferogram (3D): `'ft'` for complex FT, `'rft'` for real FT, or `'lp'` for linear prediction processing on complex data. The macro `dg2` displays 3D processing parameters in the test output page.

2D and 3D Linear Prediction

FT 1D - 1st Increment	FT Data Size	Acq Pts
Transform F2	1k	128
Full 2D Transform	1k	962

Linear prediction parameters are adjusted on the **More 2D** page, under **Linear Prediction**. The F1 check box controls the transformation process along t_1 , and the F2 check box controls the transformation process along t_2 . Using the same method of transformation is not necessary along two (or three axes). For example, you can employ a backwards linear prediction in t_2 of a 2D experiment and a forward linear prediction along t_1 , or perhaps a simple Fourier transformation along t_2 and a backwards linear prediction along t_1 .

Phasing the 2D Spectrum (Both F_1 and F_2)

The phase constants $lp1$ and $rp1$ control the phase correction along F_1 in phase-sensitive data. These should ideally be near zero in most 2D experiments. Finite pulse widths and delays in the pulse sequence can cause deviations from zero, but standard pulse sequences should properly compensate for these imperfections and make it possible to have zero $lp1$ and $rp1$. Most of the setup macros set $lp1$ and $rp1$ to zero so that the first display of the 2D spectrum will indicate the need (if any) for phase correction in F_1 . The same techniques as used in 1D phasing are employed here, with minor differences. (The methodology listed below can also be used to phase F_2 if appropriate modifications are made.)

- 1 Enter `f full` to display the full data matrix in a full chart display.
- 2 Phase the 2D spectrum: Use the horizontal cursor in the interactive display to identify a peak toward the right-hand edge of the spectrum. Note the trace number indicated at the top of the display (“memorize” this by setting `r1` equal to its value.)
- 3 Select one or more other traces at F_1 values more toward the center and left parts of the spectrum. (One easy option for spectra with a large diagonal is to use peaks on the diagonal. These will typically provide very sensitive phase corrections.) Use `r2`, `r3`, etc. to “memorize” these trace values. A minimum of two is needed, one at the far right and one at the far left.
- 4 Enter `ds(r1)`. Phase this spectrum like a 1D spectrum (for example, by using the **Phase** button in the graphical toolbar). Click the mouse on the peak displayed near the right edge of the spectrum. Phase up this spectrum (thus setting `rp1`). Do not click in the left part of the spectrum at this time.
- 5 Enter `ds(r2)`. The second trace appears. Click the mouse near the right edge of the spectrum (to fix `rp1` at the previously determined value) and do not rephase. Move the mouse to the peak at the left, click and phase it (thus setting `lp1`).
- 6 Enter `ds(r1)` to recheck `rp1`. Repeat the process again if necessary.

Use the diagonal peaks for phasing in homonuclear correlation spectra (such as NOESY, TOCSY, and ROESY).

Phase an f_1 trace exactly like a 1D spectrum if there are strong cross-peaks. Phase HMQC spectra by progressively working from right to left, with several peaks selected along the way to make sure that `lp1` does not go through an extra revolution that would induce some baseline roll.

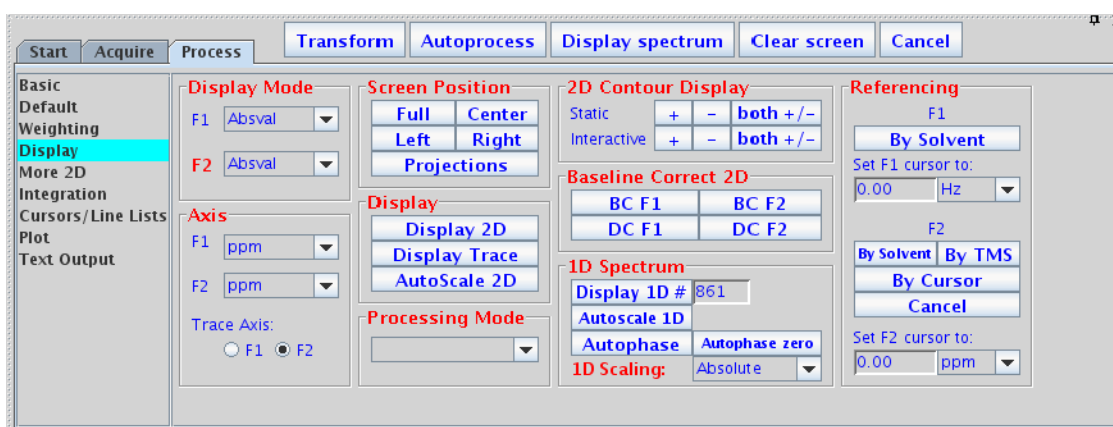
Corrections in f_2 phasing may be obvious in the 2D data when they are not in the first increment 1D spectrum. If `pmode='full'` before the 2D transform, f_2 phasing may be corrected without retransforming by setting `trace='f2'` and using the same approach as described for `f1phasing`. Transformation of the data again is necessary if `pmode=""` or `pmode='partial'`. No f_1 phasing is possible after transformation if `pmode=""`. f_1 phasing after the transform is possible (but not f_2 rephasing) if `pmode='partial'`. Do baseline corrections such as `dc2d` or `bc` only after data is properly phased in f_1 and f_2 .

Display and Plotting

This section discusses the manual mode of (non-interactive) 2D display and plotting. For more details, see [Chapter 9](#), “Printing, Plotting, and Data Output”.

Display modes

Select display modes in the **Display** page.



Display and plot limits

The center, left, right, and full set the spectrum to display (and subsequent plot) in the relevant portion of the screen (and page).

Display scaling

The `peak2d` command searches the area defined by `sp`, `wp`, `sp1`, and `wp1` in a 2D data set for a maximum intensity. It returns the maximum intensity value found, the trace number of the maximum, and the data point number of the maximum on that trace.

The **AutoScale 2D** button on the **Display** page uses `peak2d` to set up the vertical scaling and threshold for a 2D contour plot and color map display.

Grid lines

A grid of horizontal and vertical lines over a 2D display can be drawn by the `grid` macro. By default, grid lines are drawn in blue at approximately 1 cm intervals, rounded so that the intervals fall at a multiple of 1, 2, or 5 Hz or ppm. To change the defaults, enter `grid` with a different spacing (in cm) or a different color ('red', 'green', etc.); for example, `grid(2,'white')` gives white grid lines at 2 cm intervals.

The `grid` command also can define a grid, using the following syntax:

```
grid<(startf2,incrf2,startf1,incrf1,color)>
```

The arguments define the frequency and increments between grid lines in the f_2 and f_1 directions and the color of the grid lines.

The `plgrid` macro uses the same arguments as `grid`, but plots the grid instead.

Whitewashed spectra

The `dsww<(start,finish,step)>` command displays one or more spectra with whitewashing (traces in front “block” the view of traces behind them). Use the argument 'all' to display all spectra. `plww<(start,finish,step)>` plots the same spectra.

Use the **Stacked Plot** graphics button in the **Plot** page to display a stacked display of 2D spectra in the whitewash mode.

Label display

The `dssl` macro displays a label for each element in a set of stacked spectra. The label is an integer value starting with 1 and ranging to the number of spectra in the display.

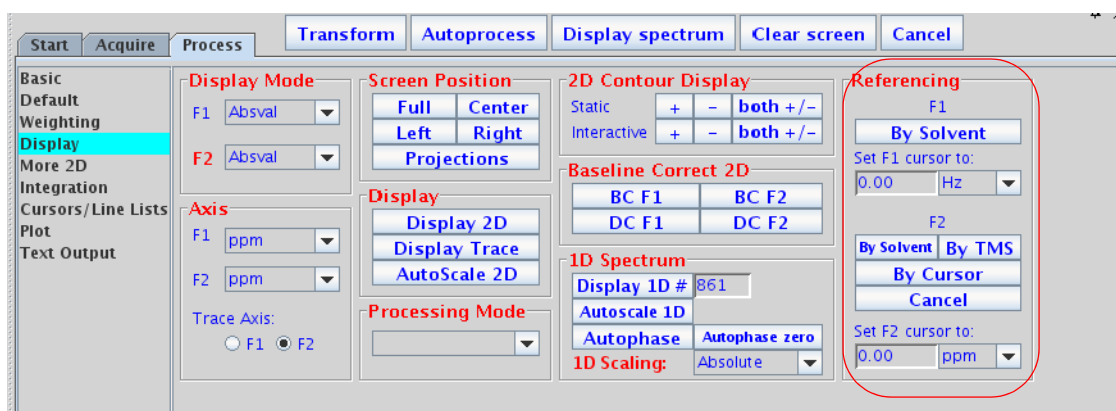
Labels can appear at incorrect positions if `wysiwyg='n'`. The positions were empirically determined for a large screen display and are not guaranteed to be correct for all displays.

The following options control the `dssl` display (more than one option can be entered as long as the options do not conflict with each other):

- 'center', 'left', 'right', 'top', 'bottom', 'above', and 'below' are keywords setting the position of the displayed index relative to each spectrum.
- 'value' is a keyword that produces a display of the values of each array element, instead of an integer index.
- 'list=xxx' produces a display of the values contained in the arrayed parameter xxx.
- 'format=yyy' uses the format yyy to control the display of each label. See the `write` command for information about formats.

2D referencing

Use the **Referencing** buttons on the **Process / Display** page to modify (as needed) the referencing of 2D spectra.



Enter a frequency (in Hz or PPM) to re-reference the indicated chemical shift axis.

Click the **Cancel** button to reset referencing along F_2 and F_1 .

Use the `centersw` macro to indicate the center of the directly detected dimension, and the `centersw1` macro to indicate the center of the first indirect dimension. Use both to center the cursors in the spectrum.

Prior to acquisition, you can use the macro `setsw1(nucleus,downfieldppm,upfieldppm)` to set the spectral width to a given spectral window.

Rotating homonuclear 2D-J spectra

The `rotate` command rotates homonuclear 2D-J data 45° (rotation in frequency-space) to line up multiplets. Use the `rotate(angle)` command with the angle argument to specify other angles.

Setting negative intensities to zero

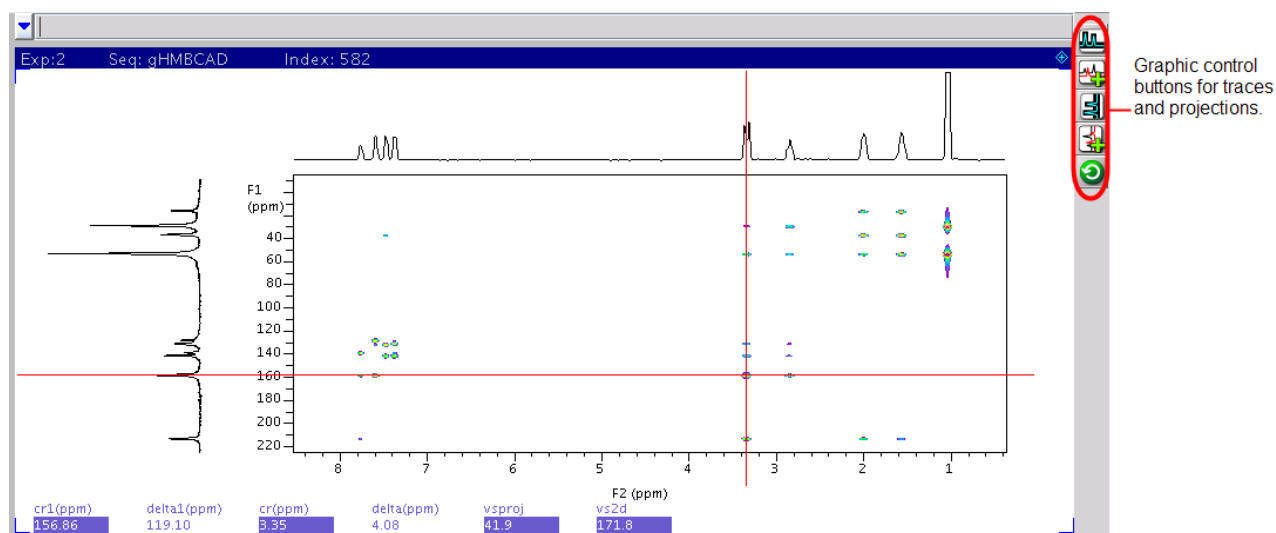
The command `zeroneg` is used for the projection of proton 2D-J spectra at 45° to strip a high-resolution proton spectrum down to a list of chemical shifts. The `zeroneg` command sets all negative intensities to zero.

Interactive 2D Color Map Display

Use the graphics control buttons and the mouse to control the display in the graphics window.

2D display

Below is an example of a 2D display with the projection graphics control buttons selected.



Controls for adjusting the color/grayscale are on the right side of the window.

The interactive display parameters are displayed across the bottom of the graphics window (assuming "fields" is checked "on" in the Frame vertical panel):

cr	Shows the current cursor position
cr1	Shows the current cursor position along the first indirectly detected dimension
delta	Shows the cursor difference
delta1	Shows the cursor difference along the first indirectly detected dimension
vs2d	Shows the vertical scale of the display
vsproj	Shows the vertical scale of the trace or projection

Interactive nD display control buttons

Table 22 Main nD display bar tools





Icon	Description
	Display color map and show common nD graphics tools
	Display contour map and show common nD graphics tools
	Display stacked spectra and show common nD graphics tools
	Display image map and show common nD graphics tools

Table 23 nD graphic tools


















Icon	Description
	One cursor in use, click to toggle to two cursors
	Two cursors in use, click to toggle to one cursor
	Click to expand to full display
	Pan and stretch
	Show trace
	Show projections
Click 	to show horizontal maximum projection across the top of the 2D display
Click 	to show horizontal sum projection across the top of the 2D display
Click 	to show vertical maximum projection down the left side of the 2D display
Click 	to show vertical sum projection down the left side of the 2D display
	Rotate axes
	Increase vertical scale 20%
	Decrease vertical scale 20%

Table 23 nD graphic tools (continued)

Icon	Description
	Phase spectrum
Click 	to select the first spectrum
Click 	to select the second spectrum
	Enter peak pick menu

Cursors

Use the left and right mouse buttons to move cursors, and the center button to adjust the vertical scale of traces, projections, and contour maps, as well as to adjust the threshold in the optional color bar. The cursors can be used to select regions for expansions of the display. The cursors can also be used to select positions to “mark” using the `ll2d('mark')` command, which displays and records spectral frequencies, maxima, intensities, and volumes.

The left mouse button adjusts the position of the 2D cursor. The corresponding frequencies are displayed at the bottom of the graphics window. Both the horizontal and vertical cursors move if the left mouse button is pressed within the 2D display box.

Above and below the box, only the vertical cursor can be moved. At the left and the right of the box, only the horizontal cursor can be moved. In addition, holding the mouse button down and then moving the mouse moves the cursor with the mouse.

The function of the center mouse button depends on the location of the cursor:

- Cursor is within the 2D display box: In gray scale images, pressing the center button sets the point to medium gray. Otherwise, for color map and contour displays, if there is no intensity at that point, the center button changes vertical scale to show intensity at that point. If there is intensity at the point, the center button changes the scale to show no intensity, then changes the parameter `vs` and redraws.

- Cursor is near an active trace and active horizontal or vertical projection: Pressing the center button changes the vertical scale of trace or projection, so that the spectrum goes through the current mouse position.
- Cursor is near the color/grayscale bar and in the color mode: Pressing the center button sets the threshold to remove low intensity peaks. If in the grayscale mode, pressing the center button sets the grayscale intensity (the right button adjusts contrast).

A second cursor pair is displayed with the right mouse button. The second pair can be moved in exactly the same way as the first pair, and is used to select a box within the 2D display. The right mouse button also switches the display into the box mode, the same as clicking on the **Box** button in the menu.

Traces

- 1 Click the **Trace** icon. A trace is displayed for the position of the horizontal cursor.
- 2 Move the horizontal cursor to change the displayed trace.
- 3 Adjust the vertical scale of the trace by clicking the middle mouse button on the trace, not in the 2D spectrum.

Exit the trace mode by displaying a box with the right mouse button, or by selecting another display mode.

Projections

- 1 Click the **Projection** icon to open the Projection graphic control buttons.
- 2 Select the type of direction (horizontal or vertical) and mode (maximum or summary).
- 3 Adjust the vertical scale of the projections with the middle mouse button.

Expanding the display

- 1 Use the Box cursors to select the region in the spectrum to expand.
- 2 Click the **Expand** icon to obtain an expanded display.

Setting the vertical scale

If a peak is expected at a certain position in the spectrum but is not visible, click once at that position with the middle mouse button. This selects a new vertical scale, so that the intensity at that point is by a factor of 2 above the threshold, and the display is redrawn.

Adjusting the threshold

If noise is visible at a certain position in a spectrum, but should be suppressed below the threshold, click once at that position with the middle mouse button. A vertical scale is calculated so that this intensity falls by a factor of 2 below the threshold, and again the spectrum is redrawn.

Treating 2D traces as 1D spectra

Enter the command `ds` after a trace has been selected in the interactive 2D display program to allow the trace to be displayed as if it were a simple 1D spectrum. All standard 1D data manipulations, including line listing, integration, etc., are then accessible for that trace. The command `ds(tracenumber)` can also be used to display an F_1 or F_2 trace, depending on the value of trace.

Interactive 2D Peak Picking

The `l12d` program is used to automatically or interactively pick peaks in 2D spectra or 2D planes of 3D spectra. The peaks can be displayed on top of the spectrum in the `dcon1` display or can be plotted using the `p112d` command.

The results of all peak-picking operations are stored in a binary file in the `l12d` subdirectory of the current experiment directory:

- The results are stored in the file `peaks.bin` for 2D spectra.
- The results are stored in `peaks_f#f#_#.bin` for 2D planes of 3D spectra, where `f#f#` denotes the orientation of the plane being picked (for example, `f1f3` or `f2f3`), and the last `#` denotes the number of the plane.



































Binary peak files can be converted to text files for printing or for export to other programs.

For each peak in a peak file, the following information is stored:

- Peak number
- Interpolated peak frequency in both dimensions
- Interpolated peak amplitude
- Full width at half-height (FWHH) in both dimensions
- Bounds of the peak in both dimensions
- Volume of the peak
- 15-character peak label
- 80-character comment

Interactive 2D peak picking buttons

Most of the above options are accessible through a series of graphics control buttons. From the 2D Display, the **Peak Picking** button brings up the **2D Peak Picking** buttons.

<p>Peak Picking Main Menu</p> <ul style="list-style-type: none">  Auto  Edit  File  Display  Return 	<p>Automatic Peak Picking</p> <ul style="list-style-type: none">  Box/Cursor  Expand/Full  Auto Peak Pick  Auto Pick Footprints  Auto Pick Peak and Footprints  Adjust for overlap  Delete all peak picks  Return 	<p>Edit Peaks</p> <ul style="list-style-type: none">  Box/Cursor  Expand/Full  Pick peaks  Un-pick peaks  Delete all peak picks  Combine peak picks  Display info - nearest peak  Integrate 2D peaks  Return
<p>Peak Files</p> <ul style="list-style-type: none">  Read Binary Peak Pick File  Read Peak Pick Text File  Write Peak Pick Text File  Write Binary Peak Pick File  Return 	<p>Display Peaks</p> <ul style="list-style-type: none">  Show Peak Picks  Show Peak Pick Numbers  Show Footprints  Show Peak Labels  Show All Peak Markers  Hide All Peak Markers  Return 	

These buttons provide access to the following menus (depending on the mode, the labels on some buttons change).

The following menu selects another **2D Peak Picking** menu. The buttons function as follows:

Table 24 2D peak picking main menu

Auto	Displays the buttons for automatically picking peaks.
Edit	Displays the buttons for interactively editing peaks.
File	Displays the buttons for manipulating peak files.
Display	Displays the buttons for controlling the display of peaks.
Return	Returns to the 2D Color Map Display buttons.

This menu provides automatic peak picking. The buttons functions as follows:

Table 25 2D peak picking automatic menu

Box/ Cursor	Selects the cursor mode.
Expand/ Full	Selects expanded or full display.
Peak	Automatically finds peaks in the 2D spectrum. If one cursor is visible, all peaks above the current threshold in the currently displayed region of the spectrum are found and marked. A peak is defined as a data point that is higher than the eight points around it. Once such a point is found, the actual peak location is determined by interpolation in both dimensions.
Volume	Automatically finds the bounds of a peak and the integral of all points within these bounds. The bounds are found by descending down the sides of a peak until the point is reached where the amplitude of a data point is less than th_{2d} times the current threshold. Thus, using a smaller value for th_{2d} will cause Volume to find and integrate a larger area for the bounds of the peaks. The peak volume is calculated by summation of all data points within these bounds. If the bounds of a peak already exist, the volume is recalculated.
Both	Picks peaks and calculates volumes. The Both button does both the peak and volume operations at once.
Adjust	Adjusts peak bounds so that none overlap. The Adjust button adjusts all peak bounds in the displayed region of the spectrum so that none overlap and recalculates peak volumes with the new peak bounds.
Reset	Deletes all peaks that have been found in the current spectrum.
Return	Displays the 2D Peak Picking buttons.

This menu provides interactive peak editing. The buttons functions as follows:

Table 26 2D peak picking edit menu

Box/ Cursor	Selects cursor mode.
Expand/ Full	Selects expanded or full display.
Mark	In <code>dcon1</code> cursor mode, this button inserts a peak at the current cursor location. In <code>dcon1</code> box mode, the cursors are taken as peak bounds, and the area inside the cursors is integrated. These peak bounds are then assigned to all peaks within the cursors that do not already have their bounds defined. If a peak without bounds does not exist inside the area defined by the cursors, the highest point within that area is found, marked as a peak, and assigned the bounds defined by the cursors.
Unmark	In <code>dcon1</code> cursor mode, this button deletes the peak nearest the cursor. In <code>dcon1</code> box mode, this button deletes peak bounds from peaks whose bounds are entirely within the area defined by the cursors.
Clear	In <code>dcon1</code> cursor mode, this button deletes all peaks in the area of the spectrum displayed in <code>dcon1</code> . In <code>dcon1</code> box mode, the Clear button deletes all peaks that are within the area defined by the cursors.

Table 26 2D peak picking edit menu (continued)

Combine	This button works only in <code>dcon1</code> box mode. It combines all peaks within the area defined by the cursors into a single peak. This combination peak is located at the average frequencies of all of the original peaks and has bounds that encompass all of the original bounds of the peaks. The volume of the combination peak is calculated by summation of all data points within its bounds. Use the Backup File button in the 2D Peak Picking File menu (see below) prior to using the Combine button to save the original peaks if this data must be kept. Crating the combination peak are permanently deletes the contents of the current file.
Label	Prompts for a 15-character label to be assigned to the peak nearest the cursor (<code>dcon1</code> cursor mode) or to all peaks within the area defined by the cursors (<code>dcon1</code> box mode). Based on the value of the parameter <code>l12dmode</code> , this label can be displayed next to the peak in <code>dcon1</code> .
Comment	Prompts for an 80-character string to be assigned to the peak nearest the cursor (cursor mode) or to all peaks within the area defined by the cursors (box mode).
Info	Prints the peak file information about the peak nearest the cursor to the text window.
Set Int	Sets the value of the peak volume.
Return	Displays the 2D Peak Picking main menu. (See Table 24 on page 321).

Table 27 2D peak picking file menu

Read	Prompts for the filename of a binary peak file and reads that file into VnmrJ. When a file is read in, the current peak file (<code>peaks.bin</code> for 2D spectra) is overwritten by a copy of the peak file that was read in.
Read Text	Prompts for the file name of a text peak file and reads that file into VnmrJ. When a file is read in, the current peak file (<code>peaks.bin</code> for 2D spectra) is overwritten by a new binary copy of the peak file that was read in.
Write Text	Prompts for a filename to write a text version of the current <code>l12d</code> peak file.
Backup File	Prompts for a file name to copy the current binary peak file. Do this occasionally when doing a significant amount of interactive peak editing, so that intermediate versions of the peak file can be recovered in the event of an error (such as inadvertently selecting the Clear or Reset button or making a mistake using the Combine button).
Return	Displays the 2D Peak Picking main menu. (See Table 24 on page 321).

Table 28 2D peak picking display menu

Show Peak/ Hide Peak	Show Peak: The "+" is hidden and this button shows a "+" at the location of each peak. Hide Peak: The "+" is shown and this button hides the "+" at the location of each peak.
Show Num/ Hide Num	Show Num: The peak numbers are now hidden, and this button shows a peak number next to each peak. Hide Num: The peak numbers are now shown, and this button hides the peak numbers.
Show Box/ Hide Box	Show Box: The box is now hidden, and this button shows a box with the area integrated to get the volume of the peak. Hide Box: The box is now shown, and this button hides this box.

Table 28 2D peak picking display menu (continued)

Show Label/ Hide Label	Show Label: The peak labels are now hidden, and this button shows a peak label next to each peak. Hide Label: The peak labels are now shown, and this button hides peak labels.
Show All/ Hide All	Show All: Displays a "+", the peak number, the peak bounds, and the peak label for each peak. Hide All: Hides all peak information.
Return	Displays the 2D Peak Picking button.

3D NMR

VnmrJ includes full support for 3D NMR, including acquisition, processing, and display.

Many of the 3D-related macros and parameters—for example, `centersw2`, `cr2`, `cr12`, `delta2`, `dmg2`, `lp2`, `lsfid2`, `phfid2`, `rfl2`, `rfp2`, `rp2`, `sp2`, `wp2`—are normally used in the same manner as their 1D and 2D counterparts and are not described further in this section.

A non-arrayed 3D experiment has two implicitly arrayed parameters: `d2` and `d3`. `d2` is associated with `ni` and `sw1`, `d3` with `ni2` and `sw2`. The order of these two arrayed parameters is such that `d2` is normally cycled the most rapidly.

An arrayed 3D experiment, such as a single 3D with “superhypercomplex” data acquisition (States-Haberhorn method applied along both t_1 and t_2), has at least three arrayed elements. By convention, such an arrayed 3D experiment is implemented using four arrayed elements: `d3` (t_2 evolution time), `phase2`, `d2` (t_1 evolution time), and `phase`. Assuming that `array='phase,phase2'` (see below), the order of arrays is such that the `phase2` array is cycled the most rapidly, followed by the `phase`, `d2`, and `d3` arrays.

3D acquisition

3D data acquisition is accomplished with pulse sequences using the parameter `d3`, which is updated according to the parameters `ni2` and `sw2`. This is analogous to incrementing `d2` according to `ni` and `sw1` for 2D NMR (of course, `d2`, `ni`, and `sw1` are active in 3D as well). In addition, the parameter `phase2` is used to control the “mode” of acquisition (hypercomplex, TPPI, or absolute value) in the third frequency domain, just like `phase` in the second domain. All of these 3D parameters are created with the macro `addpar('3d')` along with other 3D parameters, including `fiddc3d` for 3D time-domain dc correction, `ptspec3d` for region-selective 3D processing, and `path3d` for the path to the currently displayed 2D planes extracted from a 3D data set. (The macro `par3d` is functionally equivalent to `addpar('3d')`.)

By convention, 3D sequences are described with the first evolution time being known as t_1 , the second evolution time as t_2 , and the time during which data are acquired as t_3 .

After transformation, these same dimensions are called the F_1 , F_2 , and F_3 dimensions.

3D processing

Data processing includes the `ft3d` command for full 3D processing, governed by the usual parameters to control transform sizes, weighting, phasing, etc., with a “2” at the end of the parameter name signifying the third dimension. Unlike other commands, `ft3d` occurs in the background by default. That is, it is run as a separate task, leaving `VnmrJ` free to continue with other tasks (including 1D and 2D processing of the same data set). To increase the speed of 3D transforms further, the `wftt3` macro allows the software to process one dimension (the acquisition or t_3 dimension) as the data are being acquired. Also, the `ft3d` software can be configured to run on several computers simultaneously, for even greater speeds. The `killft3d` macro terminates any `ft3d` program that has been started in an experiment.

3D display

Display the data as two-dimensional planes of the 3D data set in any of the three orthogonal directions. Skew planes are not supported, nor are “full 3-dimensional” displays. One command, `getplane`, extracts the 2D planes from the 3D data set in one or more of the three orientations. After the planes are “extracted” in this manner, they are displayed with the `dplane` macro. The parameter `index2` keeps track of which plane is on display. The macro `nextpl` displays the next plane from the plane currently on view. Another macro, `prevpl`, shows the previous plane from the current plane.

The `dsplanes(start_plane, stop_plane)` macro produces a graphical 2D color or contour map for a subset of 3D planes specified by the arguments. The `dcon1` program is used to display the planes. The `plplanes` macro is available to plot a series of 3D planes.

The new concept of time-domain frequency shifting can be employed to good use in 3D NMR, where spectra in the indirectly detected directions are often “folded” by accident or by choice. The parameters `lsfrq`, `lsfrq1`, and `lsfrq2` cause the frequency of the spectrum to be shifted as part of the Fourier transformation process.

3D pulse sequences

Write a sequence that includes a d_2 and d_3 delay (these delays may also be $d_2/2$ or $d_3/2$). Use the parameters `phase` and `phase2` to select between the two orthogonal components of the hypercomplex experiment in the relevant domain (for the sequence to operate in the hypercomplex – or the hyper-hypercomplex – mode. Ensure that the experiment processes correctly by using the default processing coefficients. Write the pulse sequence so that the `phase=2` (and `phase2=2`) experiments leave the receiver unchanged (compared to `phase=1`) and either increment the phase of the pulse (or pulse sandwich) just prior to the relevant evolution time or decrement the phase of the pulse following evolution by 90 degrees (or for multiple-quantum experiments, by $90/n$).

Experiment setup

Setup is necessary in 3D experiments to position transmitters and decouplers, adjust pulse widths, etc. Just as the setup of 2D experiments can often be assisted by performing “first increment” experiments (that is, a 1D experiment that represents the first increment of the 2D), so 3D experiments can be assisted not only by 1D setup experiments, but also by “first plane” 2D experiments. To perform a 2D experiment in the `sw1` dimension, set `ni2=1` and `phase2=1` (when `ni > 1` and `phase=1,2`; or `phase=3` for TPPI experiments). This combination of parameters will perform a “normal” 2D experiment, incrementing d_2 , and the data can be processed with the `wft2da` command (or its variants).

The “third dimension” 2D experiment is performed by setting `ni=1` and `phase2=1` (when `ni2 > 1` and `phase2=1,2`; or `phase2=3`, as desired). These parameters will produce a 2D experiment in which d_3 is incremented, resulting in a spectral width `sw2`. The `wft2d` command must be given the special argument `ni2` to process this data correctly, for example, `wft2d('ni2',1,0,0,0,0,-1,0)`. Use `wft2da('ni2',1)` to transform the first $F_2 \times F_3$ plane of a 3D data set.

When processing a “first plane” 2D experiment, the axes are always labeled F_1 and F_2 because this is considered to be a 2D experiment and the axis labeling corresponds to conventions used in 2D NMR.

After setting up the 3D experiment, reset `ni`, `ni2`, `phase`, and `phase2` to their desired values. Check the value of the

parameter array and make sure that `array='phase,phase2'` and not `'phase2,phase'`, which will acquire data in the incorrect order. To ensure the correct order, always enter `phase` before `phase2`, or simply enter `array='phase,phase2'`.

Data processing

Processing of 3D NMR requires coefficients to select various components of the data to be combined to form the final data set. There are up to 40 coefficients; see the *Command and Parameter Reference*. The coefficients are normally transparent to the user, just as are the 2D coefficients. The `set3dproc` command can create a 3D coefficient file for processing 3D FID data under certain conditions.

The `ft3d` command determines from the values of `phase` and `phase2` what the expected coefficients are, based on whether a hypercomplex (“States-Haberkorn”) or TPPI experiment has been performed in a particular dimension. This assumes that the pulse sequence has been written to perform “standard” phase cycling as described above. If the data is reflected along a particular dimension, it is possible (or probable) that different coefficients are required for data processing. In this case, the `ft3d('nocoef')` form is used to allow coefficients to be specified (which are found in a text file named `coef` in the 3D experiment directory, unlike in `ft2d`, where they are given as arguments to the command). By default, `ft3d` calls the `make3dcoef` macro to create a coefficient file using the `f1coef` and `f2coef` string parameter values.

The format for the 3D coefficient file is an extension of that used for 2D coefficients. The coefficient file contains four rows of eight coefficients used to construct the t_2 hypercomplex interferograms and a final row of eight coefficients used to construct the t_1 interferogram. The actual values of the coefficients depend on the order in which the States-Haberkorn components of the 3D FID data set were collected. This order depends in turn on the values of the parameters `phase`, `phase2`, and `array`.

If TPPI phase cycling is used to collect data along one or both of the indirectly detected dimensions, instead of four data sets per (n_1, n_2) increment, there are only two or one data set(s), respectively, per (n_1, n_2) increment. If there are only two data sets per (n_1, n_2) increment, the `coef` file contains four rows of four coefficients that are used to construct the t_2 hypercomplex interferograms, and a final row of eight coefficients that are used to construct the t_1

interferogram. If there is one data set per (n_1, n_2) increment, the `coef` file contains four rows of two coefficients that are used to construct the t_2 hypercomplex interferograms and a final row of eight coefficients that are used to construct the t_1 interferograms.

Phasing a 3D data set is best accomplished using 2D transforms. In general, the recommended method in writing 3D pulse sequences is to attempt to minimize frequency-dependent phase shifts in f_1 and f_2 . Even so, there are generally small phase shifts that must be dealt with. The following steps are suggested:

- 1 Set `pmode='full'` to allow full phasing in both dimensions after a 2D transform.
- 2 Adjust `rp` and `lp` on a 1D spectrum (the first increment of the 3D), just as for 2D (for example, by typing `wft(1)`).
- 3 Enter `wft2d('ni',1,1,0,0,0,0,0,0,0,0,0,0,-1,0,0,0)` to adjust f_1 phasing (there are 11 consecutive zeros in the middle of this argument).
- 4 Fix the f_1f_3 2D spectrum (with incorrectly labeled axes). Set `trace='f1'` to adjust the f_1 phase, then set `trace='f2'` to trim the f_3 phasing. Now adjust `rp1` and `lp1` (as well as `rp` and `lp`).
- 5 Enter `wft2d('ni2',1,1,0,0,0,0,0,0,0,0,-1,0,0,0,0,0)` to adjust f_2 phasing (note that this argument has nine consecutive zeros in the middle and five zeros at the end).
- 6 Adjust f_2f_3 2D spectrum phase. Set `trace='f1'` to adjust the f_2 phasing (`rp2` and `lp2`), then set `trace='f2'` to trim the f_3 phasing if necessary.

Some pulse sequences are written to result in a 180° phase shift across the spectrum. Remember that in VnmrJ, the “origin” for phasing is defined as the right edge of the spectrum. However, in “real” terms, the actual origin of phasing (that is, the zero-frequency point) is at the center of the spectrum. If a certain `lp1` or `lp2` value is expected, such as -180° , simultaneously use a value of `rp1` or `rp2` equal to $-lp1/2$ or $-lp2/2$ (for example, 90°).

Adjust the weighting functions for the 3D transform by using the `wti` command and examine interferograms. Do so along either the t_1 or the t_2 axes. Use the same commands given above to adjust the phasing (the commands with the long series of zeros), but use `wft1d` instead of `wft2d`.

For the final transformation, the `specdc3d` parameter controls the dimensions in which a spectral drift correction is performed on the data. A three-letter value of 'ynn' gives drift correction along F_3 (the first letter) but not along F_1 (the second letter) or F_2 (the third letter); this value could be a good starting point for your efforts.

The `pmode` parameter is ignored by the 3D transformation. No phasing is possible after the 3D transform.

The 3D transformation process needs to be followed by the process of extracting the 2D planes from the full 3D data set. This can be done separately, with the `getplane` command, but most often is combined with the `ft3d` command. In general, and especially for heteronuclear experiments, the F_1F_3 and F_2F_3 planes are the most interesting. The F_1F_2 plane is not only generally less useful, but also is considerably slower to extract from the data. The recommended command to use for 3D transformation, therefore, is `ft3d('f1f3','f2f3')`, which performs the 3D transform and extracts the two interesting planes in one step.

Solvent suppression works on t_3 FIDs of 3D spectra just like in the 1D and 2D cases.

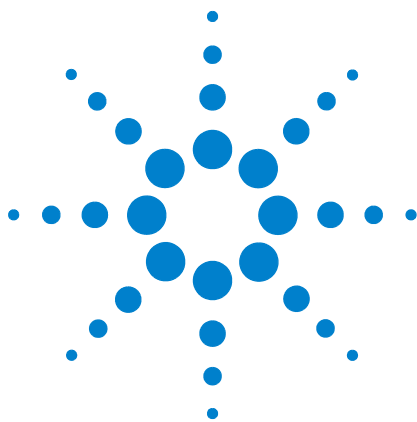
Follow the transform, set `plane='f1f3'` or `'f2f3'`. Then use the `dproj` macro to display the projection of the data on that plane, or `dplane(n)` to display the *n*th plane. The `resetf3` macro will reset parameters after a partial 3D Fourier transform.

4D NMR Acquisition

The `addpar('4d')` macro creates the parameters `ni3`, `sw3`, `d4`, and `phase3` that can be used to acquire a 4D data set (the macro `par4d` functions the same as `addpar('4d')`).

The parameter `ni3` is the number of t_2 increments. `sw3` is the spectral width along the third indirectly detected dimension. `d4` is the incremented delay, and `phase3` is the phase selection for 4D acquisition. Processing and display in 4D are currently not available in VnmrJ.

11 Multidimensional NMR



12 Indirect Detection Experiments

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This chapter describes an indirect detection experiment known as *heteronuclear multiple-quantum coherence* (HMQC). Indirect detection experiments show correlations between heteronuclei while detecting high-sensitivity protons. HMQC differs from the older heteronuclear correlation techniques that detect the low-sensitivity heteronucleus (for example, ^{13}C or ^{15}N).



Probes and Filters

Indirect detection probes

The most commonly used probes for indirect detection experiments are the Agilent Technologies, Inc. “indirect detection” NMR probes, such as the Triple Resonance, Penta, Tunable Triple, Indirect Detection, gHX Nano, Cold Probes, and others. Indirect detection probes have a ^1H coil and an X-nucleus coil with the ^1H coil positioned closer to the sample for the highest possible sensitivity for ^1H observe. When connecting cables to the probe, ignore words like “observe” and “decouple”, and think of ^1H (for observe) and X (for decouple) for connections.

Normal “broadband” probes similarly have a ^1H coil and an X-nucleus coil and can be used for indirect detection. But broadband probes have significantly lower proton sensitivity (about half that of indirect detection probes) and so are not optimum for indirect detection experiments. Nevertheless, indirect-detection data acquired on broadband probes are usually still more sensitive than direct detection heteronuclear correlation data. Four-nucleus and “Switchable” probes also have a ^1H coil and an X-nucleus coil, with the X coil closer to the sample, and can satisfy the needs for indirect detection experiments.

For more information on Agilent Technologies, Inc. NMR probes, go to www.agilent.com. Refer the manual that was shipped with the probe for installation and tuning instructions.

Filters

Bandpass filters may be needed on the transmitter, receiver, decoupler, and lock channels. Filters are part of the probe kit shipped with each indirect detection probe that Agilent sells.

Modern spectrometers normally leave the appropriate filters in place to allow you to do indirect detection “all the time” (in contrast to years ago). The details listed below are for older spectrometers and special applications.

Install a ^2H bandpass filter in the lock channel line. Expect the lock phase to change when the filter is added. This filter can be left in the system at all times; it will, however, cause

a small (about 3 dB) loss in lock sensitivity.

The following table lists part numbers for the bandpass filters supplied with indirect detection probes.

Filter	300-MHz	400-MHz	500-MHz	600-MHz	750-MHz
¹⁵ N	BE30.4-7.6-9BB	BE40-14-9BB	BE53-15-8BB	BE61-10-8BB	BE77-15-4BB
² H	BE46-4-6BB	BE61-10-8BB	BE77-3.8-8BB	BE95-12-8BB	BE115-11-6BB
¹³ C	BE75-15-8BB	BE109-22-8BB	BE135-35-8BB	BE151-40-8BB	BE188-20-7BB
³¹ P	BE135-35-8BB	BE151-40-8BB	BE175-60-8BB	BE240-100-8BB	BE301-46-8BB

NOTE

All standard specifications are given with the indirect detection filters not in place.

CAUTION

Filters used for indirect detection degrade specifications by approximately 10%.

Note that the filters used for indirect detection tend to degrade specifications approximately 10% in terms of longer pulse widths and lower signal/noise. The user thus faces a classic trade-off of performance (manually insert filters only when needed but achieve better specs) versus convenience (leave filters in place continuously and achieve worse specs). The convenience factor, of course, is non-existent if the instrument does anything other than ¹³C and ¹H, because the ¹³C bandpass filter cannot be left in place on the X line while doing ³¹P, ¹⁵N, or anything else. All standard specifications are given with the indirect detection filters not in place.

The Basic HMQC Experiment for ^{13}C

The purpose of the phase-cycled (non-gradient) HMQC experiment is to cancel or eliminate signals from proton magnetization attached to C, leaving only signals from proton magnetization attached to C, contributing to a C-H chemical shift correlation spectrum. The three basic independent mechanisms to generate this discrimination are:

Spin-Echo difference experiment

The heart of the HMQC sequence can be reduced to a heteronuclear spin-echo difference experiment that looks like [Figure 95](#).

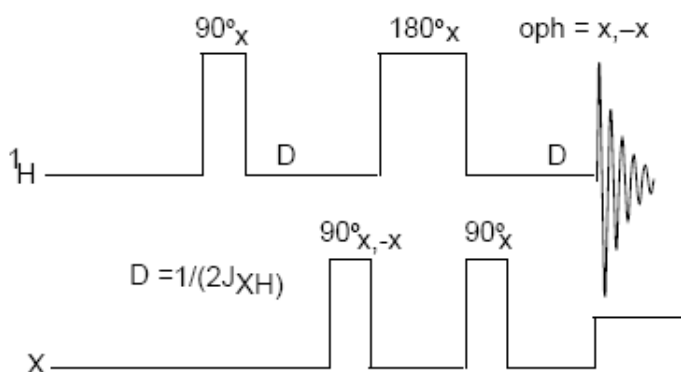


Figure 95 Heteronuclear spin-echo difference experiment

In [Figure 96](#) *a*, *b*, and *c* represent the proton magnetization attached to carbons, where *a* is the proton magnetization attached to up- ^{13}C , *b* is proton magnetization attached to ^{12}C , and *c* is proton magnetization attached to down- ^{13}C . Assume that we are at the resonance frequency of the proton magnetization attached to the ^{12}C s. In the rotating frame, the following steps (shown in [Figure 96](#)) occur:

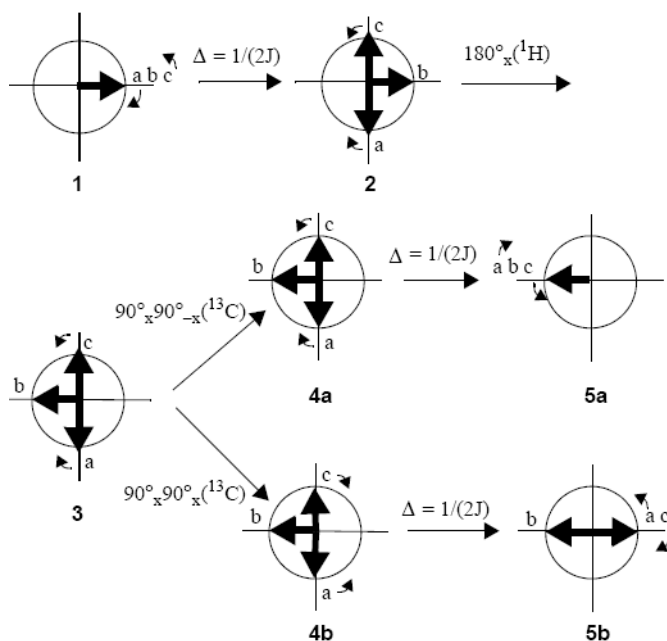


Figure 96 HMQC pulse sequence, showing movement of attached protons

- 1 The first proton 90° pulse places all proton magnetization along the y axis.
- 2 After a time $\Delta = 1/(2J)$, the *b* proton magnetization is still along the y axis, but the *a* proton magnetization is along the $-x$ axis and the *c* proton magnetization is along the $+x$ axis.
- 3 Next, the 180°_x proton pulse places the *b* proton magnetization along the $-y$ axis, but does not affect the *a* and *c* proton magnetization.
- 4 The next pulse has the following effect:
 - a The $90^\circ_x 90^\circ_{-x}$ carbon pulse is effectively a null pulse. All rotational directions are maintained.
 - b The $90^\circ_x 90^\circ_x (= 180^\circ_x)$ carbon pulse reverses the ^{13}C , which makes the *a* proton magnetization attach to the down- ^{13}C and the *c* proton magnetization attach to the up- ^{13}C , essentially reversing their rotational direction.

- 5 After another period $\Delta = 1/(2J)$, the following occurs:
- The *a*, *b*, and *c* proton magnetization are refocused along the $-y$ axis.
 - The *b* proton magnetization is still along the $-y$ axis, and the *a* and *c* proton magnetization are refocused along the $+y$ axis.

Subtracting the signal resulting from step 5b and 5a, by changing the receiver phase ϕ , results in cancellation of the *b* proton magnetization, while the signal for the *a* and *c* proton magnetization doubles.

To create a 2D experiment with information about heteronuclear chemical shifts, we introduce an evolution time t_1 that occurs between the two X-nucleus 90° pulses, as shown in Figure 97.

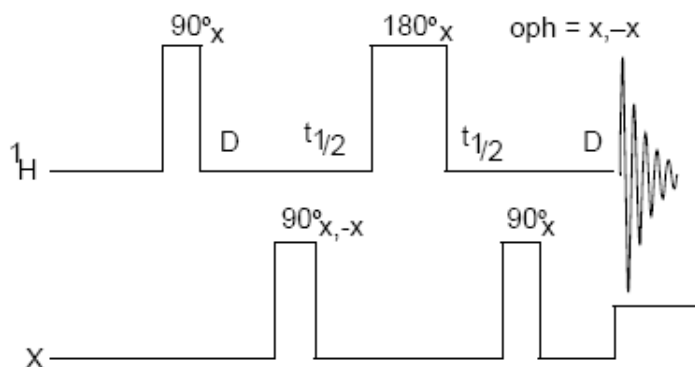


Figure 97 Evolution time added between x-nucleus pulses

In this 2D experiment, which is now a full HMQC experiment, proton magnetization attached to ^{12}C shows no different behavior and is still cancelled after two scans. Whether the ^{13}C nuclei experience a 180° pulse, a 0° pulse, or something in between depends on the time between the two 90° pulses and their rate of precession during that time (that is, their chemical shift). Therefore, this experiment produces a modulation of the intensity of the ^{13}C -bound proton magnetization, and the Fourier transform of that modulation yields the chemical shift of the ^{13}C bound to that proton.

