Spectroscopy

User Guide

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VARIAN

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Contents

Chapter 1 Introduction

Varian MR systems provide unmatched productivity for diverse chemical applications by combining easy-to-use software with outstanding performance. Push-button experiments, along with straightforward processing and data export capabilities, make the MR the best choice for compound detection, quantification and structure confirmation.

1.1 About This User Guide

This user guide contains the following information on Spectroscopy.

- Chapter 1 Introduction
- Chapter 2 Running Liquids NMR Experiments
- Chapter 3 Preparing for an Experiment
- Chapter 4 Experiment Setup
- Chapter 5 Gradient Shimming
- Chapter 6 Data Acquisition
- Chapter 7 Processing Data
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- Chapter 9 Printing, Plotting, and Data Output
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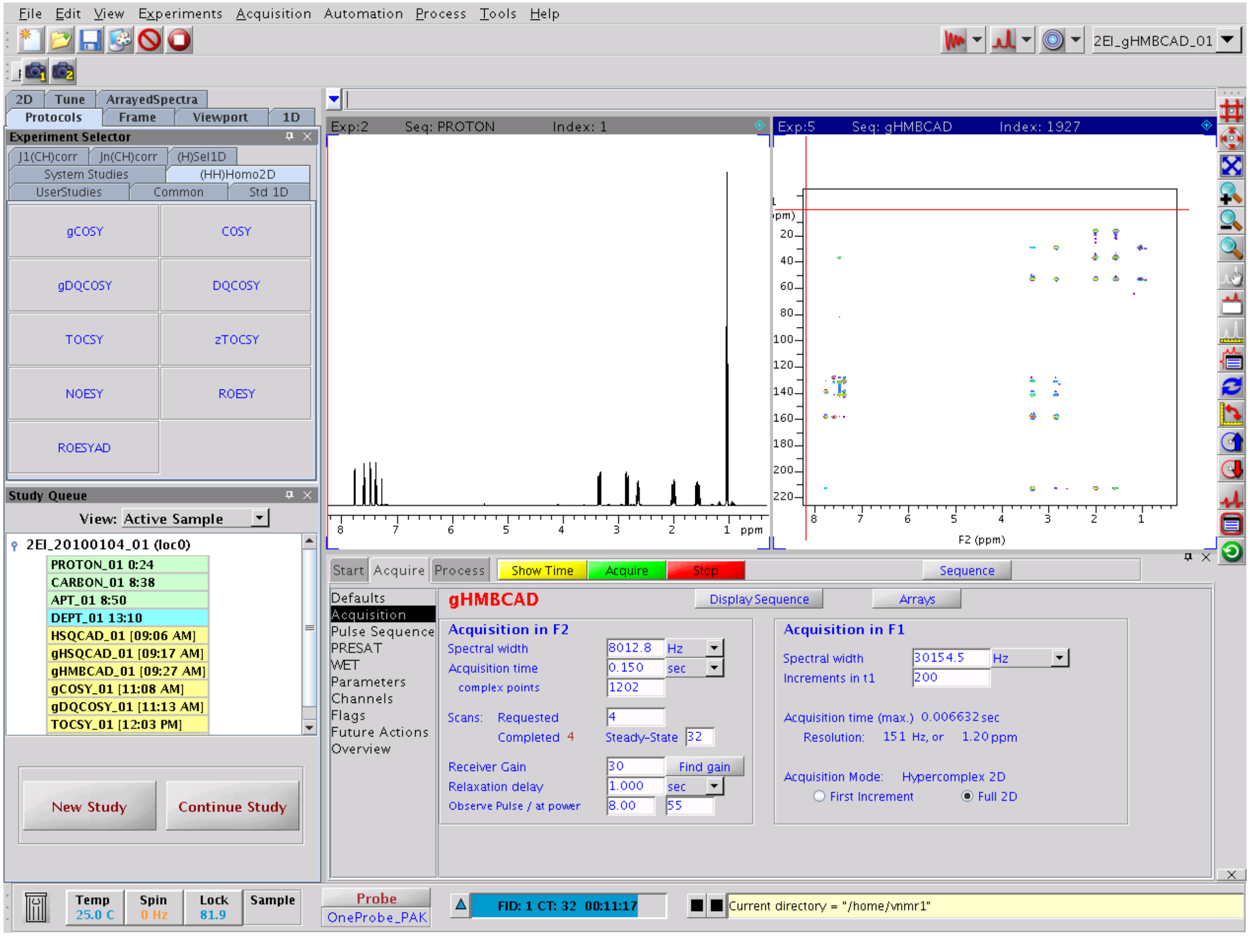
Chapter 2 Running Liquids NMR Experiments

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 *Automation User Guide*, see Figure 1.

Sections in this chapter include:

- 2.1 NMR Experiment Tasks
- 2.2 Saving NMR Data (Optional)
- 2.3 Stopping an Experiment



View: Active Sample

2EI_20100104_01 (loc0)

- PROTON_01 0:24
- CARBON_01 8:38
- APT_01 8:50
- DEPT_01 13:10
- HSQCAD_01 [09:06 AM]
- gHSQCAD_01 [09:17 AM]
- gHMBCAD_01 [09:27 AM]
- gCOSY_01 [11:08 AM]
- gDQCOSY_01 [11:13 AM]
- TOCSY_01 [12:03 PM]

gHMBCAD

Acquisition in F2

Spectral width 8012.8 Hz

Acquisition time 0.150 sec

complex points 1202

Scans: Requested 4

Completed 4 Steady-State 32

Receiver Gain 30

Relaxation delay 1.000 sec

Observe Pulse / at power 8.00 55

Acquisition in F1

Spectral width 30154.5 Hz

Increments in t1 200

Acquisition time (max.) 0.006632 sec

Resolution: 151 Hz, or 1.20 ppm

Acquisition Mode: Hypercomplex 2D

○ First Increment ● Full 2D

Temp 25.0 C

Spin 0 Hz

Lock 81.9

Probe OneProbe_PAK

FID: 1 CT: 32 00:11:17

Current directory = "/home/vnmr1"

Figure 1 VnmrJ Spectroscopy Interface

2.1 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	Refer to Prepare for an Experiment
Select an experiment	Refer to Selecting an Experiment
Set up an experiment	Refer to Experiment Setup
Acquire NMR data	Refer to Data Acquisition
Process the data	Refer to Process the Data
Display the data	Refer to Displaying FIDs and Spectra
Print the data	Refer to Printing, Plotting, and Data Output
Save the data	Refer to Saving NMR Data (Optional)

2.1.1 Prepare for an Experiment

Perform the following tasks before beginning an experiment:

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

See Preparing for an Experiment.

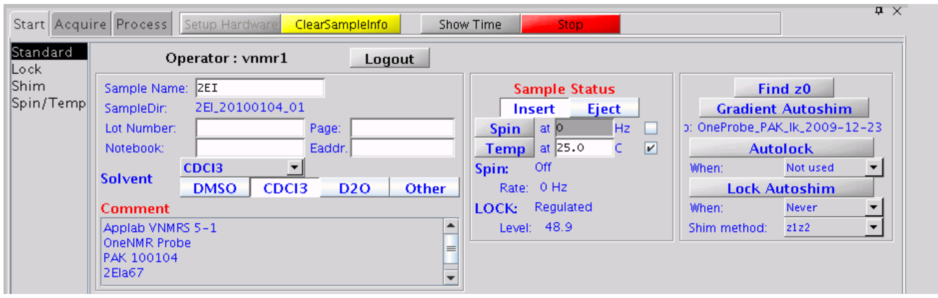
2.1.2 Select an Experiment

Select an experiment from the Protocols vertical panel or the Experiments menu, or drag-and-drop a protocol from the Locator. For more information, refer to Selecting an Experiment.

2.1.3 Set Up an Experiment

The VnmrJ experimental setup and the functions available under the Start tab are described in Experiment Setup.

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



Operator : vnmr1

Sample Name: 2EI

SampleDir: 2EI_20100104_01

Lot Number:

Page:

Notebook:

Eaddr.

Solvent: CDCl3

Comment:

Applab VNMRS 5-1
OneNMR Probe
PAK 100104
2Ela67

Spin at 0 Hz ☐

Temp at 25.0 C ☑

Spin: Off

Rate: 0 Hz

LOCK: Regulated

Level: 48.9

ɔ: OneProbe_PAK_lk_2009-12-23

When: Not used

When: Never

Shim method: z1z2

1. Select the **Standard** page.

   Fill in the information for the sample, select a **Solvent**, and enter comments. Enter a name in the **Sample Name** field to name the sample. Define the sample, if desired, by filling in the optional **Lot Number**, **Notebook**, **Page**, **Eaddr**, and **Comment** fields. Insert the sample.

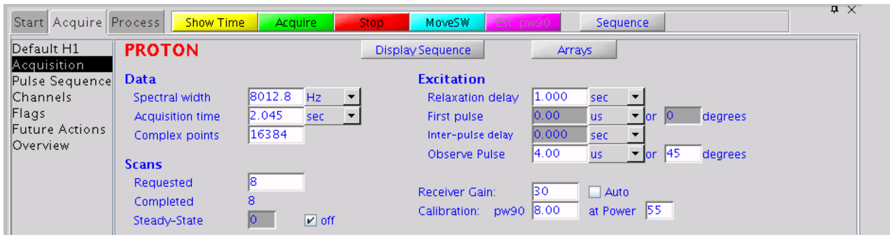
2. Regulate spinning and temperature on the **Spin/Temp** page.

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.

2.1.4 Acquire a Spectrum

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



PROTON

Data

Spectral width 8012.8 Hz

Acquisition time 2.045 sec

Complex points 16384

Scans

Requested 8

Completed 8

Steady-State 0 ☑ off

Excitation

Relaxation delay 1.000 sec

First pulse 0.00 us or 0 degrees

Inter-pulse delay 0.000 sec

Observe Pulse 4.00 us or 45 degrees

Receiver Gain: 30 ☐ Auto

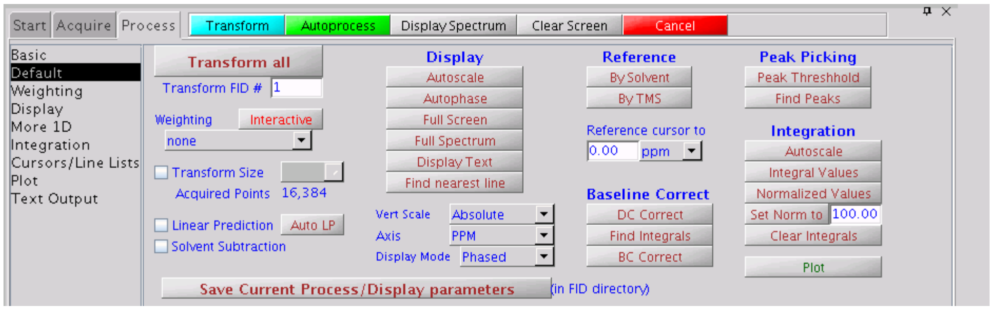
Calibration: pw90 8.00 at Power 55

1. Set up experimental parameters and post acquisition actions.

2. Click the **Acquire** button to acquire NMR data.

2.1.5 Process the Data

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



Transform FID # 1

Weighting none

☐ Transform Size

Acquired Points 16,384

☐ Linear Prediction

☐ Solvent Subtraction

Vert Scale Absolute

Axis PPM

Display Mode Phased

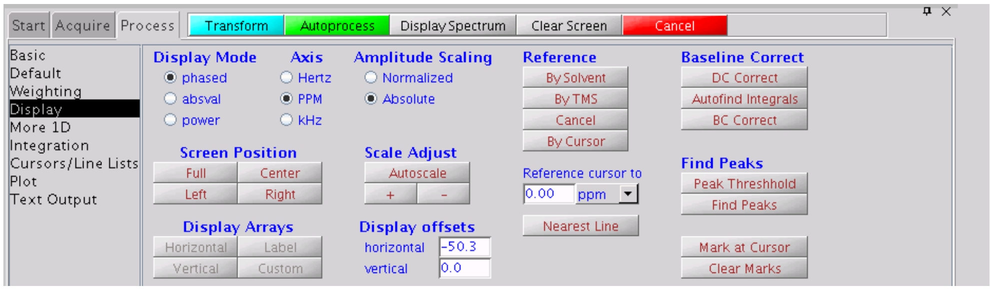
Reference cursor to 0.00 ppm

Set Norm to 100.00

(in FID directory)

2.1.6 Display the Data

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



Display Mode: ● phased ○ absval ○ power

Axis: ○ Hertz ● PPM ○ kHz

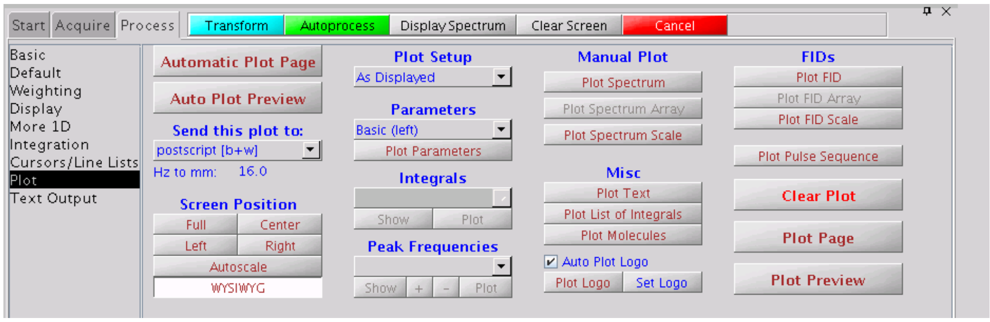
Amplitude Scaling: ○ Normalized ● Absolute

Reference cursor to 0.00 ppm

Display offsets: horizontal -50.3, vertical 0.0

2.1.7 Print or Plot the Data

VnmrJ data display is described in Printing, Plotting, and Data Output. Use the **Plot** page to create a print or plot.



Plot Setup: As Displayed

Parameters: Basic (left)

Send this plot to: postscript [b+w]

Hz to mm: 16.0

☑ Auto Plot Logo

2.2 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 on the **Acquire** tab, **Future Actions** tab, and **Save FID Now** button.
Main VnmrJ Menu Options	Click on **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 *VnmrJ Installation and Administration* manual). VnmrJ default data saving templates include DATE (producing filenames such as 20040921_01.fid), and seqfil (e.g. HMQC_01.fid).

2.3 Stopping an Experiment

There are four ways to stop an experiment:

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

Chapter 3 Preparing for an Experiment

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.

Sections in this chapter:

- 3.1 Starting VnmrJ
- 3.2 Preparing the Sample
- 3.3 Ejecting and Inserting the Sample
- 3.4 Loading a Probe File
- 3.5 Tuning Probes on Systems with ProTune
- 3.6 Tuning Probes on Standard Systems

3.1 Starting VnmrJ

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

3.2 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 shimming, or even the need to shim, is largely dependent upon the care in controlling the effects of these contact points.

3.2.1 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.

3.2.2 Setting the Sample Height

Experimentation and calculation show that the liquid column length 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; 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. Refer to 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 that 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 µL	50 mm	68 mm (65–69 mm)
600 µL	42 mm	65 mm (63–67 mm)
500 µL	36 mm	62 mm (60–64 mm)
400 µL	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.

3.2.3 Sample Position Using the Depth Gauge

Set the sample position to a repeatable position. Use the sample depth gauge provided, shown in Figure 2. (Use location 0 on the sample tray if an SMS sample changer is present.)

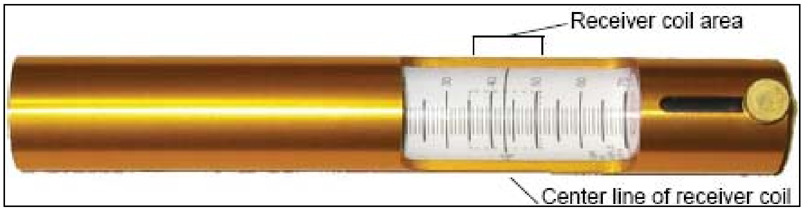


Figure 2 Sample Depth Gauge

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.

3.2.4 Sample Tubes

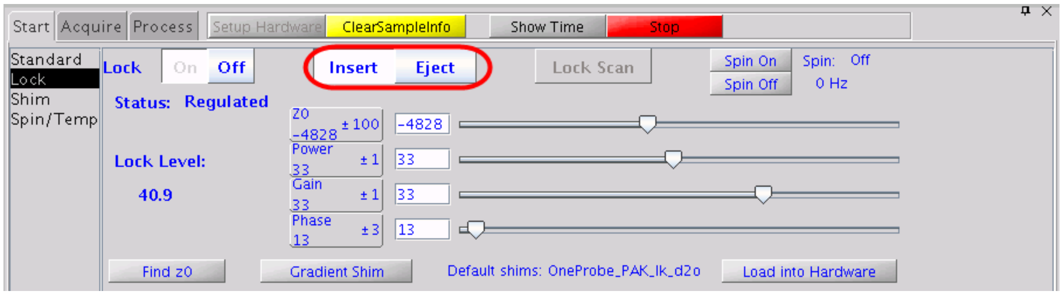
Buy the best quality NMR sample tubes and clean the outside of each tube with a solvent such as isopropyl alcohol, followed by a careful wiping with a wiper tissue before placing the tube in the probe. Fingerprints left on the sample turbine (in particular) will get transferred into the spinner housing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

3.2.5 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 (e.g., low salt to high salt) also require retuning of the probe.

3.3 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.



Status: Regulated

Lock Level: 40.9

Spin: Off 0 Hz

Z0 -4828 ±100 -4828

Power 33 ±1 33

Gain 33 ±1 33

Phase 13 ±3 13

Default shims: OneProbe_PAK_lk_d2o

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 recommended that you use computer control for maximum reproducibility and safety.

3.3.1 Ejecting a Sample

Always eject first (even if no sample is in the magnet) to start airflow to carry the sample. The eject air is turned on and 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 **Start/Standard** panel.

1. Click the **Start** tab and then the **Standard** panel.
2. 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.

Inova or Mercury:

The manual eject button is *used only in emergencies*. It does not require electrical power to eject the sample.

1. Press the black button on the top of the left leg of the magnet or on top of the Magnet Interface Box.
2. To insert a sample using this manual eject button, slowly release the black button to slowly drop the sample.

3.3.2 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.
2. Insert the sample by placing it in the top of the upper barrel.
3. Click the Start tab.
4. Select the Standard panel.
5. Click Insert.

Manual Insertion

VNMRS or 400MR:

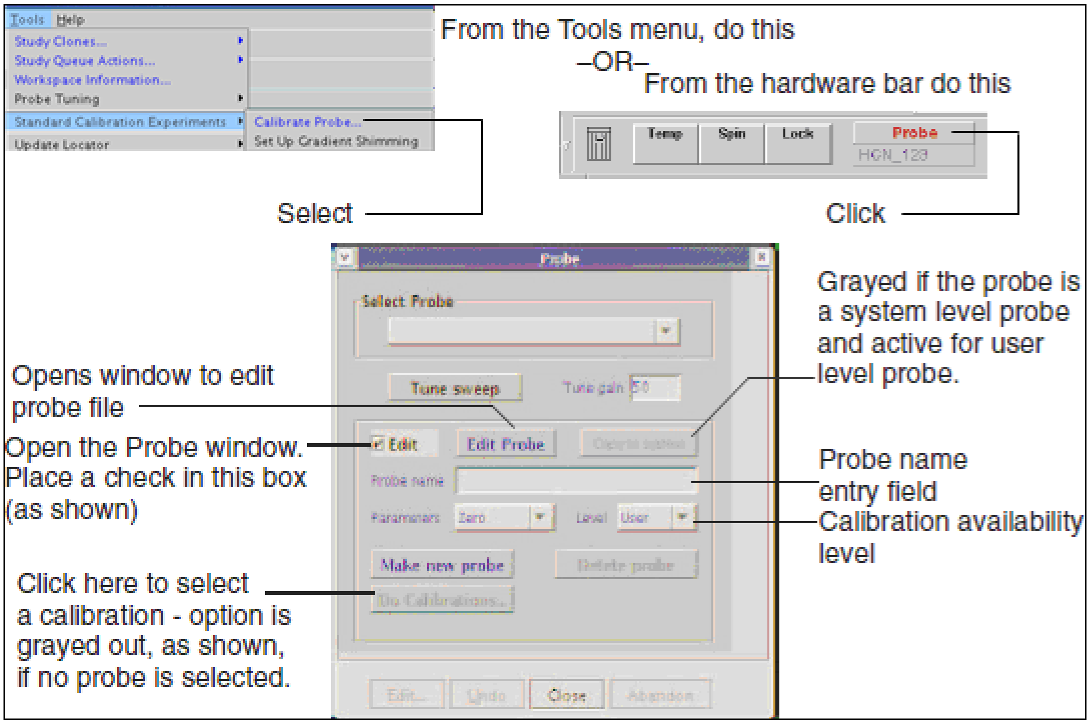
Flip down the switch at the top of the upper barrel. (Flip the switch down to insert, and up to eject)

3.4 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 Installation and Administration* manual.

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

   The probe selection window appears.
2. Click the **Select Probe** dropdown menu and select the desired probe.
3. Click Close to dismiss the window.



From the Tools menu, do this

–OR–

From the hardware bar do this

Select

Click

Grayed if the probe is a system level probe and active for user level probe.

Opens window to edit probe file

Open the Probe window. Place a check in this box (as shown)

Probe name entry field

Calibration availability level

Click here to select a calibration - option is grayed out, as shown, if no probe is selected.

Figure 3 Calibrating a Probe

3.5 Tuning Probes on Systems with ProTune

This section applies to Varian NMR Systems spectrometers equipped with ProTune.

3.5.1 Configuring for Operation with Automated Sample Handlers

- Applies to systems equipped with ProTune.
- The system must be properly configured and ProTune-calibrated. Refer to the *VnmrJ Installation and Administration* 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.

   Gradient shimming will start after each auto tune event if both auto tune and gradient shimming are selected.
3. The tune protocol (wtune) is specified in the probe file.

3.5.2 Running ProTune on Systems Not Using Automated Sample Handlers

1. Click on the **Tools** button on the main menu bar.
2. Select Tune Probe… from menu.

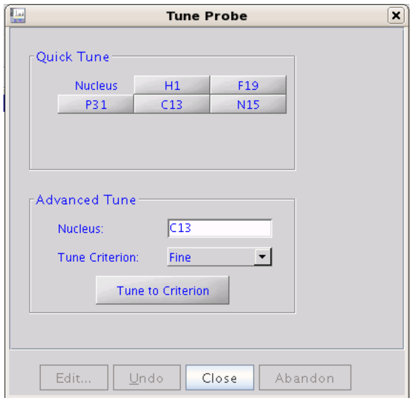


Figure 4

3. Click on a **nucleus** button next to Nucleus in the Tune region to set the tune frequency.
4. To select a nucleus not in the above list, or to select a different criterion:
   Select a criteria from the dropdown menu next to Tune Criterion in the Advanced Tune section:

   Coarse – within 5 percent of optimum pw

   Medium – within 2 percent of optimum pw

   Fine – within 0.5 percent of optimum pw

   The criteria function is available to the operator depending on value set in the parameter **`panellevel`**.

   Click on **Tune to Criterion** button.
5. Select the next nucleus and repeat; continue with the next step when all desired tuning is completed.
6. Click on **Close** to exit the Tune Probe module.

3.5.3 Remote Tuning from the ProTune Window

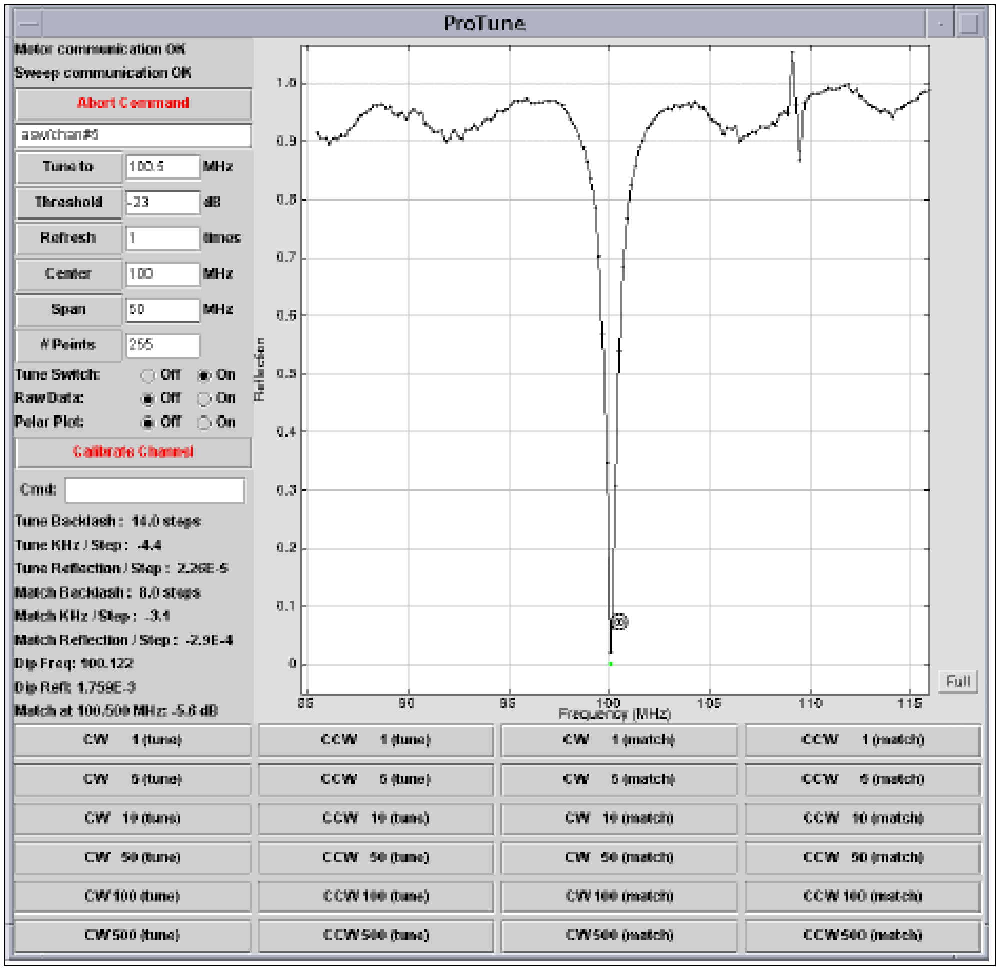
The ProTune interface window (shown in Figure 5) can also be used to tune the probe. Functions and features of ProTune are listed in Table 2.

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 6.
2. Click on the **Refresh** button.
3. Make sure that the motor and sweep communication read OK before starting manual tuning after the ProTune window appears.

   Figure 5 shows the ProTune window with RF reflection at 499.5 MHz.
4. Enter the frequency (MHz) in the Tune To box.
5. Click the **Tune To** button.



Motor communication OK
Sweep communication OK
asw/chan#5
Tune to 100.5 MHz
Threshold -23 dB
Refresh 1 times
Center 100 MHz
Span 50 MHz
Points 255
Tune Switch: ○ Off ● On
Raw Data: ● Off ○ On
Polar Plot: ● Off ○ On
Cmd:
Tune Backlash : 14.0 steps
Tune KHz / Step : -4.4
Tune Reflection / Step : 2.26E-5
Match Backlash : 8.0 steps
Match KHz / Step : -3.1
Match Reflection / Step : -2.9E-4
Dip Freq: 100.122
Dip Refl: 1.759E-3
Match at 100.500 MHz: -5.6 dB

Figure 5 ProTune Software Interface Example

HCN_123

Figure 6 Probe Name and VnmrJ Hardware Bar

The software reads the appropriate channel file and starts tuning.

6. Do the following to stop the automatic tuning and tune manually:
    a. Click the **Abort Command** button (might require several clicks) to stop.
    b. Enter the corresponding chan # of the desired tune frequency.
    c. Click the appropriate **CCW (**counterclockwise) and **CW** (clockwise) buttons to adjust the tune and match.
    d. Enter a frequency in the **Center** box and click **Refresh** to update the reflection window to center the window.
    e. Enter a frequency width in the **Span** entry box and click **Refresh** to update the reflection window to set the window span.
7. Close the ProTune window — acquisition will stop automatically. Enter `aa` on the ProTune Window Description.

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
	Text Box	Probe name and channel number
	Tune Mode On/Off	Toggle TR Switch/Relay to tune
	Raw Data Mode On/Off	Toggle window to display raw data
	Tune Freq (MHz)	Tune frequency
	Match (dB)	Criteria for successful tuning
	Track Tune (s)	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
	Number of Points	Number of the measurement points for all segments of the sweep table for each channel
	Initialize	Initialize tuning sensitivity. Run the tune and match motors and update backlash, frequency, and reflection values.
	Polar	Toggle between polar plot (imaginary and real reflection) and linear plot (reflection v. frequency). Polar plot does not work in raw data mode.
	Cmd:	Sends the command in the field directly to the motor modules.
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. This value is the same for CW and CCW movements.
	Tune/Match KHz/Step	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 Refl	Reflection the dip occurs

Feature	Button, Label, or Message	Description
	Match at Freq MHz	Value for the tuning criteria at the desired frequency
Controls and output	Tune	Tune motor control
	MATCH	Match motor control
	CC/CCW #	Clockwise or counter clockwise and number of steps to move
	Plot	Displays Reflection v. Frequency in a linear plot or imaginary and real Reflection on a polar plot. Right click to draw a box around an area of interest and expand the viewing area.
	Full	Displays the graph in full scale view; for polar plot, it shows the unit circle

3.6 Tuning Probes on Standard Systems

Typically, probes are tuned using the TUNE INTERFACE, shown in Figure 35.

3.6.1 Selecting Correct Quarter-Wavelength

When a large change is made in the frequency of the observe 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 VNMRS spectrometers and 500–900-MHz INOVA systems
- 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), usually 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.

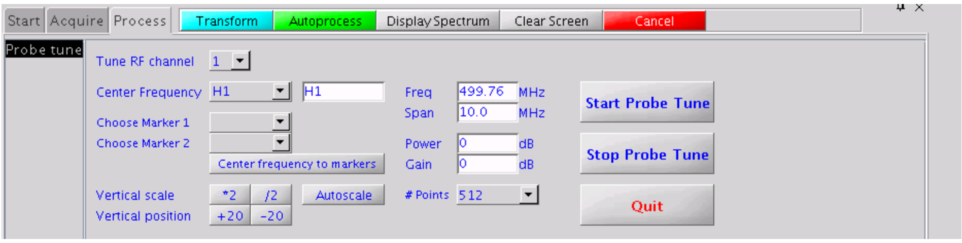
Note that ProTune probes have a universal quarter-wavelength cable that spans the entire range of X nuclei. When this cable is used, it never needs to be changed (at least when the probe is connected to ProTune).

3.6.2 Tuning Using Mtune

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

1. Click on **Tools** on the main menu
2. Select **Manual Tune Probe**... or enter `mtune` on the command line.
3. Select the center frequency of the nucleus to tune.

   For example, set H1 on channel 1.
4. Select **Start Probe Tune** on the Mtune panel, see Figure 7.



Tune RF channel 1

Center Frequency H1 H1

Freq 499.76 MHz

Span 10.0 MHz

Power 0 dB

Gain 0 dB

Points 512

Figure 7 Manual Tuning Panel

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

   A typical frequency Span for ^{1}H 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.
8. Adjust any of the parameters as needed.
9. Set one or two markers.

   Up to two frequency markers are selectable. This is useful for tuning H1 and 19F at the same time, for example with double-tuned coils like in the ATB or 4-nucleus probe families (Figure 8).

   a. Click on 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 19F at 400 MHz, as shown in Figure 8.

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

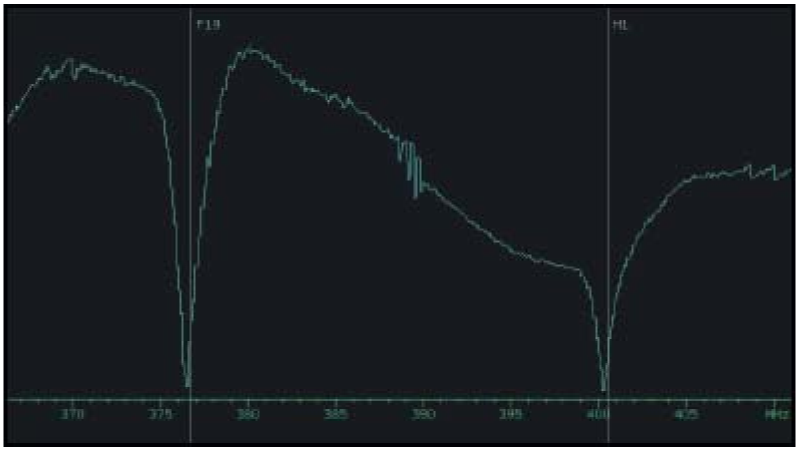


Figure 8 Frequency Span Display Showing both 19F and 1H Tune Dips

10. Click on the **Autoscale** button to fit the tuning dip vertically into the graphics canvas or scale.

11. Shift the display manually using the **Vertical scale/ Vertical Position** controls.
12. Keep the default number of acquired points, or change the number of points by selecting a new value from the **# Points** drop-down menu.
13. Increasing the number of points provides better resolution at large spectral widths. Higher resolutions will have smaller update rates.
14. Tune the other channels as follows:
15. Switch tuning off by selecting **Stop Probe Tune**.
16. Select a different channel and nucleus.
17. Switch tuning back on by selecting **Start Probe Tune**.
18. Repeat Step 1 through Step 14 for each channel.
19. Exit Mtune after tuning is done:

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

3.6.3 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 9 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 ATTEN 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 ATTEN 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 takes 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 setup 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.

   Type `su`.

   When a su executes, 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

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

3. 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.)

4. Press the **ATTEN** buttons until the readout is 6, 7, or 8.

5. Optional: Insert the appropriate sticks into the probe if necessary. Refer to the probe installation manual to choose which sticks are needed to tune to the desired nucleus.

6. Tune the probe. As the probe gets closer to being tuned, the number on the TUNE INTERFACE display will decrease.

7. 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, but around 1-2 for INOVA-generation consoles.)

8. 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.)

9. Repeat the steps above for each channel on the system.

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

Chapter 4 Experiment Setup

This chapter sequentially describes how to setup 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, manually shimming on the lock signal, shimming PFG systems, and calibrating the probe.

Sections in this chapter:

4.1 Selecting an Experiment

This section describes using the Protocol window, 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.

4.1.1 Protocols Window

1. In the left vertical windows, select the Protocols / Experiment Selector screen.
2. Click on an experiment to bring it into the active viewport. (If a study is not selected, the experiment parameters will go into the active viewport rather than into the StudyQ.

4.1.2 Main Menu Selections

Experiment

1. Click on **Experiments** in the menu bar.
2. Click on an experiment in the list to bring it into the active viewport, see Experiments Menu.
3. 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 on an experiment or data set from the Browser to a view port.
5. Click **Open** to bring it into the active viewport.

4.1.3 Locator

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

4.1.4 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 view port.

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.

4.2 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.

4.2.1 Liquids Spinner

To configure the software so that a 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.

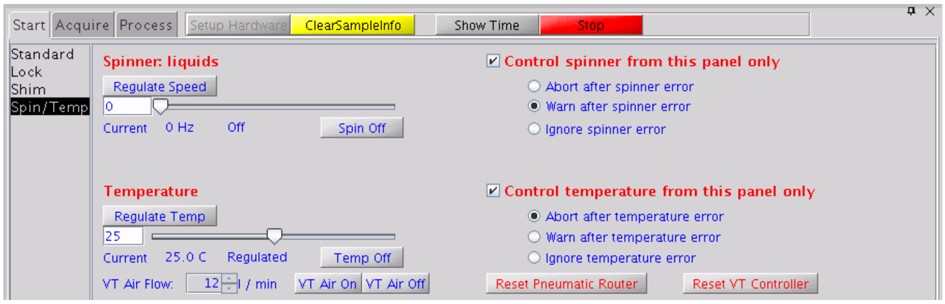
Adjust spin rate using the input window or the Acquisition window. Typical spin rates are 20 for 5-mm tubes and 15 for 10-mm tubes.

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

- The Spin chart display button on the hardware bar displays a history of the sample spin rate.
- 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** checkboxes. 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 handing for spinner and temperature by selecting one of the three options under each of the spinner and temperature sections.

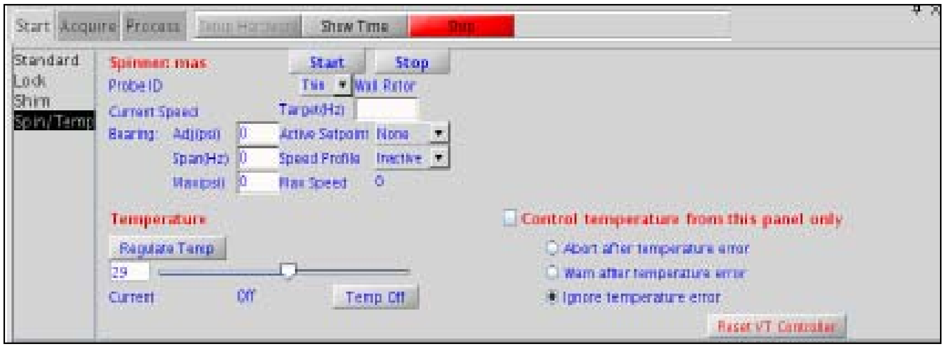
4.2.2 MAS Spinner

To configure the software so that an MASSpinner 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 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.



Probe ID Thin Wall Rotor

Current Speed

Target(Hz)

Bearing: Adj(psi) 0

Span(Hz) 0

Max(psi) 0

Active Setpoint None

Speed Profile Inactive

Max Speed 0

29

Current Off

☐ Control temperature from this panel only

○ Abort after temperature error

○ Warn after temperature error

● Ignore temperature error

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)	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. -- 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 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 the Spin button in the hardware bar will bring up a strip chart of the spinning speed with time.

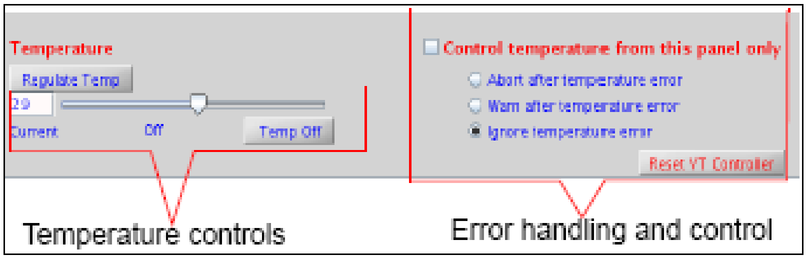
The following safety measures have been implemented for the high-speed spinner probes (e.g., MAS) to prevent rotor and stator damage:

- 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.

4.3 Setting the Sample Temperature

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

Refer to Appendix A Variable Temperature System, for more information on using the Variable Temperature module. The following steps describe a typical operation sequence:



Temperature controls

Error handling and control

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 begins the pulse sequence and data acquisition. 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 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 10º 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. Refer to the *VT Accessory Installation* manual for the NMR calibration method.

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 Varian).

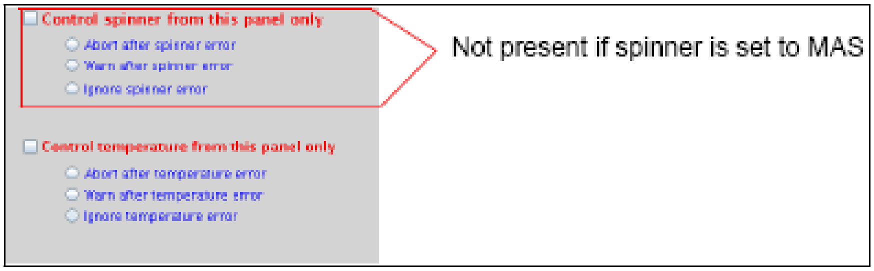
CAUTION	Operating the system with the coolant bucket filled with liquid nitrogen and with the requested temperature greater than the value of VT cutoff (vtc) results in the condensation of liquid nitrogen inside the exchanger coil tube. If the exchanger coil is then warmed above –210 C or if nitrogen 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.

4.4 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.



Not present if spinner is set to MAS

- *Control spinner/temperature from this panel only* – this 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 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.

4.5 Working with the Lock and Shim Pages

4.5.1 Mouse Control of Buttons and Sliders

To Change the Increment

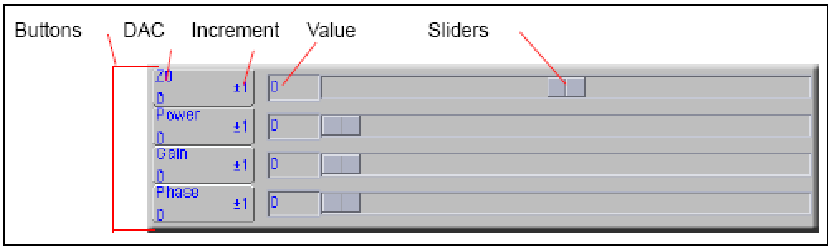
1. Middle click on the increment button until the desired value is displayed. The defaults are 1, 10, and 100.
2. To change to a customized increment value, shift/middle-click on the button, enter a new value, and press **Return**.

To Change the DAC Value

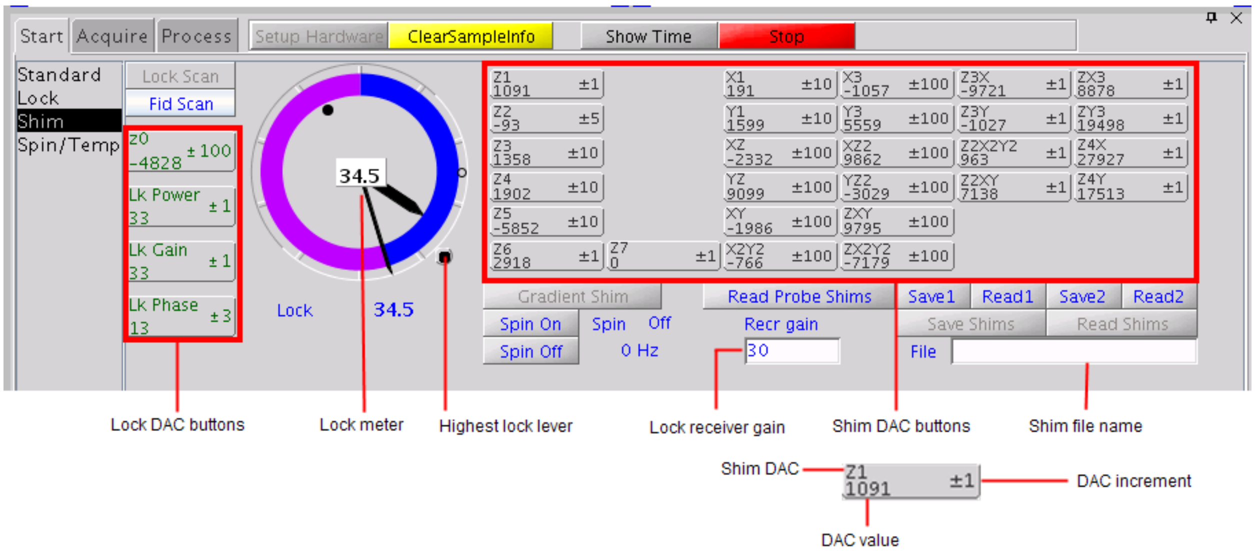
1. Enter a new value in the input window and press **Return**.
2. Alternatively, Shift/left -click on the DAC button itself. Enter a new value and press **Return**.

4.5.2 Lock Buttons and Controls

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



4.5.3 Shim Buttons and Controls



4.6 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 coincides with the lock frequency.

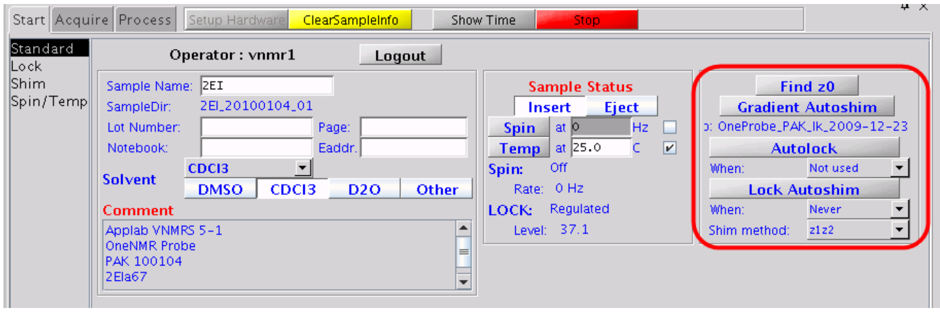
The lock level can be viewed by clicking the **Lock** button on the hardware bar.

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 can be entered as part of a macro or by using normal parameter entry (e.g., by entering lockgain=30 lockpower=24*)*. These parameters do not take effect until an su, go, or equivalent command is given.

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.

4.6.1 Finding Z0 and Establishing Lock

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



Operator : vnmr1

Sample Name: 2EI

SampleDir: 2EI_20100104_01

Lot Number:

Page:

Notebook:

Eaddr.

Solvent: CDCl3

Comment:
Applab VNMRS 5-1
OneNMR Probe
PAK 100104
2Ela67

Spin at 0 Hz ☐

Temp at 25.0 C ☑

Spin: Off

Rate: 0 Hz

LOCK: Regulated

Level: 37.1

): OneProbe_PAK_lk_2009-12-23

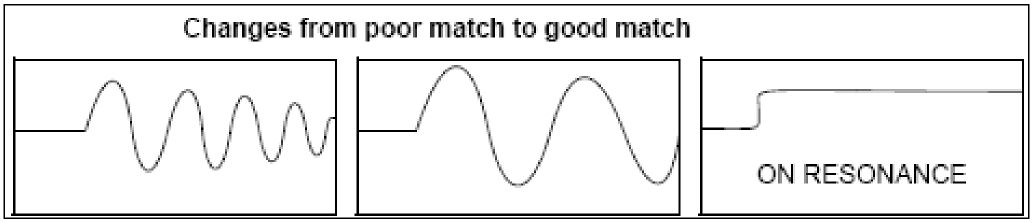
When: Not used

When: Never

Shim method: z1z2

Manual Method

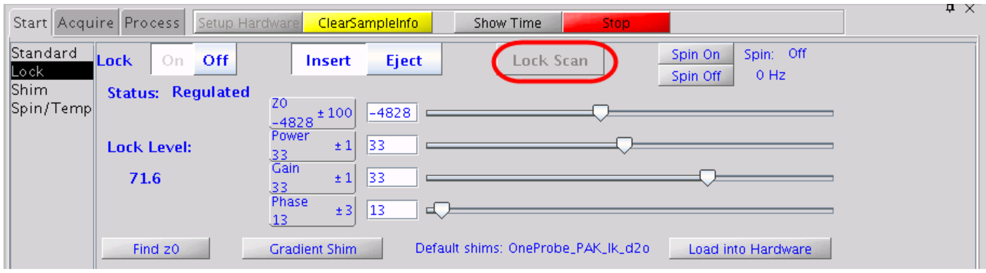
Establish lock using manual locking on the Lock page. The line that crosses the spectral window represents how close the deuterium resonance field is to the lock frequency. When the two are matched, the line should be flat (with perhaps some noise, depending on the lock gain and lock power). The greater 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 on the **Lock** page in the Start tab.
3. Click on either **Spin On** or **Spin Off**.
4. Click **Lock Scan** to open the lock display.
5. Click the **Lock Off** button. (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 the buttons.

a. 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 on 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. Click the **Lock On** button.
13. Click **Lock Scan** again to close the lock display.



Spin: Off
0 Hz

Status: Regulated

Lock Level: 71.6

Z0 -4828 -4828

Power 33 33

Gain 33 33

Phase 13 13

Default shims: OneProbe_PAK_lk_d2o

AutoLock with Probe File

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

1. Click on **Find Z0**. This button can be found on either the **Standard** page or the **Lock** page of the Start tab.

AutoLock

1. Click on the **Standard** page of the Start tab.
2. Click on either **Spin On** or **Spin Off**.

   Selection an option for the menu next to the Autolock button.
3. Click on the **Autolock** button — the spectrometer will find Z0 and make all specified adjustments.
4. Choose Find Z0 or AutoLock.
5. Clicking on the button next to opens a drop-down menu of options.

4.6.2 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.

4.6.3 Lock Control Methods

Click on the **Start** tab and select the **Standard** page to access the following lock methods and controls:

Each method is discussed in the following separate sections. Additional sections discuss error handling and lock loop time constant control.

Leaving Lock in the Current State

Set **Autolock** to **Not Used**.

When **Autolock** is set to **Not Used**, the freshly inserted sample will only lock if the new 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 **Simple**.

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 **Simple** 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**, although if the lock is not captured by this method, the system starts using the Autolock Optimization options listed below.

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 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.

4.7 Adjusting Field Homogeneity

Refer also to Chapter 5, 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 about shimming.

4.7.1 Loading Shim Values

1. Click on the **Lock** page in the Start panel
2. Click on the Load into Hardware 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)

4.7.2 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

4.7.3 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

4.7.4 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.

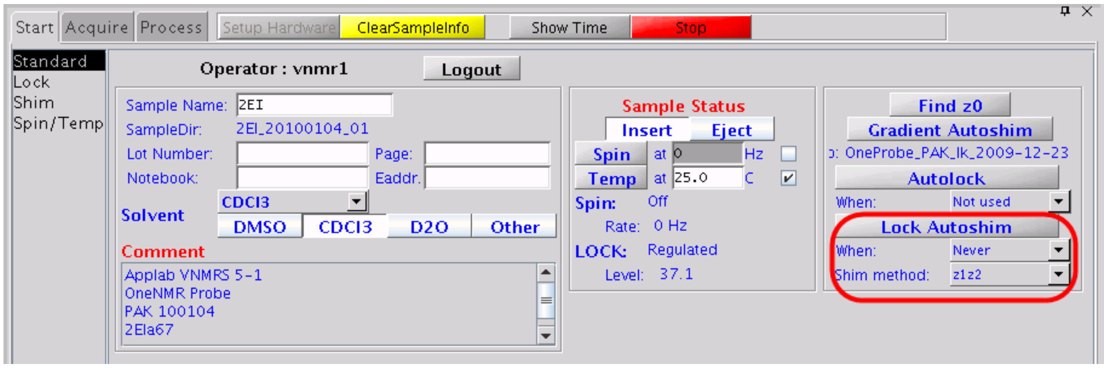
Each shim gradient is controlled by its own parameter; for example, the X1 shim gradient is controlled by a parameter named x1.

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.

4.7.5 Automated Shimming on the Lock

Refer to Autoshim Information, for more information about Autoshim.

Manual shimming on the lock is done with the controls on the Shim page Automated shimming on the lock is preferred and can be set up from the Standard page of the Start tab:



Operator : vnmr1

Sample Name: 2EI

SampleDir: 2EI_20100104_01

Lot Number:

Page:

Notebook:

Eaddr.

Solvent: CDCl3

Comment:
Applab VNMRS 5-1
OneNMR Probe
PAK 100104
2EIa67

Spin at 0 Hz ☐

Temp at 25.0 C ☑

Spin: Off

Rate: 0 Hz

LOCK: Regulated

Level: 37.1

): OneProbe_PAK_lk_2009-12-23

Autolock When: Not used

Lock Autoshim When: Never

Shim method: z1z2

Shim on Lock options menu next to **When:**

- Never — disables automatic lock shimming.
- Every sample — Shims before the start of data acquisition for each new sample.
- Every Experiment— Shims before the start of data acquisition for each new experiment.
- Every FID— Shims before the start of data acquisition for each FID.

Shim on Lock options menu next to **Shim method:**

- z1z2
- Low non-spins
- All non-spins
- All z’s
- Hi-res z’s
- Fine z1,z2
- Fine z1-z3

Autoshim is controlled by the selection made from the Shim method menu. This is a complete background Autoshim method that provides no interaction with the operator. The type of automatic shimming you should select during routine sample changes depends upon the level of homogeneity required on any particular sample, the change in sample height, and the maximum time desired for shimming.

- **z1,z2** shimming — average homogeneity needs, with long samples of identical height.
- **all z's** — variable sample heights. More time is required. The method shims first Z1, Z2, and Z4, then Z1, Z2, and Z3, and finally Z1 and Z2.

4.8 Selecting Shims to Optimize

4.8.1 Which Shims to Use on a Routine Basis

The following suggestions assist in routine shimming, especially on shim systems with a larger number of shim channels:

- Establish and maintain lineshape – Use Z to Z5, possibly Z6, X, Y, ZX, ZY, and possibly Z2X and Z2Y. The effects of Z7 and Z8 (and realistically Z6) are too small to see with the lineshape sample.
- *Shim a new lineshape sample of different geometry* – Use Z to Z5, possibly Z6, X, Y, ZX, ZY, and possibly Z2X and Z2Y.
- *Shim a new sample of the same geometry* – Use Z, Z2, and maybe Z3. (Higher field magnets are more likely to need additional Z3 corrections, and eventually even some Z4.)
- *Shim a new sample of different geometry* – Use Z to Z4 and possibly Z5, X, Y, ZX, ZY.
- *Shim for preset water suppression* – Start with a shim set that produces a good lineshape for the same sample geometry. Next, tweak Z and Z2, and then vary Z5 and Z7 to minimize the width of the base of the water (Z and Z2 may need to be tweaked if Z5 changes by more than 100 to 200 coarse units). About 80 to 90 percent of the odd-order axial-gradient induced water width is probably dominated by Z5, with Z7 and perhaps some Z3 providing the rest.

  The even-order axial shims (Z2, Z4, Z6, and Z8) affect the asymmetry of the residual water line (using presaturation). All four of these even-order axial shims can affect the final water linewidth, with Z2 and Z4 being at about the 5 mM solute level and above, Z6 being at about the 1 mM solute level, and Z8 being at about the 0.3 mM solute level. The even-order axial shims perform as expected except when the sample is less than 40 mm in length, in which case the shims still control the water linewidth but much less responsively.

  Using Z4 to narrow an asymmetric residual water line of a sample shorter than about 40 mm can destroy the base of the standard lineshape faster than the residual water signal is narrowed. The residual water resonance width is affected by magnetic susceptibility interfaces as the sample gets shorter. Iterative use of Z5-Z7 with Z6-Z8-Z4-Z2 can narrow the residual water line for samples under 40 mm but the results obtained may be hard to reproduce on subsequent samples due to an increase in sensitivity to small changes in sample geometry.

4.8.2 Shimming Different Sample Geometries

Some suggestions when moving the sample:

- *Moving the same sample up* – Z, Z3, and Z5 need to become more positive.

- *Shortening and centering (moving up) the sample* – Z2 and Z4 need to become much more positive. The trends for Z and Z3 are mixed and more complex, but they tend to become a little more negative. It appears as if Z and Z3 are driven positive as the sample is pulled up, but they are driven negative faster as the sample shortens. When shimming a lineshape sample, plan on the following changes (starting from lineshape shims for a 700 μL sample at a depth 67-68 mm):

  700 μL to 600 μL: move Z2 +50 DAC units and move Z4 +250 units.
  700 μL to 500 μL: move Z2 +200 units and Z4 +600 units.

