PROCESSING 2D SPECTRA USING VNMRJ

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1. INTRODUCTION

1.1. About this Worksheet

This worksheet describes how to manipulate, process, and analyze 2D NMR spectra acquired on any of the NMR spectrometers in the NMR facility. In addition, the steps needed to plot a processed spectrum will be described.

1.2. A Very Brief Introduction to 2D NMR

To understand how to process 2D NMR data, it is necessary to understand the basics of a 2D NMR experiment and understand some unique terminology.

1.2.1. The basics of 2D NMR spectroscopy

The basic 2D NMR pulse sequence is shown in Figure 1 and a 2D NMR experiment proceeds as follows:

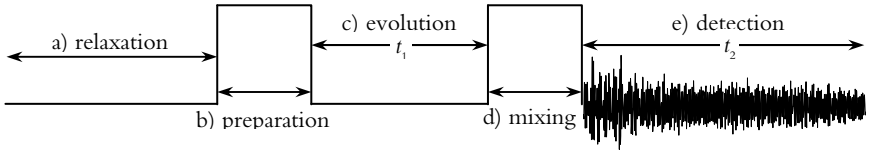


Figure 1. A schematic representation of a general 2D NMR pulse sequence.

a) Relaxation: *A relaxation delay, in which no spectrometer action is being performed, is necessary to allow the magnetization to return to equilibrium.*
b) Preparation: *A radio-frequency (rf) pulse, or series of rf-pulses are applied to the sample.*
c) Evolution: *The magnetization is allowed to evolve for a period of length t_1.*
d) Mixing: *Another rf- pulse, or series of rf-pulses are applied to the sample.*
e) Detection: *The NMR data is acquired for a length t_2.*

The pulse sequence is repeated a set number of times, known as transients or scans (ns, typically 1-4 for a 2D experiment), in the same manner as any 1D NMR experiment. In order to generate 2D data, the t_1 length is then increased

