

1 [Varian Basic 1D data acquisition Step-by-step \(for VnmrJ 4.2\)](#)

1.1 Initial set-up

Getting started

Log in to your account, then double click the **VnmrJ** icon to start the program.

Starting a new data set

“Recall the parameters” for the **vnmr pfg probe 1H** experiment by typing **UVMproton** (this is a macro).

Under **Start > Sample Info**, fill in the following details (see Figure 1.1):

→ **Sample Name**

As a best practice, researchers should start data with **ABC-N-###**, where ABC are the researcher’s initials, N is the lab notebook number, and ### is the three-digit page number of your experiment. A timestamp will be added to your sample folder, and a new data folder will be created after you hit **[enter]** ↵. OR you may save your data after it has been acquired, and leave this field blank.

→ **Solvent**

Check that the depressed solvent button matches your sample solvent, e.g.: **[CDCl₃]**. If you are using a different solvent such as D₂O, you may want to ask about tuning the spectrometer to get the best sensitivity for your spectrum.

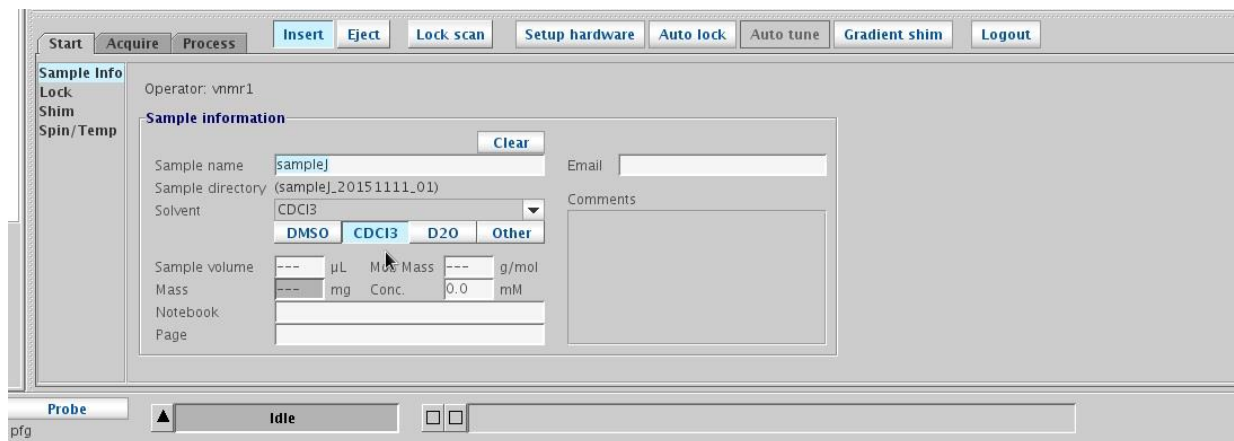


Figure 1.1: Key fields under Start>Sample Info: Sample Name, Solvent, and Comment.

Placing your sample in the instrument:

Turn on the lift air by clicking the **[Eject]** button (see Figure 1.1 above) or using the command **e**. Rest the spinner on the depth gauge and then insert the NMR sample into the spinner, making sure it is centered (especially if you don’t have ~600μl of solvent!).

Handling the glass NMR tube itself, rest the sample and spinner on the lift air in the top center of the magnet. Be careful not to push or rest on the magnet: swaying of the magnet could cause a catastrophic failure of the magnetic field and the instrument could “quench”.

Press the **[Insert]** button or use the command **i** to turn off the lift air, and the sample will drop into the spectrometer.