Thus, we detect ^{13}C chemical shifts with the intensity of proton magnetization, and simultaneously we obtain a correlation of the C and H chemical shifts. Appropriate

variations of the experiment produce long-range coupling information.

BIRD Nulling

The BIRD (Bilinear Rotation Decoupling) pulse nulling effect (Summers, Marzilli, and Bax, *JACS*, 1986, 108, 4285) is the second type of cancellation that can occur during an HMQC sequence. A particular sequence of the BIRD pulse, three pulses on the H channel and one on the X channel, inverts the z-magnetization of proton magnetization bound to C and leaves the z-magnetization of proton magnetization bound to ^{13}C unaffected. The full sequence is illustrated in Figure 104 on page 352, where $\Delta=1/2J$

After the BIRD pulse, a variable waiting period (τ in Figure 98) is inserted, allowing the ^{13}C -bound proton magnetization to relax back to equilibrium.

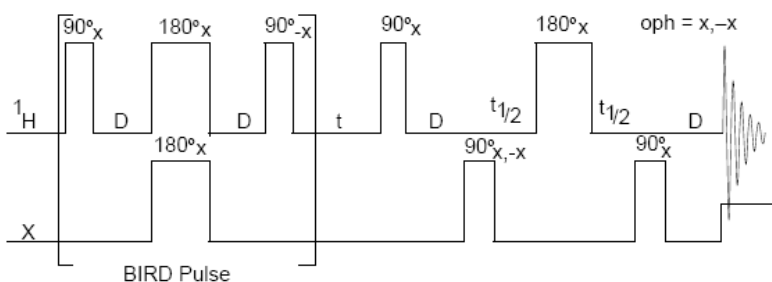


Figure 98 HMQC with BIRD pulse nulling effects

If τ is adjusted so that the C-bound proton magnetization is approximately at a null, when the remainder of the pulse sequence (the normal HMQC sequence) is executed, cancellation of the C-bound proton magnetization is enhanced (since those proton magnetization had very little magnetization at the start of the HMQC sequence). Not all proton magnetization will have the same relaxation time. The choice of τ must be a compromise; unless only one proton is involved, the additional suppression from the BIRD nulling will be a factor of two to five.

For systems that exhibit a negative NOE, such as macromolecules, cross-relaxation between the inverted proton magnetization on C and the non-inverted proton magnetization on C will decrease the intensity of the desired proton signal. The extent of this decrease can vary between 0% and 100%. Omission of the BIRD part of the sequence is advised for macromolecules.

BIRD pulse nulling is not possible when long-range indirect detection experiments (Heteronuclear Multiple-Bond Coherence, or HMBC) are performed. In this case, proton magnetization that have long-range couplings to ^{13}C are directly bonded to ^{12}C (99% of them, anyway) and the BIRD pulse nulling would lose all intensity in the proton magnetization of interest.

Transmitter presaturation for high-dynamic range signals

When high-dynamic range situations, such as observing signals in H₂O, are involved, HMQC phase cycling and/or BIRD pulse nulling may be insufficient to produce cancellation of the large proton signals. For this reason, a third mechanism, presaturation, may be necessary. Since one channel of the instrument is set to an X-nucleus like C or N, this presaturation must be accomplished with the other channel. That is, the same channel that will be applying observe pulses to the proton magnetization. During one or two different periods of the sequence (during the initial delay and during the τ delay), a change in power level and possibly frequency will be appropriate in order to perform the presaturation.

Experiment Manual Setup

HMQC and other indirect detection experiments are set up using the **Experiment Panel** or the menu system (**Experiment / Indirect Het. Corr.**). This process retrieves ^1H and ^{13}C calibrations from the active profile and generates a parameter set in which the only significant choices are how long to run the experiment (nt) and how much resolution is needed in F1 (ni). However, a step-by-step process is described in this section to detail the choices that can be made and the impact of the choices.

A recommended first natural abundance sample is the 1% 3-heptanone in CDCl_3 sample (Part No. 00-968120-93) or a similarly concentrated sample.

- 1 Insert the sample and, after shimming, leave the spinner off. Regulate the temperature if the experiment is run at a controlled temperature.
- 2 Optional – obtain a normal carbon spectrum and narrow the spectral width to the appropriate region. In some cases, the C spectrum will be too weak to observe in a reasonable amount of time. If this is the case, two approaches can be taken to set the parameters controlling the C frequency and spectral width.
 - Use the same parameters as used in similar experiments in the past on similar samples.
 - Set up a standard Carbon experiment and an appropriate solvent. The spectrum obtained should be properly referenced. Apply the appropriate knowledge of the expected chemical shift range, even if the peaks in the spectrum cannot be seen, to place two cursors where the edges of that range will be, and narrow the spectral width.
- 3 Obtain a proton spectrum and narrow the spectral width. Check the calibration of the pulse width by entering $\text{pw}=4*\text{pw ga}$. Look only at the signals near the center of the spectrum and see if they produce a null signal. If they are negative, enter $\text{pw}=\text{pw}+0.8 \text{ ga}$. If they are positive, enter $\text{pw}=\text{pw}-0.8 \text{ ga}$. Repeat until a good null is found, then enter $\text{pw}=\text{pw}/4$.
- 4 Switch to the HMQC experiment and set the relevant parameters based on the results of steps 2 and 3.
- 5 Enter $\text{phase}=1 \text{ ni}=1 \text{ dm}='nnn' \text{ null}=0 \text{ ai wexp}='wft \text{ dssh}'$. Set j to an appropriate value (normally 140 for

C-H), and set `nt` to 4 or more transients, depending on the concentration of the sample (signal to noise needs to be sufficient to see the C satellites). Now set `pwx` to an array of 0 and 90° and enter `au` to acquire two spectra. Proceed only if the two spectra are sufficiently different to be confident that the second spectrum is showing satellite peaks only and not just residual uncanceled intensity of the protons attached to ^{12}C .

If a connection is correct but the quality of the spectra is not achieved, go to Step 8 and optimize the null parameter. Then return to this step to check and optimize `pwx`. Review the checklist in “Cancellation Efficiency” on page 347 to ensure cancellation optimization.

The spectrum in Figure 99 shows the result of this experiment on a sample of 1% 3heptanone at 300 MHz, using `nt=64` `null=2.0` and `d1=2`.

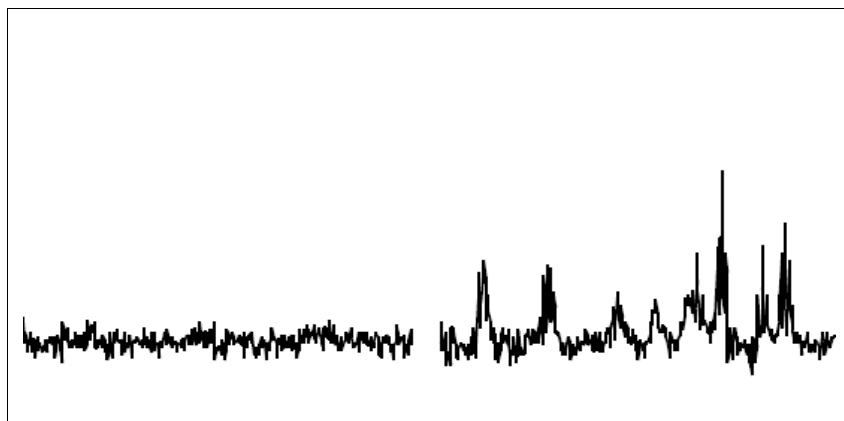


Figure 99 Verifying cancellation with `pwx=0, 90`

- 6 Run an array of `pwx` around the expected 90° value and choose the one that has the largest satellite signals.
- 7 Set `pwx` to its 90° value and enter an array of `dm='nmn', 'nny'` if decoupling is used during acquisition. These two experiments show coupled and decoupled spectra.
- 8 A value of `null=0.3` is recommended. If appropriate, optimize the parameter `null`. Set `nt=1` `ss=4` and enter an array of `null` values with at least one very short value (for example, 0.001) and one very long value (for example, 2.0). This experiment depends on the relaxation times of the spins involved. Set `at` and `d1` to the same values being used in the 2D experiment. Run the array and select the value of `null` for which either most of the

peaks, or the biggest peaks, or the peaks of most interest (choose the criterion), are approximately zero. No one value of `null` is correct for all peaks. Figure 100 shows this experiment run on a sample of 28 mg of gramicidin, with `null` arrayed over the range of values: 0.001, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 2.0. Examination of the spectra shows how different values of `null` may be chosen.

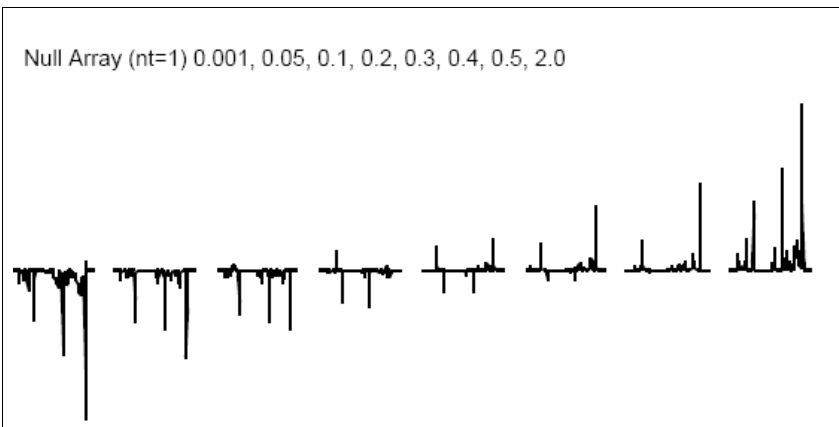


Figure 100 Optimizing the BIRD nulling time

- 9 Set up presaturation. It is necessary to observe the C-bound protons, set `nt=1 dm='nnn'`, and set `null=0` to omit the nulling period (for now at least).
 - a Enter `ga` and a proton spectrum will be observed.
 - b Move the FID to a different experiment, join the different experiment, and re-transform the data (for example, `mf(1,2) jexp2 wft`).
 - c Set the cursor on a peak that is to be removed by presaturation, and enter `n1 movetof`. Note the value of `tof` selected and copy this value back to the parameter `satfrq` (for example, `jexp1 satfrq=x`) of the original experiment.
 - d Set `satflg='yn'` and `satdly` equal to a significant time compared with `T` of the peaks (for example, `satdly=1`).
 - e To find the minimum value for which the peak will be removed (for example, `satpwr=10,7,4,1 au`), array `satpwr`.
- 10 To use presaturation, set `satflg='yy'`, reset `null`, and set `satpwr` to the determined value.

To not use presaturation, set `satflg='nn't`.

- 11 Set up the 2D experiment.
- 12 Set n_i between 128 and 256, $\text{phase}=1,2$, and n_t to an appropriate number (comparable to the value used in step 5).
- 13 Phasing in F_2 is accomplished by performing a 1D transform on the first increment with $\text{wft}(1)$ and phasing the spectrum, paying attention only to the C satellite peaks. In F_1 , the combination of the usually large spectral width and the pulse in the center of the evolution time produces large negative values for $1p1$ – unless the pulse sequence compensates for this, as modern sequences do.

Expect to see artifacts in these spectra. The residual un-cancelled signals from protons attached to ^{13}C show up as stripes parallel to the F_1 axis at the frequency of each ^1H peak. These artifacts will be larger for peaks with long T_1 , such as solvent peaks (for example, residual protons on a deuterated solvent) or methyl groups. In [Figure 101](#) they are seen at 2.4 ppm, 1.0 ppm, and 0.9 ppm.

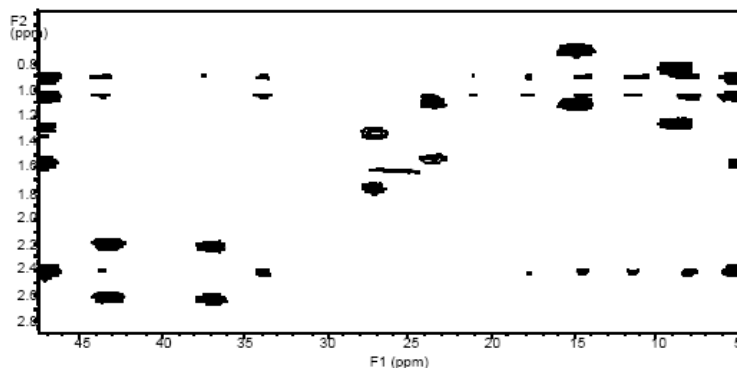


Figure 101 Coupled HMQC spectrum of 3-heptanone

Axial peak artifacts, which are common, will show up either at $f_1=0$ (the center of the spectrum in f_1) or, as in [Figure 101](#), at the edges of the spectrum in F_1 (if FAD is used), again at F_2 frequencies corresponding to each H peak and possibly through the entire spectrum. Another common artifact seen in [Figure 101](#) is the “0,0” artifact in the exact center of the spectrum. Some peaks in the ^1H spectrum, of course, will not appear in the HMQC spectrum, because they represent protons that are not bound to C (for example, protons from water or NH groups). However, this is not the case with 3-heptanone.

Multiple structures are typical with HMQC spectra. During the detection period a (that is, with H-H couplings), proton spectrum of only those protons attached to C is acquired. The proton attached to the carbon at 37.2 ppm is a quartet (it is adjacent to a CH group) and the proton attached to the carbon at 43.4 ppm is a triplet (it is adjacent to a CH group), see [Figure 102](#).

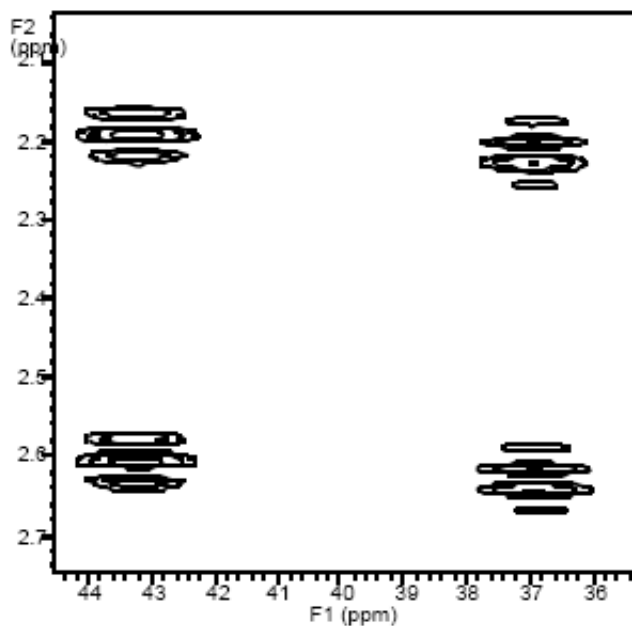


Figure 102 Expansion of coupled 3-heptanone HMQC showing multiplets

In the H spectrum itself, these two groups of protons are heavily overlapped (see [Figure 103](#)).

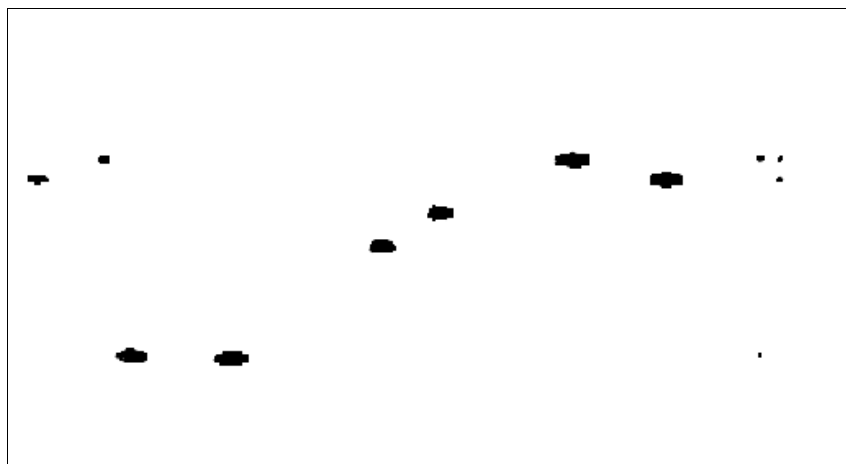


Figure 103 Decoupled HMQC spectrum of 3-heptanone

Cancellation Efficiency

Cancellation efficiency is critical because indirect detection experiments involve cancellation of non- ^{13}C -bound protons that are two orders of magnitude more intense (assuming unlabeled compounds). Cancellation efficiency depends on the fundamental stability of the system RF and the reproducibility of anything else that can affect the signal. Stability is determined by the instrumentation. A number of operating conditions that influence the quality of any cancellation experiment (NOE difference experiments are another good example) are under the control of the operator. Some of these conditions are discussed here, in the order of importance:

- Run experiments non-spinning.
- Use the highest lock power at which the lock is stable (be sure to shim with a non-saturating level, however) and keep the lock gain as low as possible, to ensure that lock is not lost during the experiment.
- Use a ^2H band-pass filter in the lock line. Interference between X-nucleus decoupling or even X-nucleus pulses can affect the lock and cause field instabilities, limiting the ability to perform cancellation experiments (if such a filter is not available, run a short-term experiment in the unlocked mode as a test).
- Use VT regulation, even at room temperature. Large changes in temperature of the environment can affect the VT gas stream. The frequency of peaks in the spectrum and of the lock resonance (which affects all peaks) is temperature-sensitive to some degree. Shimming may also change if the probe temperature varies, which can affect the lineshape.
- Be sure that the system is in thermal equilibrium. When applying large amounts of power to the system, if experiments with X-nucleus decoupling are run, the temperature of the probe, the sample, or both, are almost certain to change even with VT regulation. The best way to ensure thermal equilibrium is to set up a “dummy” experiment with *identical* conditions (in terms of duty cycle) to the actual experiment, but which runs for perhaps several minutes (easily accomplished by setting *ni* to a small number). Now, queue the real experiment to follow the dummy one, and the sample and probe are properly equilibrated.

- Ensure that the system is in an NMR steady-state by using steady-state pulses.
- Use a large value of nt . Cancellation improves with larger nt , so the relevant cancellation is that which occurs at nt comparable to what will be used in an indirect detection experiment (16 to 1024). Do not expect perfect results with $nt=2$.
- Minimize floor vibration. Install an antivibration system if this cannot be fixed by spectrometer placement.
- Use only a moderate flow of body air through the probe. Too much air can cause “rattling” from turbulent flow.
- Use lengthened pulses (attenuated RF) if there is a rise time or phase glitch problem.

Before beginning an HMQC experiment, assess the quality of the reproducibility by performing some simple difference experiments. The standard S2PUL pulse sequence is a good one to use for this purpose. The first pulse of S2PUL, controlled by $p1$, is held at a constant phase, while the receiver varies in phase. Thus, after four scans with $p1$ set to the 90° value, $p_w=0$, no signal should be seen. This can be compared to four scans with p_w set to the 90° value, $p1=0$, which produces a full signal. Taking the ratio of these two spectra gives a concrete measurement of cancellation efficiency, while repeating the null spectrum a number of times gives a measure of the reproducibility of the cancellation. Use this test to assess the value of the various steps and modifications described above, or of other differences (for example, the relative cancellation efficiency of experiments with and without X-nucleus decoupling).

Pros and Cons of Decoupling

The pros of decoupling of X during acquisition are that the spectrum is less crowded, with half as many peaks, and each peak with twice the sensitivity.

The cons of X-nucleus decoupling stems from the need to use large (up to 8 kHz) decoupling fields. This high power can cause significant heating, particularly in lossy samples. As a consequence of sample heating, experiments with X-nucleus decoupling are generally limited to relatively short acquisition times, which, in turn, may produce less resolution in f as well as less sensitivity for molecules with long T_1 . Furthermore, the heating that does occur frequently produces worse cancellation efficiency. And, finally, to prevent the buildup of heat in the sample, the duty cycle of the experiment may need to be limited to 10 to 20%, again possibly reducing sensitivity. Therefore, experiments performed without X-nucleus decoupling are perfectly reasonable, and may well be preferable.

It is important to avoid sample heating if X-nucleus decoupling is desired. This form of sample heating can be non-uniform within the sample and can cause microconvection, producing poor cancellation. Keep the acquisition time short and the overall duty cycle less than 20%.

In the sequences described here, broadband decoupling is achieved by using the acquisition computer to provide WALTZ-4 modulation of the X-nucleus channel through explicit software control. It also imposes some limitation on spectral widths and pulse widths, since the WALTZ-4 sequence (whose length is $6 * pw_{90}$) must fit in between successive data point samplings (which occur at time intervals of $1/sw$).

When WALTZ decoupling is used, the maximum power level for decoupling is the level that provides an RF field strength (in Hz) comparable to half the range of expected X shifts. The normal spread of protonated carbons is 150 ppm, which is 15 kHz on a 400-MHz system, and, consequently, a ^{13}C 90° pulse of 25 μs (corresponding to an RF field strength of 8 kHz) is adequate. The somewhat long proton pulses on broadband and switchable probes do not seem to present a problem because indirect detection experiments demand no more proton pulse power than DEPT or HETCOR.

Modern spectrometers use either GARP or WURST

12 Indirect Detection Experiments

decoupling, which offers wider decoupling bandwidths at lower powers.

¹⁵N Indirect Detection

Calibrations and operations for ¹⁵N indirect detection proceed much like it is outlined above for C. In the standard sample, 2% N-benzamide (Part No. 00-968120-97), the N satellite lines are partially obscured by other resonances in the conventional 1D spectrum. The N pulse width calibration must be done using multi-transient HMQC experiments. Use a J appropriate for NH couplings (90 Hz).

Ensure that the γB is sufficient to decouple the relatively narrow range of N chemical shifts expected in such samples. This step can be done in N work of peptides. This minimizes heating effects and improves cancellation. Typical acquisition times (αt) are 0.075 to 0.1 seconds.

Use the HMQC or HSQC pulse sequences for indirect detection experiments.

HMQC Pulse Sequence

The `hmqc<(isotope)>` macro sets up parameters for a HMQC (heteronuclear multiple-quantum coherence) pulse sequence. The optional isotope argument is the isotope number of the heteronucleus of interest, for example, `hmqc(1)` for ^1H (the default is ^{13}C). Shown below in Figure 104 is a diagram of this sequence. The first $2 \cdot \text{pwx}$ pulse on the X heteronucleus is a composite 180° consisting of $90^\circ(\nu_9) - 180^\circ(\nu_1) - 90^\circ(\nu_9)$.

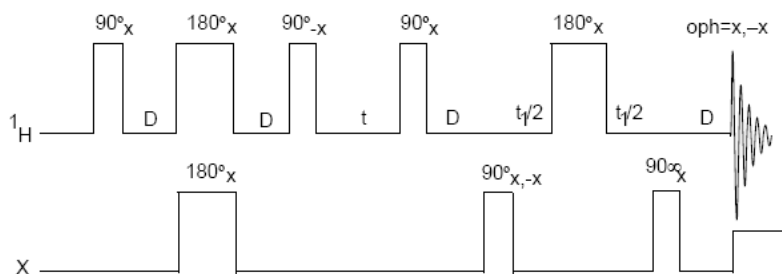


Figure 104 Basic HMQC pulse sequence

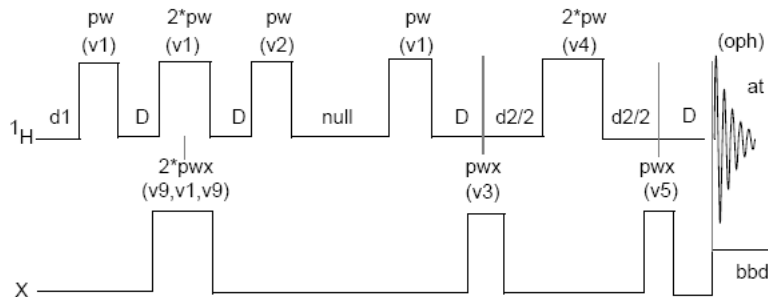


Figure 105 HMQC pulse sequence with `null<>0` and `mbond='n'`

Phase-Sensitive Aspects of the Sequence

The parameter `phase`, as in other phase-sensitive 2D experiments, controls the F_1 phase detection. Use `phase=1` for 1D setup experiments or a 2D experiment without quadrature detection in F_1 . Use `phase=1,2` for a normal 2D experiment using the States-Haberhorn-Ruben (hypercomplex) method. Use `phase=3` to acquire data with TPPI and ensure that `sw1` is twice the expected range.

The FAD, for “F1 Axial Displacement” technique (D. Marion; M. Ikura; R. Tschudin; A. Bax, *J. Magn. Reson.* 1989, 85, 393) involves a change of phase cycling that shifts the axial artifacts in a hypercomplex experiment to the edge of the spectrum, giving the hypercomplex version the benefit of TPPI with none of the disadvantages. It is also referred to as “States-TPPI.” The `hmqc` macros include FAD. Once implemented, use of the technique is transparent. Perform a standard hypercomplex experiment with `phase=1,2`.

Table 29 Parameters for HMQC pulse sequences

Parameter	HMQC
1H 90°	<code>pulse pw</code>
1H 180° pulse	derived from <code>pw</code>
1H amplifier power (if appropriate)	<code>tpwr</code>
1H frequency	<code>tn, tof</code>
1H spectral width	<code>sw</code>
X 90° pulse	<code>Pwx</code>
X 90° pulse for WALTZ decoupling	<code>1 / (4 * dmf)</code>
X 180° pulse	derived from <code>pwx</code>
X amplifier power for pulses (if appropriate)	<code>pwxlv1</code>
X amp power for decoupling (if appropriate)	<code>dpwr</code>
X frequency	<code>dn, dof</code>
X spectral width	<code>sw1</code>
Δ delay	<code>1 / (2j)</code> [if <code>j=0</code> , <code>D=0</code>]
Δ delay for BIRD nulling (if <code>null=0</code> , entire BIRD sequence is skipped)	<code>null</code>
Coupled experiment	<code>dm= 'nnn'</code>
X decoupling during acquisition.	<code>dm= 'nny'</code>

Table 29 Parameters for HMQC pulse sequences (continued)

Parameter	HMQC
Setup experiments	phase=1
Hypercomplex experiment	phase=1, 2
TPPI	phase=3
Minimum nt possible	2
Presaturation and/or multiple-bond correlation	see text
Axis parameter for proper ppm on both axes	pd

`pw` is a 90° pulse on the observed nucleus (protons) `phase=3` at power equal to `tpwr`.

`pwX` is a 90° pulse on the heteronucleus at power equal to `pwXlv1`.

`dpwr` is the decoupler power level for broadband X-decoupling.

`dmf` sets the modulation frequency ($4 \cdot \gamma B_1$) at decoupler power (`dpwr`).

`dmm` is decoupler modulation mode. `dmm='ccg'` is recommended.

`dm='nny'` activates heteronuclear broadband decoupling (recommended) during acquisition. Note that `dm` can be set to either `'nnn'` or `'nny'`, and that the duty cycle for the decoupler should be less than 20%.

`j` is the average scalar coupling constant between the protons and the heteronucleus (usually one-bond constants). `j` is 140 for ^{13}C or 90 for ^{15}N . The time Δ , shown in the pulse sequence diagram, is calculated as $1/2j$.

`null` is a WEFT-like delay used to improve the suppression of the protons connected to ^{12}C (and not to ^{13}C) that have been inverted by the preceding BIRD pulse. Try a `null` value of 0.3 for ^{13}C , 1.0 for ^{15}N , and 0 for macromolecules. To optimize, set `ss=-8` and array `null` with `nt=1` and `phase=1`. This selects the value of `null` that best minimizes the sample's signals (typically 0.2 to 0.7 seconds). If `null` is set to 0, the BIRD element is omitted from the pulse sequence.

`at` is the acquisition time (t_2 period).

`ni` is the number of t_1 increments (set up with default values for either ^{13}C or ^{15}N).

`ss` is the number of complete executions of the pulse sequence not accompanied by data collection prior to the acquisition of the real data. If `ss` is positive, `ss` steady-state pulses are applied on the first increment only; if `ss` is negative, `-ss` transients are applied at the start of each increment.

`nt` is a multiple of 4 (minimum) or multiple of 8 (recommended).

`phase=1,2` (2D hypercomplex data with hypercomplex-TPPI method) or `phase=3` 2D TPPI data). `phase=1,2` is suggested. For `phase=3`, remember that `hmqc` sets `sw1` to *twice* the desired value for heteronuclear experiments.

`satflg='yn'` gives presaturation during `satdly`, and `satflg='yy'` gives presaturation during `satdly` and `null` (not on *MERCURYplus/Vx*).

`satfrq=x` is the presaturation frequency (using the transmitter), `satdly` is the length of saturation time during the relaxation period (immediately after `d1`), `satpwr` is the power level for presaturation using the transmitter (not on *MERCURYplus/Vx*).

`hs='yn'` gives a homospoil pulse at beginning of `d1` (`length=hst`). `hs='yy'` gives a homospoil pulse at beginning of both `d1` and `null`.

`taumb` is a fixed delay associated with the multiple-bond HMQC experiment (`taumb=0.055` is recommended).

`mbond='n'` is a normal HMQC experiment. `mbond='y'` is a multiple-bond HMQC experiment (HMBC).

(1) Set `null=0` to run HMBC (`mbond='y'`); otherwise, only protons that are both long-range and short-range (one-bond) coupled to a given heteronucleus (^{13}C , for example) will not be suppressed, (2) set `dm='nnn'`, (3) set `taumb`, and (4) run the single-bond (HMQC) and multiple-bond (HMBC) experiments with `phase=1,2` or `phase=3`.

Phase cycling

The phase cycling is the following:

v1, v2, v3, v4, v5, v9 are phases for pulses. oph is the phase for receiver.

v1=x x y y

v2=-x -x -y -y

v3=x -x y -y

v4=x x y y y y -x -x

v5=x x y y x x y y

v9=y y -x -x

oph=x -x y -y

These phases are for phase=1. For phase=2, add 90° to v3. For phase=3, add $90^\circ(ix - 1)^\circ$ to v3, where ix is the increment counter.

Technique

The usual setup is to place a ^1H bandpass filter between the observe port on the probe and the $^1\text{H}/^{19}\text{F}$ preamplifier, and to place a 250-MHz lowpass LC filter and either a ^{13}C bandpass or a ^{15}N bandpass filter in the decoupler line just before the probe connection.

The experiment should be performed non-spinning and with VT regulation.

HSQC Pulse Sequence

The `hsqc` macro sets up parameters for the HSQC pulse sequence.

Parameters

`sspul='y'` selects for *trim(x)-trim(y)* sequence at the start of the pulse sequence; `sspul='n'` selects a normal experiment.

`satmode='yn'` gives presaturation during relaxation period (`satdly`) with the transmitter; `satmode='nn'` gives no presaturation during relaxation period (`satdly`); `satmode='ny'` gives presaturation during only the null period.

`satfrq` sets the presaturation frequency.

`satdly` sets the saturation time during the relaxation period.

`satpwr` sets the saturation power for all periods of presaturation with `xmtr`.

`hs='yn'` sets a homospoil pulse (`hst`) during the `d1` relaxation delay.

`null` is the delay associated with the BIRD nulling.

`tpwr` is the power level for ^1H transmitter pulses.

`pw` is a 90° transmitter pulse length for protons (the observed nucleus).

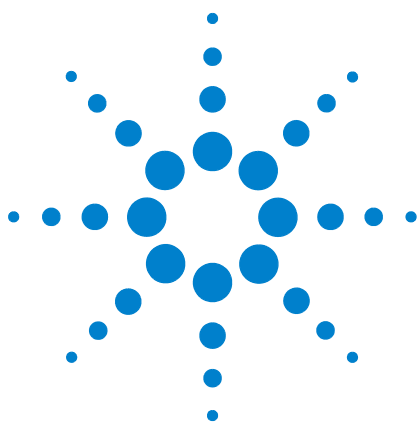
`pxlvl` is the power level for X decoupler pulses.

`px` is a 90° decoupler pulse length for X.

`jxh` is a one-bond heteronuclear coupling constant to X (in Hz).

`phase=1,2` for hypercomplex experiment with F1 quadrature (complex F1-FT).

12 Indirect Detection Experiments



13 Direct Digital Receiver (DDR)

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Application of the Direct Digital Receiver

The Direct Digital Receiver (DDR) uses a single 80 MHz ADC to digitize the NMR signal directly at the intermediate frequency (IF). Data acquired in all NMR application modes liquids, solids, and imaging benefit from flat baselines, excellent dynamic range, and reduction in artifacts associated with quadrature detection.

Quadrature detection for all NMR applications is eliminated with the Direct Digital Receiver. The NMR signal is sampled directly at the 80 MHz rate and routed through a series of digital signal processing (DSP) chips to down-sample (using proprietary algorithms) and digitally filter the data stream to produce the final spectral width.

Figure 106 shows the first increment of a presat-NOESY experiment conducted on a sample of rat urine (in 10% D₂O) using a Salt Tolerant Cold Probe and an NMR system at 600 MHz and a spectral width of 6 kHz. The flat baseline (no baseline correction) is free of artifacts arising from quadrature detection and when combined with the flat frequency response of the time-corrected digital Brickwall filters, allows for quantitative performance even in the presence of a complex biological matrix such as urine.

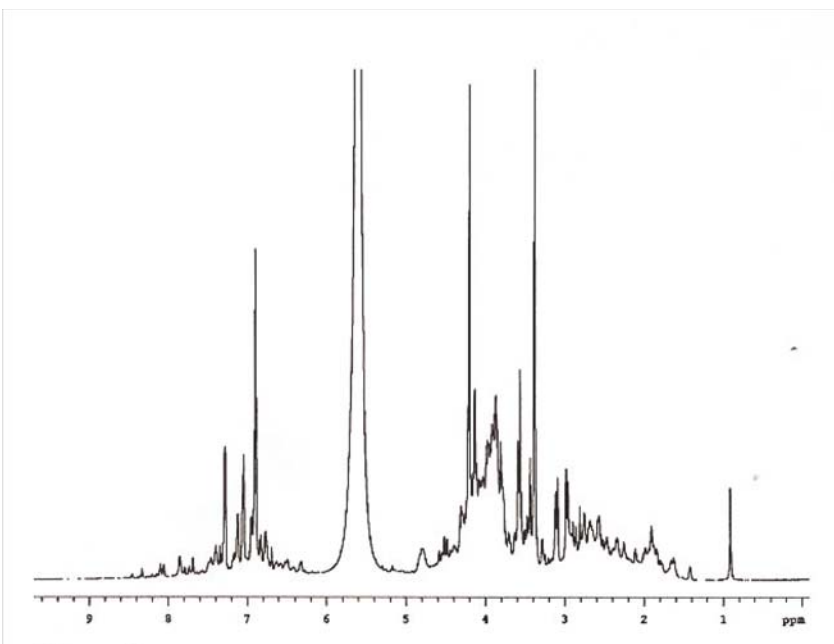


Figure 106 Presat-NOESY rat urine experiment

Figure 107 is the proton (^1H) spectrum of a paramagnetic sample, Tm-DOTP5 [Thulium(III)(1,4,7,10-Tetraazacyclododecane-1,4,7,10-teramethylene phosphonate)] in D_2O (DSS added as internal standard) acquired at 600 MHz with a spectral width of 1.25 MHz. Tm-DOTP is often used as a chemical shift reagent since Tm(III) is known to induce very large chemical shift changes.

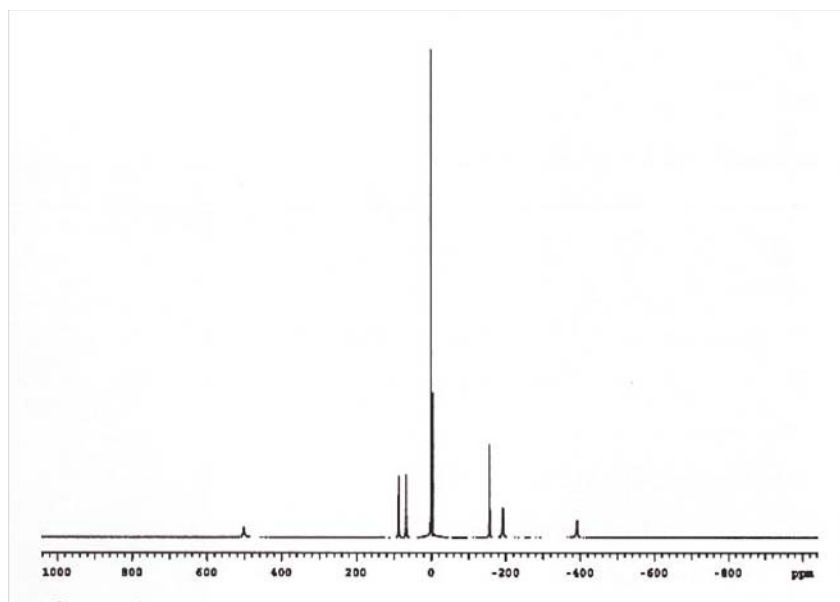


Figure 107 Paramagnetic sample of Tm-DOTP⁵

Figure 108 is the Solid state NMR spectra deuterium (^2H) NMR spectrum of malonic acid acquired at 500 MHz with a spectral width of 5 MHz.

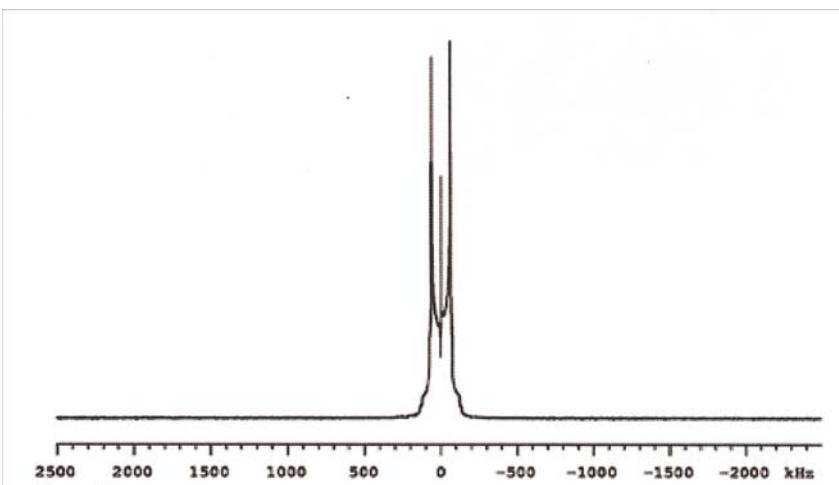


Figure 108 Malonic acid spectrum

Receiver dead time is minimized. The resulting spectrum acquired with large spectral widths has accurate lineshape and flat baseline.

[Figure 109](#) is a series of image slices of a spherical phantom acquired with varying gradient strengths and corresponding data acquisition rates. The signal-to-noise (SNR) in each of the images remains unchanged (within experimental error) when normalized for gradient strength, demonstrating the complete equivalence of the receiver response at all spectral widths. The fuzzy material seen on the sides of the images is algae growing inside the phantom.

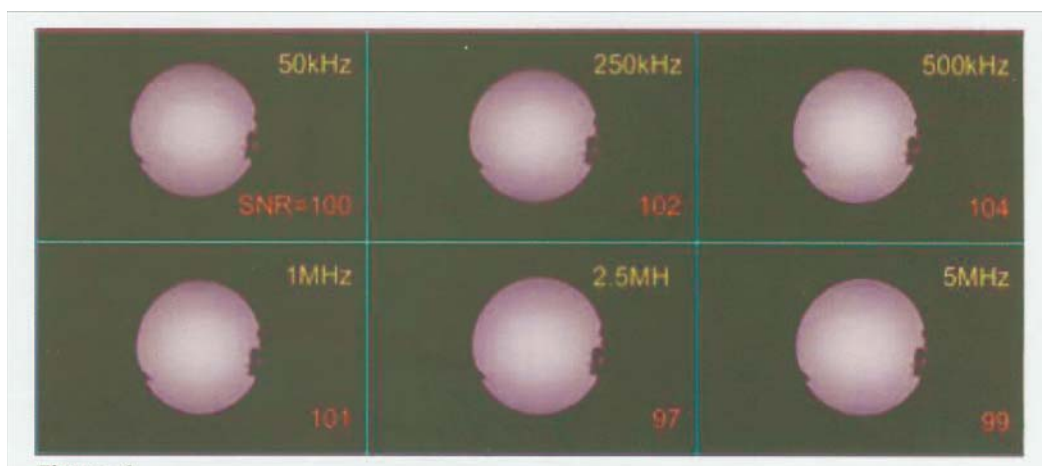


Figure 109 Image slices of spherical phantom

The spectra in [Figure 106](#) - [Figure 109](#) were acquired using a single 80 MHz ADC, no quadrature detection, and an effective spectral width of 40 MHz for the input data stream prior to down-sampling. The input data stream is reduced to the final spectral width. Processing is performed in the digital domain using digital filtering algorithms.

Direct Digital (TM) Receiver Operation

DDR parameters are automatically optimized for most applications. `alfa` and `rof2` need to be optimized for liquids probes. Some of the commonly used optimization methods, macros and parameters are:

- “Estimating minimum `alfa` and `rof2`”
- “Setting minimum `alfa` and `rof2`”
- “Optimizing receiver constants”
- “Optimizing baselines for solvent suppression”
- “Setting receiver constants for zero linear phase”
- “DDR macros and parameters”

Estimating minimum `alfa` and `rof2`

The following procedure is used to estimate a minimum `alfa` and `rof2` for a particular nucleus and set it into the probe file.

- 1 Set up a 1D experiment on a particular nucleus. For example, select **Proton** from the **Experiments** menu.
- 2 Click the **Acquire** button in the action bar to acquire a 1D spectrum.
- 3 Adjust flip angle and shims such that a signal is seen in the above step 2.
- 4 Remove the sample from the magnet.
- 5 Enter `sw = 2.5e6`, setting a wide spectral width.
- 6 Enter safe values of `alfa` and `rof2`, for example `alfa = 10` `rof2 = 50`.
- 7 Click the **Acquire** button in the action bar to acquire a FID.
- 8 Click the **Display FID** icon in the **System Toolbar** or enter `df` on the command line to display the FID.
- 9 Expand the FID scale to view the first few hundred microseconds of data.
- 10 Check if the beginning of the FID shows a damped exponential decay, typically on the order of tens of microseconds.

11 Take the following actions based upon the results:

Condition observed	Take the following action
No NMR Signal in the FID	Increase <code>nt</code> or decrease <code>alfa</code> and repeat above steps 7-10. Do not decrease <code>alfa</code> and <code>rof2</code> below their save minimum values.
FID decay does not show a single exponential decay.	Estimate the time for the signal to reach an amplitude equal to twice the noise level. Enter this value into the probe file for <code>minalfa</code> and <code>minrof2</code> .

The above procedure is recommended only if the signal is visible in step 10. If there is no signal, increase the number of transients, `nt`, or set values into the probe file as described in “[Setting minimum alfa and rof2](#)” . Decrease the values of `alfa` or `rof2` if needed.

CAUTION

Damage to the probe is possible if the safe values for `alfa` and `rof2` are exceeded. The values depend on the probe and preamp type. If you are unsure of safe values for your system, contact an Agilent representative.

Setting minimum alfa and rof2

Use the following procedure to set minimum values of `alfa` and `rof2` into the probe file for a given nucleus.

- 1 Click the **Probe** button on the hardware bar.
- 2 Select the desired probe from the **Select Probe** menu.
- 3 Select the **Edit** check box.
- 4 Set `tn` to the desired nucleus, for example `tn = 'C13'`.
- 5 Enter `addparams('minalfa',' ',tn)`.
- 6 Enter `addparams('minrof2',' ',tn)`.
- 7 Click the **Edit Probe** button.
- 8 Click the tab for the desired nucleus, for example **C13**.
- 9 Enter the minimum `alfa` value in the **minalfa** field.
- 10 Enter the minimum `rof2` value in the **minrof2** field.
- 11 Click **OK** to save the values into the probe file.

CAUTION

Safe values for `alfa` and `rof2` depend on the probe and preamp type. For room temperature probes, a typical minimum `alfa` value is 10 μ s, and a typical minimum `rof2` is 25 μ s. For cold probes, minimum `alfa` may be as much as 50 μ s, and minimum value of `rof2` may be as much as 350 μ s. If you are unsure of safe values for your system, contact an Agilent representative.

Optimizing receiver constants

Pulse sequences used in the walkup interface have automatic optimization of receiver constants. Set receiver constants for a given pulse sequence type as follows:

- 1 Set up a pulse sequence, for example select **Proton** from the **Experiments** menu.
- 2 Enter `ddrpm?` on the command line.
- 3 Enter the following on the command line if the `ddrpm` parameter does not exist:

```
create('ddrpm','string')
setenumeral('ddrpm','e','p')
```

- 4 Set `ddrpm` as appropriate for the pulse sequence:
 - Enter `ddrpm = 'p'` if the pulse sequence is a pulse type (there is no delay between the final pulse and start of acquisition).
 - Enter `ddrpm = 'e'` if the pulse sequence is an echo type (there is a delay between the last pulse and start of acquisition).
- 5 Set **pulse sequence parameters** as required (pulse width, spectral width, etc.).
- 6 Enter `setrc` on the command line.

The `setrc` macro optimizes the receiver constants using the minimum `alfa` and minimum `rof2` values in the probe file. The macro sets `lp = 0`, and sets `ddrtc` based upon `ddrpm`.

<code>ddrpm=</code>	<code>ddrtc=</code>
'p'	$\text{alfa} + \text{rof2} + 2\text{pw}/\pi$
'e'	<code>alfa</code>

Linear prediction is turned on if the `ddrtc` value is more than a dwell time. Use the **More 1D** or **More 2D** page in the **Process** folder to optimize or to disable linear prediction.

Optimizing baselines for solvent suppression

Solvent suppression and/or data subtraction pulse sequences, such as `Dqcosy` with `Presat` or `Wet1d`, typically require additional optimization as follows:

- 1 Enter `create('ddrcr','integer')`
`setlimit('ddrcr',1000,2,1) ddrcr=20.`
- 2 Enter `setLP(5).`

Filter sharpness (`ddrcr`) and linear prediction optimization may be required for some samples and for some experiments (for example, `dqcosy` on 90% water). The default filter sharpness is 75 if `ddrcr` does not exist. Use the **More 1D** or **More 2D** page in the **Process** folder to optimize linear prediction.

