Processing and Phasing Phase-Sensitive 2D Data:
(Includes gHMQC, gHSQC)

This handout contains information for the effective processing of phase-sensitive 2-dimensional data. Phase-sensitive experiments include HMQC, HSQC, and their gradient-selected variants.

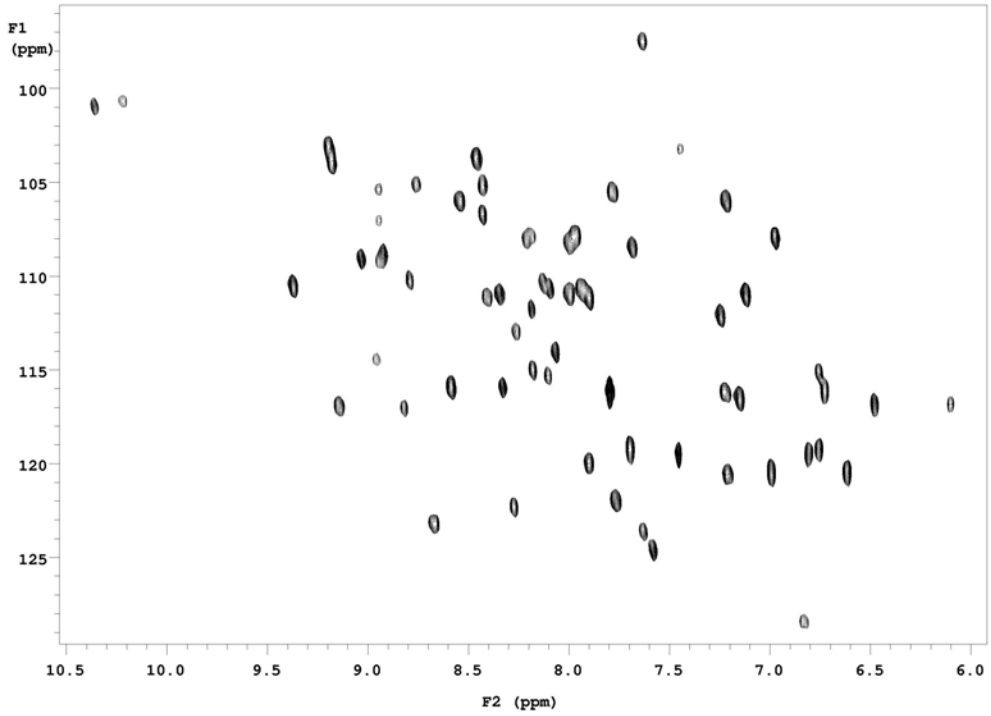

Figure 1. An example of a phase-sensitive 2D contour spectrum. This is an expansion of a ^{15}N HSQC spectrum taken on a Varian Inova 500 MHz spectrometer equipped with an indirect detection probe. The F2 dimension (^{1}H in this case) is the directly detected dimension, while the F1 dimension (^{15}N in this case) is the indirectly detected dimension.

Explanation of Types of Commands Found in this Handout:

1. The VNMR software and the UNIX operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (i.e. CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.

   EXAMPLE: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by a hitting the RETURN key.

   EXAMPLE: **su**

   Hitting the RETURN key is assumed for all bold text commands.

3. Some commands are executed by clicking a mouse button with its pointer on a 'button' found on the screen. The execution of these commands is indicated by a two-letter designation (LC {left click}, RC {right click}, or CC {center click}) followed by a word or words in shadow text that would appear in the 'button'.

   EXAMPLE: LC Main Menu

   This means to click the left mouse button with its pointer on the 'button' that says "Main Menu".

4. Some commands are executed by the mouse itself. These commands are indicated by the two-letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.

   EXAMPLE: LC (at 6 ppm)

   This means to click the left mouse button with the mouse cursor at 6 ppm.

5. Parameters are entered by typing the parameter name followed by an equal sign, the value, and a return.

   EXAMPLE: **nt=16** <rtn>

Retrieve your 2D dataset

Load your 2D data or if you have just acquired the data, save it.

Setting the Phase of the F2 dimension (Directly-Detected Dimension)

wft(1)	perform a weighted Fourier transform on the first increment
f full	display full spectrum

Manually phase the spectrum as you would a 'normal' 1D spectrum.

Manual Phasing Procedure

LC Phase	enter the interactive phasing mode

LC (click on a signal toward the right side of the spectrum about halfway vertically up the screen and adjust the phase by moving the mouse vertically while holding down the left button for coarse adjustment, or the right button for fine adjustment, of the zero-order or frequency-independent phase parameter *rp*).

LC (click on a signal toward the left side of the spectrum and adjust the phase as above to change the first order or frequency dependent phase parameter *lp*)

LC Box	exit the interactive phasing mode

NOTE: if you can't seem to phase the spectrum manually, reset both zero order and first order phases to zero by typing "**lp=Ø** <rtn>" and "**rp=Ø** <rtn>", and try again.

