I. Overview of Processing 2D Spectra in VNMR

Processing of 2D spectra can be carried out solely using the menu buttons. Alternatively, the workup of 2D spectra can be performed with great flexibility, using commands. You can also use a combination of menus and commands. The processing of 2D spectra is carried out in three basic steps. First, the spectra must be retrieved from disk and loaded into an experiment. Next, parameters that control window functions and data resolution are adjusted. Finally, the spectra (fids) are then transformed in both directions. After 2d fourier transformation, the spectra can be displayed and plotted.

Flow Chart for Transforming 2D Spectra:

Retrieve 2D data from Disk (**files** and **load** buttons on main menu)

Set desired digital resolution in the **f1** and **f2** dimensions (**fn, fn1**)

Set appropriate processing window in the **f2** dimension (**wti, lb, sb, sbs, gf, gfs**)

Transform the **f2** (acquisition) dimension (**wft1d** or **wft1da**)

Select a trace and set processing window on this trace in the **f1** dimension (use the **trace** button, **wti(trace #),** set **lb1, sb1, sbs, gf, gfs**)

Transform the **f1** or time incremented dimension (**wft2d** or **wft2da)**

Apply data massaging (e.g., **bc('f1') or bc('f2'), foldt, etc.**)

View spectrum (**dconi, dpcon, etc.)**

Plot spectrum (**pcon**)

II. Using Menus to Retrieve, Process, Display and Plot 2D Spectra

A. RETRIEVING DATA

To Retrieve a spectrum from the disk, click on **7:file**. Click on the name of the fid you wish to retrieve. Click on **Load.** The fid will be placed into the current experiment for processing. You can immediately perform a 2D transformation at this point by typing **wft2d** (for COSY, HETCOR, JRES and other magnitude 2D spectra), or **wft2da** (for NOESY, TOCSY and other phase-sensitive or absolute value 2D spectra). You can also use the **Process** button on the **MainMenu** to transform spectra once they have been retrieved from the disk.

B. PROCESS, DISPLAY AND PLOT 2D DATA USING MENUS ONLY

VNMR lets you do basic processing, display and plotting of 2D spectra solely by using the menu buttons. First, load (retrieve) a 2D data set from the disk as described above. Click on the **main menu** button and then click on the **process** button. The 2D process menu will be shown, and the buttons for this menu are described below:

2D DATA PROCESSING MENU BUTTONS:

1: Select Params	Allow selection of processing parameters using another menu.
2: Adj Weighting	Interactive weighting adjustment using the wti program.
3: Phase F2	Perform a 1D Fourier transformation of the first FID to allow phasing along the **f2** dimension (only active in phase-sensitive mode)

4: Transform F2	Perform a full set of transforms of the FIDS along the **f2** axis, allowing inspection of the interferograms. Any currently active weighting functions are used.
5: Full Transform	Perform a full set of transforms first along **f2**, then along **f1**. Any currently active weighting functions are used.

You can click on **Transform F2** to transform the spectrum only in the **f2** dimension, perform the full transform of both **f2** and **f1** by clicking on **Full Transform**, interactively adjust window functions **(Adj Weighting)** or use a second menu to set preselected processing parameter **(Select Params)**. In general, you should first set the windows for the 2D ft by selecting **Adj Weighting** or **Select Params** before you perform the 2D ft. If you click on **Select Params**, you will see the 2D parameter menu:

2D PROCESSING PARAMETER SETUP MENU BUTTONS:

1: No WT	Clear all weighting parameters.
2: Sinebell	Select sinebell weighting over whole fid length.
3: Pseudo	Select "pseudo-echo" weighting.
4: AV	Switch to absolute value mode transform.
PH	Switch to phase-sensitive mode transform.
5: FN: Small	Select Fourier number for quick transform.
6: Normal	Select Fourier number for normal size transform.
7: Large	Select Fourier number for large transform with zero filling.
6: Return	Return to 2D processing menu.