Setting receiver constants for zero linear phase

Use the following procedure to adjust `ddrtc` to obtain a flat baseline starting from a current spectrum.

- 1 Acquire a 1D spectrum.
- 2 Phase the spectrum using zero- and first-order phase correction.
- 3 Enter `setlp0.`
- 4 Reacquire the 1D spectrum. It should now be properly phased with no first-order phase correction.
- 5 Repeat steps 2 to 4 until there is no first-order phase correction (`lp = 0`).

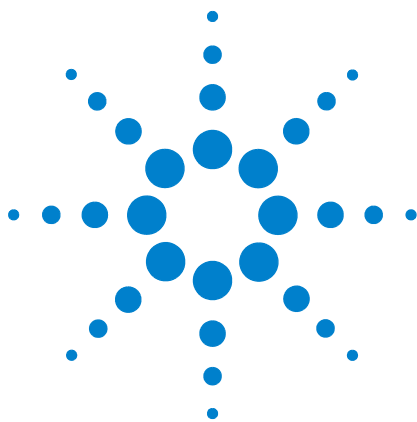
A default value of `alfa` is used if the `ddrtc` parameter does not exist.

DDR macros and parameters

Table 30 lists frequently used DDR macros and parameters.

Table 30 DDR macro and parameter table

Macro / Parameter	Description
setrc (M)	Set receiver constants
setLP (M)	Set up linear prediction in the direct dimension
setlp0 (M)	Set parameters for zero linear phase
alfa (P)	Set alfa delay before acquisition
rof2 (P)	Receiver gating time following pulse
ddrpm (P)	Direct digital receiver precession mode
ddrtc (P)	Direct digital receiver time constant
ddrcr (P)	Direct digital receiver coefficient ratio



14 Data Analysis

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This chapter describes how to analyze data by: spin simulation, deconvolution, adding and subtracting data, and analysis.



Spin Simulation

The software includes an iterative spin simulation program based on the FORTRAN program LAME, also known as LAOCOON, with magnetic equivalence added. Iven chemical shifts and coupling constants, LAME calculates the theoretical spectrum for spin-1/2 nuclei.

Up to eight closely coupled, non-equivalent spins (ABCDEFGH) can be handled. To extend the stimulation to systems such as A3B2Cd3, equivalent spins can be treated by magnetic equivalence factoring. The X-approximation can be used to handle different types of nuclei. Nuclei are treated as different types if there is at least one spare letter in the alphabet between their groups (for example, ABD and ABX are both systems using the X-approximation.) Frequencies, intensities, energy levels, and transitions can be listed, and simulated spectra can be displayed and plotted.

Parameters can be adjusted by iteration to approach a given experimental spectrum. One or several parameters can be kept constant for iterative runs and one or several parameters can be set equal to each other and held equal during the course of the iteration.

An example is provided in “[Spin simulation step-by-step](#)” on page 373. [Table 31](#) on page 377 lists specialized commands and parameters.

References for the spin simulation algorithms

- S. Castellano and A. A. Bothner-by, *J. Chem. Phys.*, 41, 3863 (1964).
- Emsley, Feeney, and Sutcliffe, eds. 1966. *Progress in Nuclear Magnetic Resonance Spectroscopy*, Vol.1, Chap. 3. Oxford: Pergamon Press.
- R. C. Ferguson and D. W. Marquardt, *J. Chem. Phys.*, 41, 2087 (1964).

Spin Selection Tab, Buttons, and Fields

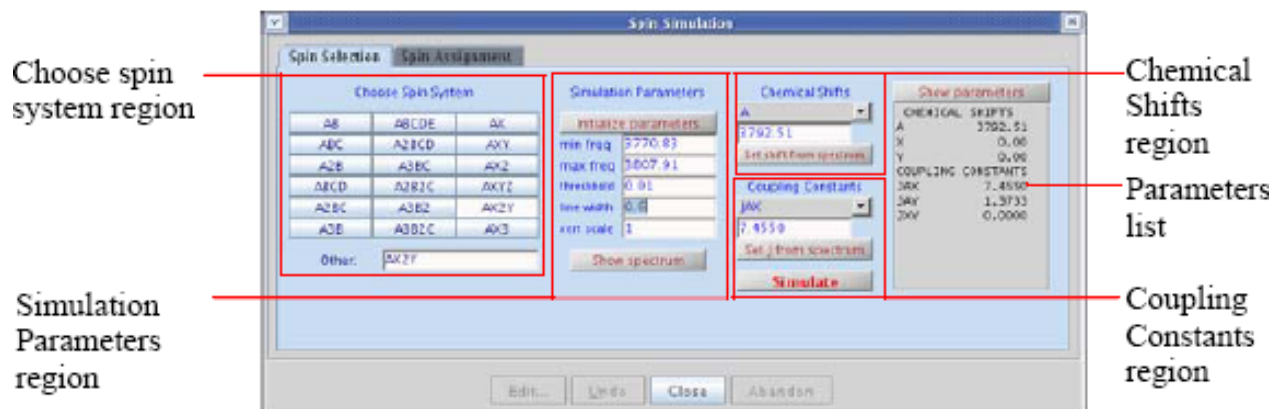


Figure 110 Spin simulation – spin selection tab

The **Spin Selection** tab has the following buttons and fields, see [Figure 110](#) as needed:

Region or button or field	Description/ Function
Choose Spin System region	
Choose Spin System option buttons	Click a button to select the spin system.
Other	Enter the spin system in this field if the required spin system is not associated with a button.
Simulation Parameters section:	
Initialize parameters button	Initializes the spin simulation parameter using the default values displayed.
Parameter fields	Enter a new value for any of these parameters. Click the Initialize parameters button to initialize the parameter fields from the current spectrum.
Show spectrum button	Display the Fourier-transform spectrum.
Chemical Shifts section:	
Chemical shift menu	Select a chemical shift from the menu (A, B etc.).
Chemical shift entry field	Enter a chemical shift value in the entry field.
Set shift from spectrum button	Set the chemical shift using the current position of the cursor in the spectrum.
Coupling Constants section:	

Region or button or field	Description/ Function
Coupling constant menu	Select a coupling constant (JAX, JAY etc.) from the menu.
Coupling constant entry field	Enter a coupling constant value in the entry field.
Set J from spectrum button	Use the current positions of the left and right cursors in the spectrum to set the coupling constant.
Simulate button	Simulate and display the spectrum using the selected spin system, chemical shifts, and coupling constants.
Show parameters button	Display the chemical shifts and coupling constants in a single list in the field below.

Spin assignment tab

The **Spin Assignment** tab has the following buttons and fields, see [Figure 111](#) as needed:



Figure 111 Spin simulation – spin assignment tab

Button/Field	Description
Spin System	Displays the spin system selection.
Show simulation button	Displays simulated spectrum.
Show spectrum button	Fourier transforms and displays the spectrum.
Make line list button	Creates a line list from the current spectrum and threshold and copies the list into the spin simulation line assignment file.

Button/Field	Description
Use line list button	Copies the current line list into the spin simulation line assignment file.
Use fitspec button	Copies the <code>fitspec</code> line list into the spin simulation line assignment file.
Show assigned lines button	Displays the spin simulation line assignment file <code>spini.la</code> .
Clear assigned lines button	Clears the line assignments.
Iterate button	Iteratively fits the simulated transition lines to the experimental lines.
# Iterations field	Displays the maximum number of iterations used for fitting the data.
Iterate field	Shows the iterate parameter containing the chemical shifts and coupling constants used in the iterative fit.

Spin simulation step-by-step

The step-by-step procedure listed here is a worked out example that includes comments at each step.

To set up the spectrum for spin simulation

- 1 Click **File** on the main menu.
- 2 Select **Open**.

The text window displays a list of directories (entries with a backslash as the last character in the name) and files (if any). The menu (near the top of the screen) displays the pathname of the current directory.

- 3 Select the **top directory (/)** from the **Look In** menu of the **File Open** dialog.
- 4 Select `/vnmr` from the file display.

The text window displays the list of subdirectories.

- 5 Select **fidlib** from the list.
- 6 Double-click the file `Proton_01.fid`.

The file is retrieved to the current experiment and viewport.

- 7 Click the **Process** tab.

- 8 Click the **Transform** button.

The graphics window displays a spectrum and the menu from the interactive spectrum display program (ds) appears.

- 9 Click the left mouse button near 7.7 ppm and the right mouse button near 7.5 ppm.

- 10 Click .

The spectrum expands to show the six-line pattern that will be simulated as an AX2Y system.

- 11 Click the **Show Spectrum** button.

The spectrum is now set up.

To simulate the spectrum

- 1 Click **Process** in the main menu.

- 2 Select **Analyze**.

- 3 Select **Spin Simulation**.

The **Spin Simulation** dialog box appears.

- 4 Click the **Spin Selection** tab.

- 5 Click the **AX2Y** button.

This picks the spin system.

- 6 Click **Initialize Parameters**.

The parameters are initialized from the spectrum and displayed.

- 7 Enter 0.6 in the **linewidth** entry field.

- 8 Click the one cursor icon on the graphics toolbar.

- 9 Move the cursor to the center of the six-line pattern.

- 10 Click the left mouse button.

- 11 Select **A** from the **Chemical Shifts** menu.

- 12 Click the **Set shift from spectrum** button.

This sets the chemical shift of spin A to the position of the cursor.

- 13 Move the cursor to the center of the left-most line.

- 14 Click the left mouse button.

- 15 Move the cursor to the center of the second left-most line.

- 16 Click the right mouse button.

17 Select **JAY** from the **Coupling Constants** menu to set the JAY coupling.

18 Click the **Set J from spectrum** button.

This sets the JAY coupling constant to match the difference frequency.

19 Click the right button on the center of the third line from the left.

20 Select **JAX** from the **Coupling Constants menu** to set the JAX coupling.

21 Click the **Set J from spectrum** button.

22 Click **Show Parameters**.

This confirms your entry of the spin system parameters.

23 Click **Simulate**.

A simulated spectrum is displayed.


To run an iterative spin-simulation

1 Click the **Spin Assignment** tab.

2 Verify that the values in the entry box are: A, JAX, and JAY.

This verifies that the iterate parameter is set correctly.

3 Enter the number of iterations in the **# iterations field**, enter 20 as a starting point.

4 Click the **Show/Hide threshold** button  to show the threshold.

Use the left mouse button to move the threshold line below the tops of the peaks.

5 Click the **Make Line List** button.

6 Click the **Assign Lines** button.

The assign macro is executed and assigns the lines from the line listing to the lines from the previous simulation.

7 Click the **Iterate** button.

This performs an iterative optimization, displays the resulting spectrum, and outputs the results of the iteration to the iterate window.

8 Click the **Spin Selection** tab.

- 9 Click the **Show parameters** button.

The listing contains the values of the A, JAX, and JAY parameters that give the best iterated fit to the experimental spectrum.

Spin simulation related commands, parameters, and files

Table 31 Spin simulation related commands and parameters

Command	Description
<code>assign*</code>	Assign transitions to experimental lines
<code>cla</code>	Clear all line assignments
<code>dga</code>	Display group of spin simulation parameters
<code>dla<'long'></code>	Displays spin simulation parameters arrays
<code>dlalong</code>	Long display of spin simulation parameter arrays
<code>dll*</code>	Displays listed line frequencies and intensities
<code>dsp<(file)></code>	Calculates the simulated spectrum (using the current value of parameter <code>slw</code> for the linewidth) and displays the spectrum using the current table of transitions and intensities. <code>dsp</code> can only be used after the spins program has been run. <code>dsp</code> with a <code>filename</code> as an argument uses the spectral information taken from that file. The result is displayed and can be modified and plotted like any other 1D spectrum.
<code>initialize_iterate</code>	Set <code>iterate</code> string to contain relevant parameters
<code>spinll<'mark'></code>	Set up a <code>slfreq</code> array
<code>spins <(options)></code>	Performs a spin simulation using the current spin system parameters when the Simulate button is clicked. The following variations are available: <code>spins('calculate','energy')</code> Puts an energy level table in the output file. <code>spins('calculate','transitions')</code> Puts a second table of transitions ordered by transition number in the output file. <code>spins('iterate')</code> Runs in an iterative mode to match experimental and calculated lines. <code>spins('iterate','iteration')</code> Lists parameters after each iteration in the output file.
<code>spsm(spin_system)</code>	Enters spin system
<code>undospins</code>	Restores spin system as before last iterative run
<code>* dll<'pos'<,noise_mult>><:lines></code> <code>assign<'mark'>, assign(transition_number,line_number)</code>	
Parameters	
<code>cla {array of real values}</code>	Calculated transition number
<code>clamp {array of real values}</code>	Calculated transition amplitude
<code>clfreq {real values}</code>	Calculated transition frequency

Table 31 Spin simulation related commands and parameters (continued)

Command	Description
clindex {array of real values}	Index of experimental frequency of a transition
iterate {string of parameters}	Parameters to be iterated
niter {1 to 9999}	Number of iterations
slfreq {real values}	Measured line frequencies
slw {0.01 to 1e6}	Spin simulation linewidth
smaxf {-1e10 to 1e10}	Maximum frequency of any simulated transition
sminf {-1e10 to 1e10}	Minimum frequency of any simulated transition
sth {0 to 1.00}	Minimum intensity threshold
svs {0 to 1e10}	Spin simulation vertical scale

Commonly used spin simulation global parameters and descriptions:

Table 32 Spin simulation global parameters

Parameter	Description
cla and clfreq	Make up a table consisting of line numbers assigned by the spin simulation program and the corresponding frequency of a measured line when the intensity of the line is above a threshold value set by the parameter th.
clamp	Stores the transition amplitude of calculated transitions when they are above a threshold set by parameter sth.
clindex	Index of experimental frequency of a transition.
slfreq	List of measured line frequencies.
sminf and smaxf	Minimum and maximum frequency limits for calculation of the final simulated spectrum. These should be set before the calculation is performed. If the Initialize Parameter button is used, sminf is initialized to sp, and smaxf is initialized to sp+wp.
sth	Minimum intensity threshold above which transitions are listed and included in the simulated spectrum. A typical value is 0.05.
svs	Maximum intensity of calculated transitions.
slw	Spin simulation line width
dga	Displays the file of simulation parameters in the Text Output window of the Process Panel .

The following commands are used to set up files for the “iterative” mode of spin simulation in which the calculated spectrum approximates an experimental spectrum.

Table 33 Iterative mode related commands

Command	Button	Description
<code>spins('iterate')</code>	Iterate	Performs the simulation in the iterative mode
<code>initialize_iterate</code>	Initialize parameters	Selects a default value for the parameter <code>iterate</code> that causes iteration of all parameters. The string parameter, <code>iterate</code> , contains a list of parameters (separated by commas) to be iterated during iterative spin simulations. Typical value is 'A,B,JAB'.
		Initializes the parameter <code>iterate</code> to a string containing parameters appropriate to the current spin system and <code>niter</code> to 20.
<code>cla</code>	Clear assigned lines	Clears the file of line assignments used for spin simulation.
<code>dla</code>	Show assigned lines	Displays the file containing the line assignments.
<code>dlalong</code>		Writes the line assignments to the file <code>spini.la</code> of the current experiment. This command is useful in more complex problems where the text window is too small for the <code>dla</code> display. <code>dlalong</code> displays the file in the text window.
<code>spinll</code>	Use Line List	Copies the frequency list from the last <code>nll</code> or <code>dll</code> line listing (contained in the parameter <code>llfreq</code>) into the simulation line frequency parameter <code>slfreq</code> . <code>spinll</code> , clears previous line assignments, and runs <code>dla</code> .
<code>spinll('mark')</code>		Places the line positions in the file <code>markld.out</code> into the parameter <code>slfreq</code> . This is useful for manual line assignment.
<code>assign</code>	Assign Lines	The nearest calculated transitions are assigned to the lines from a <code>dll</code> or <code>nll</code> listing after <code>spinll</code> places them in <code>slfreq</code> . If a frequency in <code>slfreq</code> exists with a line number, the next entry of the same frequency is assigned a unique line. Optional: Assign positive lines only. Run <code>dll('pos')</code> from the command line first. Only lines transitions greater than <code>sth</code> (typically ≥ 0.05 to prevent assignment of extremely small lines) are assigned.
<code>assign('mark')</code>		The same as <code>assign</code> , except the file <code>markld.out</code> is used instead of the <code>dll</code> listing. Use the cursor and the mark button to place the lines to be assigned in the <code>markld.out</code> file. This file is cleared by <code>mark('reset')</code> . Use <code>n1</code> to move the cursor to the center of a selected line.

Table 33 Iterative mode related commands (continued)

Command	Button	Description
<code>assign(τ#, l#)</code>		Assigns a single calculated transition number (τ #) to a line from a <code>d11</code> listing (the index is l #). Use <code>assign(τ#,0)</code> to remove the calculated transition assignment.
<code>undospins</code>		Restores a spin system as it was before the last iterative run. Chemical shifts, coupling constants, and transition assignments are returned to those existing immediately before an iterative spin simulation.

See the descriptions in the *VNMR Command and Parameter Reference*.

The `spins.list` file is an output table made by the spin simulation program. This file can be displayed by clicking on the **list** button. The following files can exist in the current experiment.

Table 34 Spin simulation files

File	Description
<code>spini.indata</code>	Line assignment input (deleted by <code>spins</code> after iterations are completed) for the <code>spins</code> program.
<code>spini.inpar</code>	List of parameters whose values are to be determined by <code>spins('iterate')</code> .
<code>spins.inpar</code>	List of initial settings of a number of spin simulation parameters.
<code>spini.la</code>	Current transition assignments for an iterative spin simulation (produced by the <code>d1along</code> command).
<code>spins.list</code>	Name of the output file. It is located in the current experiment. This file always includes the calculated transitions ordered by frequency.
<code>spins.outdata</code>	File of frequencies, amplitudes, and transition numbers from a spin simulation. It is used in calculating the displayed spectrum.
<code>spini.outpar</code>	Values of the chemical shifts and coupling constants after an iterative spin simulation.
<code>spini.savela</code>	Transition line assignments for iterative spin simulation in a format readable by the macro <code>undospins</code> .
<code>spins.stat</code>	Constants related to iteration (deleted by the <code>spins</code> program).

Deconvolution

The software supports the deconvolution of observed spectra into individual Lorentzian and Gaussian lines. Up to 2048 data points from an expansion of an experimental spectrum can be deconvoluted at one time. Up to 25 lines can be fit to this section of the observed spectrum. Each line's shape can be defined to be Lorentzian, Gaussian, or both.

The following parameters are available for each line:

- Frequency (in Hz) of line
- Intensity of line
- Linewidth (in Hz) at half-height of line
- Gaussian fraction of line: 0.0 (completely Lorentzian) to 1.0 (completely Gaussian)

All parameters can be fit at the same time, or selected parameters can be removed from the fit. In addition, a linear baseline correction is added to the fit to avoid large errors produced by base line offsets.

Deconvolution window and controls

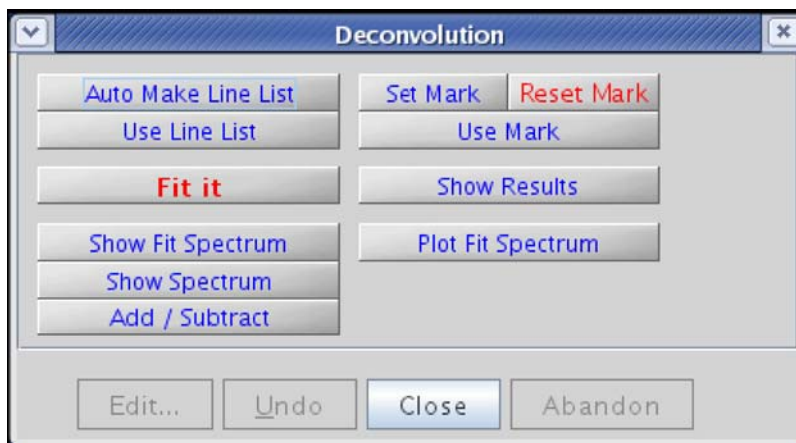


Figure 112 Deconvolution window and controls

The Deconvolution window, see [Figure 112](#), provides access to deconvolution functions via the following buttons:

Button	Function
Auto Make Line List	Create a line list from the current spectrum and threshold using the <code>nll</code> command
Use Line List	Use the current line list as a starting point for deconvolution
Set Mark	Add the current cursor position to the mark file
Reset Mark	Clear all lines from the mark file
Use Mark	Use the current mark list as a starting point for deconvolution
Fit it	Perform deconvolution on the selected line list using the <code>fitspec</code> command and display the results
Show Results	Display the deconvolution fit results in the Text Output page
Show Fit Spectrum	Display the spectrum calculated by deconvolution
Show Spectrum	Fourier-transform the current FID file and display the spectrum
Add/Subtract	Enter the add/subtract mode using the FT spectrum and fit spectrum
Plot Fit Spectrum	Plot the deconvolution fit spectrum, FT spectrum, and component analysis using the <code>plfit</code> macro

Deconvolution step-by-step

- 1 Click **File** in the main menu.
- 2 Select **Open**.
The text window displays a list of directories (entries with a backslash as the last character in the name) and files (if any). The menu (near the top of the screen) displays the pathname of the current directory.
- 3 Select the **top directory (/)** from the **Look In** menu of the **File Open** dialog.
- 4 Select **/vnmr** from the file display.
The text window displays the list of subdirectories.
- 5 Select **fidlib** from the list.
- 6 Double-click the file **Proton_01.fid**.
The file is retrieved to the current experiment and viewport.
- 7 Click the **Process** tab.

8 Select the **Default** page.

9 Select **32k** from the **Transform Size** menu.

Use a larger Fourier transform size than normal for proper digitalization of the line shape when the spectrum is to be deconvoluted. A typical transform size for deconvolution is $2 \times n_p$.

10 Click the **Transform** button.

11 Expand the six-line pattern near 7.6 ppm until it fills the center third of the display, with baseline on both sides.

12 Click the **Vert Scale** menu on the **Default** page of the **Process** folder and select **Absolute** intensity mode.

The absolute intensity mode is required for deconvolutions, simulations, etc.

13 Click **Process** in the main menu bar.

14 Select **Analyze**.

15 Select **Deconvolution**.

The Deconvolution window opens; see [Figure 112](#) on page 381.

16 Click the **Show Spectrum** button.

17 Set a threshold that lists exactly six lines.

18 Click the **Auto Make Line List** button.

19 Click the **Use Line List** button.

A line list and a file containing starting point for the deconvolution are created. The linewidth of the tallest line on the screen is measured automatically and used as the starting linewidth for the calculation.

20 Click the **Fit it** button.

The analysis is performed. This particular example is an 18 parameter fit (6 frequencies, 6 intensities, and 6 linewidths). The calculated spectrum is displayed in the graphics window when the analysis is done. Numerical results appear in the **Text Output** panel of the **Process** folder. The numerical output should be similar to this:

Number of data points:	660
Final chi square:	32611.957
Total number of iterations:	29
Successful iterations:	29
Digital resolution:	0.152 Hz/point


ITERATION HAS CONVERGED

Parameters

line	frequency	intensity	integral	Linewidth	gaussian fraction
1	3800.740	57.543	45.723	0.506	0.000*
2	3799.480	57.509	44.546	0.493	0.000*
3	3793.290	89.616	116.212	0.826	0.000*
4	3792.020	86.232	102.826	0.759	0.000*
5	3785.870	82.859	63.909	0.491	0.000*
6	3784.610	79.897	59.166	0.471	0.000*

- 21 Click the **Plot fit Spectrum** button.

The original spectrum, the calculated spectrum, and each of the component lines are plotted automatically, along with the numerical results of the calculation. At the end of this operation, the original spectrum replaces the calculated one.

- 22 Click the **Show Fit Spectrum** button to return to viewing the calculated spectrum.
- 23 Click the **Add/Subtract** button to view the original spectrum simultaneously with the calculated one.
- 24 Click the  icon on the graphics tool bar to view the difference between the measured and calculated spectra.

Using mark

It is not always possible to produce a suitable line list for starting a deconvolution. Use the **Set Mark** button following a previous spectral display (ds program) to provide a starting point. Information from marks made with a single cursor is written to the file mark1d.out and contains only a frequency and intensity. The starting linewidth is taken from the parameter slw. Information from marks made with two cursors, placed symmetrically about the center of each line at the half-height point, is written to the file mark1d.out and contains two frequencies, an intensity and an integral. The starting frequency is taken as the average of the two cursor positions. The starting linewidth is taken as their difference.

Deconvolution related commands and parameters

Table 35 lists the associated commands and parameters with a short description.

Table 35 Deconvolution commands and parameters

Commands	Description
dsp<(file)>	Display calculated spectrum
fitspec<(<'usell'><,><'setfreq'>)>	Perform spectrum deconvolution (VNMR)
fitspec	Perform spectrum deconvolution (UNIX)
mark*	Determine intensity of spectrum at a point
plfit	Plot deconvolution analysis
setgauss(fraction), setgauss(fraction*)	Set a Gaussian fraction for line shape
showfit	Display numerical results of deconvolution
usemark	Use mark as deconvolution starting point
* mark<(f1_position)><:intensity>, mark<(left_edge,region_width)><:intensity,integral> mark<(f1_position,f2_position)><:intensity> mark<(f1_start,f1_end,f2_start,f2_end)><:intensity,integral,c1,c2> mark<('trace',<options>)><:intensity,integral,c1,c2>, mark('reset')	
Parameter	Description
slw {0.01 to 1e6}	Starting default linewidth for deconvolution calculations.

Table 36 Deconvolution commands, parameters, and buttons

Command or parameter	Button	Description
fitspec	Fit it	<p>Performs spectrum deconvolution by fitting experimental data to Lorentzian and/or Gaussian line shapes. <code>fitspec</code> uses data in a file <code>fitspec.inpar</code> as a starting point for the calculation.</p> <p><code>fitspec</code> writes a text representation of the spectral data to the file <code>fitspec.indata</code>. The results of the fit are contained in a file after the calculation is finished.</p> <p><code>fitspec.outpar</code> format is identical to <code>fitspec.inpar</code>. All lines are set to have a linewidth of <code>slw</code>, and a fixed Gaussian fraction of 0. (See the <i>VNMR Command and Parameter Reference</i> for information about keyword options available with <code>fitspec</code>.)</p>
setgauss		Modifies the output of the last deconvolution (<code>fitspec.outpar</code>) and makes it the input for a subsequent analysis (<code>fitspec.inpar</code>), after first modifying the Gaussian fraction.

Table 36 Deconvolution commands, parameters, and buttons (continued)

Command or parameter	Button	Description
<code>setgauss(fraction)</code>		Use the format <code>setgauss(fraction)</code> where <code>fraction</code> is the Gaussian fraction of the line shape, a number naturally limited from 0 to 1, for example, <code>setgauss(0.4)</code> to allow this fraction to vary.
<code>setgauss(fraction*)</code>		Use the format <code>setgauss(fraction*)</code> . Suffix the <code>fraction</code> value (defined the same as above) with an asterisk and enclose the value in single quotes, for example, <code>setgauss('1.0*')</code> to fix the fraction.

Table 37 Deconvolution display and plotting commands and macros

Macro	Button	Description
<code>showfit</code>	Show Results	Displays deconvolution results and converts the data to a format suitable for printing.
<code>dsp('fitspec.outpar')</code>	Show Fit Spectrum	Displays the theoretical spectrum described by the parameters produced by a deconvolution calculation.
<code>plfit</code>	Plot Fit Spectrum	Produces a complete output plot of a deconvolution analysis, plotting the observed spectrum, the full calculated spectrum, each individual component, as well as the numerical results of the analysis.

The deconvolution program writes these text files into the user's current experiment directory.

Table 38 Deconvolution files

File name	Description
<code>fitspec.inpar</code>	Contains the starting parameters (frequency, intensity, linewidth, and Gaussian fraction) for a subsequent fitting operation. Optionally, this file contains the Gaussian fraction of the line shape. Any number followed by an asterisk (*) is held fixed during the calculation. All other parameters are varied to obtain the best fit. Edit this file before deconvolution to set different fixed Gaussian fractions for each line.
<code>fitspec.indata</code>	Contains the point-by-point intensity of the spectrum in the region of interest displayed when the fitting is begun (the part of the spectrum between <code>sp</code> and <code>sp+wp</code>).
<code>fitspec.outpar</code>	Contains the final parameters (frequency, intensity, linewidth, and Gaussian fraction) after a fit has been done.
<code>mark1d.out</code>	Contains the result of a mark operation during a spectral display. By using the Use Mark button, this file may be used as an alternative to the last line list in setting up initial guesses for a fitting operation.

Reference Deconvolution Procedures

All reference deconvolution is done from the command line. The following is a description of the fiddle program and the procedures for reference deconvolution of 1D and 2D data sets. [Table 39](#) list commands for 1D and 2D variations of the fiddle program.

Table 39 Fiddle command and variants

Commands	Description
fiddle*	Perform reference deconvolution
fiddled*	Perform reference deconvolution subtracting alternate FIDs
fiddleu*	Perform reference deconvolution subtracting successive FIDs from first
fiddle2d*	Perform 2D reference deconvolution
fiddle2D*	Perform 2D reference deconvolution
fiddle2dd*	Perform 2D reference deconvolution subtracting alternate FIDs
fiddle2Dd*	Perform 2D reference deconvolution subtracting alternate FIDs
* (option<,file><,option<,file>><,start><,finish><,increment>)	

Fiddle program options

The fiddle program and its variants, see [Table 39](#), perform reference deconvolution using a reference signal with known characteristics to correct instrumental errors in experimental 1D or 2D spectra. The main command to start the program or any of its variants can take multiple string and numeric arguments:

Syntax:

```
fiddle(option<,file><,option<,file>><,start><,finish><,increment>)
```

Arguments:

option can be the following keywords:

'alternate'	Alternate reference phase + / - (phase sensitive gradient 2D data).
'autophase'	Automatically adjust phase.
'displaycf'	Stop at the display of the correction function.
'fittedbaseline'	Use cubic spline baseline correction defined by integral regions.

'invert'	Invert the corrected difference spectrum/spectra.
'noaph'	Do not automatically adjust zero order phase of the reference region.
'nodc'	Do not use dc correction of the reference region.
'nohilbert'	Do not use Hilbert transform algorithm. Use the extrapolated dispersion mode reference signal unless 'noextrap' is also used as an option.
'normalise'	Keep the corrected spectrum integrals equal to the first spectrum.
'readcf'	Read the correction function from file; the argument file must immediately follow 'readcf'.
'satellites'	Use the satellites defined in file in the ideal reference region. The file should be in /vnmr/satellites.
'stop1'	Stop at the display of the experimental reference FID.
'stop2'	Stop at the display of the correction function.
'stop3'	Stop at the display of the corrected FID.
'stop4'	Stop at the display of the first corrected FID.
'verbose'	Show information about processing in the main window.
'writecf'	Write the correction function to file. The argument file must immediately follow 'writecf'.
'writefid'	Write out the corrected FID to file. If file does not begin with / it is assumed to be in the current working directory.

`file` – name of the file used with the 'satellites' and 'writefid' options.

`start` and `finish` – indices of the first and last array elements to be processed.

`increment` – specifies the steps in which the index is to increment. The default is to process the transformed spectra in an array.

Reference deconvolution of 1D spectra

Only spectra that contain a well-resolved reference signal dominated by a single component, not a simple multiplet, are suitable for reference deconvolution.

- 1 Fourier transform the raw FID with `ft`, preferably having zero filled (that is, set `fn` $\geq 2 \cdot n_p$). (If there are sinc wiggles, use `wft` with `gf` = `at*0.6`.)
- 2 Set the reference line to the chosen signal using the `r1` command, and then use two cursors on either side of the line to define a region of spectrum that includes all of

the reference signal, plus a little clear baseline but no other signals. This reference region will be used to define the instrumental line shape.

- 3 Decide what line shape to convert the instrumental line shape to, and set the weighting parameters accordingly. Create a 1-Hz wide Lorentzian by setting `lb` to 1 and all other weighting parameters to 'n'.

The negative consequence to signal-to-noise ratio penalty for resolution enhancement is 2 Hz wide `lb=0`, an infinitely sharp line with infinitely poor signal-to-noise is produced. A recommended strategy is to set `lb` to *minus* the expected *natural* linewidth and choose `gf` to give reasonable S/N. This strategy should convert the instrumental line shape to Gaussian. Resolution enhancement can be obtained by making `lb` more negative where signals of interest are broader than those of the reference are.

- 4 Type the `fiddle` command to carry out the reference deconvolution and display the corrected spectrum. The integral should remain unchanged. Any resolution enhancement will result in an increase in the amplitude of both signal and noise.
- 5 To save the corrected data, use the option `'writefid'` when doing the reference deconvolution. For example, to store the file `correctedfid.fid` in the current working directory, enter `fiddle('writefid','correctedfid')`.

The options `'writecf'<,file>` and `'readcf'<,file>` write and read the correction function. Performing reference deconvolution on one FID using `fiddle` with the `'writecf'` option and then use `fiddle` with `'readcf'` to process another FID applies the first correction function to the second FID. Reference deconvolution can be useful for heteronuclear lineshape correction (provided that the spectral widths for the two nuclei are in the ratio of the respective magnetogyric ratios) or for correcting spectra in which a reference signal has been suppressed (for example, an INADEQUATE spectrum can be corrected for lineshape errors by using a correction function derived from the normal carbon spectrum).

Correct a series of spectra in an arrayed or 2D experiment

Use numeric arguments:

`fiddle(1)` corrects spectrum 1, `fiddle(2,3)` spectra 2 and 3, etc.

Common satellites associated reference signals

- ^{13}C satellites
- ^{29}Si satellites
- quartet satellites (normally unresolved) from three-bond coupling to ^{13}C

^{13}C satellites are typically small enough to be ignored in samples that are not ^{13}C enriched. Referencing requiring high accuracy in which there are strong (for example ^{29}Si) satellites is accomplished by including the satellites in the specified form of the ideal reference signal by using the 'satellites' option.

The `/vnmr/satellites` directory contains the file `TMS` with details of the TMS satellite signals. The command `fiddle('satellites', 'TMS')` allows for the satellite signals when deconvoluting using TMS as a reference.

The format for satellite files consists of three real numbers in the following order:

- Separation of the satellite line from the parent signal, in Hz (0.5 JXH in the absence of homonuclear coupling).
- Intensity relative to the parent signal (natural abundance divided by the number of satellite lines [usually 2]).
- Isotope shift to high field, in ppm.

For example, the line

```
3.3          0.023          0.001
```

corresponds to a pair of satellite signals from a spin-1/2 isotope with an abundance of 4.6%, a coupling to the observed nucleus of 6.6 Hz, and an isotope shift to high field of 0.001 ppm.

Multiple lines in the satellite file account for multiple satellite signals on the parent peak.

Corrected-difference spectroscopy

Use the command `fiddled` to produce the corrected difference between successive spectra that divides `arraydim` in half. The difference spectrum is written into the second element of the pair. Because the main aim of reference deconvolution is to optimize the purity of the difference spectrum, the target line shape would be chosen to give the best possible S/N. This method corresponds to choosing a target line shape approximately twice the width of the raw

experimental signals of interest. The command `fiddleu` produces corrected differences between successive FIDs and the first FID.

Reference deconvolution of 2D spectra

The commands `fiddle2d`, `fiddle2D`, `fiddle2dd`, and `fiddle2Dd` function like the parent `fiddle` program. Because the principal objective in 2D reference deconvolution is the reduction of t_1 -noise, ideal line shape parameters are normally chosen for optimum S/N ratio rather than resolution enhancement.

- 1 Choose `fn` (preferably with `fn >= 2*np`) and `fn1`.
- 2 Type `ft` to transform the raw data (as mentioned earlier, if there is significant signal left at the end of `at`, it might be necessary to use `wft` with `gf` set).
- 3 Display the first increment with `ds(1)`, adjust the phase of the reference signal, and use `r1` to select the reference signal.

In earlier versions of `fiddle`, it was necessary to create a parameter, `phinc`, to anticipate the changes in the reference signal phase with increasing evolution time. The current algorithm automatically adjusts the phase (unless the 'noaph' option is selected). Deconvolution will set the reference signal phase as a function of t_1 to place the reference signal at frequency `rfp1` in `f1`. Therefore, remember to set `rfl1` and `rfp1` before using `fiddle2D` or the `f1` frequencies might unexpectedly change.

- 4 Define the reference region with the two cursors, and then enter the command `fiddle2D('writefid', <file>)` (or `fiddle2Dd` if a 2D difference spectrum is required, as with corrected HMBC). The 'writefid' option is essential, because `fiddle2D` alone does not store the corrected time-domain data. If phase-sensitive gradient-enhanced 2D data is to be processed, alternate FIDs will have opposite phase modulations (that is, the experimental array will alternate N-type and P-type pathways). In such a case, use the 'alternate' option.

The corrected 2D FID data can be read into an experiment and processed as normal after deconvolution is complete. The value of `arraydim` no longer matches the arrays set if `fiddle2Dd` has been used and it might be necessary to set the arguments to `wft2d` explicitly rather than using `wft2da`.

References

Further information on reference deconvolution can be found in the following literature:

Taquin, *J. Rev. Physique App.* 1979, 14, 669.

G. A. Morris, *J. Magn. Reson.* 1988, 80, 547.

G. A. Morris, D. Cowburn, *MRC* 1989, 27, 1085.

G. A. Morris, H. Barjat, T. J.Horne, *Prog. NMR Spectrosc.* 1997, 31, 197.

A. Gibbs, G. A. Morris, *J. Magn. Reson.* 1991, 91, 77.

A. Gibbs, G. A. Morris, A. Swanson, D. Cowburn, *J. Magn. Reson.* 1983, 101, 351–356.

D. N. Rutledge Ed., *Signal Treatment and Signal Analysis in NMR*, Chapter 16. Elsevier Science, 1996.

Addition and Subtraction of Data

Individual 1D spectra, FIDS, individual 1D traces from nD data sets, or spin simulated spectra can be added and subtracted. The process uses one experiment to display the results and experiment 5 as the add/subtract buffer file. Any data in experiment 5 (exp5) is overwritten.

Menu for add and subtract

Access the Add / Subtract experiments from the main menu as follows:

- 1 Click **Process** in the main menu.
- 2 Select **Add and Subtract 1D Data**.

The following sub-menu items are displayed:

Clears buffer (experiment 5): Creates experiment 5 and places current spectrum in experiment 5.

Add Second Spectrum into Buffer: Adds current spectrum (algebraically) to data in experiment 5.

Interactive add/subtract toolbar

The Interactive Add/Subtract toolbar has the following buttons (the labels on some buttons change depending on the current mode):

Each button, name (tool tip), and function is listed in [Table 40](#).

Table 40 Add/subtract toolbar buttons












Button	Name	Description
	Box	Box is shown when the display is in the cursor mode. Click to change to the box mode with two cursors.
	Cursor	Cursor is shown when the display is in the box mode. Click to change to the cursor mode.
	Select	Selects the current, addsub, or result mode. Text in the field next to active matches the color of the spectrum. Arrow colors: Green — current mode (spectrum) is selected Yellow — addsub mode (spectrum) is selected Blue — result mode (spectrum) is selected

Table 40 Add/subtract toolbar buttons (continued)

Button	Name	Description
	Expand	The third button is labeled Expand or Full , depending on the selected mode, box or cursor. When Expand appears the box mode is active, and clicking on this button expands the area between the cursors.
	Full	When Full appears, the cursor mode is active, and clicking on this button displays the full area.
	sp wp	Adjusts the start and width of the active spectrum.
	sub	The fifth button is labeled sub, min, or add. When sub appears, clicking on the button makes the result spectrum to be the difference between the current and the add/sub spectra.
	min	When min appears, clicking on the button makes the result spectrum to be a minimum intensity of either the current or the add/sub spectra.
	add	When add appears, clicking the button makes the result spectrum to be a sum of the current and the add/sub spectra.
	save	Saves the result spectrum in the add/sub spectrum in experiment 5 and returns to the last menu.
	return	Returns to the last menu without saving the result.

Interactive add/subtract

Interactive add/subtract is a multi-step process using the menu system to call the `addi` command and uses `exp5` as an add/subtract buffer file. Two spectra can be interactively added or subtracted using the menu add and subtract tools on the add/subtract tool bar; see [Table 40](#). Both horizontal displacement and vertical scale of the two spectra to be added or subtracted are under interactive control. Spectra that can be phased can be phased independently. The result can be manipulated using any of the standard software (for example, the command `p1`), including further interactive add/subtract with another data set.

To add and subtract spectra

- 1 Select any available experiment or create a new experiment. Do not use or create experiment 5.
- 2 Click **File** in the main menu.

3 Select **Open**.

The text window displays a list of directories (entries with a backslash as the last character in the name) and files (if any). The menu (near the top of the screen) displays the pathname of the current directory.

4 Select the **top directory (/)** from the **Look In** menu of the **File Open** dialog.

5 Select **/vnmr** from the file display.

The text window displays the list of subdirectories.

6 Select **fidlib** from the list.

7 Select an arrayed 1D data set such as **dept.fid**.

8 Click the **Transform** button on the action bar.



9 Click **Process** in the main menu.

10 Select **Add and Subtract 1D Data**.

11 Select **Clear Buffer** and **Add Current Spectrum** from the sub-menu.

Clears buffer (experiment 5): Creates experiment 5 and places current spectrum (spectrum 1) in experiment 5.

12 Select the spectrum to add or subtract from the first spectrum by doing either of the following:

- Click either the  or  until the desired spectrum is displayed.
- Type **ds(#)** where # is the number of the spectrum in the array.

13 Select **Add and Subtract 1D Data**.

14 Select **Add Second Spectrum** into Buffer from the sub-menu.

The default operation is to add the spectra. The current spectrum (spectrum 2) is added algebraically to data in experiment 5 (spectrum 1) and displays the three spectra as shown in [Figure 113](#).

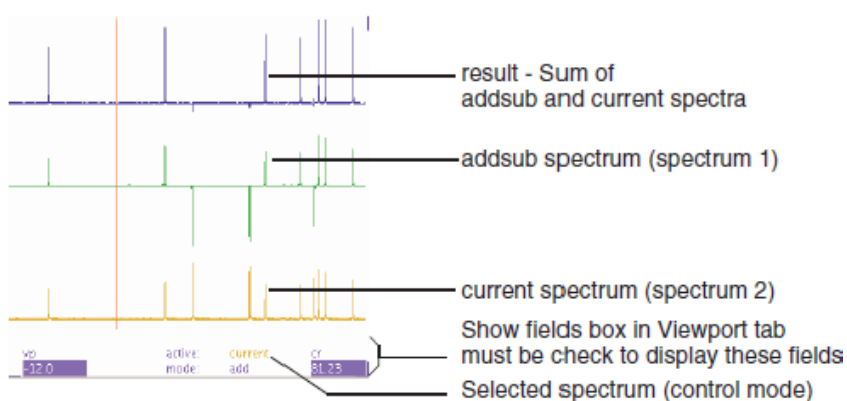




Figure 113 Add spectra and display result

- Optional: Click the  icon in the **Add/Subtract** toolbar to subtract the current spectrum (spectrum 2) from the first spectrum.


To manipulate the spectra

- Use the  button to step sequentially through the current, addsub, and result, spectra (control modes).

The mode is displayed next to active: at the bottom of the viewport. **Show fields box** in the **Viewport** tab must be selected to show the fields.

- Manipulate the spectra using the mouse:


Left mouse button positions the cursor or pair of cursors.

The left mouse button adjusts the start of the display if  is selected.

Center mouse button changes the vertical scale of the spectrum so that it goes through the current mouse position.

The horizontal position of the spectrum is adjusted if the mouse is positioned at the left edge of the spectrum.

Right mouse button positions the second cursor relative to the first cursor.


The right mouse button adjusts the width of the display if  is selected.

- Adjust any of the parameters related to the selected spectrum (except wp, it always controls all the spectra).

The spectrum can be phased, scaled, or shifted relative to the other spectrum.

- 4 Click **Process** in the main menu bar.
- 5 Select **Analyze**.
- 6 Select **Deconvolution**.

The Deconvolution window opens, see [Figure 112](#) on page 381.

- 7 Click the **Add / Subtract** button to display the resulting spectra and return to the interactive add and subtract mode.
- 8 Optional: Save the addsub spectrum after all the manipulation of the data has been completed if further operations, plotting, adding another spectrum, etc. are required.
 - a Click  to save the addsum spectrum into experiment 5. Spectrum 1, which was in the add/subtract buffer of experiment 5 is overwritten by the sum or difference spectrum. The interactive Add / Subtract routine exits.
 - b Save the result in experiment 5 to a file or move it to another available experiment.

Non interactive add/subtract

The non interactive add / subtract procedure is run from the command line. The buffer (exp5) is first cleared using `clradd`. Different FIDs or spectra are then added to or subtracted from the accumulating total by the commands `add`, `sub`, `spadd`, and `spsub`.

Adding and subtracting FIDs

The `add` and `sub` commands add and subtract the last displayed or selected FID to and from the contents of the add/subtract experiment. An optional argument allows the FID to first be multiplied by a multiplier (the default is 1.0). The parameters `lsfid` and `phfid` can be used to shift or phase rotate the selected FID before it is combined with the data in the add/subtract experiment.

A multi-FID add/subtract experiment with FIDs 1 and 2 is created with the `add` or `sub` command using the keyword

'new' as follows:

- 1 Create the add/subtract experiment with a single FID by entering the following commands from some experiment:

```
clradd select(1) add
```

- 2 Make the add/subtract experiment contain an array of two FIDs corresponding to the original FIDs 1 and 2 by entering:

```
select(2) add('new')
```

Entering the following makes the add/subtract experiment contain a single FID that is the sum of the original FIDs 1 and 2 instead of an array:

```
select(2) add
```

The `arraydim` parameter may need to be updated after constructing a multi-FID add/subtract experiment.

- 3 Type `jexp5` to join `exp5`.
- 4 Use `setvalue('arraydim',numFIDs,'processed')` to place `numFIDs` of FIDs into `exp5`.

The number of FIDs in the experiment is `numFIDs`.

Example:

```
setvalue('arraydim',12,'processed')
```

 places 12 FIDs into `exp5`.

Individual FIDs in a multi-FID add/subtract experiment can be added to and subtracted from one another. The `add` and `sub` commands without the keyword `'trace'` adds or subtracts from the first FID in the add/subtract experiment. Adding the keyword `'trace'` followed by the required index number selects specific FID to be the target of the add/subtract.