  The Z2 and Z4 changes track well with sample volume, but are relatively independent of tube depth. It is therefore easiest when changing sample geometries to make the appropriate Z2 and Z4 corrections, then adjust the more complex Z1-Z3-Z5 interactions as needed.

4.9 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.

4.9.1 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 (e.g., if the temperature has changed).
2. Click **Setup Hardware**.
3. Make sure the probe has a sample, that it is spinning at the correct speed, and that 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 deuteroacetone 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. Refer to the *Probe Installation* manual that shipped with your probe.

4.9.2 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; refer to Table 31 in Appendix B, Shimming Basics, 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.

4.9.3 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. Refer to Table 44 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”) for Z1 and Z2.

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

4.9.4 Setting the High-Order Axial Shims (Non-Routine)

Refer to Shim Interactions, 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 102, which is 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. Since 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 in the Setting Low-Order (Routine) Shims procedure.

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

4.9.5 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 Removing Spinning Sidebands (Non-Routine) procedure.
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

    Refer to 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 be 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 reexamine 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.

4.10 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.

4.10.1 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.

4.10.2 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.

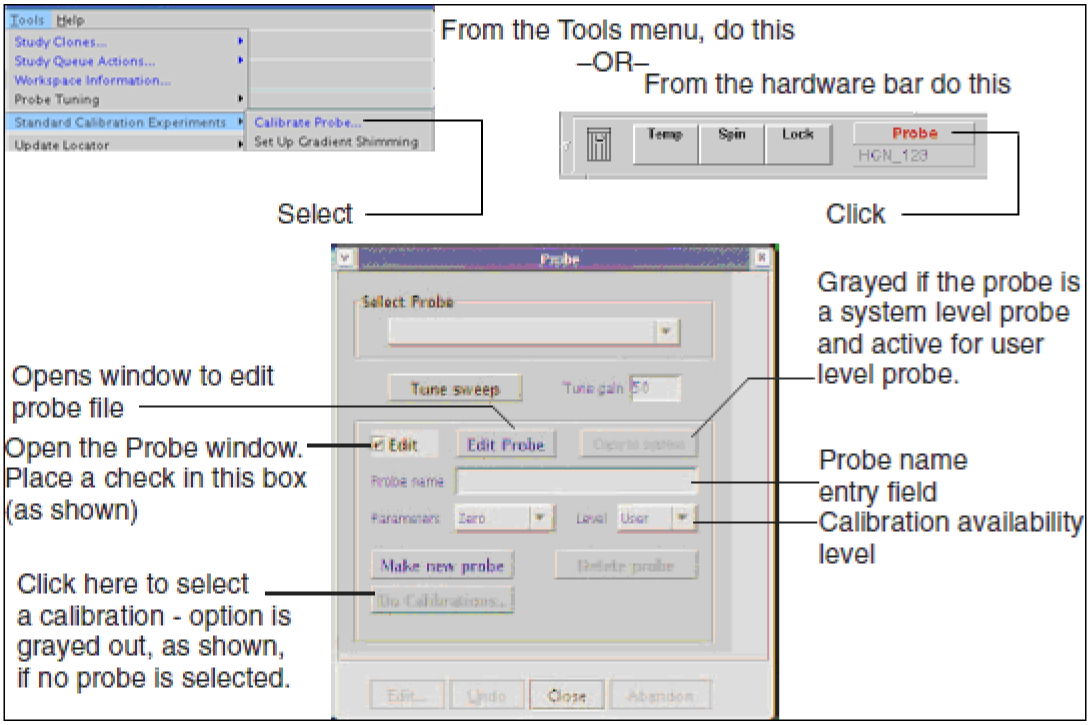
   The yellow RUN light should turn on.

4.11 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. Refer to the *VnmrJ Installation and Administration* user guide for detailed instructions on automatic and manual probe calibration procedures.

4.11.1 Using Auto Calibration Procedures

Do one of the following to start the probe calibration (see Figure 10) and open the probe window:



From the Tools menu, do this

–OR–

From the hardware bar do this

Select

Click

Grayed if the probe is a system level probe and active for user level probe.

Opens window to edit probe file

Open the Probe window. Place a check in this box (as shown)

Probe name entry field

Calibration availability level

Click here to select a calibration - option is grayed out, as shown, if no probe is selected.

Figure 10 Calibrating a Probe

Use the main menu bar:

1. Click on **Tools**

2. Select **Standard Calibration Experiments**.
3. Select **Calibrate Probe…**

Use the Hardware bar:

1. Click on the **Probe** button
2. Check the box next to **Edit** in the Probe window
3. Click the **Do Calibration** button in the probe calibration popup window
4. Select calibrations from the calibration popup window
5. Click a radio button in the Calibrate Probe window to select the calibration experiment, see Figure 11.

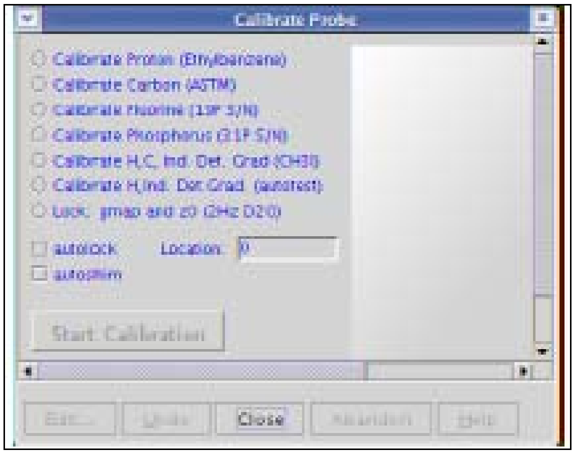


Figure 11 Calibrate Probe Window

6. Enter the location of the calibration sample.

   No entry field appears if a sample changer is not attached to system.
7. Insert the sample into the magnet manually using the insert and eject button located on the Lock page of the Start tab if a sample handler is not present or not used.
8. Select Autolock (YES or NO).
9. Click the NO button if the sample is already locked or to lock manually.
10. Select Autoshim (YES or NO).
11. Click the NO button if the sample is already shimmed or to shim manually.
12. Click the Start Calibration button.

    Follow the on line instructions and refer to the *VnmrJ Installation and Administration* user guide for detailed instructions on automatic and manual probe calibration procedures.

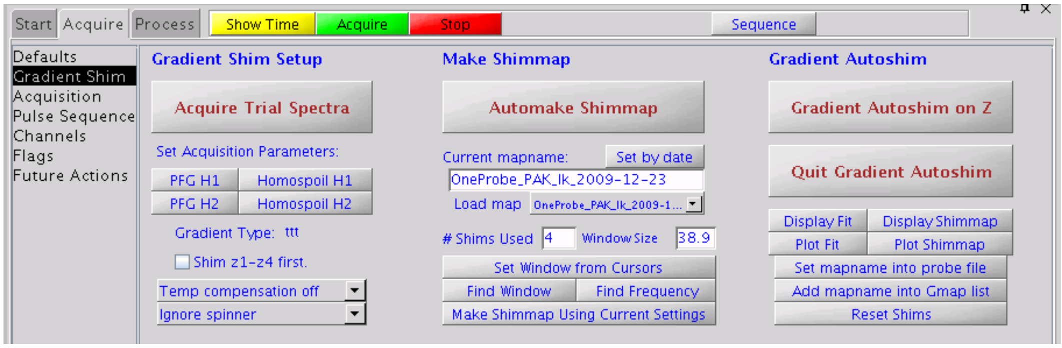
    Some probes, like the Autoswitchable and 4 nucleus probes, require additional calibrations not covered in this manual. For information on the calibration of these probes, see the installation, testing, and specifications manual for the probe.

4.11.2 Using Manual Calibration Procedures

Refer to the manual calibration section of the *VnmrJ Installation and Administration* user guide and the acceptance test procedures supplied with the probe.

Chapter 5 Gradient Shimming

Gradient autoshimming provides rapid, automatic adjustment of axial room-temperature shims. It is a reliable way to set high-order shims, which shortens 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.



Gradient Type: ttt

☐ Shim z1-z4 first.

Temp compensation off

Ignore spinner

Current mapname: OneProbe_PAK_lk_2009-12-23

Load map OneProbe_PAK_lk_2009-1...

Shims Used 4 Window Size 38.9

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; refer to the section Homospoil Gradient Type, 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. It can be performed on either 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.

Sections in this chapter:

- 5.1 Deuterium Gradient Shimming
- 5.2 Homospoil Gradient Shimming
- 5.3 Configuring Gradients and Hardware Control
- 5.4 Mapping Shims and Gradient Shimming
- 5.5 Shimmap Display, Loading, and Sharing
- 5.6 Gradient Shimming for the General User
- 5.7 Deuterium Gradient Shimming Procedure for Lineshape
- 5.8 Calibrating gzwin
- 5.9 Varying the Number of Shims
- 5.10 Variable Temperature Gradient Compensation

- 5.11 Spinning During Gradient Shimming
- 5.12 Suggestions for Improving Results
- 5.13 Gradient Shimming Pulse Sequence and Processing

5.1 Deuterium Gradient Shimming

Deuterium gradient shimming is feasible for most deuterated solvents for which 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, 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 μs.

5.2 Homospoil Gradient Shimming

5.2.1 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 (e.g., 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, i.e., for gradient shimming during automation or gradient shimming in general:

  Click on 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',gzlvl1). If the value of gzlvl1 is non-zero, homospoil is switched on; if the value of gzlvl1 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 3 for system configurations). Check your pulse sequences to ensure this time limit is not exceeded.

Table 3 Homospoil Control

Shim Supply	Homospoil Time Limit
Varian 14	20 ms/200 ms
Varian 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 available.

5.2.2 Homospoil Gradient Shimming for ^{1}H or ^{2}H

Follow the procedure in Mapping Shims and Gradient Shimming. Use **Find z0** before gradient shimming to use homospoil deuterium gradient shimming with different solvents.

5.3 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.

5.4 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. Refer to Spinning During Gradient Shimming, for instructions on spinning during gradient shimming.

5.4.1 Recommended Samples for Gradient Shimming

The following samples are recommended for gradient shimming.

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

5.4.2 Manually Calibrating the 90° Pulse for ^{1}H and ^{2}H

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 (^{2}H) 90º 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 with **Step 9**.

   Proton (1H) 90º pulse calibration:

   a. Verify that the observe nucleus is set to H1.
   b. Continue with **Step 9**.
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.

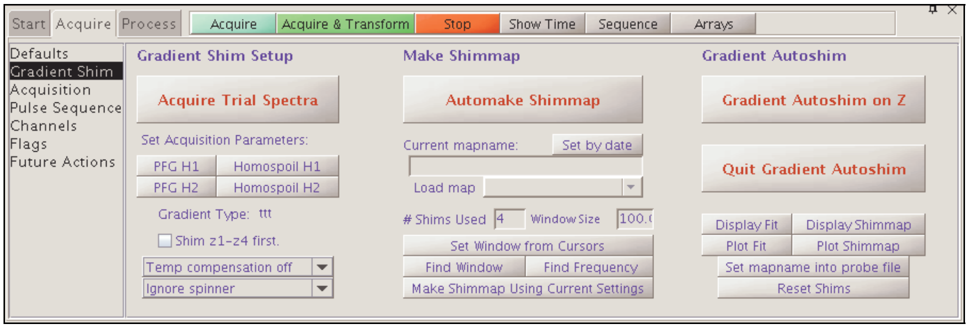
NOTE: The strong signal may cause an ADC overflow for 1H operation. If so, set gain = 0.

5.4.3 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.



Gradient Type: ttt

Shims Used 4

Window Size 100.

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).

Solvent	**Scans**	**Relaxation Delay, Sec.**	**Gain**
deuterochloroform	8-32	2	36 (18 Mercury)
dmso-d6	4-16	2	28 (10 Mercury)
D_2O	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.

    The number of shims used to make the map is controlled by the gzsize parameter (which defaults to 4).

    If the mapname already exists, a prompt appears to make a new mapname and to overwrite the current mapname.
15. After acquisition is finished, click on **Set mapname into probe file** button on the Gradient Shim page.

5.4.4 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 "gzsize" numer 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 as many gradients as were used to make the map, and the value of gzsize is ignored.

5.4.5 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.

5.4.6 Gradient Shim Commands and Parameters

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

Commands	**Description**
`gmapshim<('files'|'quit')>`	Run gradient autoshimming, quit.
`gmapsys*`	Enter Gradient Shimming setup panels, make shimmap.
`gmapz<(mapname)>`	Get parameters/files for gmapz pulse sequence.
* `gmapsys<'shimmap'<,'auto'|'manual'|'overwrite'|mapname>`	
Parameters	**Description**
`d2`	Incremented delay for 1st indirectly detected dimension.

Commands	Description
`d3`	Incremented delay for 2nd indirectly detected dimension; arrayed to two values
`gradtype*`	Gradients for x, y, and z axes
`gzlvl {DAC value}`	Pulsed field gradient strength
`gzsize {integer,1 to 6}`	Number of z-axis shims used by gradient shimming
`gzwin {0 to 100}`	Percentage of spectral window used by gradient shimming
`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'}`	

5.4.7 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 12.

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.

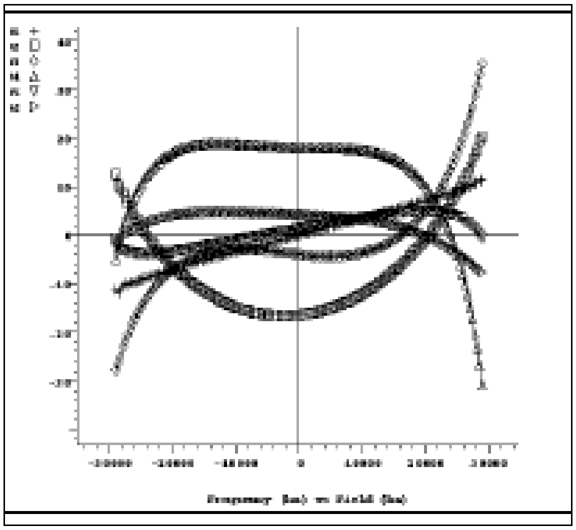


Figure 12 Shimmap Plot of Z1 Through Z6

5.4.8 How Automated Shimming Works

The shims must be mapped before gradient automated shimming is used, see Mapping the Shims, 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 13).

Shim adjustments for each iteration are also displayed in the Text Output window (see Figure 14) 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.

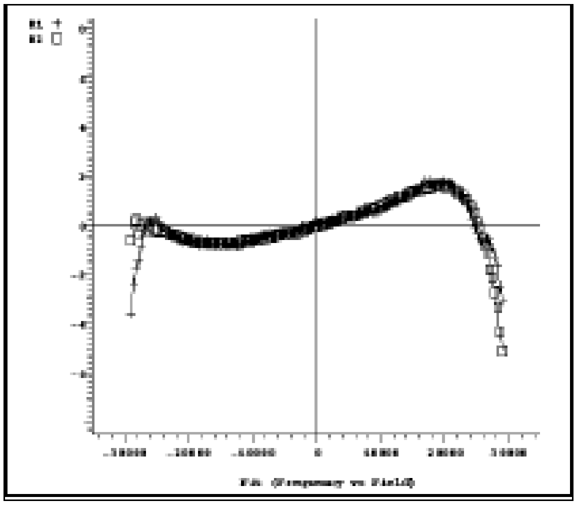
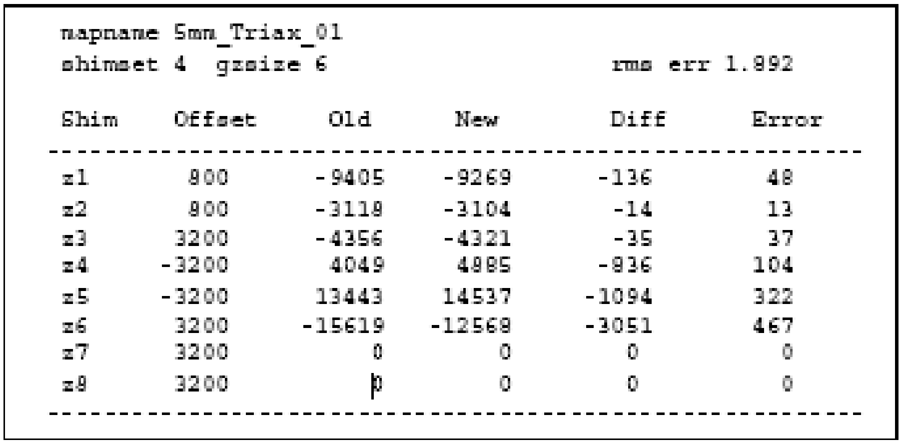