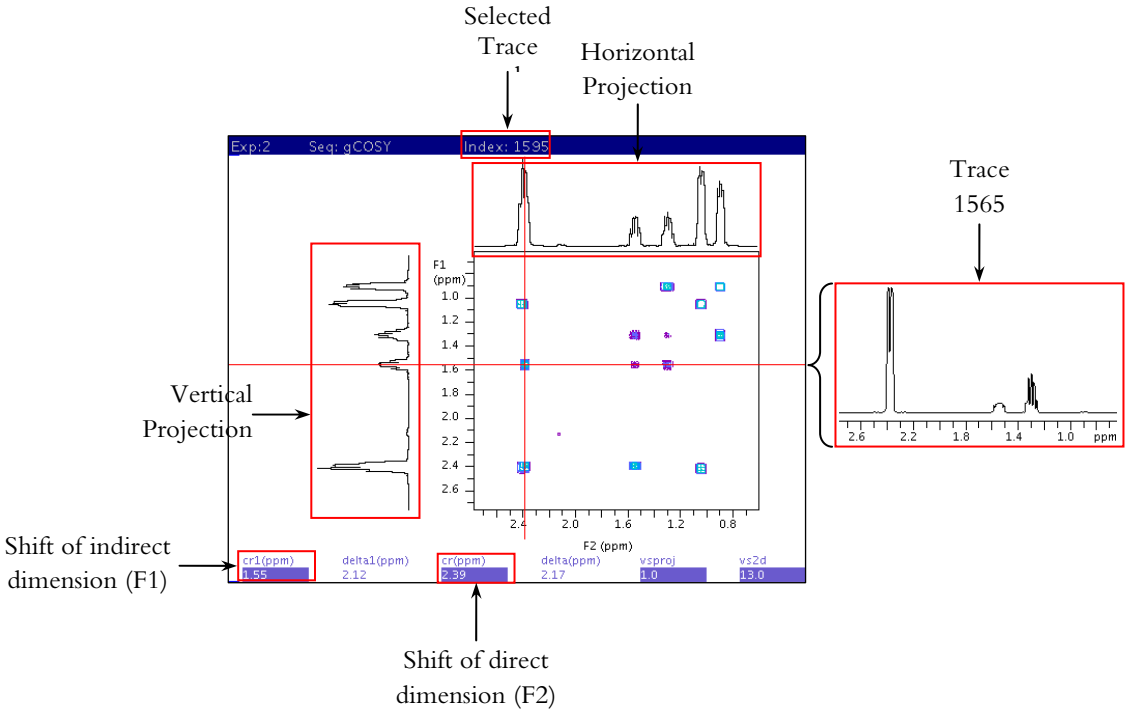


Figure 2. The gCOSY NMR spectrum of 3-heptanone in $CDCl_3$ (1% v/v) acquired on the Inova 600 with 1 scan per increment (ns=1) and 128 increments (ni=128).

by some increment, Δt, and the pulse sequence is repeated ns times. The t_1 is then increased by $2\Delta t$ and the pulse sequence is repeated ns times, then by $3\Delta t$, etc, for a total of somewhere between 64 and 512 different values of t_1.

The data is then processed to give a 2D NMR spectrum, an example of which is shown in Figure 2. The NMR spectrum consists of an indirect dimension (F1), which is usually plotted as the vertical axis, and the direct dimension (F2), which is usually plotted as the horizontal axis. The 2D NMR spectrum consists of between 128 – 512 different 1D spectra generated from the different values of t_1. These 1D NMR spectra are termed "traces" and individual traces can be viewed individually if desired for both the direct and indirect dimension. These traces can be convoluted to create a "projection" that appears at the top of the horizontal axis and the left-hand side of the vertical axis.

F1 dimension: *The indirect dimension. Data is not acquired like in a 1D spectrum; the receiver does not acquire data for this nucleus. Typically the F1 dimension is for nuclei other than ^{1}H and forms the vertical sides of a 2D spectrum.*

F2 dimension: *The direct dimension. Data is acquired in the same manner as a 1D spectrum. The receiver is turned on and a regular FID is acquired. Typically the F2 dimension is ^{1}H and is plotted on the bottom of the 2D spectrum..*

Trace: *The equivalent of a 1D spectrum produced in the 2D experiment.*

Projection: *A 1D spectrum composed by summing each individual trace or by taking the maximum intensity at a particular point from a particular trace and constructing a 1D spectrum. The projections are plotted on top of the F2 axis or to the left of the F1 axis.*

Increments: *Number of "1D spectra" obtained. The greater the number of increments, the better the resolution in the F1 dimension.*

Scans/Transients: *Number of times the "1D experiment" for a particular increment value is repeated. The greater the number of scans, the greater the signal-to-noise.*

2. OVERVIEW OF VNMRJ

This section provides an overview of the VNMRJ process panel and graphics control buttons used for manipulating and processing 2D NMR spectra. The VNMRJ screen is displayed in Figure 3.