Example:

```
select(4) add('trace',6)
```

 takes the fourth FID from the current experiment and adds it to the sixth FID in the add/subtract experiment.

The FID must already exist in the add/subtract experiment by using an appropriate number of `add('new')` or `sub('new')` commands when using the keyword `'trace'`.

Adding and subtracting spectra

Non-interactive spectral addition and subtraction uses the `spadd` and `spsub` commands. The last displayed or selected spectrum is added to (`spadd`) or subtracted from (`spsub`) the current contents of the add/subtract experiment.

Each spectrum can be optionally multiplied and shifted using the multiplier and shift arguments, respectively. For example, entering `spadd(0.75,10)` multiplies the spectrum by 0.75 and shifts the spectrum by 10 to the left. A positive shift value shifts the spectrum being added or subtracted to higher frequency, or to the left. A negative shift value shifts the spectrum being added or subtracted to lower frequency, or to the right. To shift a spectrum without multiplying it, use a multiplier of 1.0.

A multi-element add/subtract experiment with spectra 1 and 2 is created with the `spadd` or `spsub` command using the keyword 'new' as follows:

- 1 Create the add/subtract experiment with a single spectrum by entering the following commands from some experiment:

```
clradd select(1) spadd
```

- 2 Make the add/subtract experiment contain an array of two spectra corresponding to the original spectra 1 and 2 by entering:

```
select(2) spadd('new')
```

Entering the following makes the add/subtract experiment contain a single spectrum that is the sum of the original spectra 1 and 2 instead of an array:

```
select(2) spadd
```

Individual spectra in a multi-element add/subtract experiment can subsequently be added to and subtracted from. The `spadd` and `spsub` command without the keyword 'trace' adds to or subtracts from the first spectrum in the add/subtract experiment. Adding the keyword 'trace' followed by the index number of the spectrum selects that spectrum to be the target of the add/subtract. Entering `select(4) spadd('trace',6)` takes the fourth spectrum from the current experiment and adds it to the sixth spectrum in the add/subtract experiment.

The indexed spectrum must already exist in the add/subtract experiment by using an appropriate number of `spadd('new')` or `spsub('new')` commands with the 'trace' argument. Join experiment 5 (`jexp5`) and use the normal

spectral display (for example, `ds`) and plotting commands to examine the results.

Add / Subtract related commands and parameters

The add/subtract experiment related commands are listed in [Table 41](#) and parameters are listed in [Table 42](#).

Table 41 Add/Subtract related commands

Commands	Description
add*	Add current FID to add/subtract experiment
addi	Start interactive add/subtract mode
clradd	Clear add/subtract experiment
jexp1, ..., jexp9999	Join existing experiment
select*	Select a spectrum or 2D plane without displaying it
setvalue*	Set value of a parameter in a tree
spadd*	Add current spectrum to add/subtract experiment
spmin	Take minimum of two spectra in add/subtract experiment
spsub*	Subtract current spectrum from add/subtract experiment
sub*	Subtract current FID from add/subtract experiment
*	
<pre>add<(multiplier<,'new'>)>, add('new'), add('trace',index) select<('next' 'prev' selection)><:index>, select<(<'f1f3' 'f2f3' 'f1f2'><,'proj'> <,'next' 'prev' plane>)><:i> setvalue(parameter,value<,index><,tree>) spadd<(multiplier<,shift>)>, spadd('new'), spadd('trace',index) spsub<(multiplier<,shift>)>, spsub('new'), spsub('trace',index) sub<(multiplier<,'new'>)>, sub('new'), sub('trace',index)</pre>	

Table 42 Add/Subtract related parameters

Parameters	Description
arraydim {number}	Dimension of experiment
lsfid {'n',-fn/2 to ni or fn/2}	Number of complex points to left-shift np FID
phfid {'n',-360 to 360, in degrees}	Zero-order phasing constant for np FID

Regression Analysis

The process of establishing correlations between two or more variables is called *regression analysis* or *correlation analysis*. The established regression or correlation can then be used to predict one variable in terms of the others. Often, paired data indicate that a regression may have a certain functional form, but we do not want to make assumptions about any underlying probability distributions of the data.

This type of problem is often handled by the least squares curve-fitting method. Specific examples of this are used for the analysis of T_1 and T_2 NMR data and for the analysis of kinetics data. Tools for fitting arbitrary data to selected functional forms are also available.

The regression process takes a set of data pairs from the file `regression.inp` and attempts to fit a curve to the set. The implemented curves are first, second, and third order polynomials and an exponential in the form:

$$y = a1 * \exp(-x/\text{tau}) + a3$$

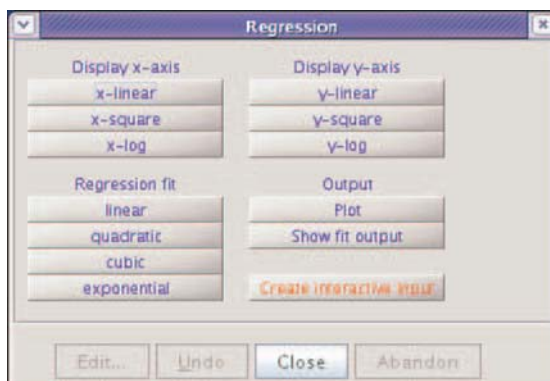
Original data may be displayed against a linear, squared, or logarithmic scales.

Regression window and controls

Access the regression window as follows:

- 1 Click **Process** on the main menu.
- 2 Select **Analyze**.
- 3 Select **Regression**.

The regression window is displayed. The functions of the buttons are as follows:



Button group	Function
Display x-axis buttons:	
x-linear	Display output with a linear x-axis
x-square	Display output with a quadratic x-axis
x-log	Display output with a logarithmic x-axis
Display y-axis buttons:	
y-linear	Display output with a linear y-axis
y-square	Display output with a quadratic y-axis
y-log	Display output with a logarithmic y-axis
Regression fit buttons:	
linear	Perform a linear regression analysis
quadratic	Perform a quadratic regression analysis
cubic	Perform a cubic regression analysis
exponential	Perform an exponential regression analysis
Output buttons:	
Plot	Plot the regression analysis
Show fit output	Display regression output
Create interactive input	The program displays a series of prompts requesting input

Regression, step-by-step using the regression window

- 1 Click **Create interactive input** button.

The program displays a series of prompts requesting the axis label titles and the data pairs.

- a Enter an X and Y pair separated by a space.
- b Enter the next X and Y pair.
- c Finish the data set by pressing the **Enter** key.
- d Respond to the prompt and press **y** to enter another data set or **n** to end the data entry.

The file `regression.inp` is created in the correct format when all the data is input and the data points and axis are displayed on the screen.

- 2 Click any one of the buttons **Display x-axis** or **Display y-axis** group.

Each button scales the display and shows either the x or y axis as labeled on the button.

- 3 Click one of the buttons in the **Regression fit** to select a fitting routine. These buttons include displaying the results using `expl`.

Fitting routine	Analyze option	Button in regression fit group
linear	'poly1'	Linear
quadratic	'poly2'	Quadratic
cubic	'poly3'	Cubic
exponential curve	'exp'	Exponential

Figure 114 on page 406 shows quadratic fittings for the data set in Table 43.

Table 43 Sample data table

Data set 1	
X value	Y value
0.0	5.0
1.0	9.0
2.0	12.0
3.0	20.0
4.0	33.0
5.0	42.0
Data set 2	
X value	Y value
0.0	20.0
1.0	22.0
2.0	27.0
3.0	29.0
4.0	37.0
5.0	45.0

Data is written to the `regression.inp` and `analyze.out` files. Contents of these files are explained in “[Contents of regression.inp file](#)” on page 412.

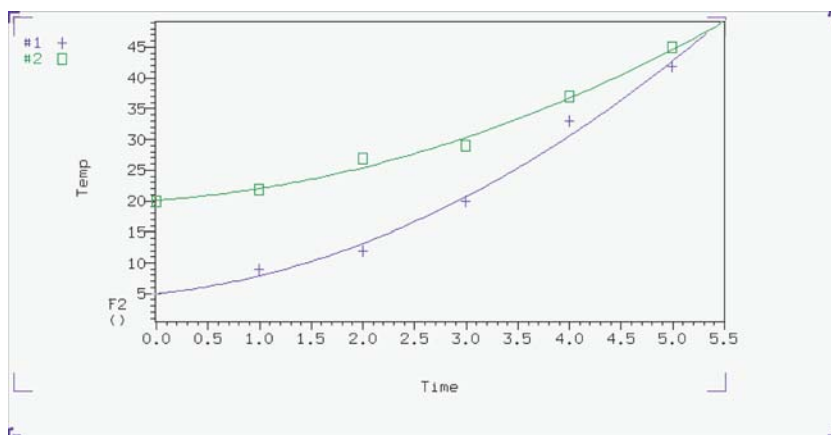


Figure 114 Display of regression fittings

- 4 Click the **Plot** button to plot the analysis.
The scale automatically to show all points (if possible).
- 5 Click the **Show fit output** button to show the results of the analysis in the **Text Output** window.

Regression, step-by-step using the command Line

- 1 Write and save the text file `regression.inp` that contains the data pairs to analyze. The next section describes the format of this file. Create it by one of the following methods:
 - Enter `rinput` on the command line.
 - The program displays a series of prompts requesting the axis label titles and the data pairs. The file `regression.inp` is created in the correct format when all the data is input. The data can be corrected using a text editor after the program writes the file.
 - Use a text editor such as `vi` or `textedit`.
 - Create a MAGICAL II macro for this purpose.
- 2 Enter the command `expl('regression')` for single data sets or `expl('regression', line#, line#, ...)` for multiple data sets.

The `expl` command uses the values in the `regression.inp` file to display a graph of the data points. It also creates the files `analyze.inp` (needed by `analyze` to run the analysis) and `expl.out` (display information for `expl`).

Optional: Use the `poly0` macro to calculate and display (as horizontal lines) the mean of the data in the file `regression.inp`.

- 3 Enter `analyze('expfit','regression',option,'list')` the command line.

The fitting routine choices for `option` are `'poly1'`, `'poly2'`, `'poly3'`, or `'exp'`.

Fitting routine	Analyze option
linear	'poly1'
quadratic	'poly2'
cubic	'poly3'
exponential curve	'exp'

The program `expfit` is called by this usage of the `analyze` command.

`expfit` creates the files `analyze.out` (used by `expl` to display the results) and `analyze.list` (a table of results).

- 4 Enter `expl` to see the results as a graph.

[Figure 114](#) on page 406 shows quadratic fittings for the data given in the example of the `regression.inp` file in the next section.

- 5 Enter `pexpl` page to plot one data set or enter `pexpl(index#,index#,...)` to plot multiple data sets.

Both `expl` and `pexpl` set the scale automatically to show all points (if possible).

- 6 Optional.

Enter the `scalegimits` macro to set limits for the scales using one of the following:

- Enter `scalegimits` with no argument to start an interactive process that prompts for the four scale limits.
- Enter `scalegimits(x_start,x_finish,y_start,y_finish)` with limits for the x-axis and y-axis as arguments.

The limits are retained as long as an `expl` display is retained. Enter `autoscale` to return to automatic scaling by `expl`.

- 7 Enter `cat(curexp+'/analyze.list')` to show the results of the analysis in the **Text Output** panel.

Contents of analyze.out file

The data input file is analyze.out, except for regression when the input file is regression.inp. The file expl.out saves certain display and plot parameters.

Values can be 2048 points maximum from a data set, 2048 points maximum from all sets displayed/plotted, 8 data sets maximum displayed, and 128 data sets maximum are read.

The following is an example of analyze.out. Numbers identify lines in the example and are not part of the actual file:

```

Line number
1          exp 7 regression
2          D1 C0 C1 C2
3          1 5 linear linear
4          Exponential Data Analysis
           time
           amp
5          NEXT 5
6          1   -248.962   22.8025   226.157
7          2           4
           3           9
           4          16
           5          25
           6          36

```

Curve types supported are listed in [Table 44](#)

Table 44 Curve type

Type	Function	Functional form
0	T_1/T_2	$(a0 - a2) \cdot \exp(-t/a1) + a2$
1	Increasing kinetics	$a0 \cdot \exp(-t/a1) + a2$
2	Decreasing kinetics	$-a0 \cdot \exp(-t/a1) + a2 + a0$
3	Diffusion	$a0 \cdot \exp(-D1 \cdot uu) + a2 \cdot \exp(-a1 \cdot D1 \cdot uu)$ where $uu = C0 + C1 \cdot t + C2 \cdot t^2$
4	None	No theoretical curve (use 'link')

Table 44 Curve type (continued)

Type	Function	Functional form
5	Linear	$a0 + a1*t$
6	Quadratic	$a0 + a1*t + a2*t^2$
7	Exponential	$a0 * \exp(-t/a1) + a2$
8	Contact time	$(a3 - (a3 - a0) * \exp(-t/a1)) * \exp(-t/a2) + a0$
9	Cubic	$a0 + a1*t + a2*t^2 + a3*t^3$

The following is a description of the numbered parts of this file:

Line number	Description
1	The keyword <code>exp</code> is followed by a number for a curve type from Table 40 Curve Type 40. The keyword <code>regression</code> , if present, indicates regression output.
2	Floating point constants <i>D1</i> , <i>C0</i> , <i>C1</i> , and <i>C2</i> , if present, are used only with the diffusion function (curve type 3).
3	An integer for the number of data sets (curves), followed by an integer for the number of data point pairs in the set. For regression, the words are scale types for the <i>x</i> and <i>y</i> axes: <code>linear</code> , <code>square</code> , and <code>log</code> .
4	Title line. Use <code>No Title</code> when a title is not desired. Two additional text lines for the <i>x</i> and <i>y</i> axis titles are present in output from regression.
5	The keyword <code>NEXT</code> identifies the start of a data set, and the integers that follow give the number of data point pairs in the data set.
6	The first integer specifies the number (usually 1) of the data point symbol used for the data set. The next three integers are the coefficients <i>a0</i> , <i>a1</i> , and <i>a2</i> (see Table 44) and must all be present, even for functions that do not use all three (for example, first-order polynomial). If a particular number is not appropriate, put any number there. <i>a3</i> must be also be present for cubic and contact time functions (curve types 7 and 8).
7	Data point pairs in the set.

Next is an example of regression.inp file for generalized curve fitting.

Line number

```

1      time
2      amp
3          0          0
4      NEXT
5          2.000000    4.000000
           3.000000    9.000000
           4.000000   16.000000
           5.000000   25.000000
           6.000000   36.000000

```

Description of this example:

Line number	Description
1	Line with text for <i>x</i> -axis label displayed by <code>expl('regression')</code> .
2	Line with text for <i>y</i> -axis label (line must not be too long, usually less than 20 characters). The first non-blank character must not be a digit.
3	Line containing an integer for the number of data sets followed by another integer for the number of pairs per data set. Both values are 0 if the number of pairs is variable.
4	A line beginning with the keyword NEXT is inserted at the start of each data set when the number of pairs per peak is variable.
5	The data pairs, listed one pair to a line.

The final example uses the 'file' argument to the `expl` command:

```

Line number
1      exp 4
2          1  5
3      time
4      1 0 0 0
5          2  4
           3  9
           4 16
           5 25

```

Description of this example:

Line number	Description
1	Keyword <code>exp</code> followed by curve type number
2	Number of data sets, followed by number of data point pairs
3	Title
4	Data point symbol number, followed by three coefficients
5	Data point pairs in the set

Contents of regression.inp file

The data input text file `regression.inp` contains a listing of axis labels and data pairs. The data file can contain up to 128 data sets. Data sets are selected by `expl` indexes (up to 6, depending upon length of data sets) with a default to the beginning data sets. The analysis is limited to 1024 data points, with the first part of larger data sets selected.

The following is an example of a `regression.inp` file that shows the format used. Numbers identify lines in the example and are not part of the actual file:

Line number

```

1          time
2          temp
3          0 0
4          NEXT
5          0.000000      5.000000
           1.000000      9.000000
           2.000000     12.000000
           3.000000     20.000000
           4.000000     33.000000
           5.000000     42.000000
4          NEXT
5          0.000000     20.000000
           1.000000     22.000000

```

```

2.000000      27.000000
3.000000      29.000000
4.000000      37.000000
5.000000      45.000000

```

Description of this file:

Line number	Description
1	X-axis label for display by <code>expl('regression')</code> . The label is optional. If used, the first non-blank character in the label must not be a digit.
2	Y-axis label for display by <code>expl('regression')</code> . The label is optional. If used, it must be less than about 20 characters and the first non-blank character in the label must not be a digit.
3	If the number of pairs per data set is fixed, this line contains an integer for the number of data sets, followed by another integer for the number of data pairs per data set. If the number of pairs per data set is variable (as in this example), both integers are set equal to 0.
4	If the number of pairs per data set is variable, a line with the word <code>NEXT</code> is inserted at the start of each data set.
5	Data pairs, one to a line, are listed for each data set, in this order: first pair of first set second pair of first set third pair of first set ... first pair of second set second pair of second set

Regression commands

Table 45 lists the commands associated with regression analysis.

Table 45 Regression commands

Commands	Button	Description
<code>analyze*</code>	Any of the buttons in the Regression fit group.	Generalized curve fitting in regression mode

Table 45 Regression commands (continued)

Commands	Button	Description
autoscale	Any of the buttons in the Output group.	Resume autoscaling after limits set by scalelimits
expfit*	Any of the buttons in the Regression fit group.	Make least-squares fit to exp. or poly. curve (Linux)
expl(<options,> line1,...>)>	Any of the buttons in the Display x-axis or Display y-axis group	Display exponential or polynomial curves
pexpl(<options,> line1,...>)>	Plot	Plot exponential or polynomial curves
poly0		Display mean of data in regression.inp file
rinput	Create iterative input	Input data for regression analysis
scalelimits*	Any of the buttons in the Output group.	Set limits for scales in regression
* analyze('expfit',xarray<,option,option,...>) expfit options <analyze >analyze.list scalelimits(x_start,x_end,y_start,y_end)		

Cosy Correlation Analysis

Cosy data chemical shift analysis window and controls



Button	Function
Create 2D Line List	Write a 2D line listing to the file <code>l12d.out</code> and displays the results in the Text Output panel.
Find Correlations	Analyze the current 2D line listing and display the results on the screen.
Redisplay	Display an interactive 2D contour map.
Plot Correlations	Analyze the current 2D line listing and plot the results.
Plot Contours	Plot the 2D contours.
Plot Page	Send plot to the printer.

Analysis of COSY Data Step-by-Step

- 1 Process and display a COSY data set.
- 2 Click **Process** from the main menu.
- 3 Select **Analyze**.
- 4 Select **Cosy Correlations...**
 Opens Cosy Correlations window if current viewport contains an appropriate data set.
- 5 Click the **Create 2D Line List** button to create a line list for the COSY data set.
- 6 Click the **Find Correlations** button display the correlations of the COSY data set on the screen.
- 7 Optional:

Click the **Redisplay** button to redisplay a normal interactive 2D contour map and make any changes or adjustments to the data as needed. Start over again at step 4.

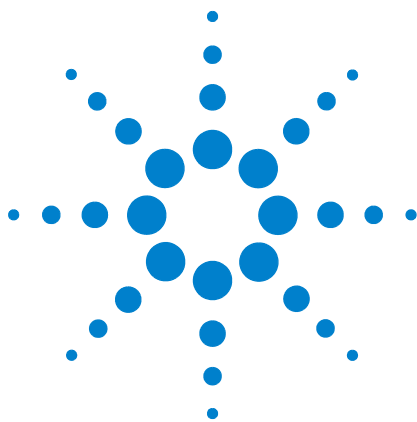
- 8 Click the **Plot Correlations** button to create plot of the line list for the COSY data set.
- 9 Click the **Plot Contours** button to create a contour plot of the COSY data set.
- 10 Click the **Plot Page** button to send the plot to the printer.

Chemical Shift Analysis

Chemical shifts are analyzed using the commands in [Table 46](#). These commands write a list of chemical shifts to the file `pcss.outpar`.

Table 46 Chemical shift analysis commands

Commands	Description
<code>do_pcss</code>	Calculate proton chemical shifts spectrum
<code>pcss</code>	Calculate and show proton chemical shifts spectrum



15 Pulse Analysis

Pbox 420

Pulse Shape Analysis 439

This chapter provides information on the Locator and File Browser.



Pbox

Pandora's Box (Pbox) software creates shape pattern files for experiments involving shaped RF pulses, composite pulses, decoupling and mixing patterns, adiabatic RF sweep waveforms, and pulsed field gradient shapes. Pbox provides the parameters (pulse width, power, dmf, dres, etc.) when the shape files are created, and this information can be extracted from Pbox shape files by macros or directly within pulse sequences. More than 160 different shapes are available from the Pbox library.

Create a new waveform

- 1 Open the **Pbox** window to access the tools for creating waveforms.
- 2 Click **Edit** on the menu bar.
- 3 Select **New Pulse Shapes**.

The Pbox window opens.

- 4 Click the **Make Waveform** tab.
- 5 Click the **New Waveform** button and the tools for creating a wave form are displayed in the **Pbox** window, see [Figure 115](#).

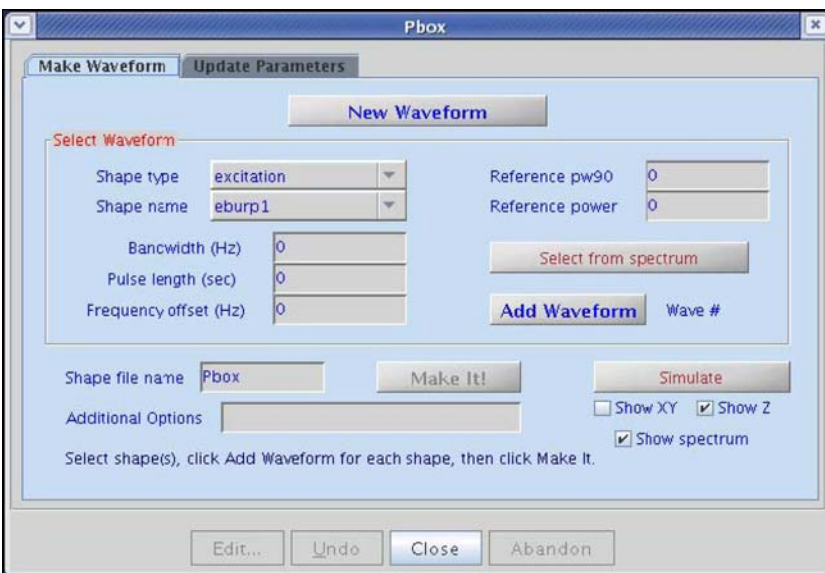


Figure 115 Pbox make waveform tab

- 6 Select the desired shape from the **Shape type** menu.

- 7 Select the desired shape name from the **Shape name** menu. The Shape name choices depend on the selected Shape type.
- 8 Set the **Reference pw90** and **Reference power**.
- 9 Set the waveform selection region as follows:
 - a Display a spectrum.
 - b Select a selective excitation band using two cursors.
 - c Click **Select** from the spectrum button. This sets the Bandwidth, Pulse length, and Frequency offset from the cursors.
 - d Optional: Explicitly enter Bandwidth, Pulse length, and Frequency offset.
- 10 Click **Add Waveform**. The **Wave #** field is updated and the **Make It!** button becomes active.
- 11 Add additional waveform shapes into a waveform by repeating steps 4 through 8 (above). The **Wave #** field updates for each selective shape added to the waveform.
- 12 Enter a name for the shape in the **Shape file name** entry box.

The field contains a default file name of Pbox.
- 13 Enter any **Additional Options** (see [Table 48](#)).
- 14 Click **Make It!** to generate and save the shape file.
- 15 Request simulations, see [Figure 116](#), as follows:

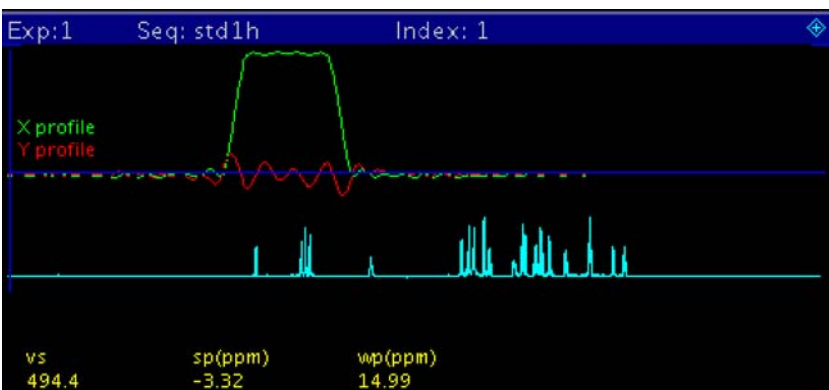


Figure 116 Shaped Pulse Simulations Reference Spectrum

- a Click the **Show XY** or **Show Z** option button.
- b Select **Show spectrum** to show the spectrum.
- c Click **Simulate**.
The simulation is displayed in the graphics window.

Update parameter values

Update the parameter values used by the waveform into the experimental parameters as follows:

- 1 Click the **Update Parameters** tab, see [Figure 117](#).

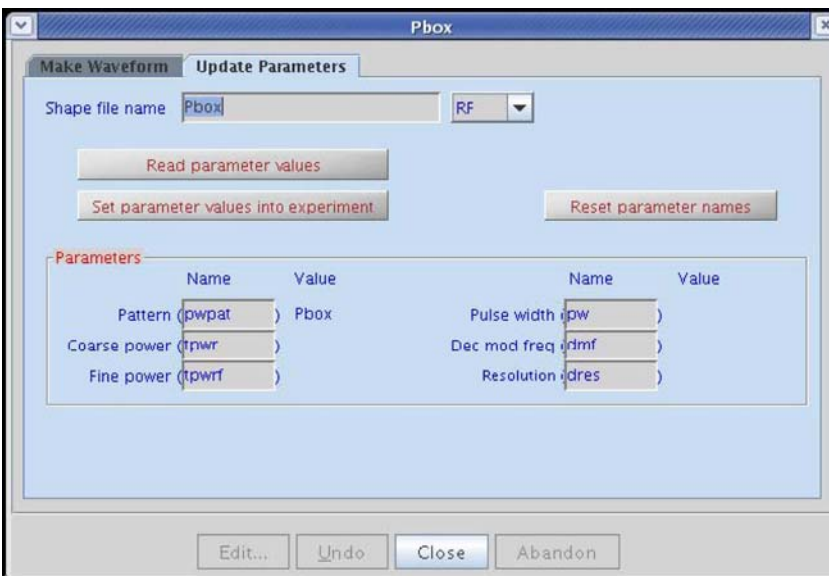


Figure 117 Pbox update parameters

- 2 Enter a shape file name in the entry box.
- 3 Click **Read parameter values** to read parameter values from the Pbox shape file.
- 4 Enter names for the parameters or use the suggested defaults for the waveform.
- 5 Click **Reset parameter names** to reset the parameter names to the default values, if desired.
- 6 Click **Set parameter values into experiment** to set the parameter values into the experimental parameters.

Parameters that do not exist are not set in the experiment. Parameter limits are not selected (especially for pulse width) – select them.

Calibrating the RF field

In the input, provide the RF field calibration data `ref_pwr` and `ref_pw90` to obtain the pulse calibration numbers in the Pbox output before waveform creation. Ensure that the RF field has been calibrated for the length of the 90° pulse at a given power level.

The RF field can be calculated at any power using spectrometers with linear amplifiers such as those on Agilent NMR spectrometer systems. Maximum accuracy for the calibration is obtained when the calibration is made as close as possible to the field used in the experiment. Provide approximate calibration data and use `cal` as an output file name for Pbox to permit an estimate of the RF field. No waveform is created and only the calibration results appear in the output.

Creating waveforms by macros

Pbox macros provide useful tools for customizing NMR experiments. The simplest way to create a shape is by using the `pxshape` macro. For example, a single band excitation pulse using the E-BURP-1 shape, covering 400 Hz, and shifted off-resonance by -880 Hz from the carrier frequency (middle of the spectrum) can be created and stored in the `alpha.RF` file as follows:

```
pxshape('eburp1 400.0 -880','alpha.RF')
```

The following steps are necessary to create multiply-selective pulses. Use the cursors if the spectrum of interest is on the screen. The parameters `ref_pwr90` and `refpwr` must be present and set correctly in the current parameter set.

- 1 Type `opx('hadamard.RF')` to open the `Pbox.inp` file and write the file header.
- 2 Select an excitation band using cursors.
- 3 Type `selex('rsnob')`.
- 4 Select the second excitation band using cursors.
- 5 Type `selex('rsnob')`.
- 6 Repeat steps 2 to 5 as many times as needed.
- 7 Type `cpx` to close the `Pbox.inp` file.
- 8 Type `dshape` to display the last created shape.

The following set of macros is used if an experimental spectrum is not available. The parameters `ref_pwr90` and `refpwr` must be present and correctly set in the current parameter set.

- 1 Type `opx('myshape')` to open Pbox and provide a file name.
- 2 Type `setwave('sech 400.0 -880.0')` to select first band at -880 Hz.
- 3 Type `setwave('sech 400.0 1240.0')` to select second band at 1240 Hz.
- 4 Type `cpx(ref_pwr90, ref_pwr)` to close Pbox.
- 5 Type `dshape` to display the shape file.

The `pbox_pw` and `pbox_pwr` macros are used to load the parameters of the last created shape file into the current experiment:

```
pbox_pw:selpw
pbox_pwr:selpwr
```

Alternatively, the calibration data is directly retrieved from the shape file provided as an argument to the `pbox_dmf` and `pbox_dres` macros:

```
pbox_dmf('ccdec.DEC'):dmf
pbox_dres('ccdec.DEC'):dres
```

where `ccdec.DEC` is the name of the decoupling shape file.

The excitation profile of shaped pulses is verified using the **Pbox Bloch** simulator. The parameters `ref_pwr90` and `refpwr` must be present and set correctly in the current parameter set.

- 1 Type `opx` to open Pbox.
- 2 Type `setwave('iburp2 400.0 -880')` to select first band at -880 Hz.
- 3 Type `setwave('iburp2 400.0 1240.0')` to select second band at 1240 Hz.
- 4 Type `pbox_rst` to reset par-s and write comments.
- 5 Type `pboxpar('name', 'test.DEC')` to define the output file name.
- 6 Type `pboxpar('bsim', 'y')` to activate the Bloch simulator.
- 7 Type `cpx` to close Pbox.
- 8 Type `dshape` to display the shape file.

9 Type `dprofile('z')` to display inversion (Mz) profile.

Pbox reads the corresponding wave file in the `wavelib` directory to determine whether the waveform being created is a 90° excitation pulse or a 180° inversion pulse, and the type of wave form required (.RF,.DEC, or.GRD). Pbox can be forced to change the waveform type by providing the required extension to the output shape file name. Modify wave files by copying it into the local user `wavelib` and editing the text file as required. See “[Pbox commands using a terminal](#)” on page 437, for a more complete description of macros.

Creating waveforms in an operating system terminal window

Creating waveforms using an operating system terminal window can be more convenient in some cases:

```
> Pbox
```

The name of the output shape file is passed as an argument:

```
> Pbox filename
```

The input data are typically stored in the `Pbox.inp` file in your `vnmrsys/shapelib` directory and are modified using standard text editors. Alternatively, most of the necessary data can be provided as arguments to the Pbox command. For example,

```
> Pbox myfile -w "eburp1 480 -1200" -p 40 -l 104
```

generates an E-BURP-1 excitation pulse covering 480-Hz-wide band and shifted -1200 Hz off-resonance using for calibration 104 μ s pw90 at 40 dB power level and stored in `myfile.RF`. Note that the name of the output shape file is always passed as the first argument.

Several other options are accepted by Pbox. For example, `-b` activates the Bloch simulator, `-c` calibrates the waveform without creating a shape file, and `-o` print out the available options. For more information, see “[Pbox commands using a terminal](#)” on page 437.

Pbox file system

Information about the waveform to be created (for example, calibration data, output file name, excitation band definition) is stored in the `Pbox.inp` text (ASCII) file in the user directory `vnmrsys/shapelib`. This file is generated whenever Pbox menus or macros are used or created by using one of

the standard text editors.

Any shape file can consist of one or several shapes that are combined into a single waveform. Each excitation band is defined by a wave definition string (a string of wave variables enclosed between delimiters {and}). There is an unlimited number of wave definition strings in a single `Pbox.inp` file. In order to simplify the input file format, the wave variables are entered without names in a strongly predefined order:

```
sh bw(/pw) ofs st ph fla trev d1 d2 d0 wrap
```

The following table describes each of the variables.

sh	Shape name as stored in wavelib
bw(/pw)	Bandwidth in Hz, or pulsewidth in sec, or both
ofs	Offset from transmitter offset or carrier in Hz
st	Spin status (0 for Mz or 1 for Mxy)
ph	Phase or phase cycle
fla	Flip angle
trev	Time reversal flag
d1	Prepulse delay
d2	Postpulse delay
d0	Delay before the pulse
wrap	Wraparound parameter

The order of parameters is listed in descending order of importance. Rarely used parameters can be omitted or defaulted by assigning a value of n (not used). The following examples are valid wave definition strings.

{qsneeze}	q-SNEEZE pulse applied on resonance. The pulse length will be internally defaulted to 5 ms.
{G3 800}	G3 pulse covering bandwidth of 800 Hz and applied on-resonance
{sech 400/0.05 -1200}	50 ms long hyperbolic secant pulse covering 400Hz and shifted off-resonance by -1200 Hz
{WURST2 2k/5m 12k n t5}	5 ms long WURST-2 decoupling pulse covering 2 kHz and shifted off-resonance by 12 kHz uses t5 phase cycle
{eburp1 450 0.0 n 180} {eburp1 450 820 1 0.0}	Two E-BURP-1 pulses mixed in a single waveform, both covering 450 Hz wide band. The first pulse is applied on-resonance with a phase of 180 deg. The second pulse is shifted to 820 Hz of-resonance, has zero phase, and is a de-excitation pulse (status 1). By default, such a pulse is time reversed.

A set of Pbox parameters can be used to define the waveform to be generated. The syntax of the Pbox.inp file is straightforward, parameter=value, for instance, name=myshape.RF, or simply name=myshape. The following table describes Pbox parameters and their default values (see “Pbox parameters” on page 433, for more details):

name=Pbox	Shape file name, the extension is optional
type=r	Shape type, r - RF, d - DEC, g - GRD
dres=9.0	Default value is stored in wavefile
steps=200	Minimum number of steps (< 64k). The default value is stored in a wave file
maxincr=30	Max phase incr, deg (<<180)
attn=i	Attenuation, i (internal), e (external) or d (nearest dB step)
sfrq=0	Spectrometer frequency, MHz
refofs=0	Reference offset, Hz (/ppm)
sucyc=d	Super Cycle, d (default), n (no), name as in wavelib/supercycles. The default value is stored in wavefile.
reps=2	Amount of reports (0-4)
stepsize=n	Size of a single step (ms)
wrap=0	Wraparound parameter (0-1)
header=y	Shape header, y (yes) n (no) i (imaging)
bsim=n	Bloch simulation, y (yes), n (no), a (add), s (subtract), 200 (time in sec)
T1=n	Relaxation time T1 (sec)
T2=n	Relaxation time T2 (sec)
dcyc=1	Duty cycle (0 - 1)
sw=0	Spectral width (Hz)
pctype=selective	pulse type (for imaging only)

The number and order of input parameters are optional.

Redefine the internally defaulted Pbox parameters by entering the default values in the .Pbox_globals file.

Parameters describing software and hardware limitations are internally pre-defined and can be redefined by the user in the .Pbox_globals file that is stored in user's home directory. The following table describes global parameters and their default

values.

shdir=\$HOME/vnmrsys/ shapelib/	default shape directory
wvdir=/vnmr/wavelib	default wave directory
maxst=65500	maximum number of steps in waveform
defnp=100	default number of steps
minpw=0.2	minimum step length, in μ s
minpwg=2.0	minimum gradient step length, in μ s
drmin=1.0	minimum dres
maxamp=1024.0	maximum amplitude
maxgr=32767.0	maximum gradient amplitude
amres=1.0	amplitude resolution
phres=0.1	phase resolution, in degrees
tmres=0.05	time resolution, in μ s
dres=9.0	default dres
maxpwr=63	maximum power level, in dB
minpwr=-16	minimum power level, in dB
maxitr=5	maximum number of iterations
maxdev=2.0	maximum deviation, in percent
cmpr=y	waveform compression
minstps=64	minimum steps in Bloch simulation
pw=0.005	default .RF and .DEC pulse length, in sec
pwg=0.001	default .GRD pulse length, in sec

The parameters of individual shapes—Gaussian, E-BURP-1, or hyperbolic secant pulse, etc.—are stored in the `wavelib` directory, which has several subdirectories, such as `excitation`, `inversion`, `refocusing`. Every individual shape is defined by a set of parameters that can be grouped in several categories.

Wave definition parameters are the following:

amf	amplitude modulation function
fmf	frequency modulation function
su	default supercycle
fla	default flip angle on resonance

pwbw	pulsewidth to bandwidth product
pwb1	pulsewidth to B1 max product
psw	pulsewidth to sweepwidth product
adb	adiabaticity on resonance
ofs	offset of excitation bandwidth
dres	default tipangle resolution, in degrees
dash	dash variable
wf	window function
st	default status
dutyc	duty cycle
c1	constant
c2	constant
c3	constant
steps	default number of steps

Wave truncation parameters are the following:

min	minimum truncation threshold (0 to 1)
max	maximum truncation threshold (0 to 1)
left	truncation from left (0 to 1)
right	truncation from right (0 to 1)
cmplx	flag, retain real (1), imag (-1) or complex(0) part of wave
wrap	wraparound factor (0 to 1)
trev	time reversal flag (yes = 1, no = 0)
srev	frequency sweep reversal flag (0 to 1)
stretch	stretching factor (≥ 0)
dcflag	dc correction, y/n

Additional parameters are usually data matrices, such as Fourier coefficients or square wave parameters, for example, length, phase, amplitude, etc. These matrices are listed without parameter names. The size of the data matrix given is defined by:

cols	number of columns
rows	number of rows

Pbox incorporates the following amplitude modulation (AM) functions:

sq	square (constant amplitude)
sqa	square wave amplitude modulation (used for "composite" pulses)
gs	Gaussian
lz	Lorentzian
sch	sech (hyperbolic secant)
hta	tanh (hyperbolic tangent)
tra	triangular amplitude (ramp)
sc	sinc function
csp	cosine power
wr	wurst (wideband uniform rate smooth truncation)
sed	seduce-1, mixture of sech and sin
qp	quadrupolar
ata	amplitude mod for CA atan frequency sweep pulse
exa	exponential amplitude
tna	tangential amplitude
fs	Fourier Series
ft	inverse Fourier Transform

Pbox incorporates the following frequency modulation (FM) functions:

ls	linear sweep (chirp)
tns	tangential sweep (tan)
ht	hyperbolic tangent sweep (tanh)
lzs	constant adiabaticity Lorentzian sweep
ca	constant adiabaticity (CA) sweep (frequency modulated frame)
cas	constant adiabaticity sweep (phase modulated frame)
cs	cosine / sine pulse frequency sweep
cs2	CA cosine square frequency sweep
ccs	CA cosine frequency sweep
sqw	squarewave phase modulation
fsw	frequency switch (step function)

fslg frequency switched as per Lee-Goldburg

Pbox parameters

The following list describes Pbox parameters.

name	Name and extension of the output shape file. If the extension is not given, the shape type is set according to the type parameter. The default name is internally set as Pbox. This can be changed in the .Pbox_globals file.
type	Shape type. Allowed values are r (.RF type), d (.DEC) or g (.GRD). If the shape type is not defined and the shape file is given without an extension, the shape file type is determined from the wave file according to the following criteria: <ul style="list-style-type: none"> • type is set to r if $pwbw > 0.0$ • type is set to d if $dres > 0.0$ • type is set to g otherwise
dres	Corresponds to dres parameter in VMNR. Active only with .DEC files.
steps	Defines the required number of steps in the waveform. The default number of steps is stored with each individual shape in the corresponding wave file. This number can be overridden by Pbox if it is smaller than the internally calculated minimum number of steps, which is necessary to maintain the functionality of the waveform. This number is defined according to the following criteria: <ul style="list-style-type: none"> • By the minimum number of steps necessary for adequate representation of the waveform (as in wave file). • If the waveform is shifted off-resonance, by the Nyquist condition (see maxincr).
maxincr	Maximum phase increment. Default is 30°. This number is active only if the waveform is shifted off-resonance or the shape itself is frequency-modulated (for example, adiabatic sweeps). In order to satisfy the Nyquist condition, maxincr should not exceed 180°, otherwise the waveform gets folded back. In fact, the degradation of performance and interference with sidebands can be observed even with a maxincr of greater than 90°, but a maxincr of less than 90° is recommended.
attn	Fine attenuation mode, which uses the following allowed values: <ul style="list-style-type: none"> i (Internal), default. Fine attenuation is implemented by internally rescaling the waveform within the amplitude range set by maxamp (0 to 1023). e (External) Fine attenuation is implemented by externally rescaling the waveform using linear modulators. The internal amplitude is set to maxamp (1023.0), and the required fine attenuator setting is produced in the output. d Attenuate to the nearest dB step by changing the pulse width, which will affect the excitation bandwidth typically within 5%, which is tolerable in most applications. The internal amplitude is set to maxamp (1023.0) 4.5i Internally attenuate to a given (4.5 kHz) B1 field strength by adjusting the pulse length. 4.5e Externally attenuate to a given (4.5 kHz) B1 field strength by adjusting the pulse length. 45i Internally attenuate, keeping course power level at a given (45 dB) power level. 45E Externally attenuate (with fine power), keeping course power level at a given (45 dB) power level. 45d Attenuate to a given (45 dB) power level by changing the pulse width. The internal amplitude is set to maxamp (1023.0).
sfrq	Spectrometer frequency in MHz.
refofs	Reference offset, usually 0.0. Can be specified if the excitation bands are shifted by or referenced to some frequency. Units: Hz, kHz, or ppm (if sfrq is defined).

sucyc	Super cycle. Allowed values are <i>n</i> (no), <i>d</i> (default), or any name of a super cycle stored in the wavelib/supercycles directory. By default, it is internally set to <i>d</i> . Super cycles can be nested by separating the names with a comma, for example, <i>t5,m4</i> represents 5 step TPG super cycle nested in four step MLEV-4 super cycle.
reps	Defines level of reporting. Allowed values are 0-4: 0=silent, 1=single line, 2=minimum, 3=medium, 4=maximum. The default is 2.
stepsize	The length of a single step in a waveform. The default units are μs . Note that stepsize disables the <i>maxincr</i> parameter.
bscor	Initiates correction for Bloch-Siegert effect in multiple band excitation, inversion or refocusing pulses. Allowed values are <i>y</i> (yes) or <i>n</i> (no, default). Active only if the number of bands is two or more. Reduces the RF interference effects (see M. Steffen, L.M.K. Vanderseypen and I.L.Chuang, <i>Abstracts of the 41st ENC</i> , p. 268, Asilomar 2000).
wrap	Wraparound parameter. It allows wrapping around the waveform. The allowed values are between 0 and 1.0.
header	Shape file header. Allowed values are <i>y</i> (yes, default), <i>n</i> (no shape file header), and <i>i</i> (imaging). Information required for imaging systems is stored in the shape file header.
bsim	Bloch simulator. Performs Bloch simulation for the given waveform at the moment of waveform generation. Allowed values are <i>y</i> (yes), <i>n</i> (no, default), <i>a</i> (add to the previous simulation), <i>s</i> (subtract from the previous simulation), and any positive integer limiting the simulation time in seconds. The default maximum length of simulation is internally set to 60 seconds and can be redefined in the <i>.Pbox_globals</i> file. Note that Bloch simulator can also be externally activated, for example, from menus or using the <i>dprofile</i> macro.
T1	Longitudinal relaxation time, T1 in seconds. Can be required by some waveforms (for example SLURP pulses). Optional for the Bloch simulation.
T2	Transversal relaxation time T2, in seconds. Can be required by some waveforms (for example, SLURP pulses). Optional for the Bloch simulation.
dcyc	Duty cycle. Usually required for homonuclear decoupling applications. Only values between 0.0 and 1.0 are active. Outside these boundaries, <i>dcyc</i> is reset to 1.0 (default).
sw	Spectral width. If given, the step size of waveform is set equal to the dwell time ($1/sw$). Recommended for H-H homo-decoupling applications. It also helps to make sure that excitation sidebands are kept outside the spectral window. Also required for Bloch simulation.
ref_pw90	Reference 90° pulse width (in μs) at <i>ref_pwr</i> . Required for calibration of waveforms. If set to 0.0, the maximum B1 field intensity (in kHz) is reported instead of the power setting.
ref_pwr	Reference power level (in dB steps). See <i>ref_pw90</i> .
pctype	Pulse type. Only necessary with imaging header. By default, set to selective.

Wave string variables

A reminder is given in `Pbox.inp` files generated by menus and macros because these parameters appear without names. The wave string variables are listed as they appear in the reminder.