1.2 Lock, spin, and shim

Optimizing your lock signal

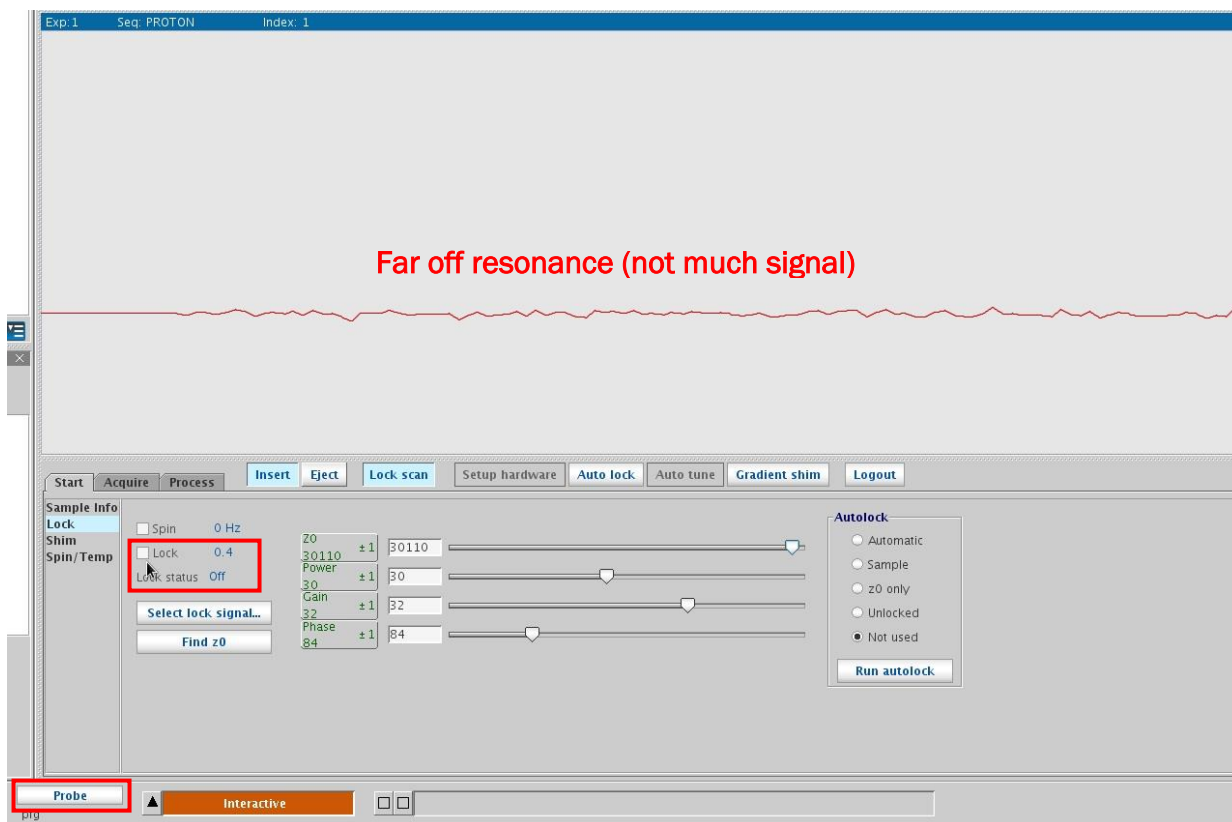
Under **Start > Lock**, click **[Lock Scan]** to find the deuterium “on resonance” signal. The display will go from being blank to showing a red line, which shape will depend on how close to ‘on resonance’ the Z0 field is set to. Start in the “unlocked” mode, so make sure the box by ‘Lock’ is unchecked. It is a good idea to begin with good shims, you will do this from the command line by typing **LoadShims** (one word; this is a macro which will read in good starting shims that are updated monthly by the manager).

Finding the “on resonance” condition can be tricky and will depend on the solvent. You will be optimizing the following fields: (see the chart posted under the monitor for values for different solvents).