Figure 13 Curve Fit Plot

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.



```
mapname 5mm_Triax_01
shimset 4  gzsize 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     4885      -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 14 Display of Shim Adjustments for Each Iteration

5.5 Shimmap Display, Loading, and Sharing

5.5.1 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 15.)

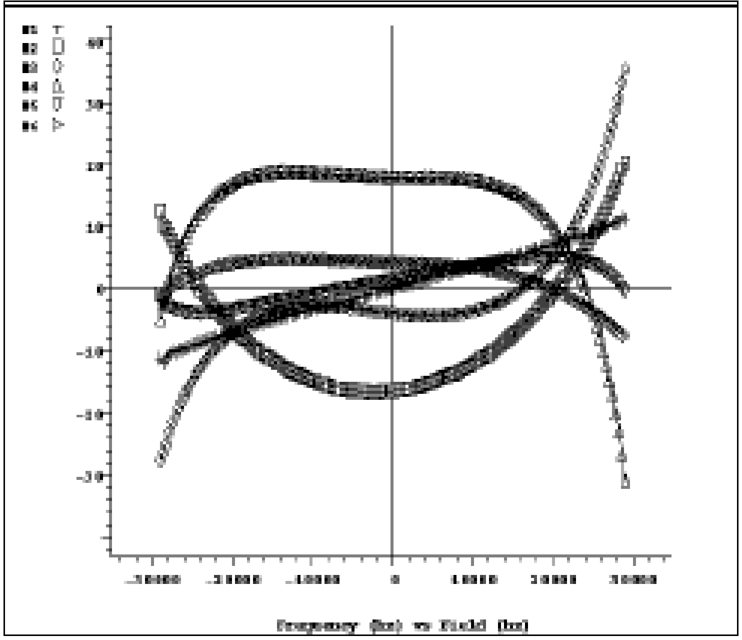


Figure 15 Shimmap Plot

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.

5.5.2 Loading a Shimmap

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

1. Click on 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.

5.5.3 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.

5.5.4 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').

5.6 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 on 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 StudyQ, select the Shim checkbox 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 on `Cancel Cmd`. Then enter **`gmapshim('quit')`** to retrieve previous data set and parameters.

5.6.1 Testing Solvents

Test gradient shimming for the solvents of interest by doing the following.

1. For each solvent of interest, do the following.
   a. Insert a sample with a solvent of interest.
   b. Select the solvent for the sample on the **Study** page.
   c. Set lock on resonance using one of the following methods.
      - Click the Find z0 button.
      - Use the controls on the **Lock** page.
   d. Click **Tools**.
   e. Select **Standard Calibration Experiments**.
   f. Select **Set up Gradient Shimming**.
   g. Click the **Acquire tab**.
   h. Select the **Gradient Shim** page.
   i. Set deuterium observe parameters as appropriate for the solvent.
   j. Click the Acquire Trial Spectra button, and wait for acquisition to complete.
   k. Click the Gradient Autoshim on Z button to test shimming if the signal-to- noise looks adequate in the spectra,.
   l. Repeat steps (g)-(i) until the shimming works properly.

2. Once autoshimming works well for the solvents of interest, do the following:
    a. Open a terminal window.
    b. Make a backup copy of the gmapz macro.
    c. Edit the bottom portion of the gmapz macro to uncomment the solvents that will be used and make required corrections as needed. By default, the entire section is uncommented.
    d. Save the edited gmapz macro.
    e. Exit the terminal window.

5.7 Deuterium Gradient Shimming Procedure for Lineshape

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

5.7.1 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).

5.7.2 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 on the main menu bar.

    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.
6. Click the **Acquire** tab.
7. Select the **Gradient Shim** page.
8. 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 setup 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 complete. 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 acquisition to complete and the message to be displayed: shimmap done!

5.7.3 Starting Z Gradient Shimming

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

5.7.4 Optimizing Non-spinning Shims

1. Shim on the lock levelonly 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.

5.7.5 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.

5.8 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.

5.8.1 Automatic Calibration of gzwin

Click on 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.

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.

5.8.2 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.

5.9 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.

5.9.1 Changing the Number of Shims Used for Gradient Shimming

1. Map the shims, see Mapping the Shims, 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 on the **Gradient Autoshim on Z** button.
4. All the shims specified in the **# Shims Used** entry box are used to optimize the field homogeneity.

5.9.2 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 (e.g., 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 on 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.

5.10 Variable Temperature Gradient Compensation

Temperature gradients or convection currents may be compensated for with the addition of a 180º 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 180º pulse.
3. Map the shims; see Mapping the Shims.
4. Start gradient shimming; see Starting Gradient Shimming.

5.11 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 Standard 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 on 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.
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.
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.

5.12 Suggestions for Improving Results

Calibrate the 90° pulse and adjust tpwr, pw, 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 df to observe this). Do one or more of the following:

- set d1 to 3 to 5 times T1
- use a smaller flip angle for pw

The phase encode delay d3 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 ^{1}H). However, longer d3 values increase the phase excursion, and can make it difficult to shim large shim corrections (especially Z1). Typical ^{1}H values are 5 to 30 ms, and typical ^{2}H values are 30 to 200 ms. If the shims are far off when making a shimmap, the second value of d3 might be too small. If this problem occurs, decrease the second value of d3 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 (e.g., 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 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 (e.g., 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 (e.g., 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_20. 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, refer to the entries for gmapshim, gmapsys, and gmapz in the *Command and Parameter Reference*.

5.13 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. Figure 16 shows the gradient shimming pulse sequence.

The gradient shimming pulse sequence in Figure 16 is shown with p1=0, in which case pw can be set to a small flip angle. If p1>0, the pulse field gradients are both set to the same sign, and p1 should be set to 180° and pw to 90°, so that RF inhomogeneities are refocused. p1=0 is usually sufficient for most cases.

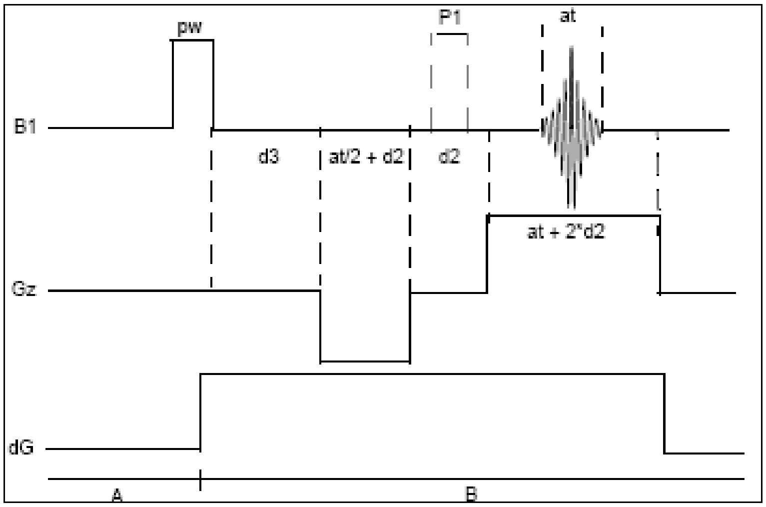


Figure 16 Gradient Shimming Pulse Sequence

Phase accumulation from all gradients present is as follows:

$$\phi = z\ Gz(-at/2 + t) + dG(d3 + at/2 + 3*d2 + t)$$

where t is the time during acquisition at, Gz 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 d3 and taking the difference in the phases:

$$\Delta\phi = \phi2 - \phi1 = dG*(d3[2] - d3[1])$$

For example, at a particular point, $\Delta\phi$ can be 2π * 100 Hz * 10 ms, or 2π radians. Thus, a pair of profiles with different d3 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:

$$\begin{aligned}\Delta(\Delta\phi) = \Delta\phi2 - \Delta\phi1 &= dG2*(d3[2]-d3[1]) - dG1*(d3[2]-d3[1]) \\ &= (dG2 - dG1)*(d3[2] - d3[1])\end{aligned}$$

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 ϕ=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 d3 arrayed. Figure 17 shows an example of mapping the z1 shim.

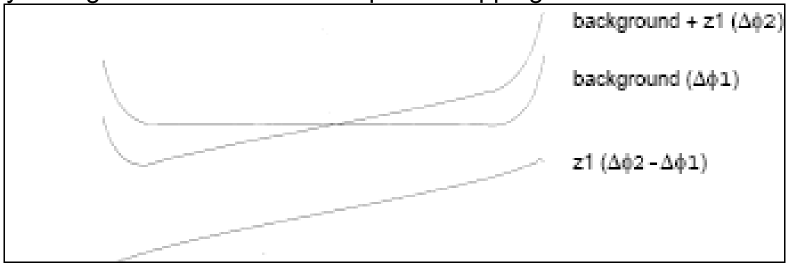


Figure 17 Mapping the z1 Shim

5.13.1 References

Van Zijl, P. C. M., et al. *J. Magn. Reson.* **1994**, *111* (Series A), 203–207.

Sukumar, S., et al. *J. Magn. Reson.* **1997**, *125* (Series A), 159–162.

Barjat, H., et al., *J. Magn. Reson.* **1997**, *125* (Series A), 197–201.

Evans C.L., et al., *J. Magn. Reson.* **2002**, *154* (Series A), 325–328.

Chapter 6 Data Acquisition

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.

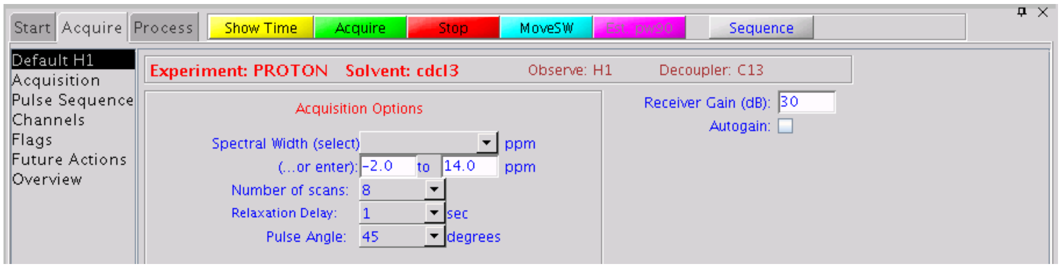
Sections in this chapter:

- 6.1 Acquiring a Spectrum
- 6.2 Acquisition Settings
- 6.3 Pulse Sequences
- 6.4 Parameter Arrays
- 6.5 Stopping and Resuming Acquisition
- 6.6 Automatic Processing
- 6.7 Acquisition Status Window

6.1 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.



Experiment: PROTON Solvent: cdcl3 Observe: H1 Decoupler: C13

Spectral Width (select) ppm

(...or enter): -2.0 to 14.0 ppm

Number of scans: 8

Relaxation Delay: 1 sec

Pulse Angle: 45 degrees

Receiver Gain (dB): 30

Autogain: ☐

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

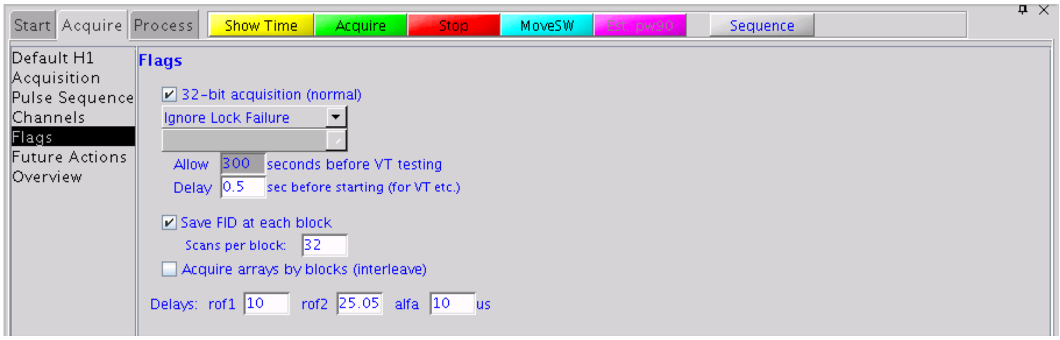
6.2 Acquisition Settings

6.2.1 Acquisition and Post Acquisition Actions

Use the Flags page of the Acquire tab to determine the data acquisition precision (32bit 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, rof 2 and alpha:` **`rof1`** is normally fixed as 10 µs. After the final pulse in each pulse sequence, the receiver is gated off for **`rof2`** µs before the acquisition begins. **`alfa`** and **`rof2`** are important where the flatness of the baseline is of concern.



Flags

☑ 32-bit acquisition (normal)

Ignore Lock Failure

Allow 300 seconds before VT testing

Delay 0.5 sec before starting (for VT etc.)

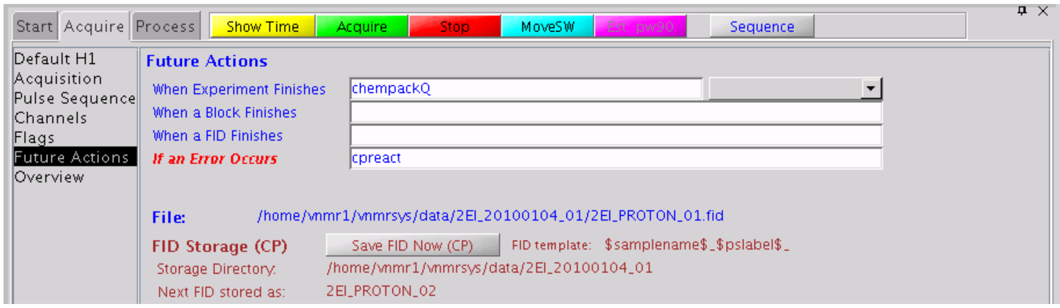
☑ Save FID at each block

Scans per block: 32

☐ Acquire arrays by blocks (interleave)

Delays: rof1 10 rof2 25.05 alfa 10 us

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.



Future Actions

When Experiment Finishes: chempackQ

When a Block Finishes:

When a FID Finishes:

If an Error Occurs: cpreact

File: /home/vnmr1/vnmrsys/data/2EI_20100104_01/2EI_PROTON_01.fid

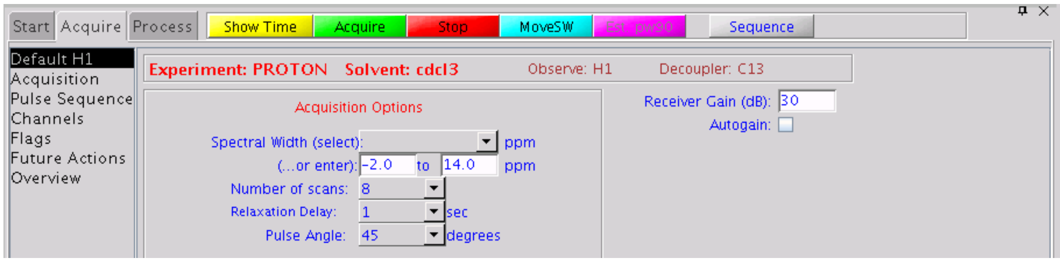
FID Storage (CP) FID template: $samplename$_$pslabel$_

Storage Directory: /home/vnmr1/vnmrsys/data/2EI_20100104_01

Next FID stored as: 2EI_PROTON_02

6.2.2 Nucleus-Specific Frequency Settings

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



Experiment: PROTON Solvent: cdcl3 Observe: H1 Decoupler: C13

Acquisition Options

Spectral Width (select): ppm

(...or enter): -2.0 to 14.0 ppm

Number of scans: 8

Relaxation Delay: 1 sec

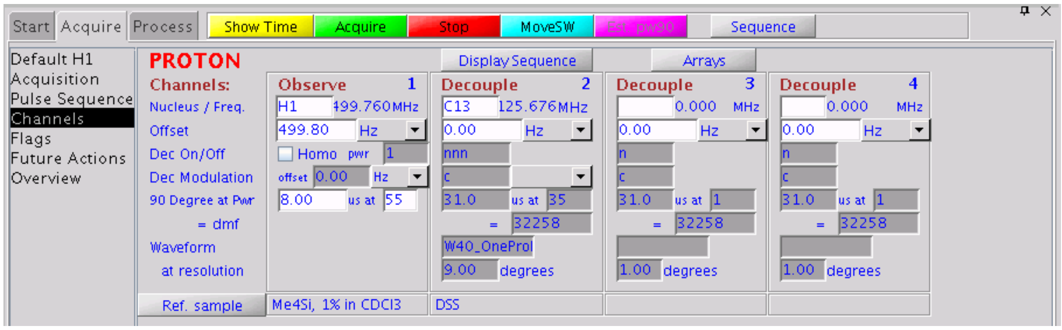
Pulse Angle: 45 degrees

Receiver Gain (dB): 30

Autogain: ☐

6.2.3 Transmitter and Decoupler Positioning

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



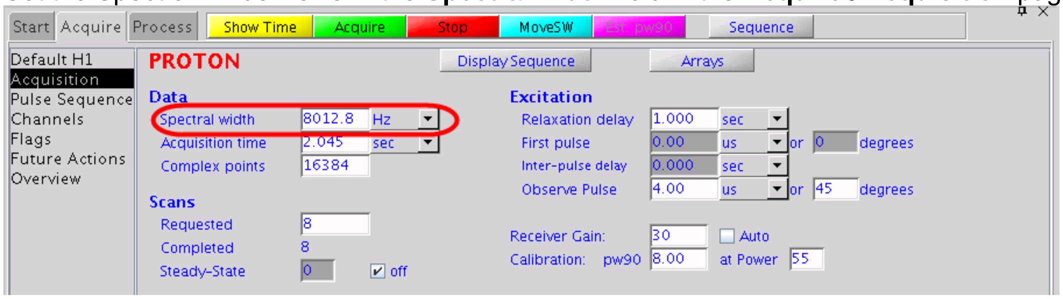
PROTON

Channels:	Observe 1	Decouple 2	Decouple 3	Decouple 4
Nucleus / Freq.	H1 499.760MHz	C13 125.676MHz	0.000 MHz	0.000 MHz
Offset	499.80 Hz	0.00 Hz	0.00 Hz	0.00 Hz
Dec On/Off	☐ Homo pwr 1	nnn	n	n
Dec Modulation	offset 0.00 Hz	c	c	c
90 Degree at Pwr	8.00 us at 55	31.0 us at 35	31.0 us at 1	31.0 us at 1
= dmf		= 32258	= 32258	= 32258
Waveform		W40_OneProl		
at resolution		9.00 degrees	1.00 degrees	1.00 degrees
Ref. sample	Me4Si, 1% in CDCl3	DSS		

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.

6.2.4 Spectral Window

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



PROTON

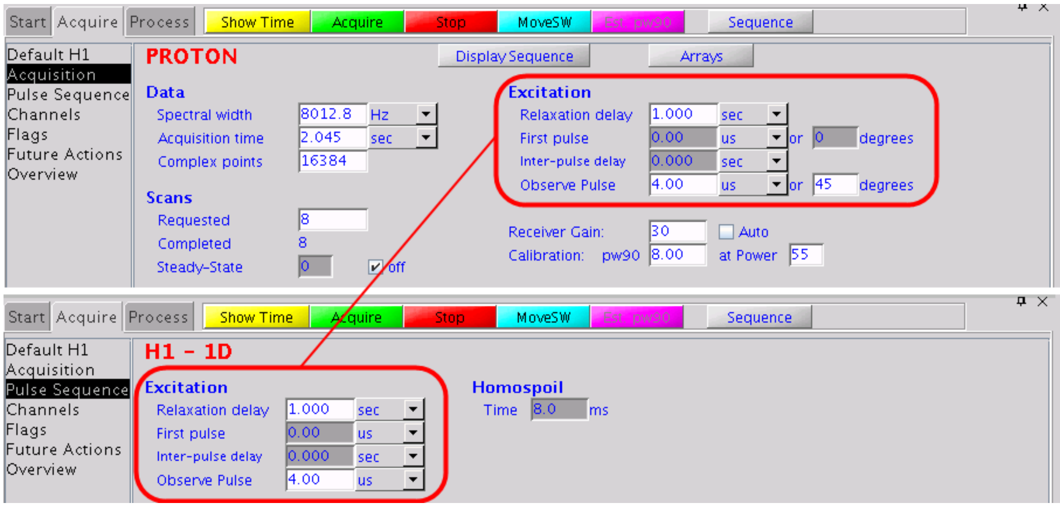
Data
Spectral width 8012.8 Hz
Acquisition time 2.045 sec
Complex points 16384

Scans
Requested 8
Completed 8
Steady-State 0 ☑ off

Excitation
Relaxation delay 1.000 sec
First pulse 0.00 us or 0 degrees
Inter-pulse delay 0.000 sec
Observe Pulse 4.00 us or 45 degrees

Receiver Gain: 30 ☐ Auto
Calibration: pw90 8.00 at Power 55

6.2.5 Pulse Sequence Settings (Standard Two-Pulse)



PROTON

Data
Spectral width 8012.8 Hz
Acquisition time 2.045 sec
Complex points 16384

Scans
Requested 8
Completed 8
Steady-State 0 ☑ off

Excitation
Relaxation delay 1.000 sec
First pulse 0.00 us or 0 degrees
Inter-pulse delay 0.000 sec
Observe Pulse 4.00 us or 45 degrees

Receiver Gain: 30 ☐ Auto
Calibration: pw90 8.00 at Power 55

H1 - 1D

Excitation
Relaxation delay 1.000 sec
First pulse 0.00 us
Inter-pulse delay 0.000 sec
Observe Pulse 4.00 us

Homospoil
Time 8.0 ms

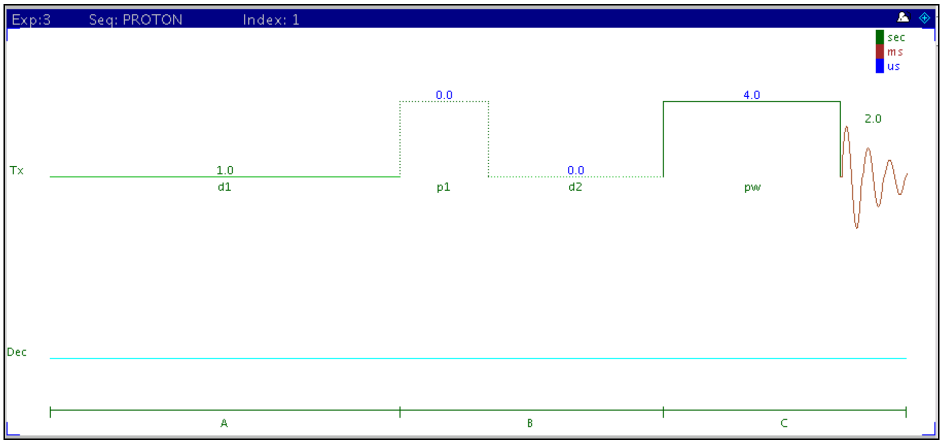
Use the Acquisition and Pulse Sequence pages to set the values for the pulse sequence.

Complex points	Generally calculated automatically when 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, (e.g., 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.

6.3 Pulse Sequences

6.3.1 Display the Pulse Sequence

1. Click on the **Acquire** tab.
2. Select the **Pulse Sequence** page
3. Click on the **Display Sequence** button to display the pulse sequence in the graphics window.



6.3.2 Standard Two-Pulse Parameters

Most experiments will be acquired using a pulse sequence known as the standard two-pulse, or S2PUL. Figure 18 shows a two-pulse sequence and the associated labels from the Acquire pages.

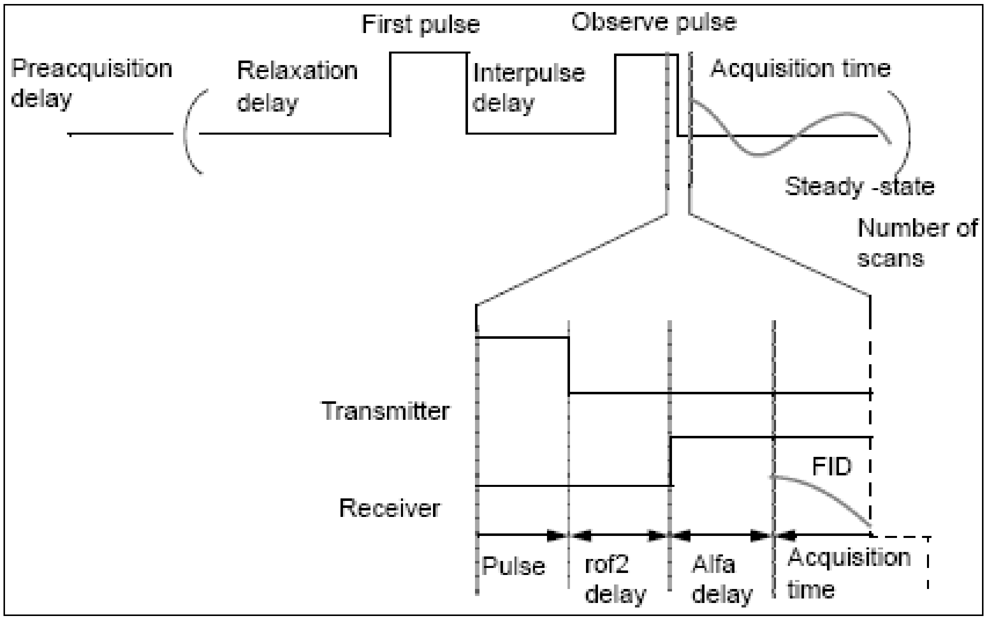


Figure 18 Acquisition Parameters for Standard Two-Pulse Sequence

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.

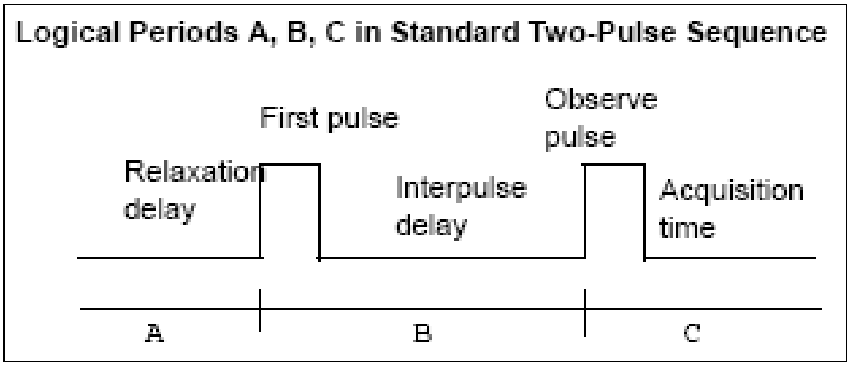
This process is repeated **Steady-State** number of times plus the **Requested Scans** field. Data is actually acquired only during the **Requested Scans** scans and not during the initial **Steady-State** scans.

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 **rof2** μs 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.

6.3.3 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).



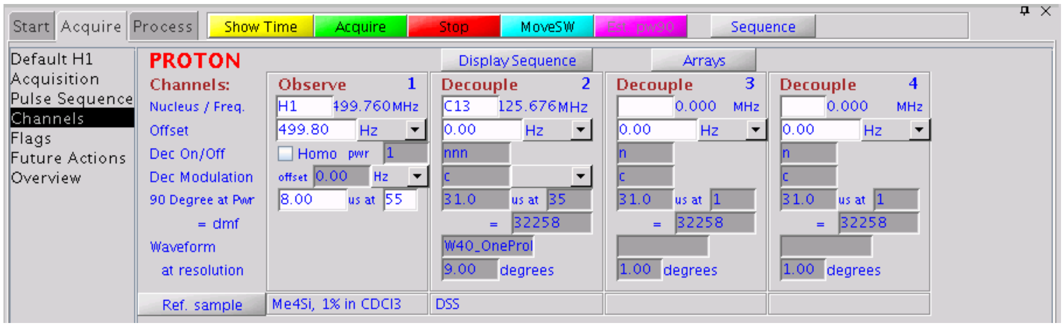
The decoupler fields in the **Channels** page use the following letters:

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.

6.3.4 Observe Transmitter and Decoupling Settings

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



Channels:	Observe 1	Decouple 2	Decouple 3	Decouple 4
Nucleus / Freq.	H1 499.760 MHz	C13 125.676 MHz	0.000 MHz	0.000 MHz
Offset	499.80 Hz	0.00 Hz	0.00 Hz	0.00 Hz
Dec On/Off	☐ Homo pwr 1	nnn	n	n
Dec Modulation	offset 0.00 Hz	c	c	c
90 Degree at Pwr	8.00 us at 55	31.0 us at 35	31.0 us at 1	31.0 us at 1
= dmf		= 32258	= 32258	= 32258
Waveform		W40_OneProl		
at resolution		9.00 degrees	1.00 degrees	1.00 degrees
Ref. sample	Me4Si, 1% in CDCl3	DSS		

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 tpwrf (or tpwrm) 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.

⚠ **CAUTION**	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.

6.3.5 Decoupler Modes

Several other efficient decoupling schemes are available from the Modulation pull-down menu, including GARP, MLEV-16, and XY32 decoupling. Refer to 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 (i.e., waveform generators).

The following values are typical for decoupling:

- Homonuclear decoupling:

`homo='y'`	Homonuclear decoupling on
`pwr=5-15`	Decoupler power level range

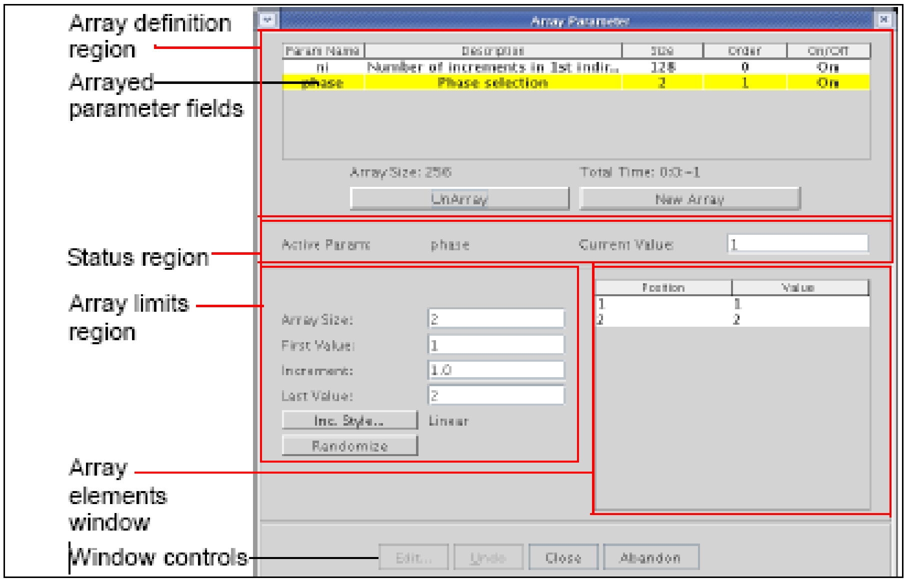
- Heteronuclear decoupling:

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

6.4 Parameter Arrays

1. Click on Acquisition on the main menu.
2. Select Parameter **Arrays…** to open the Array Parameter window to 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.



Param Name	Description	Size	Order	On/Off
ni	Number of increments in 1st indir...	128	0	On
phase	Phase selection	2	1	On

Array Size: 256   Total Time: 0:0-1

Active Param: phase   Current Value: 1

Array Size: 2
First Value: 1
Increment: 1.0
Last Value: 2
Inc. Style... Linear

Position	Value
1	1
2	2

Figure 19 Window Regions

6.4.1 Array Definition

Table 4 Arrayed Parameter Field Columns

Param Name	Defines name of arrayed parameter.
Description	Displays text description of array.
Size	Displays number of steps or increments in the array.
Order	Displays 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 5 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 list of arrayed parameters.
(Status Region)	Shows active parameter during acquisition and current value.
Array limits	Array Size field – enter the size of the array and press return. First Value – enter the starting value of the array and press return. Increment – enter the array increment and press return. Last Value – enter the ending value of the array and press return. Inc. Style … button – click and select linear or exponential. Randomize button – click to create a random array.
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	Edit—Not active. Undo—Undoes the change; restores the change (if clicked again). Close—Closes the window (and saves changes). Abandon—Closes the window and makes no changes.

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/Off**. Edit the array order (except for implicit arrays ni, ni2, etc.) to enable nested or parallel (diagonal) arrays. Only parameters of the same array size can be parallel, i.e., 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 allows 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 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.

6.5 Stopping and Resuming Acquisition

Click either the **Stop** icon ⏹ or the **Stop** button next to the Acquire button 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.

6.6 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 (e.g., `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, (e.g., 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 are 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, (e.g., 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, (e.g., wexp='wft2da').

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

6.7 Acquisition Status Window

Click on 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.

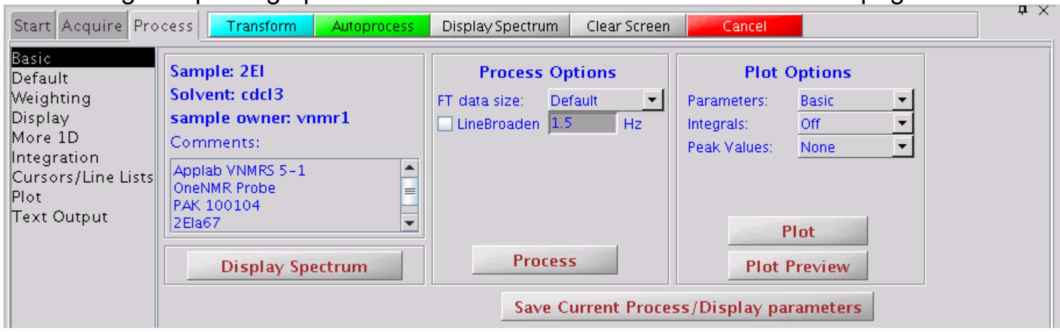
Table 6 Fields in the Acquisition Status Window

Field	**Description**
Status	Present status of acquisition. The values displayed should be self-explanatory (e. g., “Shimming”) with two exceptions: “Active” means that the acquisition computer started but the console is not active yet, and “Inactive” means that `acqstat` 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 (e. g., `exp1`, `exp2`, `exp3`)
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: O ff, Regulated, or Not Regulated (if VT is set as present and `vttype=2`)
Stored	Last time data was transferred to disk

Chapter 7 Processing Data

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.



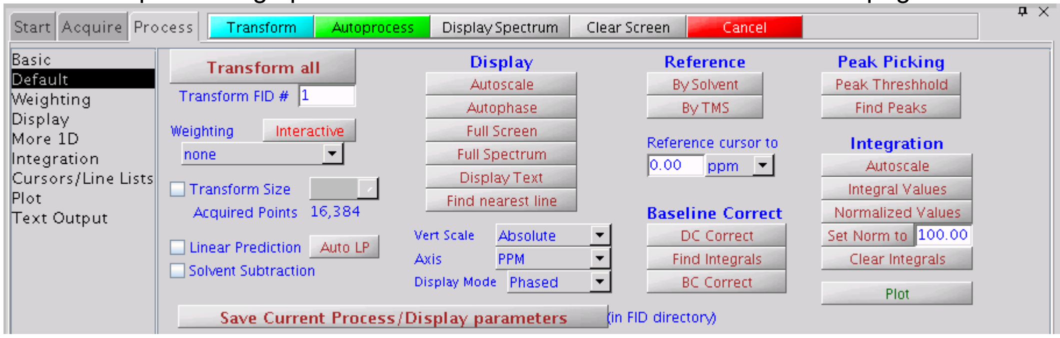
Sample: 2EI
Solvent: cdcl3
sample owner: vnmr1
Comments:
Applab VNMRS 5-1
OneNMR Probe
PAK 100104
2Ela67

FT data size: Default
☐ LineBroaden 1.5 Hz

Parameters: Basic
Integrals: Off
Peak Values: None

Figure 20 Process/Basic Page

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



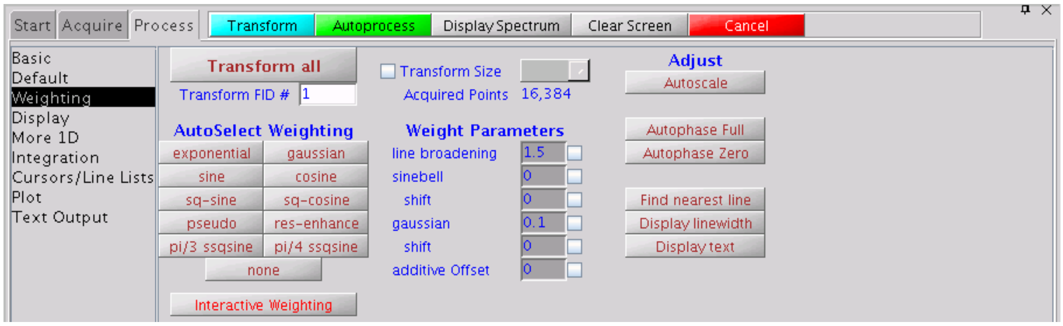
Transform FID # 1
Weighting none
☐ Transform Size
Acquired Points 16,384
☐ Linear Prediction
☐ Solvent Subtraction
Vert Scale Absolute
Axis PPM
Display Mode Phased
Reference cursor to 0.00 ppm
Set Norm to 100.00

Figure 21 Process/Default Page

Sections in this chapter:

- 7.1 Weighting Function
- 7.2 Interactive Weighting
- 7.3 Fourier Transformation
- 7.4 Phasing
- 7.5 Advanced Data Processing

7.1 Weighting Function



Transform FID # 1

☐ Transform Size

Acquired Points 16,384

line broadening 1.5 ☐

sinebell 0 ☐

shift 0 ☐

gaussian 0.1 ☐

shift 0 ☐

additive Offset 0 ☐

Figure 22 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 `sin2(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 checkbox 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.

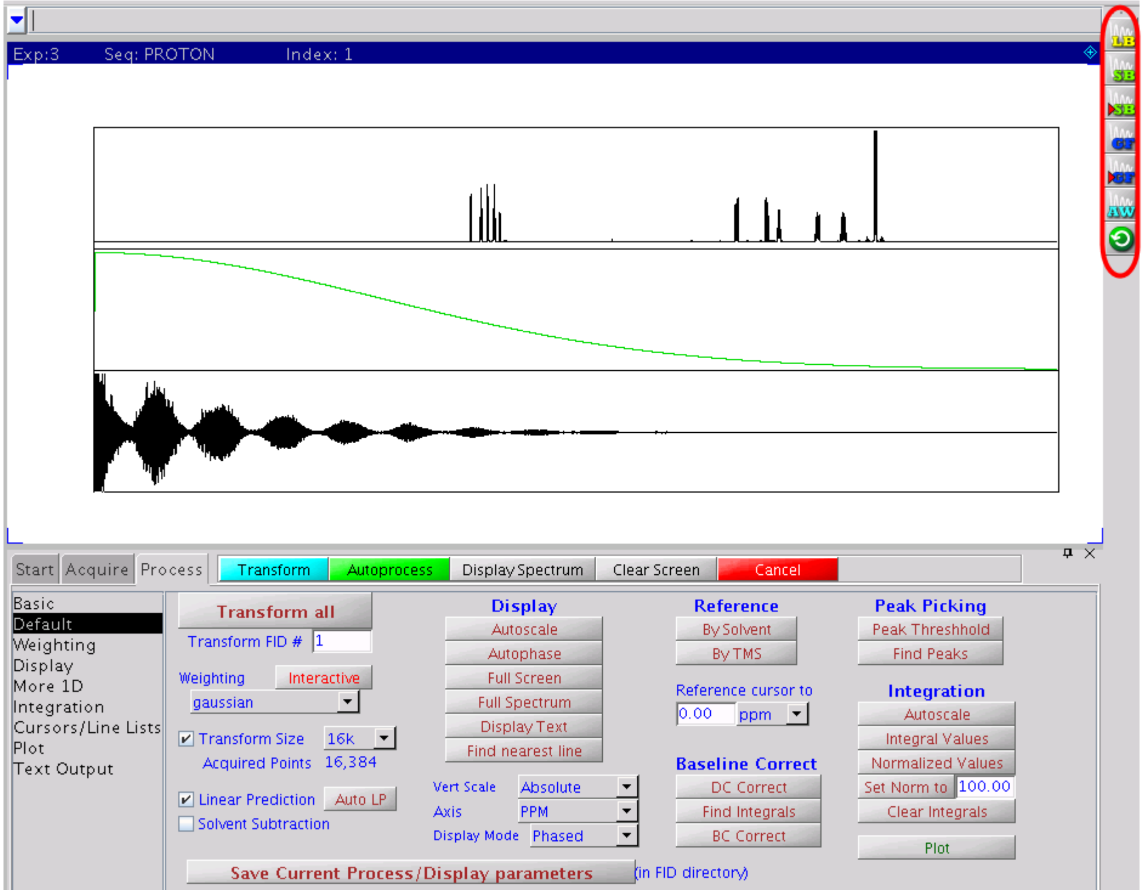
The **res-enhance** button sets defaults of a equal to 0.1 and b equal to 0.3 into the formulas lb=–0.318/(a*sw), and gf=b*sw, thereby calculating “reasonable” values for the resolution enhancement parameters lb and gf. The arguments a and b can also be selected by the user.

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

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

7.2 Interactive Weighting

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



Transform FID # 1
Weighting gaussian
☑ Transform Size 16k
Acquired Points 16,384
☑ Linear Prediction
☐ Solvent Subtraction
Vert Scale Absolute
Axis PPM
Display Mode Phased
Reference cursor to 0.00 ppm
Set Norm to 100.00

Interactive weighting buttons

Figure 23 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	LB	Selects line broadening or exponential weighting. A negative value gives resolution enhancement.
Sinebell	SB	Selects the sinebell constant. A negative value gives squared sinebell. Change sign by clicking outside the box at the left.
Shifted Sinebell	▸SB	Selects the sinebell shift constant (if sinebell is active).
Gaussian	GF	Selects the Gaussian time constant.
Shifted Gaussian	▸GF	Selects the Gaussian shift constant (if Gaussian is active).
Additive weighting	AW	Selects the additive weighting constant.
Return	↺	Returns to the previous menu.

Currently active weighting parameters can be changed by moving the mouse 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 vf). Use the center mouse button within the spectrum box to adjust spectrum intensity (vs). Use the right mouse button to turn the display of the transformed spectrum off and on. (It will be off by default whenever `fn > 25000`.)

7.3 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 (fn), 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 (np) were acquired, the first power of two greater than or equal to np will be used as fn. If fn is greater than np, or if fn is 'n' and np is not a power of two, the remaining points in the transform are filled in with values of zero (*zero-filling*). (If `np` is <10% bigger than `fn`, 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 fn and np. The number of complex data points is fn/2.

7.4 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.

7.4.1 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:

Absorption spectrum$_{\omega}$ =
$\mathit{real} * \cos\theta + \mathit{imaginary} * \sin\theta$ [Eq. 1]

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

θ = `rp + (w-w0) /sw*lp` [Eq 2]

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

The following is clear about the terms in Equation 2:

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

There are several ways in which lp and rp can be adjusted:

- Like any parameter, they can be recalled with a particular parameter set. They can also be entered directly (e.g., `lp=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°.

7.4.2 Autophase Algorithm

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

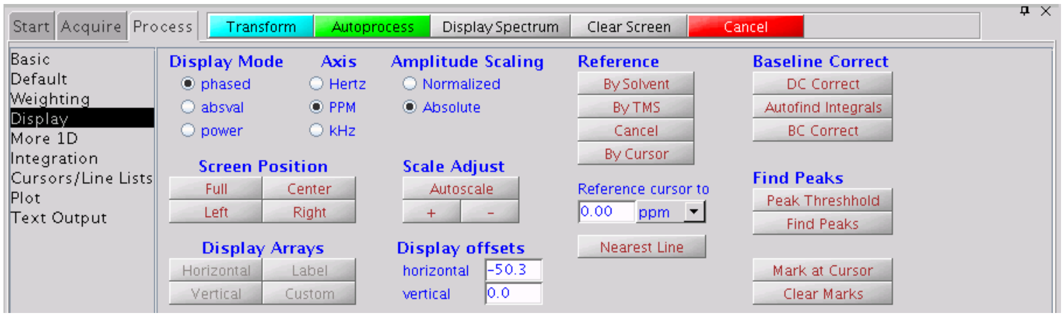
- Weighting parameters do not affect the algorithms.
- Spectra with very low signal-to-noise can be phased.
- In vivo spectra can be phased, which is very difficult for most autophasing algorithms.
- Spectra with inverted lines can be phased. Such spectra include APT or DEPT data or selectively inverted 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. Refer to the *Command and Parameter Reference* for more information about this command.

7.4.3 Spectrum Display



Display Mode: ● phased ○ absval ○ power

Axis: ○ Hertz ● PPM ○ kHz

Amplitude Scaling: ○ Normalized ● Absolute

Reference cursor to 0.00 ppm

Display offsets: horizontal -50.3, vertical 0.0

Figure 24 Process/Display Page

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

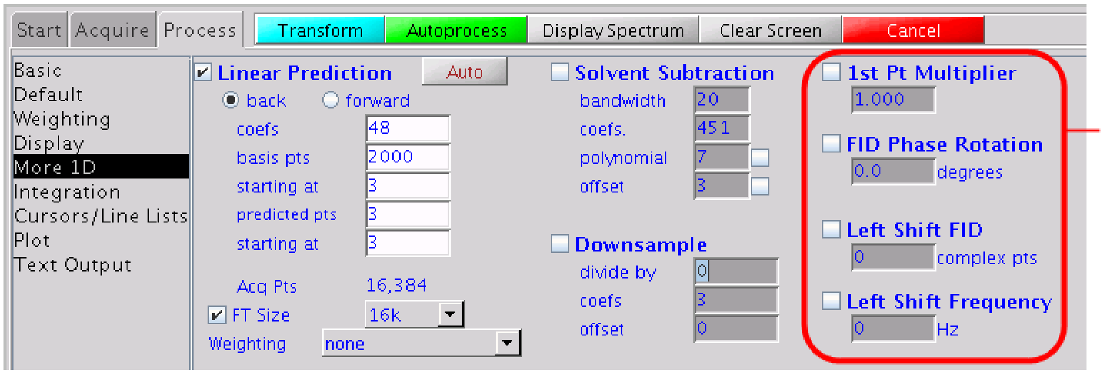
- The *phase-sensitive mode* is selected by the command ph. In this mode, the displayed spectrum is calculated using the phase parameters lp and rp. This is the most commonly used mode.
- The *absolute-value mode* is selected by the command av. In this mode, the displayed spectrum is calculated according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

  absorption spectrum(ω) = $(\textit{real}^2(\omega) + \textit{imaginary}^2(\omega))^{1/2}$
- The *power mode* 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 arctangent 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.

7.5 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.



☑ Linear Prediction: ● back ○ forward; coefs 48; basis pts 2000; starting at 3; predicted pts 3; starting at 3

Acq Pts 16,384

☑ FT Size 16k

Weighting none

☐ Solvent Subtraction: bandwidth 20; coefs. 451; polynomial 7 ☐; offset 3 ☐

☐ Downsample: divide by 0; coefs 3; offset 0

☐ 1st Pt Multiplier 1.000

☐ FID Phase Rotation 0.0 degrees

☐ Left Shift FID 0 complex pts

☐ Left Shift Frequency 0 Hz

FID manipulation options

7.5.1 FID Manipulation

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

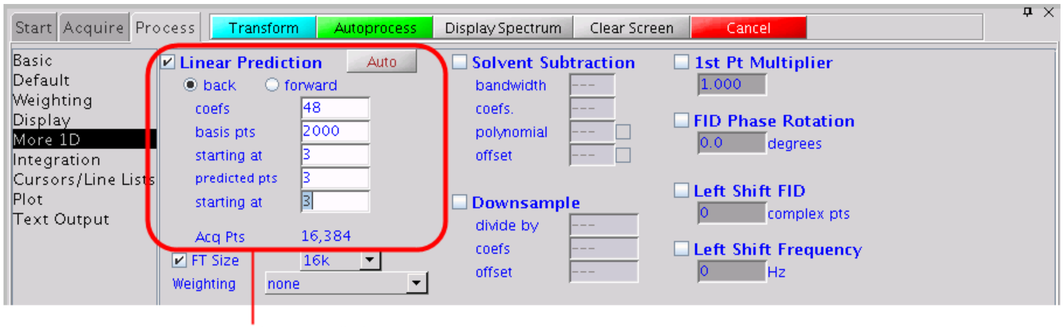
7.5.2 Data Processing Methods

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.

7.5.3 Linear Prediction

Use the Linear Prediction page to control linear prediction and to adjust its parameters.



☑ Linear Prediction
● back ○ forward
coefs 48
basis pts 2000
starting at 3
predicted pts 3
starting at 3
Acq Pts 16,384
☑ FT Size 16k
Weighting none

☐ Solvent Subtraction
bandwidth ---
coefs. ---
polynomial --- ☐
offset --- ☐

☐ Downsample
divide by ---
coefs ---
offset ---

☐ 1st Pt Multiplier
1.000

☐ FID Phase Rotation
0.0 degrees

☐ Left Shift FID
0 complex pts

☐ Left Shift Frequency
0 Hz

Linear prediction options

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 **Linear Prediction** check box in the Linear Prediction panel and clicking the **Transform** button.

Enter ft('noft') 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:

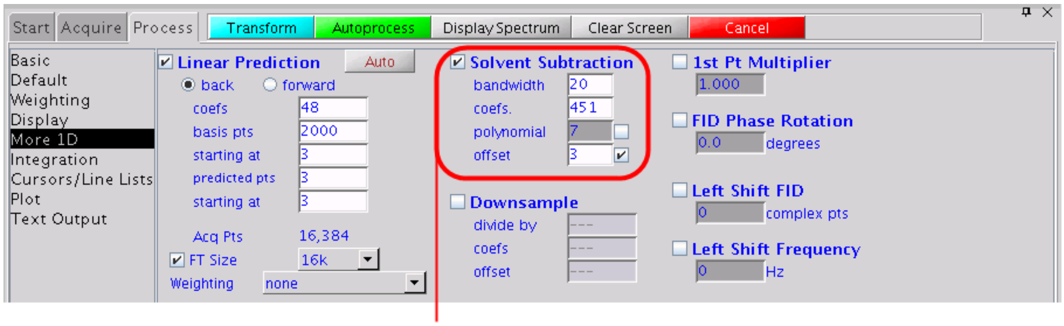
- Early points in the FID may be distorted due to a host of hardware characteristics, such as preamplifier saturation and probe ringing. Even on a perfect spectrometer, these distortions cannot always be avoided.
- The acquisition time of each FID may have been too short to allow for full decay of the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

Refer to 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.

7.5.4 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.



☑ **Linear Prediction**
● back ○ forward
coefs 48
basis pts 2000
starting at 3
predicted pts 3
starting at 3
Acq Pts 16,384
☑ FT Size 16k
Weighting none

☑ **Solvent Subtraction**
bandwidth 20
coefs. 451
polynomial 7 ☐
offset 3 ☑

☐ **Downsample**
divide by ---
coefs ---
offset ---

☐ **1st Pt Multiplier**
1.000

☐ **FID Phase Rotation**
0.0 degrees

☐ **Left Shift FID**
0 complex pts

☐ **Left Shift Frequency**
0 Hz

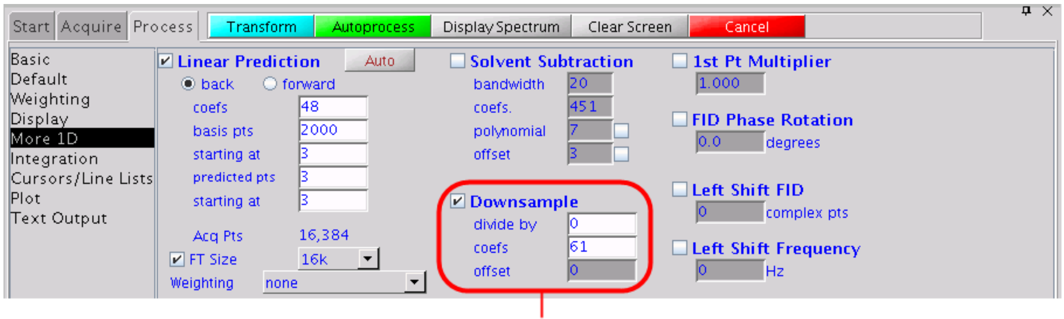
Solvent subtraction options

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 np/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.

7.5.5 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.



☑ Linear Prediction
● back ○ forward
coefs 48
basis pts 2000
starting at 3
predicted pts 3
starting at 3
Acq Pts 16,384
☑ FT Size 16k
Weighting none

☐ Solvent Subtraction
bandwidth 20
coefs. 451
polynomial 7 ☐
offset 3 ☐

☑ Downsample
divide by 0
coefs 61
offset 0

☐ 1st Pt Multiplier
1.000

☐ FID Phase Rotation
0.0 degrees

☐ Left Shift FID
0 complex pts

☐ Left Shift Frequency
0 Hz

Downsample options

- 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.

7.5.6 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. Refer to 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.

Chapter 8 Displaying FIDs and Spectra

Sections in this chapter:

8.1 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.

8.1.1 FID Display

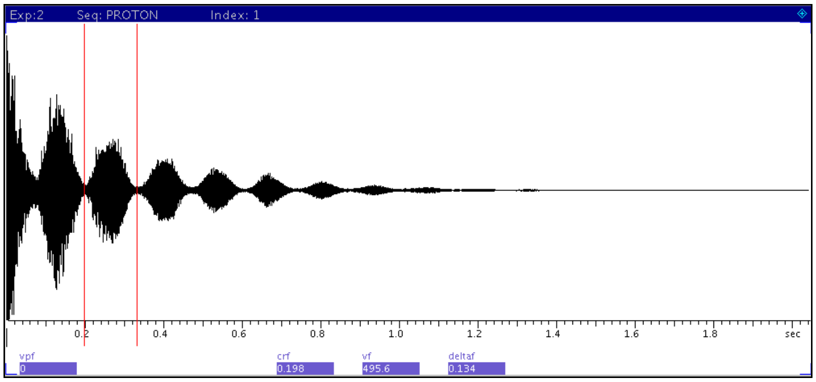
An FID is available for displaying upon completion of the acquisition of acquisition block block size). Clicking the FID button displays a FID and enables interactive manipulation of the FID display.

The FID display graphics buttons change to show that multiple FIDs can be viewed. Figure 25 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.

8.1.2 1D Spectrum Display

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

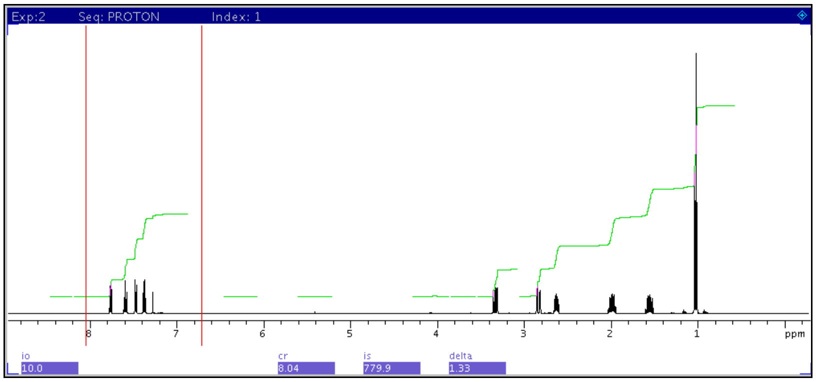


vpf 0
crf 0.198
vf 495.6
deltaf 0.134

Figure 25 Interactive FID Display

The normal spectrum display enables interactive manipulation of a single 1D spectrum. A spectrum is displayed by clicking the 1D Spectrum graphics control button or by transforming a data set.

A spectrum displays in the graphics window similar to Figure 26.



io 10.0
cr 8.04
is 779.9
delta 1.33

Figure 26 Interactive Spectrum Display

8.2 Display Tools

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

8.2.1 Interactive Display Tools

These tools are described below:

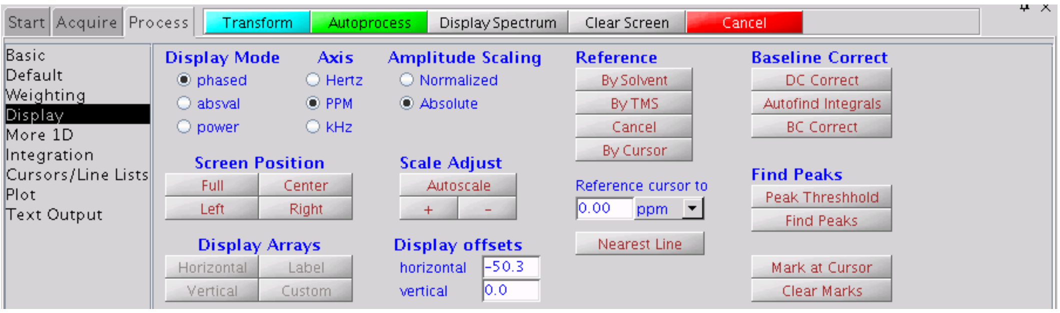
Mouse buttons	The mouse buttons correspond to the display parameters shown on the lower right part of the graphics window. The display parameter changes as different graphics control functions are selected. Typically, the left button controls the left cursor position, the middle button controls vertical 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 cursors, zooming, scales, grab & 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 graphics control bar.
2. Select the region to expand -- left click on 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.

8.2.2 Display Parameters

FID and spectral display is governed by parameters on the Display page.



Display Mode
● phased
○ absval
○ power

Axis
○ Hertz
● PPM
○ kHz

Amplitude Scaling
○ Normalized
● Absolute

Reference cursor to 0.00 ppm

Display offsets
horizontal -50.3
vertical 0.0

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.
Absval	(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 *all* 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 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.

8.2.3 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 crf or cr parameter (crf for a FID or cr 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 deltaf for a FID or delta 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 (deltaf or delta) unchanged.
Vertical scale	Click the middle mouse button to adjust the vertical scale of the FID (vf parameter) or spectrum (vs 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 vpf or vp (or vpfi if the imaginary channel) is will change.

8.2.4 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 (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.

8.3 Graphics Control Buttons

The graphics control bar for the active viewport is to the right of the graphics canvas. Use the buttons in the bar to control the interactive display in the graphics canvas.

8.3.1 Common Graphics Display Toolbar Controls

The following tools are common to 1D, nD, and fid display toolbars.

Icon	**Description**
🔍	Zoom in.
🔍	Zoom out.

	Select zoom region.
	Redraw display.
	Return to previous tool menu.

8.3.2 1D Display Spectrum Toolbar Controls

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 spectral display.
	Pan or move spectral region.
	Display integral.
	Display scale.
	Toggle threshold on or off.
	Phase spectrum.

8.3.3 Display FID Toolbar Controls

Icon	Description
	One cursor in use, click to toggle to two cursor.
	Two cursors in use, click to toggle to one cursor.
	Click to expand to full FID display.
	Pan and stretch.
	Click to show real and imaginary.
	Click to show real and zero imaginary.

	Click to show real only.
	Toggle scale on and off.
	Phase FID.

8.3.4 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.

8.3.5 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%.
	Phase spectrum.
	Click to select the first spectrum.
	Click to select the second spectrum.
	Enter peak pick menu.

8.4 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:

8.4.1 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 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.

8.4.2 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 rp.
    - Click above the horizontal cursor to increase rp (cause a clockwise rotation of the peaks).
    - Click below the horizontal cursor to decrease rp (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 `lp`.

4. Click the left or right button above or below the horizontal cursor to increase or decrease lp 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 rp and lp. 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.

8.5 Line Tools

8.5.1 Find Nearest Line and Line Resolution

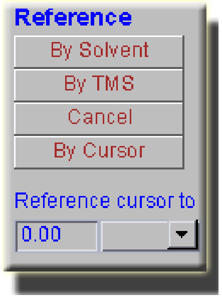
1. Place a cursor near the line of interest.
2. Select the Process page and click the **Find nearest line** button. The cursor moves to the nearest line and displays its height and frequency (in Hz and ppm) in the message window.
3. Click **Display** linewidth to display the resolution of a line, as 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.

8.5.2 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.

8.6 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 (e.g., from silicon grease) immediately to the left of the TMS line (even if they are higher than the reference line), tmsref 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-CH3 signal. Large signals within 0.6 ppm for 1H (or 6 ppm for 13C) 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 7 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.
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 *reference line reference position*.
Spectrometer frequency	The absolute frequency, in MHz, of the center of the spectrum (the transmitter position). Use the spcfrq command in order to see the accurate value of the spectrometer frequency (sfrq parameter).
Reference frequency	The frequency, in MHz, of the frequency standard, i.e., the zero position of the frequency scale, *and* the divider (unit) for the calculation of ppm scales (reffreq).

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.

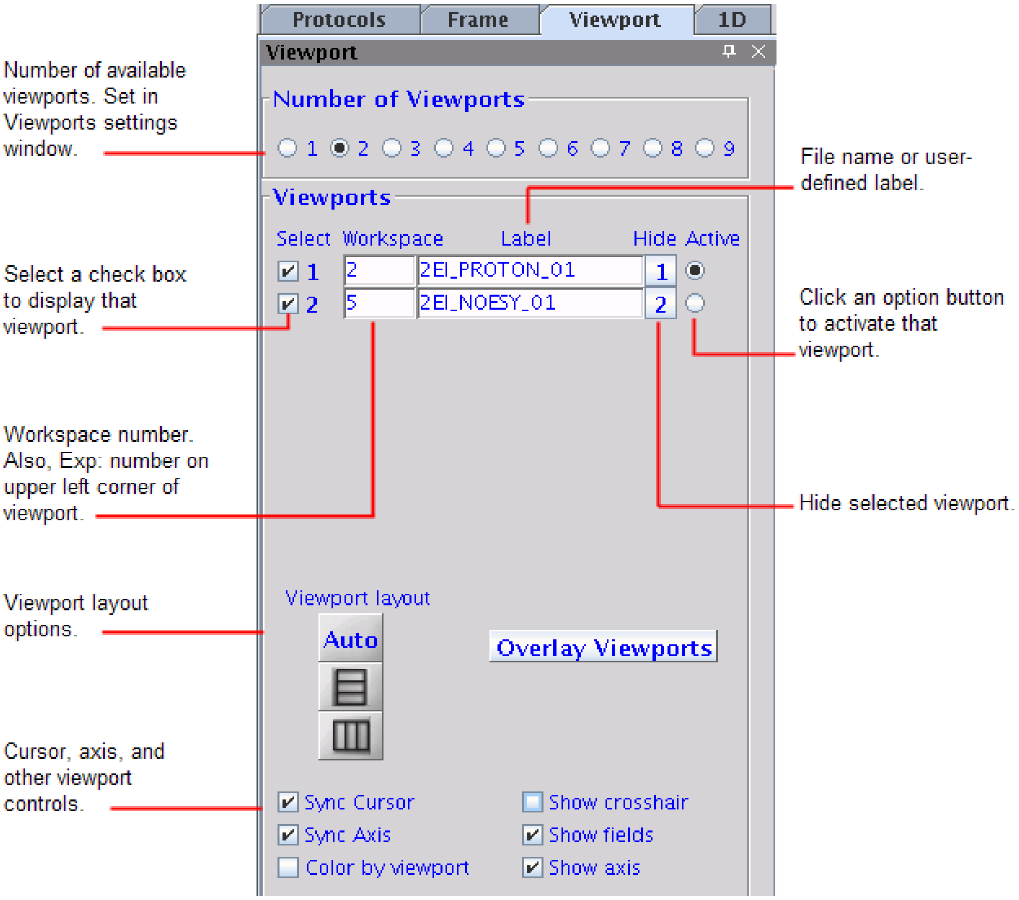
The accuracy of the solvent and TMS referencing buttons is mostly limited by the accuracy of the chemical shift of the lock resonance line, which may depend on the concentration and the chemical properties (acidity/basicity) of the components in the sample. But they should normally be accurate enough 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, provided the solvent parameter is set to the solvent that was last locked on.

8.7 Display an Inset Spectrum Using Viewport Tab

8.7.1 Viewport Tab

Click on the **Viewport** tab to display the viewport controls. If the tab is not visible, click **View** on the main menu and select **Viewport**.



Number of Viewports: ○ 1 ● 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9

Select	Workspace	Label	Hide	Active
☑ 1	2	2EI_PROTON_01	1	●
☑ 2	5	2EI_NOESY_01	2	○

☑ Sync Cursor
☑ Sync Axis
☐ Color by viewport
☐ Show crosshair
☑ Show fields
☑ Show axis

Number of available viewports. Set in Viewports settings window.

Select a check box to display that viewport.

Workspace number. Also, Exp: number on upper left corner of viewport.

Viewport layout options.

Cursor, axis, and other viewport controls.

File name or user-defined label.

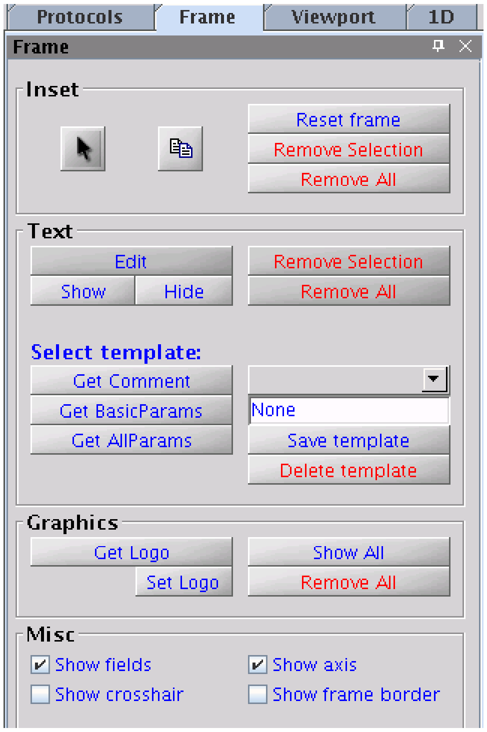
Click an option button to activate that viewport.

Hide selected viewport.

Figure 27 Viewport Tab and Controls

8.7.2 Frame Tab

The Frame tab, see Figure 28, of the viewport tab has the following tools, button, and check boxes:



☑ Show fields
☑ Show axis
☐ Show crosshair
☐ Show frame border

Figure 28 Frame Tab and Controls

Table 8 Viewport Tools

Icon	Function
↖	**Default mode** — left mouse click moves the left cursor and right mouse click moves right mouse cursor.
⧉	**Inset mode** — left mouse drag a box over a spectrum region creates an inset frame of the region. A viewport can have multiple inset frames. Exit inset mode — release mouse button.

Inset Frame Buttons

The buttons delete one or all inset frames and restore the default frame to full size.

Button	Function
Delete Inset	Delete the selected inset.
Delete all	Delete all inset frames.
Full size	Restore the default frame to its full size.

Display Check Boxes

The check boxes control optional display features.

Check box	Function
Cross hair	Display cross hair and chemical shift(s) of the cursor position when mouse is moved over the spectrum. A useful function when the fields are not shown, not in cursor mode (default mode), or when chemical shift of a peak without moving the left cursor is required while in the cursor mode.
Fields	Display cr, delta, vp etc... fields at the bottom of the viewport.
Axis	Show scale of the axis.
Show frame border	Check the box to display a box around the frame. Un-check the box to display the four corners of the selected frame as *hot spots* for resizing. No border or corner will be displayed if a frame is not selected. An empty frame is not visible until it is selected.

8.7.3 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:

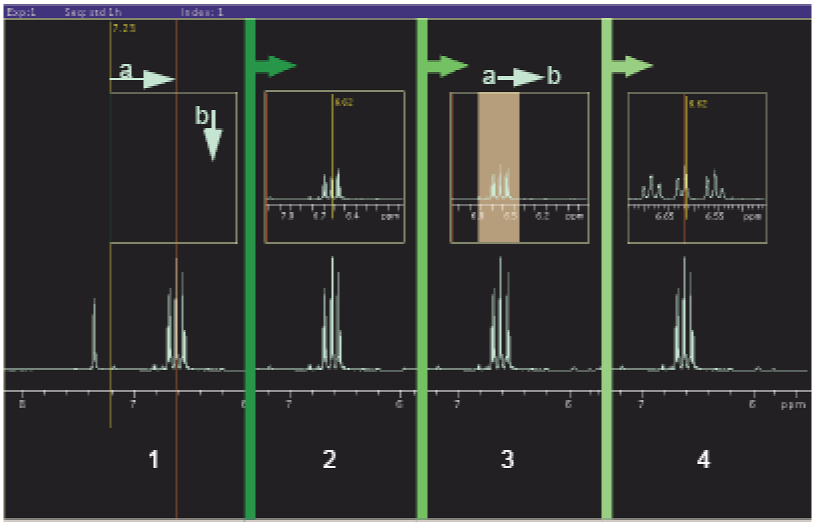


Figure 29 Creating an Inset Frame

1. Select the inset mode tool .
2. Place the cursor at the low field (left) side of the region to be expanded as shown in Figure 29 frame 1a.
3. Hold the left mouse button down and drag the inset window to the high field (right) side of the region.
4. Drag the cursor down to set the height of the inset frame as shown in Figure 29 frame 1b.
5. Release the mouse button to create the inset frame, see Figure 29 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.

   A 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).
3. Select the zoom mode tool.
4. Place the cursor at the low field (left) side of the region to be expanded as shown in Figure 29 frame 3a.
5. Hold the left mouse button down and drag the inset window to the high field (right) side of the region, Figure 29 frame 3b.
6. The region selected is indicated by a transparent gray rectangle.
7. Release the mouse button and the selected region expands to fill the inset box, Figure 29,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.

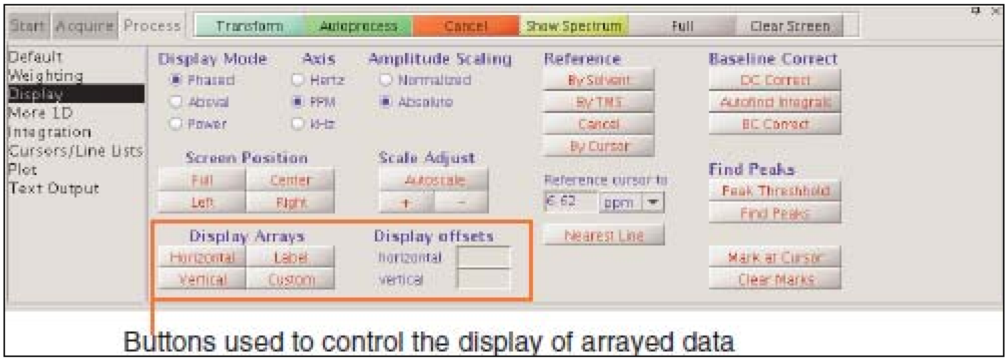
4. Hold down the left mouse button and grab the edge of the frame.
5. Drag the frame to the new position.
6. Release the mouse button when the frame is at the desired position.

8.8 Stacked 1D Display

8.8.1 Stacked Display Using the Main Menu Display

1. Click on Display on the main menu.
2. Select a display mode from the dropdown 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

8.8.2 Stacked Display Using the Display Page



Buttons used to control the display of arrayed data

1. Click on the Process tab.
2. Select the Display page.

3. Click on 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** — enter a value in mm for the separation between spectra.
    - **vertical** — enter a value in mm for the separation between spectra.

8.8.3 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 next spectrum.
←	Display 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.

8.8.4 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 next FID.
←	Display 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.

8.9 Aligning and Stacking Spectra

8.9.1 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.

8.9.2 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 for Aligning and Stacking Spectra.
3. Select viewports containing spectra to overlay by placing a check in the check box under **select**.
4. Click on the **Overlay viewports** button to overlay all selected viewports.

The Stack Spectrum button, Figure 30, is displayed below 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 Stack Spectrum button. Spectral axes are also synchronized to enable zoom and pan of the spectrum without losing the alignment.

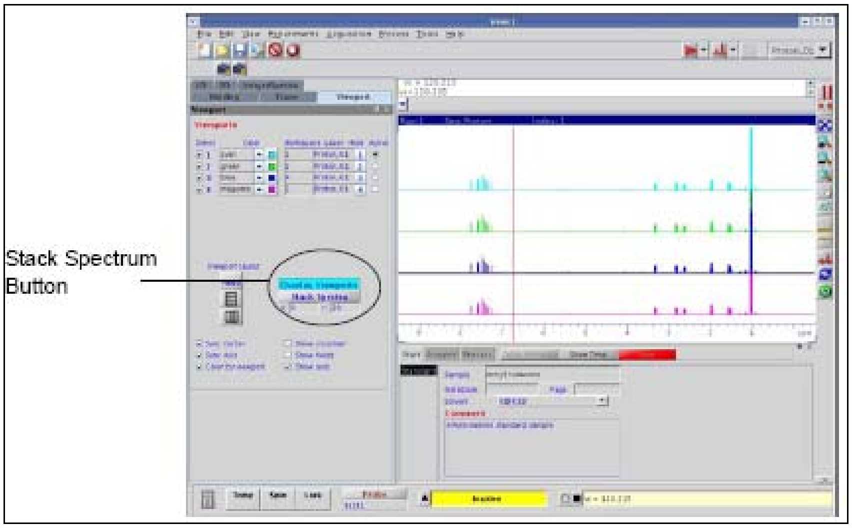


Figure 30 1D Stacked Spectra

The Align spectra button, Figure 31, 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.

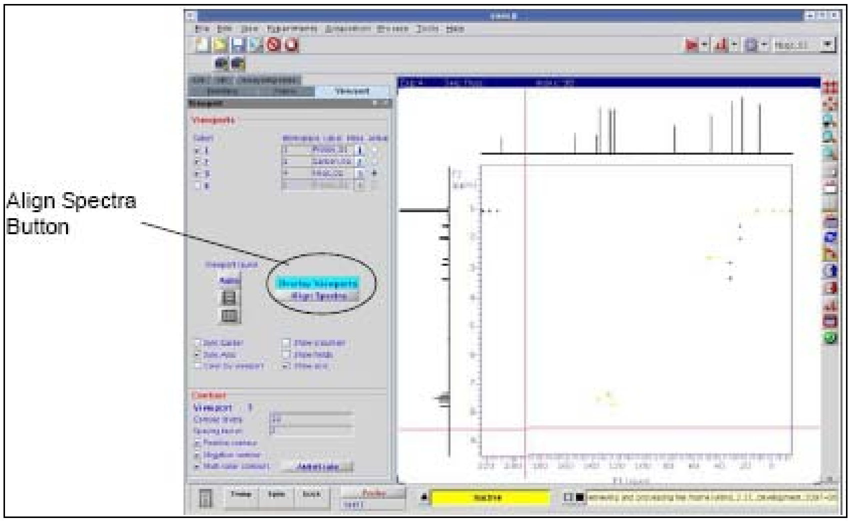


Figure 31 2D Spectra with Overlaid 1D's

The stacked spectrum button for 2Ds, Figure 32, 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.

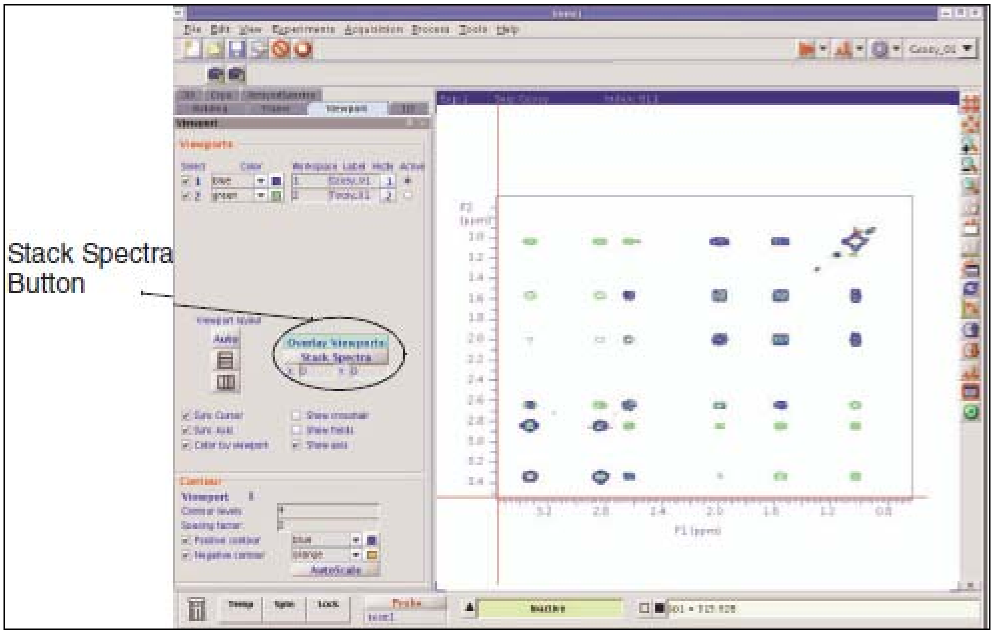
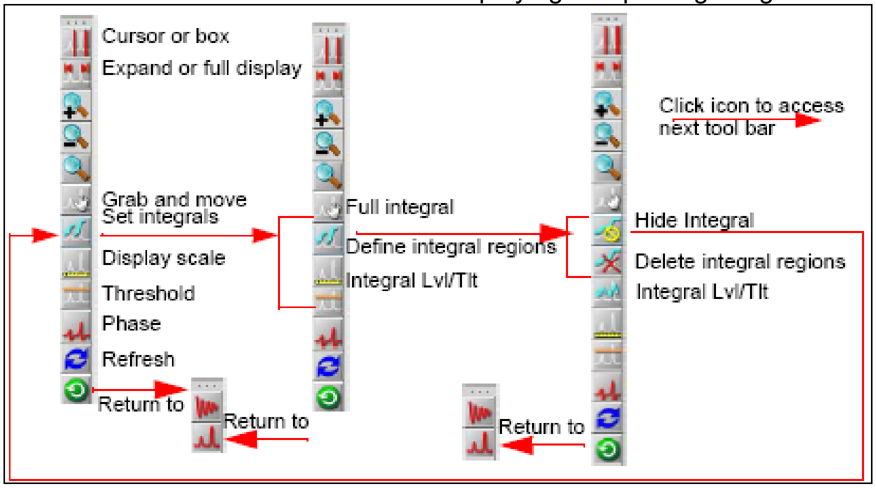
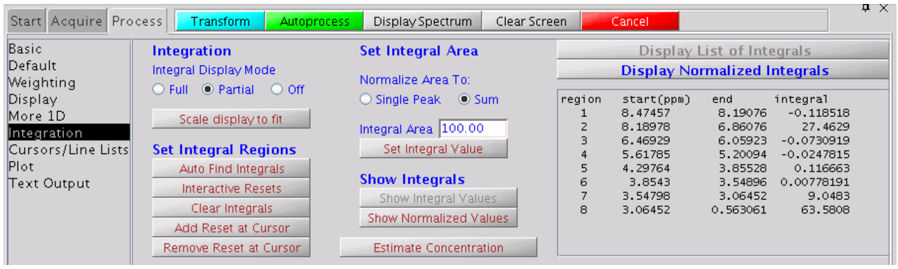


Figure 32 Stacked Overlaid 2D Spectra

8.10 Integration

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





Integral Display Mode
○ Full ● Partial ○ Off

Normalize Area To:
○ Single Peak ● Sum

Integral Area 100.00