<code>sh</code>	Shape name as in <code>wavelib</code> .
<code>bw/pw</code>	Bandwidth and/or pulsewidth. For most waveforms, only one of the two parameters is required. <code>Pbox</code> distinguishes between <code>bw</code> (in Hz), which is always greater than 1.0, and <code>pw</code> (in sec), which is always less than 1.0. Choose which of the two parameters to provide for input, because they are mutually related via the <code>pw*bw</code> product, which is stored with each individual shape in <code>wavelib</code> . Some waveforms (for example, adiabatic sweep pulses) can require both <code>bw</code> and <code>pw</code> . In such cases, both variables can be provided in a single string using the <code>"/"</code> separator. For example, <code>{WURST2 200.0/0.05}</code> denotes a 50-ms long WURST-2 pulse covering 200 -Hz-wide band. Alternatively, units can be used for clarity, for example, <code>{WURST2 0.2k/50m}</code> . If the <code>sfrq</code> parameter is defined, bandwidth can also be specified in ppm, for example, <code>{WURST 20p/5m}</code> .
<code>ofs</code>	Offset from the center of the excitation band in Hz with respect to the carrier frequency (middle of the spectrum). Note that if the <code>sfrq</code> spectrometer frequency, (in MHz) is defined, <code>ofs</code> can also be specified in ppm. In order to specify <code>ofs</code> in terms of absolute frequency, the reference offset <code>refofs</code> (that is, chemical shift value of carrier frequency) must be defined. For instance, <code>{WURST2 20p/5m 170p}</code> <code>sfrq=225.0 refofs=55p</code> .
<code>st</code>	Spin status. Defines whether the waveform is used for excitation (<code>st=0</code>), refocusing (<code>st=0.5</code>) or de-excitation (<code>st=1</code>), which, in turn, defines whether the wave starts with phase defined by <code>ph</code> (<code>st=1</code>), the <code>ph</code> occurs in the middle of the pulse (<code>st=0.5</code>), or the pulse ends with phase <code>ph</code> (status 0). In addition, the waveforms are time reversed if status is 1, as required for proper de-excitation. Undesired time reversal can be undone using the <code>trev</code> parameter. Furthermore, if several waves of different width are generated, they are bound to the beginning (<code>st=1</code>), middle (<code>st=0.5</code>), or end (<code>st=0</code>) of the waveform. The spin status of the first wave is also used by Bloch simulator as the starting magnetization.
<code>ph</code>	Phase in degrees or phase cycle (super cycle). Usually, phase is externally set in the pulse program, and this parameter is not required. Apply any phase cycle (super cycle) from <code>wavelib/supercycles</code> . The difference between this phase cycle and the <code>sucyc</code> parameter is that phase cycling is carried out before waveform mixing and is therefore independent of other Super cycles, whereas <code>sucyc</code> is applied to the final (mixed) waveform. In this way, several waves of different width can be independently phase cycled and use different super cycles.
<code>fla</code>	Flip angle, in degrees. Usually, <code>fla</code> is defined in the wave file and there are very few applications where intermediate flip angles are required.
<code>trev</code>	Time reversal flag (see <code>st</code>). Allowed values are <code>y</code> (yes) and <code>n</code> (no, default).
<code>d1</code>	Prepulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
<code>d2</code>	Postpulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
<code>d0</code>	Pre-d1 delay, in seconds. Essentially repeats <code>d1</code> . It is used only for convenience, for example, if internal duty cycle is defined in shape parameters in <code>wavelib</code> . If set to <code>'a'</code> , the wave is appended to the previous wave.

wrap	Wraparound parameter. Can take values between 0 and 1.0.
------	--

Pbox macro reference

Most selective pulse generation can be satisfied using the **Pbox** window. A set of macros is also available. The following table lists the macros in the order of decreasing importance. For additional information on Pbox macros, see the manual *Command and Parameter Reference*.

pboxvnmrj	Opens the Pbox dialog window.
opx	Opens Pbox, writes the Pbox.inp file header, and resets parameters r1-r7 and n1-n3.
selex	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also sets r1 to excitation bandwidth and r2 to offset. selex uses the pbox_bw and putwave macros.
cpx	Calls the Pbox command, which generates the specified waveform as defined by the Pbox.inp file. cpx also checks if the parameters ref_pwr and ref_pw90 exist in the current experiment and puts their values into the Pbox.inp file. If the parameters do not exist, cpx creates them and asks the user for parameter magnitudes.
setwave	Sets up a single excitation band in the Pbox.inp file. An unlimited number of waves can be combined by reapplying setwave.
putwave	Sets up a single excitation band in the Pbox.inp file. An unlimited number of waves can be combined by reapplying putwave.
pxshape	Generates a single-band waveform based on wave definition provided as a single string of wave parameters.
pboxpar	Adds a parameter definition to the Pbox.inp file.
pboxget	Extracts calibration data from the file shapefile.ext generated by Pbox or, if the file name is not provided, from the pbox.cal file containing parameters of the last created Pbox shape file. Note that the parameter is not changed by this macro if it was set to 'n' (not used)!
pbox_pw	Extracts pulse length from the file shapefile.RF generated by Pbox or, if the file name is not provided, from pbox.cal file containing parameters of the last created Pbox shape file.
pbox_pwr	Extracts the power lever from the file shapefile.ext generated by Pbox or, if the file name is not provided, from the pbox.cal file containing parameters of the last created Pbox shape file. Note that the parameter will not be changed by this macro if previously set to 'n' (not used).
pbox_pwrf	Extracts the fine power lever from the file shapefile.ext generated by Pbox or, if the file name is not provided, from the pbox.cal file containing parameters of the last created Pbox shape file. Note that the parameter will not be changed by this macro if it was previously set to 'n' (not used).
pbox_dmf	Extracts the dmf value from the file shapefile.DEC created by Pbox or, if the file name is not provided, from the pbox.cal file containing parameters of the last created Pbox shape file.
pbox_dres	Extracts the dres value from the file m_shapefile.DEC created by Pbox or, if the file name is not provided, from the pbox.cal file containing parameters of the last created Pbox shape file.

<code>pbox_name</code>	Extracts name of the last shape file generated by Pbox and stored in the <code>pbox.cal</code> file. Note that the file name extension is not stored explicitly and is not provided by this macro.
<code>dshape</code>	Displays real (X) and imaginary (Y) components of a shaped pulse. Any type of waveform (.RF, .DEC or .GRD) can be displayed.
<code>pshape</code>	Generates a single-band waveform based on wave definition provided as a single string of wave parameters.
<code>dshapef</code>	Displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in <code>pbox.fid</code> file.
<code>dshapei</code>	Interactively displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in <code>pbox.fid</code> file
<code>dprofile</code>	Displays the X, Y, and Z excitation (inversion) profile for a pulse shape generated by the Pbox software.
<code>pprofile</code>	Plots the X, Y, and Z excitation (inversion) profile for a pulse shape that has been generated with the Pbox software. If a shape name is not provided, the last simulation data stored in <code>shapelib/Pbox.sim</code> are plotted.
<code>pph</code>	Prints out the shape file header (that is, all lines starting with #).
<code>pbox_bw</code>	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also sets <code>r1</code> to excitation bandwidth and <code>r2</code> to offset. This macro is used mainly in Pbox menus and macros.
<code>pbox_bws</code>	Defines the excitation band from the position of cursors in the graphics window and reports them to the user. It also sets <code>r1</code> to excitation bandwidth and <code>r2</code> to offset. Note that the left cursor should be placed on the left side of the excitation band and the right cursor on resonance of the solvent signal. This macro is mainly used in Pbox menus and macros.
<code>pbox_rst</code>	Resets <code>r1=0</code> , <code>r2=0</code> , <code>r3=0</code> , <code>r4=0</code> , <code>n2='n'</code> , <code>n3=''</code> , and adds some standard comment lines to the <code>Pbox.inp</code> file. This macro is used in menus and other Pbox macros.
<code>pbox_files</code>	This macro is used only in conjunction with Pbox file menus.

Pbox commands using a terminal

The Pbox program is always executed when a shaped pulse is created. Any of the Pbox parameters can be used as an argument followed by the parameter value. The arguments and shortcuts listed in [Table 47](#) are available. Note that the output filename is optional and is always the first argument.

Table 47 Pbox commands and parameters

Command	Parameter	Action
Pbox*	<code>-b time</code>	Activates Bloch simulator, <code>opt=a</code> (add), <code>s</code> (subtract), or time in sec.
	<code>-c</code>	Calibrates only, does not create a shape file
	<code>-f file</code>	Sets name of the output file

Table 47 Pbox commands and parameters (continued)

Command	Parameter	Action
	-h wave	wave prints wave file header
	-I wave	wave prints wave file parameters
	-l ref_pw90	Length (in μ s) of reference pw90 pulse
	-o	Lists options
	-p ref_pwr	Reference power level (dB)
	-r file	Reshapes Pbox pulse
	-s stepsize	Defines the length (in μ s) of a single step in the waveform
	-t wave	Prints shape title from wave file
	-u userdir	Sets user home directory
	-w wavestr	Sets wave definition string
	-v	Runs in verbose mode. Also print Pbox version.
	-x	Prints all Pbox parameters.
	-value	Sets reps to value
Pxsim		Used in Pbox menus and macros for simulation of excitation profiles of shaped pulses.
Pxfid		Used by <code>dshape</code> and <code>dshapei</code> to format shape file into a FID-format text file.
Pxspy		Converts alien shapes (.RF, .DEC and .GRD) into Pbox compatible file format. Essentially converts a time-domain shape file into (frequency-domain) Fourier coefficients, which can be used to create a wave file in the <code>wavelib</code> directory.

Examples:

```
Pbox -i eburp2
Pbox newshape -wc "eburp1 450 -1280.0" -1
Pbox sel.RF -w "eburp1 420 -800" "eburp1 420 1200"
Pbox -w "eburp1 200 -1200" -attn e -pl 45 54.2 -b
Pbox tst.RF -w "esnob 20p 170p" -sfrq 150.02 -refofs 55p -refpwr 45 \
-ref_pw90 54.2
```

Pulse Shape Analysis

The pulsetool program is designed to display and examine shaped RF pulses. The standard pulse template file format is the same as for shaped pulses in /vnmr/shapelib. Data points are listed as phase amplitude time-count, where phase is in degrees, amplitude is a value between 0 and 1023, and time-count is an integer which describes the relative time duration of the step.

Starting and using Pulsetool

To start pulsetool from VnmrJ:

- 1 Click **Edit** on the menu bar.
- 2 Select **View Pulse Shapes...**

The Pulsetool starts; see [Figure 120](#) on page 441.

To start pulsetool from an operating system terminal window:

- 1 Log in a VnmrJ login account owner.
- 2 Open a **terminal window**.
- 3 Start **Pulsetool**, see [Figure 118](#), using one of these procedures:

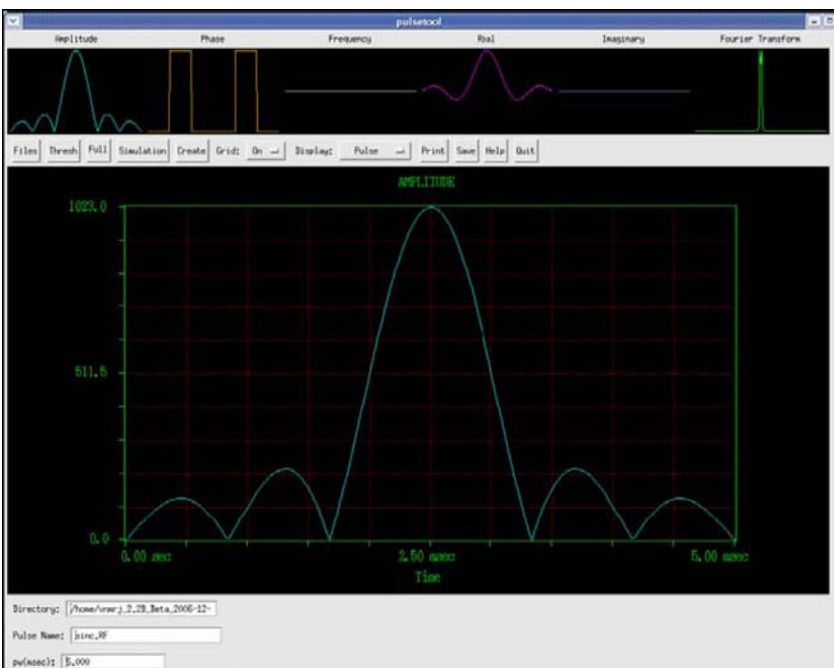


Figure 118 Pulsetool spin simulation window

- Type `pulsetool&` at the prompt to start the Pulsetool and use the file navigation tools within Pulsetool to load files.
- Type `pulsetool<-shape filepath>&` at the prompt to start the Pulsetool, and load the specified shape file (-shape) from the specified location (filepath).

Using pulsetool

Table 48 summarizes the command and parameters associated with pulse shape analysis.

Table 48 Pulse shape analysis commands and parameters

Command	Description
<code>pulsetool <-shape filepath></code>	RF pulse shape analysis
Parameters	Description
<code>phi</code>	Amount of rotation about the Z-axis
<code>theta</code>	Declination relative to XY-plane

The amplitude and phase are displayed in the small windows at the top of the display, along with the effective frequency of the pulse, the quadrature components of the pulse, and its Fourier transform. Click any graphic displayed in the small windows at the top of the screen to display in the large graphics window in the center of the screen.



Figure 119 Attribute selection

The small graphics windows at the top of the tool initially display the different attributes of the current pulse:

- Amplitude
- Phase
- Effective off-resonance frequency
- Real and imaginary quadrature components
- Fourier transform

Display any of these six windows in the large graphics window by clicking in the appropriate small window with either the left or middle mouse buttons:

- The left mouse button causes the large window to be cleared before drawing and sets the clear mode to ON.
- The middle mouse button turns off the clear mode and displays the selected attribute, overlaying any current display in the large graphics window.

Repeated selection of the small Fourier transform window will result in the large window cycling through the magnitude of the Fourier transform, the real component, and the imaginary component.

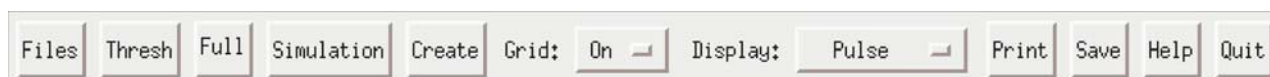


Figure 120 Control Panel Buttons

The control panel below the small windows contains the following control buttons:

Button	Function
Files	<p>Starts a file browser listing the contents of the current directory. A trailing slash "/" following a member of the list indicates a subdirectory, and an asterisk "*" an executable file. Selected from this listing by clicking on it with the left mouse button:</p> <p>File browser buttons and function:</p> <p>Done — closes file browser</p> <p>Load — reads the selected file and displays in the graphics windows. Displays an error message if the file does not correspond to the proper format for pulse template files. Comment lines beginning with the pound character "#" are ignored.</p> <p>Descriptive information about the pulse is displayed in the bottom panel—the name of the file, the number of steps in the pulse, the Fourier size required to do the FFT of the pulse, and a "power factor" calculated for the pulse. The power factor is based on the mean square amplitude of the pulse.</p> <p>Chdir — changes to and then lists the selected directory.</p> <p>Parent — changes to and then lists the parent of the current directory.</p>
Thresh	Activates the horizontal cursor. See " Cursors " on page 442.
Full	
Simulation	Opens the Bloch Simulation window. See " Simulation " on page 443.

Button	Function
Create	Opens a drop-down menu of pulse types appears after a right mouse click the button. Hold down the mouse button and select one of the pulses in the menu. Release the mouse button to load the selected pulse type. See “Creating a pulse” on page 447.
Grid:	ON/OFF menu Turns main window grid on or off.
Display:	Pulse/Simulation menu Displays the shaped pulse or, if the simulation has been run, a Block simulation of the response of the spins to the pulse
Print	Click to print the main graphics window on a PostScript printer.
Save	Saves the data currently displayed in the main graphics window to a file. <ol style="list-style-type: none"> 1 Click Save. A filename entry field and a Done button are displayed. 2 Type a filename. 3 Click Save to write the file. Repeat this as needed while in the save mode — display a different attribute in the main window; Type a new file name, and select Save. 4 Click Done to exit the save mode.
Help	
Quit	Close Pulsetool

Cursors

Interactive left, right, and horizontal cursors are available, and display a readout of position at the bottom of the large window when active. Click, using the left mouse button, anywhere on the large window to activate the left. Activate the right cursor by clicking the right mouse button anywhere to the left of the current single cursor. The right mouse button controls the position of the right cursor independently and the left mouse button moves the cursors in tandem.

The control panel button marked **Full**, when one cursor is active, changes to **Expand**, when both cursors are active. The display can be expanded to show the region between the two cursors.

NOTE

The clear mode will always be set to ON after an Expand or Full operation.

Turn off a cursor by holding down the Control key and clicking the mouse button associated with the cursor. The right cursor is turned off by clicking the right mouse button in the large window, while simultaneously pressing the

Control key.

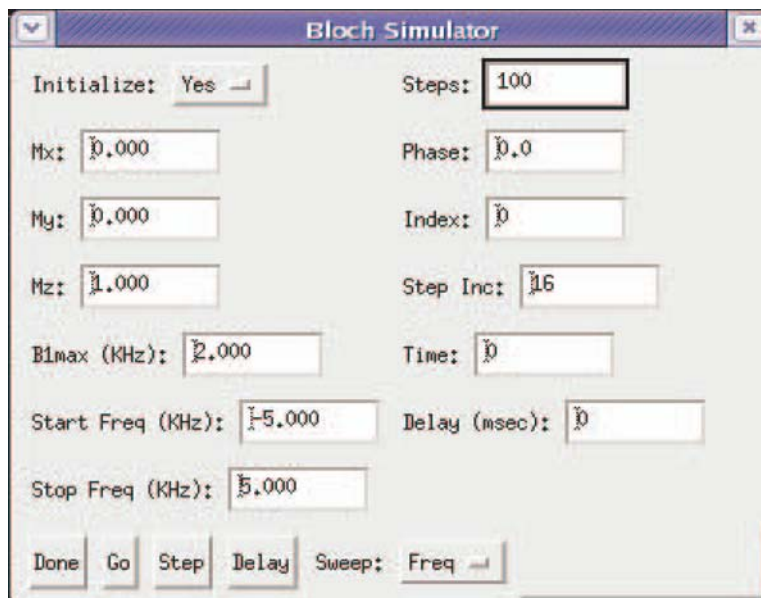
The horizontal cursor is activated with the **Thresh** button located in the control panel. When this cursor is active, it is controlled interactively with the middle mouse button. The interactive scale and reference functions that are normally controlled with the middle mouse are not available when the horizontal cursor is present. Select the **Scale** button in the control panel to turn off the horizontal cursor and reactivate the scale and reference functions (vertical scale and reference can be adjusted even with the horizontal cursor active by direct entry in the appropriate fields in the bottom panel).

Simulation

The simulation routine simulates the effects of an RF pulse by use of the classical model of nuclear spin evolution described by the Bloch equations. T_1 and T_2 relaxation effects are ignored, in which case, the evolution of a magnetization vector in the presence of an applied RF magnetic field can be evaluated by multiplication with a 3 by 3 rotation matrix. The simulation consists of repeated multiplication by such a matrix, whose elements are determined at each step by the values of amplitude and phase found in the pulse template file, and by user input values of initial magnetization, B_1 field strength, pulse length, and resonance offset. The simulation is performed over one of three possible independent variables— resonance offset, B_1 field strength, or time, and is determined by the Sweep cycle in the small button panel.

Parameters

- 1 Click the **Simulation** button in the control panel to activate the Bloch Simulation window.



- 2 The Block Simulation window contains fields for entry of all required parameters (the pulse length is taken from the value in the bottom panel of the main window).
- 3 To change, type a value for any parameter by selecting it with the left mouse button and entering a value from the keyboard. Enter values for the parameters **Mx**, **My**, and **Mz** for the starting values of the magnetization components. Their vector sum must be less than or equal to 1. Click the **Initialize** button and select **YES** or **NO**. This feature works for Frequency and B_1 sweep only, not Time.

Initialize cycle determines if the magnetization is re-initialized to the values of **Mx**, **My**, and **Mz**, or if the simulation uses the values at each point that were the result of the previous simulation. The effect of a series of pulses can be evaluated by loading the first pulse and performing the simulation with Initialize set to **YES**, loading subsequent pulse, setting Initialize to **NO**, and selecting **Go** after each pulse is loaded.

- 4 Click the **Sweep** button and select **Freq**, **B₁**, or **Time** for the sweep.

Freq

- a Type a value in the **B1max** for B₁ at the maximum pulse amplitude.
- b Type a value in the **Start Freq (KHz)** field for the starting frequency.
- c Type a value in the **Stop Freq (KHz)** field for the ending frequency.

B1

- a Type a value in the **Frequency (KHz)** field for the how far the magnetization is off resonance.
- b Type a value in the **B1 Start (KHz)** field for the starting B₁ field.
- c Type a value in the **B1 Stop (KHz)** field for the ending B₁ field.

Time

The results are displayed in the form of a projected three-dimensional coordinate system, showing the path of the magnetization over the course of the pulse.

- a Type a value in the **B1max** for B₁ at the maximum pulse amplitude.
 - b Type a value in the **Frequency (KHz)** field to see how far the magnetization is off resonance.
- 5 Accept the default values or enter values for each of the following:
 - a Steps (not available if sweep is set to **Time**) – The number of steps used in the simulation
 - b Phase – Type the phase of the pulse
 - c Index – Counter that displays the current index. No entry is required for this parameter. Setting a value of 0 and clicking on the **Steps** button updates the parameters
 - d Step Inc.– Type the number of intermediate steps to be calculated each time the **Step** button is clicked
 - e Time – Type a non-zero integer value in this field to see the 3D display drawn in real-time
 - f Delay – Pre-pulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.

Performing a simulation

- 1 Click the **Go** button.

This action enables the simulation calculations to be done and then displays the results in the first five small graphics windows, replacing (but not destroying) the pulse information being displayed. The Fourier transform information remains unaffected, so that comparisons can be made between the information displayed and the exact simulation results.

All of the display functions described elsewhere is active as well, with the simulation data. Additionally, the original pulse data is still present in the background and can be swapped into view with the Display cycle found in the main control panel.

- 2 Click the **Step** button to view the course of the magnetization at intermediate stages through the pulse based on the value in the Steps Inc. field, starting at the current value of Index.

The intermediate result is then displayed in the normal fashion.

During a **Go** simulation, a small panel containing a **Cancel** button will pop into view. Use this to stop the simulation if necessary (there may be some delay between selecting the button and the end of the process).

Do not click **Cancel** more than once.

Clicking the **Go** button with **Sweep: Set to Time** displays the results in the form of a projected three-dimensional coordinate system, showing the path of the magnetization over the course of the pulse. This display is obtained by selecting the **3D** button after first selecting the **Go** button. The left mouse button controls the viewing angle from within the canvas region delineated by the blue corner markers when the 3D display is active. This viewing angle is described by the two parameters ϕ (the amount of rotation about the Z-axis) and θ (the declination relative to the XY-plane). A “family” of trajectories can be displayed by first selecting any of the small canvases with the middle mouse button, then selecting the **3D** button. Changing either the B_1 field strength or the resonance offset followed by the **Go** button will result in display of the result without clearing the display. Select any of the small canvases with the left mouse button to reactivate the automatic clearing feature.

Creating a pulse

The pulse creation routine currently offers the following pulse types:

Square	Hermite 90	Tan swept inversion
Sinc	Hermite 180	Sin/cos 90
Gaussian	Hyperbolic secant inversion	

A file containing the pulse template for any of these pulses can be created from scratch with this utility. Alternatively, pulses can be created for examination only, using the display capabilities of pulsetool. Each pulse is generated with user-definable parameters appropriate for the pulse in question.

- 1 Click the **Create** button and hold the right mouse button down.
- 2 Select a pulse type from the menu and release the mouse button.

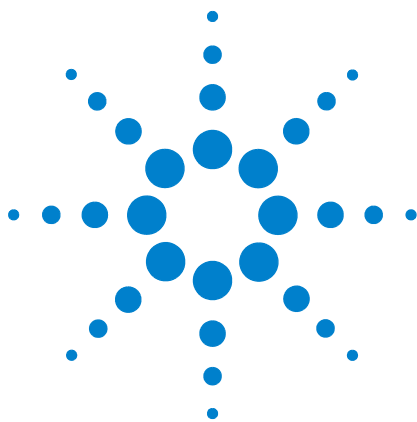
Move the mouse arrow out of the menu area and release the button if none of the choices are acceptable.

A small window appears with a brief description of the characteristics of the pulse and a set of changeable attributes values when a pulse type is selected. The number of steps in the pulse is limited to powers of 2 and can be set by clicking on the left mouse button or by holding the right mouse button down and selecting the desired value from the resulting menu. All other attributes, which vary depending on the pulse type, can be altered from their default values by first selecting the appropriate field with the left mouse button, deleting with the Delete key, and typing in the desired value (clicking on the **Return** key is not required).

- 3 Select one of the following buttons at the bottom of the window:
 - **Preview** – use the attributes as they appear on the screen to create a pulse that is loaded internally into pulsetool. All pulsetool features can then be used to examine and evaluate the new pulse. Any previous pulse information is deleted.

- **Execute** – use the attributes as they appear on the screen to create a pulse and write it to a text file. The name of the file is taken from the file name field in the Create window and written into the current directory, listed in the Directory field in the bottom panel. If a file of the same name already exists, a prompt requests confirmation to overwrite the file. An error message appears if the program is unable to write to the named file. This is generally symptomatic of not having write permission in the current directory.

No new pulse types can be added to the list. User-created shaped pulse may be examined using the **Files** button.



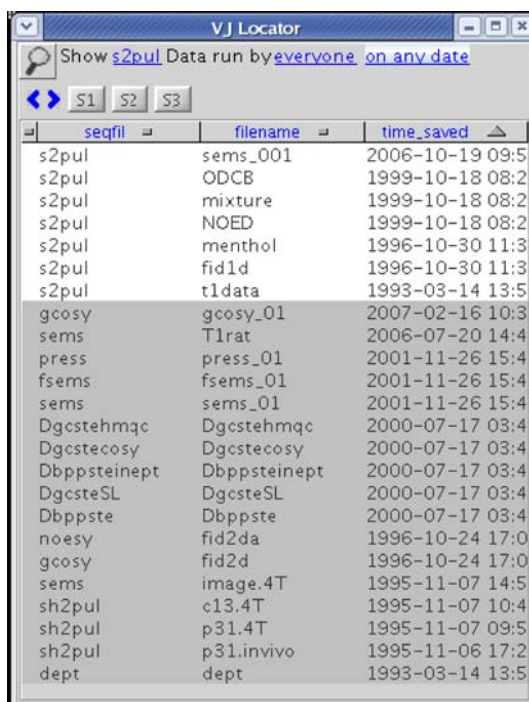
16 Locator and File Browser

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Using the File Browser [459](#)

This chapter provides information on the Locator and File Browser.

VJ Locator

The VJ Locator, [Figure 121](#), is a database browser that provides access to data sets, experiments, shim sets, commands, etc.



The screenshot shows the VJ Locator application window. At the top, there is a search bar with the text "Show s2pul Data run by everyone on any date". Below the search bar are three buttons labeled S1, S2, and S3. The main area of the window displays a table with three columns: seqfil, filename, and time_saved. The table contains 25 rows of data, with the first row highlighted in grey.

seqfil	filename	time_saved
s2pul	sems_001	2006-10-19 09:5
s2pul	ODCB	1999-10-18 08:2
s2pul	mixture	1999-10-18 08:2
s2pul	NOED	1999-10-18 08:2
s2pul	menthol	1996-10-30 11:3
s2pul	fid1d	1996-10-30 11:3
s2pul	t1data	1993-03-14 13:5
gcosy	gcosy_01	2007-02-16 10:3
sems	T1rat	2006-07-20 14:4
press	press_01	2001-11-26 15:4
fsems	fsems_01	2001-11-26 15:4
sems	sems_01	2001-11-26 15:4
Dgcstehmqc	Dgcstehmqc	2000-07-17 03:4
Dgcstecocy	Dgcstecocy	2000-07-17 03:4
Dbppsteinept	Dbppsteinept	2000-07-17 03:4
DgcsteSL	DgcsteSL	2000-07-17 03:4
Dbppste	Dbppste	2000-07-17 03:4
noesy	fid2da	1996-10-24 17:0
gcosy	fid2d	1996-10-24 17:0
sems	image.4T	1995-11-07 14:5
sh2pul	c13.4T	1995-11-07 10:4
sh2pul	p31.4T	1995-11-07 09:5
sh2pul	p31.invivo	1995-11-06 17:2
dept	dept	1993-03-14 13:5

Figure 121 VJ Locator

The Locator provides quick access to information on all or part of the disk environment. The administrator determines the scope of the Locator's actions.

The Locator works similarly to a directory or file manager by using minimal information filtering and lists of information. Three lists are shown in the Locator when terms have a boolean relationship:

- Objects that meet all criteria
- Some of the boolean terms met
- Remaining objects

The construction of the underlying Locator statement determines which list is displayed.

Within each list, the Locator displays three attributes for each object. The displayed attributes may not be in the Locator statement. Any one of the attributes can be

designated as the sort attribute. The objects in each list are sorted by the value each has for this attribute.

Locator menu and controls

The magnifying glass and the current Locator statement are at the top of the VJ Locator window, as shown in Figure 122.

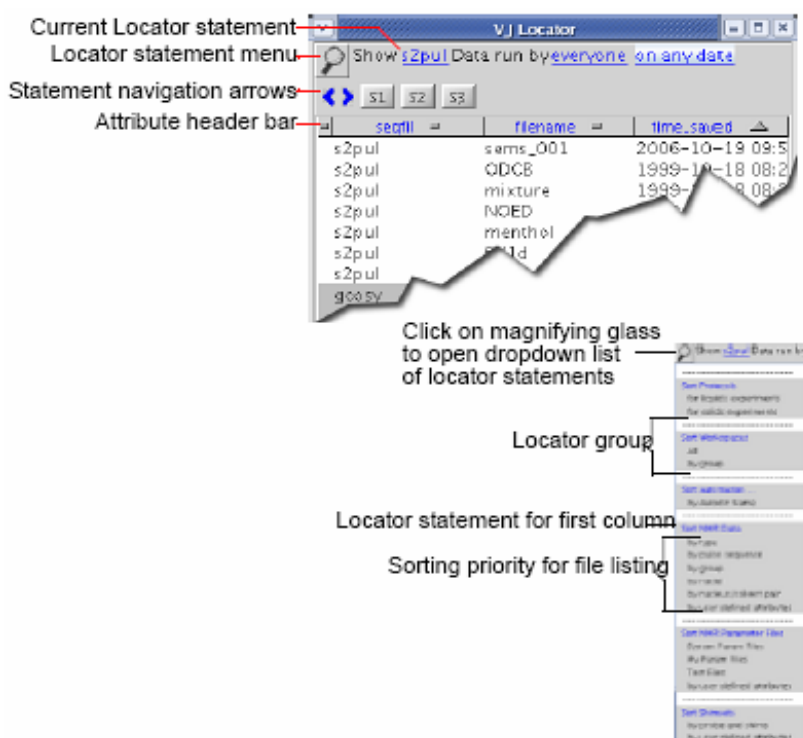


Figure 122 Locator menus and controls

Click the magnifying glass to open a menu of currently available Locator statements. This menu includes both statements provided by Agilent Technologies, Inc. and those customized and saved by the user.

Statements are Locator sentences in which a number of words or phrases are colored and underlined in a manner reminiscent of links in a web page. Each link hides a menu of choices, of which the currently displayed phrase is one. The choices available vary with the types of data currently known to the Locator.

Navigation in the locator

A pair of arrows (statement navigation arrows) below the Locator statement enables searching forward and back through past Locator operations, applying each to the current Locator environment. Thus, a set of Locator statements can be rapidly applied in a changing environment.

Attributes

The **Attribute Header** bar is below the icons. This bar enables selection of the attributes displayed and arrangement of the objects in each list in a number of ways.

seqfil	filename	time_saved
s2pul	sems_001	2006-10-19 09:5

The boundaries between the attribute labels are adjustable. Place the cursor on the boundary to adjust. When the adjust cursor appears, click and drag the boundary to its new position, and release.

Objects in the Locator are available for a number of actions. Currently, a single click selects an object. The selected object can then be dragged to another part of VnmrJ, in which case the action taken will depend on the type of object and where the object is dropped. Alternatively, double-clicking an object will cause the most likely action to occur. These actions are discussed below.

The value of an attribute might be longer than the width of the column in the Locator. When the mouse cursor rests on an attribute value, a tool tip appears for a period of time. The tool tip contains the full value of the attribute.

Attribute lists

The list of attributes in the drop-down lists is controlled by configuration files. There are three file names, for three different types of items in the locator:

- shuffler_param_list for 'vnmr data' and 'vnmr parameter' files
- study_param_list for 'study' items
- data_protocol_param_list for 'protocol' items

Each of the above mentioned files can exist for each of the

appmode types and for individual users. That is, appmode types of 'imaging', 'standard' (experimental liquids and solids), and 'walkup'. The attributes visible in the drop-down menu for each appmode type will be controlled by files in the appropriate directories. If a user does not have an individual file, the file in the appropriate appmode directories will be used. If there is no file in the imaging or walkup directories listed above, then the file in /vnmr/shuffler will be used. If users have their own individual files, the attributes listed must also be in the appmode directory file. That is, a user's files can limit the attributes shown, but cannot add to the list of attributes shown beyond the attributes in the system files.

Wildcards

Wildcards can be used in attribute values, but not for the attribute name itself. For example, 'file*' to specify the autoboot 'filename' is not allowed. Selecting an attribute of 'filename', then editing the selection value to be 'p31*' to show all files whose names start with 'p31'. '?31*' shows all files starting with any single character followed by '31', followed by 0 or more characters. The leading '?' would allow upper or lower case 'P' as well as any other character. This does not apply to dates.

The following wildcards can be used:

- '*' or '%' can be used to match any number of characters
- '?' or '_' can be used to match any single character

Configuration files

Configuration files for the locator are contained in the following directories for the different appmode types:

Interface	Directory
Standard (experimental)	/vnmr/shuffler
Imaging	/vnmr/imaging/shuffler
Walkup	/vnmr/walkup/shuffler
Individual users	\$vnmruser/shuffler

Searches

Clicking on the magnifying glass icon with the left mouse button brings up a menu of searches. Selecting one changes the search sentence displayed at the top of the Locator. The

results of the search are displayed in the list. Those items in the white part of the list satisfy the search sentence. Those in the gray part do not. For each item that is found by the search, three attributes are displayed. These correspond to the three columns in the list. Clicking on the attribute name at the top of the list with the left mouse button brings up a menu of attribute choices.

Dragging and dropping items from the locator

To select an item, click the item in the **Locator** list. The selected item can be dragged to the graphic area or the Parameter panel area for an appropriate action. For example, dragging a data set to the graphic area retrieves that data set into the current experiment workspace and can display the spectrum. Dragging a workspace to the graphic area selects that experiment workspace. Dragging an object causes the most likely action to occur.

An item can be dragged from the Locator and dropped into the holding pen. The item is then available for further selection, no matter which Locator statements are active. For example, use the Locator to inspect the available shim sets. Select the current best set and put this into the holding pen. This set of shims becomes available.

Dragging and dropping an item has an action appropriate to the context. Often the same effect can be obtained by double-clicking on an object. Some examples are:

- Drag a protocol experiment into the graphics canvas to load the experiment.
- Drag a FID from NMR data to retrieve the FID. The `process` macro can also be invoked so that the FID is transformed.
- Double-click a workspace to join that workspace. Dragging and dropping a workspace into the graphics area also joins the workspace (`jexp`).
- Double-click a parameter set to load that set in the current workspace, or drag and drop a parameter set.
- Double-click a shim set to load the shims. Dragging and dropping a shim set to the current shim buttons also loads the shims into acquisition.

- Drag either data or shims and drop them in the trash can (in the lower left portion of the hardware bar) to move the item to the trash can. Retrieve an object from the trash can by double-clicking on the trash can, selecting it, and then clicking on the **Restore items** button.

Editing file names from the locator

A new file added to the locator from within VnmrJ appears in its appropriate spot in the Locator, and in green at the top of the locator window. If one of the columns in the Locator is *filename*, click the green file name to change it.

Change the file name, click **Return** or on another line to remove the old name from the **Locator** and add the new one. The **Locator** is redisplayed to show the new name.

Locator Statements

Agilent supplies a number of Locator statements with VnmrJ. Add to or edit these statements in the following ways:

- Save the current Locator statement by clicking on **Tools** in the main menu, then **Save Custom Locator Statement**. Type a name for the statement in the Custom Locator Statement pop-up window.
- Click **Tools**, then **Delete Custom Locator Statement** to delete a Locator statement. A Custom Locator Statement Removal window appears. Select the statement from the list in the window, then click **Delete** to remove it or **Cancel** to exit the window without removing the statement.
- Sort **Protocols Entries** shows the known protocol experiments. Double-click the protocol to execute the associated macro.

Locator statements are defined in a file named `locator_statements_default.xml`.

This file can reside in the system appmode directories (see Configuration Files Configuration Files), but not in the user's individual directories.

Editable fields

Click the right mouse button on the blue and underlined items in the locator statements to show a menu of choices, and edit by clicking on them with the left mouse button. Left-clicking puts an editing cursor on the item. Place the cursor at the point to be edited and click the left mouse button. Edit the field, and then click **Return** to cause a new locator search using this edited value.

Sort workspaces

Sort all workspace in numeric order. Double-click a workspace to join the workspace.

Sort NMR Data

Entries show the known NMR data sets, but differ in the actual format of the statement as well as the initial set of attributes shown. The most comprehensive statement is the last one, **by user defined attributes and date**.

The generic statement is shown in [Figure 123](#).

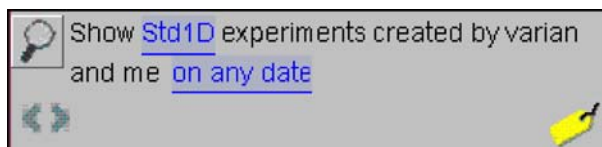


Figure 123 Generic locator statement

There are two separate underlined choices in this statement: Std1D, on any date.

Click either of the underlined phrases to produce a drop-down menu of the choices in the selected position. The menus are environment sensitive and do not display choices that do not exist.

The logic of this statement is:

Show attributes A and B of type C with additional limitations.

First, the additional limitations phrases enable selection of the owner of the data. This selection is determined by the administrator at the time a directory is made available to the Locator.

Second, the additional limitations enable reordering by date. There are various dates associated with data, for example, the time started or the time saved. Specify these date fields in any of several ways, for example, since a certain date, by changing on any date to since.

Alter the date by using the left or right arrows to decrease or increase the date by one day with each click.

All other statements supplied are simpler than the generic one. Promote statements used frequently to the top of the menu by saving them again as your local variants.

Sort NMR parameter files

The statements in this category show the list of NMR parameter sets. One major category of parameter set is **My Param Files**. Select the statements **Test Files** and by **user defined attributes** to do other selective searches. The Locator statement changes after selecting a category.

Sort shimsets

The statements in this category enable access to the saved shim sets. Note that the shim sets can be saved with a descriptive shim name provided when using the **Save Shims** button in the Shim panel.

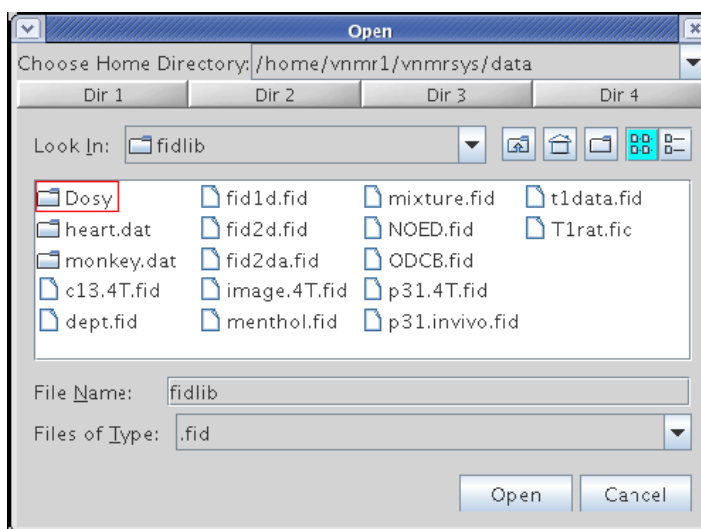
Sort command macros

The generic statement in this category enables finding a VnmrJ command or macro based on its attributes. The Locator enables reordering commands and macros by a number of attributes. Find the command to use and double-click to execute it.

Using the File Browser

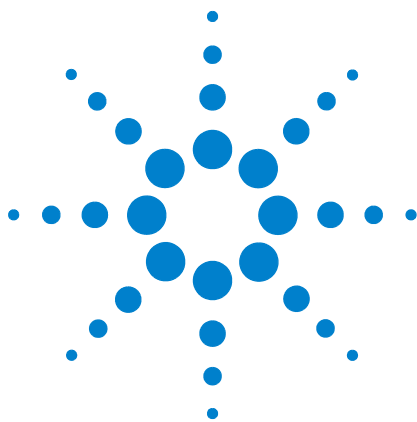
To open a File

- 1 Click **File**.
- 2 Select **Open** to open the file navigation window to locate the required file.
- 3 Click the file and click the **Open** button.



To save a File

- 1 Click **File**.
- 2 Select **Save as** to open the file navigation window to locate the required directory.
- 3 Type a file name in the Save as field and click **OK**.



17 Quantification Tools

- Quanttools Application Directory [462](#)
- The qEstimate Tool [463](#)
- Adaptive NMR [468](#)
- Automated Creation of Quantification Reports [474](#)

VnmrJ provides two tools to facilitate NMR quantitative measurements: Quanttools located in an application directory, and the qEstimate tool.

The package of programs in Quanttools provides a mechanism to assay any sample for a specific chemical substance. This method uses a user-generated template that must first be interactively created from an NMR spectrum, acquired on a standard sample. This method supports automated analysis and report generation once the user-generated template is in place. The use of internal standards is required to perform these analyses.

Use the **Determine Concentration** button, on the Integration panel in the Process tab, to provide an estimate of absolute concentration for any sample. This capability is based on the linearity of the digital receiver and does not require the use of reference signals or the addition of reference standard compounds to the NMR sample. A simple calibration must be performed once for each probe before this tool can be used, see [“Calibrating probe parameters for quantification”](#) on page 463.



Quanttools Application Directory

To access the tools:

- 1 Enable the QuantTools application directory.
- 2 Select **Applications...** from the **Edit** menu.
- 3 Select **Enabled** from the pull-down menu next to */vnmr/quanttools*.
- 4 Exit and restart VnmrJ to load the new menus and panel items, after the application directory is enabled.

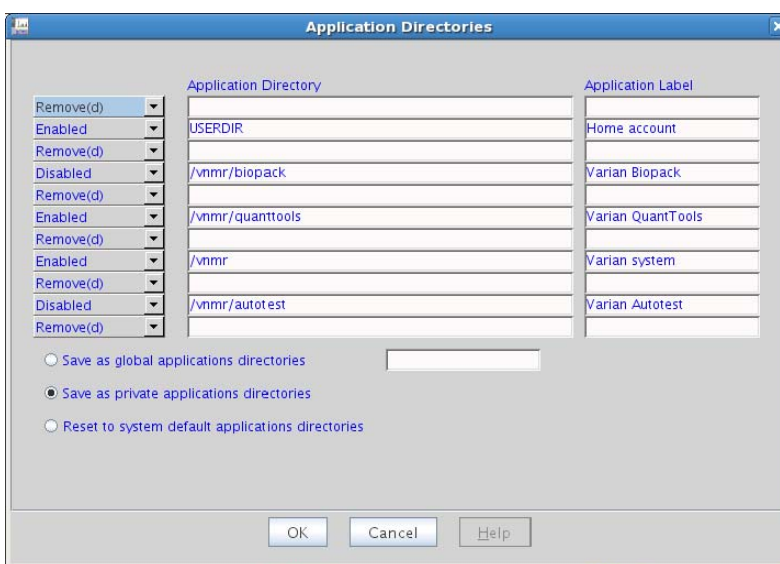


Figure 124 QuantTools Application Directory

The qEstimate Tool

Calibrating probe parameters for quantification

Perform a calibration step before using the qEstimate tool. The calibration is specific to each probe and receiver combination.

- 1 Carefully prepare a sample of known concentration. Any sample in the 40 to 100 mM range should be suitable, assuming that a.) it contains a resonance, or group of resonances, that are well separated from all other signals, b.) that resonance is reasonably sharp, and c.) the number of protons represented by that resonance is known. The ^{31}P signal to noise standard sample provided with the system can be used with reasonable results.
- 2 Insert the calibration sample and ensure that the probe is properly tuned and shimmed. Set the number of transients to 1 (that is, $nt=1$) and turn off the steady state transients (that is, $ss=0$). Set the receiver gain manually to a value that provides good signal-to-noise without giving a receiver overflow error (letting the system set the gain value automatically could introduce relaxation errors). Acquire a spectrum.
- 3 After processing the spectrum, navigate to the Integration panel under the Process tab. Establish an integral region around the resonance to be used for calibration using the Set Integral Regions tools. Make sure to include some baseline on each side of the peak in the integral region. Place the cursor within the selected integral region.
- 4 Multiply the concentration of the calibration standard by the number of protons represented under the resonance chosen in step 3, then enter that result into the Integral Area field. For example, if the standard sample was created at a concentration of 94.36 mM and the resonance used for calibration was a methyl group (that is, 3 protons), the number entered into the Integral Area field would be 283.11 (that is, $94.36 \times 3 = 283.11$). Select the **Set Integral Region** button.

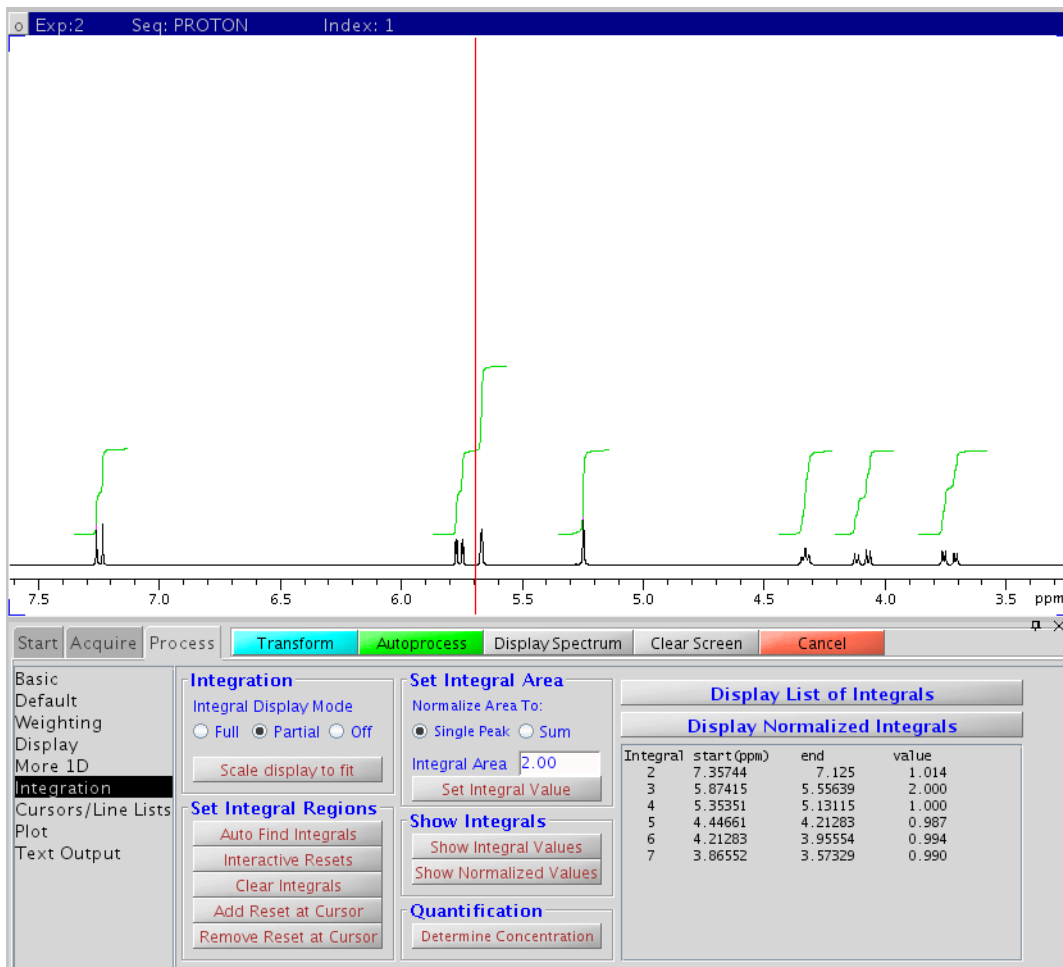


Figure 125 Setting the integral value

Setting the integral value for a calibration standard of caffeine dissolved at a concentration of 94.36 mM. The integral region for the methyl signal at approximately 3.28 ppm has been selected and a value of 283.11 has been entered into the Integral Area field.