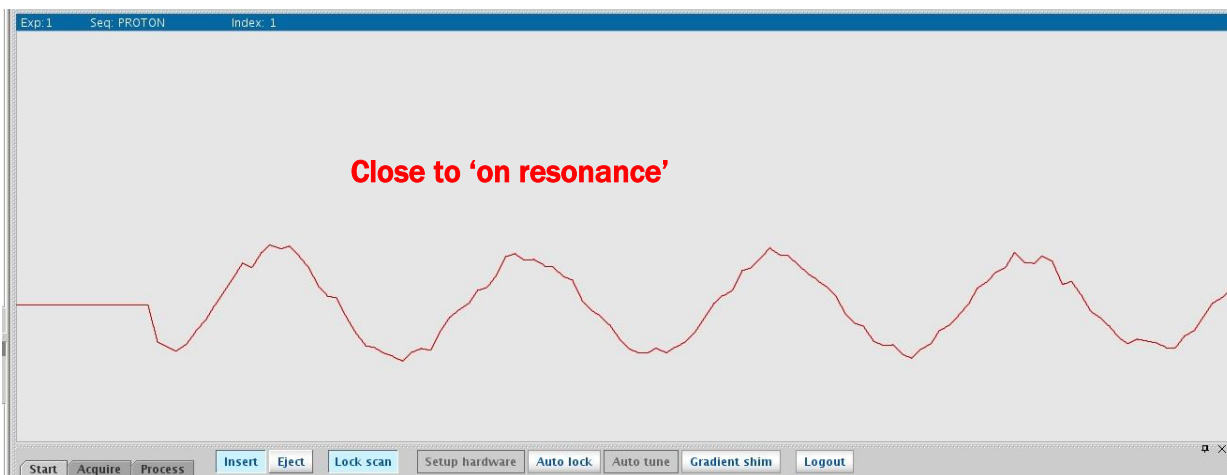
- **Field (Z0)** › will vary greatly, depending on solvent (see chart for approximate value)
- **Power** › it is important to get to the correct value, since this excites the ^2H s in the solvent
- **Gain** › increase or decrease as necessary (this is like the volume on the stereo)
- **Phase** › should be checked before and after shimming (value will be near 90)

To find ‘on resonance’, increase Lock **Power** to a few units above the recommended value for your solvent (see chart). Then increase Lock **Gain** to a few units above this value (to better enable seeing your Lock Signal).

Next we will play with **Field (Z0)** to narrow in on the ^2H resonance of your solvent. You may use the slider for course adjustments to the **Field (Z0)** value. If you are far from the ‘on resonance’ condition, you will see:



When you get close to your solvent’s resonance condition, you will start to see a sine wave. You want to move **Z0** in the direction in which you will get fewer and fewer waves.



When you get to this point, you may use the left/right mouse buttons on the Z0 panel to get to 'on resonance' where you will see 1 square wave. Middle mouse button will change increment amount, so start with +/-100.

Then increase/decrease Z0 (± 10 , then ± 1) to raise the "square wave" to the highest possible lock level. The lock level is the number to the right of 'Lock'. If you reach a lock level of 100 (ie. topped out), first reduce Power to the value for your solvent (see solvents chart).

Go back to adjusting Z0 to find the maximum lock level. If the lock signal tops out again, this time turn the Gain down then continue adjusting Z0. (Gain is similar to volume on your stereo, it only adjusts the display). Once you are at maximum possible lock level, check the box next by 'Lock' and look for Lock Status: Regulated.