Adjusting Apodization in the Indirectly Detected Dimension (F1) (Optional)

NOTE: This is usually not required, but may be necessary if you are processing prior to completing an acquisition or if you notice additional peaks of decreasing intensity on either side of your cross-peaks.

gaussian	sets up Gaussian apodization for the indirectly detected dimension
wft1da	single weighted Fourier transform of the 2D dataset. This results in a display of a transformed F2 axis (frequency domain) and a time domain F1 axis.
LC Main Menu	selects the Main Menu
LC Display	selects the Display Menu
LC Size	selects the Size options
LC Full with Traces	sets the display screen to include a contour plot and spectrum traces
LC Trace	selects the spectrum trace function. The cursor will appear on the contour plot and a trace of the spectrum along the cursor's horizontal axis will show above the contour plot.

Middle mouse click (MM) on the far left of the spectrum display near a horizontal line to increase the scale. Clicking (MM) directly on the line will decrease the scale. You will need to click (LC) Trace again.

LC and drag the cursor vertically along the plot until you find a large horizontal trace. Release the mouse button on the largest trace. In this case, it is a FID.

ds	display the trace
wti	enter the interactive weighting screen

There should be three rows on the screen.

Middle Click (MM) several times above the FID in the bottom row to increase the scale.

Click (LC) on the middle window inside the green weighting function until the left end of the weighting function ends at the left end of the FID.

Look at the spectrum displayed above the weighting function. If there is no spectrum, right click (RC) the mouse to toggle on the spectrum. The peak(s) should show no 'wiggles' on either side of the peak(s). If there are 'wiggles', LC on the weighting function to bring it to the left until the 'wiggles' are gone.

Fourier Transform the 2D Spectrum

Setup Linear Prediction

setLP1 (optional, see below)	macro that sets Linear Prediction in the first Indirectly detected dimension

NOTE: Linear prediction can be problematic if you have low S/N.

Linear Prediction

Linear prediction can be very helpful for 2D datasets with a limited number of increments (i.e. *ni* is small). Furthermore, it can be used to save time. You can acquire half or even a quarter of the number of increments (*ni*) required for your desired resolution and use linear prediction to calculate back up to the desired resolution.

Linear prediction is an algorithm that predicts new data points based on the existing data that has been acquired by assuming that each data point can be expressed as a linear combination of the preceding data points. Typically, 2- to 3-times the number of data points is reliably predicted. Linear prediction is easily implemented on Varian's spectrometers using the ***setLP1*** macro. This macro sets up linear prediction for the indirectly detected dimension. By default, it will predict 3 times the number of acquired data points. Thus, for example, if you acquired 64 increments (*ni*=64), the macro ***setLP1*** would linear predict 192 data points giving an effective total of 256 increments. Excessive noise present in the acquired data can cause extra peaks to appear in the final

2D spectrum. Therefore, care should be taken when using linear prediction. Below is a list of all the parameters associated with Linear Prediction. They are all set with the ***setLP1*** macro. The only parameter that you may want to reset is ***lpext1***, which defines how many extra points should be calculated. Setting it to only twice the number of acquired data points is not unusual.

Parameters associated with Linear Prediction:

dglp:	Macro that displays the linear prediction parameters.
lpalg1:	Specifies the linear prediction algorithm to use. This is typically set to 'lpfft', which is a least-squares calculation.
lpext1:	Specifies the number of complex time-domain points for linear prediction by which the original data is to be extended.
lpfilt1:	Specifies the number of complex linear prediction coefficients to be calculated from a specified region of time-domain data.
lpnupts1:	Sets the number of complex time-domain points to be used in constructing the least-squares matrix from which the complex linear prediction coefficients are calculated.
lpopt1:	Specifies how the specific linear prediction algorithm is to extend forward or backwards the time-domain data in the first dimension. Typically set to 'f' or forward prediction.
proc1:	Sets the type of data processing to be performed upon the interferogram. 'lp' specifies linear prediction and 'ft' specifies complex Fourier Transform.
strtext1:	Specifies inclusively the complex time-domain data point at which linear prediction data extension is to begin.
strtlp1:	Specifies the first complex time-domain data point to be used in calculating the complex linear prediction coefficients.

gaussian performs apodization

> ***NOTE****: Do not use the above command if you did the optional Apodization above.*

wft2da 2D weighted Fourier transform

If you get an error message stating 'the value of lpfilt1 is too large', you cannot use linear prediction. You can either reacquire the data with ni set to a larger value (e.g. ni=32 would be a minimum number) or you can turn off linear prediction by typing ***proc1='ft' wft2da****.*

After a brief period, a contour plot should appear on the screen. If you get an error message stating 'scale outside boundaries…' and the spectrum is not properly displayed, type ***f full dconi*** *and the spectrum should appear.*

Adjust 2D scale

LC Full displays full spectrum
vs2d? returns the value of the 2D scale

vs2d=*your desired #*	set this to a number higher or lower than the value returned from vs2d? depending on if you need to increase or decrease the scale.
dconi	redisplay contour plot

Alternatively, you can adjust the scale by:

MM (on the contour plot near a cross-peak will increase the scale to the point of the mouse click. **NOTE**: Be careful as this usually generates large scale changes.)