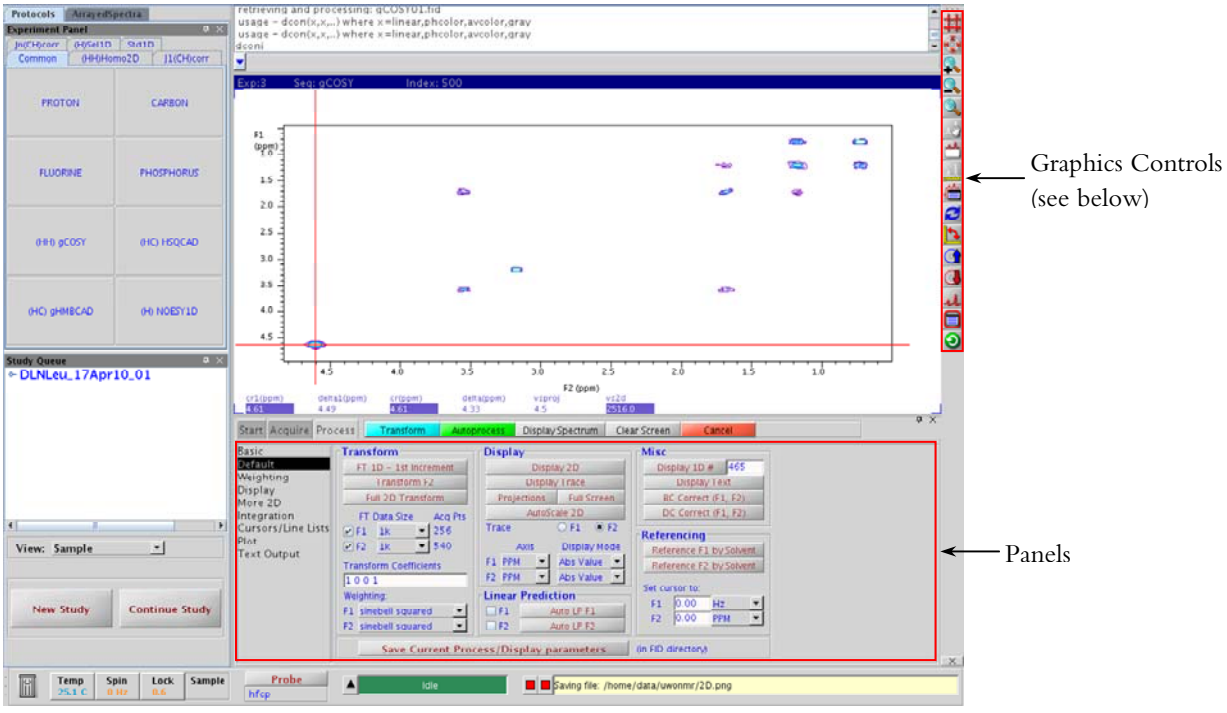


Figure 3. The **Process** panel, D**efault** page. Together the pages within the process panel and the graphics controls buttons are used to manipulate, process, save and plot 2D NMR spectra.