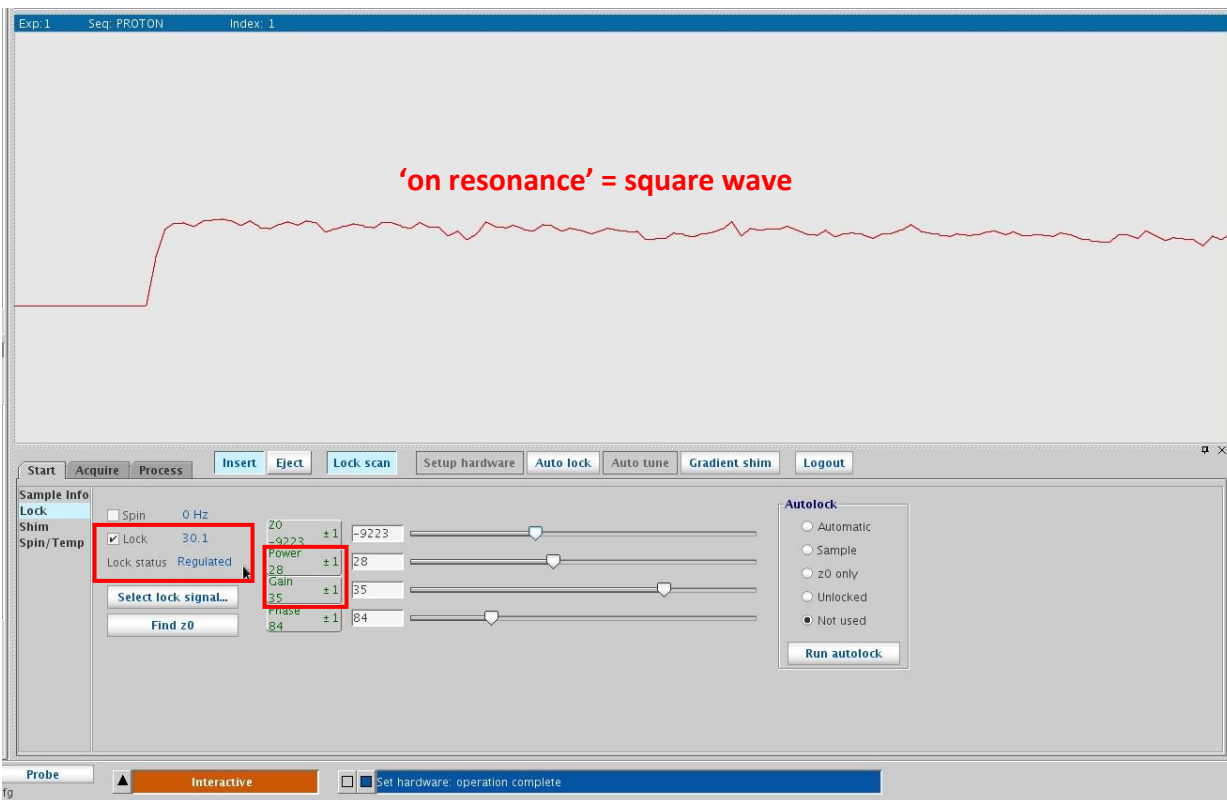


Figure 1.2: The lock display: locating the "square wave" before shimming.

Tuning and matching (optional).

If you are using a sample with high dielectric solvents, ionic solutes, or highly concentrated samples, you may want to tune and match the instrument. For routine ^1H 1D experiments this will not be necessary. However for calibration the pw90 or finding T1 values for a 2D experiment, this is recommended.

Spinning the sample (optional).

Check the box next to 'Spin'. Or you may go to **Start >Spin/Temp** tab and click **Regulate Speed**, first ensuring that the slider bar is set to 20. The green spin light will start flashing; on reaching a steady speed of 20 Hz, it will stay on.

If you do not want to spin, do not check the box next to 'Spin' and instead under **Start >Spin/Temp**, drag the spin rate slider to **zero**. Otherwise, your sample may start spinning when you start acquisition.

Spinning should NOT be attempted for:

- 2D experiments (artifacts may be introduced)
- T1 relaxation measurements; 1D NOE type experiments
- top-heavy J. Young tubes. They may break in the spectrometer upon spinning.
- NMR tubes not rated at ≥ 500 MHz (users are required to **discard** and replace!)

Shimming

Currently, the Varian cannot be shimmed automatically. SORRY!

It is the user's responsibility to learn and execute good shimming to get the best possible lineshape (symmetric, narrow, with very little broadening at the base of the peak). Becoming skilled at quick manual shimming does take a little practice and patience. Definitely start by reading in the current "optimized" shim file, by clicking on **[Probe]** at the bottom of VJ and then **[Load Shims]**, approximately the 3rd line down in the 'Probe GUI' that pops up. The most common basic shimming technique is presented below.