If the ^{31}P S/N sample is used to calibrate the system, integrate all the aromatic resonances as a group and enter the value of 727.5 into the Integral Area box. The sample is approximately 48.5 mM and the aromatic resonances represent 15 protons from 3 phenyl rings. The small amount of residual CHCl_3 also in the region does not significantly effect the calibration.

- 5 Type `qEstimate('setproBefile')` on the command line and press **Return**. This will create the parameters

required for the qEstimate function and store appropriate values for those parameters in the probe file. The parameters that are created by qEstimate('setproBefile') are H1Qgain, H1Qins, H1Qinsref, H1Qconc, and H1Qmult. H1Qmult refers to the tip angle scaling factor where a value of 1 equals a 90 degree tip angle.

Once these values are on the probefile, sample concentration is accessible by a mouse click. The results are still dependent on the physics and relaxation properties of the sample. Typically, reasonable results are obtained from normal survey ^1H spectra. More precise results can be obtained by using a single scan fid acquired with a 90 degree tip angle and fixed receiver gain.

Using Determine Concentration

When **Determine Concentration** is invoked the gain and tip angle for the current sample are noted and compared with the values used during calibration. The `ins` (concentration for the standard) parameter is loaded into the current experiment and the `insref` parameter is adjusted from the value shown on the probe file for the scaling factors defined by receiver gain and tip angle. determine the absolute concentration of an NMR sample:

- 1 Acquire a proton spectrum.
- 2 Integrate a resonance (or group of resonances) representing a known number of protons.
- 3 Click the **Determine Concentration** button in the **Integration** panel to display the **Concentration** window. This tool calculates the scale of integrals by the numbers of protons to display the sample's concentration.

For example, the methyl resonance of an isopropyl group represents 6 protons. If integration of the isopropyl methyl signals from an unknown sample yielded a value of 339.6 after clicking **Determine Concentration**, then the concentration of the sample is 56.6 mM ($339.6 \div 6 = 56.6$).

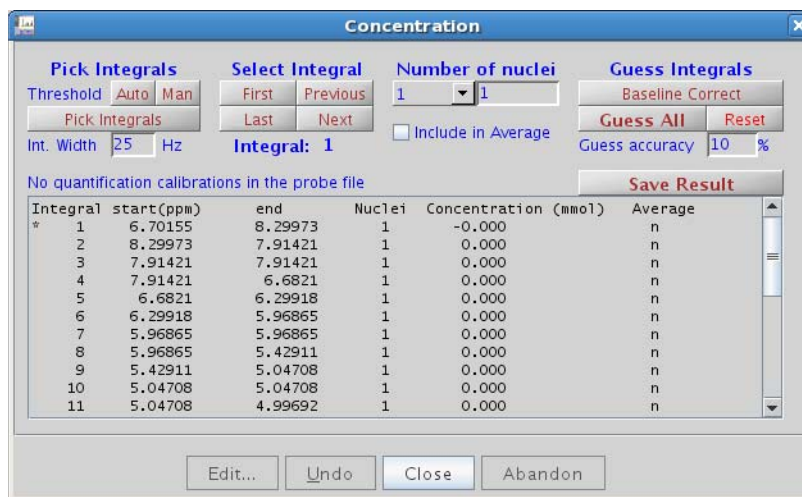


Figure 126 Concentration window

Table 49 Concentration window options

Item	Description
Pick Integrals	
Threshold Auto	Automatically sets threshold for picking peaks.
Threshold Man	Allows user to manually set threshold for picking peaks.
Pick Integrals	
Integral Width	Determines the length of the integral tails on each side of a peak.
Select Integral	
First	Click to jump to the first integral
Last	Click to jump to the last integral
Previous	Click to select the previous integral
Next	Click to select the next integral
Number of nuclei	
drop-down list and display	Enter or select the number of protons to associate with the selected integral
Include in Average	Select to include the selected integral in the average check.
Guess Integrals	
Baseline Correct	Use to perform baseline correction.

Table 49 Concentration window options (continued)

Item	Description
Guess All	Use to include all integrals into the average.
Reset	Use to reset the list of integrals if anything has changed.
Guess accuracy	Enter a tolerance which is applied to the Guess algorithm to determine if an integral should be included into the average. For example, if a signal is more than 10% different from the mean value it is disregarded.
Save Result	Select to write the sample concentration into the sample parameter file.
*	In the Integral column of the data display, this symbol displays to the left of the selected Integral.

Adaptive NMR

Adaptive NMR is a feature that was introduced with VnmrJ.1 and uses the qEstimate tool to facilitate the automatic setting of the number of scans for experiments as a function of the absolute concentration of the sample. In order to use this option three things must be done. First, the qEstimate calibration must be done. This can be done from the command line as described in “[Calibrating probe parameters for quantification](#)” on page 463. Second, the probe file needs to contain the nominal signal to noise results for ^1H and ^{13}C respectively. There is a graphical tool to assist with both steps one and two. The third requirement is to simply enable Adaptive NMR in the **Preferences/userPrefs** pane.

To access the graphical tool to help manage the introduction of qEstimate calibrations and nominal signal to noise values to the probe file choose **Setup qNMR Calibrations** from the Tools menu.

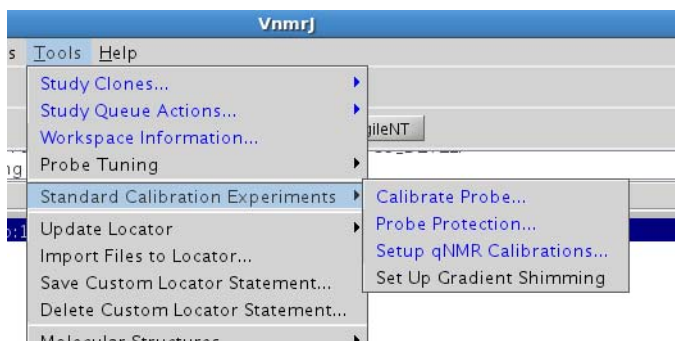


Figure 127 Standard Calibration Experiments submenu

This selection will open a small popup with it's own detailed help file as shown below.

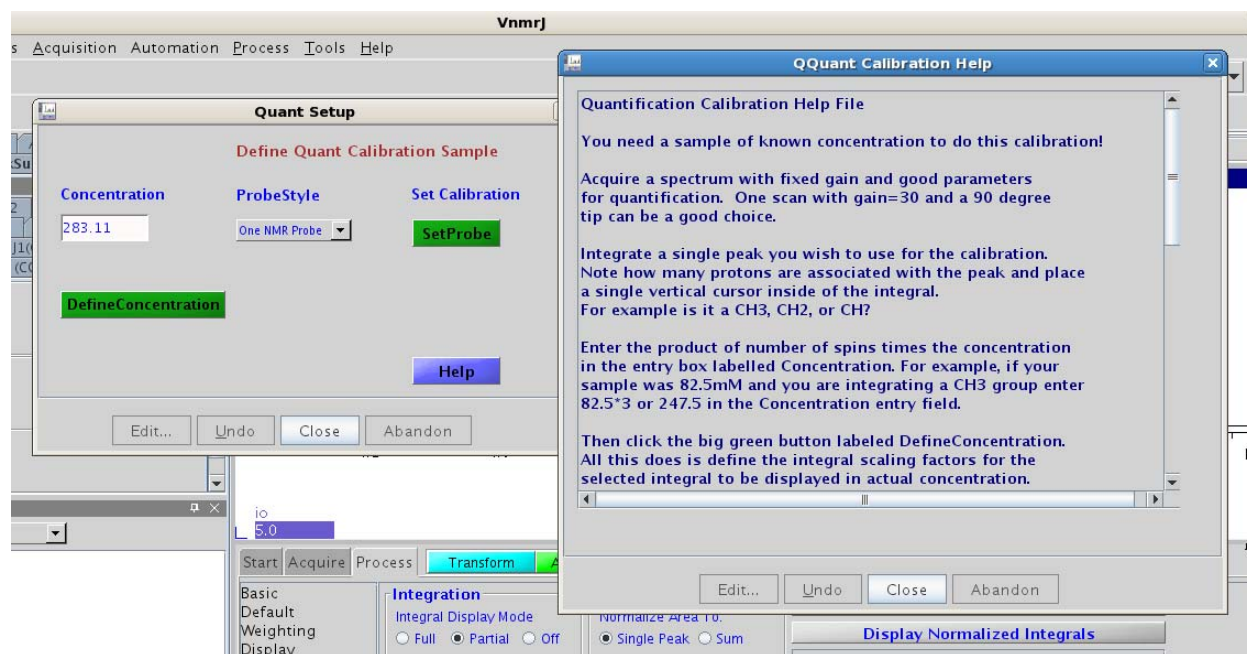


Figure 128 Setup qNMR Calibrations

This popup has two functions: the first is to scale integrations for use with qEstimate and the second is to commit the calibrations to the probe file. Note the menu labeled Probe Style. This menu has three entries to allow selection of a OnNMR, ID, or DB style probe. Once integration parameters are set in parameters with the **Define Concentration** button and a style of probe is selected the **SetProbe** button commits the values to the probe file. The following figure shows the tool in actual use. Note that a peak is integrated and that entry of 282.11 into the Concentration entry is identical to the Integral Area entry on the Integration panel. It is also important to know that clicking the **DefineConcentration** button in this Quant Setup popup is identical with selecting the **Set Integral Value** button on the Integration panel.

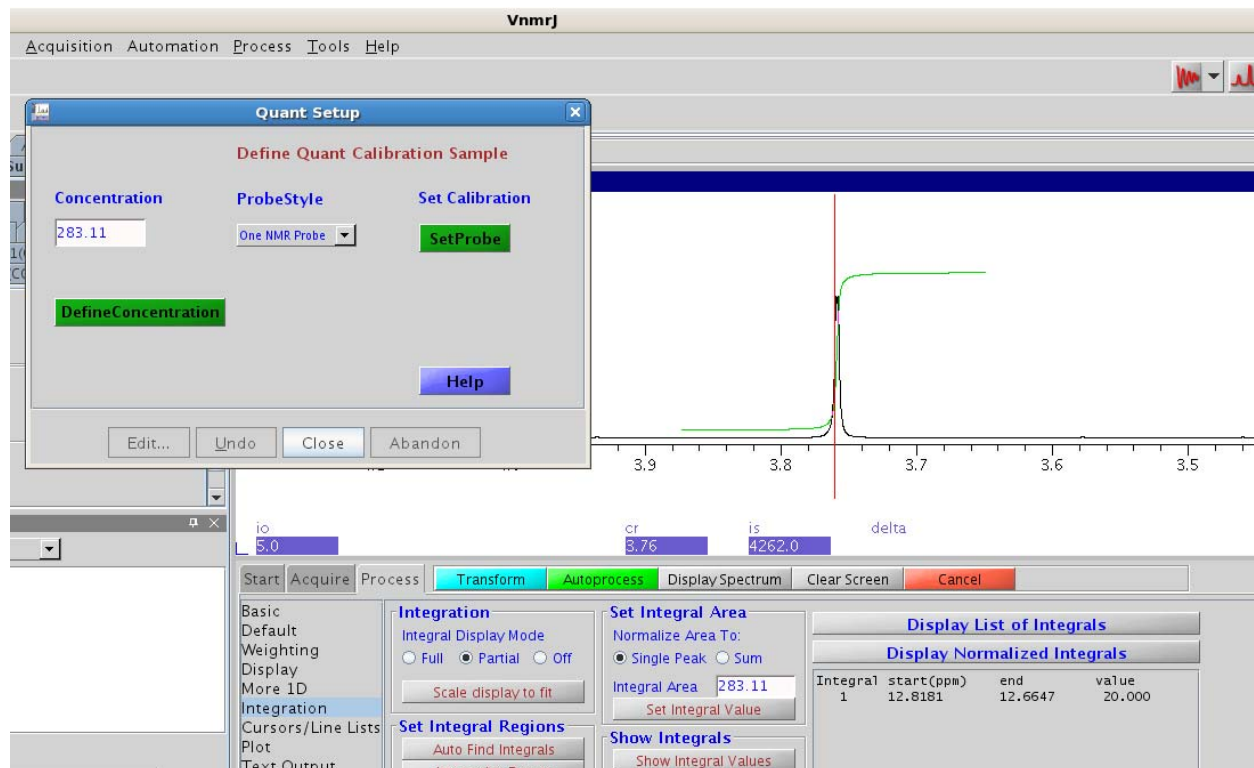


Figure 129 Quant Setup

Once the style of probe is chosen from the **ProbeStyle** menu clicking **SetProbe** is identical to typing `qEstimate ('setproBefile')` in “[Calibrating probe parameters for quantification](#)” on page 463 but with the additional step of writing a nominal `Signal2Noise` parameter to the probe file in both the H1 and C13 tabs. If your NMR console has a Proton frequency greater than 600 MHz or your style of probe is not a OneNMR, ID, or DB probe (for example it is a Cold Probe), just select any style of probe and then edit the probe file and enter the `Signal2Noise` specification in both the H1 and C13 tabs. Once the `qEstimate` scaling factors and probe `Signal2Noise` are on the probe file the system is ready for Adaptive NMR.

To enable self adaptive NMR simply enable in Preferences.

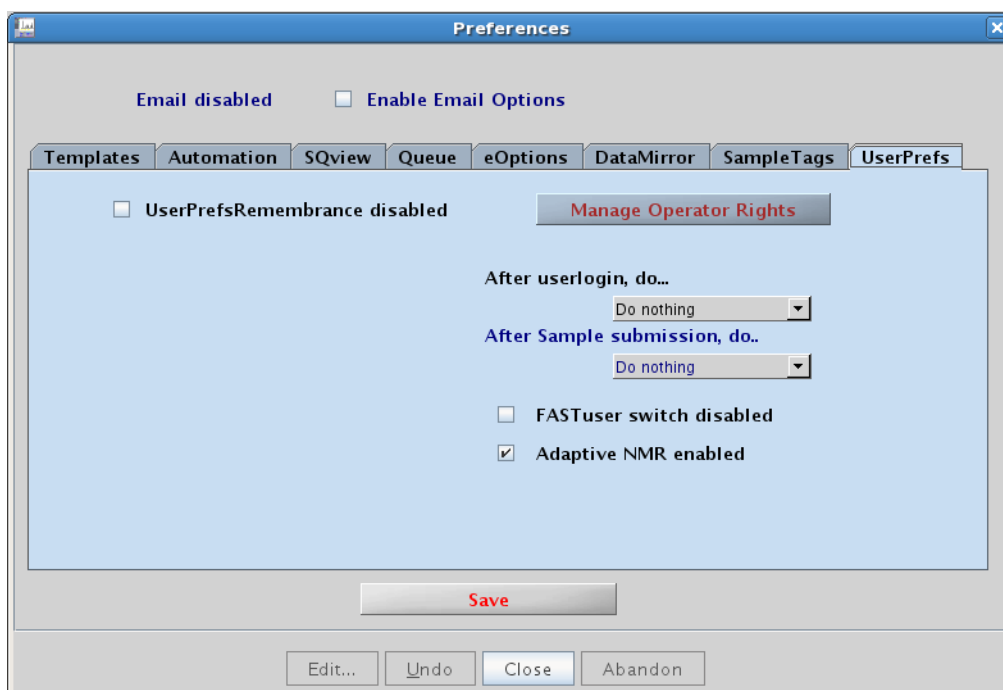
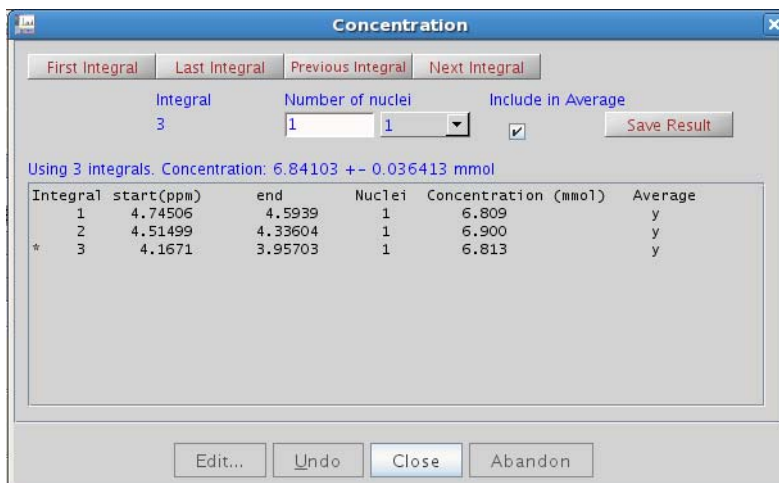


Figure 130 UserPrefs in Preferences pop-up

Once self adaptive NMR is enabled automation queues will automatically set an appropriate number of scans for most any experiment provided the sample's concentration is known or has been determined with the qEstimate tool. To facilitate this step several parameters important for qNMR have been added to the standard parameter modules. For the application of self adaptive NMR the parameter concentration now replaces the parameter lot in the standard sample entry pages. To streamline the automatic report writing mechanisms presented in the next section of this manual the parameters `intstd`, `iswt`, `sampwt`, and `volume` have also been added to the parameter tree. Those parameters are not important for self adaptive NMR; the parameter concentration is used along with the `Signal2Noise` parameters introduced to the probe file along with the qEstimate absolute concentration scaling factors. Because concentration must be known to use self adaptive NMR automation studies would typically be extended beyond a simple PROTON 1D used to survey the sample and determine the sample's concentration. The Continue Study mode of submission to the automation queue affords a simple and direct means to extend the NMR studies on any sample. Note the addition of the **Save Result** button on the

qEstimate popup tool.



Clicking **Save Result** will define the value of concentration in the saved data set so that concentration is known later when a study is continued.

There is a single administrative text file that is associated with self adaptive NMR and it is called `scancalFile` and is located in `/vnmr/adm/walkupadm`. Following are excerpts from different portions of `scancalFile`.

```
#pslabel:      tnSN          SNCa1          MinScans
HSQCAD:        430           8              2
zTOCSY:        430           6              2
CARBON:        180           70             128
HETCOR:        180           60             2
```

The interpretation of this file is as follows: For the HSQCAD experiment with a probe of a signal to noise of 430/1, 2 scans would be needed for a 8mM sample. For a 1D CARBON experiment 128 scans would be chosen for a 70 mM sample. CARBON values are selected in `scancalFile` to yield a signal to noise of about 25/1 for typical peaks. This is actually a fairly high quality carbon spectrum. If the concentration of the sample is cut by $\frac{1}{2}$, 4 times the scans are needed. Conversely, if the signal to noise of the console's probe is twice the value specified in `scancalFile` the number of scans would be decreased by a factor of 4. Note that for entries such as CARBON the choice for MinScans might need to be reduced if desired. The macro called `Agilent` interprets the sample concentration, desired experiment, of console's signal to noise to do the math to compute the appropriate number of scans. The list of experiments contained in `scancalFile` is quite extensive and the file can be easily

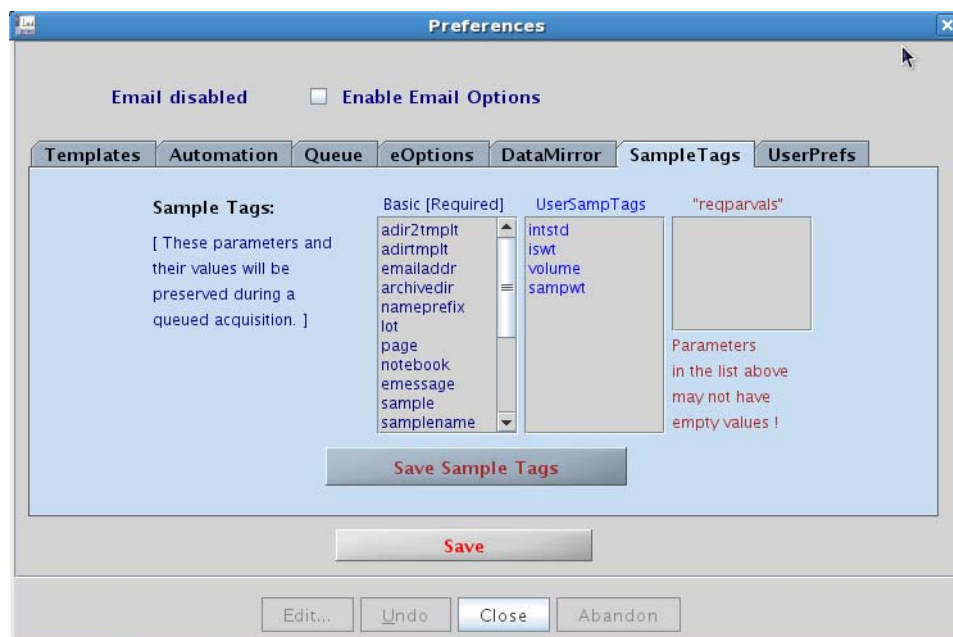
extended to more experiments or tuned so that self adaptive NMR reacts as desired. For example, changing the entry of SNCal for any experiment to a lower value would have the effect of decreasing the number of scans.

Automated Creation of Quantification Reports

A precise answer with respect to the exact purity of a substance is often required. In this situation the best solution is to add a precise amount of a reference compound and compare the integral results for the compound to that of the internal reference standard. The purpose of the reporting macro is to allow a completely automated output of results with the answer in the output rather than generating raw data for manual analysis after the fact. In order to generate a reporting macro prepare a standard sample and include a reference standard and acquire a spectrum suitable to precise quantification. Either a single scan 90° pulse experiment with fixed receiver gain or a multi-scan data set with the relaxation delay long enough to ensure complete relaxation. Four internal standards are supported, maleic acid, dimethylsulfone, dimethylfumarate, or methenamine.

Quantification requires a number of new parameters that are available via a `userfixpar` macro inside of the `quanttools` `maclib`. To use an existing `userfixpar` macro to use the automatic reporting tools in the `quanttools` applications directory, edit your local `userfixpar` macro to include what is found in the applications directory.

Once the new parameters are created, go to **Edit > Preferences > SampleTags** and add `userSamptags` field, `volume`, `sampwt`, `iswt`, and `intstd`. This step allows software to know to keep track of the values for all of these new parameters just as it does for `samplename`, `notebook`, and `page`.



In the **Sample Tags** page:

- 1 Enter the parameters.
- 2 Click **Save Sample Tags**.
- 3 Click **Save**.
- 4 Restart VnmrJ.
- 5 After the restart you can enter the quantification-specific parameters in the **Sample Info** page in the Start folder.
- 6 Click the **SetQuantPars** button to open an entry box.

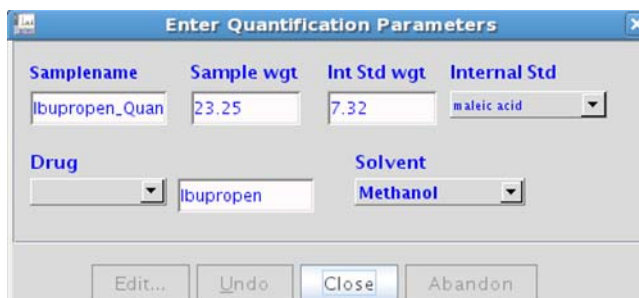
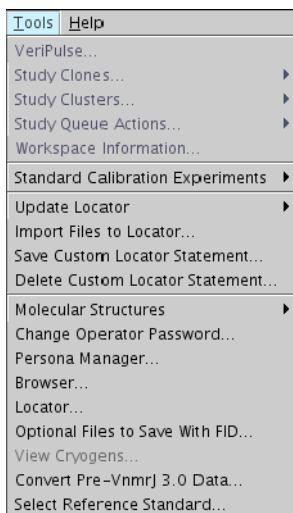


Figure 131 Enter Quantitation Parameters window

After a data set has been acquired with the reference standard of a compound of interest, navigate to the

automatic report creation menu found in the **Tools** menu. An interactive entry form and help menu opens to assist the user through the rest of the process.



The form that is opened by running the Create Quantification Report macro is shown below.

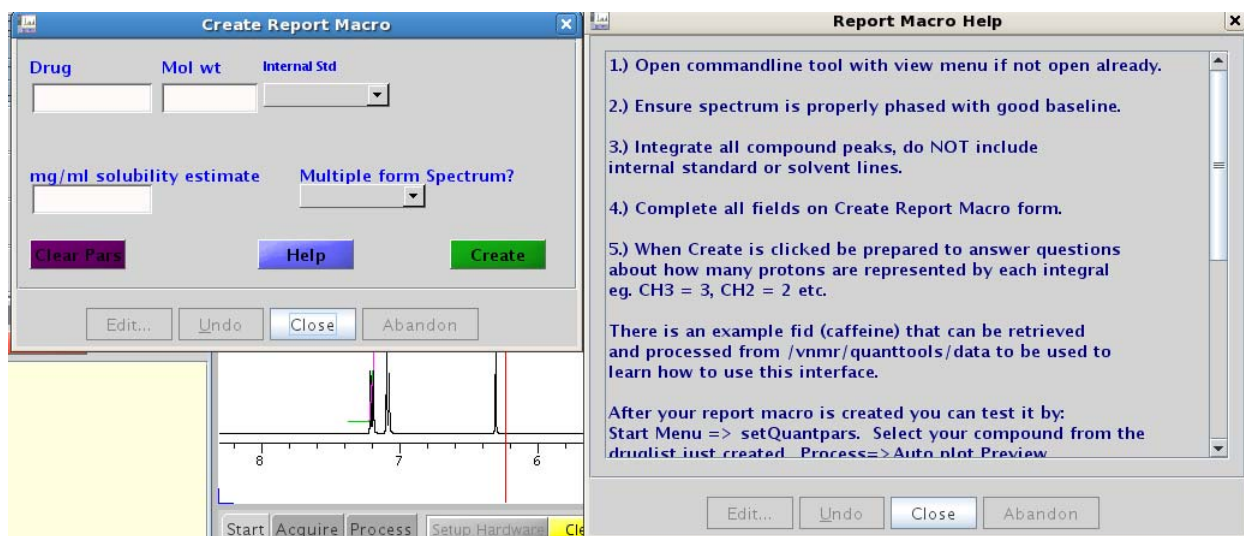


Figure 132 Create Quantification Report form

How to create a reporting macro

The following example shows how to create a reporting

macro for Ibuprofen:

- 1 First integrate the spectrum, being careful to NOT include the internal standard or the solvent peaks. The internal standard of the example was maleic acid near 6.3 ppm and the solvent was deuterated methanol with peaks near 5.0 ppm and 3.3 ppm.

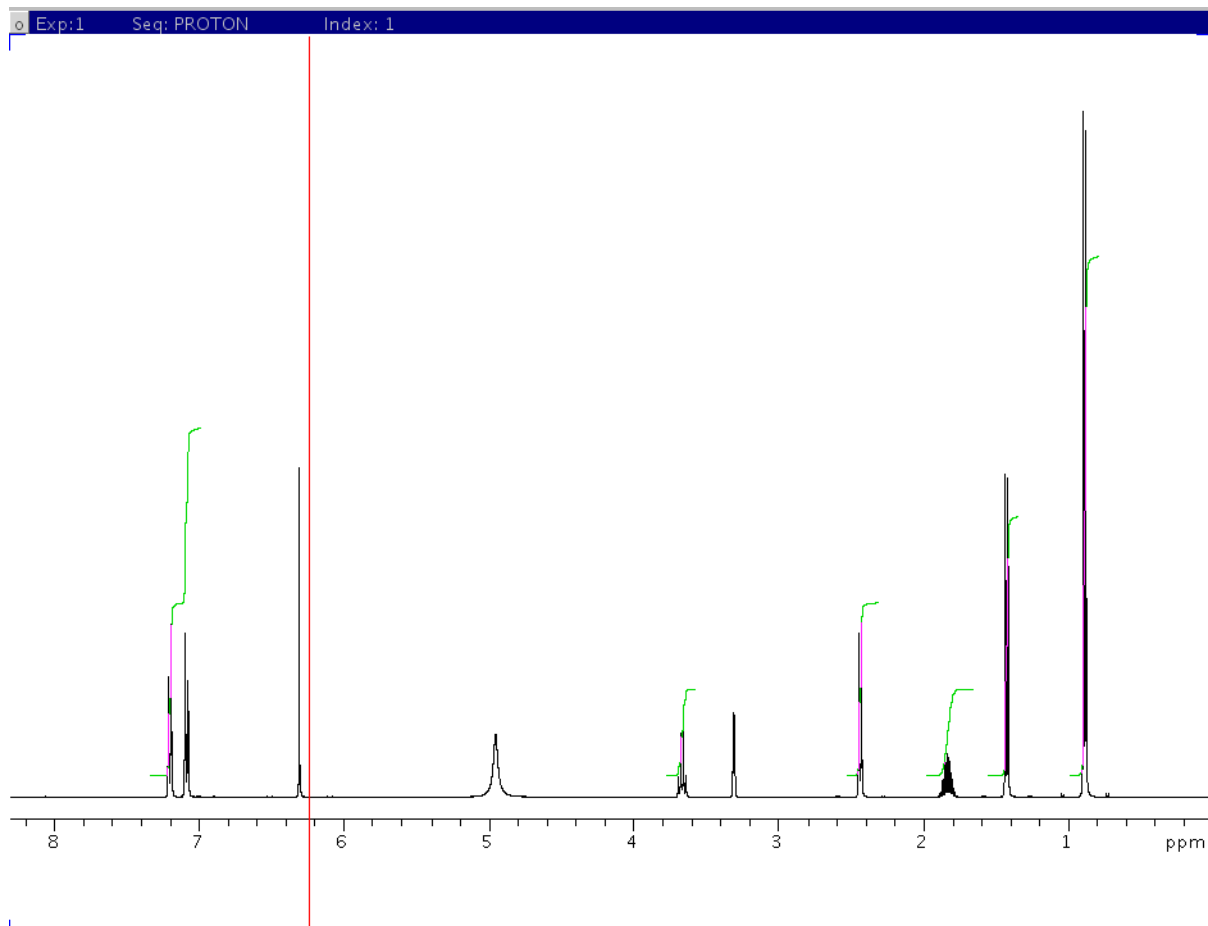
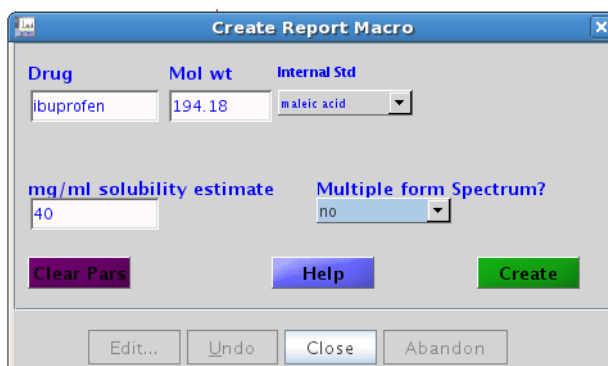


Figure 133 Integrating the spectrum

- 2 Evoke the Create Quantification Report macro in the **Tools** menu and fill out the form.



The screenshot shows a dialog box titled "Create Report Macro". It contains the following fields and controls:

- Drug:** Text input field containing "ibuprofen".
- Mol wt:** Text input field containing "194.18".
- Internal Std:** Dropdown menu with "maleic acid" selected.
- mg/ml solubility estimate:** Text input field containing "40".
- Multiple form Spectrum?:** Dropdown menu with "no" selected.
- Buttons:** "Clear Pars" (purple), "Help" (blue), "Create" (green), "Edit..." (grey), "Undo" (grey), "Close" (grey), and "Abandon" (grey).

Figure 134 Create Report Macro for ibuprofen

A guess for the solubility estimate field is sufficient.

- 3 Select **No** in the **Multiple form Spectrum** field if there is no exchange on the NMR timescale mixing populations.
- 4 Click **Create** and a cursor moves through the spectrum and you are prompted to enter the number of protons represented by each integral region.

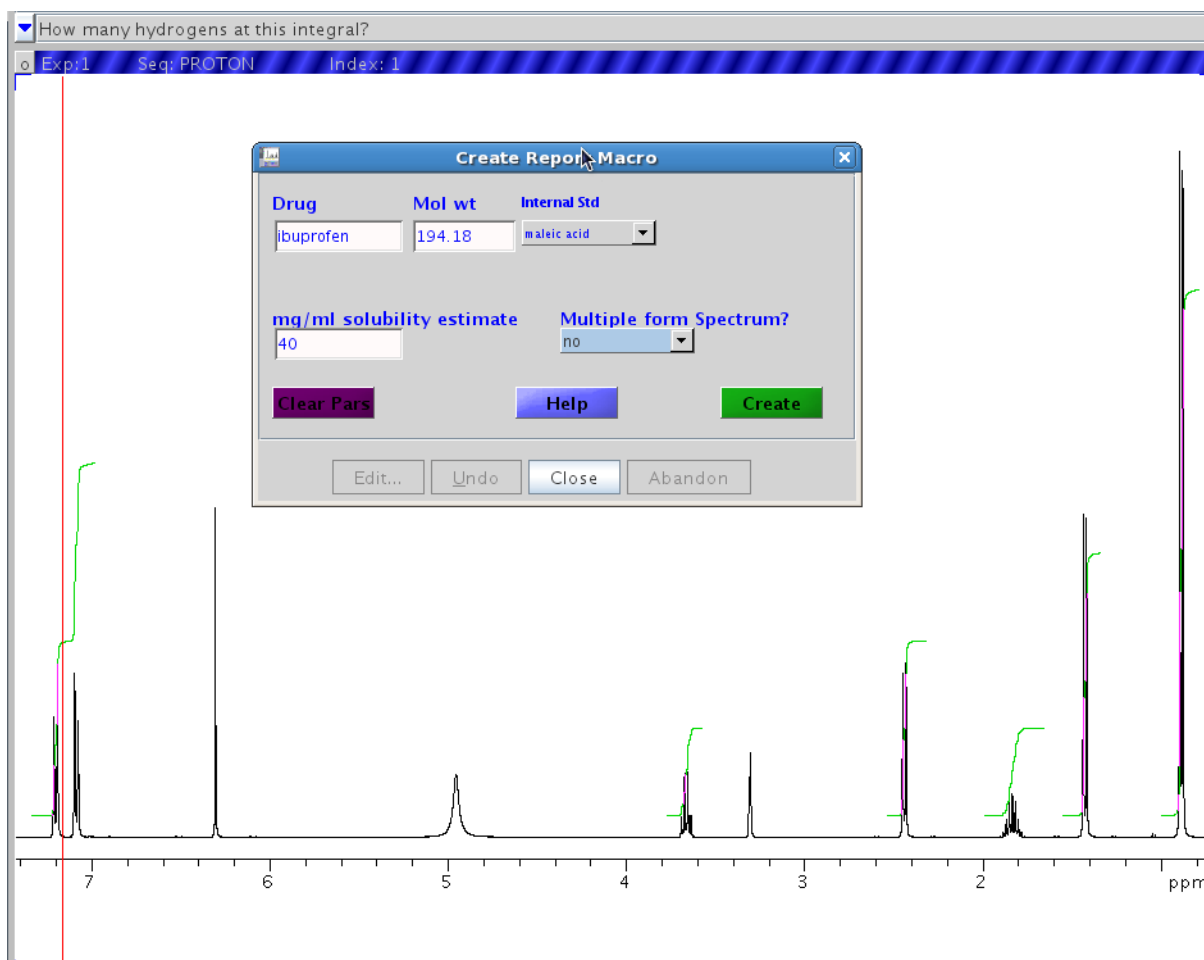


Figure 135 Filling in number of protons

After the questions are answered a macro will be created in the local user's maclib with a name based on the answers to the questions. In this example the macro will be called "quant_ibuprofen_cd3od_maleic" because the drug name was given as ibuprofen, the solvent was cd3od, and the internal standard was maleic acid.

If the purity of the internal standard is 100%, edit the macro and update the entry for \$purity on line 3 to the correct value.

Creating the report automatically adds the name of the drug to the source for a menu in the SetQuantPars tool.

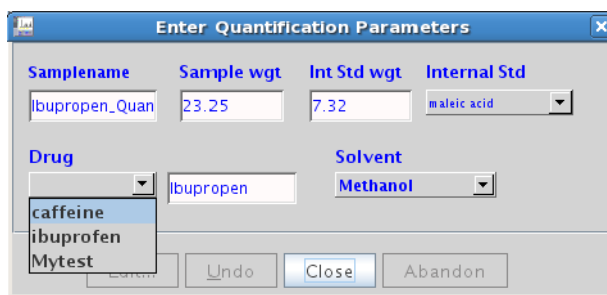


Figure 136 Enter Quantification Parameters window

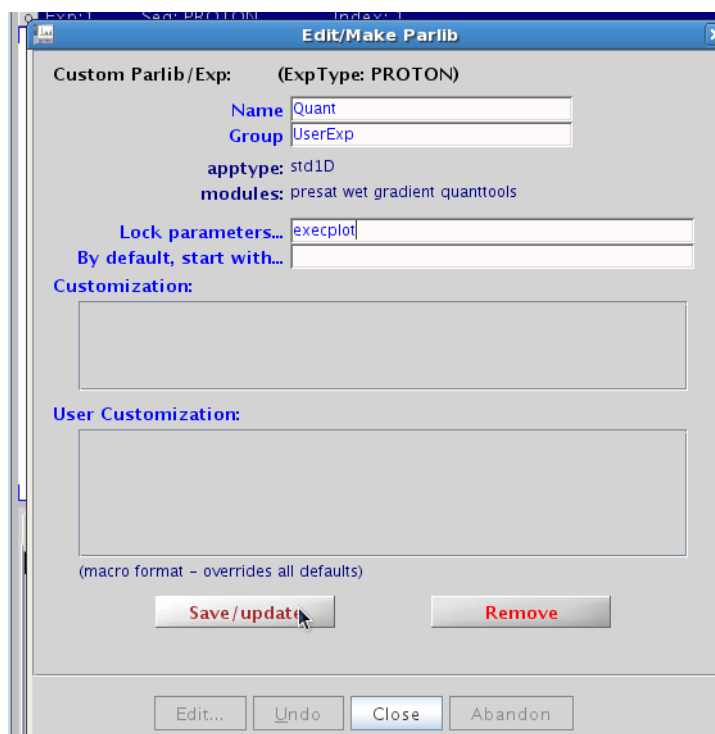
Creating an experiment button

When a purity quantification analysis calling the specific reporting macro is finished the macro will generate a custom output for that sample. There are two ways to hook into automation: the **Experiment Selector** button with output directed by the parameters entered at the time of submission, or a single **Experiment Selector** button for each drug. Both strategies require creating an experiment button.

- 1 To create a quantification-specific experiment button, begin by first acquiring a fid for the desired sample. If the parameter **execplot** does not exist (type `execplot?` on the command line) then create the parameter by entering:

```
create('execplot','string')
```

- 2 Next click **Tools > Study Clones > Edit Parlib**.



- 3 For a single **Quant Experiment** button where the reporting macro is called by the values in parameters, **drug**, **solvent**, and **intstd** assign `execplot='execquantpl'` from the command line. Then in the Edit Parlib tool add **execplot** to the Lock parameters list, enter a name for the button, and click **Save/update**.
- 4 In the example, to create a button called **Q_ibuprofen**, enter the name into the name field and assign `execplot='quant_ibuprofen_cd3od__maleic'` before opening the **Edit Parlib** tool.

The automation report output for ibuprofen is shown below.

Aug 28 2009
 NMR Frequency: 399.923 MHz
 Method: NMR-maleic/cd3od
 Solvent= cd3od Internal Standard= maleic
 Acquisition delay= 10.00 seconds
 Acquisition time= 5.11 seconds
 Sample Weight: 23.25mg
 Internal Standard Weight: 7.320mg

Ratio of internal standard to 0 PPM ref. is: 296.670

Integral Quantitation for ibuprofen at MW=206.28

7.14 ppm: 100.4% Used in Average
 3.68 ppm: 100.0% Used in Average
 2.44 ppm: 99.7% Used in Average
 1.84 ppm: 99.8% Used in Average
 1.43 ppm: 100.1% Used in Average
 0.89 ppm: 100.1% Used in Average

6 integrals meet RSD test
 Average= 100.0 +/- 0.3% with RSD= 0.3% I.S. peak
 Maleic Acid

Quantitation by Peak Height

7.21 ppm: 101.2% Used in Average
 7.19 ppm: 100.8% Used in Average
 7.09 ppm: 100.8% Used in Average
 7.07 ppm: 101.0% Used in Average
 3.67 ppm: 92.7%
 3.65 ppm: 92.9%
 2.45 ppm: 95.8% Used in Average
 2.43 ppm: 96.1% Used in Average
 1.43 ppm: 96.8% Used in Average
 1.42 ppm: 96.7% Used in Average
 0.89 ppm: 98.3% Used in Average
 0.88 ppm: 98.2% Used in Average

16 integrals and peak heights meet RSD Test
 Average= 99.1 +/- 1.9% with RSD= 1.9% (Not Reported)

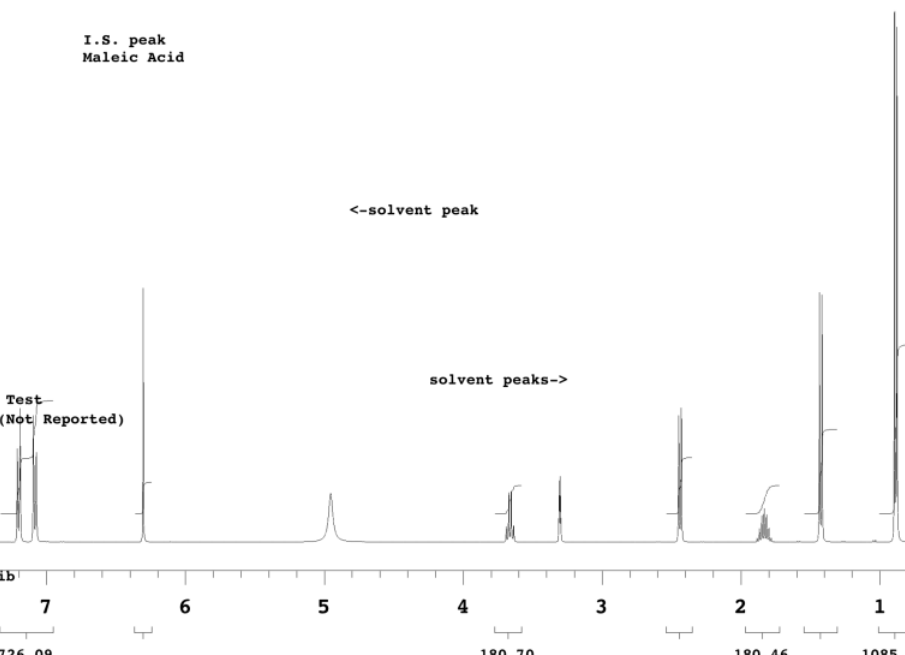
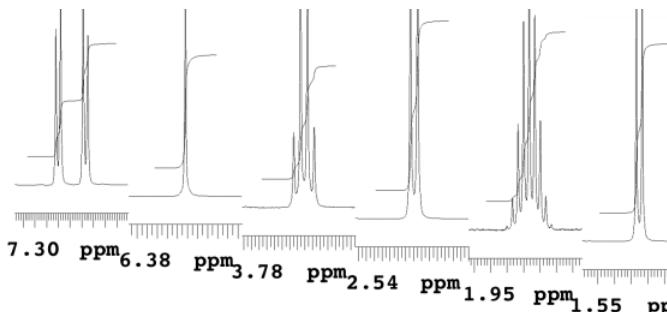
Sample Name:
 Ibuprofen_Quant
 Data Collected on:
 wendi400.varianinc.com-vnmrs400
 Archive directory:

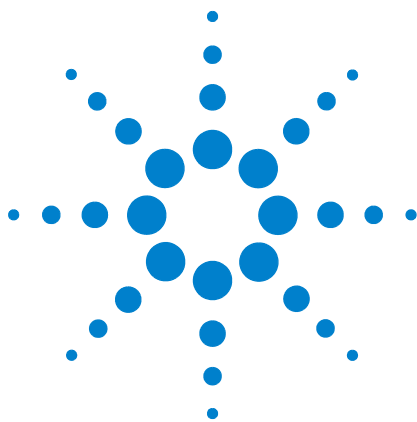
/home/vnmr1/vnmrsys/data/FOR_VJFidlib

Sample directory:
 Ibuprofen_Quant

FidFile: Ibuprofen_Quant_PROTON_01

Pulse Sequence: PROTON (s2pul) 726.09 202.30 180.70 360.44 180.46 542.61 1085.
 Solvent: cd3od
 Plot: /home/vnmr1/vnmrsys/data/FOR_VJFidlib/Ibuprofen_Quant_PROTON_01_plot01





18 Importing a Pre-VnmrJ 4 Pulse Sequence

Prerequisites for Importing Pre-VnmrJ 4 Pulse Sequences [484](#)

Protocol Creation Using Pre-VnmrJ 4 Pulse Sequences [485](#)

Many customers use pulse sequences that were created in older versions of software. These experiments will still load in VnmrJ 4 by using the command line to execute the appropriate set-up macro. Importing these sequences and their ancillary files into VnmrJ 4, however, allows these personal sequence libraries to be used while maintaining access to all the user enhancements implemented in VnmrJ 4.