Parameters can be set up to standard values with one of the buttons, or they can be typed in and observed (type, for example "fn=512"). After parameter selection, return to the 2D processing menu with the "return" button, then click on **Transform F2** or **Full Transform.** If you only transform **f2**, you can then select the weighting to be applied to the **f1** dimension by clicking on the **adj weighting** button. The transform is then completed by clicking on the **Full Transform** button.

To display 2D data using the menus, click on **main menu**, then click on **display.** You can then display a color bitmapped (interactive pixel) display, a contour map, or a gray scale image. Buttons are also available to change the size of the display, to perform various manipulations of the 2D spectrum and to plot the data.

2D DATA DISPLAY MENU BUTTONS:

1: Color Map	Display normal 2D contour display (color image or map) and allow for interactive adjustment.
2: Contour	Display a true 2D contour display, comparable to the output on a plotter. This mode will be much slower than a simple color map, and also non-interactive.
3: Image	Display gray scale image and allow for interactive adjustment.
4: Size	Select 2D display size menu (to define size of display).
5: Massage	Select 2D data manipulation menu (symmetrizations, drift corrections, rotation, intensity normalization).
6: Reprocess	Go back to the 2D processing menu.
7: Plot	Select the 2D data plotting and printing menu.
8: More	Provide more choices.

Generate a display by clicking on **color map** or **contour**. Refer to section IV for a description of the interactive bitmapped display. After generating the desired display, click on the **plot** menu button. The plot menu allows you to generate simple contour plots, image plots, parameter lists, and kick the page out of the printer or plotter.

2D DATA plotting MENU Buttons:

1: pcon	Calculate a contour plot of the current data. (Shows positive and negative peaks, if in phased mode).
2: pcon(pos)	Calculate a contour plot of the current data. (Shows positive peaks only, even in phased mode).
3: imageprint	Print a grayscale image on a dot matrix printer (LaserJet, QuietJet or InkJet; does not work on pen plotters)
4: pa	Print parameters on printer.
5: page	Plot out the currently buffered plot and change paper.
6: pap	Plot parameters in left upper corner of paper.
7: return	Return to the 2D display menu.
8: main menu	Select the main menu.

III. Processing 2D Spectra Using Commands

A. APPLYING WINDOWS AND SETTING RESOLUTION

The 2D data can be immediately transformed once loaded into an experiment. However, dramatic improvements can be made in the final result with the optimum choice of parameters that determine the digital resolution and lineshape of the final transformed 2D spectrum. You can manipulate the resolution enhancement windows that are applied to the 2D data and view the effects of changing digital resolution, before performing the complete 2D fourier transformation.

The proper sequence is to first load the 2D data into an experiment, as described earlier. Then set the resolution and windowing parameters to the desired values. You can set these values by typing them in, by using the **Process** button on the **main menu**, by using the interactive windowing functions (the **wti**) command, or any combination of these methods. Once the appropriate windowing and resolution parameters have been set, you can then perform the 2D ft of the data. You can retransform the spectrum at any point and observe the effect of different windowing and resolution settings.

Recognize that digital resolution and windowing parameters exist for both dimensions of a 2D spectrum. It is possible to transform only one dimension of the 2D data. You can then examine the resolution and windows for the remaining dimension at this halfway point in the 2D ft process.

The most relevant commands and parameters that control the 2D resolution and windowing are shown in the following table:

Window and ft the *n*th spectrum	**wft(*n*)**	Multiply the *n*th fid by the **lb, sb or gf** window and perform the fourier transformation. The *n*th spectrum will be displayed after **wft** is complete. This command is only used to transform a single fid in the acquisition (**f2**) dimension for initial examination, phasing, etc. **NOTE: wft should NOT be used for performing a 2D transformation. wft should only be used to help set windows and resolution in the first transform dimension.**