Shimming on the Lock level – routine ^1H spectra

A basic shimming technique is to use the "clock" display to reach the highest possible lock level. In general, the stronger the lock signal, the taller and narrower the peak. This is a fast method of shimming that gives decent results if starting shims are not poor and your sample has been prepared well (not too concentrated, sufficient solvent, etc).

Under **Start >Shim**, adjust the following axial shims: **Z1** and **Z2** to obtain the highest possible lock signal. If you reach a Lock level of 100, then turn the **Gain** down by one or two units and continue shimming.

Tips for basic quick shimming:

- Start with larger increments and work down (± 100 , ± 10) for **Z1**, then **Z2** (± 10 , ± 1).
- Go back to **Z1** (± 10 , ± 1) and **Z2** (± 1).
- Finally, ensure that **Z1** is the last shim you optimize! It is the most important shim.
- **Z1** and **Z2** are interdependent, so it is important that you shim them iteratively, as described.
- At some point during the process also check **Lock Phase**, to see if lock level may be incremented. If **Z2** requires large changes, it often affects **Lock Phase**.
- Remember that careful shimming will dramatically impact the quality of your data!

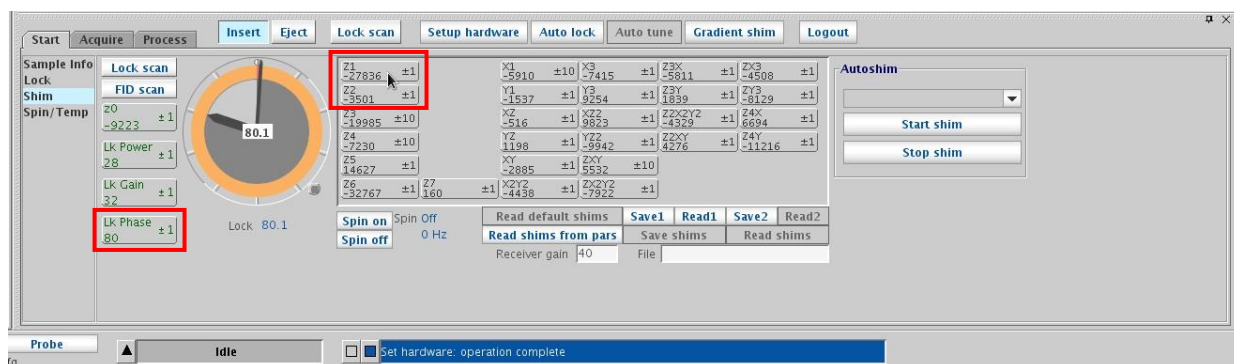


Figure 1.3: The Shim window: For basic shim adjustments the axial shims are adjusted for maximum lock signal visible in the Shim “clock” display.

Note that one can check quality of shimming by examining both the FID and the real-time transformed spectrum before gathering data. Click the [Fid Scan] button in the Shim window to access these. OR you may also judge your shim quality by acquiring 1-scan, then Fourier transforming it with the ‘wft’ command, and then looking at either the TMS peak or a sharp singlet amongst your solute peaks. The TMS peak should be a singlet and the small ²⁹Si satellites should be visible on both sides of the peak at about 0.01ppm. You may measure the linewidth of TMS by placing the cursor on the peak and typing ‘nl’ followed by ‘dc’, and then ‘dres’. This will give you the width-at-half-height. This value should be around 1.0 Hz or smaller for best shim quality (although a value of 1.5 Hz is still pretty decent).

1.3 Setting experimental parameters

Basic parameters

Under **Acquire > Default H1**, modify your basic experimental details as necessary:

- **Spectral Width (sw)**: This is the range of your spectrum; default is -2 ppm to 14 ppm
As a rule of thumb, always extend the **sw** at least 1 ppm beyond observed peaks.
- **Number of scans (nt)**: Generally 8 scans may be sufficient, depending on sample concentration. The more scans you acquire, the better your signal-to-noise ratio. So why not use nt=16 or 32 for a better spectrum, since you’ve already spent time locking and shimming (make sure to use multiples of 8!).
- **Autogain** should be checked if you would like receiver gain to be set automatically before acquisition. Or set **Receiver Gain** manually by starting with a high value for ‘gain’ (gain=50) and making sure that you don’t get an “ADC overflow” or “receiver overflow” error. If you do, set gain to a smaller number and re-acquire. You want this value as high as possible for better signal-to noise.
- **Pulse Angle**: default is 45 degrees and doesn’t need to be changed for a routine ¹H 1D spectrum.

