Automated 2D Instructions
Varian VNMRS 500 MHz NMR

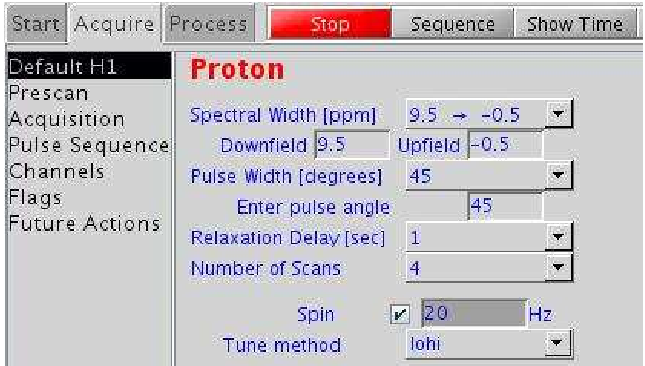
Gcosy Instructions

1. Log in to the walkup account in VNMRJ if the system isn't already logged in (password keyes208).

2. Set-up a PROTON 1-D experiment, as you would normally, with the following additions. Make sure to select the Tune option as well as Auto Lock and Gradient Shim. (If you are going to do a NOESY, it is usually best to turn off spinning. Running this survey spectrum without spinning allows you to check the homogeneity and signal to noise. Also, for a NOESY control the temperature, see below).
Submit your run to the Day queue. Display the spectrum with the full chemical shift range.

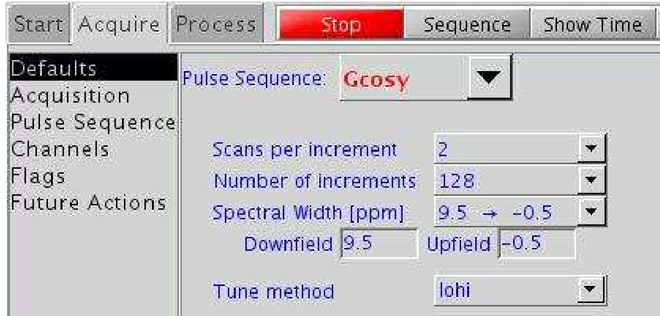
3. Switch to the Homo 2D tab in the Experiment list. In the experiment tab, choose Gcosy if spectrometer time is short and Gdqcosy if more time is available (Gdqcosy takes twice as long as Gcosy).[1] Double click on the green Proton line in the queue and click on the sample number in the autosampler display (as usual to begin the experiment set-up). Choose the Study page in the Start tab. Check that the sample name and solvent are correct. Deselect the AutoLock, Tune, and Gradient Shim options if the sample is already locked, tuned, and shimmed. (Turn off spinning for a NOESY). If you want to use the default parameters, jump to step 6.

4. Switch to the Acquire tab and the Default H1 page. Narrow the spectral width to include only the peaks of interest, by selecting the Spectral Width using the drop-down menu or the dialog boxes:



If your sample is very concentrated, you can also save time by decreasing the Number of Scans to 4.

5. Double click on the green Gcosy or Gdqcosy entry in the automation queue. Switch to the Acquire tab and the Defaults page. Check to see that the spectral width is set to the same values as your Proton spectrum from step 4. If spectrometer time is available, you might consider increasing the Scans per increment to obtain better signal to noise, especially if you have a dilute sample:



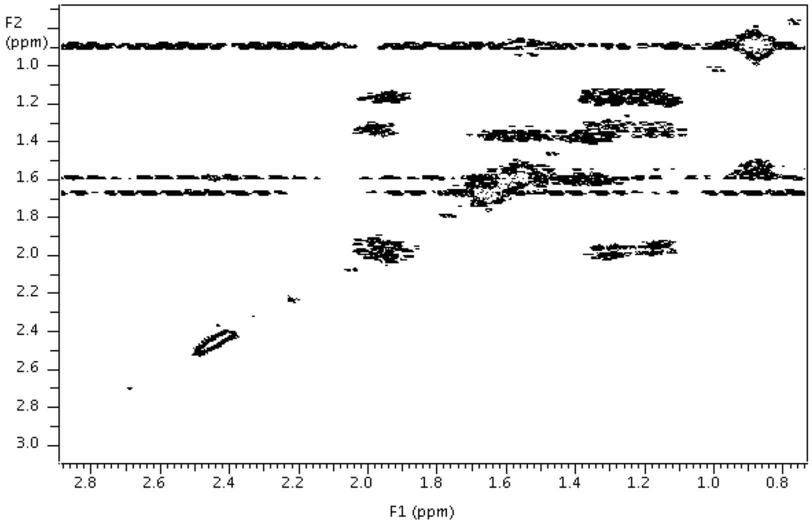
Check to make sure that the experiment will run in the time allotted by clicking on the Show Time button:

The experiment time will be displayed in the message line at the bottom right side of the VNMRJ window. If the listed time is too long, decrease the value of the Scans per increment.

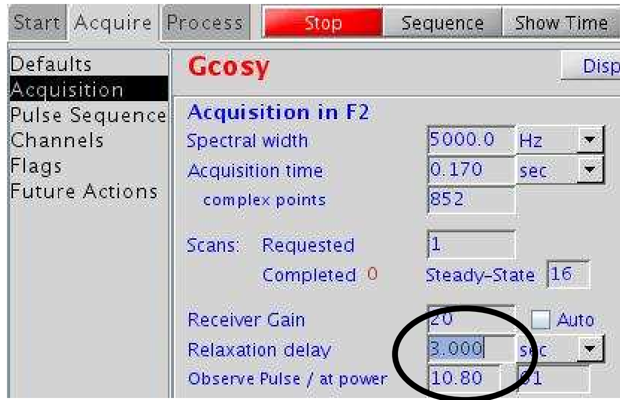
6. Submit the run to the automation queue. Continue as normal.

7. When complete, double click on the Gcosy or Gdqcosy line for your sample in the Automation queue window. After the spectrum is displayed you can edit the results using the instructions listed below.

8. The Gcosy spectrum is symmetrized by default, while the Gdqcosy is not. In unsymmetrized spectra, tall peaks often show noise ridges parallel to the F1 axis, called t1-ridges:



In the symmetrization process for Gcosy's, these t1-ridges can cause false cross peaks. Such spectra can show cross peaks for most every diagonal peak, including singlet methyls. If your Gdqcosy spectrum shows extensive t1-ridges or your Gcosy shows cross peaks for most everything, you should consider rerunning your COSY with a longer relaxation delay. The relaxation delay should be set in step 4 when you set the Scans per increment. Select the Acquisition page under the Acquire tab and type in the new Relaxation delay:



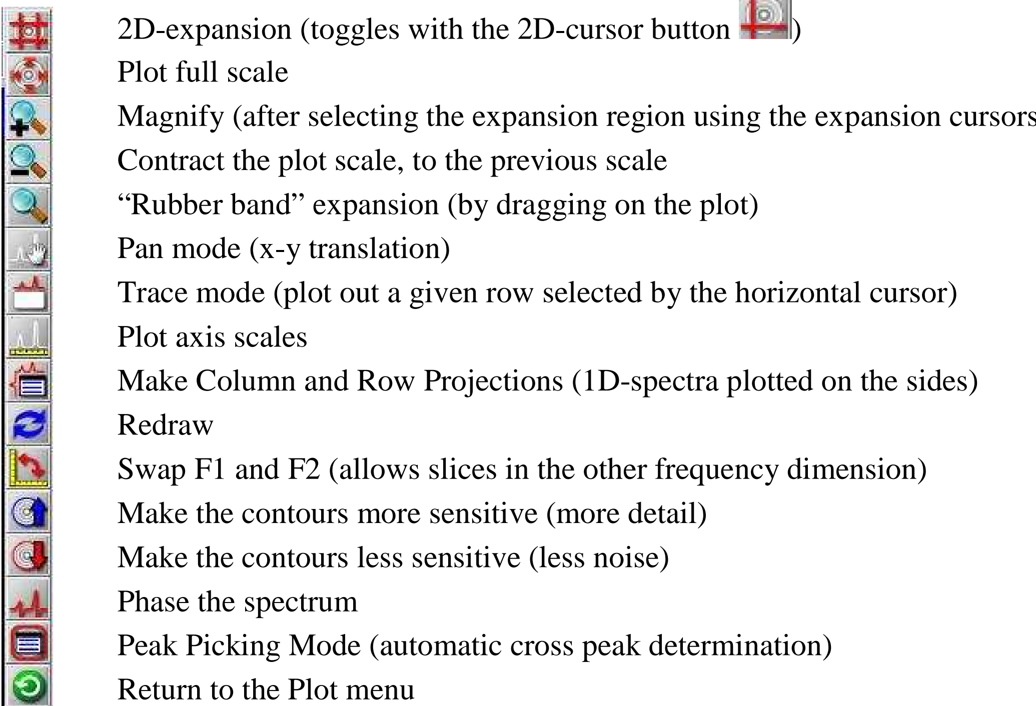
Make sure to click Show Time to ensure the spectrum will run in the time allotted.