Window and ft interactively	**wti(*n*)**	Displays the *n*th FID, the current window (exponential, sinebell, sinesquare, or Gaussian) and the spectrum resulting from FT of the *n*th FID after applying the window. The LEFT mouse button controls the position and type of window, the RIGHT mouse button turns FT of the spectrum on or off, and the MIDDLE button controls **vs** of the spectrum and FID.
Line broadening	**lb=*n*, lb1=*n***	Line broadening in Hz, as desired. The value ***n*** is in hertz. **lb** sets the line broadening for the acquisition dimension **f2**. **lb1** sets line broadening for the time incremented dimension **f1**.
sine bell	**sb=*n*, sb1=*n***	generates a sinebell (*n* is positive) or a sinebell squared (*n* is negative) window. **sb** and **sb1** apply to the acquisition (**f2**) and time incremented dimension (**f1**), respectively.
shift sinebell window	**sbs=*n*, sbs1=*n***	used to shift sinebell window in the **f2** (**sbs**) or **f1** (**sbs1**) dimensions.
gaussian windows	**gf=*n*, gf1=*n*, gfs=*n*, gfs1=*n***	generates gaussian windows and shifts in the **f2** (**gf, gfs)** or **f1** (**gf1, gfs1**) dimensions.
automatically calculate sinebell window	**sinebell('f2'** or **'f1')**	automatically calculates a sinebell window that covers the number of points in the **f2** or **f1** dimensions
automatically calculate a sine squared, shifted window	**sinesq(*n,m,*'f2'** or **'f1')**	automatically calculates a sine squared window, shifted by an amount equal to **π/*n***. The window will cover ***m*** points and will cover the **f2** or **f1** dimensions. For most phase sensitive spectra (NOESY, TOCSY, dqf-COSY, HMQC, etc.), a π/3 shifts covering **np** points in f2 or 2***ni** points in **f1** will generally give good results
fourier numbers for zero-filling	**fn, fn1**	**fn** and **fn1** determine the number of data points in the fourier transformed 2D spectrum. **fn** and **fn1** must be equal to some power of 2. The actual number of data points in the final, transformed spectrum will be equal to **fn/2** and **fn1/2** in the **f2** and **f1** dimensions, respectively.
number of acquired points and fids	**np, ni**	**np** is the number of *data points* in each of the acquired fids. The real and imaginary portions of each fid contain **np/2** points. **ni** is equal to the number of 2D time increments. This will be equal to the number of acquired *fids* in each 2D experiment if the experiment is an absolute value (non phase-sensitive) data set, e.g., a simple COSY. For most phase-sensitive 2D data, the number of fids **2* ni**.

B. A GENERAL APPROACH TO SETTING OPTIMUM 2D PARAMETERS:

Two main factors control the processing of basic 2d spectra. The first is the fourier numbers to be used in each dimension of the transform (controlled by **fn** and **fn1**). These parameters determine the digital resolution of the final transformed spectrum. The second factor is the choice of windowing applied to each fid before performing fourier transformation. Windowing, controlled by the variables **lb, lb1, sb, sb1, sbs, sbs1, gf, gf1, gfs,** and **gfs1**, determines whether the fids are multiplied by an exponential, sine, sine-squared, gaussian or shifted sine, sine-squared, or gaussian functions prior to fourier transformation in each dimension.