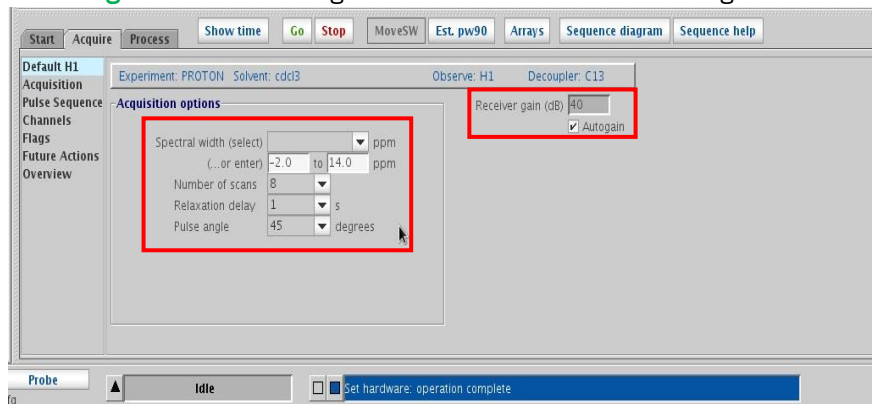


Figure 1.4: The Default 1H window with basic parameters pictured.

Under **Acquire > Acquisition**, other experimental parameters can be changed, such as:

- **Acquisition time (at)**: a longer **at** will result in better resolution, although do not exceed 20 sec!
Note: do not make the acquisition time *too* much longer than is necessary, as continuing to acquire data after the complete decay of the FID adds noise to the spectrum.
- **Relaxation delay (d1)**: (1 – 2 s is typical). This may also be accessed from **Default H1**. For a quantitative spectrum, this will typically need to be increased, depending on the T1 values of the ¹Hs in your sample.
- **Observe Pulse** (pulse angle): default is 45 degrees. This may also be accessed from **Default H1**.

You can view the full set of experimental parameters under **Acquire > Overview**. (type 'dg' for it to update)

For automatic processing options, see **Acquire > Future Actions** (Figure 1.5). By default, the **UVMproton** parameters are set to automatically process and then save data once the experiment finishes.

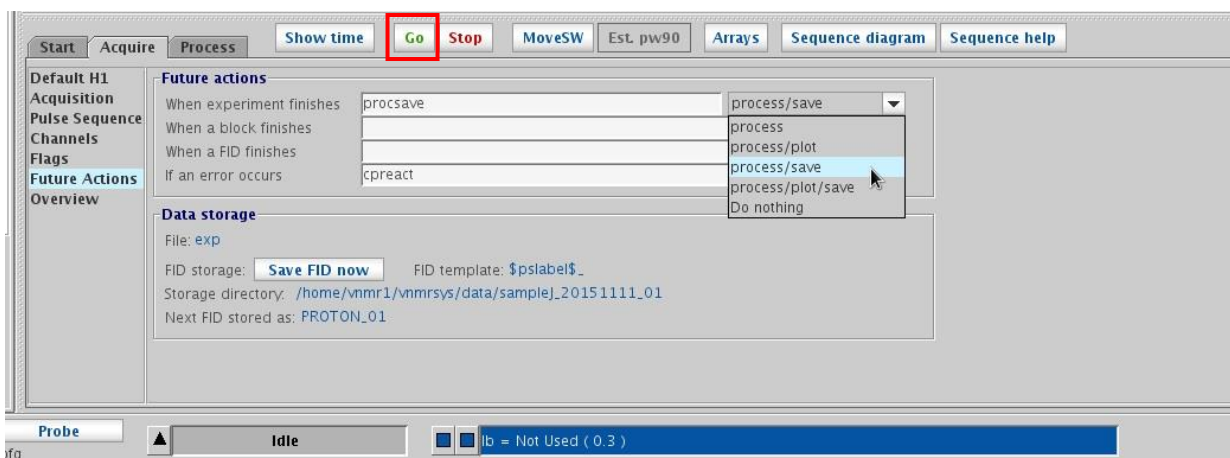


Figure 1.5: **Acquire>Future Actions** sets additional options, such as automatic processing and data saving options.