Notes:

1. Gdqcosy gives much smaller t1 ridges than Gcosy, but takes twice as long (about 6 minutes for one Scan per increment). Smaller t1-ridges make the contour levels easier to pick and give fewer spurious peaks from the symmetrization process.

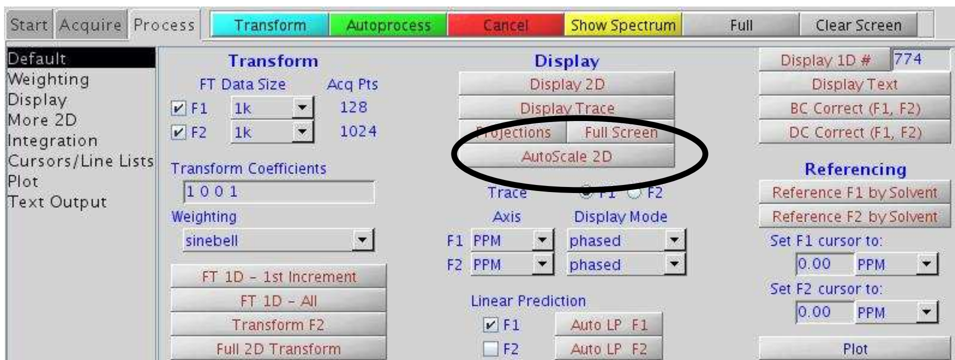
Display of 2D data in VNMRJ

The upper right corner of the screen displays a series of buttons that control the data display:



2D-expansion (toggles with the 2D-cursor button)
Plot full scale
Magnify (after selecting the expansion region using the expansion cursors)
Contract the plot scale, to the previous scale
"Rubber band" expansion (by dragging on the plot)
Pan mode (x-y translation)
Trace mode (plot out a given row selected by the horizontal cursor)
Plot axis scales
Make Column and Row Projections (1D-spectra plotted on the sides)
Redraw
Swap F1 and F2 (allows slices in the other frequency dimension)
Make the contours more sensitive (more detail)
Make the contours less sensitive (less noise)
Phase the spectrum
Peak Picking Mode (automatic cross peak determination)
Return to the Plot menu

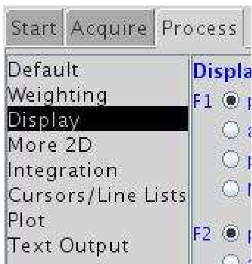
Setting Contours: To lower the contour levels to see less intense cross-peaks, click on the blue button. To increase the contour levels to see less noise, click on the red button. To reset the contours to the initial setting, switch to the Process tab and the Default Page. Press the Auto Scale 2D button:



Expanding the Display: To expand the plot region, click on the expansion cursors icon. Using the left mouse button, click along the diagonal below the region of interest. Using the right mouse button, click along the diagonal above the region of interest. Then click on the +magnifying glass icon to expand the plot scale. To return to the full 2D plot, click on the 2D-full scale button.

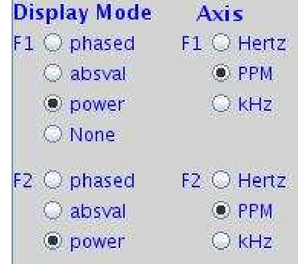
Locating Cross-Peaks: Click on the 2D-cursor icon and then click in the plot area to determine the chemical shifts of a cross peak. You can also predict the position of a possible cross-peak by moving the cursor so the vertical and horizontal lines pass through the diagonal peaks that might be coupled. The cursor button "toggles" between the expansion and 2D-cursor modes.