You can further adjust the scale by:

MM (on colored scale to the right of the spectrum. Clicking (MM) to the inside of the colors increases the number of color levels. Clicking (MM) on the color to the outside of another color will reduce the number of color levels displayed)

Phasing 2D Plot: the Directly-Detected Dimension (F2)

trace='f2'	set the horizontal axis to be F2
LC Main Menu	opens main menu display
LC Display	selects the Display Menu
LC Size	selects the Size options
LC Full with Traces	sets the display screen to include a contour plot and spectrum traces
LC Trace	selects the spectrum trace function. The cursor will appear on the contour plot and a trace of the spectrum along the cursor's horizontal axis will show above the contour plot. To increase the scale of the trace, middle mouse click (MM) above the trace.

*LC and drag to peak on the RIGHT of the spectrum. Place the cursor so that the trace displayed above the spectrum shows maximum signal. **NOTE**: Sometimes it is necessary to expand around the peak to get the cursor to the maximum signal. Look at the phase of this peak. If it is not correct, you should do the following:*

ds	display trace spectrum
f full	display full spectrum
LC Phase	enter phasing routine

Manually phase the peak as described earlier (page 2) except DO NOT attempt to phase the left side of the spectrum. Only phase the RIGHT side. This will set the zero-order phasing. When completed,

dconi	display contour plot

LC Trace	selects the spectrum trace function. The cursor will appear on the contour plot and a trace of the spectrum along the cursor's horizontal axis will show above the contour plot. To increase the scale of the trace, middle mouse click (MM) above the trace.

LC and drag on peak on the LEFT of the spectrum. Place the cursor so that the trace displayed above the spectrum shows maximum signal. ***NOTE****: Sometimes it is necessary to expand around the peak to get the cursor to the maximum signal. Look at the phase of this peak. If it is not correct, you should do the following:*

ds	display trace spectrum
LC Phase	enter phasing routine

Manually phase the peak as described earlier (page 2). ***IMPORTANT****: You MUST first click (LC) on the RIGHT of the spectrum prior to clicking on the left of the spectrum to phase. This will setup first-order phasing. When completed,*

dconi	display contour plot

The spectrum should appear with the cross-peaks having good peak shape and only in hues of orange and red. Blue hues indicate a negative intensity and more phasing is required.

If you are still seeing blue hues along the horizontal dimension, you should check the phase of the RIGHT most peak and then the LEFT most peak as described on the previous page. If these are phased correctly, then you will have to phase the intermediate peaks in the same manner working from RIGHT to LEFT until the phase is good.

Phasing 2D Plot: the Indirectly-Detected Dimension (F1)

Setting the Phasing Mode

pmode?	returns the value of ***pmode***.

If you get anything except ***pmode='full'*** *from the above command, you will need to set pmode by typing the following:*

pmode='full'	set the phasing mode to full, which allows interactive phasing of the indirectly-detected dimension

If you had to reset the phasing mode, you will need to reprocess the data by typing:

wft2da	2D weighted Fourier transform

Rotating the Spectrum

You will need to rotate the spectrum so that the axes are swapped (i.e. F1 axis displayed vertically instead of horizontally) and you can easily phase the other dimension. To do this, you;

trace='f1'	sets the trace or horizontal axis to F1
dconi	displays contour plot

The procedure for phasing this dimension is identical to that described previously (see page 6). When completed, the spectrum should have little or no blue peak intensities.

INTERACTING WITH THE CONTOUR PLOT

Display the Contour Plot

LC Redraw	redraws the contour plot

Or

dconi	draws the contour plot

Expanding the Contour Plot

dconi	draws the contour plot
LC	(on the bottom left of where you wish to expand)
RC	(on the top right of the region you wish to expand)
LC Expand	expands region

Rotating the Spectrum

Sometimes you will need or desire to rotate the spectrum so that the axes are swapped (i.e. F1 axis displayed vertically instead of horizontally). To do this, you;

trace?	returns the current value of trace. Note this value.

*If the **trace?** command gives **trace='f1'**, then*

trace='f2'	sets the trace or horizontal axis to F2
dconi	displays contour plot

*If the **trace?** command gives **trace='f2'**, then*

trace='f1'	sets the trace or horizontal axis to F1
dconi	displays contour plot