1.4 Data acquisition

To start acquiring data, click the green **[Go]** button (see above Figure 1.5).

If you need to stop acquisition, click the red **[Stop]** button or use the “abort acquisition” command **aa**; this will only save the data until the last ‘bs’ acquired (block size; bs=4 for ‘UVMproton’).

You will be able to track the acquisition status and remaining time in the status bar at the bottom of the window.

Note that if you are using the **UVMproton** experimental parameters, basic data processing and saving is done automatically by default (IF you use the ‘Go’ button to acquire).

When the experiment is finished, your spectrum will display with automatic phasing having completed. The FID is saved to the folder:

`/home/username/vnmrsys/data/ABC-N-###_datestamp/`

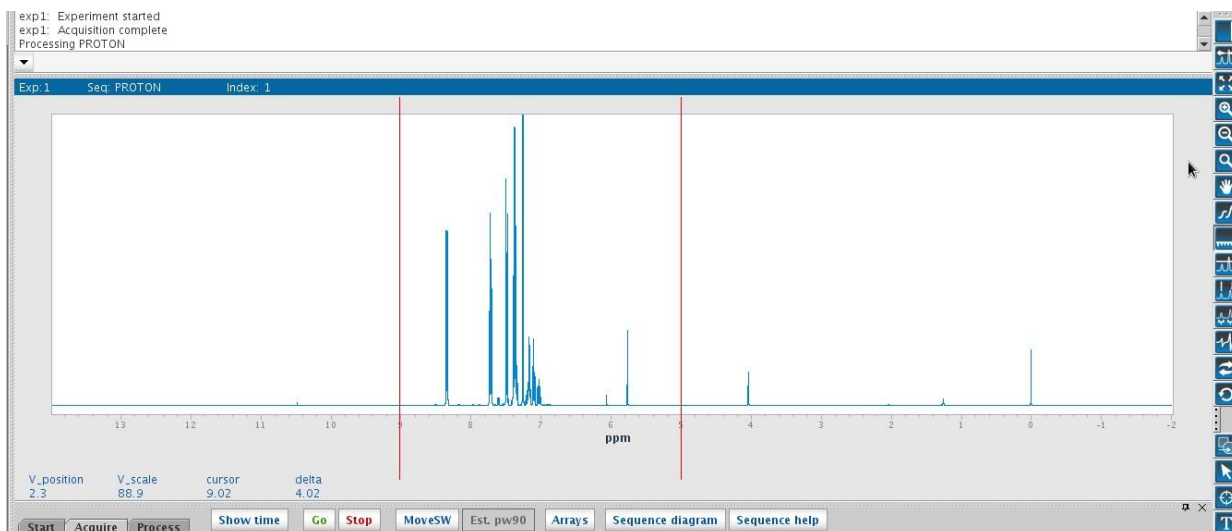






Figure 1.6 After acquisition, the processed, phased, and saved spectrum displays.




1.5 View, process, print, save (OR use MNova on computer in room A217)

Basic processing:

If automatic phasing is not satisfactory, use the phasing button  on the right-hand side of the window to start interactive phasing. Centered around the region of interest, left-click-and-drag to adjust zero-order phasing. Right-click-and-drag to adjust first-order phasing (opposite side of spectrum).

Use left/right mouse buttons to define regions and buttons on the right    to **zoom in/out/full**.

Under **Process > Integration**, the integral under your cursor may be calibrated to a desired value.