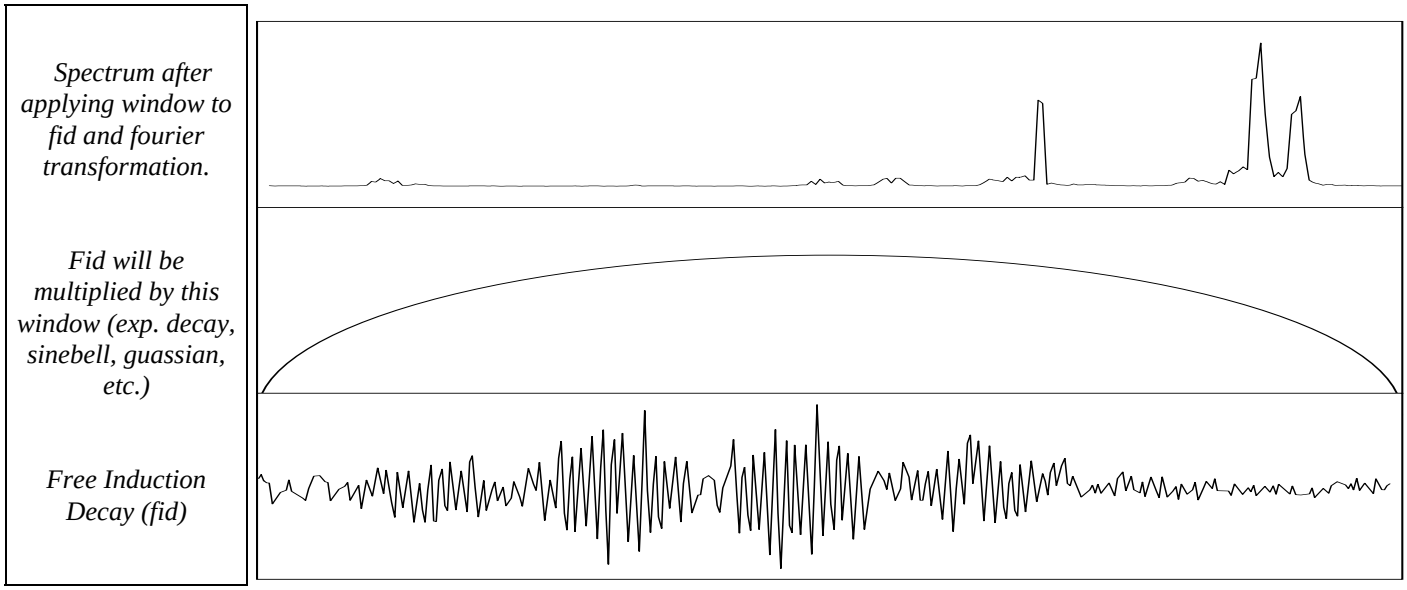
1) Determine Desired Digital Resolution. The digital resolution is the number of hertz/point along each axis in the 2D spectrum. Each axis can have a different digital resolution. In most cases, the digital resolution for each dimension is equal to the sweep width of the dimension divided by half the fourier number of the dimension (**sw**/0.5***fn** in the **f2** dimension, **sw1**/0.5***fn1** in the **f1** dimension). You can improve the digital resolution of the final spectrum by fourier

transforming a larger number of points than you acquire, which is called zero-filling the spectrum (Please refer to the handout on zero-filling for a detailed description of zero-filling).

The spectrum will be zero-filled whenever **fn>np** (or **fn1>2*ni)**. As a general rule, zero-filling will improve the resolution between two individual peaks upto the point where **fn** ≤ **np*2**^{3}. If **fn>np**, then the **f2** (acquisition) dimension will be zero-filled. automatically to **fn** points. Generally, if **fn1>ni*2**, the time-incremented dimension will be zero-filled. Zero-fill spectra if you need to improve the final resolution of 2D spectra, but do not zero-fill more than is necessary. Zero-filling can rapidly lead to massive (100 MBtye to GigaByte) data sets that can require many hours to process and display.

2) Next, determine the appropriate window function to be applied to the fid before fourier transformation. This topic will be covered in detail for 1D spectra in a separate handout. The discussion here will be limited to basic choices to be made in analyzing 2D spectra.

The command **wti** will allow you to interactively view and apply windows to the fid. In an experiment containing a 2D spectrum, type **wft(1)**. This will transform the first acquired fid in the **f2** dimension. Next, type **wti(1)**. You will see the following display:



The **wti** function lets you interactively view the fid, apply a window function to the fid (either an exponential, sinebell, shifted sinebell, gaussian, shifted gaussian, squared sinebell or shifted squared sinebell), fourier transform the fid, and view the effects of the window on the resulting spectrum, in real time. Use the **lb, sb, sbs, gf, and gfs** menu buttons to turn on or off each of the various window functions. Then, place the mouse arrow near the green window function, and click the left mouse button to change the shape of the window. Place the mouse arrow in the top box and click the right mouse button to turn the display of the fourier transformed spectrum on or off (this can save time when transforming spectra with very large fourier numbers (**fn**).

