

BARE BONES GUIDE TO gradientCOSY on the Varian Inova-400&500 (with PFG probe)

This guide is written assuming proficiency in basic operation of the Varian NMR instrument. You should be experienced in performing basic 1-dimensional NMR experiments before attempting to perform 2D experiments on your own. Please ask for help the first time you perform this, to minimize your frustration (if for no other reason).

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Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<angle brackets> are used to designate a key to be pressed (i.e. **<Ret>** for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

LMB will be used to designate the Left Mouse Button

MMB will be used to designate the Middle Mouse Button

RMB will be used to designate the Right Mouse Button

Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press **<Return>** afterwards.

- 1) Make sure you are working in Exp:1 or Exp:2 (jexp1, or jexp2).
- 2) Acquire a normal ¹H NMR spectrum, setting NT=4.
- 3) Zoom-In on the region containing peaks of interest (Note: if you zoom-in on a region with peaks outside that region, you will have *folded* peaks in your spectrum...this can be OK, or it can be a problem; depending upon where the folded peaks land).
- 4) Type **movesw** , and this will set the spectral width and offset to match your selected window.
- 5) Type **ga** to acquire a new spectrum, then phase it.
- 6) Save this spectrum in your directory, as a 1-D trace for plotting beside your 2D contour plot.
- 7) Type **mp(1,100) jexp100** (assuming you are working in Exp: 1, substitute "2" if you are starting in Exp:2). You should now be in Exp:100
- 8) Type **gCOSY** to load the gCOSY parameters.
- 9) Set NT (# transients):
 - a) If your sample is very concentrated, you can use **nt=1** (this takes the minimum amount of time).
 - b) For the best spectrum with minimal artifacts, use **nt=2**.
 - c) Use **nt=4** for more dilute samples (will take 2x as long as (b)).
- 10) Set the number of increments in the *t1* dimension:
 - a) Type **ni?** , and note the current value for ni.
 - b) ni should be 128 by default, You can type **ni=256** . This improves resolution, but it takes longer.
- 11) Type **time** to see how long the experiment will take (*optional*).
- 12) Type **go** to start the experiment.
 - a) After ~32 or more transients, you can type: setLP1<Ret>, sinebell<Ret>, wft2d<Ret> to see if your spectrum is coming along nicely. Otherwise, you can wait until the acquisition is completed.
- 13) If you must stop the acquisition before it is done (i.e., you run out of time), always stop the 2D experiment by typing: **sa('nt')** . This will stop the experiment at the end of the current FID.
- 14) Type **svf** , followed by your filename, to save the data for processing.
- 15) Type **jexp1** , eject your sample, and re-insert the reference. Be sure to turn the spinner back on in **[Acqi]**, and lock/shim on the reference before you sign-out.

Processing and Plotting the 2D-COSY spectrum:

- 1). Select your printer/plotter and paper size first.
- 2). Type **jexp1** to join Exp:1, and load the 1D-¹H spectrum from step 8 on the previous page.
- 3). Type **jexp2** (or any other valid Exp:#), and load the 2D-COSY spectrum
- 4). Type **setLP1** to activate forward linear prediction (improves resolution and quality).
 - a). Type **fn?<Ret>**, and note the value for fn. If greater than 2048, the processing will take a very long time.
 - b). If $fn > 2048$, type: **fn=2k<Ret>** then **fn1=fn <Ret>**.
- 5). type **sinebell** to set the window-function parameters.
- 6). type **wft2d** to process the data.
- 7). type **foldt** to symmetrize the spectrum (recommended). This removes artifacts, but can reduce the information content of the spectrum in some cases.
- 8). If the contour plot is not centered, type **center** followed by **dconi**.
- 9). NOTE: the command **dconi** is analogous to “ds” for 1D spectra. If you lose your 2D image, and you want to view it, type **dconi** to re-display the 2D spectrum. You don’t need to re-type wft2d unless you want to try different processing parameters. In fact, re-processing every time will waste a lot of your time!
- 10). Adjusting the contour plot:
 - a). *click* the **MMB** on any spot in the 2d-display, to scale the spectrum so that spot just touches the *floor* of the contour plot. *Clicking MMB* on a taller peak will reduce the *vs* to make smaller peaks disappear.
 - b). Alternatively, you can *click MMB* on the colored scale (on the right) to reduce the minimum level displayed. I recommend using (a), rather than (b).
 - c). You can fine-tune the peaks displayed peaks by *clicking* on [**vs+20%**] or [**vs-20%**].
- 11). Plotting: there are lots of ways to do this...this is the recommended way.
 - a). Plotting with your high-resolution 1D spectrum (in Exp: 1) displayed along the sides. Problem: If your 1D spectrum has very tall peaks, the size of your 2D plot will be reduced in size (sometimes, very small).
 - 1). Type **plcosy(15,1.5,1)**. This means: draw 15 contours, separated by 1.5 level units, and the 1D spectrum that is in Exp: 1 on the sides.
 - a) Note: if your 1D spectrum is very small due to intense singlets, you can multiply the 1D trace by a factor of “n” by typing: **plcosy(15,1.5,1,n)** (i.e. plcosy(15,1.5,1,5) will multiply the 1D trace intensity by 5x).
 - 2). If using BIG (11”x17”) paper, substitute “plcosybig” for “plcosy”
 - b). If you want to print to a PostScript file instead of to the printer, do the following:
 - 1). Substitute “**plcosy_nopg**” for “plcosy” above
 - 2). After executing **plcosy_nopg(15,1.5,1,n)**, type **page(‘filename.ps’)** and your plot will be saved in your directory as a PostScript file under the name “filename.ps”. Of course, use your own filename instead of “filename”.

-R.Shoemaker