Prerequisites for Importing Pre-VnmrJ 4 Pulse Sequences

- 1 An "experiment" is assumed to have a setup macro, a parlib entry and a pulse sequence. If there are other supporting files, such as templates, supporting macros, psg file, shapes, etc., it is assumed that they are all compatible with VnmrJ 4 (i.e., no overlap or conflict with existing files).
- 2 If the setup macro uses the `psgset` command to retrieve parameters from the parlib entry, then the macro must be modified to use `Autopsget` command instead of `psgset`.
- 3 The set-up macro should not explicitly set `wexp`. The value of `wexp` will be set by the queuing mechanism, so any changes made by the user's set-up macro will be overwritten.
- 4 It is assumed that the pulse sequence will compile, execute, and process without any errors using the automation tools in VnmrJ 4. This should be tested by issuing the user's set-up macro on the command line, clicking the **Acquire** button to start data collection, and clicking the **Autoprocess** button to process the completed data set.

Protocol Creation Using Pre-VnmrJ 4 Pulse Sequences

Once the experiment has been shown to load and execute from the command line, creating a protocol for that experiment is a simple process. For example, assume that the user wanted a RELAYH experiment in VnmrJ 4:

- 1 Execute "rtppar('RELAYH') exptime:ACQtime REQexp=' ' apptype=' ' svp(file)" on the command line. This will ensure that the parameter set is updated with all VJ3 unique parameters.
- 2 Next, select **MainMenu > Tools > StudyClones > Command Protocol Buttons**. This will open the Protocol pop-up panel. The information entered into this panel will be used to create the protocol.

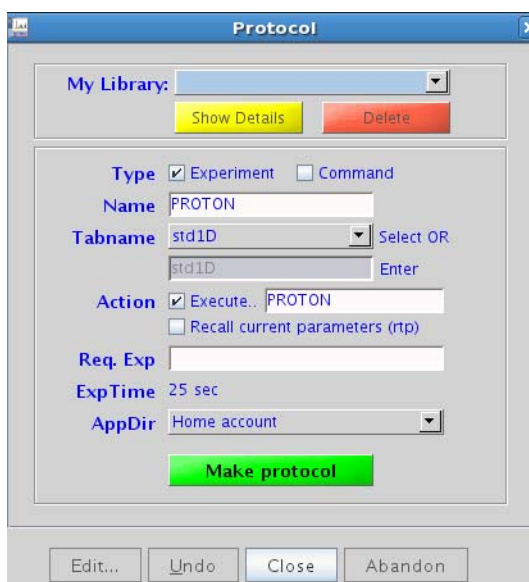
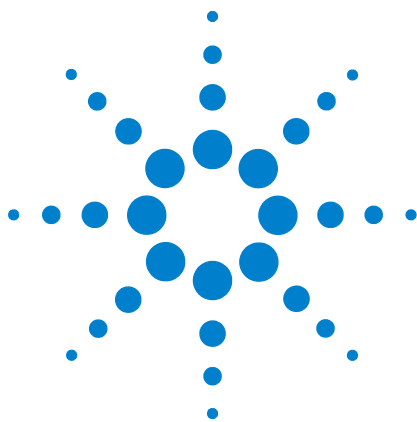


Figure 137 Protocol window

- 3 Confirm that the **Experiment** check box is selected.
- 4 Fill in the name to be used for the protocol. In our example, the name of the protocol will be RELAYH.
- 5 Choose the tab in the Experiment Selector where the new protocol will appear by selecting an existing tab from the drop-down menu, or select **New Tabname** from the list and fill in the tabname field to create a new tab.
- 6 Confirm that the **Execute** check box is selected.

- 7 Fill in the field with the name of the macro used to set up the experiment.
- 8 Fill in the **Req Exp** field with a rational starting experiment(s) as desired.
 - a Leaving this field blank will cause the new protocol to load from the default set-up macro (based on the value of tn) when used in the Study Queue, or it will load on top of the parameters in the current workspace when used in Review mode.
 - b By filling in this field, the new protocol will check for the presence of the specified experiments and operate on the last such parameter set that exists in the sample queue, or it will add the required experiment to the queue if one doesn't already exist. This is the default behavior for the protocols supplied with the system and is the recommended action.
- 9 Choose where the new protocol will be saved from the **App Dir** drop-down menu.
- 10 Click **Make Protocol**.

This will create a new XML file and the new protocol will now appear in the **Experiment Selector**. Assuming that the **Required Experiments** field was populated appropriately, the protocol will behave as do all the standard system protocols. Adding the new protocol to a Study Queue will preserve the sample tags and locked parameters (that is, sw, pw, solvent suppression parameters, etc.) from the last valid starting data set in that queue, and the node can be customized as desired.



19 Non-Uniform Sampling (NUS)

Enabling NUS and Defining a Sampling Schedule 488

Processing NUS Data 491

Non-Uniform Sampling (NUS) is an approach to improve spectral resolution or reduce measurement time by changing the scheme used to sample the indirect dimension of an NMR experiment. Conventionally, the indirect dimension is measured by a series of uniformly spaced increments. In an alternative NUS scheme, some fraction of these linearly-spaced increments are randomly skipped. This kind of NUS scheme is called NUS on a uniform grid.

Note: The *MercuryPlus* and *MercuryVx* systems do not support NUS.



Enabling NUS and Defining a Sampling Schedule

- 1 Select the **Enable non-uniform sampling** check box on the Acquire > NUS parameter panel.

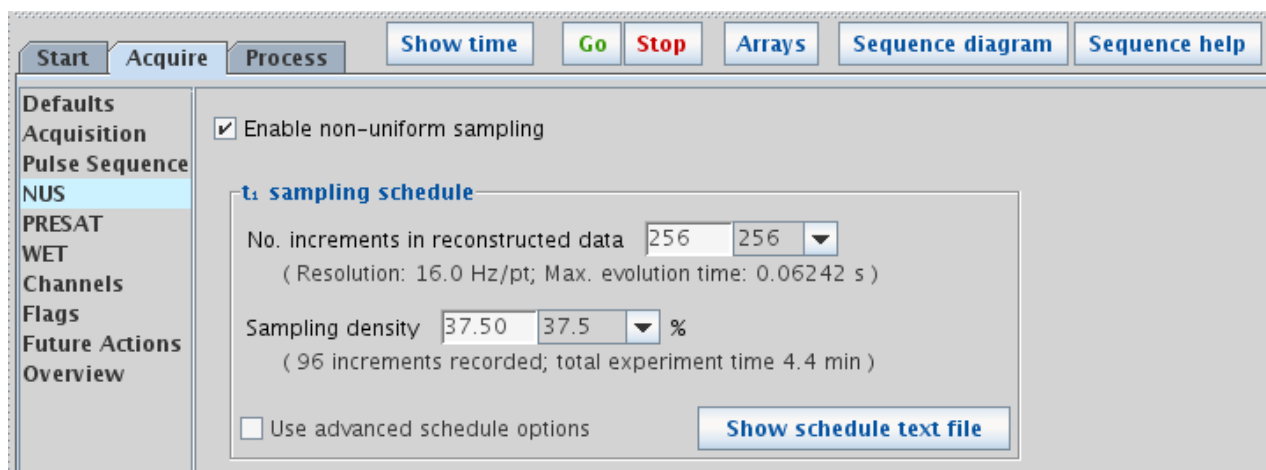


Figure 138 NUS parameter panel.

- 2 Select the **Number of increments** in the reconstructed (i.e. final) 2D dataset.
- 3 Select the **Sampling density** percentage for the number of increments. The number of increments actually recorded is the number of increments in the reconstructed dataset multiplied by the sampling density. The default sampling density is 50%.
- 4 Click **Show schedule text file** to display the sampling schedule text file.

Using advanced schedule options

- 1 Select **Use advanced schedule options** to access the **Schedule builder** and **Schedule weighting** tools.
- 2 Select **Sequential** to create a schedule in which the t_1 evolution time is increased monotonically.

- 3 Select **Weighted** to create a weighted sampling schedule based on selected functions.
 - Select **Use Pbox** to access a drop-down list of weighting functions.

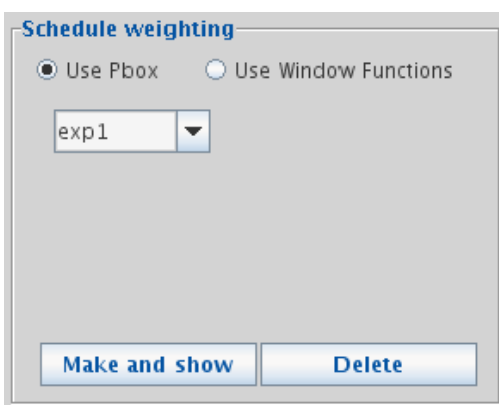


Figure 139 Pbox Schedule weighting

- a Click **Make and show** to display a graphical representation of the selected Weighted sampling schedule on the Graphics Canvas.
 - b Click **Build and show** to build the selected weighting sampling schedule and view the graphical representation in the Graphics Canvas.
- Select **Use Windows function** to access alternative scheduling parameters.

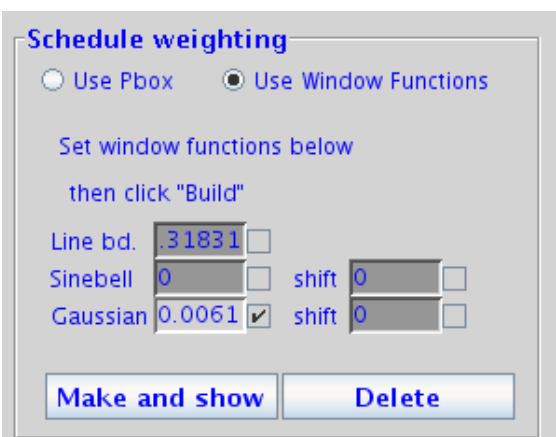


Figure 140 Window Functions Schedule weighting

- a Select the check boxes to the right of each field to

19 Non-Uniform Sampling (NUS)

adjust the parameter.

- b** Click **Make and show** to display a graphical representation of the selected Weighted sampling schedule on the Graphics Canvas.
- c** Click **Build and show** to build the selected weighting sampling schedule and view the graphical representation in the Graphics Canvas.

Processing NUS Data

Click **Process** in the NUS Processing group on the Process > Default parameter panel. By default, NUS data are processed using the CLEAN algorithm.

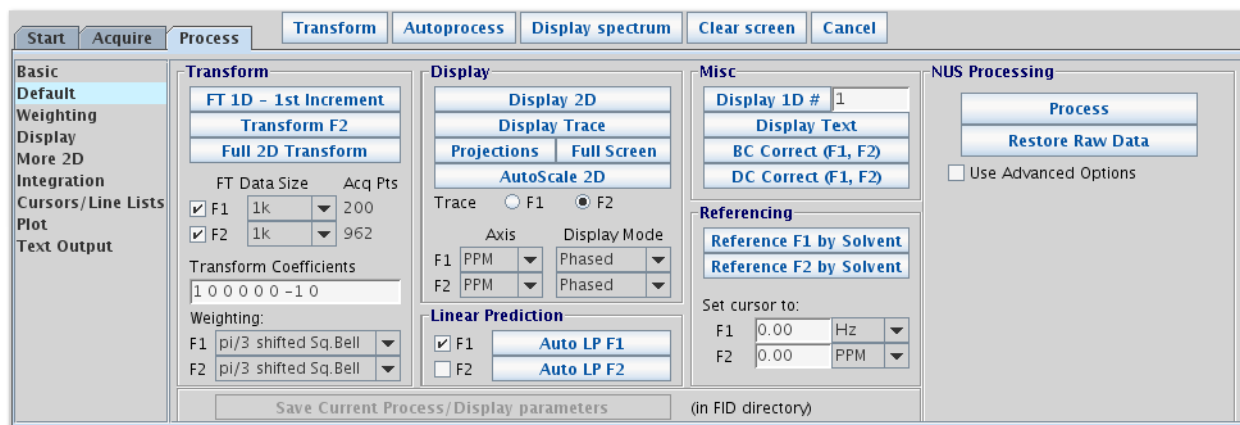


Figure 141 NUS Processing on the Process > Default parameter panel.

To CLEAN processing or to use IST processing, select **Use Advanced Options**.

Advanced CLEAN Processing

Select **Use Advanced Options** to access additional NUS processing options. [Figure 142](#) below shows the options applicable to CLEAN processing.

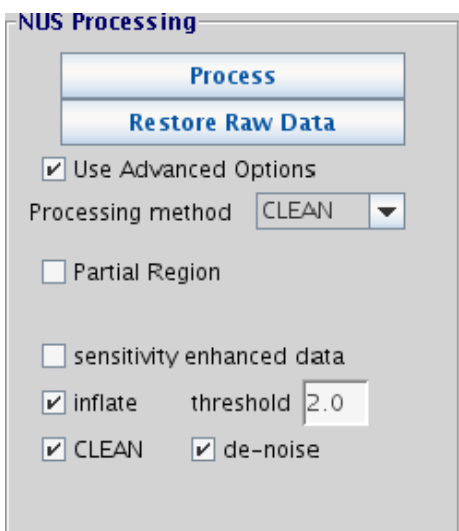


Figure 142 Advanced CLEAN options

Table 50 CLEAN processing options

Field	Description
Partial Region	This option allows the user to reduce the portion of the spectrum used for processing. This greatly accelerates the processing for datasets by excluding portions of the spectrum that do not include signals. If unchecked, the entire spectral window is used by the subsequent processing. Note that when the check box is activated, the current position of the cursors is read. Therefore, if the cursors are subsequently moved to a new position, the selection menu must be used again or the check box reactivated so that the correct position of the cursors is registered.
Sensitivity enhanced data	Check this box to indicate that the data were recorded using the so-called "sensitivity enhanced" (Rance-Kay) scheme. If this box is unchecked, no sensitivity enhancement processing is used.
Inflate	NUS data will be augmented by placing zeros in the "holes" where no t_1 data were recorded. This processing is known as "inflation".
CLEAN	Process the data using the CLEAN algorithm. If unchecked, conventional multi-dimensional Fourier transform can still be applied except the sampling artifacts are not suppressed in any way.
Threshold	Allows setting of the relative threshold with respect to the noise level for CLEAN processing. A higher threshold speeds up the processing whereas a lower number makes the result more accurate.

Table 50 CLEAN processing options

Field	Description
De-noise	Activates the de-noising option in CLEAN processing. If activated, the residual noise is not added to the final spectrum. This reduces the appearance of the t_1 -noise-like artefacts in the final spectrum which helps when displaying and plotting spectra with high dynamic range. Note however, that depending on the "threshold" setting, the noise floor may contain individual noise spikes that can resemble weak peaks and the line shape may appear somewhat distorted, particularly in non-constant time experiments. Some weaker peaks may be lost.

IST Reconstruction for NUS Data

VnmrJ now includes options for processing this kind of NUS data using the Iterative Soft Thresholding method (IST). The processing is performed "behind the scenes" using the NMRPipe spectral processing engine.

As noted, in this type of NUS acquisition, some fraction of increments which are normally measured in a conventional linearly-sampled spectrum are skipped. Such data can be processed using a conventional Fourier Transform (FT) by filling the missing values with zero. But as shown in [Figure 144](#), this is not optimal. When an FT is applied directly to NUS data, the resulting spectrum will have noise-like artifacts, even if the original data is noise free. The pattern of the apparent noise is actually determined by the NUS sampling scheme. Furthermore, the amount of this apparent noise increases with the size of the spectral signals. IST attempts to reduce this apparent noise by gradually reducing the size of the signals.

IST in VnmrJ 4

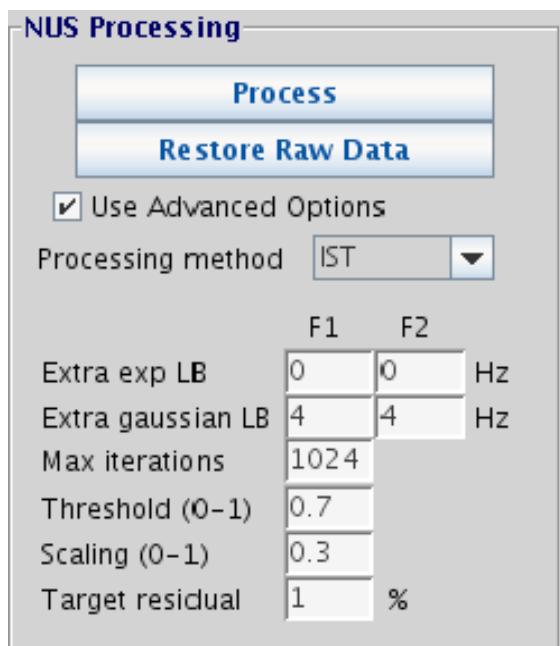


Figure 143 NUS IST on the Process > Default parameter panel

NOTE

The default values for the following parameters will be suitable for many cases.

Table 51 IST processing options

Field	Description
Extra Line Broadening (exp or gaussian)	Choose either exponential or gauss. The default value is 4Hz; useful values 0.0 to twice the linewidth. NUS reconstructions may still have artifacts much like truncation artifacts in conventional spectra. Furthermore, since IST builds a spectrum iteratively by addition, there may be small but visible distortions to lineshapes, especially at the base of signals. For these reasons, the IST reconstruction will often give best results if some additional line broadening is applied during the reconstruction. The IST parameters allow for either exponential or Gaussian line broadening, specified in Hz. Choose one or the other type of line broadening according to whether desired signals should be more Lorentzian in shape, or more Gaussian. The amount of line broadening will depend on the experiment, and will commonly be on the order of the natural linewidth.
Max iterations	The default value is 1024; 1 or more allowed. Useful values are 200-2000.

Table 51 IST processing options

Field	Description
Threshold (0-1)	The default value is 0.70; 0.0 to 1.0 allowed. Useful values are 0.70-0.99. This fraction is intended to be larger than the largest NUS artifacts. Since these artifacts can be more than half the size of the largest signals, the Threshold parameter must usually be greater than 0.5. Larger values will cause slower convergence, but might give better results in cases such as high-dynamic range spectra.
Scaling (0-1)	The default value is 0.30; 0.0 to 1.0 allowed. Use values are 0.10 - 0.99. As for the Threshold parameter, larger values will cause slower convergence, but might give better results in cases such as high-dynamic range spectra.
Target residual %	The default value is 1.0; useful values are 0.1 to 10.0. Smaller values will cause slower convergence, but can give better artifact suppression. Data with low dynamic range might still give good results with a larger Target residual.

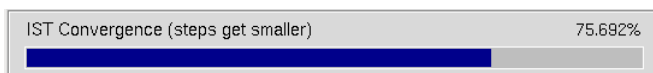
The IST method builds a spectrum iteratively, by generating a conventional Fourier transform spectrum at each step. At each iteration, a threshold is selected based on a fraction of the current largest signal in the spectrum. This threshold is chosen so that it will always be larger than the largest NUS artifacts. Then, all the intensities above that threshold are reduced by a constant factor. The details of the VnmrJ IST procedure are as follows:

- 1 Start with NUS time-domain data T, and empty spectrum S.
- 2 Generate spectrum A from T by applying a conventional FT.
- 3 Choose a threshold, which is the **Threshold** value times the maximum intensity of spectrum A. The intent is that this threshold should be larger than any NUS artifact.
- 4 Generate spectrum B from A by reducing the data above the threshold by the **Scaling** value.
- 5 Accumulate the difference A - B into spectrum S.
- 6 Generate new time-domain data T: Inverse FT spectrum B, and set points not acquired in the NUS schedule to zero.
- 7 Repeat steps 2-6 until the residual in spectrum B is small, or until **Max iterations** is reached.
- 8 At this point, spectrum B contains whatever signals have not already been captured in the accumulated spectrum S.

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Make a final IST result by adding residual B into the accumulated IST spectrum S.

About the convergence test for step 7: a percent convergence value is computed based on the maximum intensity in the current iteration relative to the maximum intensity in the first iteration. This percentage must drop below the **Target residual** parameter for convergence. During IST processing, the progress of the calculation will be shown with a bar graphic like the one shown here. The bar graphic will stay on the screen after the IST calculation is finished. The progress bar can be dismissed at any time via the usual "X" icon in the window border, either during the IST calculation, or after it is complete. Dismissing the progress bar during the calculation will not stop the IST processing.



Because of the fractional scaling at each iteration, the steps towards convergence get smaller and smaller as the IST calculation proceeds. Towards the end of the IST calculation, the steps will usually be smaller than 1%.

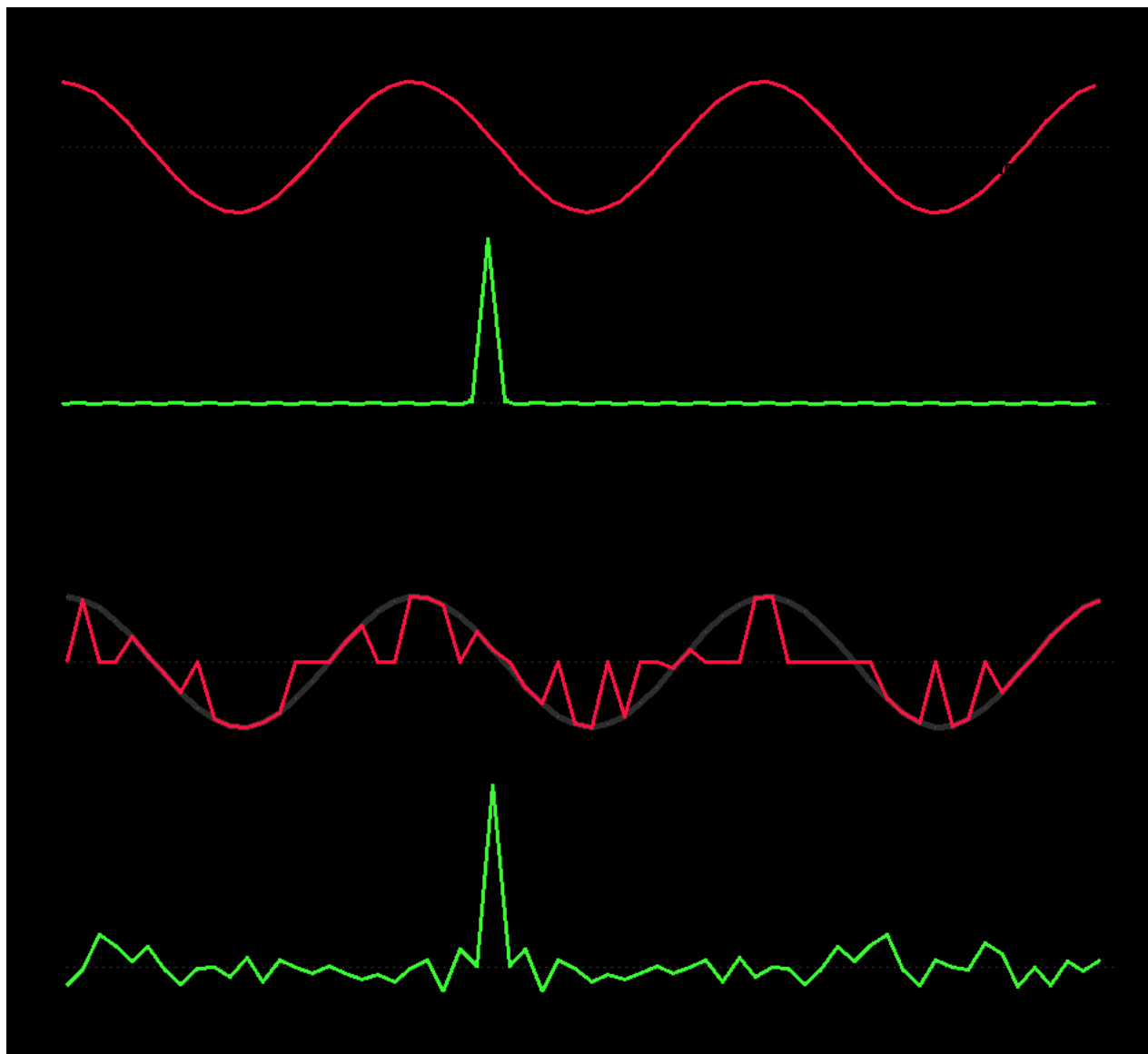
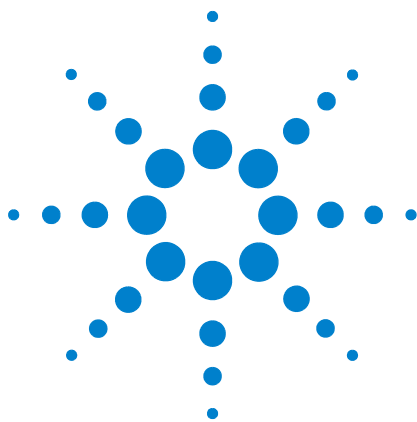


Figure 144 Limitations of conventional FT for NUS Data

The top shows conventional uniformly-sampled time-domain data (red) and its corresponding conventional FT spectrum (green). Below, the same data, but Non-Uniformly Sampled with half the number of points. As shown, the conventional FT of NUS data is equally sharp as the fully-sampled spectrum, but the NUS FT spectrum has noise-like artifacts, even though the original data in this case is noise-free. So, in order to take full advantage of the resolution and measurement time improvements of NUS, a method other than conventional FT must be used.

19 Non-Uniform Sampling (NUS)



A Status Codes

VT Setup	500
VT Startup	501
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VT Controller Safety Circuits	508

This chapter describes startup and operation of the optional variable-temperature (VT) unit. A VT unit is available for Agilent Technologies, Inc. NMR spectrometers to regulate the sample temperature. A thermocouple senses the temperature of the gas flowing over the sample; this temperature is then continuously displayed both in the software and on the optional remote status unit. The controller compares the user-requested value with the current probe temperature and changes the heater current accordingly. The VT controller then reports the temperature of the gas flow and status to the spectrometer through a serial port at the rear of the console.



VT Setup

Use the System Settings window to configure the spectrometer for the VT accessory and to Type a VT (variable temperature) cutoff value. The VT cutoff (Edit / System settings / VT cutoff) determines which of the two gas streams in the system is supplied to the probe. (If the requested temperature is above the VT cutoff, it uses the high-temperature gas. If the requested temperature is below the VT cutoff value, it uses the low-temperature gas.)

- 1 Open the System Configuration window (Edit / System settings / System config) if the VT controller is off and it cannot be turned on.
- 2 Check that the VT Controller label is set to Present.
- 3 Open the System settings window (Edit / System settings) and type an appropriate value for VT cutoff and click **OK**.

Set the VT cutoff to a temperature near the ambient VT gas temperature (normally VT cutoff is correct and need not be changed). Based upon the value of VT cutoff compared to the typed temperature, the system selects either the high-temperature or low-temperature VT gas flow.

VT Startup

Starting up the VT unit requires the following steps:

- 1 Switch the VT unit on using the unit power switch (on the back of the spectrometer).
- 2 Reset the VT controller, if the system power has been off or the VT unit has been disconnected from the probe by pressing the **POWER** switch to turn the unit OFF, then press **POWER** again to turn it ON. The VT controller also can be reset with the **Reset VT** button on the **Spin/Temp** page in the **Start** tab.

CAUTION

Use either dry nitrogen gas or dry air for VT and probe operation. A mixture of nitrogen gas and air can cause spikes in the baseline adjacent to the large peaks in the spectra. The use of air as the VT gas is not recommended for temperatures above 100°C. Such use will destructively oxidize the heater element and the thermocouple.

- 3 Use dry nitrogen gas if the requested temperature is over 100°C or below the dew point (or below 0° C, whichever is higher).

Otherwise, air may be used as the VT gas. If the requested temperature is below -40°C, dry nitrogen gas is recommended for cooling the bearing, spinner, and decoupler. This prevents moisture condensation in the probe and spinner housing.

The source of heating or cooling gas is not automatically selected. To use nitrogen, attach a nitrogen gas source to the VT system. The same is true when using air. The VT system only selects the routing of the gas flow.

- 4 Use the flow control meter on the magnet leg to adjust the flow to about 10 LPM (as shown on the flow gauge).
- 5 Place a sample that can be handled at ambient temperature and be placed in the probe, NMR lock obtained, and field homogeneity adjusted. Samples that cannot be handled at ambient temperature should wait until the system reaches the requested temperature.

Temperature Array

If an array of sample temperatures is to be used, set a pre-acquisition delay (pad) that allows sufficient time for the sample to equilibrate after a temperature change. The system will then wait pad between each temperature before starting data acquisition. Delays of several minutes are optimum because the sample will take longer to equilibrate than it takes the VT controller to stabilize the heating/cooling gas at the setpoint.

- 1 Open the **Acquire / Acquisition** page, and click the **Arrays** button – or select **Acquisition / Parameter arrays**.

This opens the Array Parameter window.

- 2 Click **New Array** and type `temp` in the Param Name column.
- 3 Specify Array Size, First Value, Increment, and Last Value.
- 4 Click **Close**.
- 5 Set the pre-acquisition delay in the **Acquire / Flags** page.
- 6 Delay __ sec before starting (for VT etc.)

Operating Considerations

Use the following recommendations to help achieve better VT performance.

- The spectrometer system was designed and tested with a VT gas flow rate of about 10 – 15 LPM. Sizable deviation from this rate can result in significant inaccuracy in temperature calibration and reduce the attainable temperature limits.
- Initial cool-down of the exchanger and transfer tubing after the coolant is added increases the initial time required to reach regulation (about 5 to 10 minutes for -40°C with liquid nitrogen). This may be longer than the `vtwait` parameter (this is the longest time the `su` command will wait for the temperature to equilibrate).
- Below -40° , use dry nitrogen gas for the spinner and bearing air supply to avoid moisture and frost buildup on the spinner housing and turbine. Should this happen, the sample spinning can become erratic or stop altogether.
- Every sample has some amount of temperature gradient along the vertical axis. Minimize the gradient by not filling the sample tube more than about 25 to 32 mm (1 to 1.25 in.) for VT samples, by inserting a vortex plug or glass wool plug in the tube just above the sample solution, and by placing the liquid column at the probe-coil cType lines. The plug reduces refluxing of the solvent in the upper portion of the tube. Any mass movement, such as refluxing or convection, can seriously degrade resolution and lock stability.
- Above 100°C , use dry nitrogen gas to reduce oxidation of the heater and thermocouple.
- High-power decoupling adds heat to the sample. (It does so internally – within the sample – meaning that the temperature readout does not reflect this heating.) The increase in temperature depends on the dielectric of the solution and the power level. Under these conditions, the temperature accuracy can be affected. If necessary, reduce decoupler power and use a more efficient decoupling mode.

- Overnight or long-term unattended VT operation at low temperatures is hampered by the fact that the usual coolant, liquid nitrogen, provides only about 1 to 2 hours of operation on a single fill of the coolant bucket. Some other coolant that lasts longer can be used if the operating temperature does not require the low temperature of liquid nitrogen. A common alternative is a mixture of dry ice and isopropyl alcohol (or ethylene glycol). (Do not use acetone – it will dissolve the bucket!) Another option is to cool the fluid indirectly by a refrigerating device such as isopropyl alcohol or ethylene glycol cooled. Do not use aromatic, ketone, or chlorinated solvents (including acetone) in the coolant bucket, because these solvents will dissolve the standard polystyrene bucket.
- On older spectrometers, the ability of the VT unit to achieve temperature stability is affected by the stability of the room temperature. For best results, the room temperature should be made as stable as possible.
- An optional setup to help stabilize the VT gas supply is to run the VT gas through a heat-exchanger coil in a water bath at a regulated temperature. For best results, use an ice bath to cool down the VT gas to between 5°C and 10°C, and keep the flow as stable as possible for experiments below 40°C. Generally, the VT gas supply temperature should be a minimum of 10°C below the set temperature of the VT controller to get the best performance of the VT controller and heater in the probe.
- A temperature calibration curve must be made for each probe if you want an exact determination of sample temperature. All data, such as gas flow rates, must be noted. Samples of ethylene glycol are used for high-temperature calibration, and samples of methanol are used for low-temperature calibration.
 - a Obtain a spectrum after bringing the sample to the desired temperature and allowing sufficient time for equilibration. Note that these samples have no lock solvent, and usually require additional shimming due

to their short sample length (to reduce temperature gradients).

- b** Display two cursors and align them on the two resonances in the spectrum.
- c** Type `tempcal('e')` if the sample is ethylene glycol. If the sample is methanol, type `tempcal('m')`.
- d** The temperature is calculated and displayed based on the difference in frequency between the cursors.

VT Error Handling

Select how VT errors are handled in the **Spin/Temp** page under the **Start** folder.

Interlock selections	Wait setting
(Start folder, Spin/Temp page)	(Acquire folder, Flags page)
<input checked="" type="checkbox"/> Control temperature from Setup panel only <input checked="" type="radio"/> Abort after temperature error <input type="radio"/> Warn after temperature error <input type="radio"/> Ignore temperature error	<input type="checkbox"/> Stop on VT failure Allow <input type="text" value="300"/> seconds before VT testing Delay <input type="text" value="0.5"/> sec before starting (for VT etc.)

Abort after temperature error	The VT regulation light is monitored during the course of the experiment, and if it starts to flash (regulation lost), the current data acquisition is stopped. The acquisition does not resume automatically if regulation is regained. With Abort selected, a maximum limit is imposed on the time that the system waits for regulation to be established (<code>vtwait</code> , the "wait before testing setting"). This time is independent of the delay setting (<code>pad</code>). If regulation is not established after the wait time (<code>vtwait</code> , normally set to 300 seconds), the system displays the message VT FAILURE and does not proceed with the experiment. If the regulation problem is later corrected, the experiment can be resumed.
-------------------------------	---

Warn after temperature error	The VT regulation light is monitored during the course of the experiment, and if it starts to flash (regulation lost), a warning is generated, but acquisition is not stopped.
------------------------------	--

Ignore temperature error	The temperature interlock feature is turned off.
--------------------------	--

The temperature interlock selections (Abort, Warn, or Ignore) and VT wait time (Acquire folder, **Flag** page) check VT operation and stop the experiment if temperature regulation is lost.

The lost regulation causes error processing to occur for both the Abort and Warn selections, thus providing a user-selectable mechanism to respond to VT failure.

The interlock operation does not apply when VT regulation is temporarily lost as a result of a programmed temperature change in an experiment where temperature is an array. The VT gas flow has a sensor and an interlock on VNMRS and 400MR consoles. Additionally, the heater is protected if gas flow stops by an internal temperature limit sensor that turns off the heater current before the element overheats. Any experiment in progress is stopped if **Abort** is selected because a loss of gas flow will result in a loss of regulation. Only the

sample is left unprotected if VT gas stops.

CAUTION

Do not run unattended a sealed sample of highly volatile materials that must be kept cold to avoid excessive pressure buildup. The undetected loss of VT gas or exchanger coolant could result in the rupture of the sample tube and damage to the probe components.

VT Controller Safety Circuits

The VT controller includes safety circuits to avoid damage to the heating element and probe. The following error conditions produce an error code:

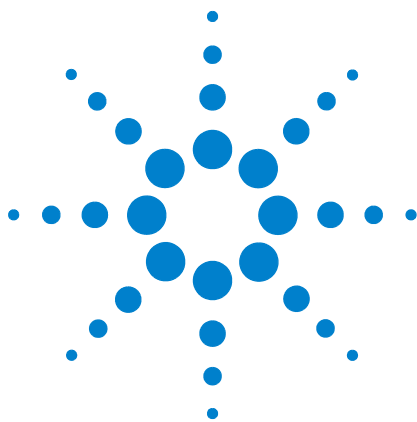
- Open circuit in the thermocouple circuit
- Open circuit, short circuit, or over-temperature at safety senso
- Short circuit or software/microprocessor failure at the output transistor

Over-temperature at the safety sensor initially turns off the heater. If this method fails to correct the condition within 5 seconds, either the gas flow has been interrupted or an output transistor failure has occurred, whereupon a protective relay operates, isolating the heater from the control electronics. Failure of any of the sensors also results in this relay operating.

Once the protective relay has operated, the output will remain off. A power-down and power-up cycle of the VT controller is required to release the relay.

The over-temperature circuit can be inadvertently tripped if the VT is started at a below ambient temperature and the temperature is increased greater than 70°C. If the circuit is tripped, reset it by turning the VT off and on, then change to the desired temperature in 50°C steps.

Excessive heat requirements that cause the current to remain near the maximum can also trip the second circuit. Therefore, when using liquid nitrogen for cooling and when operating above 0°C, reduce the gas flow rate to between 8 and 9 LPM. Reset will also occur if the VT cable is removed from the probe while the VT is on.



B Shimming Basics

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The room-temperature shims coils, which are commonly referred to as just "the shims", are a set of coils inside the magnet's room-temperature bore that induce small changes in the shape of the magnetic field. Each shim produces a specific change in the magnetic field that can be easily shown. The approximate shapes of the axial gradients (spinning shims) are shown in [Figure 145](#) to provide a visual reference for the interactions of the shims.

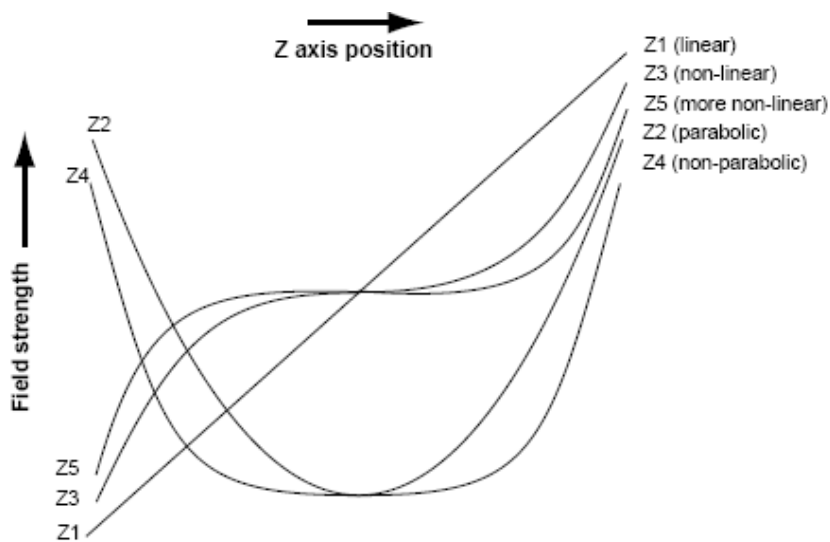


Figure 145 Approximate shape of axial gradients

Understanding the effect of various shims on the symmetry of the resonance is important in simplifying the shimming



B Shimming Basics

process. The following two points must be considered:

- The effect of a given shim on the spectral lineshape
- How the shims interact with each other

Understanding how the shims interact is critical to simplifying the task of shimming. Pure shim gradients produce a very specific and predictable effect on the magnetic field and, to a lesser extent, on the resonance lineshape.

Shim Interactions

The following sections show theoretically predicted changes in lineshape caused by changes in shim DAC values. Shim sets with pure shims, such as the Agilent Ultra•nmr shims, follow the theoretically predicted response very closely. Other shim systems, with more interactions between shims, produce different results.

Theoretically perfect lineshape and effect of Z1 shim

Figure 146 shows a theoretically perfect lineshape (at left) produced in a perfectly homogeneous field (at right). The magnetic field shape appears as a flat line, indicating that the magnetic field does not change across the length of the sample.

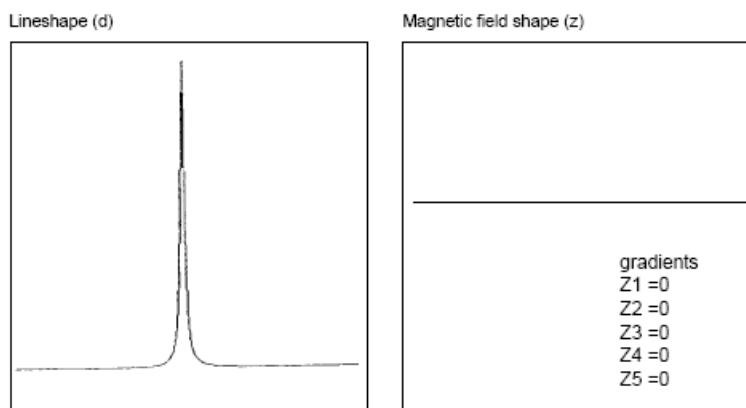


Figure 146 Theoretically perfect lineshape

Figure 147 shows how changing the linear shim Z1 affects both the lineshape (left) and the magnetic field (right).

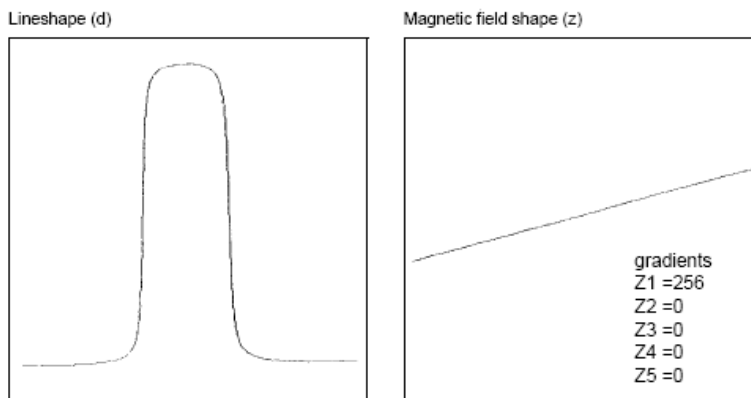


Figure 147 Effects of linear shim Z1

Effects of even-order shims Z2 and Z4

[Figure 148](#) shows the effect of the even-order shims, Z2 and Z4, on the lineshape. Notice that a positive mis-adjustment of both shims produces an upfield tail on the peak. If Z2 and Z4 are misadjusted in the negative direction, the asymmetry occurs on the downfield side of the peak. The difference between Z2 and Z4 is in the height of the asymmetry. The Z2 shim causes asymmetry higher on the peak than Z4.

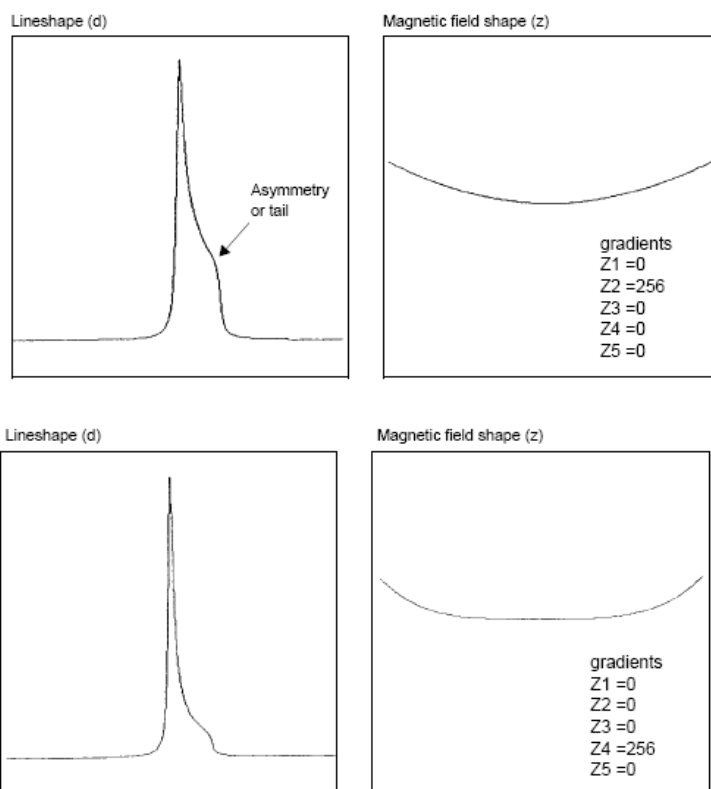


Figure 148 Effects of even order (parabolic) shims Z2 and Z4

Effects of odd-order shims Z3 and Z5

Figure 149 shows the effects of the odd-order shims Z3 and Z5 on the lineshape. The odd-order shims cause broadening of the peak and therefore affect resolution. The Z5 shim is unavailable on systems with 13-channel shim sets (shimset=1).

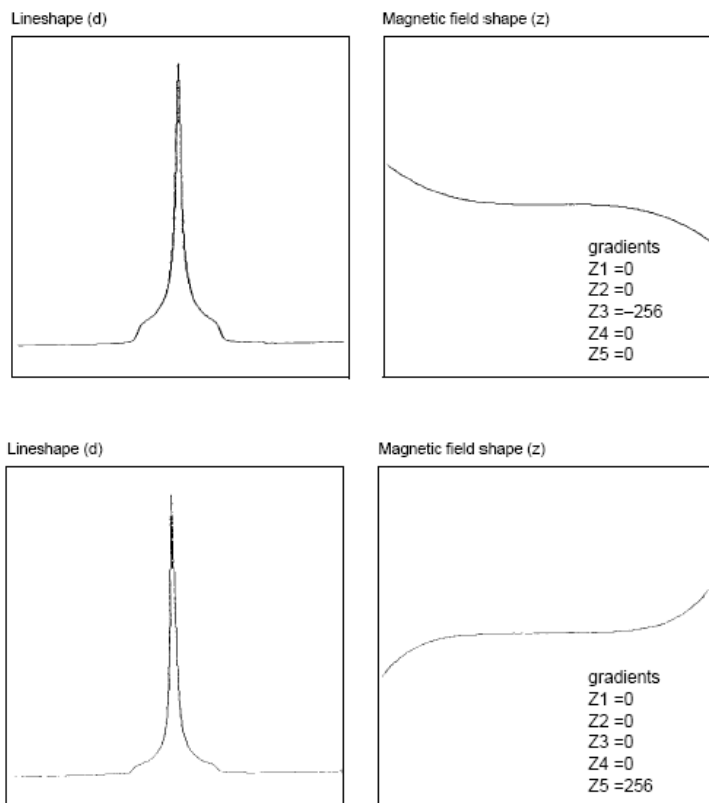


Figure 149 Effect of odd order (non-linear) shims Z3 and Z5

Effects of improperly adjusted shims

Figure 150 shows two examples of the effects when more than one shim is improperly adjusted. This is the typical case with real samples. The complex lineshapes make simple visual analysis difficult. A procedure for correcting the shims that can be used as a guide when adjusting shims is provided later in this section.