```
region   start(ppm)     end      integral
  1      8.47457      8.19076   -0.118518
  2      8.18978      6.86076     27.4629
  3      6.46929      6.05923  -0.0730919
  4      5.61785      5.20094  -0.0247815
  5      4.29764      3.85528    0.116663
  6       3.8543      3.54896  0.00778191
  7      3.54798      3.06452      9.0483
  8      3.06452     0.563061     63.5808
```

8.10.1 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 on 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 on another region of the spectrum, outside the vertical cursors.

   A new horizontal cursor displays at the mouse arrow, two new vertical cursors display on either side of the mouse arrow, and a single vertical cursor displays in the middle of the region where lvl was being updated. The mouse now controls the first-order baseline correction parameter tlt.

4. Right or left click above or below the horizontal cursor to increase or decrease tlt, and change lvl 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 lvl and tlt. 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 display.

   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 lvltlt. This parameter is a multiplier, with a default value of 1.0, for the size of the changes. To make larger changes, make lvltlt larger than 1.0. To have finer control, set lvltlt 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.

8.10.2 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 on the Process tab.
4. Select the Integration page.
5. Click on 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 on **Auto Find Integral**s to automatically set the integral resets and display the data as set by the Integration Display Mode radio button.
7. Click on **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 on one of the following radio buttons under the **Normalize Area to: page** region.

    Single Peak — select the region or peak under the cursor as the reference and set 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 on 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 on the Show Integral Values to display the values of the integral regions on the screen below the spectrum.

      Click on Display Lists of Integrals to list the display regions and the value of the integral over each region.
    - **Normalized Integral Values**

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

      Click on 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 on the Process tab.
4. Select the Integration page.

5. Click on 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 on 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 on the left mouse button.
   f. Repeat Step b through Step e until all the required integral regions are defined.
7. Click on 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 on one of the following radio buttons under the Normalize Area to: page region.

      Single Peak — select the region or peak under the cursor as the reference and set 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 on 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 on the **Show Integral Values** to display the values of the integral regions on the screen below the spectrum.

     Click on **Display Lists of Integrals** to list the display regions and the value of the integral over each region.
   - Normalized Integral Values

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

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

8.10.3 Command Line Equivalents for VnmrJ Interface Driven Integration

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

1. Click on 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:

8.10.4 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 lvl and a first-order baseline correction parameter tlt. The cdc command turns off this correction. The results of the dc or cdc command are stored in the dcg parameter, which can be queried (dcg?) to determine whether drift correction is active. If active, dcg=''; if inactive, dcg='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, refer to 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.

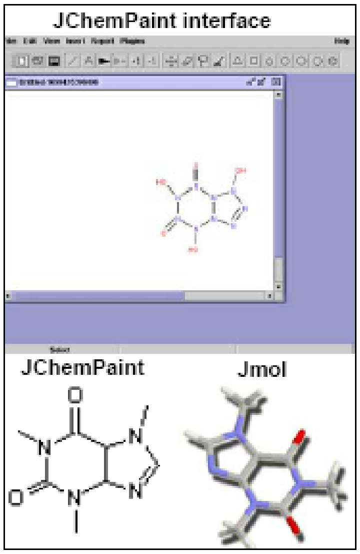
The pir command plots digital integral values below the spectrum, using the integral blanking mode in which only every other integral is plotted. The command pirn plots the normalized integral values in an analogous fashion.

8.11 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.



8.11.1 Running JChemPaint

1. Click on Tools.
2. Select Molecular Structures.
3. Select JCh**emPaint menu**.

Refer to http://jchempaint.sourceforge.net for documentation.

8.11.2 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 on **Tools** on the main menu.
4. Select Open, browse to the file, and drag it to the VnmrJ graphics screen.

8.11.3 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:

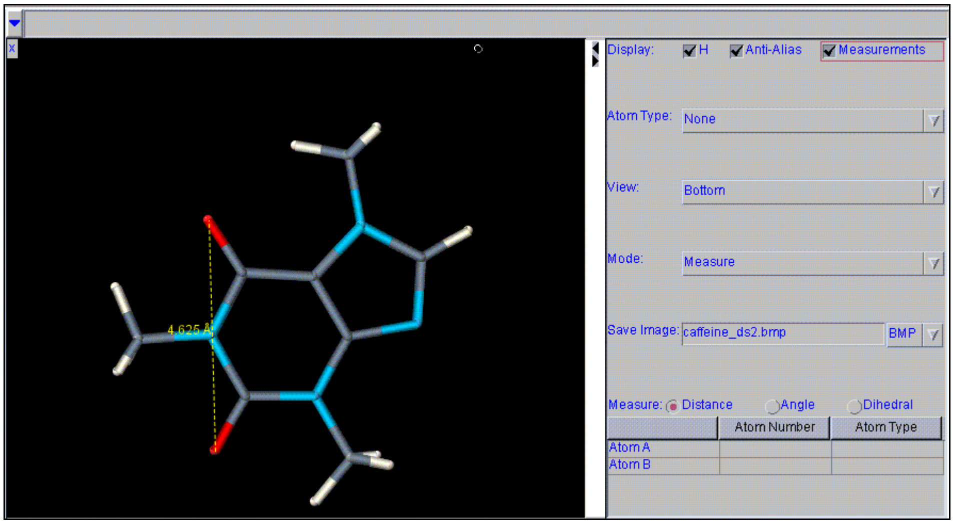
1. Click on Tools on the main menu.
2. Select Browser, browse to the mollib directory:
   /vnmr/mollib or ~*username*/vnmrsys/mollib
3. Select the appropriate files and drag them to the VnmrJ graphics window:
   - Molecule file -- select a file from the mollib directory that ends in the mol extension.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

8.11.4 Jmol Interface in VnmrJ

VnmrJ provides some Jmol tools to view a molecule.



Display: ☑ H ☑ Anti-Alias ☑ Measurements
Atom Type: None
View: Bottom
Mode: Measure
Save Image: caffeine_ds2.bmp BMP
Measure: ● Distance ○ Angle ○ Dihedral

Menu	Description
Display	H– Displays hydrogen atoms. Anti-Alias – Turns on anti-aliasing and smooths the graphics display. Measurements – Display measurements.
Atom Type	Display the atoms with atomic symbols, atom types, atom numbers, or nothing.
View	View the molecule from front, tom, 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. Refer to Measuring a Molecule.
Save Image	Saves the molecule image as BMP, JPEG, PPM, PNG, or PDF. The image is saved in the directory ~username/vnmrsys/mollib/icons with the name entered in the field. Refer to Saving a Molecule Image.

Measuring a Molecule

1. Select the measure mode: distance, angle, dihedral.
2. Click on 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 on the ☒ to exit Jmol.

8.11.5 Full-Featured Jmol

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

Refer to http://jmol.sourceforge.net/ for Jmol documentation.

8.11.6 Licenses for JChemPaint and Jmol

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

Chapter 9 Printing, Plotting, and Data Output

This chapter describes how users can print and plot data.

Sections in this chapter:

- 9.1 Printing of the Graphics
- 9.2 Plotting
- 9.3 Plot Designer
- 9.4 Color Printing and Plotting
- 9.5 Sending a Plot via email
- 9.6 Pasting text into a Text Editor or other Application
- 9.7 Advanced Printing Commands
- 9.8 Advanced Plotting Commands

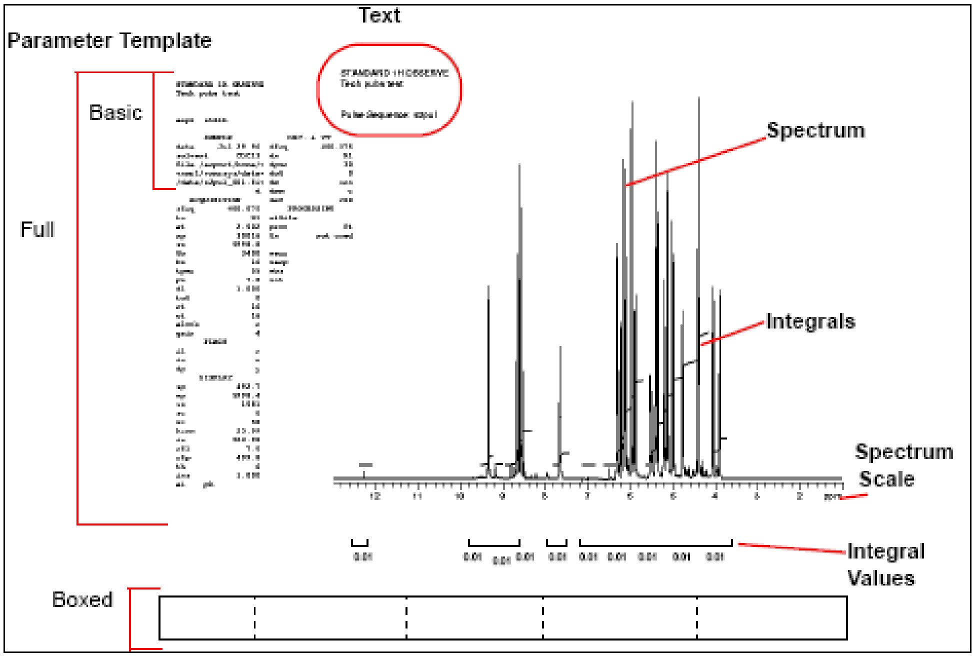


Figure 33 A general printout with several objects identified

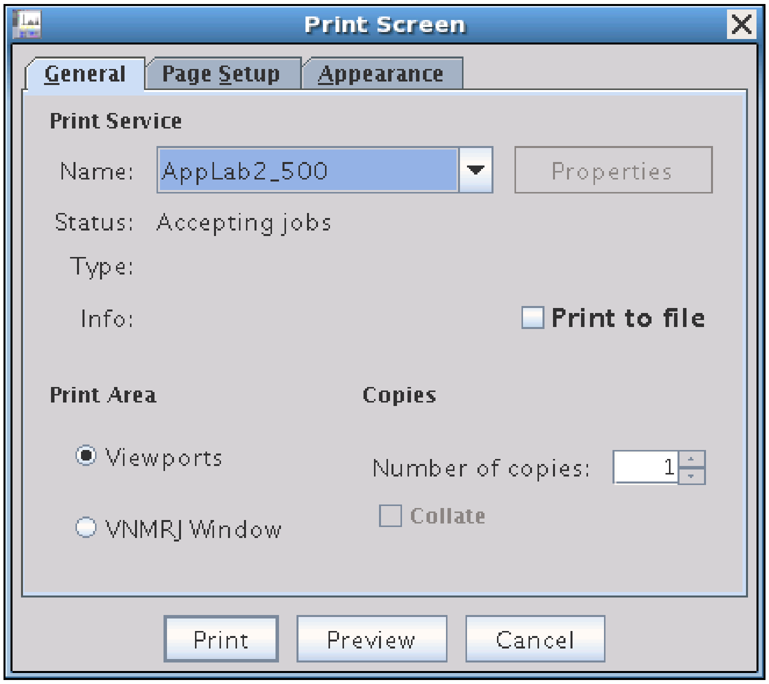
9.1 Printing of the Graphics

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 34 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.



Print Service

Name: AppLab2_500

Status: Accepting jobs

Type:

Info:

☐ Print to file

Print Area

● Viewports

○ VNMRJ Window

Copies

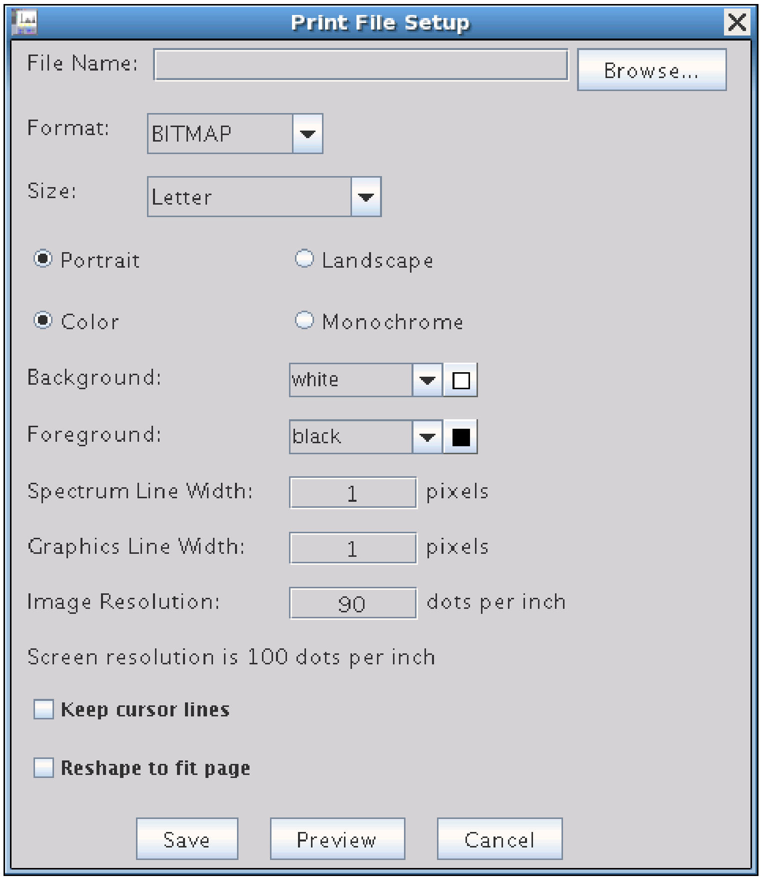
Number of copies: 1

☐ Collate

Figure 34 General tab of the Print Graphics pop-up

9.1.1 Printing a file

Select the **Print to file** check box. Figure 35 shows the pop-up that appears when the **Print to file** check box is selected and the **Print** button is clicked.



File Name:

Format: BITMAP

Size: Letter

● Portrait ○ Landscape

● Color ○ Monochrome

Background: white

Foreground: black

Spectrum Line Width: 1 pixels

Graphics Line Width: 1 pixels

Image Resolution: 90 dots per inch

Screen resolution is 100 dots per inch

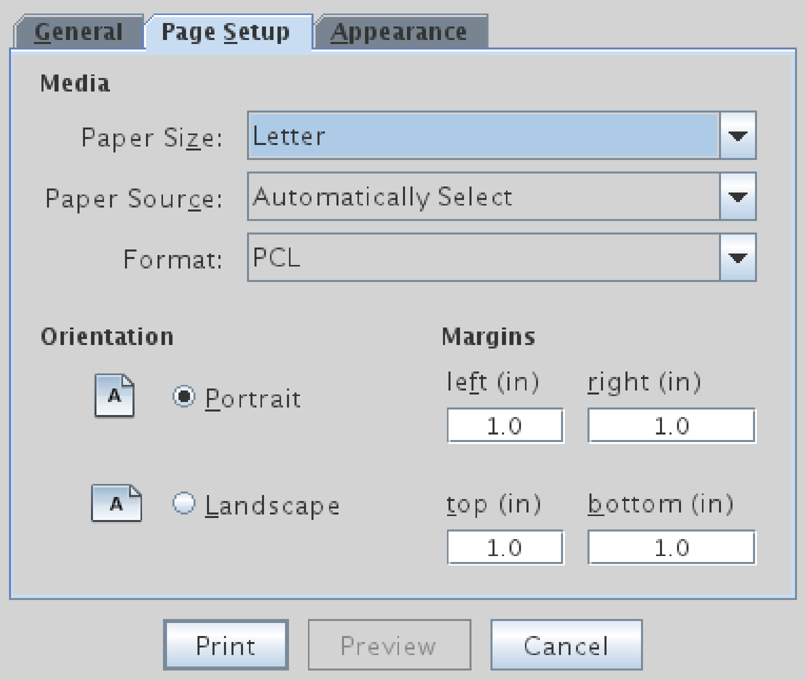
☐ **Keep cursor lines**

☐ **Reshape to fit page**

*Figure 35 The **Print to File** pop-up*

- **File Name**: name of the file to be saved
- **Browse…** button: allows one to browse to a folder to set the location of where the file will be saved.
- **Format**: a dropdown menu choices of various outputs
- **Size**: a dropdown menu choices for paper size
- Orientation selection: a 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**: enter a line width in pixels
- **Graphics Line Width**: enter a line width in pixels
- **Image Resolution**: enter the desired image resolution in dots per inch
- **Screen resolution**: displays the current screen resolution in dots per inch
- **Keep cursor lines**: select check box to include cursor lines as they appear in the graphics area
- **Reshape graphics to fit paper**: select check box to resize graphics to fit paper size
- **Save** button: saves output to File name
- **Preview** button: starts Adobe Acrobat Reader and displays (without saving) output
- **Cancel** button: closes Print to File pop-up (without saving)

The **Page Setup** and **Appearance** tabs (Figure 36 and Figure 37) allows 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.



Media

Paper Size: Letter

Paper Source: Automatically Select

Format: PCL

Orientation

● Portrait

○ Landscape

Margins

left (in) 1.0

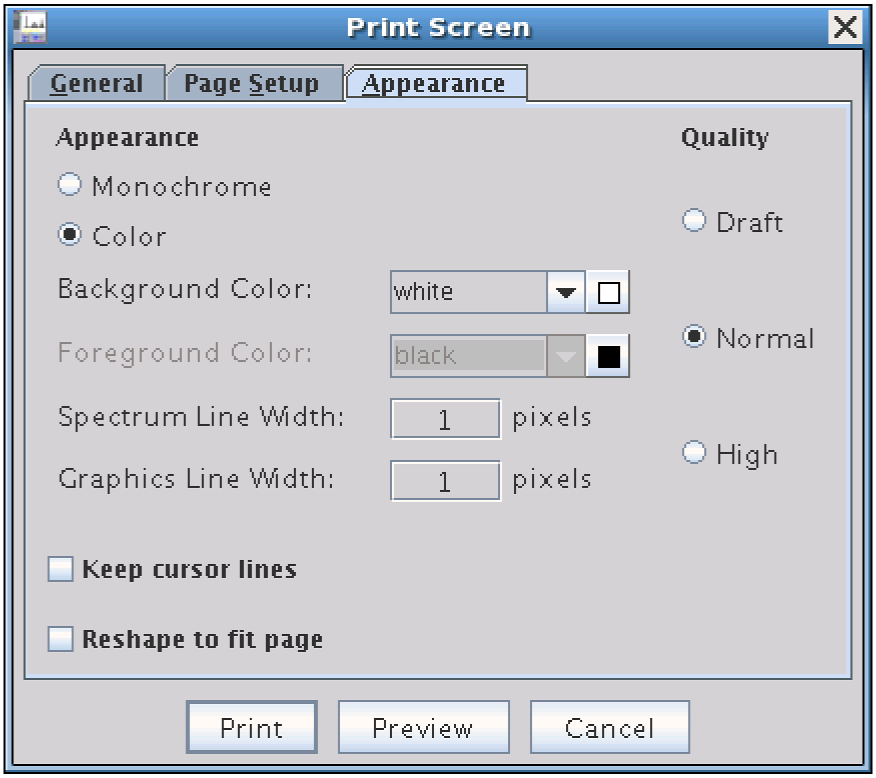
right (in) 1.0

top (in) 1.0

bottom (in) 1.0

Figure 36 The Page Setup tab of the Print Graphics pop-up

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



Appearance

○ Monochrome

● Color

Background Color: white ☐

Foreground Color: black ■

Spectrum Line Width: 1 pixels

Graphics Line Width: 1 pixels

☐ Keep cursor lines

☐ Reshape to fit page

Quality

○ Draft

● Normal

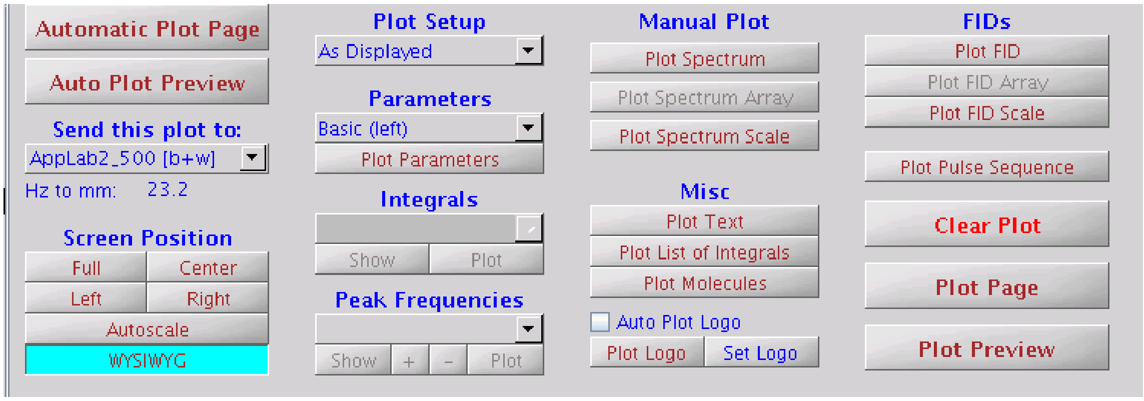
○ High

Figure 37 The Appearance Setup tab of the Print Graphics pop-up

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

9.2 Plotting

Plotting is based around the concept of a plot file. Setting up and submitting a plot can be done from the vertical and horizontal panels and from the command line. The **Plot** parameter page is accessed from the **Process** tab after the spectrum or FID is displayed. Items selected on the **Plot** parameter page (Figure 39 and Figure 39) are added to a temporary plot file, and the **Plot Page** button submits the plot file to the plotter.



Send this plot to: AppLab2_500 [b+w]

Hz to mm: 23.2

Plot Setup: As Displayed

Parameters: Basic (left)

☐ Auto Plot Logo

Figure 38 The Plot Parameter page for a 1D data set

To Plot:	Select:	Click:
Pulse sequence		Plot Pulse Sequence
FID		Plot FID, Plot Page
FID and scale		Plot FID, Plot FID Scale, Plot Page
Spectrum		Plot Spectrum, Plot Page
Spectrum and scale		Plot Spectrum, Plot Spectrum Scale, Plot Page
Spectrum, scale, and text		Plot Text, Plot Spectrum, Plot Spectrum Scale, Plot Page
Spectrum, scale, and parameters	Parameter Template option	Plot Spectrum, Plot Spectrum Scale, Plot Page
Spectrum, scale, and peak frequencies	Peak Frequencies option	Plot Spectrum, Plot Spectrum Scale, Plot Page
Spectrum, scale, and integrals		Plot Spectrum, Plot Spectrum Scale, Integrals Plot, Plot Page
Spectrum, scale, and integrals, integral values	Integrals option	Plot Spectrum, Plot Spectrum Scale, Integrals Plot, Plot Page
Parameters only	Parameter Template option	Plot Page
Text only		Plot Text, Plot Page
Peak frequencies only	Peak Frequencies option	Plot Page
Integrals only	Integrals option	Integrals Plot, Plot Page
Scaled integral values only	Integrals option	Integrals Plot, Plot Page
Normalized integral values only	Integrals option	Integrals Plot, Plot Page
Molecules only		Plot Molecules, Plot Page
Logo only		Plot Logo, Plot Page
Using default settings to the printer		Automatic Plot Page
Using default settings to Adobe Reader for preview		Auto Plot Preview

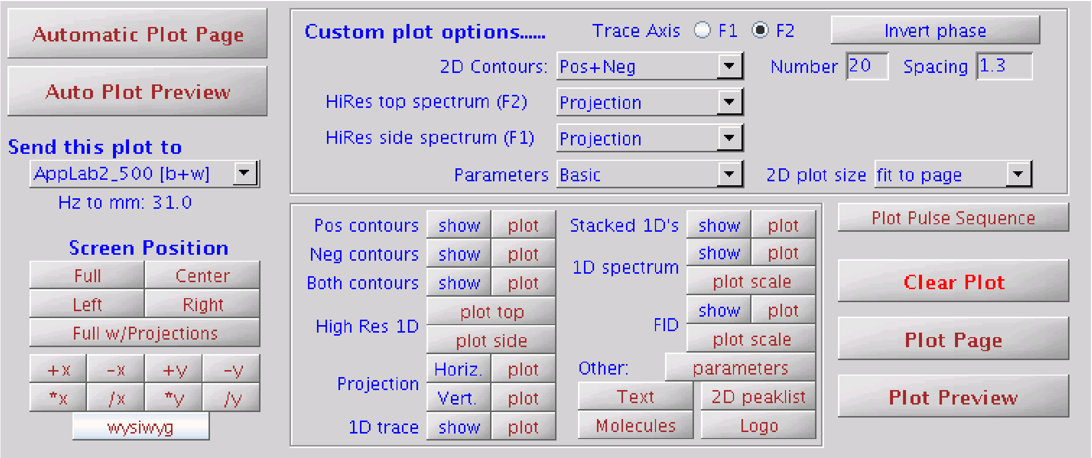


Figure 39 The Plot Parameter page for a 2D data set

The **Clear Plot** button removes the plot file. The **Plot Preview** button starts Adobe Reader (Figure 40) and displays the output of the plot file. Some of the menu options in Adobe Reader are available for use. The **Automatic Plot Page** button executes the `plot` macro then the `resetplotter` macro and **Auto Plot Preview** button executes the macro `pageview('auto')` to generate the output. The PlotView pop-up (Figure 41) 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.

The **Plot** parameter page for a 2D data set contains both **show** and **plot** buttons, **plot** buttons add to a temporary plot file, **show** displays in the graphics area.

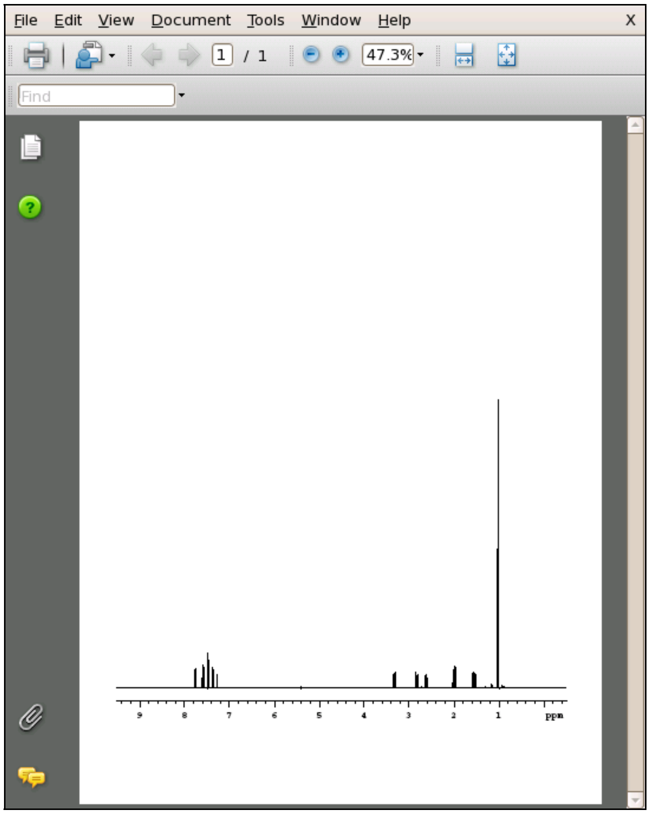
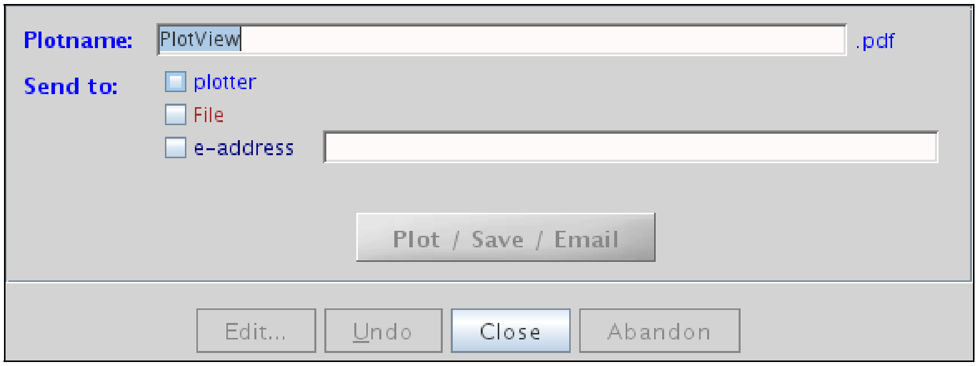


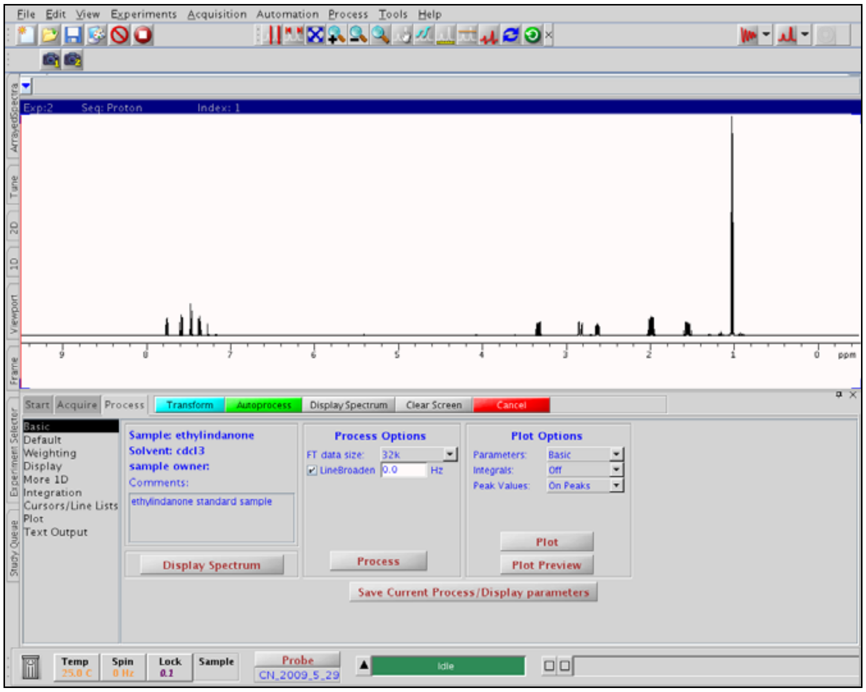
Figure 40 Preview of the plot file initiated from pressing the Plot Preview button


Plotname: PlotView .pdf

Send to:
☐ plotter
☐ File
☐ e-address

Figure 41 The PlotView pop-up

The **Basic** parameter page (Figure 42) contains a **Plot** and a **Plot Preview** buttons; both have the same function as the **Automatic Plot Page** and **Auto Plot Preview** buttons found on the **Plot** parameter page.


Exp:2 Seq: Proton Index: 1

Sample: ethylindanone
Solvent: cdcl3
sample owner:
Comments: ethylindanone standard sample

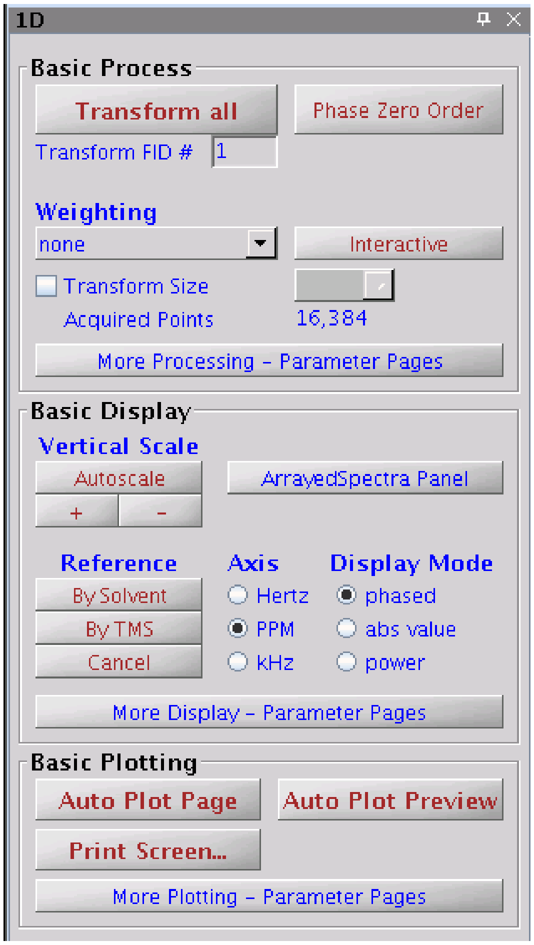
FT data size: 32k
☑ LineBroaden 0.0 Hz

Parameters: Basic
Integrals: Off
Peak Values: On Peaks

Temp 25.0 C
Spin 0 Hz
Lock 0.1
Probe CN_2009_5_29
Idle

Figure 42 The Basic Parameter Page for a 1D data set

The **1D** vertical parameter page contains four buttons in the Basic Plotting group. **Auto Plot Page** does the same function as the **Automatic Plot Page** button in the **Plot** parameter page, the **Auto Plot Preview** button does the same function as the **Plot** parameter page, and the **Print Screen…** button does the same function as **File** / **Print Screen…** The More **Plotting – Parameter Pages** button opens the horizontal panel and displays the **Plot** parameter page.



Transform FID # 1

Weighting: none

☐ Transform Size

Acquired Points 16,384

Axis: ○ Hertz ● PPM ○ kHz

Display Mode: ● phased ○ abs value ○ power

Figure 43 The 1D Vertical Parameter page

9.3 Plot Designer

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.

9.3.1 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.

9.3.2 Using Plot Designer

Select a viewport and process the data set for plotting.

Start the Plot Designer program as follows:

1. Click **File**
2. Select **Create a Plot Design**.

   The Plot Designer window opens; see Figure 44.

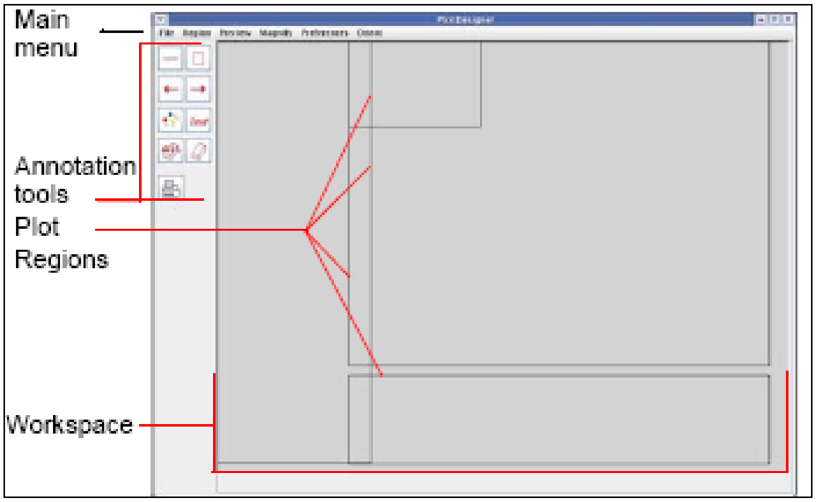


Figure 44 Plot Designer with Current Default Template

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

deptB	dicom.default	chemParray	chemP1d
basic2D	oneD	Whitewash	ChemP2d

   e. Place a check in **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.
   f. Click **Open**.
4. Select **Preview** from the Plot Designer Menu.
5. 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.

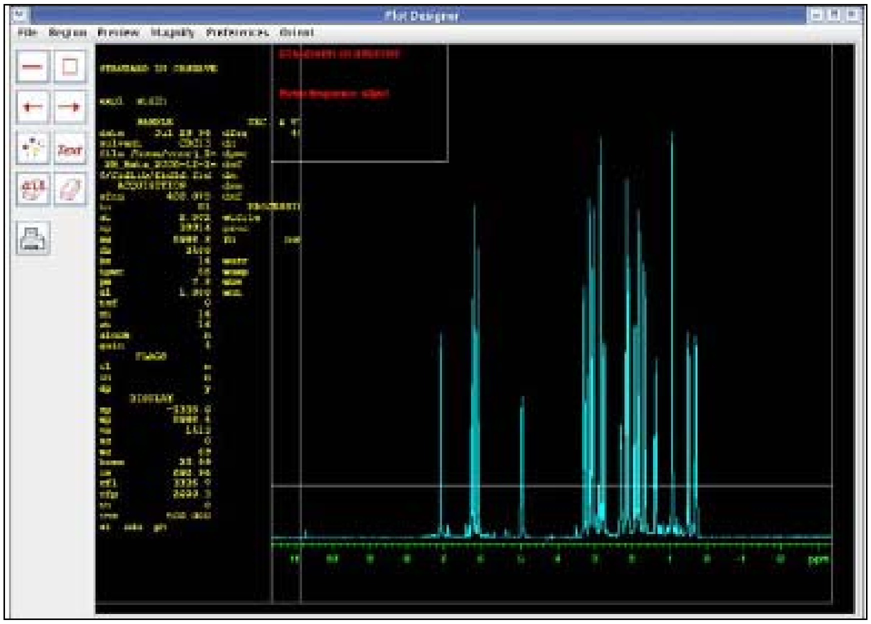


Figure 45 Default oneD Template with Imported Data

9.3.3 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.
   - Delete all the regions: see Clearing all Regions from the Workspace Permanently.
   - Add a new region: see Adding a Region.
   - Edit an existing region: double on a region, click on **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.
   - Add text and graphics elements: see Adding and Editing Text and Graphics Elements.

9.3.4 Deleting a Region

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

9.3.5 Restoring a Single Deleted Region

Click **Region-Undelete**.

9.3.6 Clearing all Regions from the Workspace Permanently

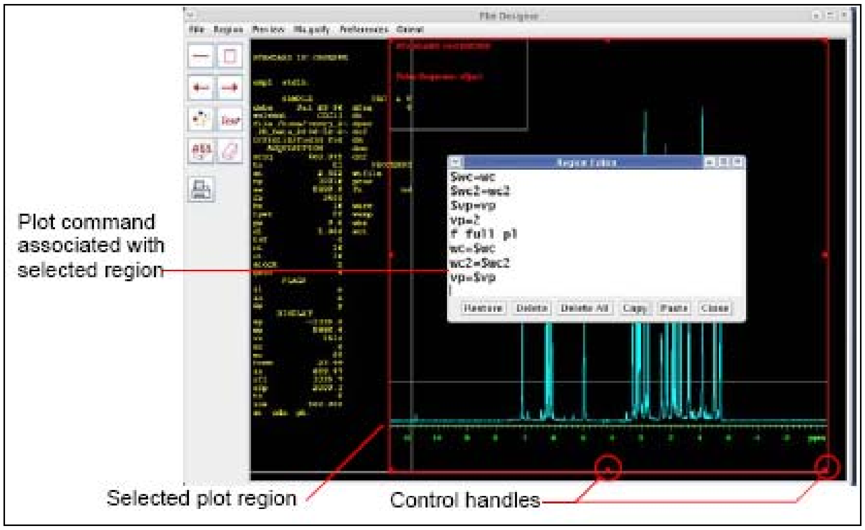
Click **Delete All**: no undelete. Regions removed with Delete All cannot be restored with Undelete.

9.3.7 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

9.3.8 Editing Plotting Commands in a New or Existing Plot Region

1. Double click on 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 currently support plotting command is allowed.



```
$wc=wc
$wc2=wc2
$vp=vp
vp=2
f full pl
wc=$wc
wc2=$wc2
vp=$vp
```

Figure 46 Editing a Plot Region Commands

The Region Editor window control buttons as listed in Table 9 are found.

Table 9 Region Editor Buttons

Button	**Function**
Restore	Applies the original template to a region. Restore 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.

Button	Function
Paste	Inserts copied text in the input area.
Close	Exit the Region Editor.

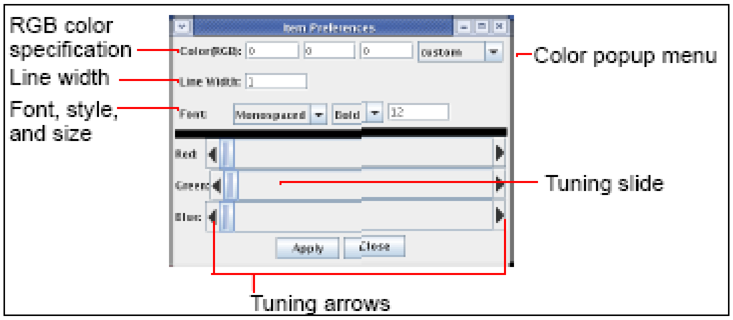
9.3.9 Resizing and Moving Plot Regions and Objects

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 46) on the border, and dragging it to the new size.

9.3.10 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 46.



Color(RGB): 0 0 0 Custom
Line Width: 1
Font: Monospaced Bold 12

Figure 47 Item Preferences Window

Click **Region-Preferences** to open this window or click on the **Item Preferences** tool , described in Plot Designer Tools.

9.3.11 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.

9.3.12 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 **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.

9.3.13 Changing Colors

1. Change the **color of a line** by doing the following:
2. Highlight the line or region.
3. Click **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.
4. Click **Apply** when the required colors are selected.
5. Place the cursor anywhere in the workspace and click once to apply the color change.

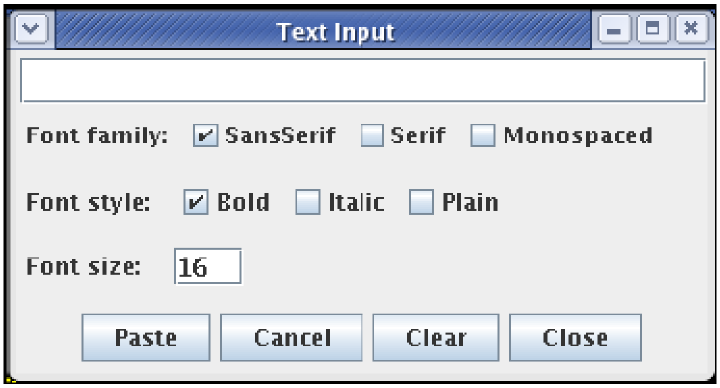
9.3.14 Adding Text

Do the following to add text into your design:

1. Click text input tool Text 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 text input tool Text to open the Text Input window shown in Figure 47.
3. Select a Font family and Font style, and enter a Font size.
4. Click **Put** to paste the text in the workspace.



Font family: ☑ SansSerif ☐ Serif ☐ Monospaced

Font style: ☑ Bold ☐ Italic ☐ Plain

Font size: 16

Figure 48 Text Input Window

9.3.15 Changing Font Color

Repeat the procedure given in Changing Colors, to change font colors.

9.3.16 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: Click the box next to Use this template as default to make the file the default template. The default template automatically loads when Plot Designer is started.
4. Click **Save** to store the template in $vnmruser/templates/plot directory.
5. A warning is displayed if the saved template overwrites a current template. Click **Cancel** to not replace the file.
6. Quit the Plot Templates window by clicking on **Close**.

9.3.17 Plot Designer Tools

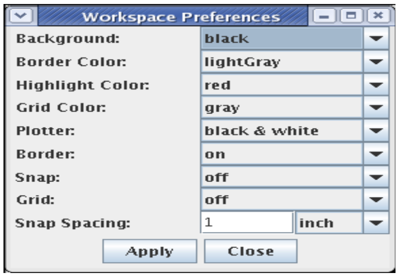
Plot designer tools listed in Table 10. Press and hold down the left mouse button and drag the cursor in the workspace to use a drawing tool.

Table 10 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 for a description of its properties.
Text	Text Input	Adds text into the design and controls the size and appearance of the text; see Adding Text .
ALL ALL	Erasers	The ALL eraser removes all objects The eraser tool removes selected objects. See also Adding and Editing Text and Graphics Elements, Adding a Region, and Deleting a Region.
🖶	Print	Prints a file.

9.3.18 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.



Background: black
Border Color: lightGray
Highlight Color: red
Grid Color: gray
Plotter: black & white
Border: on
Snap: off
Grid: off
Snap Spacing: 1 inch

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 11 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 indicate that it is selected.
Grid Color	Changes the color of the grid.
Plotter	Select 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.

9.3.19 Changing the Shape of the Plot Designer Window

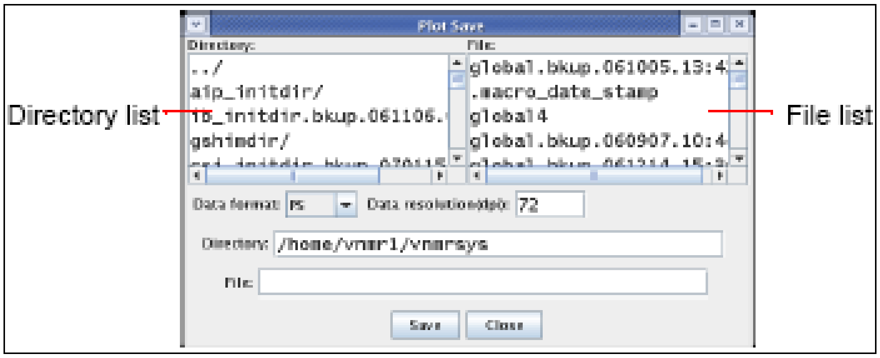
Plot Designer can be viewed in two orientations, Landscape or Portrait (which is the default orientation). Change the shape of the Plot Designer window in the Orient menu.

9.3.20 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.

9.3.21 Saving a Plot File

Do the following procedure to save a plot:



Data format: PS

Data resolution(dpi): 72

Directory: /home/vnmr1/vnmrsys

Figure 49 Plot Save Window

1. Click **File** in the Main Menu, then **Save Data** to open the Plot Save window shown in Figure 49.
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 12 lists the formats that are available.

Table 12 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

Format	Description
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.

9.3.22 Printing a Plot

Click the print tool. 🖨

9.3.23 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.

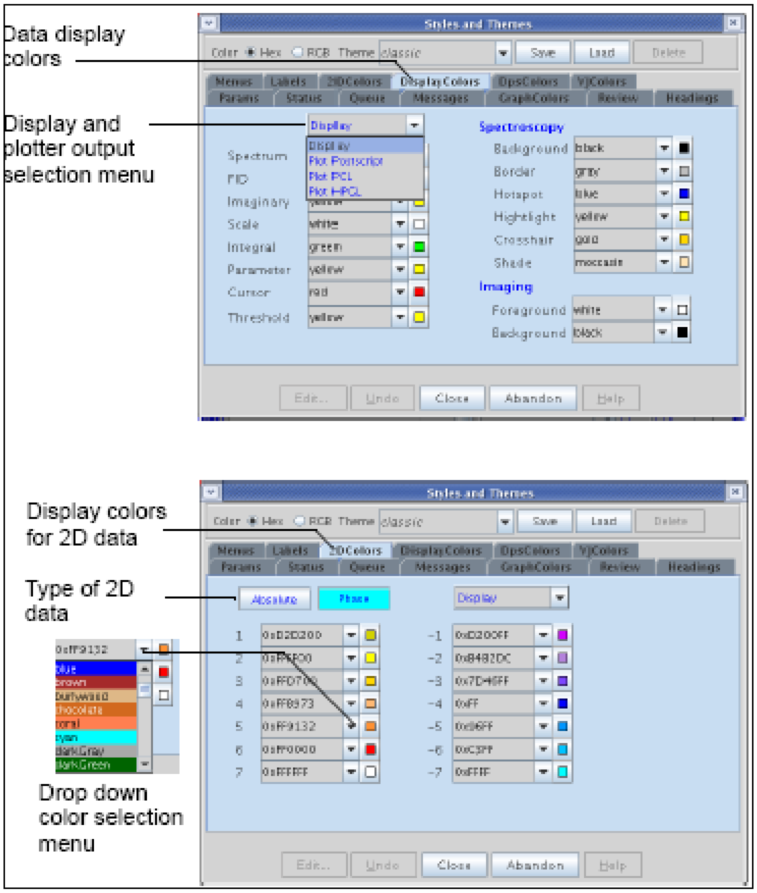
9.4 Color Printing and Plotting

Printer and Plotter color output is defined using the Styles and Themes window, which provides access to the display colors and the VnmrJ interface colors.

9.4.1 Setting Colors

1. View the current settings or define new color settings as follows:
2. Click **Edit**.
3. Select **Display options…**

   The Style and Themes window opens; see Figure 50.



Color ● Hex ○ RGB Theme classic

Imaginary yellow
Scale white
Integral green
Parameter yellow
Cursor red
Threshold yellow

Background black
Border gray
Hotspot blue
Highlight yellow
Crosshair gold
Shade moccasin

Foreground white
Background black

1 0xD2D200
2 0xFFFF00
3 0xFFD700
4 0xFF8973
5 0xFF9132
6 0xFF0000
7 0xFFFFFF

-1 0xD200FF
-2 0xB482DC
-3 0x7D46FF
-4 0xFF
-5 0x96FF
-6 0xC3FF
-7 0xFFFF

Figure 50 Styles and Themes for Display and 2D Colors

4. Select **Display** tab to set the colors for the spectra, axis, parameters, etc.
5. Click **Display and Output** selection drop down menu.
6. Select the output device: Display, Plot Postscript, Plot PCL, or Plot HPGL.
7. Select a color or keep the current color for each display or function shown.
8. Enter a **name** in the field next to the Save button to save the selection to a user defined file or continue with the next step to overwrite the current file.
9. Click **Save** to save the color selections to the specified file.
10. Click **2D colors**.
11. Click **Phase** to set the colors for this 2D display mode.
12. Select the output device: Display, Plot Postscript, Plot PCL, or Plot HPGL.
13. Select a **color** or keep the current color for each contour level.
14. Click **Absolute** to set the color for this 2D display mode.
15. Select a **color** or keep the current color for each contour level.
16. Enter a **name** in the field next to the Save button to save the selection to a user defined file or continue with the next step to overwrite the current file.
17. Click **Save** to save the color selections to the specified file.

9.4.2 Loading a Color File

To retrieve a color file:

1. Select a theme file from the T**heme** drop-down menu.
2. Click **Load**.

9.4.3 Changing or Renaming a Color File

To change the colors in a file:

1. Select a theme file from the T**heme** drop-down menu.
2. Click **Load**.
3. Follow procedure in Setting Colors.
4. Click **Save** to save the file.

To change the name of a color file:

1. Select a **theme file** from the Theme drop-down menu.
2. Click **Load**.
3. Enter a **new name** in the field next to the Save button.
4. Click **Save** to save the file.
5. Optional: To delete the file with the old name, see Removing a Color File.

9.4.4 Removing a Color File

To remove a color file from the list:

1. Select a **theme file** from the Theme drop-down menu.
2. Click **Load**.
3. Click **Delete**. The deleted file is removed from the bottom list box.
4. Click **OK**, when prompted, to delete the file, or **Cancel** to keep the file.

9.4.5 Closing the Color Selection Window

Click **Close** to exit the window.

9.5 Sending a Plot via email

After selecting the options to and clicking the **Plot Preview** button in the Plot Parameter page.

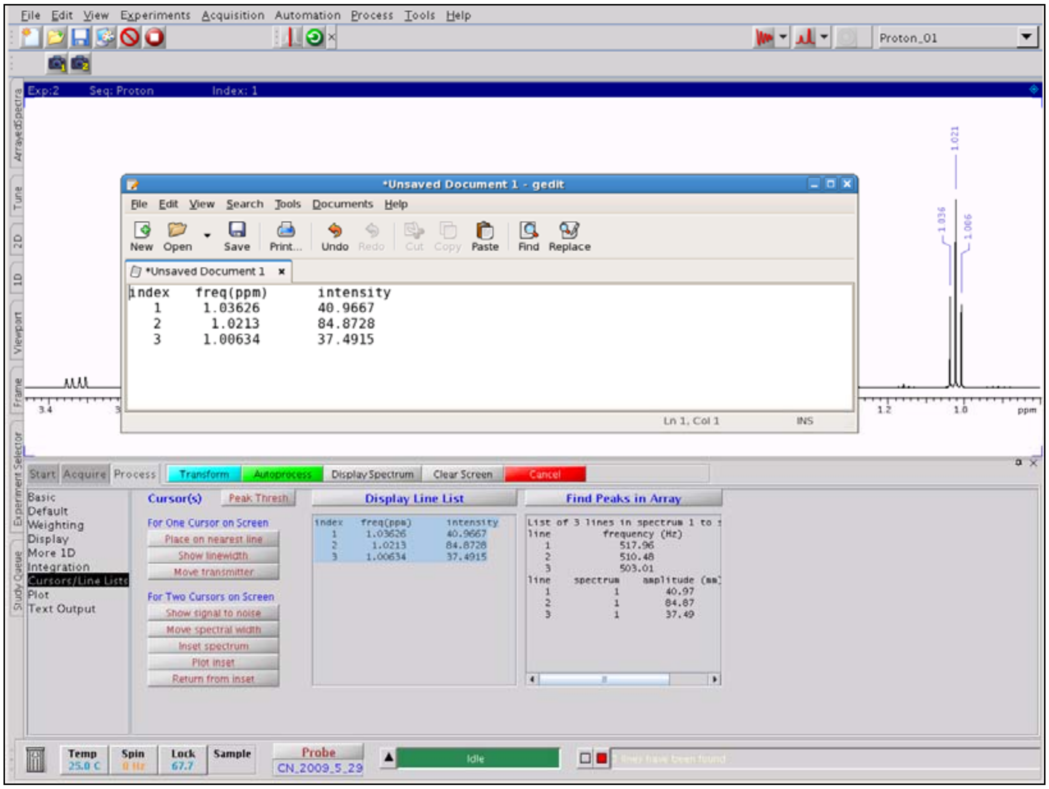
1. Enter a **Plot name** in the PlotView pop-up.
2. Check **File**.
3. Check **e-address**.
4. Enter valid email addresses in the entry field of e-address.
5. Press the **Plot / Save / Email** button.
6. Press the **Close** button when done.

There are advanced macros which can be issued from the command line (**eplot**, **epage**, **espec**, **fplot**, **fpage**, **efid**, **esampledir**) which can be used to send the output to an email address. See the *Command and Parameter Reference* for details on the usage of the macros.

9.6 Pasting text into a Text Editor or other Application

Text output which appears in the Integration, Cursors / Line Lists / Text Output Parameter pages can be pasted into a text editor or other application as shown in Figure 51, to be saved or used elsewhere.

1. Highlight the text to be pasted by clicking the left mouse button and dragging the mouse to the end of the desired text.
2. Release the mouse button at the end of the desired text. The selected text is highlighted indicating what has been selected.
3. Start the text editor or application and place the mouse cursor on the active document.
4. Click the middle mouse button to paste the highlighted text into the text editor.



```
index     freq(ppm)        intensity
   1      1.03626          40.9667
   2       1.0213          84.8728
   3      1.00634          37.4915
```

```
List of 3 lines in spectrum 1 to
line          frequency (Hz)
   1               517.96
   2               510.48
   3               503.01
line      spectrum      amplitude (mm)
   1             1           40.97
   2             1           84.87
   3             1           37.49
```

Figure 51 Contents of the Display Line List text box copied into the Linux gedit text editor.

9.7 Advanced Printing Commands

Printing from within VnmrJ is initiated with the `printon` command. All output which normally appears in the text output window is saved and when the `printoff` command is issued, sent to a printer. 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, tl, t2, etc.
- Any other information that some program or macro may write to the text window.

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 that `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 user's print job is deleted from the print queue.

Table 13 Printer-Associated Commands and Parameters

Commands	Parameter
`killprint printoff«'clear'lfile»`	Stop print job and remove from print queue
`printon`	Stop sending text to printer & start print operation
`ptext (file)`	Direct text output to printer Print out a text file
`showplotter`	Display currently defined plotters and printers
`showprintq`	Display print jobs in print queue
`vnmrprint*`	Print text files (UNIX)
`* vnmrprint`	
`printer` {string}	Printer device

9.8 Advanced Plotting Commands

9.8.1 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')` only plots positive peaks. 0ther arguments for noise suppression (`noise_mult`) and label positioning work the same as the dpr command.

The `pll` 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 `pll<(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='yariable'` 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)>` plots automatically 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 VNMR Command and Parameter Reference* .

9.8.2 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 +/-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 14 Plotting-Associated Commands and Parameters

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”

Commands	Descriptions
`setpen<(maxpen,max_num_pen)>`	Set maximum number of HP pens
`setplotdey<:plotter_type>`	Return characteristics of a named plotter
`showplotq`	Display plot jobs in plot queue
`showplotter`	Display currently defined plotters and printers
`yrmrplot <file>`	Plot files (UN IX)
`*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>)>`	

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 H P plotter or Postscript device
y0 { number, in mm}	Y -zero position of H P plotter or Postscript device
hzmm { number}	Scaling factor for plots
pltmod*	Plotter display mode
sp { number, in H z}	Start of plot
wp { number, in H z}	Width of plot
*** pltmod { ’ off’ ,’ fixed’ ,’ full’ ,’ standard’ ,’ user’ ,’ variable’ }**	

Chapter 10 Advanced 1D NMR

This chapter describes how to work with 1D NMR liquids experiments.

Sections in this chapter:

- 10.1 Working with Experiments
- 10.2 Multi-FID (Arrayed) Spectra
- 10.3 T1 and T2 Analysis
- 10.4 Kinetics
- 10.5 Filter Diagonalization Method (FDM)

10.1 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 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 **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 on 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 popup 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.

10.2 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 Varian software.

10.2.1 Arrayed Parameters

Use the Array Parameter window (called from the **Acquisition/ Parameter arrays…** menu; see Parameter Arrays) 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; i.e., temp=30,50,70,90 or pw=5,10,15,20,25). Alphanumeric parameters can also be arrayed (i.e., 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.

10.2.2 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 (e.g., three different values of decoupler power dpwr are used for each of the four different values of the decoupler offset dof).

10.2.3 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, 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, ...x_ny_1, x_1y_2, x_2y_2,...x_my_2...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,...x_1y_n, x_2y_1, x_2y_2,...x_2y_n,...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, ...x_ny_n, with n experiments being performed.

Entering one or more arrayed parameters automatically sets up array. It is necessary to enter 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.

10.2.4 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 array with a parameter name as an argument, (e.g., array('pw')) still starts an interactive mode, but the program only asks for the remaining three items of information. Alternatively, 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.

10.2.5 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 indicate 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 (e.g., pw=10) to reset an arrayed parameter to a single value. The array parameter is automatically modified to reflect this change.

10.2.6 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).

10.2.7 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 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'`.

10.2.8 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).

10.2.9 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 (i.e., 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 (i.e., 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 pl 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 pl 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.

10.2.10 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 (e.g., pw=5) or use some other small value.
3. Enter 1 for the value of nt (e.g., nt=1).
4. Obtain a spectrum and phase it properly. Set d1 to 5* T1 (or set it to a long value like d1=10 if you do not know the T1).
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 instensity mode is also set by Process / Display / Amplitude Scaling / Absolute.
7. Transform and display the stacked data after the experiment finishes acquisition (e.g., **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.

10.3 T_1 and T_2 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 (d1) then inverted with a 180° pulse (p1), given a variable time to return to equilibrium (d2), and finally given a monitoring 90° pulse (pw) to measure their peak height as a function of d2. Under most circumstances the behavior of the peak heights as a function of d2 will be exponential and this exponential time constant is the T_1.

10.3.1 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 p1, pw, d1, and an array of values for d2 or by enter
2. Using the dot1 macro. dot1 sets up all parameters to perform a T_1 experiment, including d1, pw, p1, nt, and an array of d2 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 on the **T1_measure** protocol button, or use the menu as follows:
    a. Click on **Experiment**.
    b. Select **H1 Relax. Measurements**.
    c. Select **T1 Measurements**.
    d. Fill in the information on the Standard 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.

10.3.2 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** / **T_1 Analysis** panel, and follow the directions listed there.
2. Display the last spectrum of the (or ds(1) of theT1 arrayed dataset.
3. Phase this spectrum properly.
4. Select a threshold and adjust the threshold line position.
5. Enter dpf, or dll, or click on 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 (e.g., fp(1,3)), only the peak heights of the corresponding lines are measured.
7. Type t1 to run the T_1 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.

10.3.3 Analyzing the Data

T_1 and T_2 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 T_1 and T_2, or kinetic analysis. Optional input of line numbers as arguments allows you to display of 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.

10.3.4 T_1 Data Workup: Step-by-Step

The following procedures accomplish a manual T_1 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.`

10.4 Kinetics

The arraying capability of the VnmrJ software provides for the acquisition of data for the study of kinetics.

10.4.1 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.

10.4.2 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.

10.4.3 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 on 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.

10.5 Filter Diagonalization Method (FDM)

Filter Diagonalization Method is an optional package that must be purchased and installed. Installation requires a password.

Filter Diagonalization Method (FDM) is a non-Fourier data-processing method that extracts spectral parameters (peak positions, line widths, amplitudes, and phases) of Lorentzian lines directly from the time-domain signal by fitting FID data to a sum of damped complex sinusoids. The spectral parameters (saved in curexp/datdir/fdm1.parm) are also called “line list” and are used to construct an “ersatz” spectrum of the NMR data.

FDM is slower than Fast Fourier Transform, but it offers better resolution in the case of truncated signals and it has the option of processing only a selected spectrum region. FDM has the potential to work well with corrupted data, and the potential to produce a line list with each line representing a true NMR peak.

FDM reads input parameters from a file created by the fdm1 macro, using default (optimal) values. Change any of the parameters from the command line as needed. Table 15 lists fdm1 parameters. If the spectrum is not referenced with rl, the reference rfl is also read from curpar in the local parameter set. The section Changing Local FDM Variables describes how to override the default setting.

In most cases, the only parameters that need to be set are the number of data points to be used and the spectrum window to be processed. The default setting uses half of the FID data or 3000 data points, whichever is smaller.

Table 15 fdm1 Parameters

Parameter	Description
`Cheat`	No cheat if cheat=1, lines are narrower if cheat<1
`Cheatmore`	No cheatmore if cheatmore=0
`Error`	Error threshold for throwing away poles
`Fdm`	1 for fdm, −1 for dft
`Gamm*`	Smoothing width (line broadening)
`Gcut`	Maximum width for a pole
`Idat`	−4 for ASCII complex FID file, −5 for FID file
`Kcoef`	`kcoef>0`; use “complicated” `dk(k)`. −1 always preferred
`Nb*`	Number of basis function in a single window
`Nbc*`	Number of coarse basis vectors
`Nsig*`	Number of points to use, 3000 is ok
`Nskip*`	Number of points to skip
`Rho`	rho=1 is optimal
`ssw`	A test parameter.
`t0`	Delay of the first point.
`theta`	Overall phase of FID (rp in radians).
`wmin`	Minimum spectrum frequency in hertz.
`wmax`	Maximum spectrum frequency in hertz

10.5.1 Using FDM

The following steps describe how to do normal activities such as phasing, zooming in, zooming out, and processing a spectrum window with the fdm1 macro.

1. Display the FID data and use the right mouse button to select the data points to be used by FDM.
2. Process the data with ft (it uses all FID points), then display and reference the spectrum.
3. Place the cursor on a region of interest, zoom in on it, then type fdm1 or **Process**....**1D FDM**...from the main menu. Selecting and not zooming in on a region causes the whole spectrum in display to be processed.

4. A new menu appears with Start and Abort buttons. The calculation might take a few seconds to a few minutes depending on the number of data points used and the size of spectrum window to be processed. To abort the process, click on **Abort**. The process is finished when the message *FDM Execution Stopped Successfully* is displayed. If the process is finished, display the spectrum.

10.5.2 Reprocessing a Spectrum

The Start button is displayed in the Process...1D FDM... drop-down menu. Use this button to reprocess a spectrum.

10.5.3 Changing Parameters

Relevant fdm1 global parameters are displayed on the 1D FDM... popup window with current values. These parameters can be changed. The value of a global parameter is saved to curpar, and it remains the same until changed from the parameter panel or a new assignment using the command line is made. The parameters can also be changed from the fdm1 command line as described in the section Changing Local FDM Variables.

10.5.4 FDM Global Parameters

The following FDM parameters are global.

- Nsig is the number of FID points to use. Initialize it with the right mouse button position (crf + deltaf) *sw. If Nsig=0, half of the FID data points or 3000, whichever is smaller, is used. Nsig can be changed from the command line Nsig=nnnn, the right mouse button (when the FID is displayed), or the command line fdm1('Nsig',nnnn). In general, the more peaks, the more data points it takes to fit the spectrum. Check the reliability of the FDM method by changing Nsig a few times, and reprocess the data to see if the result is the same.
- Nskip is the number of data points to skip at the beginning of a FID. By default, zero points are skipped. In some cases, baseline can be improved by skipping the first one or two points.
- Nb is the number of basis functions (poles) used to fit each of the windows in an FDM calculation. The default is 10. FDM breaks down the specified window into smaller windows. In general, bigger Nb gives better results, especially better baseline. Sensible values for Nb are between 10 and 50.
- Nbc is the number of additional poles (coarse basis functions) to be used. The default is zero, but setting Nbc to an integer larger than zero (typically 4-10) might improve the baseline.
- Gamm is the smoothing width (line broadening). The default is 0.2*sw/Nsig, which is about a tenth of the FT resolution. Typical values are 0.1 to 1.0.

Using bigger Nsig, Nb, Nbc, or a spectral window significantly slows down the calculation.

10.5.5 Changing Local FDM Variables

FMD parameters that are not commonly used are set as fdm1 local variables. These parameters are listed with global parameters in Table 15. Local variables can be changed only from the command line. Parameter values are lost after the completion of the macro. To use a value again, it must be reentered; otherwise, fdm1 sets the value to the default. To change more than one local variable, enter the variables from the same command line.

Any of the FDM parameters in the fdm1 command and both global and local variables can be changed. Values entered from the fdm1 command override the default, the change from the **1D FDM** panel, and the value selected with the cursor. Enter command line arguments by giving the parameter name in single quotation marks and a value separated by a comma, for example:

```
fdm1('cheat',0.8)
fdm1('Nsig',3000)
fdm1('Nsig',3050)
fdm1('Nb',20)
fdm1('Nbc',10,'Nb',20)
fdm1('Nsig',3000,'Nb',20,'Gamm',0.5)
fdm1('wmin',-1600,'wmax',1600)
fdm1('wmin',-1600,'wmax',0)
```

`cheat` is a factor multiplied to the line width. There is no cheat when `cheat=1`; lines are narrower when `cheat<1`.

`wmin` is the minimum spectrum frequency in Hz. The default is `sp+rfl-sw/2`. `wmin` is the upper field.

`wmax` is the maximum spectrum frequency in Hz. The default is `wmin+wp`. `wmax` is the lower field.

The center of the full spectrum is zero.

10.5.6 Seeing Parameter Values

Parameters are set to their default values. Normally, it is not necessary to change these parameters. Values of local fdm1 parameters can not be queried with echo. To see the values of all parameters used, look in the fdm1.inparm file created by the fdm1 macro in the datdir directory of the current experiment.

10.5.7 FDM References

J. Chen and V. A. Mandelshtam, *J. Chem. Phys.* (2000) **112:** 4429-4437.

V. A. Mandelshtam, *J. Magn. Reson.* (2000) **144:** 343-356.

H. Hu, A. A. De Angelis, V. A. Mandelshtam and A.J. Shaka, *J. Magn. Reson.* (2000) **144:** 357-366.

Chapter 11 Multidimensional NMR

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; the number of different experiments).

Sections in this chapter:

11.1 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 once 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.

11.1.1 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 d2. This time is normally calculated automatically from the number of increments (the parameter ni) and 1/sw1. The value of ni determines if a 2D experiment will be run. The initial value of d2 (at the start of the experiment) is usually set to zero but can be set to any value.

A d2 array does not appear in the display da (e.g., d2 is “implicitly” arrayed). Only the first value of d2 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 transform 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 (e.g., sw1, lb1, fn1), 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.

11.1.2 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, *are* expected to appear in absorption mode in both directions , i.e., Heteronuclear 2D-J.
- A 2D experiment in which the data are 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, so 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.

11.2 2D Experiment Set Up

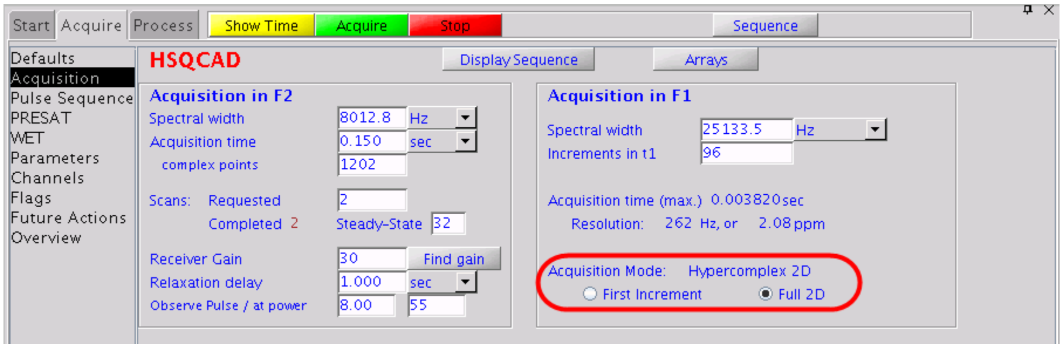
1. Drag-and-drop or double click the desired experiment, using the Experiment Selector.
2. Use the Experiments menu – which offers more options for customization.
3. Use the Locator to list the available 2D experiments:
    a. Click the Locator Statements button (magnifying glass icon), and select Sort 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).

11.3 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.

11.3.1 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.



HSQCAD

Acquisition in F2

Spectral width 8012.8 Hz
Acquisition time 0.150 sec
complex points 1202
Scans: Requested 2
Completed 2 Steady-State 32
Receiver Gain 30
Relaxation delay 1.000 sec
Observe Pulse / at power 8.00 55

Acquisition in F1

Spectral width 25133.5 Hz
Increments in t1 96
Acquisition time (max.) 0.003820 sec
Resolution: 262 Hz, or 2.08 ppm
Acquisition Mode: Hypercomplex 2D
○ First Increment ● Full 2D

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.

11.3.2 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: d2 (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-Haberkorn	d2=0	phase=1
	d2=0	phase=2
	d2=1/sw1	phase=1
	d2=1/sw1	phase=2
TPPI (non-arrayed)	d2=0	phase=3
	d2=1/sw1	phase=3
	d2=2/sw1	phase=3
	d2=3/sw1	phase=3

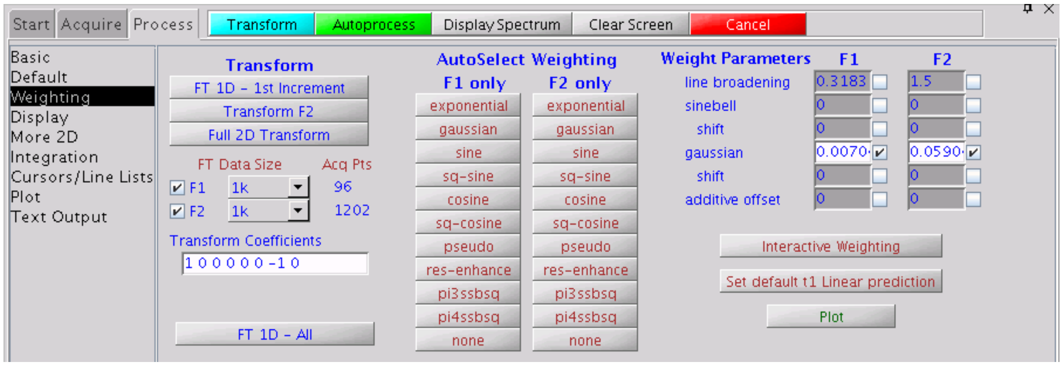
Not all pulse sequences have the TPPI method incorporated. The acquisition status window displays a count of the current FID and the number of completed transients (ct) in that FID. The current FID number is the *total* count of completed FIDs to this point, including all arrays. Since the phase parameter 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.

11.4 Weighting

This section describes weighting functions for processing 2D experiments.

11.4.1 2D Weighting Parameters

The 2D weighting parameters used for processing the t_1 domain (the interferogram) or first indirectly detected dimension (ni) are set on the Process page in the Process panel and are analogous to weighting parameters for 1D experiments.



FT Data Size		Acq Pts
☑ F1	1k	96
☑ F2	1k	1202

Transform Coefficients
1 0 0 0 0 0 -1 0

Weight Parameters	F1		F2	
line broadening	0.3183	☐	1.5	☐
sinebell	0	☐	0	☐
shift	0	☐	0	☐
gaussian	0.0070	☑	0.0590	☑
shift	0	☐	0	☐
additive offset	0	☐	0	☐

Non-phase-sensitive (absolute-value and power) 2D experiments: "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.

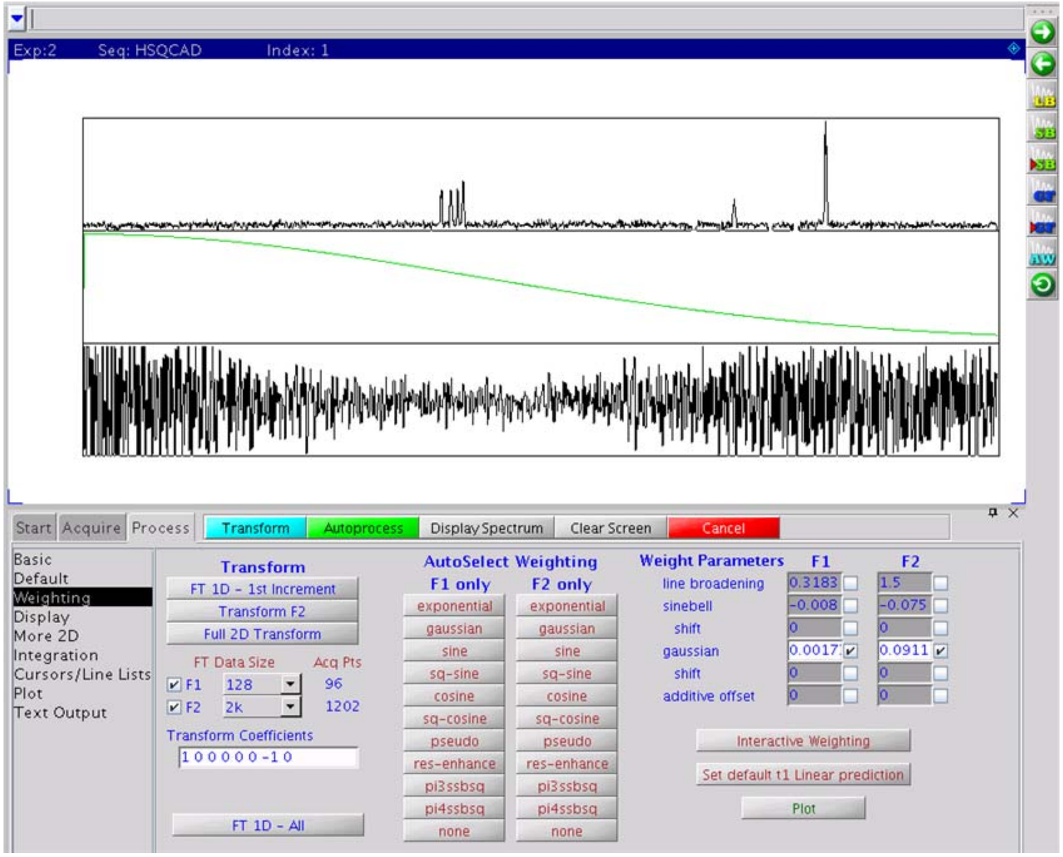
Weighting	AutoSelect Weighting Button
pseudo-echo	Pseudo
sinebell	Sine
sinebell-squared	sq-sine

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.

11.4.2 Interactive Weighting

The Interactive Weighting button which 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; e.g., both the ni and ni2 dimension). The currently active element or trace is used in adjusting the weighting parameters.



FT Data Size		Acq Pts
☑ F1	128	96
☑ F2	2k	1202

Transform Coefficients: 1 0 0 0 0 0 -1 0

Weight Parameters	F1		F2	
line broadening	0.3183	☐	1.5	☐
sinebell	-0.008	☐	-0.075	☐
shift	0	☐	0	☐
gaussian	0.0017	☑	0.0911	☑
shift	0	☐	0	☐
additive offset	0	☐	0	☐

The following graphics toolbar buttons control these parameters used with interactive weighting:

Check Box	Icon	Function
Line broadening	LB	Selects line broadening or exponential weighting. A negative value gives resolution enhancement.
Sinebell	SB	Selects the sinebell constant. A negative value gives squared sinebell.
Shifted Sinebell	SB	Selects the sinebell shift constant (if sinebell is active).
Gaussian	GF	Selects the Gaussian time constant.
Shifted Gaussian	GF	Selects the Gaussian shift constant (if Gaussian is active).
Additive weighting	AW	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 check 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.)

- 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 either command must be executed before 2D FT.

11.5 Baseline and Drift Correction

Use the Linear Prediction and Display pages in the Process tab to correct baseline and drift.

11.5.1 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 lp 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 lp 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 lp is rendered approximately 0 after acquisition. This macro requires an existing spectrum, properly phased (it looks at lp), 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, lp should now be approximately 0 when the spectrum is properly rephased. This macro requires an existing spectrum, properly phased (it looks at lp), 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 lp is rendered approximately 0 when the spectrum is reacquired. This macro requires an existing spectrum, properly phased (it looks at lp), and it must be followed by a reacquisition of the spectrum.

11.5.2 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 (i.e., VNMRS) 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 (i.e., Inova) 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.

11.5.3 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.

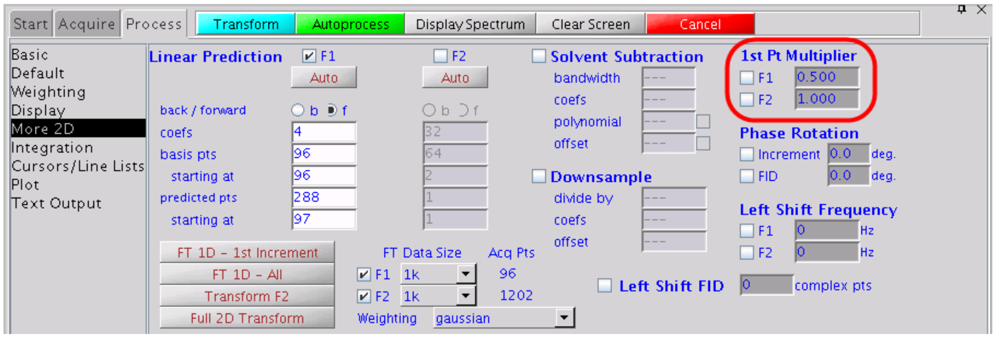
The effect of using the F2 value in 1st Pt Multiplier is to perform a linear baseline correction on all F_2 data, reducing negative-going ridges along F_2 in phase-sensitive 2D data. This correction is not needed in experiments such as COSY where the FID starts at zero and grows or in absolute-value 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 value and try again.

    Usually, only a few tries are required before the proper value of F2 1st Pt Multiplier is found.



Linear Prediction ☑ F1 ☐ F2
back / forward ○ b ● f ○ b ○ f
coefs 4 32
basis pts 96 64
starting at 96 2
predicted pts 288 1
starting at 97 1

FT Data Size Acq Pts
☑ F1 1k 96
☑ F2 1k 1202
Weighting gaussian

☐ Solvent Subtraction
bandwidth ---
coefs ---
polynomial --- ☐
offset --- ☐

☐ Downsample
divide by ---
coefs ---
offset ---

1st Pt Multiplier
☐ F1 0.500
☐ F2 1.000

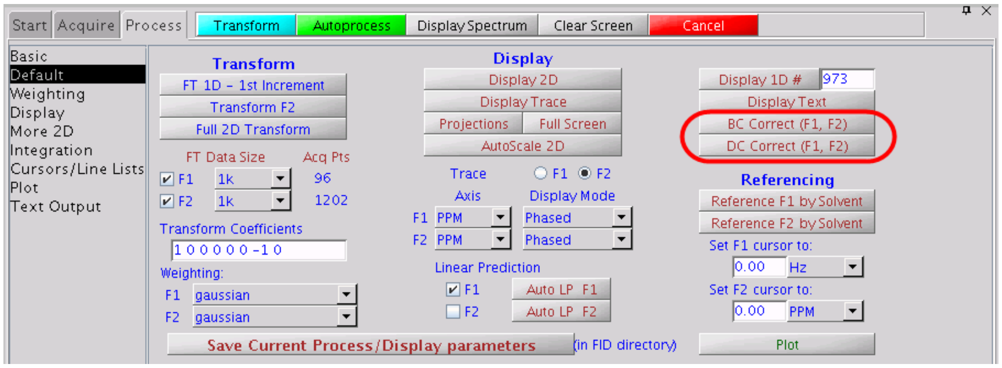
Phase Rotation
☐ Increment 0.0 deg.
☐ FID 0.0 deg.

Left Shift Frequency
☐ F1 0 Hz
☐ F2 0 Hz

☐ Left Shift FID 0 complex pts

11.5.4 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.



FT Data Size Acq Pts
☑ F1 1k 96
☑ F2 1k 1202
Transform Coefficients 1 0 0 0 0 0 -1 0
Weighting:
F1 gaussian
F2 gaussian

Display 1D # 973
Trace ○ F1 ● F2
Axis Display Mode
F1 PPM Phased
F2 PPM Phased
Linear Prediction
☑ F1
☐ F2

Set F1 cursor to: 0.00 Hz
Set F2 cursor to: 0.00 PPM

11.5.5 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 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 using 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.

11.6 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).

4*n* coefficients must be supplied if there are *n* data sets to be transformed, as in typical phase-sensitive experiments. The first 2*n* coefficients are the contributions to the real part of the interferogram, alternating between real and imaginary parts of the successive data sets. The next 2*n* 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 allows the addition, subtraction, etc., of eight 2D data sets (that is, eight different phase cycles). See the macro wft2dac for more information.

11.6.1 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 F2 regions that are to be transformed along t_1. Partition the integral of the spectrum into several regions. The even numbered F_2 regions, e.g., 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.

11.6.2 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*.

11.6.3 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 checked.
- The solvent subtraction (both the zfs and lfs options) is both turned off if the bandwidth is blank.

11.6.4 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.

11.6.5 2D Processing of 3D Data

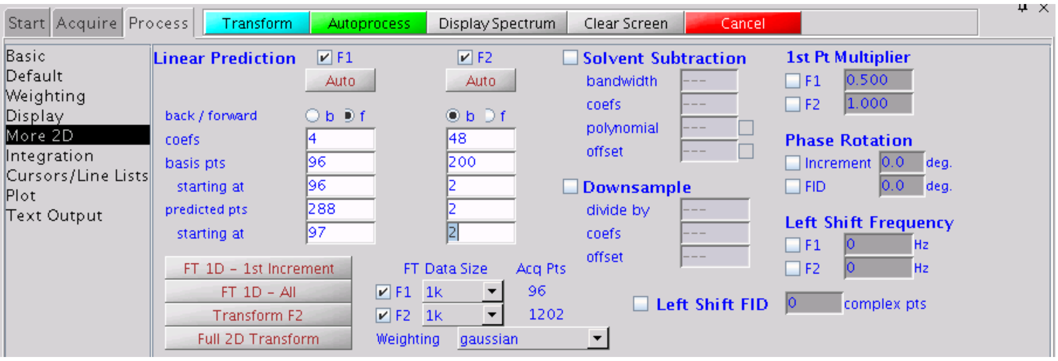
Acquisition and full processing of 3D data requires the parameters ni2 and sw2 (d3 is the delay increment in the ni2 dimension). 2D processing of “slices” of the 3D data matrix is accomplished using the following commands:

`ft2d('ni2')`	Transforms non-arrayed 2D data that have been collected with ni2 and sw2 (instead of ni and sw1). The addpar('3d') macro creates the necessary processing parameters for the ft2d('ni2') operation (par3d functions the same as addpar('3rf')).
`ft2d('ni',#)`	Selectively transforms selected np–ni 2D plane within a non-arrayed 3D data set; # is an integer that can range from 1 to ni2.
`ft2d('ni2',#)`	Selectively transforms selected np–ni2 2D plane within a non-arrayed 3D data set; # is an integer that can range from 1 to ni.
`wft2d`	Acts the same as ft2d, 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-Haberkorn 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 t_1-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.

11.7 2D and 3D Linear Prediction



Linear Prediction ☑ F1 ☑ F2

	F1	F2
back / forward	○ b ● f	● b ○ f
coefs	4	48
basis pts	96	200
starting at	96	2
predicted pts	288	2
starting at	97	2

	FT Data Size	Acq Pts
☑ F1	1k	96
☑ F2	1k	1202

Weighting gaussian

☐ **Solvent Subtraction**
bandwidth ---
coefs ---
polynomial --- ☐
offset --- ☐

☐ **Downsample**
divide by ---
coefs ---
offset ---

☐ **Left Shift FID** 0 complex pts

1st Pt Multiplier
☐ F1 0.500
☐ F2 1.000

Phase Rotation
☐ Increment 0.0 deg.
☐ FID 0.0 deg.

Left Shift Frequency
☐ F1 0 Hz
☐ F2 0 Hz

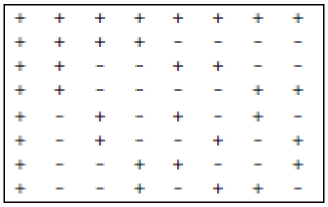
Linear prediction parameters are adjusted on the Linear Prediction page. The F1 checkbox controls the transformation process along t_1, and the F2 checkbox controls the transformation process along t_2. Using the same method of transformation is not necessary along two (or three axes). For example, you 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.

11.8 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.

11.8.1 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 52.



+	+	+	+	+	+	+	+
+	+	+	+	-	-	-	-
+	+	-	-	+	+	-	-
+	+	-	-	-	-	+	+
+	-	+	-	+	-	+	-
+	-	+	-	-	+	-	+
+	-	-	+	+	-	-	+
+	-	-	+	-	+	+	-

Figure 52 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.

11.8.2 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.

11.8.3 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.

11.8.4 Setting Up a Homonuclear 2D Hadamard Sequence

1. Acquire a 1 D spectrum (^{1}H or other nucleus).
2. Process and phase the spectrum.
3. Enter editht on the command line to open the Edit HT Freq dialog window.
4. Enter a line width in the Min line width entry box.
5. Press the Create Line List button and edit the line list.
6. Click on Save HT Frequencies button to save the frequency list.
7. Load the Hadamard experiment `TocsyHT`.

   Either use the Experiments menu or enter the macro on the command line.
8. Edit the Hadamard frequencies using the Edit HT Freq dialog window.
9. Start acquisition.
10. Process using the Fu**ll 2D Transform** button on the Default Process page or enter **`wft2da`** on the command line.

11.8.5 Setting up a Heteronuclear 2D Hadamard Sequence

1. Acquire a 1 D spectrum (^{13}C or other nucleus).
2. Process and phase the spectrum.
3. Enter editht on the command line to open the Edit HT Freq dialog window.
4. Enter a line width in the Min line width entry box.
5. Press the Create Line List button and edit the line list.
6. Click on Save HT Frequencies button to save the frequency list.
7. Acquire a 1D 1H spectrum and adjust the spectral width and decoupling as desired.
8. Load the Hadamard experiment HsqcHT.

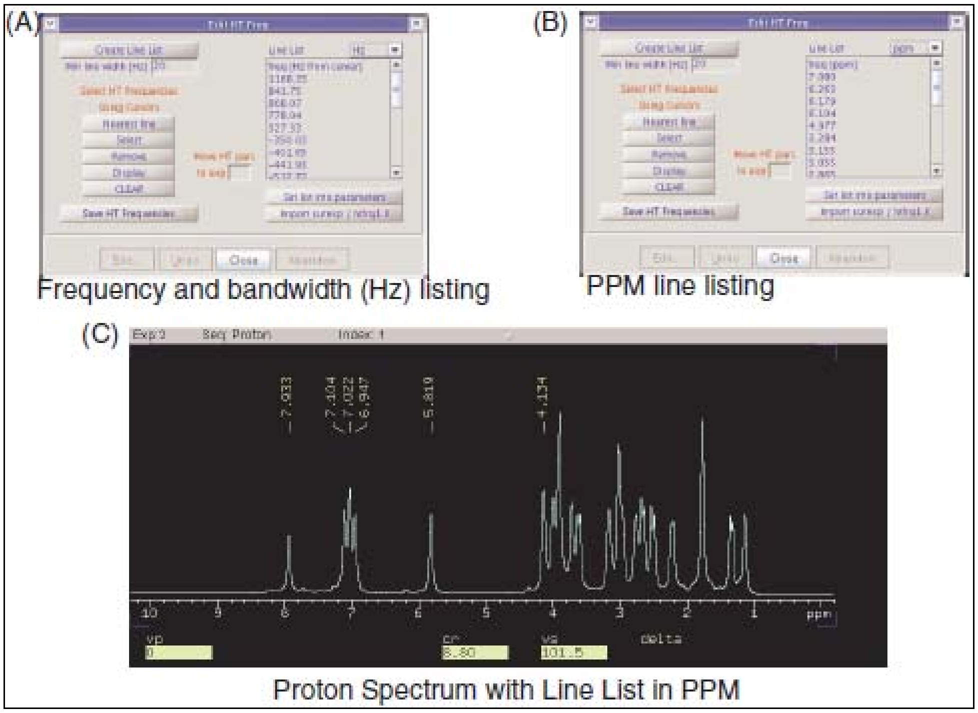
   Either use the Experiments menu or enter the macro on the command line.
9. Edit the Hadamard frequencies using the Edit HT Freq dialog window.
10. Start acquisition.
11. Process using the Full 2D Transform button on the Default Process page or enter wft2da on the command line.

11.8.6 Editing or Creating Hadamard Frequencies

1. 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.

   Two line listings are available; see Figure 53.
3. Click on the drop down menu next to Line List and select one of following:
   - Select **Hz** to display the line list and frequencies in Hz from the center of the spectrum. The bandwidth is also displayed if it is arrayed.
   - Select the **ppm** from the drop down menu to display the frequencies in ppm.

4. Click **Display** to show the Hadamard frequencies.
5. Create or edit a Hadamard frequency list using the Edit HT Freq window buttons.



Frequency and bandwidth (Hz) listing

PPM line listing

Proton Spectrum with Line List in PPM

Figure 53 Spectrum and Edit HT Freq Window

Table 16 Edit HT Freq Window Buttons

Button	**Function**
Create Line List	Processes the spectrum using the line broadening specified in field next to Min line width [Hz] to smooth out multiplet structures. Peak thresholds are adjusted automatically and a line list is created.  Min line width [Hz] 20 Line List: Hz freq [Hz from center] 1168.35 841.75 808.07 778.04 327.33 -350.03 -401.65 -441.93 -517.72 The line list is set into the parameter `htfrq1`. Only frequencies separated by the minimum line width are used.
Nearest line	Places the cursor on the nearest line.
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 dpf in units set by the axis parameter.

CLEAR	Clears all frequencies from the frequency list.
Save HT Frequencies	Saves the current frequency list as a Hadamard line list for the current nucleus (tn). 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.
Hz/ppm menu	Select Hz or ppm to display the line list in Hz or ppm. The line list is displayed in Hz from the center of the spectrum if Hz is selected.
Set list into parameters	Sets the changes from the Line List text entry window into the parameters htfrq1 and htbw1.
Import list from curexp	Copies a line list file from curexp into the current line list, and sets the line list into the parameters. The file name: /export/home/vnmr1/vnmrsys/exp2/htll.htfrq1 has the same format as the Line List in the text entry window.

11.8.7 Commands, Macros, and Parameters

For more information on the following commands, macros, and parameters used in Hadamard Spectroscopy, refer to the *VnmrJ Command and Parameter Reference* manual.

Command/Macro	**Description**
`dll`	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)
`nll`	Fine line frequencies and intensities (C)
`SelexHT`	Set up the SelexHT experiment (M)
`sethtfrq1`	Set a Hadamard frequency list from a line list ((M)
`TocsyHT`	Set up the TocsyHT experiment (M)
`HsqcHT`	Set up the HsqcHT 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)

Command/Macro	Description
`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 ni (P)
`ni`	Number of increments in 1st indirectly detected dimension (P)
`htofs1`	Hadamard offset in ni (P)
`proc1`	Type of processing on ni interferogram (P)
`fn1`	Fourier number in 1st indirectly detected dimension (P)
Optional Parameters	**Description**
`htbw1`	Hadamard pulse excitation bandwidth in ni (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 ni (P)
`htcal1`	RF calibration flag for Hadamard waveforms in ni (P)
`htpwr1`	Power level for RF calibration of Hadamard waveforms in ni (P)
`nimax`	Maximum limit of ni (P)

11.8.8 Reference

E. Kupce and R. Freeman, “Two-dimensional Hadamard spectroscopy”, *J. Magn. Reson.* (2003), **162**:300-310.

11.9 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 macro 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 F2 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 F1 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.

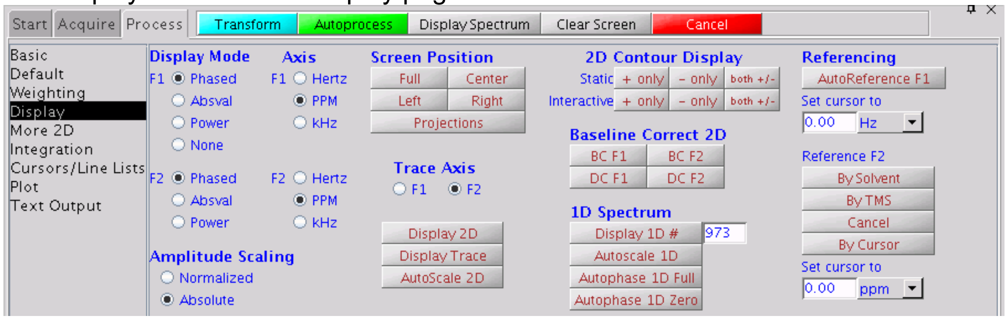
Corrections in f_2 phasing may be obvious in the 2D data when they are not in the first increment 1D spectrum. If pmode='full' before the 2D transform, f2 phasing may be corrected without retransforming by setting trace='f2' and using the same approach as described for f1phasing. Transformation of the 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 are properly phased in f_1 and f_2.

11.10 Display and Plotting

This section discusses the manual mode of (non-interactive) 2D display and plotting. For more details, see the chapters on Display and Output.

11.10.1 Display Modes

Select display modes in the Display page.



11.10.2 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).

11.10.3 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.

11.10.4 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.

11.10.5 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.

11.10.6 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.

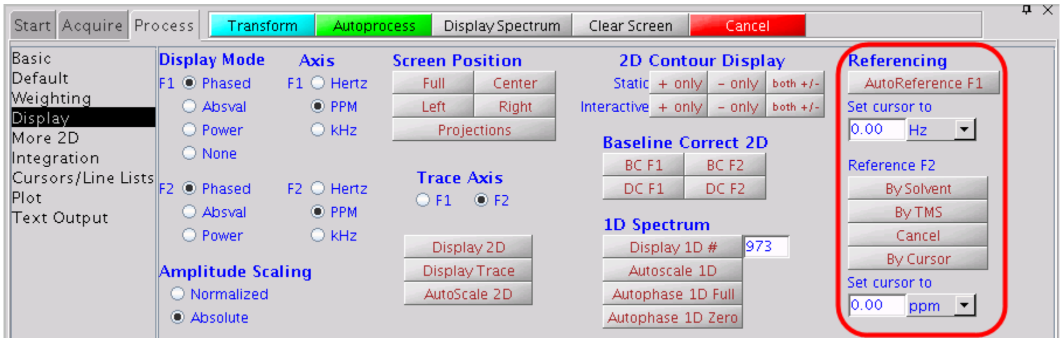
Labels can appear at incorrect positions if wysiwyg='n'. The positions were empirically determined for a large screen display and are not guaranteed to be correct for all displays.

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.

11.10.7 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.

11.10.8 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.

11.10.9 Setting Negative Intensities to Zero

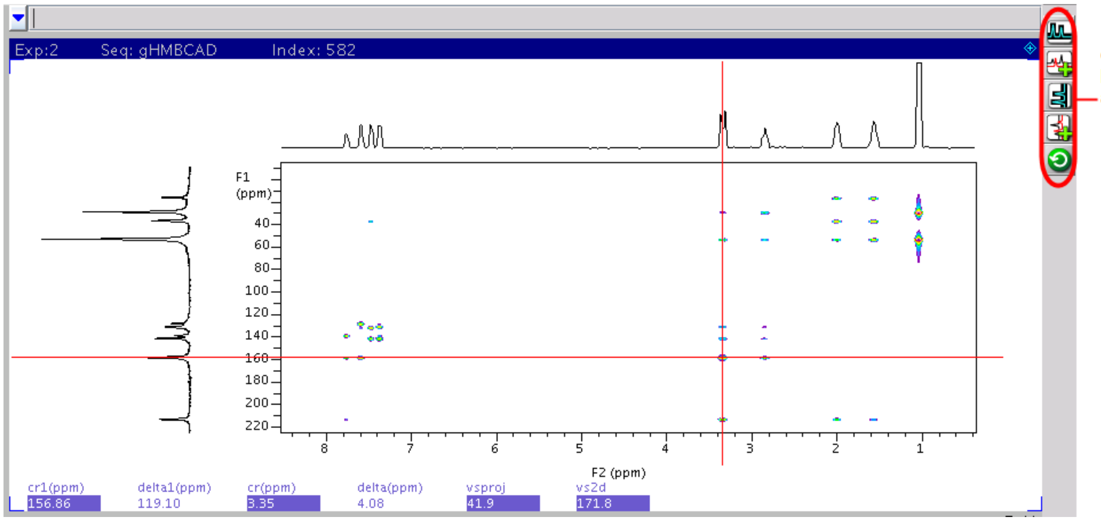
The command zeroneg is used for the projection of proton 2D-J spectra at 45° to strip a high-resolution proton spectrum down to a list of chemical shifts. The zeroneg command sets all negative intensities to zero.

11.11 Interactive 2D Color Map Display

Use the graphics control buttons and the mouse to control the display in the graphics window.

11.11.1 2D Display

Below is an example of a 2D display with the projection graphics control buttons selected.



Exp:2 Seq: gHMBCAD Index: 582

cr1(ppm) 156.86
delta1(ppm) 119.10
cr(ppm) 3.35
delta(ppm) 4.08
vsproj 41.9
vs2d 171.8

Graphic control buttons for traces and projections.

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.

11.11.2 Interactive nD Display Control Buttons

Table 17 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 18 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%.
	Phase spectrum.
	Click to select the first spectrum. Click to select the second spectrum.
	Enter peak pick menu.

11.11.3 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 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.

11.11.4 Traces

1. Click the Trace icon. A trace displays 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.

11.11.5 Projections

1. Click the Projection icon to open the Projection graphic control buttons.
2. Select type of direction (horizontal or vertical) and mode (maximum or summary).
3. Adjust the vertical scale of the projections with the middle mouse button.

11.11.6 Expanding the Display

1. Use the Box cursors to select the region in the spectrum to expand.
2. Click on the Expand icon to obtain an expanded display.

11.11.7 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.

11.11.8 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.

11.11.9 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.

11.12 Interactive 2D Peak Picking

The ll2d 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 dconi display or can be plotted using the pll2d command.

The results of all peak-picking operations are stored in a binary file in the ll2d 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 (e.g., f1f3 or f2f3), and the last # denotes the number of the plane.

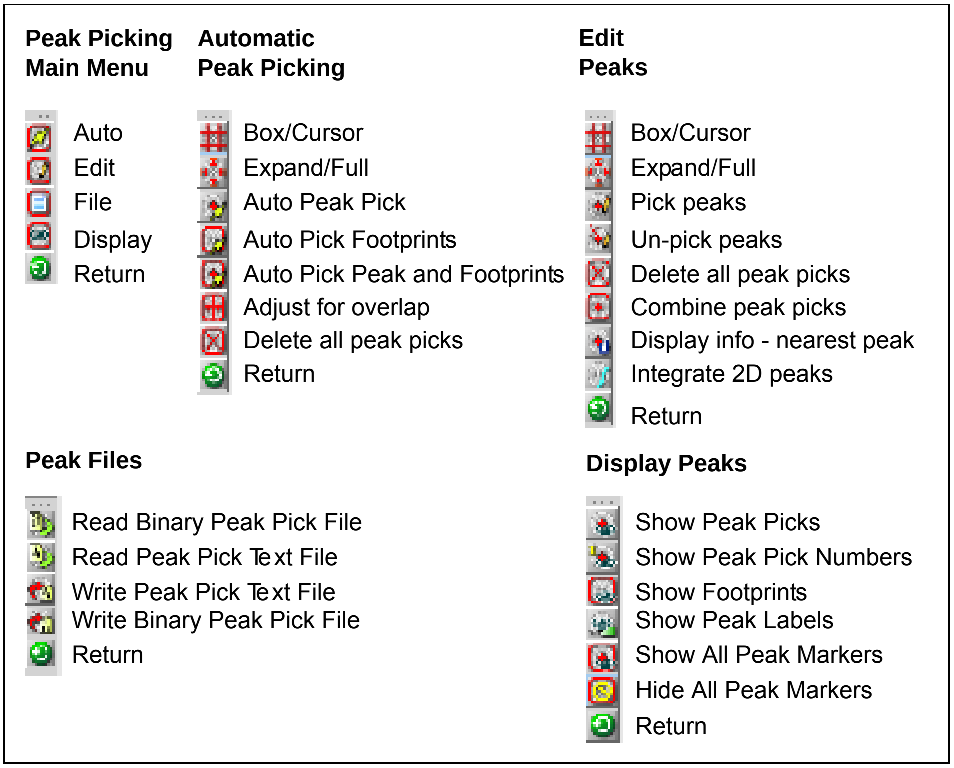
Binary peak files can be converted to text files for printing or for export to other programs.

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 dimension
- Volume of the peak
- 15-character peak label
- 80-character comment

11.12.1 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.



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 19 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 20 2D Peak Picking Automatic Menu

Box/ Cursor	Selects 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 `th2d` times the current threshold. Thus, using a smaller value for `th2d` will cause `ll2d` 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 21 2D Peak Picking Edit Menu

Box/ Cursor	Selects cursor mode.
Expand/ Full	Selects expanded or full display.
Mark	In dconi cursor mode, this button inserts a peak at the current cursor location. In dconi 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 dconi cursor mode, this button deletes the peak nearest the cursor. In dconi box mode, this button deletes peak bounds from peaks whose bounds are entirely within the area defined by the cursors.
Clear	In dconi cursor mode, this button deletes all peaks in the area of the spectrum displayed in dconi. In dconi box mode, the Clear button deletes all peaks that are within the area defined by the cursors.
Combine	This button works only in dconi 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 (dconi cursor mode) or to all peaks within the area defined by the cursors (dconi box mode). Based on the value of the parameter ll2dmode, this label can be displayed next to the peak in dconi.

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 above).

Table 22 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 (peaks.bin 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 (peaks.bin 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 ll2d 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 above).

Table 23 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.
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.

11.13 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, crl2, 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-Haberkorn method applied along both t1 and t2), has at least three arrayed elements. By convention, such an arrayed 3D experiment is implemented using four arrayed elements: d3 (t2 evolution time), phase2, d2 (t1 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.

11.13.1 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.

11.13.2 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.

11.13.3 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 dconi 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.

11.13.4 3D Pulse Sequences

Write a sequence that includes a d2 and d3 delay (these delays may also be d2/2 or d3/2). 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, b y 90/*n*).

11.13.5 Experiment Set Up

Set up is necessary in 3D experiments to position transmitters and decouplers, adjust pulse widths, etc. Just as the set up of 2D experiments can often be assisted by performing “first increment” experiments (i.e., 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 d2, 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 phase=1 (when ni2 > 1 and phase2=1,2; or phase2=3, as desired). These parameters will produce a 2D experiment in which d3 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',1,0,0,0,0,0,-1,0). Use wft2da('ni2',1) to transform the first F2 X F3 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

11.13.6 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 are 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 *(*ni,ni2) increment, there are only two or one data sets, respectively, per (ni*,*ni2) increment. If there are only two data sets per *(*ni*,*ni2) 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 (ni*,*ni2) 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 (e.g., by typing wft(1)).
3. Enter wft2d('ni',1,1,0,0,0,0,0,0,0,0,0,0,0,–1,0,0,0) to adjust f1 phasing (there are 11 consecutive zeros in the middle of this argument).
4. Fix the f1f3 2D spectrum (with incorrectly labeled axes). Set trace='f1' to adjust the f1 phase, then set trace='f2' to trim the f3 phasing. Now adjust rp1 and lp1 (as well as rp and lp).
5. Enter wft2d('ni2',1,1,0,0,0,0,0,0,0,0,0,–1,0,0,0,0,0) to adjust f2 phasing (note that this argument has nine consecutive zeros in the middle and five zeros at the end).
6. Adjust f2f3 2D spectrum phase. Set trace='f1' to adjust the f2 phasing (rp2 and lp2), then set trace='f2' to trim the f3 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 (i.e., 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 (e.g., 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 is probably 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 nth plane. The resetf3 macro will reset parameters after a partial 3D Fourier transform.

11.144D 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.

Chapter 12 Indirect Detection Experiments

This chapter describes one 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).

Sections in this chapter:

12.1 Probes and Filters

12.1.1 Indirect Detection Probes

The most commonly used probes for indirect detection experiments are the Varian, 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 ^{1}H coil and an X-nucleus coil with the ^{1}Hcoil positioned closer to the sample for the highest possible sensitivity for ^{1}H observe. When connecting cables to the probe, ignore words like “observe” and “decouple” and think of ^{1}H (for observe) and X (for decouple) for connections.

Normal “broadband” probes similarly have a ^{1}H 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 that direct detection heteronuclear correlation data. Four-nucleus and “Switchable” probes also have a ^{1}H 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 Varian, Inc. NMR probes, go to the NMR probe product pages at www.varianinc.com. Refer the manual that shipped with the probe for installation and tuning instructions.

12.1.2 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 Varian 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 ^{2}H 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 nonexistent if the instrument does anything other than ^{13}C and ^{1}H, because the ^{13}C bandpass filter cannot be left in place on the X line while doing ^{31}P, ^{15}N, or anything else. All standard specifications are given with the indirect detection filters *not* in place.

12.2 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:

12.2.1 Spin-Echo Difference Experiment

The heart of the HMQC sequence can be reduced to a heteronuclear spin-echo difference experiment that looks like Figure 54.

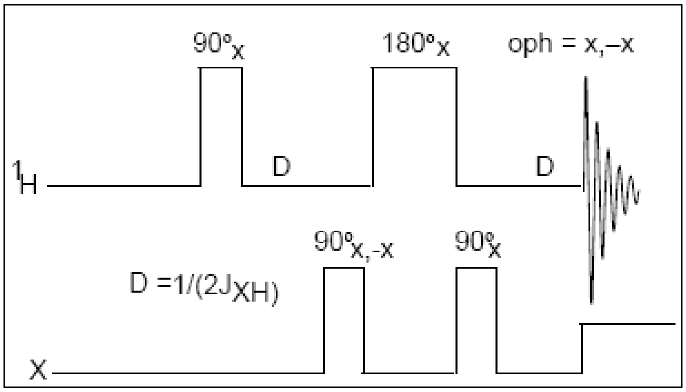


Figure 54 Heteronuclear Spin-Echo Difference Experiment

In Figure 55*a*, *b*, and *c* represent the proton magnetization attached to carbons, where *a* are the proton magnetization attached to up-^{13}C, *b* are proton magnetization attached to ^{12}C, and *c* are proton magnetization attached to down-^{13}C. Assume that we are at the resonance frequency of the proton magnetization attached to the ^{12}Cs. In the rotating frame, the following steps (shown in Figure 55) occur:

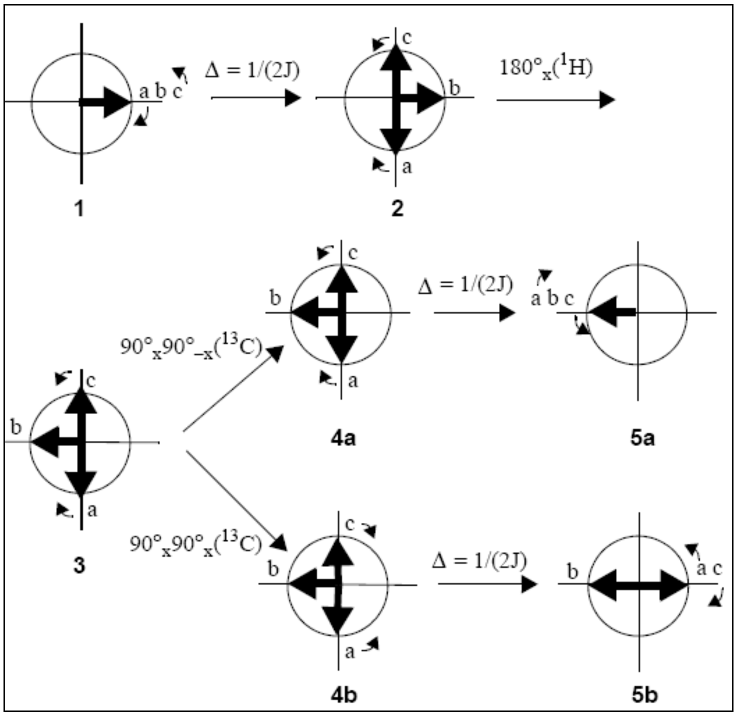


Figure 55 HMQC Pulse Sequence, Showing Movement of Attached Protons

1. The first proton 90° pulse places all proton magnetization along the *y* axis.
2. After a time Δ = 1/(2J), the *b* proton magnetization are still along the *y* axis, but the *a* proton magnetization are along the –*x* axis and the *c* proton magnetization are 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°$_X$90°$_{-X}$ carbon pulse is effectively a null pulse. All rotational directions are maintained.
    b. The 90°$_X$90°$_X$ (= 180°$_X$) 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 Δ = 1/(2J), the following occurs:
    a. The a, b, and c proton magnetization are refocused along the –y axis.
    b. The b proton magnetization are 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 oph, 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 56.

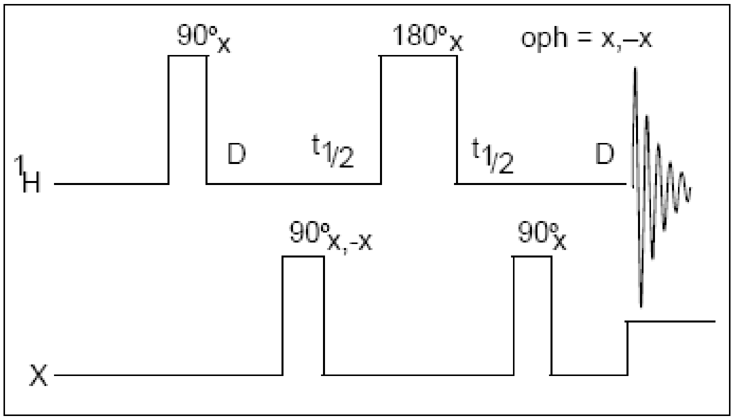


Figure 56 Evolution Time Added Between X-Nucleus Pulses

In this 2D experiment, which is now a full HMQC experiment, proton magnetization attached to ^{12}C show no different behavior and are 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 (i.e., 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.

12.2.2 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 13C unaffected. The full sequence is illustrated in Figure 69, where Δ=1/2J

After the BIRD pulse, a variable waiting period (τ in Figure 57) is inserted, allowing the ^{13}C-bound proton magnetization to relax back to equilibrium.

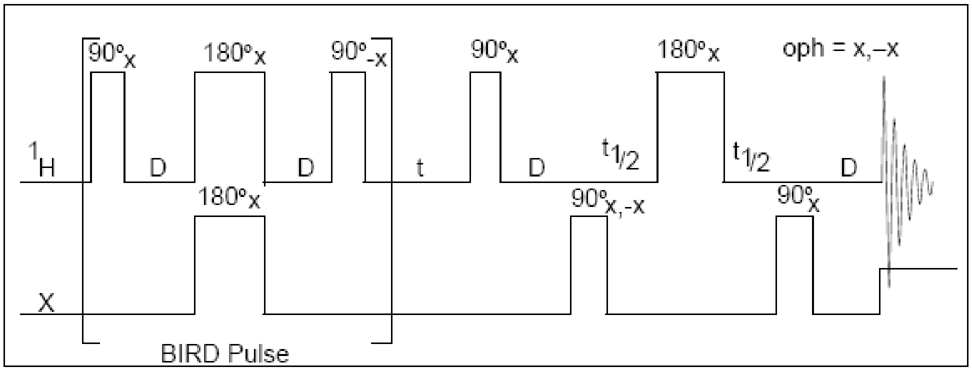


Figure 57 HMQC with BIRD Pulse Nulling Effects

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.

12.2.3 Transmitter Presaturation for High-Dynamic Range Signals

When high-dynamic range situations, such as observing signals in H_2O, 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.

12.3 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 ^{1}H and ^{13}C calibrations from the active probefile 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. Two approaches can be taken to set the parameters controlling the C frequency and spectral width if this is the case.
    - 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 `pw=4*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 `pw=pw+0.8 ga`; if they are positive, enter `pw=pw-0.8 ga`; repeat until a good null is found, then enter `pw=pw/4`.
4. Switch to the HMQC experiment and set the relevant parameters based on the results of steps 2 and 3.
5. Enter `phase=1 ni=1 dm='nnn' null=0 ai wexp='wft 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 to ensure cancellation optimization.

   The spectrum in Figure 58 shows the result of this experiment on a sample of 1% 3heptanone at 300 MHz, using nt=64 null=2.0 and d1=2.

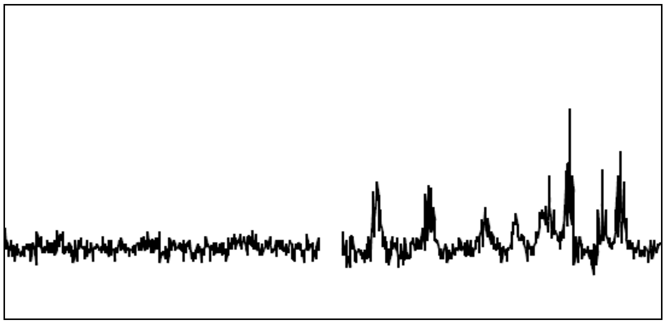


Figure 58 Verifying Cancellation with `pwx=0, 90`

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='nnn','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 (e.g., 0.001) and one very long value (e.g., 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 59 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.

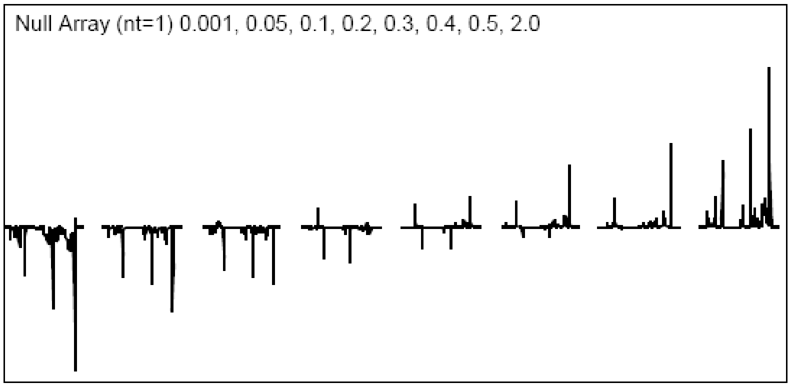


Figure 59Optimizing the BIRD Nulling Time

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).
    c. Enter ga and a proton spectrum will be observed.
    d. Move the FID to a different experiment, join the different experiment, and re-transform the data (e.g., mf(1,2) jexp2 wft).
    e. Set the cursor on a peak that is to be removed by presaturation, and enter nl movetof. Note the value of tof selected and copy this value back to the parameter satfrq (e.g., jexp1 satfrq=x) of the original experiment.

f. Set satflg='yn' and satdly equal to a significant time compared with T of the peaks (e.g., satdly=1).

g. To find the minimum value for which the peak will be removed (e.g., 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 `ni` between 128 and 256, **`phase=1,2`**, and `nt` 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 `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 lp1 – 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 (e.g., residual protons on a deuterated solvent) or methyl groups. In Figure 60 they are seen at 2.4 ppm, 1.0 ppm, and 0.9 ppm.

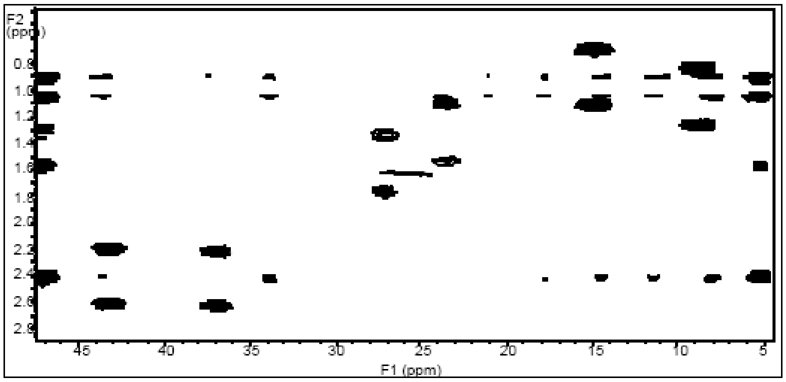


Figure 60 Coupled HMQC Spectrum of 3-Heptanone

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 60, 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 60 is the “0,0” artifact in the exact center of the spectrum. Some peaks in the ^{1}H spectrum, of course, will not appear in the HMQC spectrum, because they represent protons that are not bound to C (e.g., protons from water or NH groups). This is not the case with 3-heptanone, however.

Multiple structures are typical with HMQC spectra. During the detection period a (i.e., 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’s adjacent to a CH group), see Figure 61.

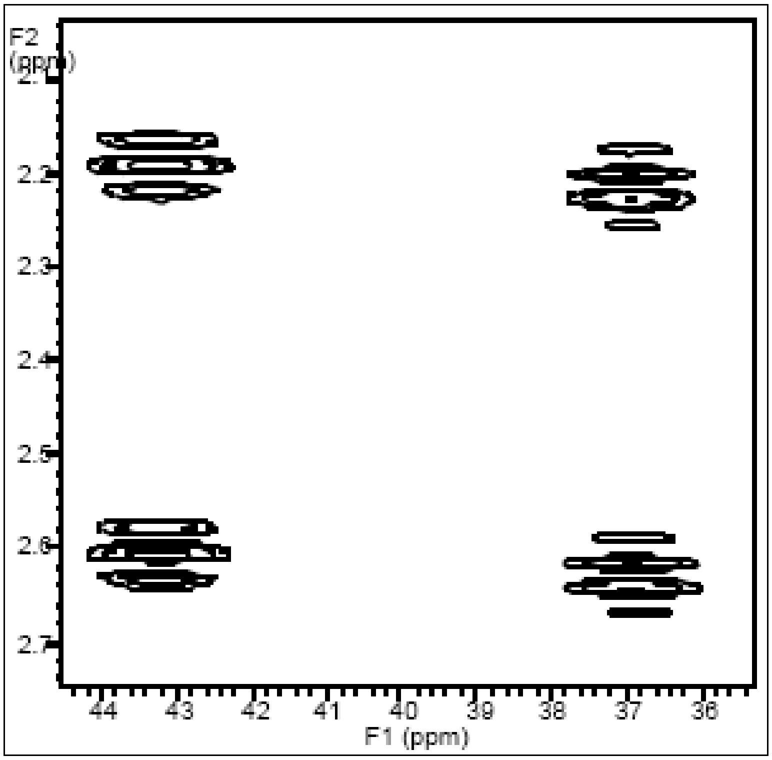


Figure 61 Expansion of Coupled 3-Heptanone HMQC Showing Multiplets

In the H spectrum itself, these two groups of protons are heavily overlapped (see Figure 62).

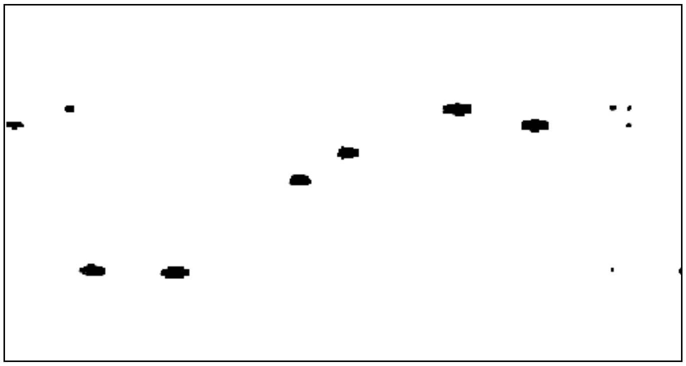


Figure 62 Decoupled HMQC Spectrum of 3-Heptanone

12.4 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 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 ^{2}H 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 the system is in thermal equilibrium. 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, is 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 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, pw=0, no signal should be seen. This can be compared to four scans with pw 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).

12.5 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*pw90) 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 does 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 decoupling, which offers wider decoupling bandwidths at lower powers.

12.6 ^{15}N 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 (at) are 0.075 to 0.1 seconds.

Use the HMQC or HSQC pulse sequences for indirect detection experiments.

12.7 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 ^{1}H (the default is ^{13}C). Shown below Figure 63 is a diagram of this sequence. The first 2*pwx pulse on the X heteronucleus is a composite 180 consisting of 90(v9) – 180(v1) – 90(v9).

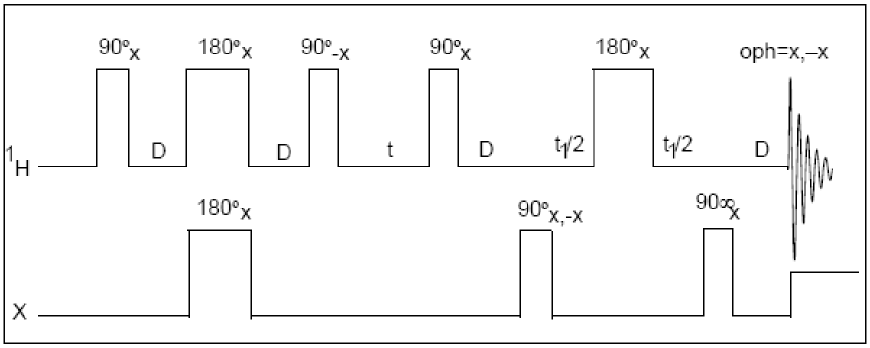


Figure 63 Basic HMQC Pulse Sequence

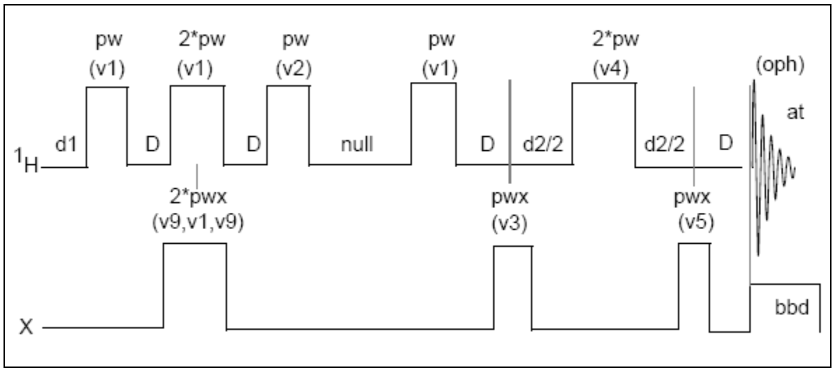


Figure 64 HMQC Pulse Sequence with `null`<>0 and `mbond='n'`

12.7.1 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-Haberkorn-Ruben (hypercomplex) method. Use phase=3 to acquire data with TPPI and ensure sw1 is twice the expected range.

The FAD, for “F1 Axial Displacement” technique (Marion, D.; Ikura, M.; Tschudin, R.; Bax, A. *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 24 Parameters for HMQC Pulse Sequences

Parameter	HMQC
1H 90°	**`pulse pw`**
1H 180° pulse	derived from **`pw`**
1H amplifier power (if appropriate)	**`tpwr`**
1H frequency	**`tn, tof`**
1H spectral width	**`sw`**
X 90°pulse	**`Pwx`**

Parameter	**HMQC**
X 90°pulse for WALTZ decoupling	`1/(4¥dmf)`
X 180°pulse	derived from **`pwx`**
X amplifier power for pulses (if appropriate)	**`pwxlvl`**
X amp power for decoupling (if appropriate)	**`dpwr`**
X frequency	**`dn, dof`**
X spectral width	**`sw1`**
Δ delay	**`1/(2j) [if j=0, D=0]`**
τ delay for BIRD nulling (if null=0, entireBIRD sequence is skipped)	**`null`**
Coupled experiment	**`dm='nnn'`**
X decoupling during acquisition.	**`dm='nny'`**
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 **`pwxlvl`**.

`dpwr` is the decoupler power level for broadband X-decoupling.

`dmf` sets the modulation frequency (4*γB_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`.

12.7.2 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*(`ix` - 1) ° to `v3`, where `ix` is the increment counter.

12.7.3 Technique

The usual setup is to place a ^{1}H bandpass filter between the observe port on the probe and the ^{1}H/^{19}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.

12.8 HSQC Pulse Sequence

The hsqc macro sets up parameters for the HSQC pulse sequence.

12.8.1 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 ^{1}H transmitter pulses.

`pw` is a 90° transmitter pulse length for protons (the observed nucleus).

`pwxlvl` is the power level for X decoupler pulses.

`pwx` 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).

Chapter 13 Solids Experiments

Sections in this chapter:

13.1 Solids Pulse Sequences and VnmrJ

The section describes pulse sequences and protocols used for solid-state NMR experiments in VnmrJ versions 3.0 and later. These sequences include basic experiments for cross-polarization, single-pulse experiments, and experiments such as MQMAS, REDOR, HETCOR, and WISE. This section includes proton multi-pulse acquisition CRAMPS sequences such as BR24 and windowed PMLG.

All of the solids sequences described here use a new set of consistent parameter definitions. These experiments can be set up from either set-up macros or protocols on the command line, set up from the Experiments pull-down menu or by drag-and-drop from protocols in the Experiment Selector.

Solids parameter sets are not consistent with parameter sets for other non-solids sequences, including very basic sequences such as `s2pul.c`, older solids sequences such as xpolar1.c, and those sequences of the older SolidsLib for UNITYINOVA. The sequence onepul.c replaces s2pul.c for solids (or xpolarv1.c with xpol = 'n') and the sequence tancpx.c replaces xpolarv1.c with xpol = 'y'. All the functions of xpolarv1.c (TOSS, relaxation measurements, and dipolar dephasing) are distributed among individual sequences. Sequences with multiple functions are avoided.

Solids pulse sequences use a new logical method of naming parameters based upon parameter groups. Each parameter group refers to a single NMR function (for example cross polarization or TPPM decoupling) and each parameter group has it's own interface on the Pulse Sequence page of the Acquire tab for the sequence. A pulse-sequence module (a set of C-functions in the pulse-programming language that executes the NMR function in a pulse sequence) is also associated with each parameter group.

The VnmrJ solids sequences do not use legacy parameter names such as pw, tpwrm, or the older ad hoc names for solids such as dipolr, level1, or evolve. VnmrJ solids sequences do not use the status() command for decoupling and the associated parameters, dm, dmm, dres, dmf and dseq. Equivalent choices for solids decoupling methods (CW, TPPM, SPINAL64 and off) are made through the decoupler parameter group and module. More information about parameter groups and pulse-sequence modules can be found in the User Programming manual.

Descriptions of the sequences are included in this chapter. The same information can be obtained through the Sequence Manual button on the Text page of Process tab or the man(seqfil) command on the command line.

13.2 Running a Solids Experiment

The following is a generalized procedure for running a solids experiment. Steps in the procedure are referenced to sections in this and other manuals where more detailed information or procedures are provided. Use these procedures only if the spectrometer is set up for solids experiments and all probes have been installed, tested, and calibrated.

Set the user profile to **AllSolids** before starting this procedure if the Experiment Panel does not contain solids related protocols, see Loading a Protocol.

1. Do one of the following procedures:
    - Systems operating in either the liquids or solids mode and the last used probe is either unknown or a liquids probe:

    a. Load any liquids probe file.

    b. Click on the Start tab.

    c. Select the Spin/Temp page

    d. Click on the VT Air Off button

    e. Type su on the command line.

    f. Continue with **Step 2**.

    - A System that operates only as a solids systems or the last probe used was a solids probe.

    g. Continue with **Step 2**.
2. Load the sample into the rotor and install the probe in the magnet.

    Refer to the probe installation and operation manuals and related rotor packing manuals.
3. Load the **Settancpx** protocol to initialize the workspace for solids experiments.

    See Initializing a Workspace for Solids Experiments.

4. Load the correct probe file.

   See Setting Up a Probe File for a Solids Probe.

5. Do one of the following:
   - Recall a data set acquired with the protocol to be used (if such a data set exists) into the workspace initialized for solids.

     The data set contains the last used calibrations and shim sets. The referenced adamantane spectrum that was saved during the probe calibration procedure is a good initial set up, see Shimming with Adamantane.
   - Load the protocol for the experiment to be run and set the parameters as needed.

6. Optional: Calibrate the system if the values in the recalled data set or the probe file are not known to be correct or to update the file. Follow the procedure in Calibration of $^{13C\text{-}1H}$ CPMAS Probes, using a probe designed for cross polarization experiments.

7. Set the sample temperature regulation, see Setting the Temperature.

8. Set the MAS Spin rate, see Setting the MAS Spin Rate.

9. Set up for either:
   - One pulse experiments see Setting up Single-Pulse Experiments .
   - Cross polarization experiments, see Setting up Cross Polarization .

10. Set up the decoupler parameters, see Setting Up Decoupling.

11. Set up the acquisition parameters, see Acquisition Parameters and Other Pages.

12. Tune the probe, see Tuning a Solids Probe.

13. Acquire the data.

14. Reference the spectrum, see Referencing for Solids.

15. Save the data.

16. Do one of the following:
    - Select the next experiment from the Experiments menu to load the protocol into the current workspace for data acquisition.
    - Click on a Protocol in the Experiment Selector to load the protocol into the current workspace.

17. Lower the probe from the magnet to change the sample or to change to another probe as follows:
    a. Bring the sample to room temperature by adjusting the temperature setting on the MAS Spin/Temp panel, refer to Setting the Temperature.

    b. Press the Temp Off button to turn the temperature regulation off when the probe reaches room temperature.

    c. Stop spinning the rotor. Press the Stop button on the MAS Spin/Temp panel.

    d. Lower the probe from the magnet.

    e. Change samples and begin again at **Step 2**.

13.3 Initializing a Workspace for Solids Experiments

A workspace must be initialized with standard solids parameters before any solids experiments are run.

1. Load the probe file corresponding to the probe in use, see Loading a Probe File.

   Create a probe file if one does not exist for the probe; see Setting Up a Probe File for a Solids Probe and *VnmrJ Installation and Administration* user guide for instructions on creating a probe file.

2. Load the protocol Settancpx either from the Experiment Selector (see Figure 65) or from the Experiments drop down menu (see Figure 66) to initialize a workspace for the solids experiments.

   The protocol Settancpx retains the values of tn, dn, dn2, etc. and uses the rtp command to load the parameter set, Settancpx.par, from /vnmr/parlib and replace all remaining parameters.

   Settancpx loads basic calibrations for requested nuclei from the probe file if a probe file is defined. Alternatively, load an existing dataset derived from an experiment initiated with Settancpx using the Open window in the File pull-down menu. Unpredictable system behavior results if a solids protocol is run in a workspace with parameters that have not been initiated for solids with Settancpx.

   The solids1d apptype has a single protocol Settancpx that is used to initialize a workspace. All remaining solids protocols are apptype solidsseq1d. Protocols of apptype solidsseq1d preserve calibrations in the workspace and can be run in any order.

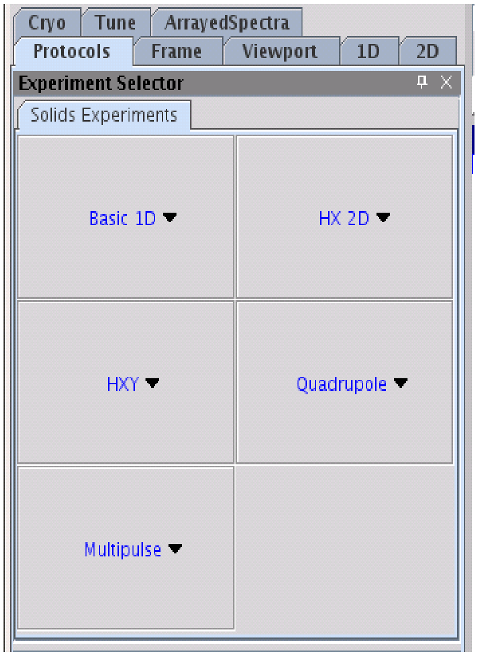


Figure 65 Solids Protocols Tabs

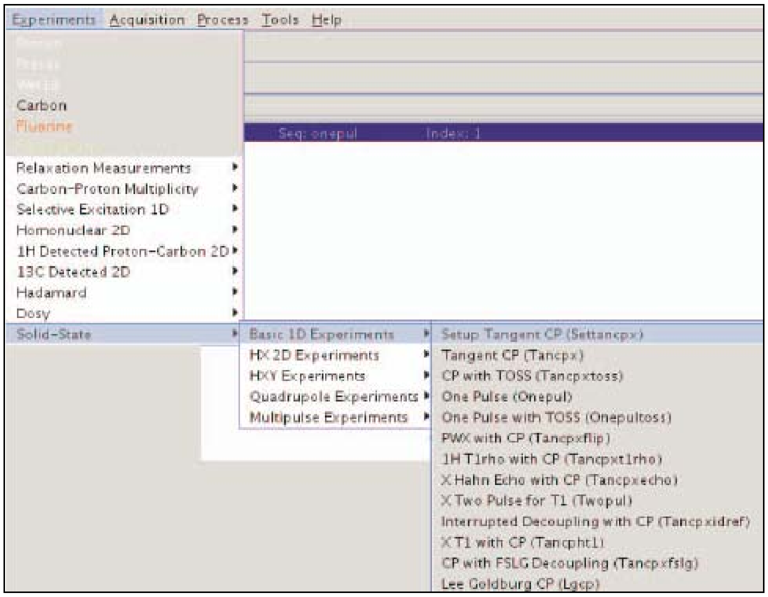


Figure 66 Solids Experiments and Menus

3. Calibrate the probe beginning with the Settancpx protocol.

   Refer to Calibration of 13C-1H CPMAS Probes, for a CP-MAS probe calibration procedure.

4. Save a standard dataset with calibrations and begin all experiments with that dataset.

13.4 Loading a Protocol

Protocols can be loaded from either the Experiment Selector or the Solid-State tab of the Experiments pull-down menu.

13.4.1 Using the Experiments Pull-down Menu

1. Click on Experiments on the main menu.
2. Select Solid-State.
3. Select an experiment group, see Figure 66.
4. Click on the specific protocol to load; see next section.

13.4.2 Using the Experiment Selector

The VnmrJ system administrator must assign the user profile `AllSolids` to use the Experiment Selector. Only solids protocols are displayed. The `AllLiquids` profile is required to display the liquids protocols. Refer to the *VnmrJ Installation and Administration* user guide for instructions on assigning profiles and creating custom profiles.

1. Select the **Protocols** tab from the vertical panel tabs.
2. Click a **Protocol Type** type on the **Experiment Selector**, see Figure 65 .
3. The tabs of the Experiment Selector are organized according to experiment type.
4. Select a **Protocol** from the menu and drag it to the active viewport.
5. The experimental protocol is loaded in the current active viewport as a foreground process.

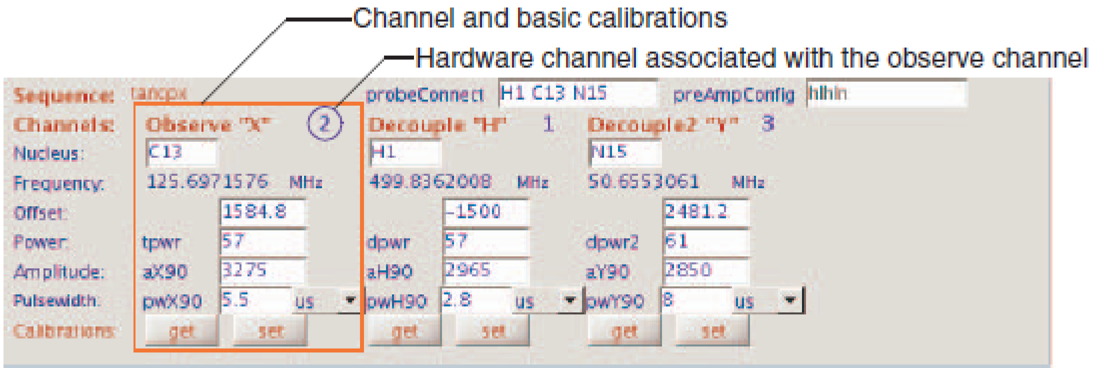
13.5 Setting Up a Probe File for a Solids Probe

The Channels page of the Acquire tab shows the values of the nucleus and basic calibrations for each pulse-sequence channel obs, dec, dec2, etc., up through a potential of 5 channels. The basic parameter calibrations and functions are listed in Table 25.

Table 25 Basic Solids Calibration Parameters

Channel type (synthesizer)	**Offset: (synthesizer offset)**	**Channel Identifier**	**Power: (course power)**	**Amplitude: (fine power)**	**Pulsewidth:**
obs	tof	X	tpwr	aX90	pwX90
dec	dof	H	dpwr	aH90	pwH90
dec2	dof2	Y	dpwr2	aY90	pwY90
dec3	dof3	Z	dpwr3	aZ90	pwZ90
dec4	dof4	W	dpwr4	aW90	pwW90

Basic calibrations for each channel and number of the hardware channel associated with the channel are contained on the Channels page, see Figure 67.



Sequence:	tancpx		probeConnect	H1 C13 N15	preAmpConfig	hlhln
Channels:	Observe "X" ②		Decouple "H" 1		Decouple2 "Y" 3	
Nucleus:	C13		H1		N15	
Frequency:	125.6971576 MHz		499.8362008 MHz		50.6553061 MHz	
Offset:		1584.8		-1500		2481.2
Power:	tpwr	57	dpwr	57	dpwr2	61
Amplitude:	aX90	3275	aH90	2965	aY90	2850
Pulsewidth:	pwX90	5.5 us	pwH90	2.8 us	pwY90	8 us
Calibrations:	get	set	get	set	get	set

Figure 67 Channels Page and Basic Calibrations

The first column contains the standard name for each channel, see Table 28. The second column contains the standard name of the synthesizer offset. All parameters use channel identifiers (column three), upper-case X, H, Y, Z and W, that correspond to obs, dec, dec2, dec3, and dec4. Column four, the coarse power, is the second parameter. Fine power, associated with the linear modulator, is the third parameter. The fourth parameter (column five) is the standard pulse-width calibration based on the power settings.

Click on the Set button for each channel to save changes to the probe file for the set of basic calibrations for each nucleus to the probe file. The current value of the nucleus is attached to the values in the probe file. If needed, a new nucleus will be added to the probe file. An acknowledgement that the data is saved appears in the Message window.

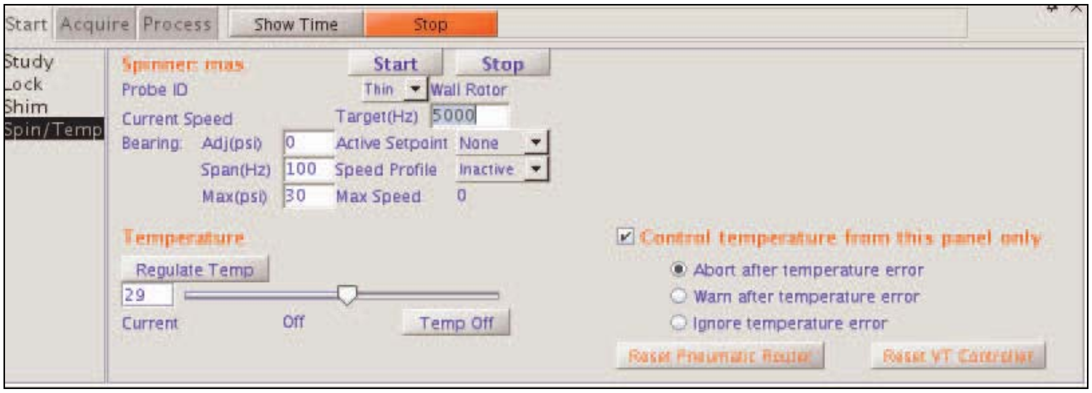
Click on the Get button for each channel to retrieve calibrations from the probe file. An error message appears in the Message Window if calibrations are not present. The current nucleus for the channel is used to choose the calibrations.

Use the Set and Get functions when the observe and decouple channel nuclei are interchanged.

The Settancpx protocol loads basic calibrations from the probe file if the probe file is defined and the nuclei are present in the file. The basic calibrations are the only values stored in the probe file by solids protocols.

13.5.1 Setting the MAS Spin Rate

The MAS spin rate is controlled through the MAS Spin/Temp page of the Start tab, see Figure 68. VnmrJ sends commands to the MAS Automated Speed Controller by way of the console master controller.



Probe ID: Thin Wall Rotor
Current Speed
Target(Hz): 5000
Bearing: Adj(psi): 0
Active Setpoint: None
Span(Hz): 100
Speed Profile: Inactive
Max(psi): 30
Max Speed: 0
29
Current: Off
☑ Control temperature from this panel only
● Abort after temperature error
○ Warn after temperature error
○ Ignore temperature error

Figure 68 Solids Spin/Temp page with: spintype ='mas'

Varian T3 probes provide the name of the module (e.g." 3.2 MM PENCIL") to the MAS automated speed controller so that the appropriate values are set for start-up and regulation. The module name is also sent to VnmrJ and it is displayed on the MAS Spin/Temp page as Probe ID. VnmrJ uses the module name with underscores between words to automatically set the probe parameter.

13.5.2 Loading the Probe File

Do one of the following:

- Select and load the probe file corresponding to the probe in use as follows:
    a. Click on **Probe** on the hardware toolbar to open the Probe pop-up window.
    b. Select a probe from probes shown in the drop down menu.
    c. Click **Close** to load the probe and exit the Probe pop-up window.
- Create the probe file for the probe in use if one does not exist.
    a. Click on **Probe** on the hardware toolbar to open the Probe pop-up window.
    b. Check the **Edit** box.
    c. Enter a name for the probe.
    d. Click the **Make new probe** button.
    e. Click on the **Edit Probe** button to open the Probe Edit pop-up.
    f. Click on the **Probe** tab.
    g. Enter the following information into the probe file:

Probe File Field	Value	Parameter Set
Probespintype	mas	`spintype`
Probespinmax	Maximum rotor speed in Hz	`spinmax`

Press return after typing each entry into the specified field.

The probe name and these entries are required to display the **MAS Spin/Temp** page:

h. Click **OK** to save and close the pop-up.

i. Select any **probe** from the probes shown in the drop down menu.

j. Select the **name** of the solids probe from the dropdown menu.

   The probe name must be explicitly selected after the probe file has been created.

k. Click **Close** to load the probe file and exit the pop-up window.

13.5.3 Starting the Rotor

1. Set the MAS automated speed controller to AUTO mode.
2. Verify that the correct module name is shown on the MAS Spin/Temp page.

   Load (or reload) the correct probe file for the installed probe if the module name is not correct.
3. Select **Thin** or **Std** to select the correct Max Speed.
4. Set the **Target(Hz)** for spinning.
5. Press **Start** on the MAS **Spin/Temp** page.
6. Wait for the speed to regulate.

   The LOCKED light on the controller is on when spinning is in regulation. The current speed is displayed on the MAS **Spin/Temp** page and on the controller.

13.5.4 Changing the Rotor Speed

1. Enter a new **Target(Hz)** value.
2. Press return.
3. Wait for the speed to regulate.

13.5.5 Stopping the Rotor

Press **Stop** to stop the rotor.

13.5.6 Setting the Bearing Pressure

The bearing pressure is set automatically based on the value of **Bearing: Max(psi)** as roughly a linear function of the **Target(Hz)** spin rate.

Make a fine adjustment for the bearing as follows:

1. Set Bearing: **Span = 0**
2. Set the Bearing: Adjust(psi) to the change in pressure, positive or negative.
3. Reset Span = 100 to lock the new bearing pressure in place.

   The new bearing pressure can be read from the display on the MAS Automated Speed Controller.

13.5.7 Starting the Rotor Manually

Start a rotor manually and then put it in regulation as follows:

1. Set the **Target(Hz)** on the MAS **Spin/Temp** page.
2. Set the knobs for the drive and bearing pressure valves on the controller counter clockwise.
3. Press the Auto/Manual button on the controller to put it in manual mode.
4. Obtain stable spinning near the desired speed using the knobs.
5. Press the Auto/Manual button again to put the rotor in regulation.
6. Reset the pressure valves counterclockwise.
7. Stop the rotor as follows:
8. Press **Stop** button on the MAS **Spin/Temp** page.

   It is not possible to return to manual mode while the rotor is spinning.

The **Start** and **Stop** buttons on the MAS Automated Speed Controller have the same function as the **Start** and **Stop** buttons on the **MAS Spin/Temp** page.

13.6 Setting the Temperature

The temperature of the rotor is controlled through the VT stack, which sits in the upper bore of the magnet and must be installed before starting solids VT operations. The VT stack replaces the liquids upper barrel and mates to the top of the probe for wide-bore magnets. The VT stack sits inside the liquids upper barrel in narrow-bore magnets. A heater and thermocouple are present at the exit of the VT stack to provide temperature-controlled gas to the module. Both bearing and drive gas for MAS spinning are maintained at ambient temperature and are separate from the gas used for VT operation. Low temperature cold gas is supplied to the VT stack using a gas cooler, such a cold trap in a liquid nitrogen Dewar or an FTS unit.

13.6.1 Starting Temperature Regulation

A solids/liquids NMR system pneumatics router has separate gas regulation systems for solids and liquids and a common regulator valve for the VT-gas pressure. Solids gas flow is controlled with a manual valve below the flow meter. A typical flow rate is 40 lpm.

Start temperature regulation as follows:

1. Load the Solids Probe file if it is not already loaded.

   The solids meter is selected by loading a solids probe file and entering `su`.

2. Click on the Start tab.

3. Select the Spin/Temp page.

   Solids gas flow is set from the solids **Spin/Temp** page, see Figure 68. A set of LED's are lit on the selected pneumatic router meter.

4. Set the value of the parameter vtc from the command line to a value above the highest expected temperature that requires cooling gas.

   A typical value for vtc is 5 ºC to 10 ºC below the ambient room temperature or the temperature of the VT supply gas.

   The choice of gas cooling or the bypass is determined by the parameter vtc, the variable temperature cutoff. A set temperature below vtc chooses gas cooling and a set temperature above vtc chooses the bypass. Choose a value of vtc in the dataset that corresponds to the desired operation.

   The standard protocol **Settancpx** automatically resets vtc = -30. To change the default value of vtc, save a copy the parameter set, Settancpx.par, with the new value of vtc in ~/vnmrsys/parlib. The new parameter set will be loaded each time the protocol **Settancpx** is loaded by the current user. The change does not affect any of the other users. Make the change available to all users by logging in as the system administrator, vnmr1, and saving Settancpx.par with the new value of vtc in /vnmr/parlib. The system administrator can edit the file
   /vnmr/parlib/Settancpx/procpar
   and change the entry for vtc:
   vtc 1 1 250 -100 0.1 2 1 2 1 64 1 `-30`
   using vi or any ASCII text editor supplied with the operating system to reflect the new cut off temperature by changing the value of entry -30 at the end of the vtc line to a new value such as 15. The new entry reads:
   vtc 1 1 250 -100 0.1 2 1 2 1 64 1 `15`
   The entries for vtc of primary interest are:
   vtc 1 1 [`upper temperature limit`] [`lower temperature limit`] [`precision`] 2 1 2 1 64 1 [`bypass cutoff`]

5. Enter `su` on the command line.

   Press **Pneumatics Reset** button once or twice if the set up (`su`) does not proceed.

   Liquids flow may persists after selection of solids. Do the following if this happens:

   a. Load a liquids probe file.

   b. Press the VT Air Off button on the Spin/Temp panel.

   c. Reload the solids probe file.

6. Move the **Temperature** slider control left or right on the **Spin/Temp** page until the required temperature (ºC) is displayed in the field to the left of the slider or enter the temperature regulation **value**, in ºC, in the field to the left of the slider on the **Spin/Temp** page and press return.
7. Press the **Regulate Temp** button.

   Press the **Reset Pneumatics Router** and/or **Reset VT Controller** buttons before proceeding if the temperature regulation does not set up.
8. Place a check in the box for **Control Temperature from This Panel Only**.
9. Check on an error reporting radio button.

   The radio button choices below **Control Temperature from This Panel Only** are:

   - Abort after temperature error
   - Warn after temperature error
   - Ignore temperature error

   The parameter temp is a passive indicator of the temperature set in the panel when the **Control Temperature from This Panel Only** check box is checked. The current value of the parameter temp is used to set the temperature at su or at the start of acquisition if the **Control Temperature from This Panel Only** is not checked.

13.6.2 Stopping Temperature Regulation

Press the **Temp Off** button.

1. Reselect a liquids probe and press the VT Air **Off** button on the **Spin/Temp** panel.
2. Turn **Off** the solids gas-flow valve when selecting liquids.

13.6.3 Recovering from a VT Fault

The solids VT stack is equipped with a high temperature bypass that bypasses the installed gas cooler supplying ambient temperature gas to the stack. Narrow bore VT stacks have a gas-flow sensor, a probe-stack integrity sensor, and a bore-temperature sensor that may fault under a variety of error conditions. The temperature controller is disabled and the gas flow is switched to bypass if a fault condition detected. A small amount purge gas is vented through the gas cooler to keep it from freezing up.

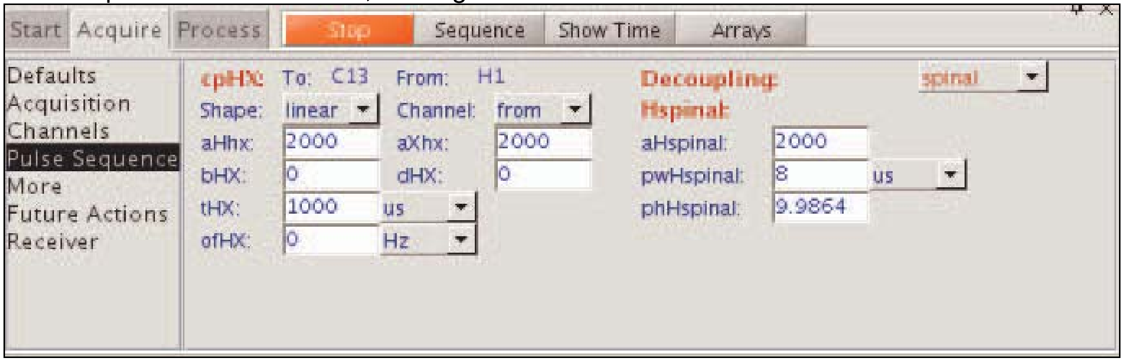
1. Correct the problem causing the fault condition.
2. Press the Reset Pneumatics Router and Reset VT Controller buttons.
3. Reset the temperature to resume operation.

13.7 Setting up Single-Pulse Experiments

Single-pulse excitation is run using the **Onepul** protocol. The protocol uses the calibrated 90-degree pulse pwX90 from the Channels page of the Acquire tab and provides acquisition with a standard Decoupling parameter group, see Setting Up Decoupling. Decoupling parameters are located on the Pulse Sequence page.

13.8 Setting up Cross Polarization

Cross polarization is run using the **Tancpx** protocol. The protocol uses the **cpHX** parameter group for constant, linear, or tangent-ramped cross polarization. The **cpHX** parameter group is located on the Pulse Sequence page of the Acquire tab and it used in any pulse sequence where cross polarization is needed, see Figure 69.



cpHX: To: C13 From: H1
Shape: linear
Channel: from
aHhx: 2000
aXhx: 2000
bHX: 0
dHX: 0
tHX: 1000 us
ofHX: 0 Hz
Decoupling: spinal
Hspinal:
aHspinal: 2000
pwHspinal: 8 us
phHspinal: 9.9864

Figure 69 Tancpx Pulse Sequence page of the Acquire tab

Calibrate the probe, see Calibration of $^{\text{13C-1H}}$ CPMAS Probes.

Tancpx provides excitation with the basic calibrated 90-degree pulse pwH90 from the Channels page and acquisition with standard Decoupling parameter group located on the Pulse Sequence page, see Setting Up Decoupling.

The **cpHX** parameter group and upper-case channel identifiers specify the channels involved in cross polarization. The first character is the channel from which the starting polarization is derived and the second is the channel that is polarized. The **cpHX** parameter group used with **Tancpx** indicates cross polarization from the dec channel to the obs channel.

The Pulse Sequence page of the Acquire tab for **Tancpx**, see Figure 69, contains two parameter groups: **cpHX** for cross polarization and **Decoupling** for proton decoupling. These parameter groups are used in all sequences that contain cross polarization and proton decoupling functions.

Menu or Parameter	**Description**
Shape menu	Values are: const, linear, or tan. Pulse shape used on one of the two channels for the `cpHX` parameter group.
Channel menu	Values are: from — channel used to apply the shape to the decoupler channel. to — channel used to apply the shape to the observe channel.
`aHhx` parameter	The median amplitude of the decoupler channel and `aXhx` is the median amplitude of the observe channel.
`dHX` parameter	The +/- amplitude excursion of a linear or tangent ramp about the median value and has no effect on constant CP.
`bHX` parameter	Curvature factor for tangent ramps only. bHX must be greater than 0 (zero). bHX > 10000.0 applies a linear ramp. bHX < 1.0 applies a tangent ramp that is nearly constant with extreme excursions at the beginning and end. Values in between provide tangents of various curvatures.

`tHX` parameter	Contact time.
`ofHX` parameter	Frequency offset applied to the shaped channel as designated by the Channel menu choice.

The CP parameter group generates it's offset with a phase ramp and the offset adds or subtracts from the value of the synthesizer offset (tof or dof) for the channel.

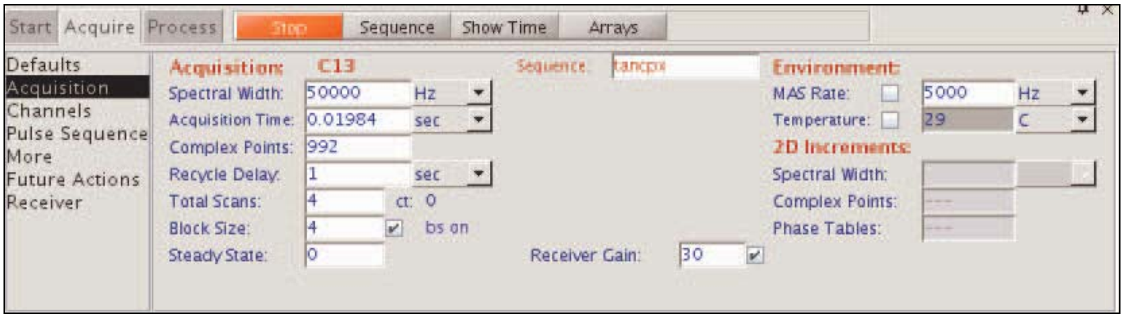
13.9 Setting Up Decoupling

Decoupling during acquisition is carried out in nearly all pulse sequences with the **Decoupling** parameter group on the Pulse Sequence page of the Acquire tab, see Figure 81. Select from the choices: **TPPM**, **SPINAL64**, **CW decoupling** or **decoupler off**.

Parameter or Group	**Description**	
Decoupling parameter	Menu choices TPPM or SPINAL determine which of the **Htppm** or **Hspinal** parameter groups is displayed.	
TPPM	aHtppm	Decoupling amplitude.
	pwHtppm	Width in microseconds of each of the two pulses (usually just less than a 180-degree pulse).
	phHtppm	The +/- phase of each of the two pulses. The sign of the phase is that of the first pulse.
SPINAL	aHspinal	Decoupling amplitude
	pwHspinal	Width in microseconds of each of the two pulses. (usually just less than a 180-degree pulse).
	phHspinal	The +/- phase of each of the two pulses of the base cycle. The sign of the phase is that of the first pulse.
	SPINAL	Applies +/- pulse pairs with a phase of +/-1.0, +/-1.5 and +/-2.0 times phHspinal in a 64-member supercycle.
CW decoupling	**On** with either the TPPM or the SPINAL parameter groups when phHtppm or phHspinal is 0.0. **Off** either the TPPM or the SPINAL parameter groups when aHtppm or aHspinal is 0.0. The decoupling module explicitly associates a decoff() command with an amplitude of 0.0 to gate the decoupler fully off. The typical application with an amplitude of 0.0 only sets the linear modulator to full attenuation.	

13.10 Acquisition Parameters and Other Pages

The Acquisition page of the Acquire tab provides a customized display of the standard 1D Acquisition parameters and the Receiver gain, see Figure 70.



Acquisition: C13

Spectral Width: 50000 Hz

Acquisition Time: 0.01984 sec

Complex Points: 992

Recycle Delay: 1 sec

Total Scans: 4 ct: 0

Block Size: 4 ☑ bs on

Steady State: 0

Sequence: tancpx

Receiver Gain: 30 ☑

Environment:

MAS Rate: ☐ 5000 Hz

Temperature: ☐ 29 C

2D Increments:

Spectral Width:

Complex Points: ---

Phase Tables: ---

Figure 70 Acquisition Parameters - Solids

Field	Parameter	Description and Settings
Spectral Width	`sw`	Spectral width, menu select units: Hz, kHz, MHz, or ppm
Acquisition Time	`at`	Data acquisition time, menu selections: sec, ms, or µs.
Complex Points	`np`	Total number of points collected during the acquisition time.
Recycle Delay	`d1`	First delay in the sequence, menu selections: sec, ms, or µs.
Total Scans	`nt`	Total number of transients collected.
Block Size	`bs`	Enter number of scans for block size and place a check in the check box to enable. Remove the check from the box to set bs='n', not used, and the block to Total Scans, nt.
Steady State	`ss`	Number of dummy scans run before starting data acquisition
Receiver Gain	`gain`	Gain of the receiver. Set the value from 0 to 30 in the field. Place a check in the box to turn off the autogain function. Do not use autogain with solids
MAS Rate	`srate`	Rotor spin rate, menu select units: Hz, kHz, MHz, or ppm. srate, identifies the spin rate for automatic calculation of synchronized delays in some sequences. The srate parameter is not the same as the set point for the MAS Automated Speed Controller on the Spin/Temp page and must be set manually to a value equal to the spin rate. A check box next to srate sets the parameter hsrotor. Checking this sets the value of srate automatically from the measured spin rate at run time and cannot be used to track the spinning rate while the sequence is running.
Temperature	`temp`	Temperature regulation point, select C (centigrade) or K (Kelvin)
Other		2D parameters that are specific to a sequence. The fields are grayed out if the field is not defined. Use par2d if the 2D parameters are required.

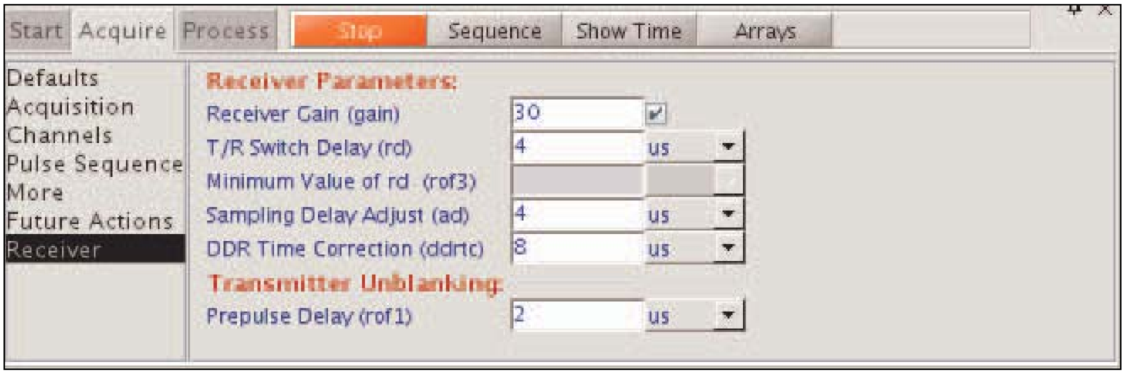
13.10.1 Default and More Pages

The Defaults page of the Acquire tab is empty. The More page is used in some sequences to provide space for additional parameter groups that do not fit on the Pulse Sequence page. Content of these pages is specific to the pulse sequence.

13.10.2 Other Pages and Tabs

The Acquisition, Channels, and Receiver pages that are set up when the **Settancpx** protocol is loaded have content that is specific to solids sequences. The Future Actions page, Start tab, and Process tab are common to all liquids and solids protocols.

13.11 Receiver Parameters for Solids



Receiver Parameters:

Receiver Gain (gain) 30 ☑

T/R Switch Delay (rd) 4 us

Minimum Value of rd (rof3)

Sampling Delay Adjust (ad) 4 us

DDR Time Correction (ddrtc) 8 us

Transmitter Unblanking:

Prepulse Delay (rof1) 2 us

Figure 71 Receiver Page of the Acquire tab, Solids

The Receiver page of the Acquire tab contains parameters that affect the receiver gain, the dead time and phase correction, see Figure 71. The dead time is the time after the pulse or cross polarization before acquisition of the first data point.

All solids pulse sequences make use of explicit code for acquisition and avoid using rof2 and alfa. The parameter ddrtc controls the digital receiver time correction and is explicitly defined in solids data sets. Solids protocols do not automatically set ddrtc. The user must set it manually as described below.

Field name	**Parameter**	**Values / Description**
Receiver Gain (gain)	`gain`	Values between 0 and 60, default is 30. Gain can be as low as 0 for very strong signals as for proton experiments. A gain greater than 30 is usually of no benefit.
T/R Switch Delay (rd)	`rd`	Values range from 2.0 and 4.0 μs for ^{1}H or ^{13}C to many hundreds of microseconds for low-gamma nuclei with acoustic ringing. The application of rd in solids sequences is analogous to that of the rof2 in other sequences. Set rd to avoid probe ring-down in the first few points of the FID. The delay follows the last pulse or the cross polarization and precedes opening the T/R switch.
Minimum Value of rd (rof3)	`rd rof3,if defined`	Controls the time between the opening of the T/R switch and the receiver turn-on. The value of rof3 can be set greater or less than 2.0 μs. The default time is 2.0 μs if rof3 is not defined.

		Multi-pulse sequences explicitly set the value of rof3 so that it can be set less than 2.0 μs. The value associated with rof3 for multi-pulse sequences such as BR24 is part of the parameter group of the multi-pulse waveform and is labeled with the prefix r3. Always set rof3 = r3 when running multiple-pulse sequences.
Sampling Delay Adjust (ad)	**`ad`**	Values between 4.0 and 10 μs. The application of ad in solids sequences is analogous to that of alfa in other sequences. Typically the T/R switch (if used) is opened at the beginning of the delay and the receiver is turned on 2.0 μs later. In this case ad cannot be less than 2.0 μs. The first point is acquired at the end of the delay.
DDR Time Correction (ddrtc)	**`ddrtc`**	Controls automatic back-prediction of the FID in the digital receiver. The first-order phase correction lp will be approximately 0.0 if ddrtc = rd + ad. Fine tune ddrtc to obtain lp = 0.0. The parameter ddrtc corrects the phase for all spectral widths of 1.25 MHz and less. A correct setting of ddrtc is very effective for automatic phasing, but it does not necessarily produce a flat baseline. One can expect a flat baseline if the value of ddrtc < 1/(2.0*sw) (a usual situation for CPMAS but not for larger spectral widths) and if the true first point of the FID is not distorted.

First-point distortion of the baseline resulting from time correction has characteristic hooks at the outer edges. The spectrum appears to sit in a trough or on a pedestal. Ringing in the back-prediction calculation by bad first points in the FID is the typical cause. The ringing may extend well beyond the first points so this problem cannot be corrected with linear prediction.

Fix the first point distortion by increasing rd until the first point is good. Estimate the first point distortion and set rd by observing the FID with sw = 5.0e6. Choose the new value of rd and then obtain the data with the original spectral width.

Mitigate the effect of a first-point distortion by decreasing the slope of the digital-filter cut-off using the parameter ddrcr as follows:

1. Create the parameter ddrcr (if it does not already exist) by entering the following on the command line:

   ```
   create('ddrcr')
   ```

2. Set ddrcr to a value in the range of 5 to 10.

   The default value (if the parameter does not exist) is 75 and produces steep filters. A lower number decreases the slope of the filters and mitigates the ringing created by bad first points.

   Linear prediction can readily correct the baseline with a value of:
   `ddrtc >1/(2.0*sw)`
   and a good first point.

The prepulse delay, rof1, used in liquids sequences to unblank the transmitter and to preset the phase of pulses, is displayed but it is not generally used in solids sequences. Solids sequences explicitly preset the phase of pulses at the beginning of the previous delay and the transmitters are continuously unblanked making the pre-pulse delay unnecessary. A pre-pulse delay for multi-pulse sequences, such as BR24, is part of the parameter group of the multi-pulse waveform and is prefixed r1.

13.12 Tuning a Solids Probe

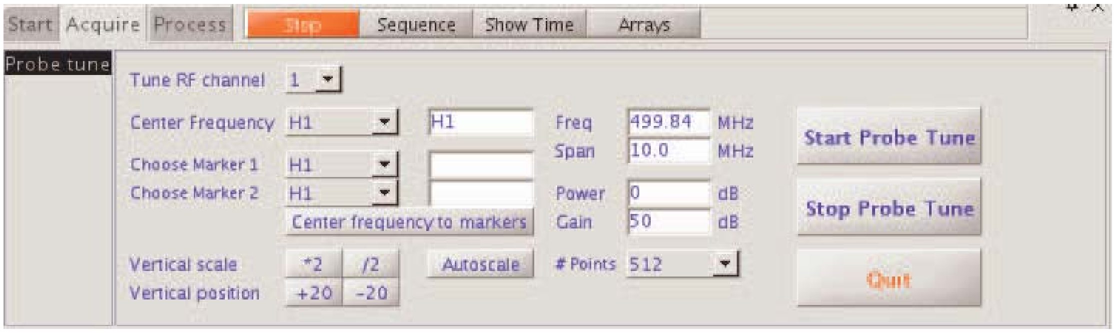
Solids experiments require frequent probe tuning, usually for each new rotor. Varian NMR Systems provide in-line 30-db directional couplers to tap off the reflected power of each channel. A set of reversible 50-db directional couplers are available as an option for solids systems. Probe tuning is carried out with the standard **Tune Sweep** function of the Probe pop-up window or with the Tunerp protocol associated with the group of solids sequences.

13.12.1 Low Power or In-Line Tuning Using Tune Sweep

Tune the probe using the in-line tune (or low power) method as follows:

1. Install the 30-db couplers correctly with the arrow pointing **towards** the **Front End**. Be sure that the arrows on the reversible 50-db couplers point towards the **Front End**, not the **Probe**.
2. Click on the Probe button.
3. Click on the Tune Sweep button in the probe pop-up window.

   The Probe tune page replaces the pages in all of the tabs, see Figure 72.
4. The Tune RF channel menu displays the hardware channel to be tuned. The default nuclei selections are: **1H** for channel 1, **13C** for channel 2, and channels 3 and 4 undefined. The nuclei and frequencies that appear on the Probe tune page are unrelated to those in the Workspace.
5. Select the **Channel** to be tuned.



Tune RF channel 1
Center Frequency H1 H1
Choose Marker 1 H1
Choose Marker 2 H1
Freq 499.84 MHz
Span 10.0 MHz
Power 0 dB
Gain 50 dB
Points 512

Figure 72 Tune Panel - Solids

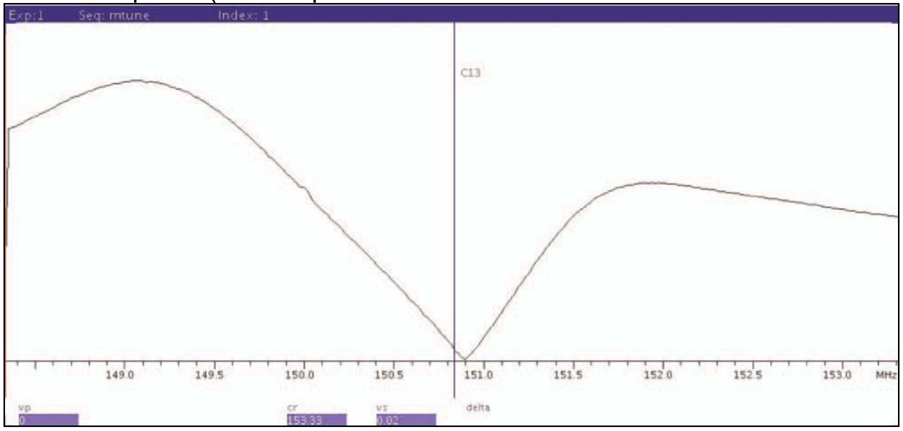
6. Select **Center Frequency** by entering the nucleus from the menu or enter the frequency in the field next to **Freq**.
7. Choose a value (between 1MHz and 10 MHz) in the field next to **Span**.
8. Click the **Start Probe Tune** button.
9. Press **Autoscale** to obtain a useful display.
10. Increase the power from 0 to 10 db if the signal-to-noise of the pattern is low.

    The tune pulse sequence does not accept powers above 10 db to protect the probe.
11. Decrease the gain if the pattern clips.
12. Adjust the **Vertical** scale and the **Vertical** position of the pattern with the buttons or with the center mouse button.
13. Adjust the **Span** to the region of the tuning dip and adjust the vertical scale as needed.

The large sinusoidal changes of the tuning baseline are expected for tuning in-line with a directional coupler. These sinusoidal changes are the result of standing waves on the input cables between the preamplifier and the probe caused by the effectively infinite impedance of the probe outside the region of the tuning dip.

Some systems have channels with high-power low-band preamplifiers. These preamplifiers use passive diodes that cut off the input tune signal if it falls to too low a value and in some frequency ranges the pattern has no amplitude. Increase the gain to 10 on the channel if this occurs. The gain may still be too low to observe a tune dip. Bypass the preamplifier by connecting the cable on the XMTR port of the preamp to the cable attached to the PROBE port if increasing the gain is not enough. The amplitude of the pattern is affected by the presence of filters in-line with the input cables.

14. Tune the probe (see the probe manual to determine the correct knobs on the probe).



Exp:1 Seq: mtune Index: 1

vp 0

cr 153.33

vs 0.02

Figure 73 Tuning Pattern, Solids

The tuning pattern, see Figure 73, is a dip of the reflected power near zero when the probe is tuned. The width of the dip approximates the bandwidth of the probe.

15. Press Stop Probe Tuning to halt tuning.
16. Press Quit to return to the data in the Workspace.

13.12.2 High-power tuning

See High Power Pulse Tuning (Tunerp) for tuning with pulses using the Protocol **Tunerp** and the reversible 50-db directional couplers.

13.13 Shimming and Referencing for Solids

13.13.1 Shimming

The field homogeneity for a MAS probe is usually optimized with a sample of adamatane. Follow the procedure in Shimming with Adamantane.

13.13.2 Referencing for Solids

Referencing for Solids is complicated by the fact that a solids probe has no lock channel and there is no lock solvent. As a result the lock frequency is not necessarily on resonance. VnmrJ automatic referencing uses the setref command, the file /vnmr/nuctables/nuctabref, and is dependant upon the lock being on resonance.

Set the lock frequency on resonance as follows:

1. Recall a referenced ^{13}C spectrum of adamantane.
2. Click on the Start tab.
3. Select the Lock page.
4. Click on the Lock OFF radio button
5. Enter 0.0 in the field next to the Z0 button if the value in the field it is no 0.0.
6. Insert a rotor containing adamatane and install the probe.
7. Shim using the adamantane signal.
8. Acquire a spectrum and reference the higher ppm line to 38.3 ppm.
9. Put a cursor on the line and type calclockfreq(38.3p).
10. Note the value of lockfreq in the message window.
11. The value of lockfreq is set to at least the fifth place after the decimal point.
12. Acquire a spectrum and reference the higher ppm line to 38.3 ppm (see Figure 74).

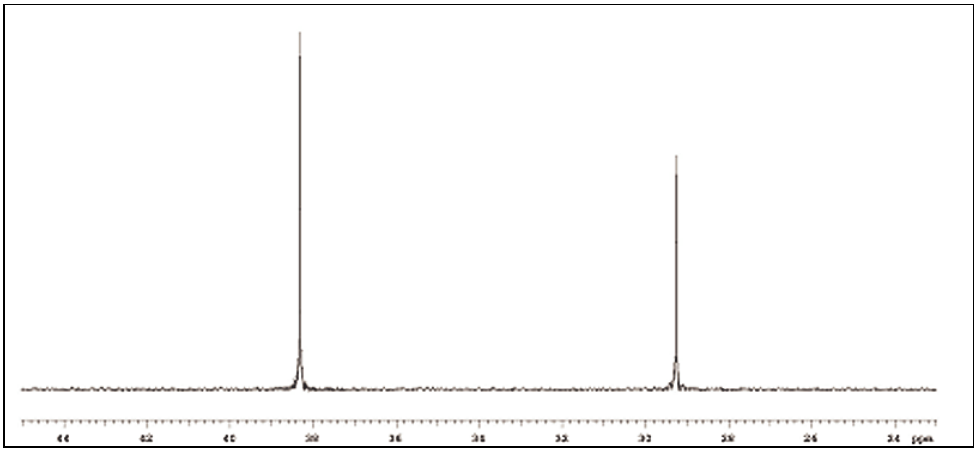


Figure 74 Adamantane Reference Spectrum

13. Reacquire the spectrum and type setref.
14. Set the correct value for lockfreq into the configuration parameters in the vnmr1 user. The value of lockfreq is lost when logging out of the user if it is not saved.

The correct value of the lock frequency depends upon the choice of the lock solvent. Choose a constant value of solvent (for example CHCL3) to be used for solids data. Referencing remains correct until the magnet has drifted outside the solids lineshape, usually after a couple of days.

The reference display of VnmrJ does not correct itself if the spectral width sw or the offset tof is reset. Re-establish the reference based on the lock with setref after changing these parameters.

13.14 Using probeConnect with Solids

The transmitter associated with each pulse-sequence channel is displayed to the right of the channel name in the **Channels** page of the **Acquire** tab, see Figure 67.

A two-channel Varian NMR System is always configured with channel 1 as the high-band transmitter and channel 2 as the low-band transmitter if the sfrq > 0.85 times the proton frequency. Otherwise, the transmitters are reversed. The system always sets the correct band based on the nucleus.

Varian NMR System with more than two channels and a pulse sequence that uses more than two channels must use the parameter probeConnect. The probeConnect parameter does not need definition if only channels 1 and 2 are used. Channels 3 and 4 can be configured as either high-band or low-band. Entry boxes for **probeConnect** and **preAmpConfig** are on the top line of the **Channels** page (Figure 67). The value of **probeConnect** is initially undefined, as indicated by dashes in the box.

13.14.1 Creating the probeConnect Parameter

1. Verify the existence of the probeConnect parameter as follows:
   a. Enter `probeConnect` on the command line.
   b. Continue with Step 2 if the message in the message box is:

      Variable “probeConnect” doesn’t exist

   c. Skip to Step 3 if there is no message reporting that the variable does not exist.
2. Entering the following on the command line:

```
create('probeConnect','string','global')
```

3. Enter the nucleus associated with each channel in order, channel 1, channel 2, etc. and separate each entry with a space.

   The entries in the **probeConnect** field identify the nuclei that are associated with each hardware channel. Change the entries in the **probeConnect** field to correspond to a new probe-tuning configuration.

13.14.2 Using probeConnect During the Decoupler Calibration Process

1. Set the decoupler nuclei one by one in the Nucleus: field of the Observe "X" section of the Channels page of the Acquire tab and determine the basic calibrations using the Onepul experiment.
2. Click the Set button to enter the calibration into the probe file.
3. Return each nucleus to its proper decoupler channel when the calibrations are complete.
4. Click the Get button to reload the decoupler calibrations.

13.15 Using preAmpConfig with Solids

The value of preAmpConfig indicates which type of preamplifier, high-band or low-band, is associated with each hardware channel. The default is preAmpConfig = 'hlnnn' where 'h' means high-band, 'l' means low-band and 'n' means no preamplifier. The characters of preAmpConfig are the hardware channels. The third to fifth characters of preAmpConfig should be changed to their correct values if preamplifiers are present on channels 3 to 5.

13.16 Using hipwrampenable for Solids

A Varian NMR System with high-power (kilowatt) amplifiers contains relay boards to select high power for solids or low power for liquids. The default selection for the relay board is low power by default unless the parameter hipwrampenable is created. High power is defined as the specified amplifier output (usually a kilowatt, but a bit less for high-band amps at 600 MHz and above). Low power is defined as 50 Watts for high-band and 300 Watts for low band. High power NMR Systems use the high-band driver to provide the 50 Watts and they attenuate input of the low-band kilowatt amp to provide the 300 Watts.

1. Verify the existence of the hipwrampenable parameter as follows:
    a. Enter **`hipwrampenable`** on the command line.
    b. Continue with Step 2 if the message in the message box is:

       Variable "hipwrampenable" doesn't exist
    c. Skip Step 2 if there is no message reporting that the variable does not exist.
2. Entering the following on the command line:

```
create('hipwrampenable','string','global')
```

The parameter hipwrampenable is a global parameter and applies to all data sets in a user and can only be accessed from the command line.

`'y'` enables high power or the RF channel.

`'n'` enables low power and disables high power for the RF channel.

A character is required for each hardware channel up to the total number of channels numrfch. The value of numrfch is 3 for system with three RF channels and high power on channel 1 and low power on the remaining channels is specified as:

```
hipwrampenable='ynn'
```

13.17 Using Amplifier Blanking and Unblanking for Solids

All solids sequences are written to maintain amplifiers on all channels as unblanked throughout the pulse sequence, except during acquisition. This convention is different from the convention used by the Varian NMR System for liquids sequences (and older solids sequences). Continuous unblanking maintains each amplifier temperature on or hot to provide greater amplifier stability.

Solids sequences execute the unblank command on the obs channel before the recycle delay d1 and the sequence ends with an unblank after the acquisition. The same pattern of blanking and unblanking is repeated for the decouplers. Liquids sequences (and older solids sequences) execute the unblank on the obs channel before the first pulse or turn it on and off before and after the pulses. The decouplers have no blank and unblank commands and they use the decoupler amplifiers in continuous mode.

Solids sequences provide the option to blank decouplers during acquisition. The decoupler amplifiers must be set to pulsed mode by setting the decoupler channel ampmode to 'p' instead of 'd'. All solids data sets contain the ampmode parameter for this purpose. Blanking dec2 or dec3 during acquisition when using a 3-channel HXY probe delivers better performance.

Very high stability also requires that amplifiers remain on between experiments. Consult the *VnmrJ Installation and Administration* user guide to set solids mode. The solids mode has no effect on the state of the amplifiers when the sequence is running.

13.18 Calibration of ^{13}C-^{1}H CPMAS Probes

Use the following calibration procedure for ^{13}C-^{1}H CPMAS probes and VnmrJ 30C released sequences. Use the signal to noise test to verify the calibrations and as a routine system performance check.

These procedures use the Onepul and Tancpx protocols and related sequences.

13.18.1 Sample Preparation

Pack 2 rotors as follows:

- 50% (by weight or volume) adamantane and potassium bromide (Adamatane/KBr).
- Full rotor of hexamethylbenzene (HMB).

13.18.2 Setting Up the Workspace for Calibration

Do one of the following:

- A calibration data set does not exist:
  a. Select the protocol **Settancpx** from the Basic ID Experiments directory of the Experiment Panel pull-down menu.
  b. Set `tn = 'C13'` and `dn = 'H1'`.

     The **Settancpx** protocol loads any existing calibrations from the probe file. Do not use the calibrations that are shown unless they are know to be correct.
- A calibration data set does not exit:
  Load a previous ^{13}C-^{1}H spectrum or parameter set for the particular solids probe configuration to be used, if one is available.

13.18.3 Tuning the Probe

1. Verify that all cables are correctly connected to the probe.
2. Place the adamantane/KBr rotor in the module.
3. Raise the probe into the magnet.
4. Spin the sample at 5.0 kHz.
5. Press the Probe button on the lower bar.
6. Click on Tune Sweep in Probe pop-up window.
7. Tune Sweep starts the mtune program and loads the Tune Panels.
8. Set Channel 1 to H1 using the dropdown menu.
9. Click Start Probe Tune.
10. Follow the procedures in Tuning a Solids Probe.
11. Set Channel 2 to C13 using the dropdown menu.
12. Click Start Probe Tune.
13. Follow the procedures in Tuning a Solids Probe.
14. Repeat the tuning of channels 1 and 2 iteratively until the optimal tuning is obtained.
15. Press Quit Probe Tune to return the workspace.

13.18.4 Calibrating the ^{1}H pw90

1. Load the **Onepul protocol** from the Experiment Selector or select it from the Experiment menu on the main menu bar.
2. Click the **Acquire** tab.
3. Click the **Acquisition** page.
4. Type the following values in the associated fields:

Field	**Value**
Spectral Width	`1.0e5 Hz`
Acquisition Time	`0.003 s`
Recycle Time	`5.0 s`
Gain	`0.0`
Scans	`1`

5. Click the **Channels** page.
6. Enter `H1` in the **Nucleus:** field for the **Observe "X"** channel.
7. Enter `C13` in the **Nucleus:** field for the **Decouple "H"** channel.
8. Remove any entries in the **Nucleus:** fields for the **Decouple2 "Y"** and **Decouple3 "Z" channels** if these fields are present.
9. Set the starting calibrations if necessary.

⚠ CAUTION	Excessive power can damage to the probe and amplifiers. Verify the type and placement of installed attenuators on the system before setting tpwr>54. Some system managers choose to use attenuators on the input of the transmitter to allow a tpwr setting higher than 54 with a high power amplifier.

- Low-power system (100 Watts ^{1}H)

Field	**Value**
tpwr	`63`
aX90	`2000`
pwX90	`4.0 µs.`

- High-power system (nominally 1000 Watts ^{1}H)

Field	**Value**
tpwr	`54`

aX90	`2000`
pwX90	`4.0 µs.`

10. Click the **Acquire** tab.
11. Select the Pulse Sequence page.
12. Select the TPPM decoupling option.
13. Set aHtppm = 0.0 to turn off the decoupler.
14. Set phHtppm = 0.0 to set CW decoupling for future tests.
15. Click on the Acquire button to start the experiment
16. Put a cursor on the center peak and type movetof on the command line to move the transmitter on resonance.
17. Array pwX90 from 1.0 to 25.0 µs in steps of 1.0 µs.
18. Click on the **Acquire** button to obtain the array.

    The data will look similar to Figure 75.

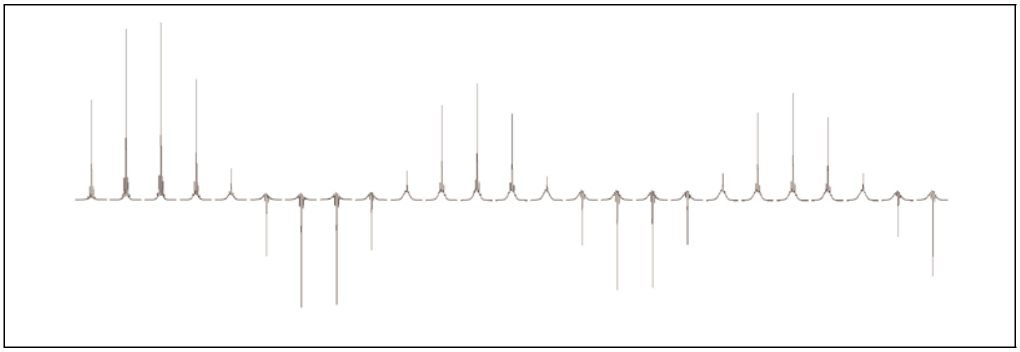


Figure 75 CPMAS pwX90 1H Calibration Array

19. Estimate the 90-degree pulse as one-half the 360-degree minus the 180-degree pulse. Ignore the broad line. It is probe 1H background.
20. Adjust the value of **aX90** to obtain a 90-degree 1H pulse **pwX90** equal to the 1H target pulse width or pulse specification.
21. Reduce **tpwr** and increase **aX90** as required to place **aX90** in the range of **3500** to **4095**. Lowering **tpwr** by 6 db increase **aX90** by 2 fold.
22. Save the data and record the values.
23. The value of aX90 serves as the maximum value for **aHtppm** or **aHspinal**.
24. Readjust the value of **aX90** to obtain a 1H 90-degree 1H pulse pwX90 equal to the 13C pulse specification, see Figure 76.
25. Save the data and record the values.

    Use this ^{1}H amplitude for cross polarization as **aHhx** in the **cpHX** parameter group.

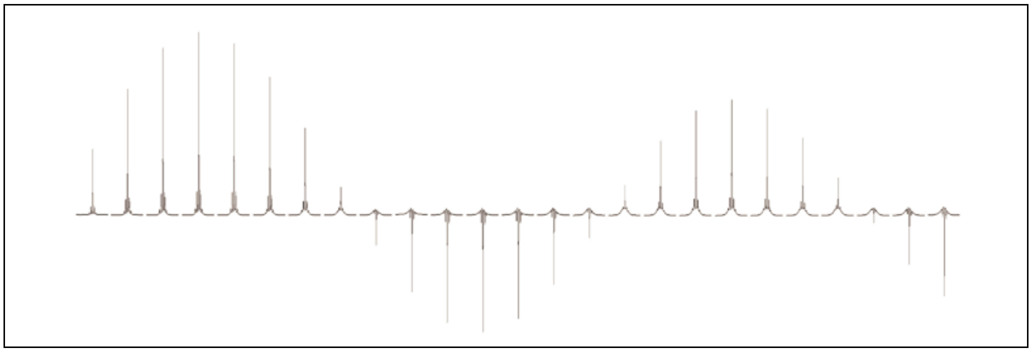


Figure 76 CPMAS pwX90 for 4.0 /s 1H 90-degree pulse

CAUTION	Damage to the probe can result if this field strength is exceeded. This field strength is the maximum that should be used in cross polarization. Calibrate a cross-polarization amplitude lower than the specification as required by the experiment or general operating practice. A ^{1}H pulse width of 4.0 to 5.0 μs works for most experiments and the lower power reduce wear and tear on the probe.

26. Enter these second ^{1}H basic calibrations on the Channels page of the Acquire tab.
27. Press the **Set** button for **obs** and **dec** on the Channels page to save the ^{1}H calibration and the existing ^{13}C numbers in the probe file.

13.18.5 Calibrating the ^{13}C pw90

Continue in the same workspace using the Onepul protocol and maintain the Adamantane/KBr sample at 5.0 kHz.

1. Click on the on the Acquire tab.
2. Select the Acquisition page.
3. Set the following:

Field	**Value**
Spectral Width	`5.0e4 Hz`
Acquisition Time	`0.02 s`
Recycle Time	`5.0 s`
Gain	`30`
Scans	`1`

4. Click on the **Channels** page.
5. Enter **C13** in the **Nucleus**: field for the **Observe "X"** channel.
6. Enter **H1** in the **Nucleus**: field for the **Decouple "H"** channel.
7. Press the **Get** button for **Observe**: and **Decouple**: to retrieve the most recent 1H and 13C calibrations from the probe file under the correct nucleus settings.

8. Set the starting calibrations for 13C if necessary.

⚠ CAUTION	Excessive power can damage to the probe. Verify the type and placement of installed attenuators on the system before setting tpwr>54. Some system managers choose to use attenuators on the input of the transmitter to allow a tpwr setting higher than 54 with a high power amplifier.

- Low-power system (300 Watts ^{13}C)

Field	Value
tpwr	`63`
aX90	`2000`
pwX90	`4.0 µs.`

- High-power system (nominally 1000 Watts ^{1}H)

Field	Value
tpwr	`54`
aX90	`2000`
safety limit -ON	`54`

9. Click on the Acquire tab.
10. Select the Pulse Sequence page.
11. Select the TPPM decoupling option.
12. Set aHtppm = aH90 value and use the aH90 from the Channels page.
13. Set phHtppm = 0.0 for CW decoupling.
14. Enter lb = 50 to set the line broadening to 50 Hz.
15. Click on the Acquire button.
16. Place the transmitter between the two peaks of Adamantane.
17. Put one cursor on each peak.
18. Enter split movetof on the command line.
19. Reduce the Spectral Width to 5 to 10 kHz (600 MHz and above).
20. Array pwX90 from 1.0 to 25.0 µs in steps of 1.0 µs.
21. Click on Acquire button to acquire the arrayed data.
22. The result is similar to Figure 77.

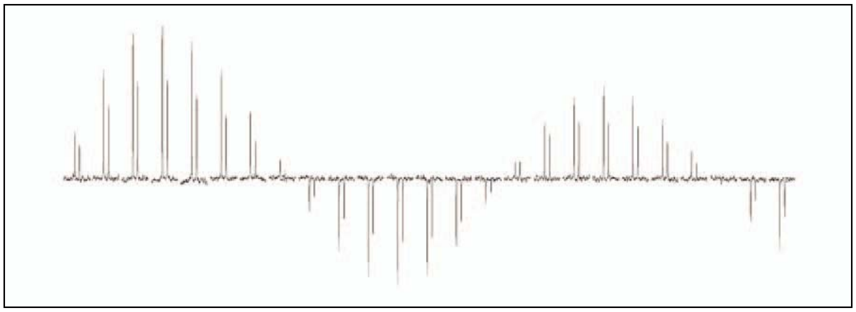


Figure 77 CPMAS pwX90 for 4.0 ⁄s 13C 90-degree pulse

23. Adjust the value of aX90 to obtain a 90-degree 13C pulse pwX90 equal to the 1H values. The values may be either the 13C specification or a lower value as determined for the second 1H calibration.
24. Reduce tpwr and increase aX90 as required to place aX90 in the range of 3500 to 4095. Lowering tpwr by 6 db increase aX90 by 2 fold.
25. Save the data and record the values.
26. The value of aX90 is used for cross polarization as aXhx in the cpHX group.
27. Enter the 13C basic calibrations on the Channels page.
28. Press the **Set** button for **Observe:** and **Decouple:** on the Channels page to save the ^{13}C and ^{1}H calibrations in the probe file.

13.18.6 Magic-Angle Adjust with KBr

Continue in the same workspace using the Onepul protocol and the Adamantane/KBr sample at 5.0 kHz.

1. Click on the **Acquire** tab.
2. Select the Acquisition page
3. Set the following:

Field	**Value**
Spectral Width	`1e5 Hz`
Acquisition Time	`0.02 s`
Recycle Delay	`0.2 s`
Gain	`30`
Scans	`16`

4. Select on the **Channels** page.
5. Enter **Br79** in **Nucleus**: field for the **Observe "X"** channel and be sure the following transmitter number is **2**.
6. Do not readjust probe tuning for 79Br.
7. Click on the **Pulse Sequence** page.
8. Select the **TPPM** decoupling option.

9. Set **aHtppm = 0** to turn off decoupling.
10. Click on the **Acquire** button.
11. Place the cursor on the tall center band.
12. Type `movetof` command.
13. Click on the **Acquire** button.

    The result is similar to Figure 78.

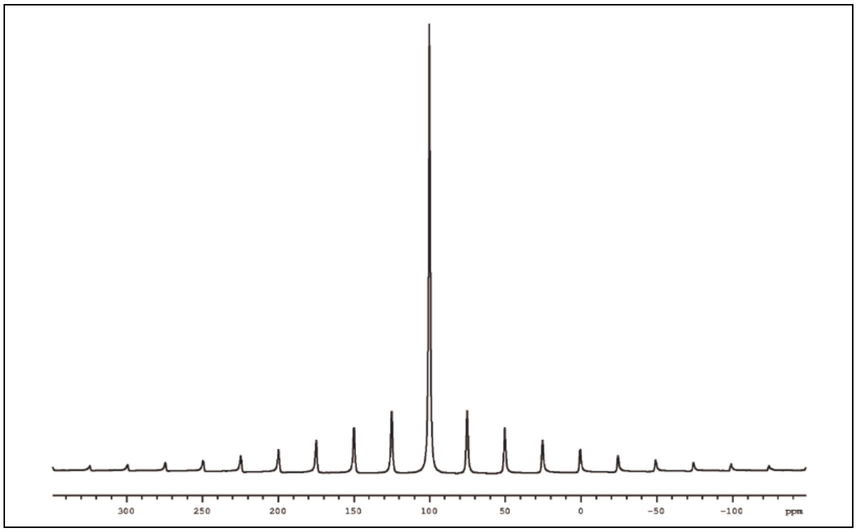


Figure 78 Spectrum of KBr to Optimize the Magic Angle

14. Enter the **Interactive** mode with the **Fidscan** button on the **Shims** page of the **Start** tab.
15. Adjust the magic-angle adjust-knob or screwdriver-adjust on the bottom of the probe to maximize the number of spikes (rotational echoes).

    The result is similar to Figure 79A when the rotor angle is adjusted match the magic angle. The rotational echoes have the same T2 as the central line and the magic angle is optimal.

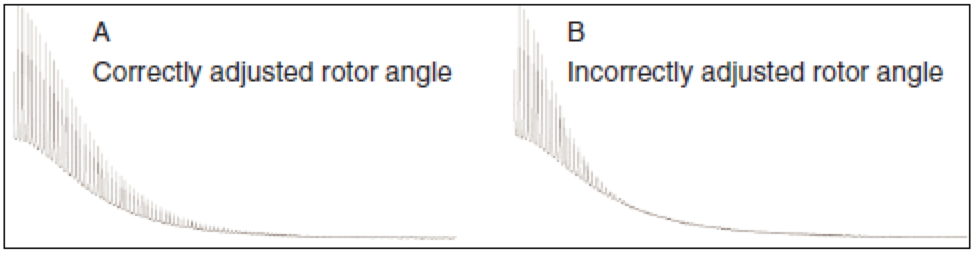


Figure 79 KBr FID for Magic Angle Adjustment

    Improperly set rotor angle produces echoes that decay more quickly and the result is similar to Figure 79B.

16. Save the data.
17. Continue with Shimming with Adamantane.

13.18.7 Shimming with Adamantane

Continue in the same workspace using the Onepul protocol and the Adamantane/KBr sample at 5.0 kHz.

1. Click on the **Acquire** tab.
2. Select the Acquisition page.
3. Set the following parameters:

Field	**Value**
Spectral Width	`5e3 Hz`
Acquisition Time	`0.1 s`
Recycle Delay	`5 s`
Gain	`30`
Scans	`1`

4. Select the Channels page.
5. Enter C13 in the Nucleus: field for the Observe "X" channel.
6. The transmitter will remain set to **2**. The ^{13}C calibrations were not changed and should remain from the previous step. Press the **Get** button for both the **Observe "X"** and **Decouple "H"** channels to retrieve the calibrations or if there is any doubt that the calibrations shown are not correct.
7. Click on the **Pulse Sequence** page.
8. Select **TPPM** decoupling.
9. Set **aHtppm = aH90/3.0**, use the value of **aH90** determined in Step 24 of Calibrating the ^{1H} pw90.

⚠ CAUTION	Adamantane shimming will ultimately use an Acquisition Time = 0.4 s. Probe damage will result if the amplitude of decoupling with acquisition time exceeds aH90/2.0. Do not exceed the probe specification for the maximum time the decoupler can be on at an amplitude greater than aH90/2.0. That number is usually 50 ms.

10. Set **phHtppm = 0** for CW decoupling.
11. Enter **0.0** in the field next to **line broadening** under **Weighting Parameters** and remove checks from the boxes next to any of the other weighting parameters. This sets lb = 0.0 and places a check in the box next to line broadening field.
12. Click on the **Acquire** button to acquire the data.

    A spectrum similar to Figure 80B is a typical example of data acquired when the field homogeneity is poor (poorly shimmed field). A well-shimmed field produces a spectrum of Adamantane, see Figure 80A, with a line width between 0.005 and 0.025 ppm (2 to 10 Hz at 400 MHz). The left hand line is referenced at 38.3 ppm.

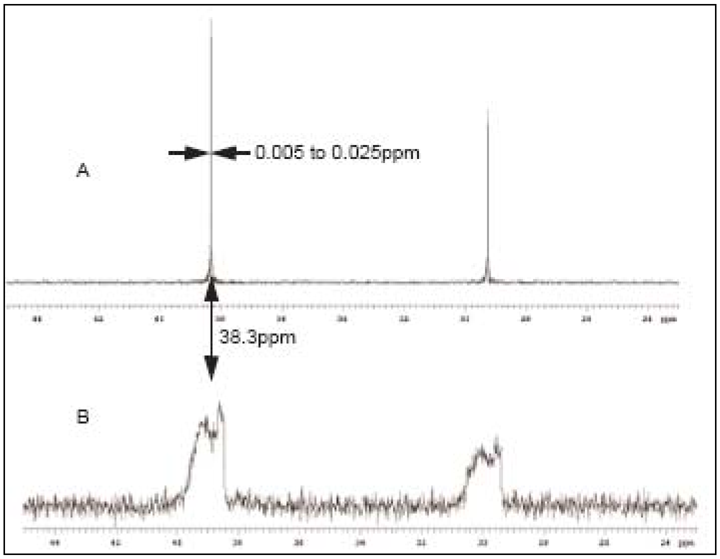


Figure 80 Adamantane Spectra

13. Place the transmitter between the two lines of Adamantane.
    a. Select 2 cursors and place them on the two lines of Adamantane.
    b. Enter `split movetof` on the command line.
    c. Click on the **Acquisition** page.
14. Set the spectra width as follows:

System Frequency	**Spectral Width**
500 MHz and lower	5 kHz
600 MHz and above	10 kHz

15. Click on the **Acquire** button to acquire the data.
16. Click on the **Channels** page.
17. Set an **array** of the decoupler offset **dof** from **-10000 Hz to 10000 Hz** in steps of **1000 Hz**.
18. Click on the **Acquire** button to acquire the data.

    Figure 81 shows the decoupler offset dependence of a shimmed Adamantane sample. The decoupler must be on resonance.

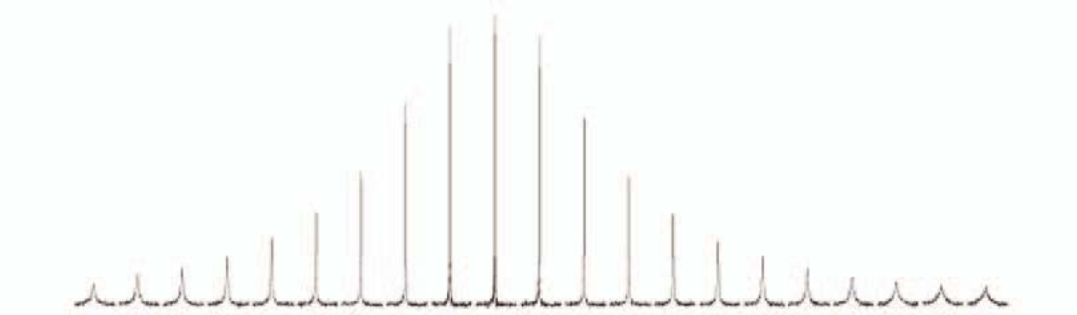


Figure 81 Optimizing Decoupler Offset for Adamantane

19. Enter the value of **dof** corresponding to the spectrum with the greatest peak height.
20. Click on the **Acquisition** page.
21. Set the following:

Field	**Value**
Recycle Delay	`5.0 s`
Scans	`4.`

22. Click on the **Acquire** button.
23. Use **Fid Scan** on the **Shims** page of the **Start** tab to obtain repeating display of the Adamantane spectrum.
24. Optimize the shims to obtain a minimum line width.
25. Typical shims are X1, Y1, Z1 and Z2.
26. Remove the hump at the base of the line with XY and X2Y2.
27. Record a well-shimmed Adamatane spectrum similar to Figure 80A and save the shims with svs on the command line.
28. Continue with Matching the Hartmann-Hahn Condition.

13.18.8 Matching the Hartmann-Hahn Condition

To set up Tancpx:

1. Change samples.
    a. Replace the Adamantane/KBR sample with the HMB sample.
    b. Adjust probe tuning.
    c. Set spin to 3300 Hz or the value in the probe specification sheet.

2. Load the **Tancpx** protocol using either of the following:
    - Use the Experiment Selector.
    1. a. Click on the Basic tab of the Experiment Selector.
    2. b. Click on **Tancpx**
    - Use the Solid-State tab.
    1. a. Click on **Experiments**.
    2. b. Select **Solid-State**.
    3. c. Select **Basic 1D Experiments**.
    4. d. Click on **Tancpx**.
3. Click on the **Acquire** tab.
4. Click on the **Acquisition** page.
5. Set the following:

Field	**Value**
Spectral Width	`5.0e4`
Acquisition Time	`0.02 s`
Recycle Delay	`5.0 s`
Gain	`30`
Scans	`4`

6. Click on the Channels page.
7. Enter ^{13}C in the Nucleus: field for the Observe "X" channel.
8. Enter ^{1}H in the Nucleus: field for the Decouple "H" channel.
9. Press the Get button for both the Observe "X" and Decouple "H" channels to retrieve the ^{13}C and basic ^{1}H basic calibrations from the previous steps.
10. Click on the Pulse Sequence page.
11. Select tppm form the Decoupling: drop-down menu.
12. Select const from the Shape menu in the cpHX: parameter group.
13. Set the following parameters in the fields in the cpHX: parameter group

Field (Parameter)	**Set or Type the Following Value in the Field**
`aHhx`	calibrated ^{1}H amplitude (aH90 from the Channels page)
`aXhx`	calibrated ^{13}C amplitude (ax90 from the Channels page)
`tHX`	7000 μs.
`aHtppm`	calibrated ^{1}H amplitude (aH90 from the Channels page)
`phHtppm`	0.0 for CW decoupling.

14. Click on the Acquire button.
15. Set the transmitter offset tof to place the tall methyl line in the center of the spectrum.
16. Place a cursor on the line and type movetof on the command line.

To adjust the ^{13}C Match Condition:

1. Array **aXhx** about +/- 1000 units.
2. Click on the Acquire button.
3. Enter the value of aXhx producing the maximum signal in the field next to aXhx: on the Pulse Sequence page.

   An example of a 12 step aXhx array is shown in Figure 82.

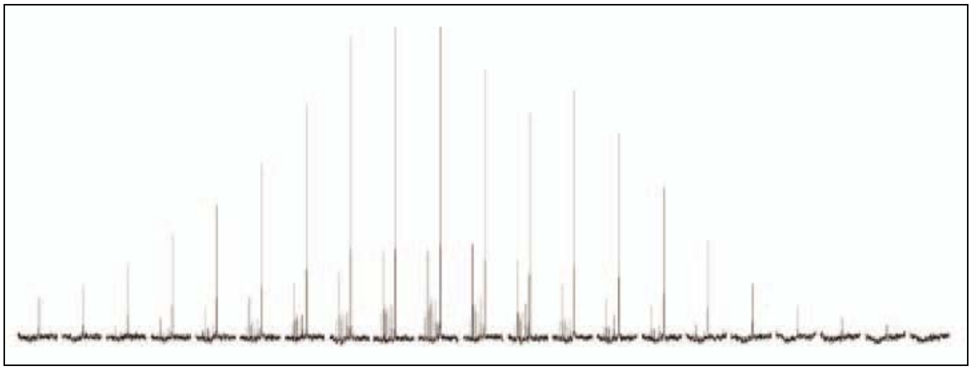


Figure 82 Array of `aXhx` for 13C Hartmann Hahn Match

To make the fine adjustment to **pwH90**

1. Select the Channels page.
2. Array pwH90 from 1.0 to 25.0 µs in steps of 1.0 µs.
3. Click on the Acquire button.
4. Enter the value of pw90 producing the maximum signal in the field next to pwH90: on the Channels page.
5. Continue with HMB Signal-to-Noise

13.18.9 HMB Signal-to-Noise

1. Set an array of the number of scans, with 10 repeated values of 4.
2. Click on the Acquire button.
3. 10 spectra are acquired with the optimum values from Matching the Hartmann-Hahn Condition.
4. Save the data.
5. Set two cursors with delta = 100p for each spectrum and place the right cursor at the right edge of the spectrum.
6. Type dsnmax on the command line to measure the signal-to-noise ration.
7. Record the value.
8. Average 10 values.

Typical array is shown in Figure 83.

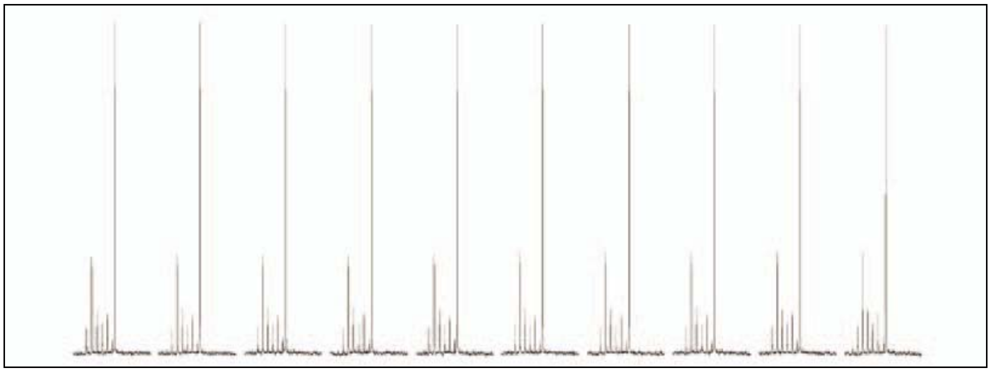


Figure 83 HMB Signal to Noise, 10 repetitions

9. Continue with Create a Starting Data Set.

13.18.10 Create a Starting Data Set

1. Load the data from HMB Signal-to-Noise .
2. Place the cursor at 100 ppm.
3. Enter movetof.
4. Set the transmitter offset at 100 ppm.
5. Click on the Acquire button.
6. Reference the methyl line of the spectrum at 17.3 ppm.
7. Set the cursor on the methyl line.
8. Enter rl(17.3p) on the command line.
9. Click on the Acquire button.
10. Set Shape = 'linear' and dHX about 200 in the cpHX parameter group.
11. Optional: set ramped cross polarization.
12. Set the receiver parameters correctly and ddrtc = ad + rd.
13. Click on the Acquire button.
14. Save the dataset.

A standard HMB spectrum centered at 100 ppm, see Figure 84, obtained with ramped cross polarization. This spectrum is a starting point for all ^{13}C CPMAS experiments.

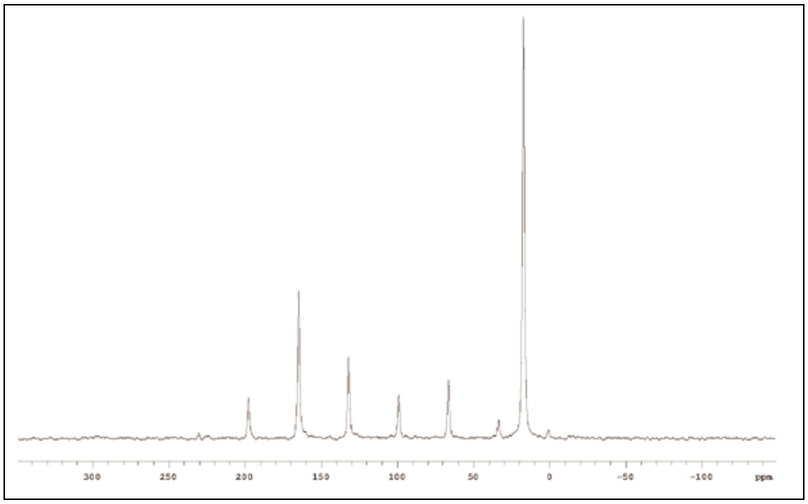


Figure 84 A standard HMB spectrum centered at 100 ppm

13.19 Basic 1D Experiments

13.19.1 Constant, Linear, or Tangent Cross Polarization (CP) (Tancpx)

Constant, linear, or tangent-ramped cross polarization (CP) between H and X with a choice of SPINAL64 or TPPM decoupling. Used for calibration of the Hartmann-Hahn Match and calibration of the 90º pulse width pwH90 with X detection.

Protocol	**Sequence**	**Apptype**
`Tancpx`	`tancpx.c`	`solidsseq1d`

13.19.2 One Pulse (Onepul)

Single-pulse preparation with a choice of SPINAL64 or TPPM decoupling. Used for calibration of the 90 pulse width pwX90.

Protocol	**Sequence**	**Apptype**
`Onepul`	`onepul.c`	`solidsseq1d`

13.19.3 Lee-Golburg CP (Lgcp)

Lee-Goldburg cross polarization (CP) between H and X with a choice of SPINAL or TPPM decoupling. Used for selective CP with suppression of homonuclear dipolar interactions and for setup of Lee-Goldburg HETCOR.

Protocol	**Sequence**	**Apptype**
`Lgcp`	`lgcp.c`	`solidsseq1d`

13.19.4 One-Pulse with TOSS (Onepultoss)

Four pulse TOSS side-band suppression is applied to X after single-pulse excitation, with a choice of SPINAL64 or TPPM decoupling. Used to obtain sideband-free spectra.

Protocol	Sequence	Apptype
`Onepultoss.c`	`Onepultoss.c`	`solidsseq1d`

13.19.5 pwX90 Measurement with CP (Tancpxflip)

Constant, linear or tangent-ramped cross polarization (CP) between H and X with an X flip-back pulse and a choice of SPINAL64 or TPPM decoupling. Used for calibration of pwX90 with CP and X detection.

Protocol	Sequence	Apptype
`Tancpxflip`	`tancpxflip.c`	`solidsseq1d`

13.19.6 CP with FSLG Decoupling (Tancpxfslg)

Constant, linear or tangent-ramped cross polarization (CP) between H and X followed by FSLG decoupling during acquisition. Used to evaluate FSLG decoupling for use in HETCOR. Tancpxfslg automatically sets up FSLG based upon calibrated values of aH90 and pwH90, using the macro reset_fslg.

Protocol	Sequence	Apptype
`Tancpxfslg`	`tancpxflslg.c`	`solidsseq1d`

13.19.7 H T_1 with CP (Tancpht1)

An H T_1 measurement using inversion recovery followed by X detection with constant, linear or tangent-ramped cross polarization (CP) between H and X with a choice of SPINAL64 or TPPM decoupling. Used for proton T_1 measurements with CP.

Protocol	Sequence	Apptype
`Tancpht1`	`tancpht1.c`	`solidsseq1d`

13.19.8 Interrupted Decoupling with CP (Tancpxidref)

Interrupted decoupling for suppression of the signals of protonated X nuclei. Follows constant, linear or tangent -ramped cross polarization (CP) between H and X with a choice of SPINAL64 or TPPM decoupling. Provides a refocusing pulse, synchronized with two rotor periods to avoid a large first order phase correction. Used to edit protonated X nuclei from a CPMAS spectrum.

Protocol	Sequence	Apptype
`Tancpxidref`	`tancpxidref.c`	`solidsseq1d`

13.19.9 X $T_{1\rho}$ with CP (Tancpxt1rho)

Measurement of the X $T_{1\rho}$ with a spinlock following constant, linear or tangent-ramped cross polarization (CP) between H and X, with a choice of SPINAL64 or TPPM decoupling. Used for measurement of the X $T_{1\rho}$ with CP preparation and X detection.

Protocol	**Sequence**	**Apptype**
`Tancpxt1rho`	`tancpx1rho.c`	`solidsseq1d`

13.19.10 CP with TOSS (Tancpxtoss)

Protocol	**Sequence**	**Apptype**
`Tancpxtoss`	`tancpxtoss.c`	`solidsseq1d`

13.19.11 X Two Pulse for T_1 (Twopul)

A two-pulse X experiment with a delay d2 and with a choice of SPINAL64 or TPPM decoupling. Used for inversion recovery T_1 measurements.

Protocol	**Sequence**	**Apptype**
`Twopul`	`twopul.c`	`solidsseq1d`

13.19.12 X Hahn Echo with CP (Tancpxecho)

Constant, linear or tangent-ramped cross polarization (CP) between H and X followed by a Hahn echo with a choice of SPINAL64 or TPPM decoupling. Used for simple measurements of T_2 and to avoid ring down for static samples. The first delay must be synchronized with the rotor if this sequence is used with MAS.

Protocol	**Sequence**	**Apptype**
`Tancpxecho`	`tancpxecho.c`	`solidsseq1d`

13.20 HX2D Experiments

13.20.1 2Q-1Q with CP and C7 Mixing (C7inad2d)

1Q-2Q correlation using C7 mixing, with TPPM or SPINAL decoupling. A sequence to correlate X nucleus pairs with double quantum filtering. F1 is the evolution of double-quantum coherence and F2 is single quantum chemical shift. The spectral appearance is similar to the INADEQUATE experiment. Polarization (along "Z") is created with a constant, linear or tangent ramped CP followed by a flip-back pulse on X. C7 is used to recouple double quantum coherence and a second pulse returns polarization to X. TPPM or SPINAL decoupling is applied during acquisition and CW decoupling with an amplitude aHmix is applied during C7.

Protocol	Sequence	Apptype
`C7inad2d`	`c7inad2d.c`	`solidsseq1d`

13.20.2 H to X HECTOR (Hetcorlgcp2d)

H to X HETCOR using FSLG during F1 followed by a Lee-Goldburg CP, with SPINAL64 or TPPM decoupling. Frequency switched Lee Goldburg decoupling is used during F1 to suppress the proton homonuclear dipolar interaction. HETCOR is used to resolve the proton chemical shift spectrum and to assign X-H pairs.

Protocol	Sequence	Apptype
`Hetcorlgcp2d`	`hetcorlgcp2d.c`	`solidsseq1d`

13.20.3 PISEMA (Pisema2d)

A sequence to correlate X chemical shift with the X-H dipolar interaction in the rotating frame. PISEMA is used to characterize ^{15}N-^{1}H dipolar interactions for static, oriented membrane protein samples.

`reset_pisema` – This macro uses the calibrated values of pwH90 and aH90 to calculate the initial parameters for the fslgH waveform and sets up 2D parameters.

reset_pisema2d is run from the protocol.

Protocol	Sequence	Apptype
`Pisema2d`	`pisma2d.c`	`solidsseq1d`

13.20.4 WISE (Wisentancp2d)

2D correlation between the X chemical shift and the H wideline spectrum using constant, linear or tangent-ramped cross polarization (CP) between H and X with a choice of SPINAL64 or TPPM decoupling. Used for separation of the proton wideline spectra of XH pairs with tHmix = 0. Used for spin diffusion measurements with tHmix > 0.0.

Protocol	Sequence	Apptype
`Wisetancp2d`	`wisetancp2d.c`	`solidsseq1d`

13.21 HXY Experiments

13.21.1 One Pulse REDOR with XY8 on X and Y (Redor1onepul)

Rotational Echo Double Resonance (REDOR) with one-pulse preparation, XY8 decoupling with pulses alternating on X and Y, with a choice of SPINAL64 or TPPM decoupling during both acquisition and REDOR evolution. Used to measure XY bond distances for organic materials where one-pulse preparation is preferred.

Protocol	**Sequence**	**Apptype**
`Redor1onepul`	`redor1onepul.c`	`solidsseq1d`

13.21.2 CP REDOR with XY8 on X and Y (Redor1tancp)

Rotational Echo Double Resonance (REDOR) with constant, linear or tangent-ramped preparation, XY8 decoupling with pulses alternating on X and Y, with a choice of SPINAL64 or TPPM decoupling during both acquisition and REDOR evolution. Used to measure XY bond distances for organic materials.

Protocol	**Sequence**	**Apptype**
`Redor1tancp`	`redor1tancp.c`	`solidsseq1d`

13.21.3 One Pulse REDOR with XY8 Y with X Inversion (Redor2onepul)

Rotational Echo Double Resonance (REDOR) with one-pulse preparation, XY8 decoupling with pulses on Y, and a refocusing pulse on X, with a choice of SPINAL64 or TPPM decoupling during both acquisition and REDOR evolution. Used to measure XY bond distances for inorganic and organic materials where one-pulse preparation is preferred.

`redor2onepul` is the favored REDOR sequence if homonuclear coupling or quadrupole coupling is present for the X nuclei. Rotationally synchronized pi pulses interfere with the refocusing of these interactions into rotational echoes and dephase the magnetization. redor2onepul has only one refocusing pulse on X.

Protocol	**Sequence**	**Apptype**
`Redor2onepul`	`redor2onepul.c`	`solidsseq1d`

13.21.4 CP REDOR with XY8 on Y with X Inversion (Redor2tancp)

Rotational Echo Double Resonance (REDOR) with constant, linear or tangent-ramped preparation, XY8 decoupling with pulses on Y, and a refocusing pulse on X, with a choice of SPINAL64 or TPPM decoupling during both acquisition and REDOR evolution. Used to measure X-Y bond distances for inorganic and organic materials.

`redor2tancp` is the favored REDOR sequence if homonuclear coupling or quadrupole coupling is present for the X nuclei. Rotationally synchronized pi pulses interfere with the refocusing of these interactions into rotational echoes and dephase the magnetization. redor2tancp has only one refocusing pulse on X.

Protocol	**Sequence**	**Apptype**
`Redor2tancp`	`redor2tancp.c`	`solidsseq1d`

13.22 Quadrupole Experiments

13.22.1 3Q-1Q MQMAS with Z-filter (Mqmas3qzf2d)

3-quantum multiple-quantum MAS with a third Z-filter pulse and a choice of SPINAL64 or TPPM decoupling. Used to obtain a 2D MQMAS spectrum for all spins 3/2 to 9/2.

Protocol	Sequence	Apptype
`Mqmas3qzf2d`	`mqmas3qzf2d.c`	`solidsseq1d`

13.22.2 5Q-1Q MQMAS with Z-filter (Mqmas5qzf2d)

5-quantum multiple-quantum MAS with a third Z-filter pulse and a choice of SPINAL64 or TPPM decoupling. Used to obtain a 2D MQMAS spectrum for all spins 5/2 to 9/2.

Protocol	Sequence	Apptype
`Mqmas5qzf2d`	`mqmas5qzf2d.c`	`solidsseq1d`

13.22.3 Quadrupole Echo (Ssecho1d)

A two-pulse solid or quadrupole echo with a choice of SPINAL64 or TPPM decoupling. Used primarily for wideline deuterium NMR.

Protocol	Sequence	Apptype
`Ssecho1d`	`ssecho1d.c`	`solidsseq1d`

13.23 Multipulse Experiments

13.23.1 BR24 with Quadrature Detection (Br24q)

X acquisition with interleaved BR24. Quadrature phase cycle.

Quadrature BR24 multiple-pulse acquisition for high resolution proton NMR. This sequence uses a variable-length preparation pulse and fine-phase array to preparation are the magnetization in quadrature perpendicular to the multiple-pulse axis of precession (1,1,1). The four variable length pulses are calculated assuming that `pwXprep` has a 90-degree flip angle. BR24 has a cycle time of 36 tau with 24 pulses per cycle.

Set `phXprep` = -45 degrees. `Br24q` is used with slow spinning (< 2500 Hz).

Protocol	Sequence	Apptype
`Br24q`	`br24q.c`	`solidsseq1d`

13.23.2 MREV8 with Quadrature Detection (Mrev8q)

Quadrature MREV8 multiple-pulse sequence is used for acquisition for high resolution proton NMR. The sequence uses a variable-length preparation pulse and fine phase array to prepare the magnetization in quadrature perpendicular to the multiple-pulse axis of precession (sqrt(1/2),0,sqrt(1/2)).

The four variable-length pulses are calculated assuming that pwXprep has a 90-degree flip angle. MREV8 has a cycle time of 12 tau with 8 pulses per cycle. Set **`phXprep = 0`** degrees. Mrev8q is used with slow spinning (< 2500 Hz).

Protocol	**Sequence**	**Apptype**
`Mrev8q`	`mrev8q.c`	`solidsseq1d`

13.23.3 Semiwindowless WaHuHa (Swwhh4)

Semi-windowless WaHuHa (SWWHH4) multiple-pulse acquisition for high resolution proton NMR. This sequence uses 90-degree preparation pulses on X and -X offset by -45 degrees to prepare the magnetization perpendicular to the multiple-pulse axis (1,1,1). SWWHH4 has a cycle time of 6 tau with 4 pulses per cycle. Set phXprep = -45 degrees. SWWHH4 is used with medium speed spinning (8.0 to 14.0 kHz).

Protocol	**Sequence**	**Apptype**
`Swwhh4`	`swwhh4.c`	`solidsseq1d`

13.23.4 High Power Pulse Tuning (Tunerp)

A pulse sequence for pulse tuning through the directional couplers in the VNMRJ display. Tunerp is used for high-power pulsed tuning and for characterization of phase transient. It provides a phase detected output of the pulse that can be displayed in phased or absolute value mode. Launch Tunerp with the **Fidscan** button on the Shims page in Setup tab.

Measure the ratio of forward to reflected power when using Tunerp. The standard directional couplers are wired to measure reflected power only. The arrow on the side of the coupler should point back toward the Front End to measure reflected power. To measure forward power, reverse the coupler so that the arrow points toward the probe.

A second optional bidirectional coupler is available on some systems. With this coupler reverse the direction of the arrow by turning the knob on the top.

Protocol	**Sequence**	**Apptype**
`Tunerp`	`tunerp.c`	`solidsseq1d`

13.23.5 Windowed PMLG-N (Wpmlg1d)

Quadrature, windowless PMLG-N (wPMLG) is used for multiple-pulse acquisition. The sequence uses a composite preparation pulse involving a quadrature phase-shifted 90 and a 54.7-degree pulse along Y to prepare the magnetization perpendicular to the multiple-pulse axis of precession (sqrt(2/3), 0 sqrt(1/3)). The recycle time for wPMLG is:

```
tauXwpmlg = 2.0*(4.0*pwX90)*sqrt(2/3) + B
```

where pwX90 is the 90-degree pulse and B is the window time.

Protocol	Sequence	Apptype
`Wpmlg1d`	`wpmlg1d.c`	`solidsseq1d`

13.23.6 2D F1 and Windowed PMLG-N (Wpmlg2d)

2D homonuclear correlation with PMLG in F1, a spin diffusion mixing period and quadrature windowed PMLG (wPMLG) for multiple-pulse acquisition in F2. This sequence uses the composite preparation pulse to put the magnetization along -Y perpendicular to the PMLG axis of precession. After F1 PMLG, a tilt pulse returns precession to the XY plane and puts the Y axis along Z for cosine peaks and the X axis for sine peaks. A delay of tXmix allows mixing by spin diffusion. A quadrature wPMLG sequence similar to wpmlg1d.c then samples the Z axis magnetization in F2. PMLG and wPMLG are used with moderate-speed spinning (8 to 14 kHz).

Protocol	Sequence	Apptype
`Wpmlg2d`	`wpmlg2d.c`	`solidsseq1d`

13.23.7 XX Tuneup (Xx)

X pulses with interleaved acquisition. Used for multipulse calibration of pwX90. Usually performed with a liquid sample or a sample of RTV silicon rubber with slow spinning. Xx can also be used to examine sampling windows and set the blank/unblank delays r1Xxx and r2Xxx.

Xx subtracts probe ring-down with a two-scan (0,2) phase cycle of the preparation pulse and the receiver. The xmxX has 0 phase for all scans. The oscillating pattern will not be symmetric about zero due to a constant signal from probe ringing and background For very short values of tau and when probe proton background is present. Use 2 scans to see the signal without ringing.

Protocol	Sequence	Apptype
`Xx`	`xx.c`	`solidsseq1d`

13.23.8 XmX Tuneup (Xmx)

X pulses with interleaved acquisition and alternating phase. Used for multi-pulse calibration to minimize phase transient. Usually performed with a liquid sample or a sample of RTV silicon rubber with slow spinning. Xmx can also be used to examine sampling windows and set the blank/unblank delays r1Xxmx and r2Xxmx.

Xmx subtracts probe ring-down with a two-scan (0,2) phase cycle of the preparation pulse and the receiver. The xmxX sequence has 0 phase for all scans. The oscillating pattern is not symmetric about zero for very short values of tau and when probe proton background is present due to a constant signal from probe ringing and background. Use 2 scans to see the signal without ringing.

Protocol	Sequence	Apptype
Xmx	xmx.c	solidsseq1d

Chapter 14 Data Analysis

This chapter describes how to analyze data by: spin simulation, deconvolution, adding and subtracting data, and different analysis.

Sections in this chapter include:

- 14.1 Spin Simulation
- 14.2 Deconvolution
- 14.3 Reference Deconvolution Procedures
- 14.4 Addition and Subtraction of Data
- 14.5 Regression Analysis
- 14.6 Cosy Correlation Analysis
- 14.7 Chemical Shift Analysis

14.1 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 (e.g., 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.

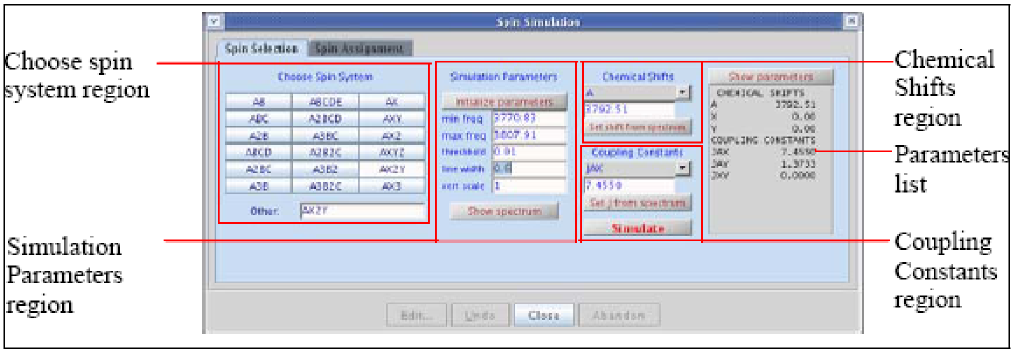
Parameters can be adjusted by iteration to approach a given experimental spectrum. One or several parameters can be kept constant for iterative runs and one or several parameters can be set equal to each other and held equal during the course of the iteration.

An example is provided in Spin Simulation Step-by-Step. Table 26 lists specialized commands and parameters.

References for the spin simulation algorithms:

- Castellano, S. and Bothner-by, A.A., 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.
- Ferguson, R.C. and Marquardt, D.W.;, J. Chem. Phys., 41, 2087 (1964).

14.1.1 Spin Selection Tab, Buttons, and Fields



Other: AX2Y

min freq 3770.83
max freq 3807.91
threshold 0.01
line width 0.6
vert scale 1

A
3792.51

JAX
7.4550

```
CHEMICAL SHIFTS
A          3792.51
X             0.00
Y             0.00
COUPLING CONSTANTS
JAX         7.4550
JAY         1.3733
JXY         0.0000
```

Figure 85 Spin Simulation – Spin Selection Tab

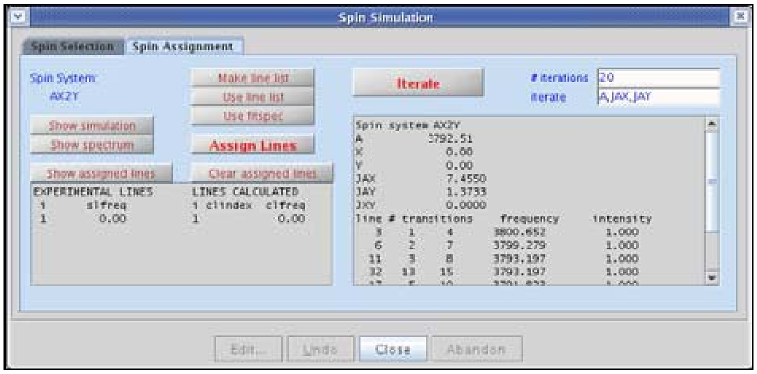
The Spin Selection Tab has the following buttons and fields, refer to Figure 85 as needed:

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.

Region or Button or Field	Description/ Function
Coupling Constants section:	
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.

14.1.2 Spin Assignment Tab

The Spin Assignment Tab has the following buttons and fields, refer to Figure 86 as needed:



Spin System: AX2Y

iterations: 20

iterate: A,JAX,JAY

```
EXPERIMENTAL LINES
 i      slfreq
 1        0.00
```

```
LINES CALCULATED
i clindex  clfreq
1            0.00
```

```
Spin system AX2Y
A           3792.51
X              0.00
Y              0.00
JAX          7.4550
JAY          1.3733
JXY          0.0000
line # transitions    frequency     intensity
   3    1    4        3800.652        1.000
   6    2    7        3799.279        1.000
  11    3    8        3793.197        1.000
  32   13   15        3793.197        1.000
```

Figure 86 Spin Simulation – Spin Assignment Tab

Button/Field	Description
Spin System	Displays the spin system selection.
Show simulation button	Display simulated spectrum.
Show spectrum button	Fourier transform and display the spectrum.
Make line list button	Create a line list from the current spectrum and threshold and copy the list into the spin simulation line assignment file.
Use line list button	Copy the current line list into the spin simulation line assignment file.
Use fitspec button	Copy the fitspec line list into the spin simulation line assignment file.
Show assigned lines button	Display the spin simulation line assignment file spini.la.
Clear assigned lines button	Clear the line assignments.

Button/Field	Description
Iterate button	Iteratively fit 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.

14.1.3 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 on the file Proton_01.fid.
7. The file is retrieved to the current experiment and viewport.
8. Click the **Process** tab.
9. Click the **Transform** button.
10. The graphics window displays a spectrum and the menu from the interactive spectrum display program (ds) appears.
11. Click the left mouse button near 7.7 ppm and the right mouse button near 7.5 ppm.
12. Click 🔍 .

    The spectrum expands to show the six-line pattern that will be simulated as an AX2Y system.
13. 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 **Spin Selection Tab**.
5. Click **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 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.

14.1.4 Spin Simulation Related Commands, Parameters, and Files

Table 26 Spin Simulation Related Commands and Parameters

Command	Description
`assign*`	Assign transitions to experimental lines
`cla`	Clear all line assignments
`dga`	Display group of spin simulation parameters
`dla<('long')>`	Display spin simulation parameters arrays
`dlalong`	Long display of spin simulation parameter arrays
`dll*`	Display listed line frequencies and intensities
`dsp<(file)>`	Calculates the simulated spectrum (using the current value of parameter slw for the linewidth) and displays the spectrum using the current table of transitions and intensities. dsp can only be used after the spins program has been run. dsp with a filename 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.
`initialize_iterate`	Set iterate string to contain relevant parameters
`spinll<('mark')>`	Set up a slfreq array

Command	**Description**

`spins <(options)>`	Performs a spin simulation using the current spin system parameters when the Simulate button is clicked. The following variations are available:
	`spins('calculate','energy')`
	Puts an energy level table in the output file.
	`spins(calculate','transitions')`
	Puts a second table of transitions ordered by transition number in the output file.
	`spins('iterate')`
	Runs in an iterative mode to match experimental and calculated lines.
	`spins('iterate','iteration')`
	Lists parameters after each iteration in the output file.

`spsm(spin_system)`	Enter spin system
`undospins`	Restore spin system as before last iterative run
`* dll<('pos'<,noise_mult>)><:lines>` `assign<('mark')>, assign(transistion_number,line_number)`	
Parameters	
cla `{array of real values}`	Calculated transition number
clamp `{array of real values}`	Calculated transition amplitude
clfreq `{real values}`	Calculated transition frequency
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 27 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`	Sores 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 28 Iterative Mode Related Commands

Command	Button	Description
`spins('iterate')`	Iterate	Performs the simulation in the iterative mode
`initialize_iterate`	Initialize parameters	Selects a default value for the parameter iterate that causes iteration of all parameters. The string parameter, iterate, contains a list of parameters (separated by commas) to be iterated during iterative spin simulations. Typical value is 'A,B,JAB'. Initializes the parameter iterate to a string containing parameters appropriate to the current spin system and niter to 20.
`cla`	Clear assigned lines	Clears the file of line assignments used for spin simulation.
`dla`	Show assigned lines	Displays the file containing the line assignments.

Command	**Button**	**Description**
`dlalong`		Writes the line assignments to the file spini.la of the current experiment. This command is useful in more complex problems where the text window is too small for the dla display. dlalong displays the file in the text window.
`spinll`	Use Line List	Copies the frequency list from the last nll or dll line listing (contained in the parameter llfrq) into the simulation line frequency parameter slfreq. spinll, clears previous line assignments, and runs dla.
spinll(‘mark’)		Places the line positions in the file mark1d.out into the parameter slfreq. This is useful for to manual line assignment.
assign	Assign Lines	The nearest calculated transitions are assigned to the lines from a dll or nll listing after spinll places them in slfreq. If a frequency in slfreq exists with a line number, the next entry of the same frequency is assigned a unique line. Optional: Assign positive lines only. Run dll(‘pos’) from the command line first. Only lines transitions greater than sth (typically ≥0.05 to prevent assignment of extremely small lines) are assigned.
`assign(‘mark’)`		The same as assign except the file mark1d.out is used instead of the dll listing. Use the cursor and the mark button to place the lines to be assigned in the mark1d.out file. This file is cleared by mark(‘reset’). Use nl to move the cursor to the center of a selected line.
`assign(t#,l#)`		Assigns a single calculated transition number (t#) to a line from a dll listing (the index is l#). Use assign(t#,0) to remove the calculated transition assignment.
`undospins`		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.

Refer to 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 29 Spin Simulation Files

File	**Description**
spini.indata	Line assignment input (deleted by spins after iterations are completed) for the spins program.
spini.inpar	List of parameters whose values are to be determined by `spins('iterate')`.
spins.inpar	List of initial settings of a number of spin simulation parameters.
spini.la	Current transition assignments for an iterative spin simulation (produced by the dlalong command).
spins.list	Name of the output file and it is located in the current experiment. This file always includes the calculated transitions ordered by frequency.
spins.outdata	File of frequencies, amplitudes, and transition numbers from a spin simulation. It is used in calculating the displayed spectrum.
spini.outpar	Values of the chemical shifts and coupling constants after an iterative spin simulation.
spini.savela	Transition line assignments for iterative spin simulation in a format readable by the macro undospins.
spins.stat	Constants related to iteration (deleted by the spins program).

14.2 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.

14.2.1 Deconvolution Window and Controls

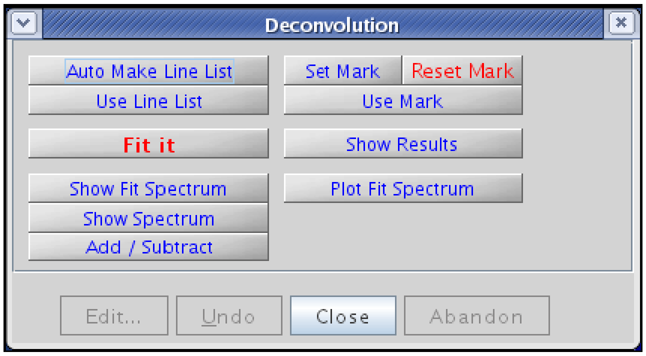


Figure 87 Deconvolution Window and Controls

The Deconvolution window, see Figure 87, 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 nll 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 fitspec 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 plfit macro

14.2.2 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*np.
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 87.
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 on − icon on the graphics tool bar to view the difference between the measured and calculated spectra.

14.2.3 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.

14.2.4 Deconvolution Related Commands and Parameters

Table 30 lists the associated commands and parameters with a short description.

Table 30 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 31 Deconvolution Commands, Parameters, and Buttons

Command or Parameter	Button	Description
`fitspec`	Fit it	Performs spectrum deconvolution by fitting experimental data to Lorentzian and/or Gaussian line shapes. fitspec uses data in a file fitspec.inpar as a starting point for the calculation. `fitspec` writes a text representation of the spectral data to the file fitspec.indata. The results of the fit are contained in a file after the calculation is finished. fitspec.outpar format is identical to fitspec.inpar. All lines are set to have a linewidth of slw, and a fixed Gaussian fraction of 0. (Refer to the *VNMR Command and Parameter Reference* for information about keyword options available with fitspec.)
`setgauss`		Modifies the output of the last deconvolution (fitspec.outpar) and makes it the input for a subsequent analysis (fitspec.inpar), after first modifying the Gaussian fraction:

Command or Parameter	Button	Description
`setgauss(fraction)`		Use the format setgauss(fraction) where fraction is the Gaussian fraction of the line shape, a number naturally limited from 0 to 1, for example, setgauss(0.4) to allow this fraction to vary.
`setgauss(fraction*)`		Use the format setgauss(fraction*). Suffix the fraction value (defined the same as above) with an asterisk and enclose the value in single quotes, for example, setgauss('1.0*') to fix the fraction.

Table 32 Deconvolution Display and Plotting Commands and Macros

Macro	Button	Description
showfit	Show Results	Displays deconvolution results and converts the data to a format suitable for printing.
dsp('fitspec.outpar')	Show Fit Spectrum	Displays the theoretical spectrum described by the parameters produced by a deconvolution calculation.
plfit	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 33 Deconvolution Files

File name	Description
fitspec.inpar	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.
fitspec.indata	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 sp and sp+wp).
fitspec.outpar	Contains the final parameters (frequency, intensity, linewidth, and Gaussian fraction) after a fit has been done.
mark1d.out	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.

14.3 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 34 list commands for 1D and 2D variations of the fiddle program.

Table 34 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>)`	

14.3.1 Fiddle Program Options

The fiddle program and its variants, see Table 34, 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; 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.

14.3.2 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 (i.e. set fn >= 2*np). (If there are sinc wiggles, use wft with gf = at*0.6.)
2. Set the reference line to the chosen signal using the **`rl`** 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 (e.g., 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 (e.g. ^{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.

14.3.3 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 t1-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 **`rl`** 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 t1 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 (i.e., 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.

14.3.4 References

Further information on reference deconvolution can be found in the following literature:

Taquin, J. *Rev. Physique App.* **1979**, *14***,** 669.

Morris, G. A. *J. Magn. Reson.* **1988***, 80***,** 547.

Morris, G. A.; Cowburn, D. *MRC* **1989**, *27***,** 1085.

Morris, G.A., Barjat, H., Horne T.J., *Prog. NMR Spectrosc.***1997**, *31*, 197.

Gibbs, A; Morris, G. A. *J. Magn. Reson.* **1991**, *91***,** 77.

Gibbs, A.; Morris, G. A.; Swanson, A.; Cowburn, D. *J. Magn. Reson.* **1983,** *101*, 351–356.

Rutledge, D. N. Ed. *Signal Treatment and Signal Analysis in NMR*, Chapter 16. Elsevier Science, 1996.

14.4 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.

14.4.1 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.

14.4.2 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 35.

Table 35 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.
	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.

Button	Name	Description
➕	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.

14.4.3 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 35. 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 (e.g., the command pl), 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 88.

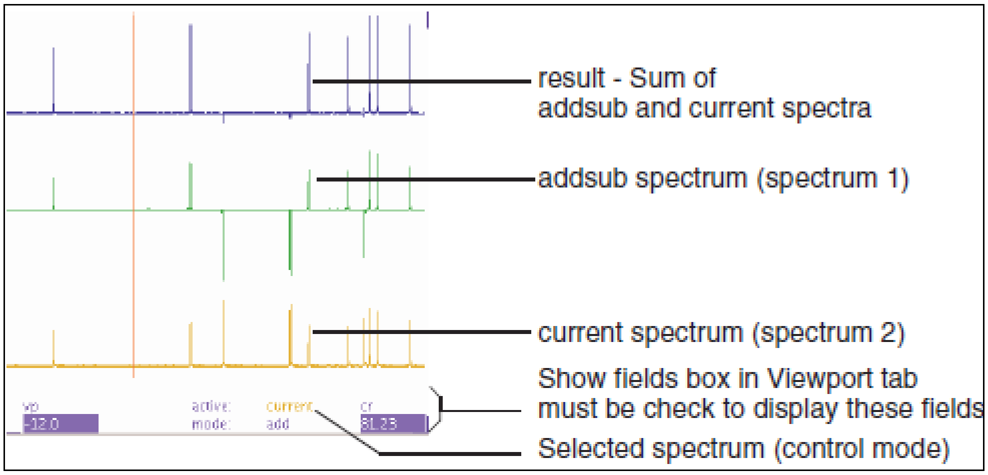


Figure 88 Add Spectra and Display Result

15. Optional: Click − icon in the Add/Subtract toolbar to subtract the current spectrum (spectrum 2) from the first spectrum.

To manipulate the spectra:

1. 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 checked to show the fields.

2. 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.

3. 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 87.

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.
    b. 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.
    c. Save the result in experiment 5 to a file or move it to another available experiment.

14.4.4 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.

14.4.5 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.

1. Type **`jexp5`** to join exp5.
2. 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'.

14.4.6 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 value of shift shifts the spectrum being added or subtracted to higher frequency, or to the left. A negative value of shift 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 can 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 (e.g., ds) and plotting commands to examine the results.

14.4.7 Add / Subtract Related Commands and Parameters

The add/subtract experiment related commands are listed in Table 36 and parameters are listed in Table 37.

Table 36 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.