Pick peaks by setting a minimum **peak threshold**: click the peak-minimum button  and drag the yellow bar on your spectrum to the desired minimum peak height. Type **dpf** to display peaks above that value. Return to the standard spectrum mode by clicking the return button  and then the view spectrum button .

→ NOTE: If for some reason you do not see your most recent spectrum, type **wft** or **ds**.

Plotting your spectrum:

The printer on the Varian NMR spectrometer was removed, so you will not be able to print directly from this computer. You may however save a *.pdf file and e-mail it to yourself. It is highly recommended that you process your data in Mestrelab instead, since processing is much more straightforward in that program (you may use the computer across the hall, in room A-217).

Under **Process > Basic**, first click **[Process]** then click **[Auto Preview]** to print a PDF of your spectrum (specify whether you would like to display integrals and peaks in the drop-down menus above the button).

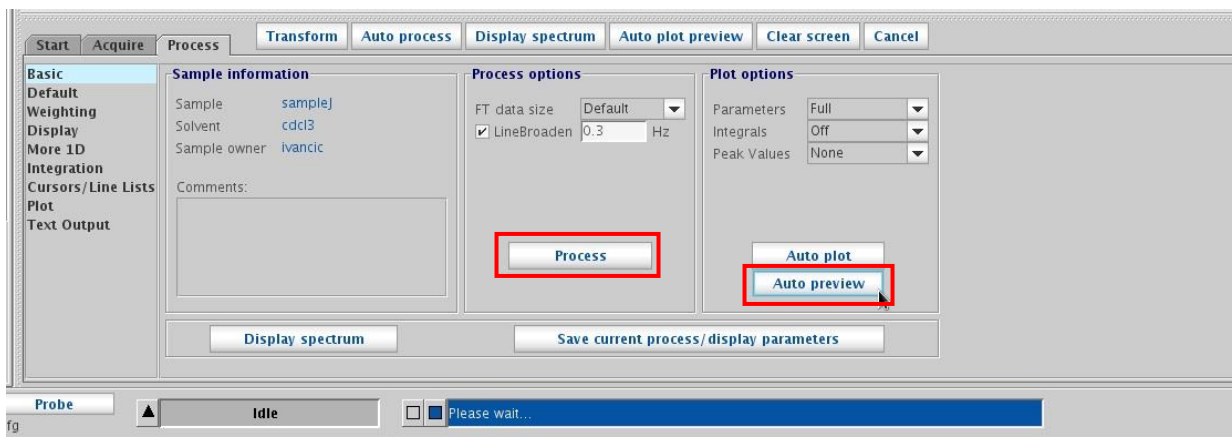


Figure 1.7 Basic plot options after acquisition and processing

Click the **Send to:** File check box, which saves the PDF to the folder:

`/home/username/vnmrsys/data/ABC-N-###_datestamp/plots/.`



Figure 1.8: Saving your plot to PDF using the [Plot Preview] button

As of October, 2015, the printer on the Varian has been removed. Either save your data to a *.pdf file and FTP it to your personal computer or e-mail it to yourself OR go use the computer across the hall in A-217 to process your data in MestreNova.

► If you did not use the **UVMproton** method or you didn't click the green 'Acquire' button to start acquisition, your data may not have automatically saved. Click **File** and then **Save As** to specify a file name and location for your data manually.

Final steps

Turn lock and spin off from **Start >Lock**, by unchecking 'Spin' and 'Lock'.

Turn the lift air on by clicking **[Eject]** or typing **e**, and carefully remove your sample from the instrument.

Turn the lift air off by clicking **[Insert]** or typing **i**

Exit the program by clicking: **File>Exit VnmrJ** or by typing **exit**. **DO NOT use the 'X' in the upper right corner!!!**

Log out of your account by clicking **System>Log out username**. Remember to take your sample tubes and printouts when you go.

Back up your data

► Remember to **download your data folder** onto a USB Key or remotely using FTP software such as WinSCP. Use your instrument-specific login name and password with this FTP address: **132.198.10.87**