

## COSY on the U400

### PRELIMINARY INFORMATION

- pw90***: The default *pw90* (currently 20.0  $\mu$ s, determined using the 0.1% ETB standard) is usually adequate for this experiment, unless the physical properties of your sample are very different from those of the standard due to, for example, the presence of ionic or paramagnetic materials or the use of a very different solvent. Refer to handout UVU423 for the determination of *pw90*, if it is required. If a new one is determined, remember to change the parameter *pw90* to the newly determined value.
- T1***: If you can not make a reasonable guess of the longest T1 of your sample, it may be necessary to measure it in order to set the relaxation delay *d1* correctly. If *d1* is too short, your COSY spectrum may appear as if every peak is coupled to every other peak. If *d1* is too long, time will be wasted. Refer to handout UVU 438 for instructions on T1 measurement.
- Sample spinning**: non-spinning of the sample is recommended to minimize T1 noise and other artifacts in the COSY spectrum.
- Optimization of acquisition parameters — *sw*, *ni*, *d1*, and *nt***:
  - sw***: It is essential that *sw* be optimized in order to maximize digital resolution and to save time.
  - ni***: Usually *ni* is set to between 128 and 512, depending on *sw1* and the digital resolution desired in the *f1* dimension {Hz/pt(*f1*)=*sw1*/*ni*}. In general, *ni* should be set so that Hz/pt(*f1*)  $\geq 10$ . A number of *sw1*-*ni* combinations that satisfy the above condition are given below for your reference:
 

<b>sw1</b> (ppm)	20	15	10	8	6	4
<b>ni</b> (for Hz/pt=10)	800	600	400	320	240	160
- d1***: Ideally  $d1 = \{1.3 \times T1(\text{longest}) - a\}$ . *d1* is usually set to 1 to 2 sec because for most medium-size molecules, their proton's T1's are typically around 1 sec, and *a* for COSY is normally around 0.3 sec. If these assumptions do not hold for your compound, change the *d1* accordingly.
- nt***: Set *nt* to the smallest multiple of 4 that would give reasonable signal-to-noise in the 1D spectrum. Note that doubling *ni*, *nt*, or *d1* will approximately double the experiment time. In general, within a given experiment time, one should minimize *nt* and maximize *ni* to obtain the best quality data.
- VT***: With long acquisitions (longer than 4 hours), consider running with VT on at 25 - 30°C to avoid or minimize artifacts due to room temperature fluctuation.
- Calculation of experiment time**: The time calculated by the command **time** and the *Time Remaining* in the Acquisition Status window (shown only after the acquisition is started) may not be accurate, and could underestimate the length of a 2D experiment by as much as 25%. You should take this into account when setting up the experiment.

### 1. COLLECT A 1D 1H SPECTRUM

**chdir** <rtn>

enter your username at the prompt

**jexp1** <rtn>

collect the 1D spectrum in exp1

Select a standard parameter set and retrieve an appropriate shim file.

Lock and shim on the sample as usual. When done with shimming, enter:

**nt=1** <rtn>

set *nt* for a spectrum that can be phased and referenced properly; *nt*=1 is usually sufficient

**ga** <rtn>

start acquisition with autogain

**f full aph** <rtn>

full display and autophase

**nl rl( )** <rtn>

set reference

Optimize the spectral window for the COSY experiment. Use the two cursors to include the signals of interest plus 10% of empty baseline on either side of the spectrum, then enter:

<b>movesw</b> <rtn>	reset <i>sw</i> and <i>tof</i> as defined by the two cursors
<b>gain='y'</b> <rtn>	set gain value to that selected by autogain above
<b>nt=_____</b> <rtn>	set <i>nt</i> for a reasonable S/N 1D 1H spectrum
<b>ga</b> <rtn>	start acquisition
<b>f full aph</b> <rtn>	full display and autophase

Inspect the spectrum to make sure that it contains all the signals of interest and that folding over, if any, does not cause a problem. Also check and make sure that the spectrum is phased and referenced correctly. If everything is OK, save the data as usual.

## 2. SET UP THE COSY EXPERIMENT — **docosy**

NOTE: **docosy** is an interactive macro that will take you through the setup of a COSY experiment. In this handout, the dialog will appear in **this font**, and the symbol ¶ signifies that a response is required. Most messages will appear in the Status Window, while inputs are entered *via* the Input Window. Window arrangement on the left side of the screen is, from top to bottom, Status, Input, Menu, Graphics, and Text.

### 2A. Enter the Macro

In *exp1* where the 1D 1H spectrum has been acquired, enter:

<b>docosy</b> <rtn>	set up a COSY in <i>exp2</i> (2 is the default <i>exp#</i> )
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NOTE: If you wish to do the COSY in a different experiment, you should enter **docosy(exp#)**, where **exp#** is a number between 1 and 9, excluding 5. Avoid doing COSY in current experiment or *exp1*.