As an alternative to the interactive windowing routine **wti**, you can use the following commands to create precisely defined windows, such as a sinebell covering **np** points, shifted by $\pi/4$ radians.

define a sinebell window	**sinebell('f1' or 'f2',n)**	the **sinebell** command by itself will generate an unshifted sinebell window covering **np** points in **f2** or **ni*2** points in **f1.** Use the optional parameters **f1** or **f2** to define the dimension to apply the window. The optional parameter **n** can be set to any desired number of points, e.g., 2048, or a calculated value, e.g., **np**, **ni, 0.5*ni,** etc.)
define a sine-squared window, unshifted or shifted	**sinesq(n,m,'f1' or 'f2')**	the **sinesq** command will generate a sine-squared window, shifted by **π/n** radians, covering **m** points, in the **f1** or **f2** dimensions. For example, the command **sinesq(4,2048,'f2')** will generate a sine-squared window covering 2048 points in the **f2** dimension, shifted by **π/4** radians.

Guidelines for optimum windows: Sinebell windows reduce the intensity of the leading edge of the fid and increases intensity in the later portion of the fid. This manipulation will artificially narrow the base of the nmr signals and will significantly enhance resolution, at the expense of sensitivity. For 2D data that are displayed as magnitude (non-phase sensitive) spectra, one should generally start with unshifted sinebell windows matched to the length of the data in both dimensions. This is particularly important for all basic COSY spectra. *With few exceptions,* you should always use unshifted **sinebell** windows matched to the number of points in each dimension when you are processing COSY spectra. For COSY spectra, use **sinebell('f2',np)** for the **f2** dimension and **sinebell('f1',2*ni)** for the **f1** dimension of non-phase sensitive COSY data. You will generally get good results when you process all other 2D spectra using **sinesq** windows. To improve sensitivity, these windows can be shifted by **π/(3 to 8 radians)** and should generally cover the number of points in each of the data dimensions. For example, **sinesq(4,np,'f2')** for the **f2** dimension and **sinesq(4,ni*2,'f1')** for the **f1** dimension will give decent results for many phase-sensitive 2D data.

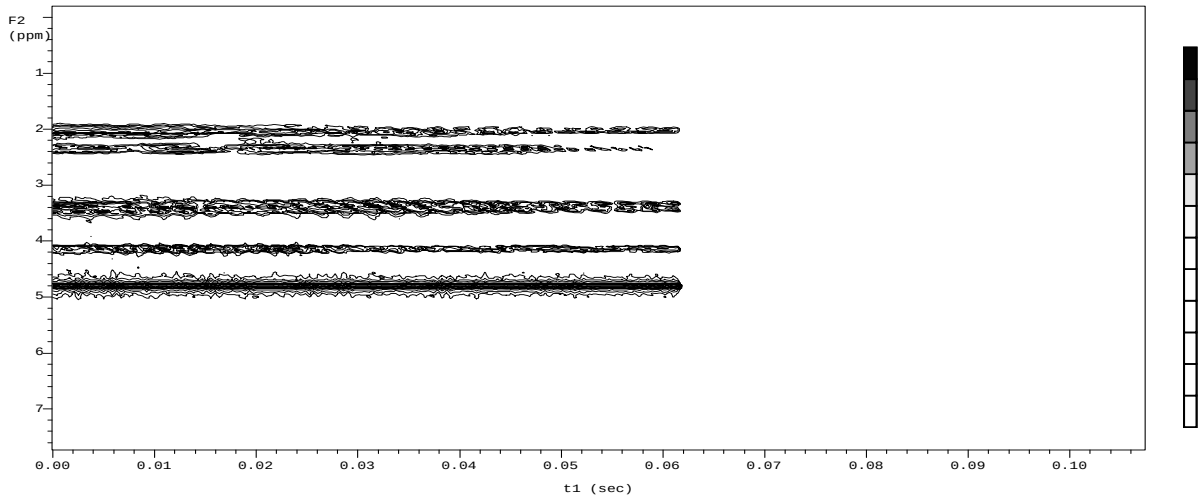
C. Fourier Transforming the Acquisition (f2) Dimension and the Time-Incremented (f1) Dimension