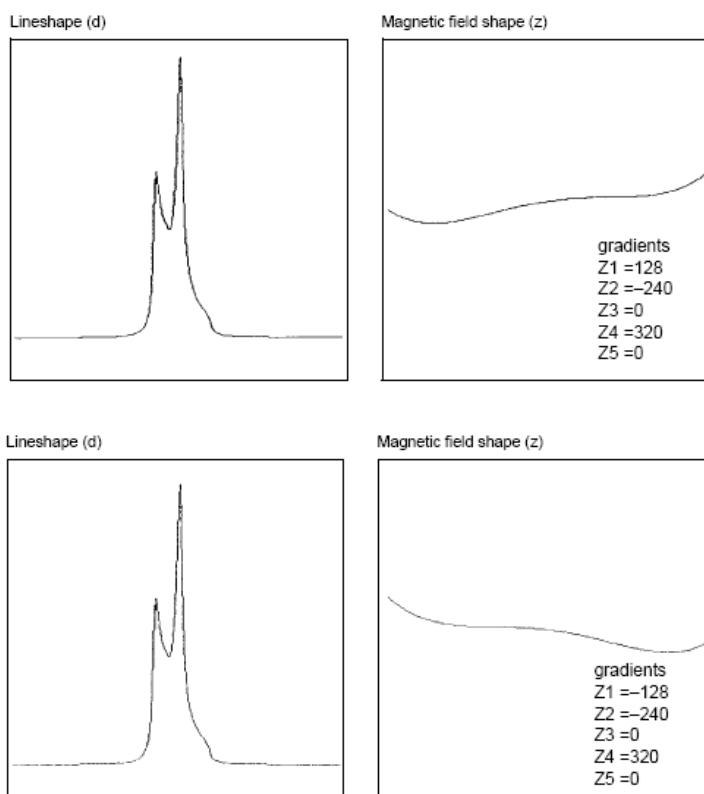


Figure 150 Effects of misadjusted shims

Effects of Non-Spin Shims

Figure 151 shows the effect of the non-spin shims on the spectrum (note that Z3X and Z3Y are not available on 13- or 14-channel shim systems). If set wrong, the first-order non-spin shims (X, Y, ZX, and ZY) can cause first-order spinning sidebands. XY and X2-Y2 can cause second-order spinning sidebands. High-order non-spin shims can cause a broad peak base.

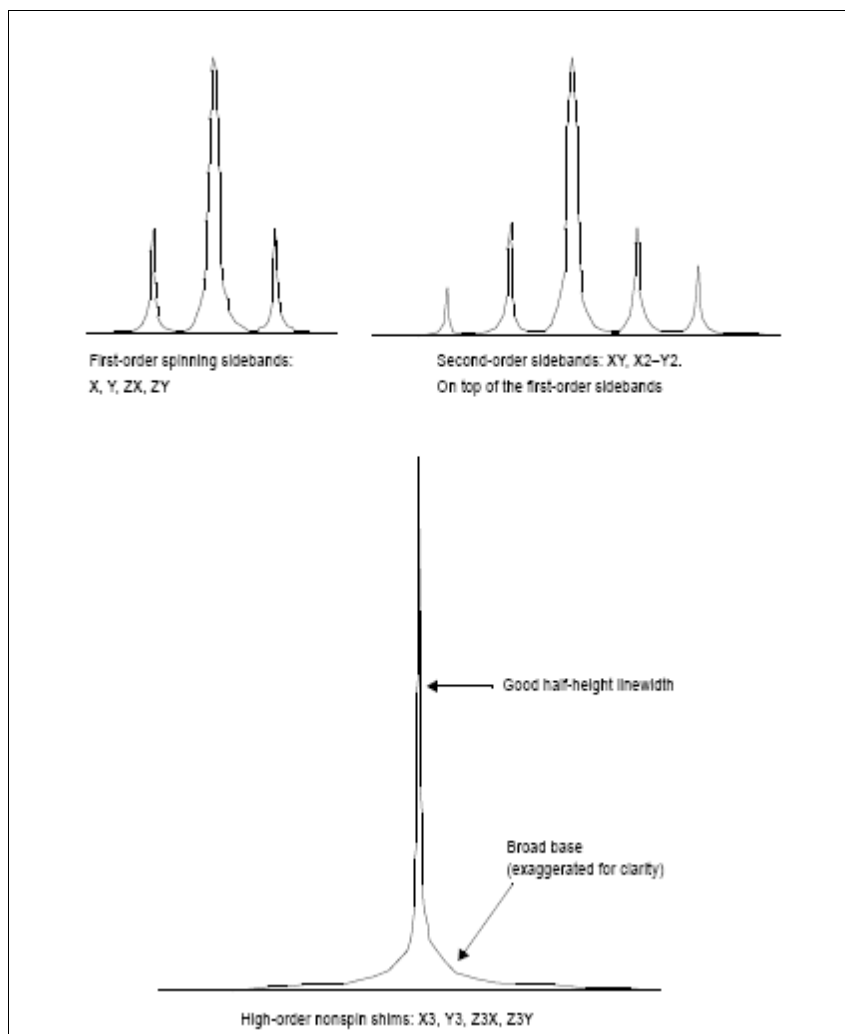


Figure 151 Effects of nonspin shims

Summary of shim interactions

Table 52 lists some lineshape effects associated with shims. Note that the 13-channel shim systems (shimset=1) do not have Z5, Z3X, ZXY, etc., and that the 14-channel shim systems (shimset=10) have Z5 but do not have Z3X, ZXY, etc.

Table 52 Lineshape effects and their associated shims

Lineshape effect	Shims
Split peak	Z4 and Z1
Asymmetry greater than half-way up	Z2
Asymmetric foot	Z4
Symmetric feet and or low broad base	Z5
Symmetrically broad base	Z3
Spinning sidebands	Low-order radials X1, Y1
Symmetric broad base	High-order radials X3, Y3, etc

Typical interactions for axial shims:

- Z1 and all other axial shims, to some extent
- Z2 and Z1
- Z3 and Z1
- Z4 and Z2 (with large delta Z4s: Z4 and Z3)
- Z5 and both Z3 and Z1 (Z5 not available on 13-channel shim systems)

Autoshim Information

Gradient autoshimming is the preferred method for shimming. There are also other (slower) methods that use the lock signal. These methods are Hardware and Background Autoshim.

Hardware autoshim

Hardware autoshim methods vary according to the system that is involved. They are used primarily to maintain a well-shimmed homogeneity over long runs.

The `hdwshim` parameter enables the commands `su`, `go`, `ga`, or `au` to turn on and off “hardware” autoshimming, which is done using a software emulation of hardware autoshim. Shimming is active only while a pulse sequence is executing:

- `hdwshim='y'`: shimming is active only during the first delay of the pulse sequence
- `hdwshim='p'`: shimming is active only during the first presaturation pulse, defined as a change in power level followed by a pulse (for example, `presat.c`)

Shimming during subsequent delays or presaturation pulses can be activated by using the `hdwshiminit()` statement before the delay or presaturation pulse. Shimming uses the `z1` shim by default.

If the parameter `hdwshimlist` is created, shimming will be done on only the specified list of shims. Only the following shims are allowed:

`z1, z1c, z2, z2c, x1, y1`

Shimming is done in the order of `z1, z1c, z2, z2c, x1, y1`, regardless of the order in which the shims are used in `hdwshimlist`, and is performed on each shim in intervals of 20 seconds. The fine shims (`z1, z2, x1, and y1`) are recommended for routine use.

Using the input window

- Type `hdwshim='y' su`.

Hardware shimming starts at the next acquisition – a during the first delay – and stops when acquisition is complete.

- Type `hdwshim='p' su`.

Hardware shimming starts at the next acquisition – during the first presaturation pulse – and stops when acquisition is complete.

- Type `hdwshim='n' su` to turn off hardware shimming.

Method autoshim

Method Autoshim is controlled by the parameter `method` and the command `shim`. This is an Autoshim method that provides no interaction with the operator. The shimmethod (`method`) that should be used during routine sample changes depends upon the level of homogeneity required, the change in sample height between samples, and the maximum time desired for shimming.

- For samples that are either long or all of identical height, and with average homogeneity needs: a simple `z1z2` shimming is usually sufficient (`method='z1z2'`).
- For samples where the sample height might vary: the method `allzs` has been found to be the most reliable, at the expense of greater time spent in shimming. This method shims first Z1, Z2, and Z4, then Z1, Z2, and Z3, and finally Z1 and Z2.

Using the input window

Type `method=file shim`, where `file` is the name of a file in the directory `shimmethods` (for example, `method='z1z2' shim`).

Two shimmethods directories can exist. A user can have a private copy of shimmethods in a personal shimmethods directory. A system-wide set of shim methods is also located in the `/vnmr/shimmethods` directory. The user's private library is searched first for a given method. If the method is not found in the user's directory, then the directory `/vnmr/shimmethods` is searched.

Shimming methods can be used in succession or strung together. For example, Typing `method='z1z2' shim shim` would cause

the method in the file `z1z2` (Z1, Z2 shimming) to be used, as indicated by its code, twice in succession, and Typing `method='z1z2' shim method='nsm' shim shim shim` would cause the first method to be used once and the second method three times.

Shimming criteria for method autoshim

Two aspects of Autoshim must in some way be specified by the user. One is the resolution of the starting point—good or bad? If good, only small changes need to be made to the shim settings to find the optimum, if bad, larger changes are necessary. The second is how good must the final resolution be? Clearly, the better the desired resolution, the smaller the steps that Autoshim must take as it approaches the maximum, in order to find the absolute maximum to within a specified degree.

As shown in [Table 53](#), there are five criterion values: B (bad), L (loose), M (medium), T (tight), and E (excellent). (The lower-case letters are used when Typing criterion values into a shim method, discussed below).

Table 53 Permissible shimming criterion values

Criterion	Meaning	Recommended Usage
B or b	Bad	No decent starting shim values available
L or l	Loose	Extreme change in sample height
M or m	Medium	Typical sample change
T or t	Tight	Resolution desired above average
E or e	Excellent	Resolution desired less than 0.2 Hz

A full criterion consists of two letters, for example, `L > M` indicates a loose starting criterion (the shims are expected to be far from their desired values) and a medium ending criterion (end with “normal” shim quality). Tight and excellent are only used for extremely high resolution, where the beginning resolution is very nearly that desired.

Specify the starting and ending points as `L > M` if poor resolution is suspected and improvement is needed without spending the time necessary to get excellent resolution.

The duration of automatic shimming is a function of these criteria. Therefore, try to make an informed choice with respect to the resolution needed and, in particular, for FID shimming, the choice of acquisition time as specified in the

parameter table. An acquisition time of 2 seconds gives a limiting *digital* resolution of 0.5 Hz, a resolution that would be inconsistent with shimming to a tight criterion. In the interactive shimming mode using `acqi`, only the most important criteria are accessible to the user: L > M, M > M, M > T, and T > T (B and E are inaccessible).

The starting criterion should never affect the final result, only the time taken to get the result. If the starting criterion is specified as T, for example, and the optimum shim is far off, this shim will eventually be found. The search may take longer than if a starting criterion of L had been specified.

Shim methods for autoshim

Table 54 lists standard two-character codes for shim gradient combinations.

Table 54 Codes for standard shim gradient combinations

Standard code	Gradients	Hexadecimal code
z1	Z1C	0000008
z2	Z2C	0000020
z3	Z3	0000040
z4	Z4	0000080
z5	Z5	0000100
zq	Z1C,Z2C	0000028
zt	Z1C,Z2,Z3	0000068
zb	Z1C,Z2C,Z4	00000A8
za	Z1C,Z2C,Z3,Z4,Z5	00001E8
ze	Z2C,Z4	00000A0
zo	Z1C,Z3,Z5	0000148
zc	Z1C,Z2C,Z5	0000128
zm	User-selected gradients	User-Typeed
tx	X,Z1	0010004
ty	Y,Z1	0020004
t1	X,Y,Z1	0030004
t2	X,Y,XY,YZ,X2,Y2,Z1	03B0004
tz	X,Y,XZ,YZ,Z1	0270004
tt	X,Y,XZ,XY,X2Y2,YZ,Z1	03F0004

Table 54 Codes for standard shim gradient combinations (continued)

Standard code	Gradients	Hexadecimal code
t3	X,Y,XZ2,YZ2,Z1	5030004
t4	X,XZ,X3,XZ2,Z1	4450004
t5	Y,YZ,Y3,YZ2,Z1	1A20004
t6	XY,X2Y2,ZX2Y2,ZXY,Z1	A180004
t7	X,Y,XZ,XY,X2Y2,YZ,X3,Y3, YZ2,ZX2Y2,XZ2,ZXY,Z1	FFF0004
ta	X,Y,XZ,XY,X2Y2,YZ,YZ2,XZ 2,1	53F0004
tm	User-selected gradients	User-typed

A shim method consists of a text string contained in a file within the `shimmetholds` directory of either the `VnmrJ` system or a user. That text string is interpreted as a series of instructions describing the shimming method. Commands in elements include:

- Turn on and off the spinner
- Set maximum shim time per element
- Set the delay between lock level samplings
- Specify the gradients to be shimmed and the criterion used for shimming

A complete method consists of one or more elements, separated by commas and terminated with a semicolon (for example, `f,ry,t600,szq:cmm;`). The element setting for specific gradient or gradients to be shimmed has the syntax `sxx:cyz`, where `s` identifies the shim part of the form, `xx` is a two-character code for a specific shim gradient or gradient combination, `c` identifies the criterion part of the form, `y` is the starting criterion, and `z` is the desired ending criterion.

See the description of the `shimset` parameter in the *VnmrJ Command and Parameter Reference* for a list of shims in each type of shim set.

The following examples show the meaning of a few standard shim methods:

- `szq:cmm;` means set shims Z1C and Z2C with a medium to medium criterion.
- `sza:clm;` means shim all Z gradients with a loose to medium criterion.

- `szt:clm,szb:clm,szq:cmm`; means shim Z1C, Z2, and Z3 with a loose to medium criterion, then shim Z1C, Z2C, and Z4 with a loose to medium criterion, and lastly shim Z1C and Z2C with a medium to medium criterion.

User-defined shim methods for autoshim

The shim methods supplied with the system are based on a series of “standard” coil groupings. Automatic shimming operation using other groups of shims than are provided is supported by creating user-defined shim methods. For example, shimming Z1, Z3, and Z4 while holding Z2 fixed.

Certain combinations of shim coils can be selected by constructing a 7-digit hexadecimal (base 16) number based upon the following shim coil chart:

8	4	2	1	8	4	2	1	8	4	2	1	8	4	2	1	8	4	2	1	8	4	2	1	8	4	2	1
ZX _Y	XZ ₂	ZX ₂ Y ₂	YZ ₂	Y ₃	X ₃	YZ	X ₂ Y ₂	XY	XZ	Y ₁	X ₁							Z ₅	Z ₄	Z ₃	Z ₂ C	Z ₂	Z ₁ C	Z ₁	Z ₀		

To construct a method for this example, first notice in the diagram that Z1 is represented by a 1 in the first digit (on the far right) and that Z4 and Z3 are represented by a 8 and 4, respectively, in the second digit, which gives a total of 12 (or C in hexadecimal notation). The rest of the digits are 0 because no other shimming is desired. Thus, the seven-digit hexadecimal number representing Z1, Z3, and Z4 is 00000C4. This number is then prefixed by *zm* or *tm* (the two are equivalent) making *szm00000C4* the method desired.

Some examples of user-selected methods:

- `stm0A30004:clm;` means shim Z1, X1, Y1, YZ, Y3 with loose to medium criterion.

The hexadecimal code for each standard coil grouping is listed in the third column of [Table 54](#) on page 521.

The following codes enable control of other aspects of automatic shimming:

- `l` sets shimming on the lock instead of the FID (default).
- `f` sets shimming on the FID instead of the lock.
- `f:0,90` sets shimming on the FID with limits for the FID evaluation range. Full range is 0 to 100 percent of the duration of the FID. Sensitivity to higher-order spinning gradients is increased with a start of 0 and a finish limit of about 5 or 10, which weights the evaluation to the front of the FID.
- `ry` (rotation yes) turns the spinner on.

- `rn` (rotation no) turns the spinner off.
- `dx` sets a delay x hundredths of seconds between lock samplings. Variations in lock solvent T_1 and T_2 relaxation times affect the ability of automatic shimming to attain good resolution in reasonable times. If too short, automatic shimming will not be performed properly. If too long, the shimming will become unacceptable in duration. `dx` allows setting an appropriate delay and can be used one or more times within a text string. If no entry is made using `dx`, the system automatically measures the lock response and sets a delay accordingly.
- `tx` sets the maximum shimming time to x seconds. Once `tx` is set, it governs all future shim elements within a method string, just as `dx` governs the lock sampling interval for all shim elements until changed. If `tx` is not set, the shimming will proceed based on internal program criteria.
- `q` recalls an algorithm's internal parameters so that shimming starts quickly. `q` is a background autoshim that keeps the magnetic field at an optimum during experiments of long duration. Shimming is performed at the time `wshim` instructs. Only the portion of the shim methods following the letter `q` is executed after the experiment's first increment. Any shim method may follow `q`. However, the `sz1` (Z1 only) and `szq` (Z1, Z2) are the most effective. Multiple shim methods may follow `q`, but time effectiveness is reduced.

Methods may be typed into the `shimmethods` file using a text editor such as `vi`. The macros `newshim` and `stdshim` provide an interactive method of defining shim methods. Note that unlike normal text files, which have unrestricted size, the maximum text file size for a shim method is 128 characters.

The following examples show complete user-defined shim methods:

- `szq:cmm, rn, stz:cmm, ry, szq:cmm`; means shim Z1C, Z2C with medium to medium criterion, turn off spinner, shim X, Y, XZ, YZ, Z1 with medium to medium criterion, turn on spinner, and then shim Z1C, Z2C again with medium to medium criterion throughout.
- `d50, szq:cmm, d150, sza:cmm`; means to sample every 0.5 seconds while shimming Z1C, Z2C, and then to sample every 1.5 seconds.

B Shimming Basics

- `t60,szq:cmm,t240,sza:cmm`; means shim Z1C, Z2C for 60 seconds maximum, then shim all Z gradients for a maximum of 4 minutes. Use medium to medium criterion throughout.
- `f,ry,t600,szq:cmm`; means turn on spinner and FID shim Z1C, Z2C with medium to medium criterion for 10 minutes maximum (not available on *GEMINI 2000*).
- `t60,sza:cmm,q,t30,sz1:cmm`; (with `wshim='f20'`) means initially shim on all Z gradients for 60 seconds, then shim Z1. After every 20 FIDs, shim Z1 for 30 seconds.
- `sza:cmm,q,t30,szq:cmm`; (with `wshim='f10'`) means initially shim on all Z gradients (with no time out) and then perform a Z1, Z2 shim for 60 seconds every 10 FIDs.

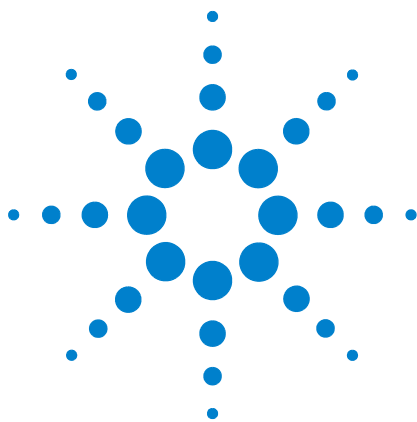
Homogeneity Commands and Parameter

Command	Description
dgs	Display shim and automation parameter group
diffshims(shimfile1,shimfile2)	Compare two sets of shims (VNMR)
diffshims shimfile1 shimfile2	Compare two sets of shims (Linux)
dshim<(file)>,dshim('method' 'help')	Display a shim "method" string
gmapshim<('files' 'mapname' 'quit')>	Start gradient autoshimming
gmapsys	Setup gradient shimming
newshm	Interactively create shim method
readallshims	Read all shims from hardware
readhw(par1,par2,...)<:var1,var2,...>	Read acquisition hardware values
rts(file)<:status>	Retrieve shim coil settings
setallshims	Set all shims into hardware
sethw*	Set acquisition hardware values (shim-related)
shim	Submit an Autoshim experiment
stdshm	Interactively create a shim method
Svs(file)<:status>	Save shim coil settings
*sethw(<'wait' 'nowait',>par1,va1<,par2,va2,...)	

Parameters

gmap_findtof {'n','y'}	Find tof before start of gradient shimming
gmap_z1z4 {'n','y'}	Gradient shim z1-z4, then higher-order shims
hdwshim {'n','y','p'}	Hardware shimming (if available)
hdwshimlist {'z1','z1z2x1y1'...}	List of shims for hardware shimming
load {'n','y'}	Load status of displayed shims
method {file in shimmetholds}	Autoshim method
shimset {1,2,3,...14}	Type of shim set
shimspath {absolute path}	Path to user's shims directory
wshim {'n','e','s','g','f','f#'}	Conditions when to shim
x1, y1, z1,...	Shim gradients X1, Y1, Z1...
z0 {-2048 to 2047, -32768 to 32767}	Z0 field position

B Shimming Basics



C Liquids Spectra

Ethylindanone	530
Azithromycin	535
Clindamycin	536
Paclitaxel	537
Sucrose	538
Vitamin B12	539
3-Acetyl-2-Fluoropyrimidine	540

Following is a short description of the liquids spectra found in the /vnmr/fidlib directory.



Ethylindanone

	<i>Sample</i>	Ethylindanone (M = 160), 106 mM in CDCl ₃
	<i>Spectrometer</i>	ProPulse 500 + OneNMR probe
	<i>Directory</i>	/vnmr/fidlib/Ethylindanone
	<i>Molecule</i>	/vnmr/molib/2-ethyl-1-indanone.mol
Ethylindanone_PROTON_01	30 s	1D ¹ H (8 scans)
Ethylindanone_PROTON_02	1 min 0 s	1D ¹ H without/with homonuclear decoupling (homo='y','n' 2×4 scans)
Ethylindanone_PROTON_03	6 s	1D ¹ H (1 scan)
Ethylindanone_CARBON_01	8 min 44 s	1D ¹³ C (256 scans)
Ethylindanone_INVREC_01	19 min 42 s	1D Inversion Recovery to measure T1 relaxation (see "Process->T1 Analysis" panel for analysis options and results, array: 10×4 scans)
Ethylindanone_CPMGT2_01	8 min 29 s	1D CPMG (Carr-Purcell-Meiboom-Gill) experiment to measure T2 relaxation (see "Process->T2 Analysis" panel for analysis options and results, array: 9×4 scans)
Ethylindanone_APT_01	8 min 57 s	1D ¹³ C APT (Attached Proton Test) (256 scans)
Ethylindanone_DEPT_01	2 min 20 s	1D DEPT (DEPT-135, CH & CH ₃ positive, CH ₂ negative, 64 scans)
Ethylindanone_DEPT_02	8 min 55 s	1D DEPT (full editing, separate CH ₃ , CH ₂ , CH and all CH _x spectra, quaternary carbons suppressed, 4×64 scans)
Ethylindanone_DEPT_03	26 min 20 s	1D DEPT (full editing including quaternaries, separate CH ₃ , CH ₂ , CH and CQ spectra, 8×64 scans)
Ethylindanone_CRAPT_01	8 min 55 s	1D CRAPT (CRISIS APT, 256 scans)
Ethylindanone_PS1D_01	10 min 5 s	PureShift 1D
Ethylindanone_HOMODEC_01	21 s	1D ¹ H with homonuclear decoupling and presaturation of irradiated signal (HOMODEC sequence, 4 scans) Selected band: 150 Hz @ 3.328 ppm
Ethylindanone_CARBONecho_01	8 min 45 s	1D ¹³ C CARBONecho pulse sequence (256 scans)
Ethylindanone_gCOSY_01	3 min 10 s	2D gradient COSY (128 increments × 1 scan)
Ethylindanone_COSY_01	5 min 42 s	2D COSY (128 increments × 2 scans)
Ethylindanone_gDQCOSY_01	8 min 30 s	2D gradient double quantum filtered COSY (200 increments × 1 scan)
Ethylindanone_DQCOSY_01	32 min 1 s	2D double quantum filtered COSY (200 increments × 4 scans)
Ethylindanone_TOCSY_01	17 min 27 s	2D TOCSY (TOtal Correlation SpectroscopY) (200 increments × 2 scans)

Ethylindanone_zTOCSY_01	18 min 30 s	2D TOCSY (TOtal Correlation SpectroscopY) with a zero quantum filter (200 increments × 2 scans)
Ethylindanone_ROESY_01	57 min 52 s	2D ROESY (500 ms spinlock, 128 increments × 8 scans)
Ethylindanone_ROESYAD_01	58 min 2 s	2D ROESY (500 ms adiabatic spinlock, 128 increments × 8 scans)
Ethylindanone_NOESY_01	1 h 8 min	2D NOESY (800 ms mixing) with a zero quantum filter (128 increments × 8 scans)
Ethylindanone_zCOSY_01	21 min 56 s	2D z-COSY for long-range H–H coupling determination (512 increments × 1 scan)
Ethylindanone_HSQCAD_01	8 min 48 s	2D 1H–13C HSQC with adiabatic pulses (96 increments × 2 scans)
Ethylindanone_gHSQCAD_01	8 min 48 s	2D 1H–13C gradient HSQC with adiabatic pulses (96 increments × 2 scans)
Ethylindanone_gHSQCAD_02	4 min 44 s	2D 1H–13C gradient HSQC with adiabatic pulses (128 increments × 2 scans, NUS 50%)
Ethylindanone_gHSQCAD_03	3 min 43 s	2D 1H–13C gradient HSQC with adiabatic pulses (128 increments × 2 scans, NUS 37.5%)
Ethylindanone_gHSQCAD_04	2 min 42 s	2D 1H–13C gradient HSQC with adiabatic pulses (128 increments × 2 scans, NUS 25%)
Ethylindanone_HSQC_01	8 min 14 s	2D 1H–13C HSQC (96 increments × 2 scans)
Ethylindanone_gHSQC_01	8 min 14 s	2D 1H–13C gradient HSQC (96 increments × 2 scans)
Ethylindanone_HMQC_01	8 min 8 s	2D 1H–13C HMQC (96 increments × 2 scans)
Ethylindanone_gHMQC_01	8 min 11 s	2D 1H–13C gradient HMQC (96 increments × 2 scans)
Ethylindanone_HETCOR_01	4 h 59 min	2D 13C–1H HETCOR (128 increments × 64 scans)
Ethylindanone_gHETCOR_01	4 h 59 min	2D 13C–1H gradient HETCOR (128 increments × 64 scans)
Ethylindanone_ASAPHMQC_01	1 min 49 s	2D 1H–13C ASAP-HMQC (very fast 2D HMQC, 96 increments × 4 scans)
Ethylindanone_gHMBCAD_01	1 h 7 min	2D 1H–13C gradient HMBC with adiabatic pulses (200 increments × 8 scans)
Ethylindanone_gHMBCAD_02	33 min 33 s	2D 1H–13C gradient HMBC with adiabatic pulses (200 increments × 8 scans, NUS 50%)
Ethylindanone_gHMBCAD_03	25 min 19 s	2D 1H–13C gradient HMBC with adiabatic pulses (200 increments × 8 scans, NUS 37.5%)
Ethylindanone_gHMBCAD_04	17 min 5 s	2D 1H–13C gradient HMBC with adiabatic pulses (200 increments × 8 scans, NUS 25%)
Ethylindanone_gHMBC_01	1 h 7 min	2D 1H–13C gradient HMBC (200 increments × 8 scans)
Ethylindanone_gH2BCAD_01	51 min 51 s	2D 1H–13C gradient H2BC (2-bond specific long-range correlation) with adiabatic pulses (160 increments × 8 scans)
Ethylindanone_gH2BC_01	51 min 49 s	2D 1H–13C gradient H2BC (2-bond specific long-range correlation) (160 increments × 8 scans)
Ethylindanone_CIGAR_01	1 h 9 min	2D 1H–13C CIGAR (HMBC-type) experiment (200 increments × 8 scans)

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Ethylindanone_CIGARAD_01	1 h 9 min	2D 1H-13C CIGAR (HMBC-type) experiment with adiabatic pulses (200 increments × 8 scans)
Ethylindanone_HMBC_01	1 h 6 min	2D 1H–13C HMBC (200 increments × 8 scans)
Ethylindanone_gHMBCmeAD_01	2 h 13 min	2D 1H–13C gradient HMBC with adiabatic pulses (multiple-bond correlation with multiplicity editing: subspectra available on the "Process->Default" panel, 200 increments × 8 scans)
Ethylindanone_gHMBCRELAY_01	43 min 44 s	2D 1H-13C gradient relay-HMBC (process as 2J(CH) or nJ(CH) subspectrum on the Process->Default panel, 128 increments × 8 scans)
Ethylindanone_NOESY1D_01	4 min 22 s	1D selective NOESY (DPFGSE) zero quantum filtered, 800 ms mixing time, irradiation band: 150 Hz @ 3.328 ppm (array: 8, 64 scans without/with mixing time)
Ethylindanone_ROESY1D_01	3 min 54 s	1D selective ROESY (DPFGSE), 300 ms spinlock time, irradiation band: 150 Hz @ 3.328 ppm (array: 8, 64 scans without/with spinlock time)
Ethylindanone_TOCSY1D_01	57 s	1D selective TOCSY (DPFGSE), 80 ms TOCSY mixing time, irradiation band: 150 Hz @ 3.328 ppm (array: 8, 16 scans without/with mixing time)
Ethylindanone_zTOCSY1D_01	59 s	1D selective TOCSY with a zero quantum filter (DPFGSE), 80 ms TOCSY mixing time, irradiation band: 150 Hz @ 3.328 ppm (array: 8, 16 scans without/with mixing time)
Ethylindanone_selexcit_01	56 s	1D selective DPGSE experiment, irradiation bands: 150 Hz @ 3.328 ppm, 150 Hz @ 1.553 ppm (16 scans)
Ethylindanone_stepNOESY1D_01	58 s	1D dual-selective TOCSY–NOESY, TOCSY (STEP): off/on (stepmix=0,0.08), STEP band: 150 Hz @ 3.328 ppm, subsequent NOE irradiation: off (array: 2 × 8 scans)
Ethylindanone_stepNOESY1D_02	4 min 16 s	1D dual-selective TOCSY–NOESY, TOCSY (STEP): on (stepmix=0.08), STEP band: 150 Hz @ 3.328 ppm, subsequent NOE irradiation: on (mixN=0.5) NOE band: 120 Hz @ 2.831 ppm (64 scans)
Ethylindanone_bsHSQCAD_01	3 min 13 s	2D band selective 1H–13C HSQC with adiabatic pulses (120–140 ppm selected in F1, 32 increments × 2 scans)
Ethylindanone_bsgHSQCAD_01	3 min 14 s	2D band selective 1H–13C gradient HSQC with adiabatic pulses (120–140 ppm selected in F1, 32 increments × 2 scans)
Ethylindanone_bsgHMBC_01	21 min 52 s	2D band selective 1H–13C gradient HMBC with adiabatic pulses (120–140 ppm selected in F1, 32 increments × 2 scans)
Ethylindanone_EXSIDE_01	2 h 47 min	2D 1H-13C EXSIDE (determination of long-range C–H coupling constants), selected bands: 150 Hz @ 3.328 ppm, 150 Hz @ 1.553 ppm (768 increments × 4 scans)

Ethylindanone_bsHSQCNOESY_01	48 min 54 s	2D band selective 1H–13C gradient HSQC–NOESY with adiabatic pulses (120–140 ppm selected in F1, 32 increments × 32 scans)
Ethylindanone_bsHSQCROESY_01	52 min 32 s	2D band selective 1H–13C gradient HSQC–ROESY with adiabatic pulses (120–140 ppm selected in F1, 32 increments × 32 scans)
Ethylindanone_bashdROESY_01	7 min 39 s	2D band selective ROESY (7–8 ppm selected in F1, 400 ms spinlock, 32 increments × 4 scans)
Ethylindanone_bashdTOCSY_01	3 min 36 s	2D band selective TOCSY (7–8 ppm selected in F1, 80 ms spinlock, 32 increments × 2 scans)
Ethylindanone_bashdNOESY_01	9 min 34 s	2D band selective NOESY (7–8 ppm selected in F1, 800 ms mixing time, 32 increments × 4 scans)
Ethylindanone_c2hsqcse_01	15 min 54 s	2D 1H–13C CRISIS-2 HSQC (shaped pulses on both high and low band channel) with sensitivity enhancement (96 increments × 4 scans)
Ethylindanone_c2hsqc_01	8 min 16 s	2D 1H–13C CRISIS-2 HSQC (shaped pulses on both high and low band channel, 96 increments × 2 scans)
Ethylindanone_gc2h2bcme_01	1 h 18 min	2D 1H–13C CRISIS-2 gradient H2BC (2-bond specific long-range correlation, shaped pulses on both high and low band channel) with multiplicity editing: subspectra available on the "Process->Default" panel (C/CH2 and CH/CH3, 102 increments × 8 scans)
Ethylindanone_gc2h2bc_01	39 min 4 s	2D 1H–13C CRISIS-2 gradient H2BC (2-bond specific long-range correlation, shaped pulses on both high and low band channel, 120 increments × 8 scans)
Ethylindanone_gc2hmbcme_01	2 h 13 min	2D 1H–13C CRISIS-2 gradient HMBC (shaped pulses on both high and low band channel, multiple-bond correlation with multiplicity editing: subspectra available on the "Process->Default" panel (C/CH2 and CH/CH3, 200 increments × 8 scans)
Ethylindanone_gc2hmbc_01	1 h 7 min	2D 1H–13C CRISIS-2 gradient HMBC (shaped pulses on both high and low band channel, 200 increments × 8 scans)
Ethylindanone_gc2hmbc_02	33 min 32 s	2D 1H–13C CRISIS-2 gradient HMBC (shaped pulses on both high and low band channel, 200 increments × 8 scans , NUS: 50%)
Ethylindanone_gc2hmbc_03	25 min 18 s	2D 1H–13C CRISIS-2 gradient HMBC (shaped pulses on both high and low band channel, 200 increments × 8 scans , NUS: 37.5%)
Ethylindanone_gc2hmbc_04	17 min 5 s	2D 1H–13C CRISIS-2 gradient HMBC (shaped pulses on both high and low band channel, 200 increments × 8 scans , NUS: 25%)
Ethylindanone_gc2hsqcse_01	8 min 18 s	2D 1H–13C CRISIS-2 gradient HSQC (shaped pulses on both high and low band channel) with sensitivity enhancement (96 increments × 2 scans)

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Ethylindanone_gc2hsqc_01	8 min 16 s	2D 1H–13C CRISIS-2 gradient HSQC (shaped pulses on both high and low band channel, 96 increments × 2 scans)
Ethylindanone_HSQCADTOXY_01	22 min 19 s	2D 1H–13C HSQC–TOCSY with adiabatic pulses (80 ms spinlock, 128 increments × 4 scans)
Ethylindanone_gHSQCADTOXY_01	1 h 28 min	2D 1H–13C gradient HSQC–TOCSY with adiabatic pulses (80 ms spinlock, 128 increments × 16 scans)
Ethylindanone_HSQCCTOXY_01	22 min 22 s	2D 1H–13C HSQC–TOCSY (80 ms spinlock, direct correlations inverted, 128 increments × 4 scans)
Ethylindanone_gHSQCCTOXY_01	1 h 28 min	2D 1H–13C gradient HSQC–TOCSY (80 ms spinlock, direct correlations inverted, 128 increments × 16 scans)
Ethylindanone_HMQCTOXY_01	22 min 13 s	2D 1H–13C HMQC–TOCSY (80 ms spinlock, direct correlations inverted, 128 increments × 4 scans)
Ethylindanone_gHMQCTOXY_01	1 h 27 min	2D 1H–13C gradient HMQC–TOCSY (80 ms spinlock, direct correlations inverted, 128 increments × 16 scans)
Ethylindanone_HETERO2DJ_01	1 h 13 min	2D HETERO2DJ (JCH-resolved, 64 increments × 32 scans)
Ethylindanone_HOMO2DJ_01	6 min 24 s	2D HOMO2DJ (J-resolved, 64 increments × 2 scans)
Ethylindanone_dqcosyHT_01	45 s	2D Hadamard DQCOSY (45 Hz HT bandwidth, 16 increments × 2 scans)
Ethylindanone_gcosyHT_01	25 s	2D Hadamard gCOSY (45 Hz HT bandwidth, 16 increments × 1 scan)
Ethylindanone_hetcorHT_01	10 min 13 s	2D Hadamard HETCOR (45 Hz HT bandwidth, 16 increments × 32 scans)
Ethylindanone_hmbcHT_01	11 min 41 s	2D Hadamard HMBC (45 Hz HT bandwidth, 16 increments × 32 scans)
Ethylindanone_hsqcHT_01	40 s	2D Hadamard HSQC (45 Hz HT bandwidth, 16 increments × 2 scans)
Ethylindanone_hsqctocsyHT_01	2 min 31 s	2D Hadamard HSQCCTOCSY (20 Hz HT bandwidth, 16 increments × 8 scans)
Ethylindanone_noesyHT_01	4 min 9 s	2D Hadamard NOESY (45 Hz HT bandwidth, 16 increments × 8 scans)
Ethylindanone_roesyHT_01	2 min 23 s	2D Hadamard ROESY (45 Hz HT bandwidth, 16 increments × 8 scans)
Ethylindanone_tocsyHT_01	39 s	2D Hadamard TOCSY (45 Hz HT bandwidth, 16 increments × 2 scans)

Azithromycin

	<i>Sample</i>	Azithromycin dihydrate antibiotic (M = 784) 10 mg in CDCl ₃
	<i>Spectrometer</i>	400-MR + OneNMR probe
	<i>Fid Directory</i>	/vnmr/fidlib/Azithromycin
	<i>Molecule</i>	/vnmr/molib/Azithromycin.mol
Azithromycin_PROTON_01.fid	4 s	1D 1H (1 scan)
Azithromycin_ASAPHMQC_01.fid	1.5 min	2D 1H-13C ASAP-HMQC (fast 2D HMQC)
Azithromycin_gHSQCAD_01.fid	6 min	2D 1H-13C HSQC
Azithromycin_CARBON_01.fid	1 h 16 min	1D 13C (2000 scans)
Azithromycin_gDQCOSY_01.fid	9 min	2D gradient double quantum filtered COSY
Azithromycin_zTOCSY_01.fid	11 min	2D z-TOCSY with zero quantum suppression
Azithromycin_gHMBCAD_01.fid	25 min	2D 1H-13C gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Azithromycin_gHMBCAD_02.fid	1 h 40 min	2D 1H-15N gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Azithromycin_gH2BCAD_02.fid	44 min	2D 1H-13C gradient H2BC with adiabatic pulses (2-bond correlation, 8 Hz)
Azithromycin_NOESY_01.fid	2 h 59 min	2D NOESY with zero quantum suppression

Clindamycin

	<i>Sample</i>	Clindamycin phosphate antibiotic (M = 425) 10 mg in D2O
	<i>Spectrometer</i>	400-MR + OneNMR probe
	<i>Directory</i>	/vnmr/fidlib/Clindamycin
	<i>Molecule</i>	/vnmr/molib/Clindamycin.mol
Clindamycin_PROTON_01.fid	4 s	1D 1H (1 scan)
Clindamycin_CARBON_01.fid	178 min	1D 13C (2048 scans)
Clindamycin_PHOSPHORUS_01.fid	2 min	1D 31P shows large (phosphate) and medium signals
Clindamycin_PHOSPHORUS_02.fid	30 min	1D 31P shows large, medium and small signals (only the small ones show up in the 1H-31P gHMBCAD)
Clindamycin_gDQCOSY_01.fid	16 min	2D gradient double quantum filtered COSY
Clindamycin_ASAPHMQC_01.fid	1 min	2D 1H-13C ASAP-HMQC (fast 2D HMQC)
Clindamycin_gHSQCAD_01.fid	8 min	2D 1H-13C gradient HSQC with adiabatic pulses and multiplicity editing
Clindamycin_gHMBCAD_01.fid	1 h 6 min	2D 1H-13C gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Clindamycin_gHMBCAD_02.fid	21 min	2D 1H 31P gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Clindamycin_gHMBCAD_03.fid	11 h 13 min	2D 1H-15N gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)

Paclitaxel

<i>Sample</i>	Paclitaxel (Taxol) (M = 854) 10 mg in CDCl ₃
<i>Spectrometer</i>	400-MR + OneNMR probe
<i>Directory</i>	/vnmr/fidlib/Paclitaxel
<i>Molecule</i>	/vnmr/molib/Paclitaxel.mol

Paclitaxel_PROTON_01.fid	4 s	1D 1H (1 scan)
Paclitaxel_ASAPHMOC_01.fid	1 min 32 s	2D 1H-13C ASAP-HMOC (fast 2D HMOC)
Paclitaxel_gDQCOSY_01.fid	21 min	2D gradient double quantum filtered COSY
Paclitaxel_gHSQCAD_01.fid	8 min	2D 1H-13C gradient HSQC with adiabatic pulses and multiplicity editing
Paclitaxel_zTOCSY_01.fid	24 min	2D z-TOCSY with zero quantum suppression
Paclitaxel_CARBON_01.fid	77 min	1D 13C (2048 scans)
Paclitaxel_NOESY_01.fid	3 h 50 min	2D NOESY with zero quantum suppression
Paclitaxel_gHMBCAD_01.fid	2 h 49 min	2D 1H-13C gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Paclitaxel_gHSQCAD_02.fid	42 min	2D 1H-15N gradient HSQC with adiabatic pulses and multiplicity editing
Paclitaxel_gHMBCAD_02.fid	5 h 36 min	2D 1H-15N gradient HMBC with adiabatic pulses (multiple bond correlation, 8 Hz)
Paclitaxel_gH2BCAD_01.fid	1 h 27 min	2D 1H-13C gradient H2BC with adiabatic pulses (2-bond correlation, 8 Hz)

Sucrose

Sample: Sucrose (Saccharose) (M = 342), saturated solution (~4-5 M) in D2O
Spectrometer: 400-MR + OneNMR probe
Directory: /vnmr/fidlib/Sucrose
Molecule: /vnmr/molib/Sucrose.mol

Sucrose_PROTON_01.fid	6 s	1D 1H (1 scan)
Sucrose_CARBON_01.fid	4 s	1D 13C (1 scan)
Sucrose_gHETCOR_01.fid	2 min 41 s	2D 13C-1H gradient HETCOR (13C detection)
Sucrose_ASAPHMQC_01.fid	13 s	1H-13C ASAP-HMQC (fast 2D)
Sucrose_ADEQUATEAD_1.fid	1 h 40 min	2D ADEQUATE with adiabatic pulses
Sucrose_INADEQUATE_01.fid	5 h 19 min	2D INADEQUATE (13C-13C double quantum correlation)
Sucrose_INADEQUATEAD_01.fid	5 h 19 min	2D INADEQUATE with adiabatic pulses (13C-13C double quantum correlation)

Vitamin B12

Sample: Vitamin B12
(5'-Desoxyadenosyl-Cobalamin) (M = 1580),
6 mg in 9:1 H₂O:D₂O

Spectrometer: VNMRS-500 + OneNMR probe

Directory: /vnmr/fidlib/VitaminB12

Molecule: /vnmr/mollib/VitaminB12.mol

VitaminB12_PROTON_01.fid	10 s	1D 1H (4 scans)
VitaminB12_PRESAT_01.fid	40 s	1D 1H PRESAT water suppression (8 scans)
VitaminB12_PRESAT_02.fid	40 s	1D 1H PRESAT with PURGE water suppression, PURGE cycle = 2 (8 scans)
VitaminB12_PRESAT_03.fid	40 s	1D 1H PRESAT with PURGE water suppression, PURGE cycle = 4 (8 scans)
VitaminB12_wet1D_01.fid	31 s	1D 1H wet1D water suppression (8 scans)
VitaminB12_gDQCOSY_01.fid	24 min 15 s	2D gradient double quantum COSY with PURGE water suppression
VitaminB12_zTOCSY_01.fid	36 min 48 s	2D z-TOCSY (Total Correlation SpectroscopY along z-axis), with zero quantum suppression and PURGE water suppression
VitaminB12_NOESY_01.fid	1 h 57 min	2D NOESY with (500 ms mixing time), with zero quantum suppression and PURGE water suppression
VitaminB12_gHSQCAD_01.fid	21 min 32 s	2D 1H-13C gradient HSQC with adiabatic pulses and PURGE water suppression
VitaminB12_gHSQCAD_02.fid	42 min 44 s	2D 1H-15N gradient HSQC with adiabatic pulses and PURGE water suppression
VitaminB12_gHMBCAD_01.fid	1 h 26 min	2D 1H-13C gradient HMBC with PURGE water suppression
VitaminB12_gHMBCAD_02.fid	5 h 43 min	2D 1H-15N gradient HMBC with PURGE water suppression

3-Acetyl-2-Fluoropyrimidine

<i>Sample</i>	3-Acetyl-2-Fluoropyrimidine (M = 271), 10 mg in 9:1 H ₂ O:D ₂ O	
<i>Spectrometer</i>	VNMRS-500 with dual high-band channels + AutoX-HFX triple resonance probe	
<i>Directory</i>	/vnmr/fidlib/3-Acetyl-2-Fluoropyrimidine	
<i>Molecule</i>	/vnmr/mollib/3-Acetyl-2-Fluoropyrimidine.mol	
3-Acetyl-2-Fluoropyrimidine_PROTON_01.fid	6 s	1D 1H (1 scan) without/with 19F decoupling
3-Acetyl-2-Fluoropyrimidine_FLUORINE_01.fid	4 s	1D 19F (1 scan) without/with 1H decoupling
3-Acetyl-2-Fluoropyrimidine_FH_gHETCOR_01.fid	2 min 45 s	2D 19F-1H gHETCOR
3-Acetyl-2-Fluoropyrimidine_gDQCOSY_01.fid	3 min 56 s	2D 19F-19F gradient DQCOSY
3-Acetyl-2-Fluoropyrimidine_gHSQCAD_01.fid	2 min 38 s	2D 19F-13C gradient HSQC with adiabatic pulses and dual 13C & 1H decoupling
3-Acetyl-2-Fluoropyrimidine_gHSQCAD_02.fid	5 min 15 s	2D 1H-13C gradient HSQC with adiabatic pulses and dual 13C & 19F decoupling
3-Acetyl-2-Fluoropyrimidine_gHMBCAD_01.fid	42 min 47 s	2D 1H-13C gradient HMBC with adiabatic pulses and 19F decoupling
3-Acetyl-2-Fluoropyrimidine_CARBON_01.fid	4 h 36 min	1D 13C (2048 scans): a) without b) with 19F c) with 1H and d) with dual 19F & 1H decoupling
3-Acetyl-2-Fluoropyrimidine_FH_HOESY_01.fid	53 min 36 s	2D 19F-1H HOESY (heteronuclear NOE)



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