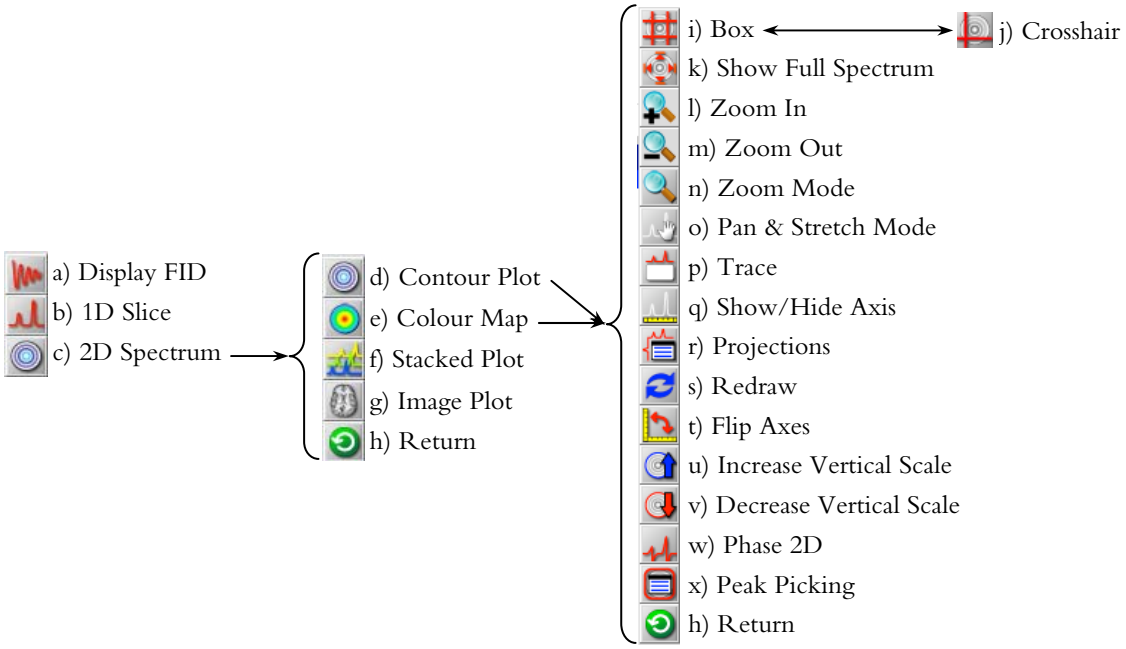


Figure 4. An overview of the graphics controls used to manipulate 2D NMR spectra.

The graphics controls buttons are displayed in Figure 4. The text below describes the various functions of the graphics controls buttons.

a) Display FID: *Displays individual FIDs.*
b) 1D Spectrum: *Displays 1D slices and enters 1D processing mode.*
c) 2D Spectrum: *Displays the 2D spectrum and enters 2D processing mode.*
d) Contour Plot: *Displays the 2D spectrum as a contour plot (i.e. similar to a topographic map).*
e) Colour Map: *Displays the 2D spectrum as a colour map (i.e. intensities distinguished by colours).*
f) Stacked Plot: *Displays the 2D spectrum as a series of stacked 1D spectra (not frequently used.)*
g) Image Plot: *Displays an image (not used).*
h) Return: *Returns to previous graphics control menu.*
i) Box: *Toggles between box and crosshair. The box is used to select a specific region in the spectrum. (See section)*
j) Crosshair: *Toggles between crosshair and box. The crosshair is used to select specific peaks in the spectrum.*
k) Show Full Spectrum: *Zooms out to show the entire spectrum. (See section)*
l) Zoom In: *Used to zoom into a selected region of the spectrum. (See section)*
m) Zoom Out: *Used to zoom one level out and see a broader view of the spectrum. (See section)*
n) Zoom Mode: *Used to highlight & zoom in to a specific area of the spectrum. (See section)*
o) Pan & Stretch Mode: *Used to move the spectrum upfield or downfield, and zoom in and out. (See section)*
p) Trace: *Shows individual 1D traces/spectra. (See section)*
q) Show/Hide Axes: *Displays or hides the chemical shift scale.*
r) Projections: *Enters the projections submenu where projections can be displayed along the F1 and F2 axes. (See section)*
s) Redraw: *Refreshes the spectrum and in current zoom-mode.*
t) Rotate: *Flips the F1 and F2 axes.*
u) Scale +20%: *Increases the vertical scale by 20%.*

v)  Scale -20%: *Decreases the vertical scale by 20%.*
w)  Phase 2D: *Enters 2D phase-mode and displays the 2D phasing submenus. (See section)*
x)  Peak Picking: *Enters the 2D peak picking mode and displays 2D peak picking submenus.*

3. OPENING YOUR SPECTRUM

1) From the VNMRJ main screen go to **File → Open…**.
2) Locate the directory where the spectrum is saved; your data should be in /home/data/group_name/your_name/.
3) Double click on the .fid file of the 2D NMR spectrum you would like to process.

4. SPECTRUM MANIPULATION AND REFERENCING

4.1. Adjusting the Vertical Scale

Click on  to increase the vertical scale or  to decrease the vertical scale.

4.2. Zooming In and Out

There are several ways in which to zoom into and out of a specific chemical shift region of interest.

4.2.1. Using the zoom mode button

1) Click on  .
2) In the spectrum, click and hold the left mouse button and move the mouse in whichever direction you desire. The selected region will turn light purple.
3) Release the left mouse button once the region of interest is selected.

4.2.2. Using the box, zoom-in and zoom-out buttons

1) Click on  .
2) In the spectrum, use the left mouse button sets the position of the bottom left-hand corner of the box.
3) In the spectrum, use the right mouse button sets the position of the top right-hand corner of the box.
4) Click on  to zoom in on this area of the spectrum.
5) Click on  to zoom out of this area of the spectrum.