```
*
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)
```

Table 37 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

14.5 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/tau) + a3`

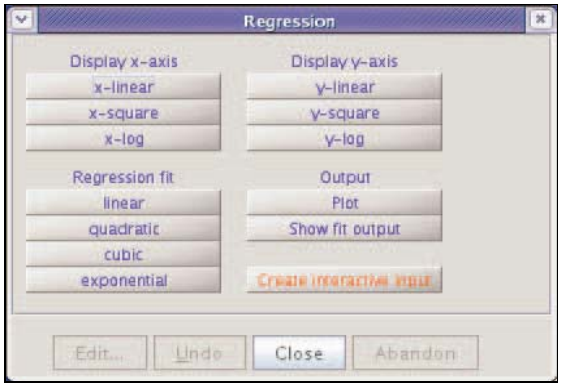
Original data may be displayed against a linear, squared, or logarithmic scales.

14.5.1 Regression Window and Controls

Access the regression window as follows:

1. Click on **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.

Button Group	**Function**
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.

14.5.2 Regression, Step-by-Step Using the Regression Window

1. Click on **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 on any one of the buttons **Display x-axis** or **Display y-axis** group.

   Each buttons scales the display and shows either the x or y axis as labeled on the button.

3. Click on one of the buttons in the **Regression fit** to select a fitting routing. 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 89 shows quadratic fittings for the data set in Table 38.

Table 38 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 and Contents of “analyze.out” File.

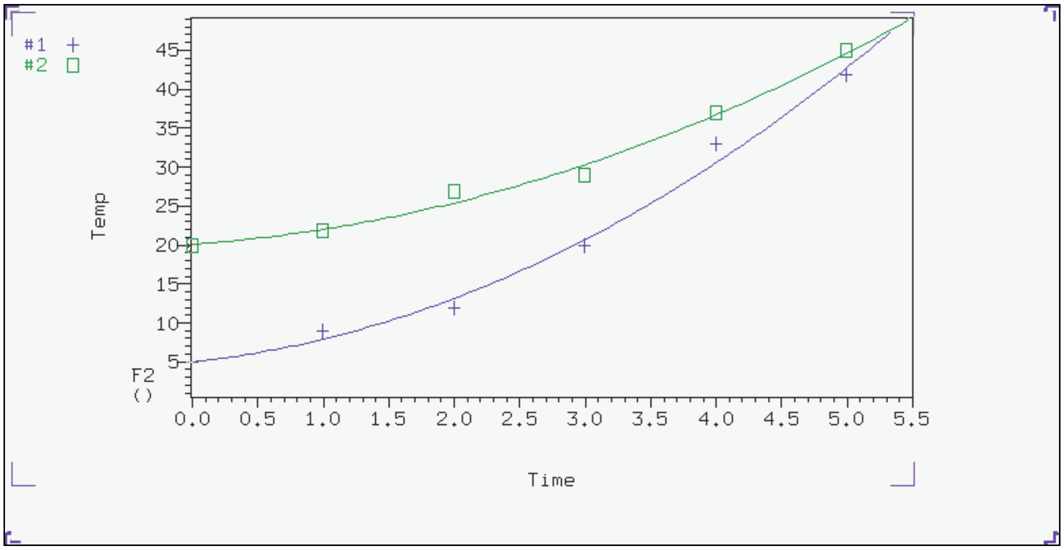


Figure 89 Display of Regression Fittings

4. Click on the **Plot** button to plot the analysis.

   The scale automatically to show all points (if possible).

5. Click on the **Show fit output** button to show the results of the analysis in the Text Output window.

14.5.3 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 89 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 **`scalelimits`** macro to set limits for the scales using one of the following:

   - Enter **`scalelimits`** with no argument to start an interactive process that prompts for the four scale limits.
   - Enter **`scalelimits(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.

14.5.4 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 39

Table 39 Curve Type

Type	Function	Functional Form
0	T1/T2	_(a0 – a2)*exp(–t/a1) + a2_
1	Increasing kinetics	_a0*exp(–t/a1) + a2_
2	Decreasing kinetics	_–a0*exp(–t/a1) + a2 + a0_
3	Diffusion	_a0*exp(–D1*uu) + a2*exp(–a1*D1*uu)_ where $uu=C0+C1*t+C2*t^2$
4	None	No theoretical curve (use 'link')
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 exp is followed by a number for a curve type from Table 39. The keyword regression, if present, indicates regression output.
2	Floating point constants _D1_, _C0_, _C1_, and _C2_, 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 _x_ and _y_ axes: `linear, square`, and `log`.
4	Title line. Use No Title when a title is not desired. Two additional text lines for the _x_ and _y_ axis titles are present in output from regression.
5	The keyword NEXT 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 _a0, a1_, and _a2_ (see Table 39) and must all be present, even for functions that do not use all three (e.g., first-order polynomial). If a particular number is not appropriate, put any number there. _a3_ 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 *x*-axis label displayed by expl('regression').
2	Line with text for *y*-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
            6    36
```

Description of this example:

Line number	**Description**
1	Keyword exp 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.

14.5.5 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 `expl('regression')`. 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 `expl('regression')`. 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 `NEXT` is inserted at the start of each data set.

Line number	Description
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

14.5.6 Regression Commands

Table 40 lists the commands associated with regression analysis

Table 40 Regression Commands

Commands	Button	Description
`analyze*`	Any of the buttons in the Regression fit group.	Generalized curve fitting in regression mode
`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)
```

14.6 Cosy Correlation Analysis

14.6.1 Cosy Data Chemical Shift Analysis Window and Controls

Button	**Function**
Create 2D Line List	Write a 2D line listing to the file ll2d.out 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.

14.6.2 Analysis of COSY Data Step-by-Step

1. Process and display a COSY data set.
2. Click on **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 on the **Create 2D Line List** button to create a line list for the COSY data set.
6. Click on the **Find Correlations** button display the correlations of the COSY data set on the screen.
7. Optional:

   Click on the **Redisplay** button to redisplay an normal interactive 2D contour map and make any changes or adjustments to the data as needed. Start over again at **Step 4**.
8. Click on the **Plot Correlations** button to create plot of the line list for the COSY data set.
9. Click on the **Plot Contours** button to create a contour plot of the COSY data set.
10. Click on the **Plot Page** button to send the plot to the printer.

14.7 Chemical Shift Analysis

Chemical shifts are analyzed using the commands in Table 41. These commands write a list of chemical shifts to the file pcss.outpar.

Table 41 Chemical Shift Analysis Commands

Commands	Description
`do_pcss`	Calculate proton chemical shifts spectrum.
`pcss`	Calculate and show proton chemical shifts spectrum.

Chapter 15 Pulse Analysis

Sections in this chapte include:

- 15.1 Pandora's Box
- 15.2 Pulse Shape Analysis

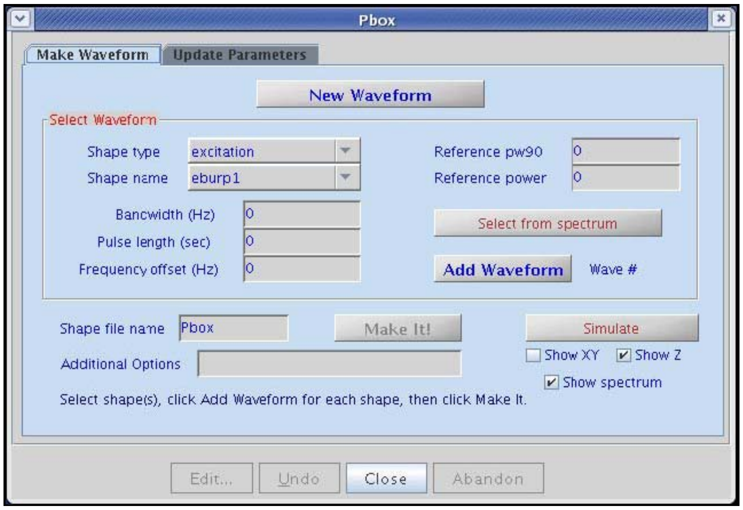
15.1 Pandora's Box

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 at from Pbox shape files by macros or directly within pulse sequences. More than 160 different shapes are available from the Pbox library.

15.1.1 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 90.



Shape type: excitation
Shape name: eburp1
Bandwidth (Hz): 0
Pulse length (sec): 0
Frequency offset (Hz): 0
Reference pw90: 0
Reference power: 0
Shape file name: Pbox
Additional Options:
☐ Show XY ☑ Show Z
☑ Show spectrum

Select shape(s), click Add Waveform for each shape, then click Make It.

Figure 90 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 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 updates 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** (refer to for Table 42, Pbox Commands and Parameters).
14. Click **Make It!** to generate and save the shape file.
15. Request simulations, see Figure 91, as follows:

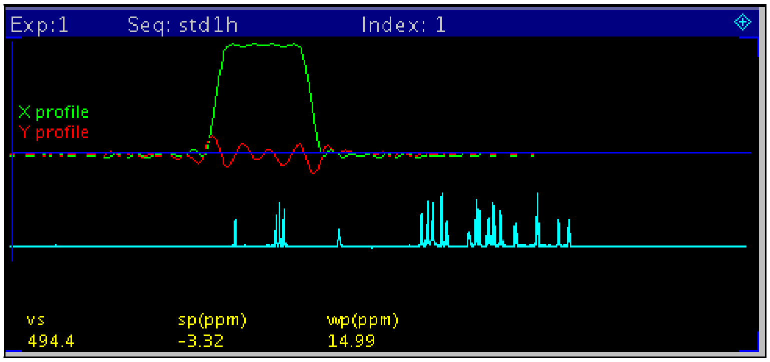


Figure 91 Shaped Pulse Simulations Reference Spectrum

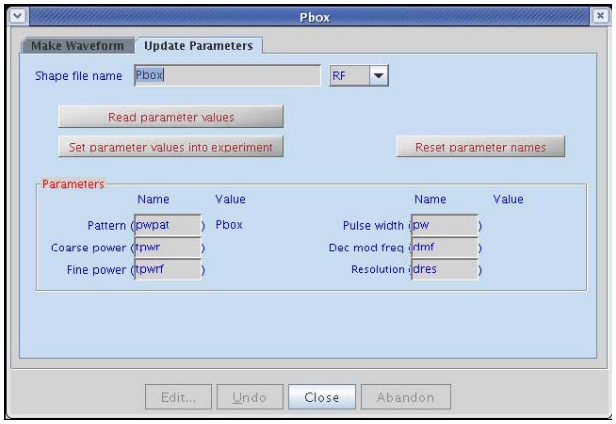
    a. Click a **Show XY** or **Show Z** option button.
    b. Select **Show spectrum** to show the spectrum.
    c. Click **Simulate**.

    The simulation displays in the graphics window.

15.1.2 Update Parameter Values

Update the parameter values used by the waveform into experimental parameters as follows:

1. Click the **Update Parameters** tab, see Figure 92.



Shape file name: Pbox (RF)

Name		Value	Name		Value
Pattern	pwpat	Pbox	Pulse width	pw	
Coarse power	tpwr		Dec mod freq	dmf	
Fine power	tpwrf		Resolution	dres	

Figure 92 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 to use 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 checked (especially for pulse width) — check them.

15.1.3 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 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 Varian 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.

15.1.4 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_pw90, 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 conveniently 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 section 15.1.10 Pbox Commands Using a Terminal, for a more complete description of macros.

15.1.5 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 μs 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 15.1.10 Pbox Commands Using a Terminal.

15.1.6 Pbox File System

Information about the waveform to be created (e.g., 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, 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)
ptype=selective	pulse type (for imaging only)

The number and order of input parameters is 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 μs
minpwg=2.0	Minimum gradient step length, in μs
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 μs
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 B1max product
`pwsw`	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, e.g., 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

15.1.7 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: • 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: • 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 (e.g., 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: 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 n (no), d (default) or any name of a super cycle stored in the wavelib/supercycles directory. By default, it is internally set to d. Super cycles can be nested by separating the names with a comma, for example, t5,m4 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 maxincr parameter.

bscor	Initiates correction for Bloch-Siegert effect in multiple band excitation, inversion or refocusing pulses. Allowed values are y (yes) or n (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, *Abstracts of the 41st ENC*, 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 y (yes, default), n (no shape file header) and 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 y (yes), n (no, default), a (add to the previous simulation), s (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.Pbox_globals file. Note, that Bloch simulator can also be externally activated, e.g., from menus or using the dprofile macro.
T1	Longitudinal relaxation time, T1 in seconds. Can be required by some waveforms (e.g. SLURP pulses). Optional for the Bloch simulation.
T2	Transversal relaxation time T2, in seconds. Can be required by some waveforms (e.g., 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 dcyc 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 ref_pwr. 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 ref_pw90 .
ptype	Pulse type. Only necessary with imaging header. By default, set to selective.

15.1.8 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.

`sh`	Shape name as in wavelib.
`bw/pw`	Bandwidth and/or pulsewidth. For most waveforms, only one of the two parameters is required. Pbox distinguishes between bw (in Hz), which is always greater than 1.0, and pw (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 pw*bw product, which is stored with each individual shape in wavelib. Some waveforms (e.g., adiabatic sweep pulses) can require both bw and pw. In such cases, both variables can be provided in a single string using the "/" separator. For example, {WURST2 200.0/0.05} denotes a 50-ms long WURST-2 pulse covering 200 -Hz-wide band. Alternatively, units can be used for clarity, e.g., {WURST2 0.2k/50m}. If the **`sfrq`** parameter is defined, bandwidth can also be specified in ppm, e.g., {WURST 20p/5m}.
`ofs`	Offset from the center of the excitation band in Hz with respect to the carrier frequency (middle of the spectrum). Note that if the **`sfrq`** spectrometer frequency, (in MHz) is defined, ofs can also be specified in ppm. In order to specify ofs in terms of absolute frequency, the reference offset refofs (i.e., chemical shift value of carrier frequency) must be defined. For instance, {WURST2 20p/5m 170p} sfrq=225.0 refofs=55p.
`st`	Spin status. Defines whether the waveform is used for excitation (st=0), refocusing (st=0.5) or de-excitation (st=1), which, in turn, defines whether the wave starts with phase defined by ph (st=1), the ph occurs in the middle of the pulse (st=0.5), or the pulse ends with phase ph (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 trev parameter. Furthermore, if several waves of different width are generated, they are bound to the beginning (st=1), middle (st=0.5), or end (st=0) of the waveform. The spin status of the first wave is also used by Bloch simulator as the starting magnetization.
`ph`	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 wavelib/supercycles. The difference between this phase cycle and the sucyc parameter is that phase cycling is carried out before waveform mixing and is therefore independent of other Super cycles, whereas sucyc 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.
`fla`	Flip angle, in degrees. Usually, fla is defined in the wave file and there are very few applications where intermediate flip angles are required.
`trev`	Time reversal flag (see `st`). Allowed values are y (yes) and n (no, default).
`d1`	Prepulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
`d2`	Postpulse delay, in seconds. Normally not required. If defined, it disables the internal wave shifting according to the spin status.
`d0`	Pre-d1 delay, in seconds. Essentially repeats d1. It is used only for convenience, e.g., if internal duty cycle is defined in shape parameters in wavelib. If set to 'a', the wave is appended to the previous wave.
`wrap`	Wraparound parameter. Can take values between 0 and 1.0.

15.1.9 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, refer to 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 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.

pbox_name	Extracts name of the last shape file generated by Pbox and stored in the pbox.cal file. Note, that the file name extension is not stored explicitly and is not provided by this macro.
dshape	Displays real (X) and imaginary (Y) components of a shaped pulse. Any type of waveform (.RF, .DEC or .GRD) can be displayed.
pshape	Generates a single-band waveform based on wave definition provided as a single string of wave parameters.
dshapef	Displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in pbox.fid file.
dshapei	Interactively displays the real (X) and imaginary (Y) components of last generated shaped pulse, stored in pbox.fid file
dprofile	Displays the X, Y, and Z excitation (inversion) profile for a pulse shape generated by the Pbox software.
pprofile	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 shapelib/Pbox.sim are plotted.
pph	Prints out the shape file header (i.e., all lines starting with #).
pbox_bw	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. This macro is used mainly in Pbox menus and macros.
pbox_bws	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. Note, 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.
pbox_rst	Resets r1=0, r2=0, r3=0, r4=0, n2='n', n3='', and adds some standard comment lines to the Pbox.inp file. This macro is used in menus and other Pbox macros.
pbox_files	This macro is used only in conjunction with Pbox file menus.

15.1.10 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 42 are available. Note that the output filename is optional and is always the first argument.

Table 42 Pbox Commands and Parameters

Command	Parameter	Action
`Pbox*`	**`-b time`**	Activates Bloch simulator, `opt=a` (add), `s` (subtract), or time in sec.
	`-c`	Calibrate only, do not create a shape file
	`-f file`	Sets name of the output file.
	`-h wave`	`wave` prints wave file header.
	`-I wave`	`wave` prints wave file parameters
	`-l ref_pw90`	Length (in µs) 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 µs) 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 `dshape` and `dshapei` 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 `wavelib` 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
```

15.2 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.

15.2.1 Starting and Using Pulsetool

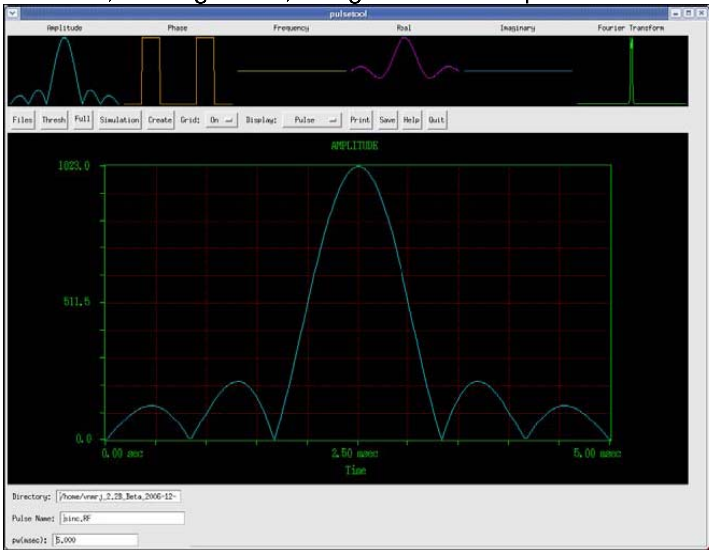
To start pulsetool from VnmrJ:

1. Click **Edit** on the menu bar.
2. Select **View Pulse Shapes…**

   The Pulsetool starts, see Figure 105.

To start pulsetool from an operating system terminal window:

1. Log in a VnmrJ login account owner.
2. Open a **terminal window**.
3. Start **Pulsetoo**l, see Figure 93, using one of these procedures:



Directory: /home/vnmrj_2.2B_Beta_2006-12-

Pulse Name: sinc.RF

pw(msec): 5.000

Figure 93 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`).

15.2.2 Using Pulsetool

Table 43 summarizes the command and parameters associated with pulse shape analysis.

Table 43 Pulse Shape Analysis Commands and Parameters

Command	**Description**
`pulsetool <-shape filepath>`	RF pulse shape analysis
Parameters	**Description**
`phi`	Amount of rotation about the Z-axis
`theta`	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 on 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.

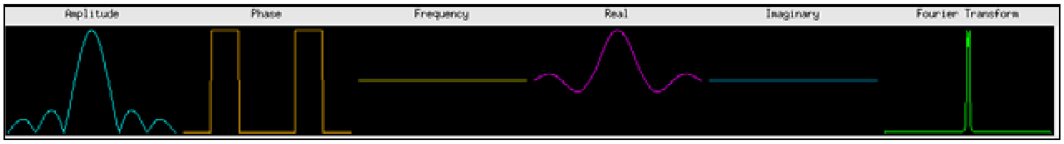


Figure 94 Attribute Selection

The six 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 95 Control Panel Buttons

The control panel below the small windows contains the following control buttons:

Button	**Function**
Files	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: File browser buttons and function: Done — closes file browser Load — selected file is read, and displayed in the graphics windows. An error message is displayed if the file does not correspond to the proper format for pulse template files. Comment lines beginning with the pound character “#” are ignored. 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. Chdir — changes to and then lists the selected directory. Parent — changes to and then lists the parent of the current directory.
Thresh	Activates the horizontal cursor. Refer to Cursors
Full	
Simulation	Opens the Bloch Simulation window. Refer to Simulation.
Create	Opens a dropdown menu of pulse types appears after a right mouse click on 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. Refer to Creating a Pulse.
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.
	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 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

15.2.3 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 that 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.

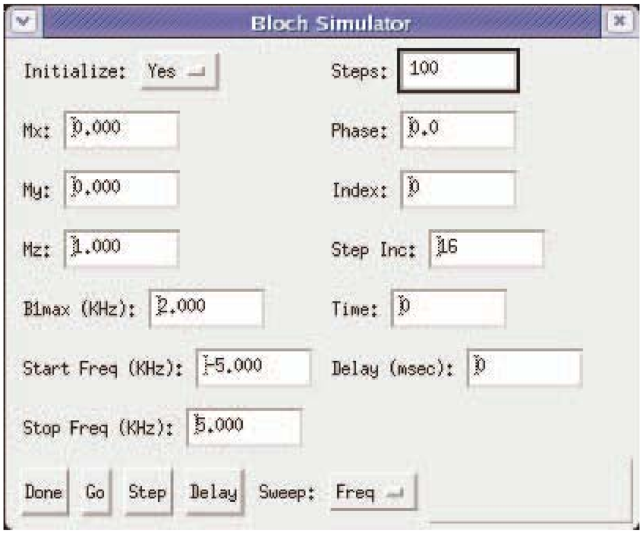
The horizontal cursor is activated with the Thresh button located in the control panel. When this cursor is active, it is controlled interactively with the middle mouse button. The interactive scale and reference functions normally controlled with the middle mouse are not available when the horizontal 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).

15.2.4 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.

15.2.5 Parameters

1. Click the **Simulation** button in the control panel to activate the Bloch Simulation window.



Initialize: Yes
Mx: 0.000
My: 0.000
Mz: 1.000
B1max (KHz): 2.000
Start Freq (KHz): -5.000
Stop Freq (KHz): 5.000
Steps: 100
Phase: 0.0
Index: 0
Step Inc: 16
Time: 0
Delay (msec): 0
Sweep: Freq

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 or type a value for any parameter by selecting it with the left mouse button 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 on 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 on the **Sweep:** button and select **Freq**, **B_1**, or **Time** for the sweep.
   - **Freq**
     a. Type a value in the B1max for B_1 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 B_1 field.
     c. Type a value in the B1 Stop (KHz) field for the ending B_1 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 B_1 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 or 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 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 nonzero 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.

15.2.6 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 phi (the amount of rotation about the Z-axis) and theta (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.

15.2.7 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 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 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.

Chapter 16 Locator and File Browser

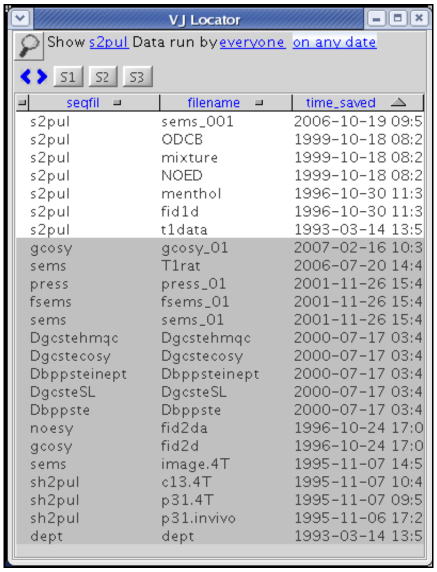
This chapter provides information on the Locator and File Browser.

Sections in this chapter include:

- 16.1 VJ Locator
- 16.2 Locator Statements
- 16.3 File Browser

16.1 VJ Locator

The VJ Locator, Figure 96, is a database browser that provides access to data sets, experiments, shim sets, commands, etc.



Show s2pul Data run by everyone on any date

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
Dgcstecosy	Dgcstecosy	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 96 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:

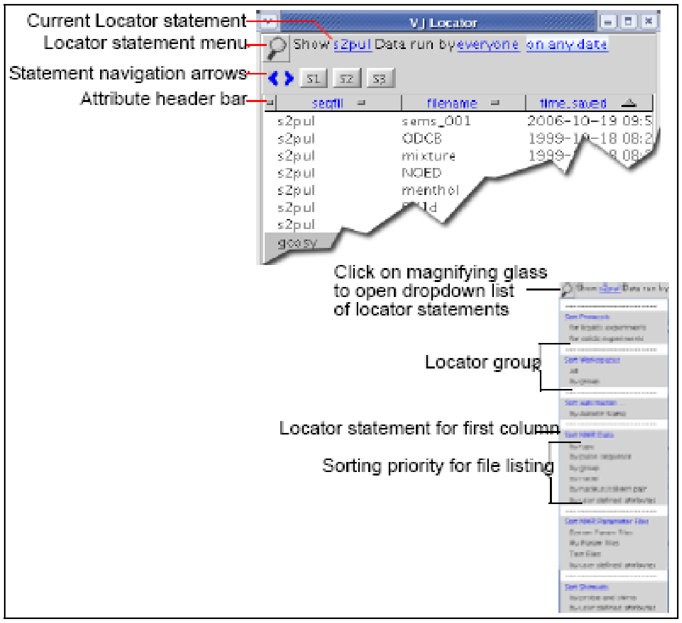
- Objects that meet all criteria
- Some of the boolean terms met
- Remaining objects

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.

16.1.1 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 97.



Show s2pul Data run by everyone on any date

Figure 97 Locator Menus and Controls

Click on the magnifying glass to open a menu of currently available Locator statements. This menu includes both statements provided by Varian, Inc. and those customized and saved by the user.

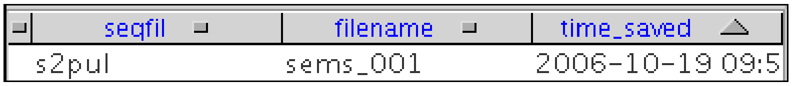
Statements are Locator sentences in which a number of words or phrases are colored and underlined in a manner reminiscent of links in a web page. Each link hides a menu of choices, of which the currently displayed phrase is one. The choices available vary with the types of data currently known to the Locator.

16.1.2 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.

16.1.3 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 mouse 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, a double click on 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.

16.1.4 Attribute Lists

The list of attributes in the dropdown lists are 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 dropdown 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 attributes shown, but cannot add to the list of attributes shown beyond the attributes in the system files.

16.1.5 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 file 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

16.1.6 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

16.1.7 Searches

Clicking 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 the attribute name at the top of the list with the left mouse button brings up a menu of attribute choices.

16.1.8 Dragging and Dropping Items from the Locator

To select an item, click on 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 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 the trash can, selecting it, and then clicking the Restore items button.

16.1.9 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 on the green file name to change it.

Change the file name, click **Return** or click on another line to remove the old name from the Locator and add the new one. The Locator redisplays to show the new name.

16.2 Locator Statements

Varian 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 popup window.
- Click on **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 on **Delete** to remove it or **Cancel** to exit the window without removing the statement.
- Sort **Protocols Entries** shows the known protocol experiments. Double click on 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), but not in the user's individual directories.

16.2.1 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.

16.2.2 Sort Workspaces

Sort all workspace in numeric order. Double -click on a workspace to join the workspace.

16.2.3 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 98.

Show Std1D experiments created by varian and me on any date

Figure 98 Generic Locator Statement

There are two separate underlined choices in this statement: Std1D, on any date.

Click on either of the underlined phrases to produce a dropdown menu of the choices in this 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.

16.2.4 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.

16.2.5 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 by when using the Save Shims button in the Shim panel.

16.2.6 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.

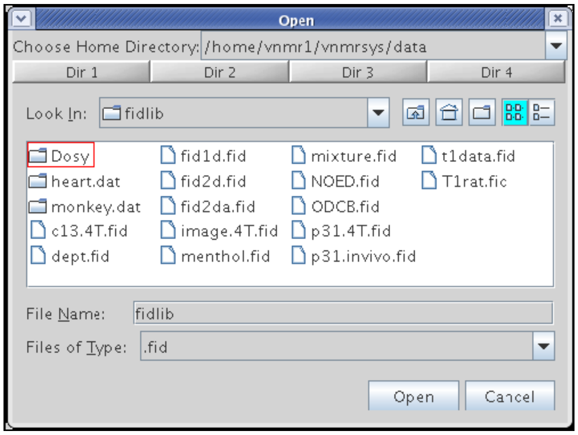
16.3 File Browser

File Browser Buttons and Drop Down Menus

16.3.1 Open a File

Button	**Description**
⬆	Go up one level in the directory tree.
⌂	Go to user's home directory.
🗀	Make a new folder in the current directory.
☷	Show list of files and directories at the current directory level.
☰	Show details of files and directories at the current directory level.
Open	Open selected file. Load into current experiment if it is a VnmrJ data file, sequence, or parameter set.
Save	Save file with the name shown in the **File Name:** field using the extension shown in the **Files of Type:** field.
Cancel	Cancel selection and close the file browser.

1. Click **File**.
2. Select **Open** to open the file navigation window to locate the required file.
3. Click the file and click **Open** button.



Choose Home Directory: /home/vnmr1/vnmrsys/data

Look In: fidlib

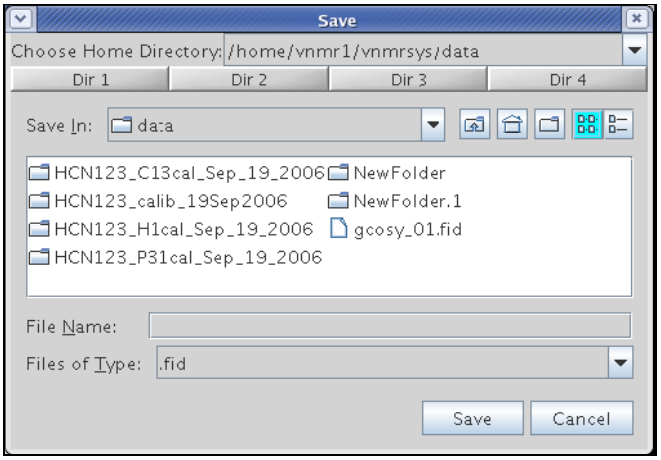
Dosy
heart.dat
monkey.dat
c13.4T.fid
dept.fid
fid1d.fid
fid2d.fid
fid2da.fid
image.4T.fid
menthol.fid
mixture.fid
NOED.fid
ODCB.fid
p31.4T.fid
p31.invivo.fid
t1data.fid
T1rat.fic

File Name: fidlib

Files of Type: .fid

16.3.2 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**.



Choose Home Directory: /home/vnmr1/vnmrsys/data

Save In: data

Files of Type: .fid

Appendix A Variable Temperature System

This chapter describes startup and operation of the optional variable-temperature (VT) unit. A VT unit is available for Varian, 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.

Sections in this appendix include:

- A.1 VT Setup
- A.2 VT Startup
- A.3 Temperature Array
- A.4 Operating Considerations
- A.5 VT Error Handling
- A.6 VT Controller Safety Circuits

A.1 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 Typeed temperature, the system selects either the high-temperature or low-temperature VT gas flow.

A.2 VT Startup

The VT hardware must be installed and calibrated as described in the *VT Accessory Installation* manual. Starting up the VT unit takes 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.

A.3 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 set point.

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.)

A.4 Operating Considerations

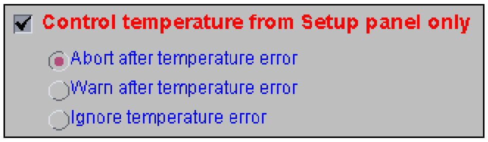
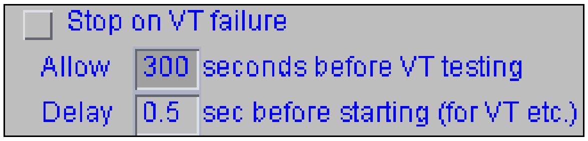
Use the following recommendations to help achieve better VT performance.

- 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

A.5 VT Error Handling

Select how VT errors are handled in the Spin/Temp page under the Start folder.

Interlock selections (Start folder, Spin/Temp page)	Wait setting (Acquire folder, Flags page)
 ☑ Control temperature from Setup panel only ● Abort after temperature error ○ Warn after temperature error ○ Ignore temperature error	 ☐ Stop on VT failure Allow 300 seconds before VT testing Delay 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 (`vtwait`; the "wait before testing setting"). This time is independent of the delay setting (`pad`). If regulation is not established after the wait time (`vtwait`; 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.

A.6 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 sensor.
- 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.

Refer to the *VT Accessory Installation* manual for system failure analysis.

Appendix B Shimming Basics

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 99 to provide a visual reference for the interactions of the shims.

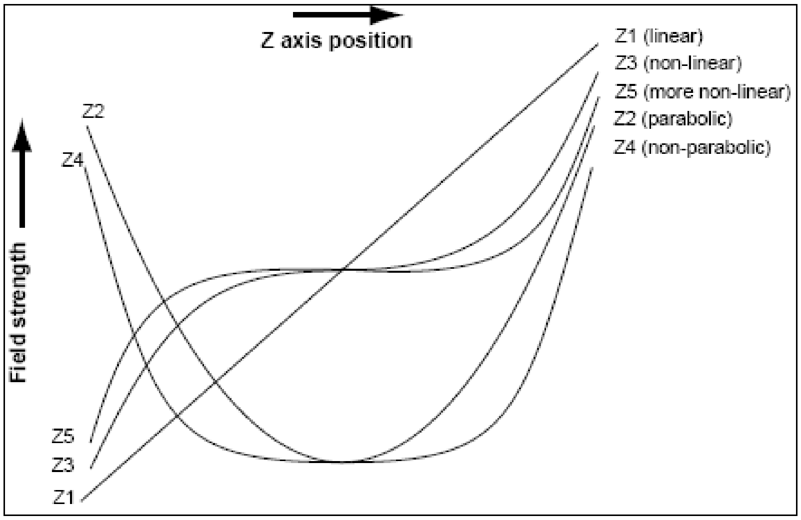


Figure 99 Approximate Shape of Axial Gradients

Understanding the effect of various shims on the symmetry of the resonance is important in simplifying the shimming 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.

Sections in this appendix include:

- B.1 Shim Interactions
- B.2 Autoshim Information
- B.3 Homogeneity Commands and Parameter

B.1 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 Varian Ultra•nmr shims, follow the theoretically predicted response very closely. Other shim systems, with more interactions between shims, produce different results.

B.1.1 Theoretically Perfect Lineshape and Effect of Z1 Shim

Figure 100 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.

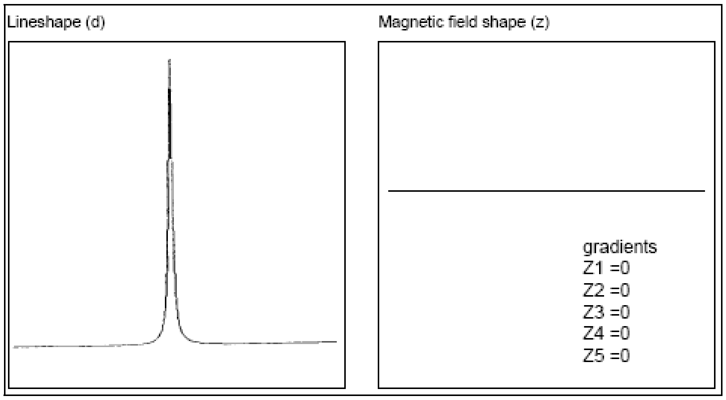


Figure 100 Theoretically Perfect Lineshape

Figure 101 shows how changing the linear shim Z1 affects both the lineshape (left) and the magnetic field (right).

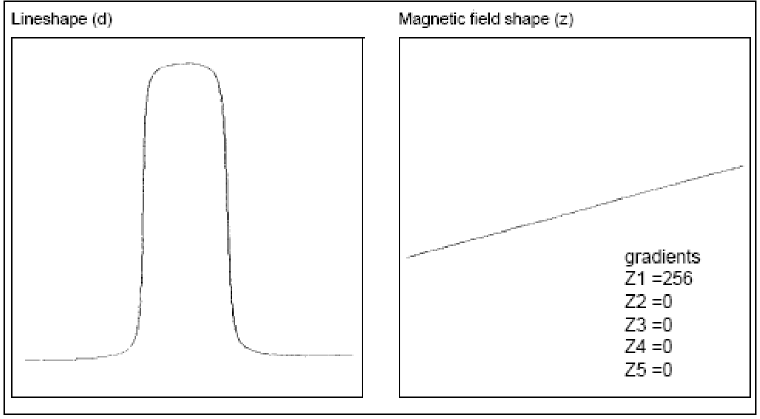


Figure 101 Effects of Linear Shim Z1

B.1.2 Effects of Even-Order Shims Z2 and Z4

Figure 102 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.

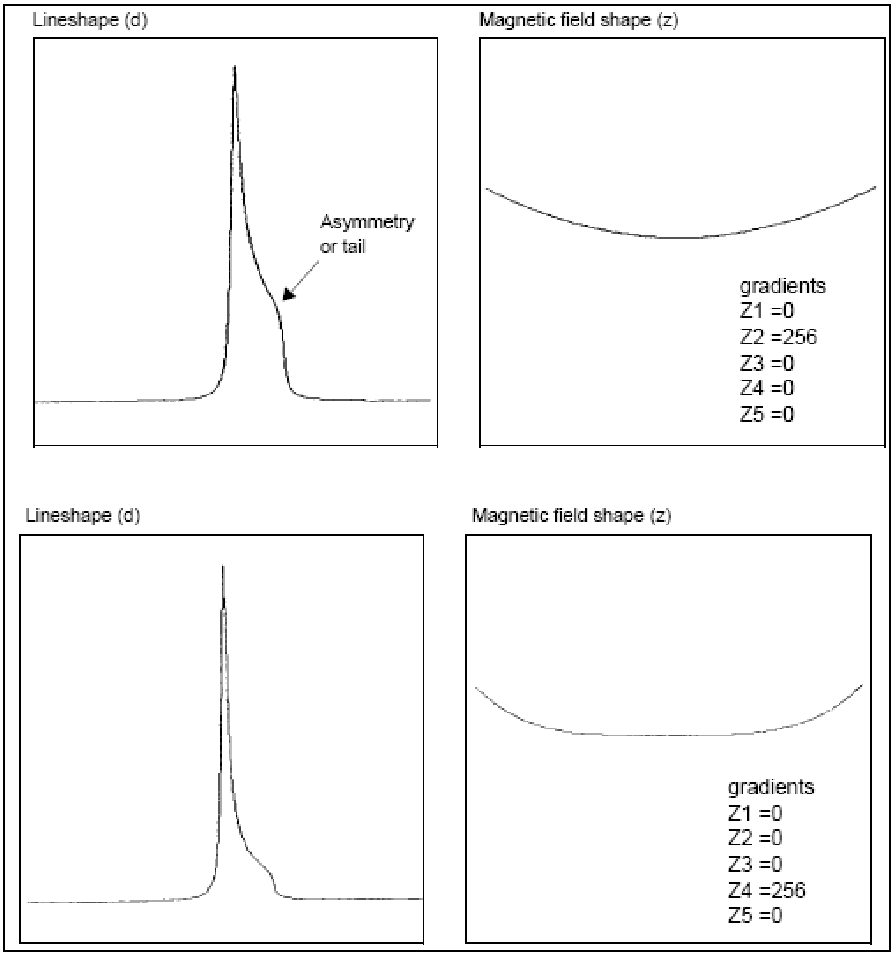


Figure 102 Effects of Even Order (Parabolic) Shims Z2 and Z4

B.1.3 Effects of Odd-Order Shims Z3 and Z5

Figure 103 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).

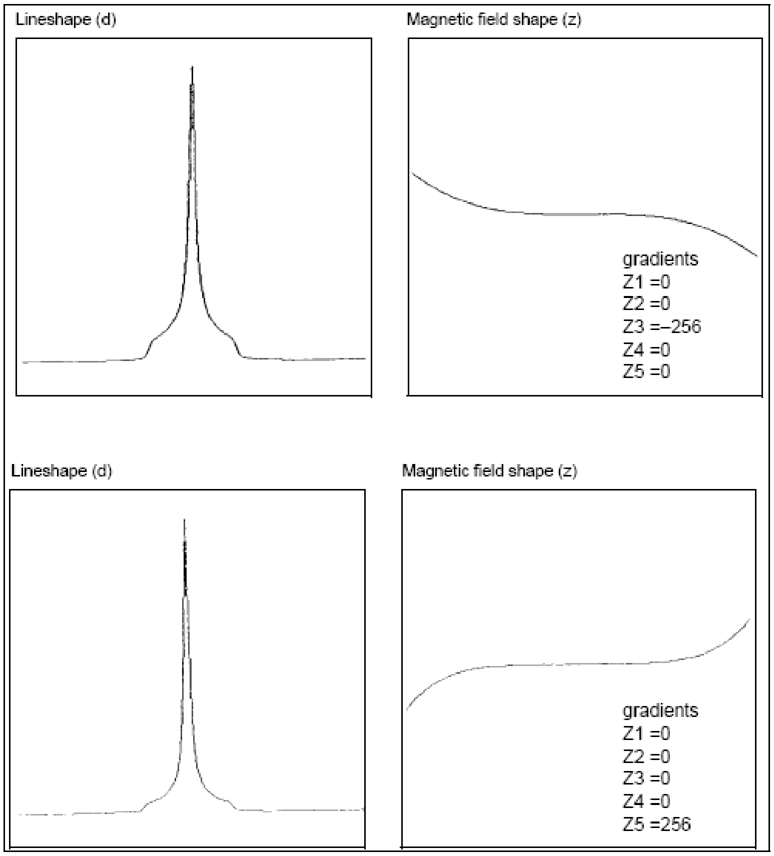


Figure 103 Effect of Odd Order (Non-Linear) Shims Z3 and Z5

B.1.4 Effects of Improperly Adjusted Shims

Figure 104 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.

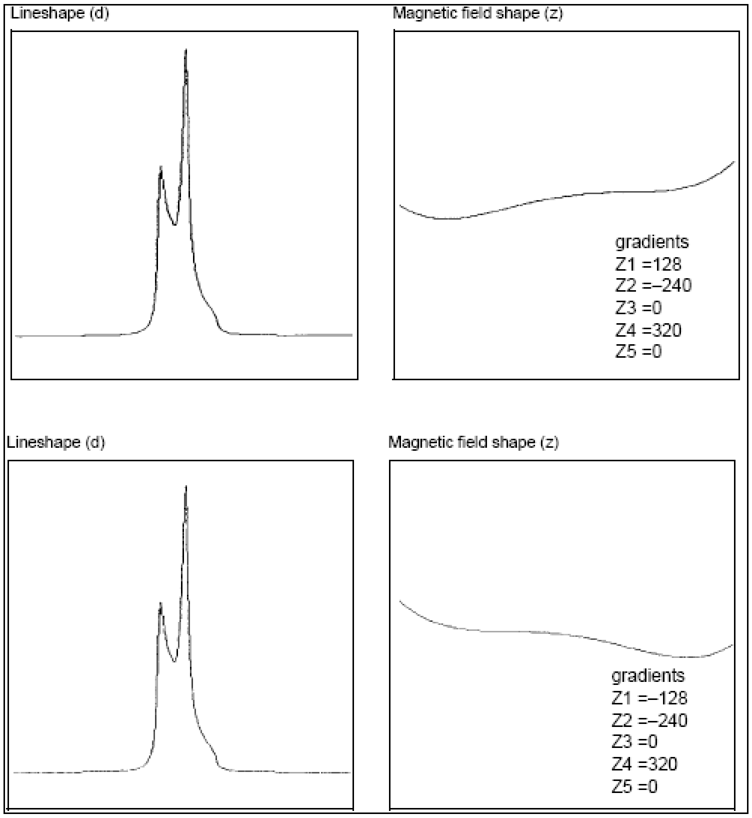


Figure 104 Effects of Misadjusted Shims

B.1.5 Effects of Non-Spin Shims

Figure 105 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.

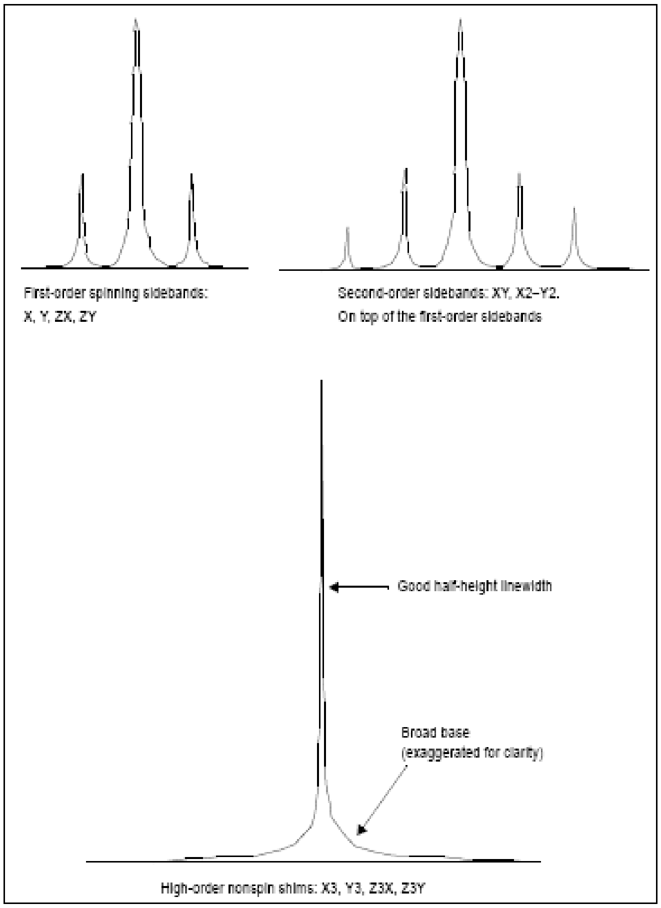


Figure 105 Effects of Nonspin Shims

B.1.6 Summary of Shim Interactions

Table 44 lists some lineshape effects associated with shims. Note that 13-channel shim systems (shimset=1) do not have Z5, Z3X, ZXY, etc., and that 14-channel shim systems (shimset=10) have Z5 but do not have Z3X, ZXY, etc.

Table 44 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)

B.2 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.

B.2.1 Hardware Autoshim

Hardware autoshim methods vary according to which system 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 (e.g., 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.

B.2.2 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.

B.2.3 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.

B.2.4 Using the Input Window

Type `method=file shim`, where `file` is the name of a file in the directory shimmethods (e.g., 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, Typeing 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 Typeing method='z1z2' shim method='nsm' shim shim shim would cause the first method to be used once and the second method three times.

B.2.5 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 be the final resolution? 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 45, there are five criterion values: B (bad), L (loose), M (medium), T (tight), and E (excellent). (The lower-case letters are used when Typeing criterion values into a shim method, discussed below).

Table 45 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 at 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.

B.2.6 Shim Methods for Autoshim

Table 46 lists standard two-character codes for shim gradient combinations.

Table 46 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
t3	X,Y,XZ2,YZ2,Z1	5030004
t4	X,XZ,X3,XZ2,Z1	4450004

Standard Code	Gradients	Hexadecimal Code
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 shimmethods 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 (e.g., 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.

Refer to 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.

B.2.7 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
ZXY	XZ2	ZX2Y2	YZ2	Y3	X3	YZ	X2Y2	XY	XZ	Y1	X1	—	—	—	—	—	—	—	Z5	Z4	Z3	Z2C	Z2	Z1C	Z1	Z0	—

To construct a method for this example, first notice in the diagram that Z1 is represented by a 4 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 46

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 perform 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 Typeed into the shimmethods file using a text editor such as vi. The macros newshm and stdshm 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 while
- 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.

B.3 Homogeneity Commands and Parameter

Command	**Description**
`dgs`	Display shim & 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(fi`le)<:status>	Save shim coil settings

Command	Description
`*sethw(<'wait'|'nowait',>par1,val1<,par2,val2,...)`	

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 shimmethods}`	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