You can begin the 2d transformation once the window functions for the acquisition dimension **f2** and the digital resolution for both the **f2** and **f1** are set. Type **wft1d** to transform the **f2** or acquisition dimension only. The intermediate 2d spectrum will be displayed and you can then set the windows for the time-incremented dimension. Finally, type **wft2d** to carry out the transformation of both the **f2** and **f1** dimensions. (Note: use the commands **wft1da** or **wft2da** if you are processing phase-sensitive spectra).

If you haven't determined the windows to be applied when transforming the time-incremented dimension **f1**, then you should transform the **f2** dimension first (**wft1d**). If the windows for the **f1** dimension have been determined, e.g., with the **sinebell** or **sinesq** commands, you can perform the fourier transformation of both dimensions at this point (**wft2d**).

transform the acquisition **(f2)** dimension only	**wft1d** or **wft1da**	Use **wft1d** to transform the **f2** (acquisition) dimension of magnitude mode spectra (e.g., COSY simple HETCOR, COLOC, most 2D-J resolved, etc.). Use **wft2da** to transform all absolute value, phase-sensitive 2D data sets, typically all n-quantum filtered experiments (dqf-cosy, mqf-cosy), all indirect-detection experiments (hmqc, hmbc, hmqc-tocsy, etc.), most NOE and spin-locking experiments (NOESY, ROESY, tocsy, etc), and phase-sensitive COSY experiments.
transform both the **f2** and **f1** dimensions	**wft2d** or **wft2da**	Use **wft2d** to perform the complete transformation of both the **f2** and **f1** dimensions of magnitude mode spectra. **wft2da** is used to transform both dimensions of all absolute value, phase-sensitive spectra.

If you transform a spectrum halfway by typing **wft1d** (or **wft1da**) you will see a bitmapped display of the result, as shown in the following figure:



You can select a trace of data from the halfway transformed spectrum by clicking on the **trace** menu button. Use the **left** mouse button to place the horizontal crosshair directly on one of the peaks. The individual data points for that peak, acquired as a function of increasing time will be extracted and shown above the bitmapped display. The data point corresponding to this peak in the **f2** dimension is given by the index number, shown in the VNMR message/status window. Make a note of this number. Say the number is **513**. Now type **wti(513)** and this trace of data will be placed into the interactive windowing program. Determine the desired window for processing the **f1** time-incremented dimension. Finally, complete the transform of this dimension by typing either **wft2d** or **wft2da**. Use **wft2d** for non-phase sensitive spectra. Use **wft2da** for all phase-sensitive spectra. At this point, the 2D spectrum will be displayed.