Positive and Negative Peaks: Some 2D spectra have only positive peaks and some, like phase sensitive COSY's (Gdqcosy), have positive and negative peaks. The Ghsqc spectra from the automation system plots CH_3 and CH peaks as positive and CH_2 peaks as negative. Sometimes Gcosy spectra are easier to see with only positive peaks plotted. Switch to the Display page under the Process tab:



You can plot only positive peaks by clicking on Interactive: "+ only" and only negative peaks by clicking on "– only". To return to the normal display, remember to click on "both +/-" so that you don't neglect a cross peak.

Another approach to simplifying phase sensitive spectra is to display the spectra in power mode. The result is sometimes easier to interpret, but switching the display to power mode removes the phase sensitive information and broadens the cross peaks. Power mode doesn't work well for cross peaks close to the main diagonal. To switch to power display mode click on the power buttons in the Display Mode list. You will then need to lower the contour levels.



Gcosy Without Symmetrization: The automated processing for Gcosy spectra forces the cross peaks to be symmetrical through a process called symmetrization. In the symmetrization process for Gcosy's, intense t1-ridges can cause false cross peaks. To verify that a weak cross peak in a Gcosy spectrum is real, it is advisable to check the unsymmetrized version. To see the unsymmetrized version, click on the Transform button:

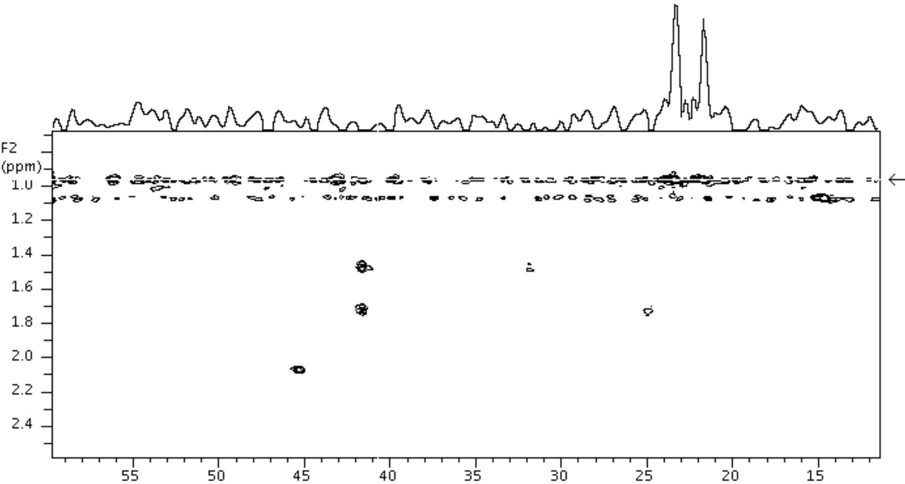
Displaying Row and Column Projections: To plot projections click on the button. The 2D-plot screen doesn't display the high resolution spectra at the sides of the 2D-plot. However, row and column projections can play the same role, except at poorer resolution. The function of each button is described below. With some spectra, projections made from the maximum along each row or column work best. With other spectra, projections made by summing the intensity along each row or column are clearer. Try both.

- Column projection using the maximum point along each column (top)
- Column projection using the sum along each column (top)
- Row projection using the maximum point along each row (side)
- Row projection using the sum along each row (side)
- Return

After making a projection, you can rescale the vertical axis by clicking the middle mouse button in the corresponding projection plot area (not the 2D-plot area). Column projection vertical rescaling is available immediately after clicking on one of the column projection buttons. Row projection rescaling is available immediately after clicking on one of the row projection buttons. When finished, click the Return button to continue.

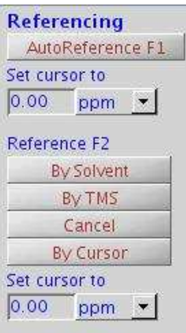
Plotting: After you have expanded the 2D-spectrum and adjusted the contours, choose the Plot page from the Process tab and click on AutoPlot.

Trace Displays: To display single rows of the 2D-data matrix, click on the Trace display button. Use the 2D-cursor to select a row for display. As you click on different rows, the row spectrum appears on top of the 2D display area. Traces are useful for measuring the intensity of different cross peaks. Traces are also useful for seeing cross peaks that occur in t1-ridges. A trace from the t1-ridge at 0.95 ppm in an HMQC spectrum is shown below as an example.