After entering the macro, the following menu will be displayed in the text window at the bottom of the screen:

COSY MENU	
1)	np=1024, ni=256, nt=4, d1=1, time=20min
2)	np=2048, ni=512, nt=4, d1=1, time=40min
3)	np=1024, ni=128, nt=16, d1=1, time=40min
4)	np=1024, ni=256, nt=16, d1=1, time=1.5hr
5)	np=2048, ni=512, nt=16, d1=1, time=3.0hr
6)	Manual entry of np, ni, nt, and d1

The suggested **np** and **ni** value will also be shown in the Status Window, which is calculated using the current spectral width to give a digital resolution of 5 Hz/pt in the *f2* dimension and 10 Hz/pt in the *f1* dimension. This is for your reference in choosing a suitable COSY setup.

### 2B. Choose a Setup

Enter your choice of setup at the following prompt in the Input Window:

Enter your choice (1-6) from the menu below: ¶

If 1 to 5 is chosen, the parameters will be set automatically.

If 6 is chosen, the following dialog will appear:

Enter the following parameters:

np= ¶  
ni= ¶  
nt= ¶  
d1= ¶  
Is everything OK? (y/n) ¶

*If answered no, you will be prompted to re-enter these parameters.*

NOTE: nt will be rounded off to the nearest multiple of 4.

## 2C. Save the Data

*Finally, you are given a choice of saving the COSY data automatically when it is finished:*

Save COSY data when done? (y/n) ¶

*If answered yes, the current directory will be shown in the Status Window and the dialog will appear:*

Are you in the correct directory? (y/n) ¶

*If answered no, you will be prompted to enter your username, and the working directory will be changed to your fids directory. Then, the dialog continues:*

Enter file name to save: ¶

*If the file already exists:*

File exists! overwrite? (y/n) ¶

*If answered no, you will be prompted to enter a new file name.*

## 2D. Start the Experiment

The **docosy** macro will terminate at this point and allow you to check the parameters before starting the COSY experiment with either **au** or **go**:

**au** — start COSY experiment, and if you answered yes to **Save COSY data when done?**, it will save and process the data at the conclusion of the experiment. If you answered no, it will process the data only, and no data will be saved. **IMPORTANT:** If the experiment is terminated with **aa** or **sa** (see below) before it is finished, you will have to manually save and/or process the data.

**go** — start COSY experiment, with no actions taken at the conclusion of the experiment.

NOTE: Once the experiment is started, it may be aborted by clicking the Abort Acq button or typing the **aa** command *if no data is to be retained*, such as when you discover that a mistake has been made in the setup of the experiment, or when it has become apparent that the experiment is useless. *If data retention is desired*, such as when sufficient signal-to-noise has been obtained or when you have run out of time, you should use the **sa('fid')** command which will stop the acquisition at the next complete FID. At this point, you should save the data manually because automation will not work when the experiment is terminated before its completion.

NOTE: You may examine the data in the middle of the acquisition and in the same experiment the data is being acquired by typing **cosyproc** (see Part 3A below). Be aware that a 2D spectrum processed before its completion will have a lower resolution in the *f1* dimension (or both dimensions if data is symmetrized) because fewer FID's are being used. Therefore, it is recommended that you wait until at least half of the number of FID's have been collected before processing and examining the data.

*When the experiment is finished, save and/or process the data if it is not yet saved and/or processed. Then proceed with the displaying and plotting of the data as described below.*

### 3. PROCESSING, DISPLAYING, AND PLOTTING OF COSY DATA

NOTE: Since processing and plotting of 2D data require a significant amount of time, it is recommended that you move these tasks to the SPARC2 data station. This will not only free up the spectrometer for other users, but will also save you time and research money, because the SPARC2 has a faster computer and a much lower charge rate (approximately half that of the U400).

#### 3A. Processing

**cosyproc** <rtn> macro to process COSY data

This macro will do a weighted 2D Fourier transformation and, if the data is square (*i.e.*,  $fn=fn1$  and  $sw=sw1$ ), symmetrize the data as well. It will also attempt to adjust the vertical scale and the threshold properly and display a square color map with room for traces or projections. It gives the same display as **cosydisp** or **cosyds** below.

*To process the COSY data manually, enter:*

**wft2d** <rtn> weighted 2D Fourier transformation  
**foldt** <rtn> (optional, requires  $fn=fn1$  and  $sw=sw1$ ) symmetrization of COSY data

#### 3B. Displaying

*a. Adjust the dimensions of the 2D display:* (not needed if data is processed with **cosyproc**)

**cosydisp** <rtn> macro to adjust  $vs$  and  $th$  and display a square 2D color map with room for projections  
- OR -  
**cosyds** <rtn> macro to display a square 2D color map without changing  $vs$  or  $th$

NOTE: If a different display is desired, see Part 3.C.c below.