IV. Display and Manipulation of 2D Spectra

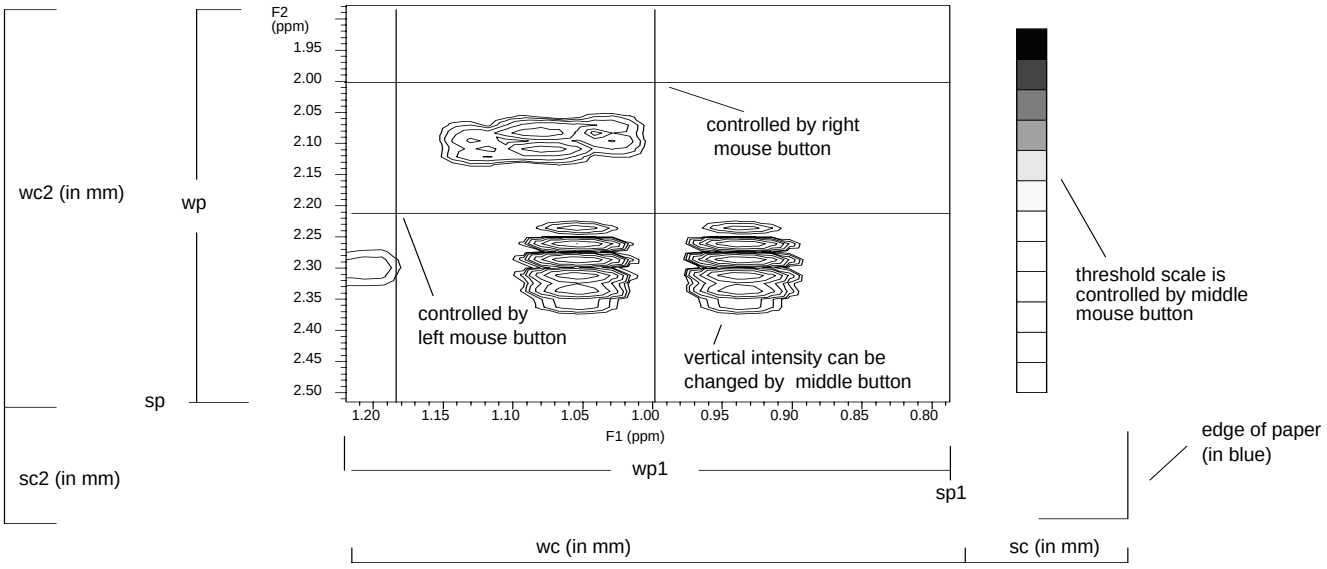
2D NMR spectra can be displayed as a bitmap (pixel) display, as a topological, contour-mapped (raster or interpolated) display, or as a stacked plot of each individual spectrum making up the 2D experiment. The 2d data can be expanded and manipulated in real time. The parameters controlling the region of the spectrum that is displayed can be precisely controlled, as can the parameters that control the physical dimensions of the display. The primary 2d display commands are summarized in the following table:

generate interactive bitmapped display	**dconi(options)**	**dconi** generates a bitmapped contour display. The **middle** mouse button controls the threshold scale at the right of the display. The **left** button controls the left cursor. The **right** mouse button controls the **right** cursor. **dconi** can accept several options, including **'gray', 'linear',** and **'dpcon'**, for gray-scale image, linear level spacing, and interpolated contour display.

display interpolated contour (topological) display	**dpcon(options)**	**dpconi** draws contours that are interpolated between points in the 2d spectrum. **dpconi** accepts several options, i.e., **dconi('display type',number of levels,spacing of levels). display type** can be **'pos', 'neg',** or **'linear',** corresponding to positive or negative peaks, linear vertical spacing of contour levels, respectively**.** The **number of levels** can be 1 to 50. The **spacing of levels** is the spacing between the vertical intensity of contours and is in powers of 2 or fractional powers of 2. **dcon('pos',20,1.3)** would generate a contour map showing only positive peaks, containing 20 contours separated by 1.3 powers of 2 in intensity.
vertical scale of display	**vs=*n***	**vs** is equal to the tallest peak in the 2D spectrum, in ***n*** millimeters. **vs** interacts with **dconi** to control the colors in the 2D display (reduce **vs** if you only see white peaks, increase **vs** is you cannot observe peaks).
threshold of display	**th=*n***	**th** controls the threshold of the 2d display. Peaks below the threshold are not shown. The intensity of peaks above the threshold are shown in changing colors and are drawn at successive powers of 2 in intensity. **th** and **vs** interact, i.e., increasing the threshold by 1 is equivalent to dividing the vertical scale by 2.
region of spectra that is displayed	**sp, sp1, wp, wp1**	**sp** and **wp** define the start and width of the displayed plot along the **f2** dimension, in ppm or hertz. **sp1** and **wp1** do the same along the **f1** dimension
physical size of the spectrum	**sc, wc, sc2, wc2**	**sc** and **wc** define the physical size of the plot, in mm, in the horizontal axis of the display. **sc2** and **wc2** control the vertical dimensions of the plot or display.
display the full spectrum	**f**	**f** will set **sp, sp1, wp** and **wp1** to their maximum values. The complete 2d spectrum will be displayed the next time **dconi** or **dpcon** is typed.
display the spectrum at the right side, left side or center of the display	**right, left,** or **center**	**right, left** and **center** will automatically calculate changes to **sc, sc2, wc,** and **wc2** that will place the spectrum at the right, left or center of the display. This is useful for leaving room for traces or parameters on plots and can be used to quickly make side-by-side plots.
expand the current plot to full screen	**full** or **max**	**max** will set the physical size of the plot to cover the complete page. **full** will leave some room for a trace to be displayed along the top of the page.
Save display	**s1, s2, etc.**	Saves the *parameters* that define the current 2D display (**wp, sp, wc, sc, vs, wp1, sp1, wc2, sc2**). You can save upto nine different display sets by typing **s1** to **s9**.
Recall a display	**r1, r2, etc.**	Recalls one of the nine possible sets of display parameters and regenerates the 2D display.