To see traces for columns instead of rows, first click on the Swap F1 and F2 button.

Setting the Chemical Shift Reference for 2D-Spectra: Sometimes the automation routines don't set the chemical shift reference correctly. Switch to the Display page under the Process tab. If your sample has TMS, click on the AutoReference F1 and Reference F2 By TMS button. If your sample doesn't have TMS try the Reference F2 by Solvent button:



When you reference by solvent, the system tries to find the residual solvent peak for the chosen solvent to use as the chemical shift reference (e.g. 7.26 ppm for $CDCl_3$). You can also reference the F1 axis By Solvent from the Default Process page (see the Default page layout shown above). If all else fails, position the cursor at 0 ppm for both axes and press the By Cursor button. If you don't know where 0 ppm is, position the cursor on a known peak, type the chemical shift of that peak into the "Set cursor to" dialog box and then press By Cursor.

Manual Probe Tuning

When: Before any experiment that requires an exact 90° pulse, for example, Dept or a Gcosy.

Procedure:

1. Take a normal automated 1-D spectrum for the nucleus of interest. This first spectrum sets the appropriate experimental parameters for the observation frequency.

2. In VNMRJ, pull down the Tools menu, slide right on Probe Tuning and choose Manual Tune Probe.

3. If you are not in the spectrum view mode click on the [O] button at the top-left of the Autosampler page.

4. To tune the proton probe, choose Rf channel 1. To tune the ^{13}C or other low frequency nucleus, choose Rf channel 2. Click on Start Probe Tune to plot the reflected power from the probe as a function of frequency. The goal of tuning is to minimize the reflected power at the operating frequency of the spectrometer. The operating frequency is denoted with a vertical blue (or magenta line) in the middle of the display. Click on the vertical line to change its color.

For the proton side of the probe:

5. Find the knob on the **left-most** controller labeled “H-tune”, which controls the tuning capacitor. Note the position of the mark on the tuning knob. Draw the orientation in your lab book so that if something goes wrong you can return the knob to the starting position.

6. Adjust the tuning control to move the dip to the center of the vertical line on the computer display. Adjust the Matching control to give the lowest and sharpest dip possible.

7. On the computer display, at the right side in the middle, click on the Stop Probe Tune button.

8. Click on Quit.

For the ^{13}C side of the probe:

In step 4, above, choose Rf channel 2 in VNMRJ and use the match and tuning controls on the **middle** controller.

Temperature Control

To determine the current temperature in the probe:

1. The current temperature is shown in the indicator box on the lower left side of the VNMRJ screen.

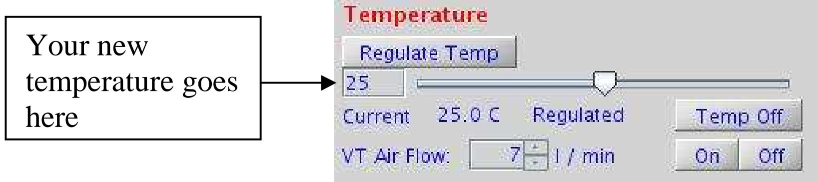
To Control the temperature in the probe (above room temperature):

The probe temperature is usually controlled at 25°C, so under normal circumstances you won't need to make any changes. If the temperature isn't regulated use the following instructions.

Equilibrate at the new sample temperature before submitting your run to the sample queue.

Make sure to keep the temperature well below the boiling point of your solvent, or you risk a tube explosion in the probe.

1. From the Start tab, select the Spin/Temp page. Normally, the VT Air Flow is set to 7 L/min:



2. Normally, the temperature will already be regulated as indicated by the "Regulated" indication to the right of the Current temperature setting. If already regulated, then all you need do is type in the new temperature in the dialog box to the left of the slider. If the temperature is not currently regulated, after typing in your new desired temperature, click the Regulate Temp button.

3. <u>Let the temperature stabilize at the new temperature</u> and then submit your sample to the queue.

4. When finished, **<u>equilibrate the probe at 25°C</u>** and then run ethylbenzene. Leave the temperature regulated at 25°C.

5. Contact the system administrator for temperatures below room temperature.