*b. Scaling the 2D display manually:*

*Option 1:* place cursor on the weakest signal you want to see and click the middle mouse button. This will rescale and update the 2D display so that the selected signal is just visible. Then adjust the threshold with the middle mouse button on the colored threshold vertical bar to obtain the desired display.  
*Option 2:* **vs**=\_\_\_ <rtn> enter an estimated  $vs$  value  
**dconi** <rtn> redisplay the 2D spectrum using the new  $vs$  value  
then adjust the threshold as above to obtain the desired display.

*c. Expanding a region:*

LC (at lower left-hand corner of the region of interest)  
RC (at upper right-hand corner of the region of interest)  
LC 3:Expand expand the enclosed region

#### 3C. Plotting

NOTE: If you are plotting to the Zeta plotter, set the pen to about 1 cm from the perforation.

*a. Plotting with 1D spectra on the sides (Fig. 1 - 3):*

**cosyplot**(#,#) <rtn> macro to plot COSY data with levels=7, spacing=2, and 1D 1H spectrum in  $expl$

The vertical scale of the 1D plots can be adjusted by providing the two optional arguments with the command:

† First argument —  $vs$  scaling factor for the 1D plot on the top of the contour plot, *i.e.*, the vertical scale as adjusted by **vsadjh** will be multiplied by this factor.

† Second argument —  $vs$  scaling factor for the 1D plot on the left side of the contour plot

*You will be prompted to enter a plotting option depending on the plotter:*

For *LaserJet\_150R*: **plotting options: 1)default, 2)as displayed: ¶**

For *Zeta*: **plotting options: 1)default, 2)as displayed, 3)one page: ¶**

\*If you enter 1, the 2D display will be adjusted to the default setting before plotting (i.e.,  $wc=wc2=120$ ,  $sc=10$ , and  $sc2=0$  for *LaserJet\_150R*, and  $wc=wc2=160$ ,  $sc=10$ , and  $sc2=0$  for *Zeta*). See example in Fig. 1.

\*If you enter 2, the spectrum will be plotted as displayed. See example in Fig. 3.

\*If you enter 3 (for *Zeta* only), the 2D display will be adjusted and plotted on one page. See example in Fig. 2.

You will also have a choice of plotting with or without text and/or parameters:

plot with: 1)text & parameters, 2)text only, or 3)none? ¶

**b. Plotting with different contour levels, spacing, and 1H exp# (Fig. 4):**

**cosypl(##,##,##,##,##) <rt>** macro to plot COSY data with user's input of contour levels, spacing, and 1D 1H exp#.

† First argument — number of contour levels to be plotted. 5 is usually sufficient, 7 is the default.

† Second argument — spacing (in mm) between contour levels, default is 2.

† Third argument — 1D 1H experiment number. If not provided, the following dialog will appear:

**plot without 1D spectra on the sides? (y/n) ¶**

If answered no, you will be prompted to enter the experiment number that contains the 1H spectrum.

† Fourth argument — vs scaling factor for the 1D plot on the top of the contour plot, default is 1.

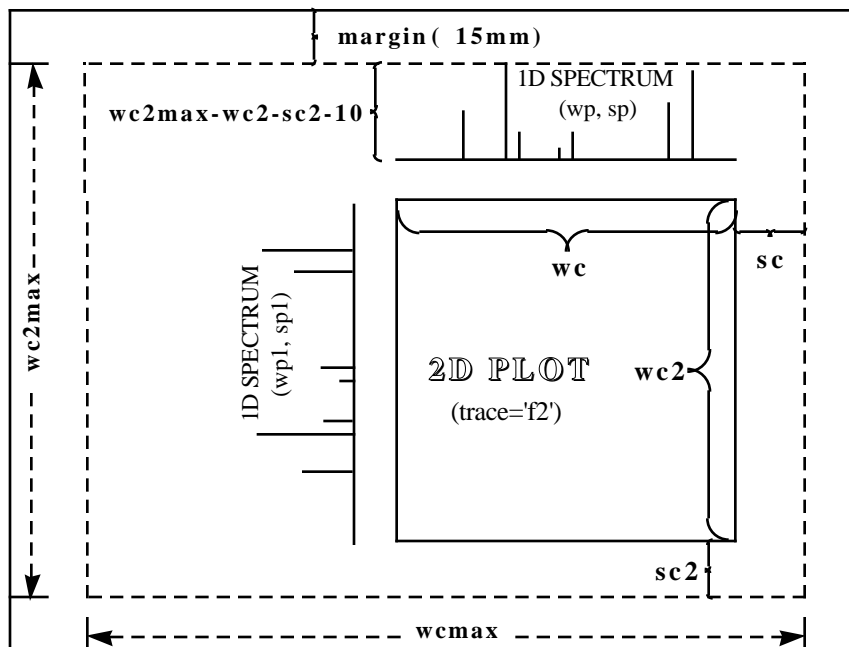
† Fifth argument — vs scaling factor for the 1D plot on the left side of the contour plot, default is 1.