4.2.3. Using the pan & stretch mode button

1) Click on  .
2) In the spectrum, click and hold the right mouse button
3) To manipulate the vertical axis, move the mouse down to zoom in or move the mouse up to zoom out. Release the right mouse button when the desired region is in view.
4) To manipulate the horizontal axis, repeat steps 1 and 2, then move the mouse left to zoom in or move the mouse right to zoom out. Release the right mouse button when the desired region is in view.

4.2.4. Using the show full spectrum or reset to full display buttons

At any point you are able to return to the original full spectrum by clicking on either  or  .

4.3. Moving the Spectrum Upfield or Downfield

When zoomed in to a particular region, you may move the spectrum downfield or upfield in either axis using the pan & stretch mode button.

1) Click on  .
2) In the spectrum, click and hold the left mouse button.
3) In the horizontal axis, move the mouse left to view a downfield region or move the mouse right to view an upfield region. Release the left mouse button when the desired region is in view.

4) In the vertical axis, repeat steps 1 and 2, then move the mouse up to view a downfield region or move the mouse down to view an upfield region. Release the left mouse button when the desired region is in view.

4.4. Phasing

4.4.1. Manual phasing

The spectrum will be autophased using the VNMRJ software. While the phasing is usually sufficient, manual phasing is sometimes necessary. If manual phasing is required, then do the following:

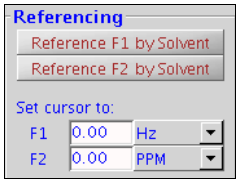
1) Go to the **Process** panel, **Plot** page.
2) Under the **Screen Position** section, click on Full w/Projections to re-size the spectrum appropriately.
3) In the Graphics Controls, click on  . This brings up the phasing sub-menu:

Cursor/Box: *Toggles between crosshair and box.*
Zoom In: *Used to zoom into a selected region of the spectrum.*
Trace: *Shows individual 1D traces/spectra. (See section)*
Redraw: *Refreshes the spectrum, clearing any traces, peak-picks, and projections..*
Rotate: *Flips the F1 and F2 axes*.
Spectrum 1: *Stores the selected trace as spectrum 1.*
Spectrum 2: *Stores the selected trace as spectrum 2.*
Phase Selected Spectra: *Reads in selected spectrum 1 for phasing.*
Return: *Returns to the main menu.*

4) Left-click on the spectrum and move the mouse up or down to select different traces. The trace will be displayed above the spectrum and can be re-sized using the middle mouse button.
5) When a suitable trace with high signal-to-noise is selected, click on  to store the trace as spectrum 1.
6) If desired, repeat step 4, then click on  to store the a different trace as spectrum 2.
7) Click on  . This reads in the trace stored as spectrum 1 for phasing.
8) Left click and hold the mouse button down on the largest peak. A set of red vertical lines will appear on either side of the peak.
9) Drag the mouse up and down until the peak is in phase. (If required, phase the left-most peak the same way).
10) Release the mouse button when finished.
11) To exit phase mode, click  .
12) To return to the main menu, click  again.

4.5. Chemical Shift Referencing

VNMRJ automatically references the spectrum based on the lock signal of the chosen solvent, but periodically the referencing is incorrect. If re-referencing the spectrum is necessary, there are three different ways this can be accomplished.



4.5.1. Using the Reference By Solvent Button

1) Go to the **Process** panel, **Default** page.
2) Under the **Referencing** sub-heading click on: Reference F1 by Solvent to automatically reference the F1 dimension.
   Reference F2 by Solvent to automatically reference the F2 dimension.

4.5.2. Manually Referencing: Method 1

1) Go to the **Process** panel, **Default** Page.