After the 2D transform is completed, you can display the spectrum by typing **dconi**. In **dconi,** the middle mouse button controls the threshold for viewing peaks in the bitmapped display. To change the threshold, place the mouse on the color bar scale at the right of the spectrum. Hold the middle mouse button down and move the mouse up or down. The middle mouse button will also change the vertical scale (or intensity) of peaks. Place the mouse on a peak and click the middle mouse button. This will lower the vertical scale of the spectrum. Do the same thing on a region containing only noise. The vertical scale will be increased. In **dconi**, the left mouse button controls the left and bottom crosshairs. You can click the right mouse button to generate a second pair of crosshairs. The position of the right and top crosshairs is controlled by the right mouse button. The two pairs of crosshairs can be used to 'box' any region of the 2d spectrum.

Expand (or zoom in on) this boxed region by pushing the **expand** menu button. Push the **full** menu button to display the complete 2d spectrum. You can switch between displaying one or both pairs of crosshairs by pressing the **box/cursor** menu buttons.



sp, sp1, wp, wp1 control which region of the 2d spectrum is displayed. These are in hertz or ppm.
sc, sc1, wc, wc1 control the physical size and location of the 2d plot. These are in mm.

Use the cursors, **th** and **vs** to define the region you want to plot, and hide noise in the display. You can also change from a bitmapped display to the interpolated contour display by typing **dpcon.** As described in the previous table, **dpcon** can be used to view positive or negative contours and control both the number of contours and the spacing between contours. **dpcon** itself can be passed as an option to **dconi**, in order to view contour displays interactively (type **dconi('dpcon')** to get an interactive contour map display).

V. Plotting 2D Spectra

Spectra will be plotted with the same spectral region, vertical scale, threshold and physical size as shown in the display. The principal plot command is **pcon**, which will generate a contour plot. You can control the numbers of levels plotted and the spacing between levels with the **pcon** command. More than one 2D plots can be placed on a single page. Plots are sent into a buffer and will be sent to the same page until the **page** command is given. For example, two different regions of a 2d display can be saved (with the commands **s1** and **s2**). Typing **r1 left pcon r2 right pcon page** will recall each of the 2d regions and plot each region side-by-side on the same page.

plot contours to paper	**pcon('pos'** or **'neg', #levels,spacing)**	**pcon** by itself will plot 4 contour levels, each spaced by a power of 2 apart in vertical intensity. For phase-sensitive spectra, the options **'pos'** and **'neg'** allow you to plot only positive or negative peak contours, respectively. The option **#levels**, input as an integer, defines how many levels you can plot. The minimum is one, the maximum 50. The **spacing** option is the vertical separation between levels, in powers of two. The minimum value is 1.01, allowing for very close spacing of levels. **pcon(20,1.3)** can often give dramatic improvement in the presentation of many 2D spectra.
finish plots, eject page	**page('filename')**	The **page** command will send all plots to the plotter and eject the page. Adding the option **'filename'** will divert the plot output to a file, instead of the plotter. This can be used to generate a file containing hpgl (HP plotter language) or postscript output that can be inserted directly into word processing and drawing programs on PC's or Macs.