NOTE: Plotting options for this macro are the same as **cosyplot**. In addition, if you decided NOT to plot the 1D spectra on the sides, the default settings are  $wc=wc2=160$ ,  $sc=10$ , and  $sc2=0$  (See Fig. 4) for the *LaserJet\_150R*, and for *Zeta*, the default settings are the same as the one page plot:  $wc=wc2=160$ ,  $sc=220$ , and  $sc2=0$ .

**c. Plotting with different displaying and plotting parameters (Fig. 3):**

NOTE: Due to the symmetry of COSY data ( $fn1=fn$ ,  $sw1=sw$ ), it is preferable to plot the COSY spectrum as a square as adjusted by the **cosyproc**, **cosydisp**, or **cosyds** macro. However, if desired, the size and shape of the 2D plot can be adjusted by changing the 2D displaying and plotting parameters, as illustrated in the diagram below and the examples in Figure 1 to 4 at the end of this handout.

Diagram: 2D Displaying and Plotting Parameters



**For LaserJet\_150R:**

$wcmax = 250$   
 $wc2max = 180$

**Default plot:**

$wc = wc2 = 120$   
 $sc = 10, sc2 = 0$

**For Zeta:**

$wcmax = 400$   
 $wc2max = 240$

**Default plot:**

$wc = wc2 = 160$   
 $sc = 10, sc2 = 0$

**One page plot:**

$wc = wc2 = 120$   
 $sc = 215, sc2 = 0$

To plot, set  $wc$ ,  $wc2$ ,  $sc$ , and  $sc2$  according to your specifications, making sure that you have enough room for the 1D plots if you want them. Then redraw the 2D display with the **dcon1** command, and plot the spectrum using either the **cosyplot** or **cosypl** macro with the **as displayed** option.

d. Plotting with grid (Fig. 3):

**grid** <rtm> display grid (NOTE: **grid**(#) will change spacing between grid lines to # cm, default is 1)  
**dcon** <rtm> (without it, the cursor cross will be plotted along with the grid)  
**plgrid** <rtm> plot grid (*plgrid*(#) works the same way as *grid*(#))  
**cosyplot** or **cosypl** <rtm> (IMPORTANT: you must choose the **as displayed** plotting option)

e. Plotting with correlation map (Fig. 4):

Obtain the desired display as described above and enter:

**l12d** <rtm> perform a 2D line listing  
**acosy** <rtm> analyze COSY spectrum and display correlation map and connection table. Readjust *vs* and *th* if needed.  
NOTE: if plotter='LaserJet\_150R', the message "No room to display COSY connection table" will appear and no table will be displayed. Ignore this and proceed with the plotting.

**pacosy** <rtm> analyze COSY spectrum and plot correlation map and connection table

**cosyplot** or **cosypl** <rtm> (IMPORTANT: you must choose the **as displayed** plotting option)

As you can see from the example in Fig. 4, the COSY auto analysis often misses some quite strong signals while picking up others that are not even there.

f. Plotting with projections:

i) Plot the 2D contours: adjust the 2D display as before and enter:

**pcon** <rtm> plot the 2D contours  
**dconi** <rtm> enter interactive 2D display

ii) Plot the horizontal projection:

LC 3:Proj select projection menu  
LC 1:Hproj(max) select horizontal projection  
CC (on the projection) adjust vertical scale of projection, if necessary  
LC 5:Plot plot horizontal projection  
(Make sure the Plot button blinks with the click, otherwise the command may not have been taken.)

iii) Plot the vertical projection:

LC 3:Proj select projection menu  
LC 3:Vproj(max) select vertical projection  
CC (on the projection) adjust vertical scale of projection, if necessary  
LC 5:Plot plot vertical projection  
(Make sure the Plot button blinks with the click, otherwise the command may not have been taken.)  
**page** <rtm> send plots to the plotter

g. Plotting traces from the 2D dataset:

**dconi** <rtm> enter interactive 2D display mode  
LC 2: Trace select trace menu  
LC (on peak of interest) select a trace  
CC (on the trace) adjust vertical scale of the trace, if necessary  
**ds** <rtm> display the trace

The trace can now be displayed and plotted just like a normal 1D spectrum. Adjust **wc**, **sc**, and **vs**, then plot it as usual with **pl pscale page** <rtm>, for example.

REFERENCES:

Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: 1987; pp183-243.

Sanders, J. K. M.; Hunter, B. K. *Modern NMR Spectroscopy*; Oxford Press: 1987; pp108-113.