2) If necessary, click on  to bring up the crosshair.
3) Place the crosshair on the peak to be referenced. The selected chemical shift will be displayed on the bottom of the spectrum as cr(ppm) for the F2 dimension and cr1(ppm) for the F1 dimension.
4) Under "Set cursor to", select ppm from the drop down menu
5) Enter the appropriate chemical shift value for the peak in the F1 window or the F2 window to reference the F1 and F2 dimensions, respectively, and hit enter.

4.5.3. Manually Referencing: Method 2

1) Go to the **Process** panel, **Plot** page.
2) Under the **Screen Position** section, click on Full w/Projections to re-size the spectrum appropriately.
3) The dimension you would like to reference must be the horizontal axis. If necessary, click on  to swap the vertical and horizontal axes and bring your desired dimension onto the horizontal axis.
4) Click on  .
5) Left-click on the spectrum and move the mouse up or down to select different traces. The trace will be displayed above the spectrum and can be re-sized using the middle mouse button.
6) Type `ds` in the command line to read the trace as a 1D spectrum.
7) If necessary, click on  to bring up a single cursor.
8) Place the cursor on the peak you would like to reference.
9) Go to the **Process** panel, **Default** page.
10) Under "Set cursor to", select ppm from the drop down menu.
11) If you are referencing the F1 dimension (look for cr1 at the bottom of the spectrum), enter the appropriate chemical shift value for the peak in the F1 window and hit enter. If you are referencing the F2 dimension, enter the appropriate chemical shift value for the peak in the F2 window and hit enter.
12) Click on Display 2D to return to the 2D spectrum.

4.6. Displaying Projections

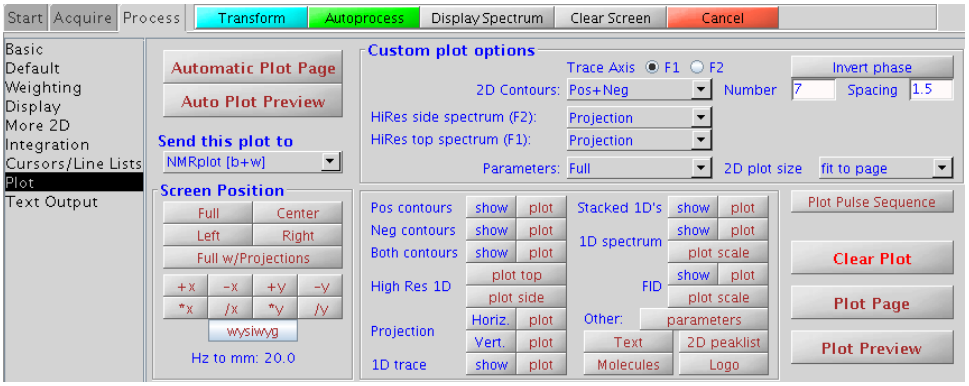
Projections are constructed 1D spectra that can be viewed on the top of the horizontal axis or the left-side of the vertical axis. These projections aid with the interpretation of the 2D spectra.

1) Go to the **Process** panel, **Plot** page.
2) Under the **Screen Position** section, click on Full w/Projections to re-size the spectrum appropriately.
3) Click on  to enter the projection submenu:
   -  Horiz proj (max): *Displays the maximum projection on the top horizontal axis*
   -  Horiz proj (sum): *Displays the summation projection on the top horizontal axis*
   -  Vert proj (max): *Displays the maximum projection on the left vertical axis*
   -  Vert proj (sum): *Displays the summation projection on the left vertical axis*
   -  Cancel: *Returns to main menu*
4) Click on whichever horizontal projection you would like to display. In general the summation projections are better than the maximum projections.
5) Middle-click above the horizontal projection to increase the vertical scale or below the projection (but above the axis of the 2D spectrum) to decrease the vertical scale.
6) Click on whichever vertical projection you would like to display.
7) Middle-click above the vertical projection (i.e. to the left) to increase the vertical scale or below the projection (i.e. to the right but above the axis of the 2D spectrum) to decrease the vertical scale.
8) When finished, click on  to return to the main menu.

5. PLOTTING

5.1. Automatic Plotting

1) Go to the **Process** panel, **Plot** page. Before sending the spectrum to the printer, you may choose how you would like the NMR parameters, integrals and peak frequencies printed.



2) Under the **Custom plot options** section, choose which peaks you would like displayed using the drop-down list next to 2D contours. The options given include:

Pos+Neg	prints all peaks in the 2D spectrum (recommended)
Pos Only	prints only the peaks with positive intensity.
Neg Only	prints only the peaks with negative intensity.

3) Under the **Custom plot options** section, choose which style of spectrum will be printed along the F2 dimension using the drop-down list next to HiRes side spectrum (F2). The options given include:

saved 1D fid	prints the appropriate saved 1D spectrum if the spectrum exits in the data folder
Projection	prints the summation projection
other workspace	prints the 1D spectrum that is open in another workspace/experiment number
None	does not print a spectrum

4) Under the **Custom plot options** section, choose which style of spectrum will be printed along the F1 dimension using the drop-down list next to HiRes side spectrum (F1). The options are the same as for the F2 dimension (see step 3).

5) Under the **Custom plot options** section, choose which style of spectrum will be printed along the F2 dimension using the drop-down list next to Parameters. The options given include:

Basic	prints the most important acquisition and processing parameters (recommended)
Full	prints all acquisition and processing parameters
Horiz. Box	does not work
Vert. Box	does not work
None	prints no acquisition or processing parameters

6) Once plot settings have been adjusted click on Automatic Plot Page.

5.2. Manual Plotting

More control of what is plotted is achieved if you manually plot the data. You may print the FID, pulse sequence, etc. Simply click the buttons labeled plot to plot the desired item, then Plot Page to send it to the printer. To print the same spectrum as the automatic plotting, follow the procedure below.

1) Go to the **Process** panel, **Plot** page.
2) Select which peaks you would like printed.
   Positive and negative peaks: click on plot located next to Both contours.
   Positive peaks only: click on plot located next to Pos contours.
   Negative peaks only: click on plot located next to Neg contours.
3) Under the **Custom plot options** section, choose which style of spectrum will be printed along the F2 dimension using the drop-down list next to HiRes side spectrum (F2). The options given include:

saved 1D fid	prints the appropriate saved 1D spectrum if the spectrum exits in the data folder
Projection	prints the summation projection
other workspace	prints the 1D spectrum that is open in another workspace/experiment number

   Then click on plot side.
4) Under the **Custom plot options** section, choose which style of spectrum will be printed along the F1 dimension using the drop-down list next to HiRes stop spectrum (F1). The options given include:

saved 1D fid	prints the appropriate saved 1D spectrum if the spectrum exits in the data folder

Projection	prints the summation projection
other workspace	prints the 1D spectrum that is open in another workspace/experiment number

Then click on plot top.

5) Select the plot parameters under the *Parameters* section as described in 5.1, step 5, then click on parameters.
6) Click on Plot Page to send the plot to the printer.

6. PREPARING GRAPHICS/POSTSCRIPT FILES

VNMRJ saves pictures (or graphics) as a postscript file, which is a high-quality LINUX graphics file. Once saved, you may FTP the postscript file to your own computer and edit the file directly in graphics programs such as CorelDraw, Adobe Illustrator or Adobe Photoshop. Alternately, if you have Adobe Distiller installed or use a Mac, if you double-click on the postscript file it will be automatically converted to a PDF file.

1) Go to the **Process** panel, **Plot** page.
2) Under the Send this plot to: section select Postscript [b+w] 

.
3) Select all spectra parameters as described in section 5.2.
4) In the command line, change the directory by typing `cd('/home/data/group_name/username'),` where *groupname* and *username* are replaced with your particular values. The plot will be saved in this directory.
5) In the command line, type `page('filed name.ps')` to create a postscript file, where *filename* is replaced by whatever you would like to call the plot.