2 Dimensional NMR
User's Brief Guide

(This handout presumes your thorough familiarity with the 1D handout commands and their use!)

Options

There are a vast number of 2D experiments to choose from. We describe here the explicit setup and processing for COSY (the H,H kind) and HMQC (the C,H kind). The setup and processing are pretty general and with skills learned from one of these experiments you can advance to others. After you become comfortable running COSY and HMQC, you should consider the following other 2D experiments:

DQFCOSY (COSYDQFPHSW)
a much cleaner version of COSY greatly reducing the diagonal intensity and selecting only the Double Quantum contributions to the FID. For special searches one could even do triple quantum to give very clean 2Ds showing only peaks from three spin systems.

COSYTP,COSYST (COSYPHSW)
Phase sensitive COSY from which one can get J couplings and assign which couplings are due to which protons in complex multiplets

HMBC (HMBCGPND)
heteronuclear multiple bond correlation. One can see the connections from a proton to carbons two and sometimes three bonds away. Often useful for relieving structural ambiguities.

TOCSY (MLEVPHSW)
'total' correlation: that is, all the spins in a system of connected spins.

NOESY,ROESY (NOESYPHSW,ROESYPHSW)
gives through space coupling of protons and from the NOEs or ROEs the distance can be calculated

Acquisition Set-up

It is assumed that the sample is locked and well shimmed.

1. open any data file.
2. type *edc* to create a new file. Click Experiment, then click Select. In the pop-out window, find the small "Source" window on the top-right and select /opt/…/user. Choose the suitable experiment: for COSY, pick COSY1; for HMQC, pick HMQC1.
3. check *execute getprosol*.
4. Now your file will have all the needed standard parameters to run a 2D spectrum. You can change parameters if you wish (details discussed below)
5. You could run *expt* command to estimate experiment time. Then you can adjust *ns* and *td* (1st dimension) to suit your available time on the spectrometer.
6. *atma*
7. *rga*
8. *zg*
9. After a number of slices have been acquired, you can type *xfb* to get a preliminary look of your 2D spectrum. The vertical dimension may not look highly resolved yet.
10. If you like, you can type *halt* to stop the experiment before it finishes. After completion, type *xfb* to transform the final signal.

A 2D data is comprised of a number of 1D data. Each 1D data is called a slice. *1 td* displays the number of slices, while *ns* displays the number of scans for each slice. The number of slices dictates the resolution of peaks in the first dimension (also called the f1 dimension or indirect dimension). The default is usually 128 or 256, which is usually sufficient for most general purposes. You will need to adjust *ns* so that your experiment can finish within your reserved slot.

Acquisition parameters can be viewed and changed by *eda.* In eda window, each column or dimension has a TD (number of acquired points in FID) and an SW (spectral width centered at O2 and O1 respectively) . The first column is the f2 dimension, the second column is f1.

Processing parameters are stored in *edp* window. Important parameters to change include the number of data point (SI) and window functions in both f1 and f2 dimensions.

Parameters that you might want to play with

<u>ns (number of scans per slice)</u>

Generally for COSY for a 2-5mg sample there is plenty of signal for the default single scan per slice, i.e. NS=1. Obviously if you are short on material and have to run a 0.2mg sample you will have to increase NS by something like (2/0.2)**2 = 100 to get the same signal to noise as with 2 mg. The same goes with HMQC - one with 75mg of sample can keep the run time down to 20 minutes or so.

1 td

Resolution in f1 dimension = sw(Hz)/# slices. The # slices = *1 td*, that is, td in the second (f1) column in the parameters displayed by the command *eda* in a 2D file. Note that td can be any number and need not be 2^n. This also means that one can process the 2D spectrum during acquisition even though not all the slices have been acquired yet.

sw and o1p, o2p

In order to maximize the resolution in the f1 dimension in a limited instrument time slot, we MUST restrict the spectral width to where there are peaks. Load a 1D file or run a preliminary 2D spectrum, then find the ppm range of interest. Write down the center position and the width of this window (both in ppm).

Then type *eda* and enter the acquisition menu. Find the blanks for sw (F1) and enter the new value. For COSY(H,H) you MUST change the sw in both the f2(1^{st} column) and f1 (2^{nd} column) in the parameter set displayed by *eda*. For HMQC you only want to change the carbon dimension SW. Changing the proton dimension SW will not save you any time.

For COSY, there is only one nucleus of interest, Nucleus 1. Enter the new spectrum center ppm value in the o1p blank. For HMQC, the carbon nucleus is called Nucleus 2 and the offset is called o2p although it is in the 1^{st} dimension.

rg

The default rg may already be good enough for you. If the signal seems too big or too small on the screen, you can try to adjust it. Thus you must either do *rga* just as in 1D to adjust the receiver gain.

The fid will almost always be truncated at the end of the window because one is sacrificing resolution for time. Unless you have special need do not adjust aq at this point.

Some 2D programs such as NOESY have an FID which grows in intensity with t_1 so later fids are more intense than the early ones! For COSY and HMQC this is not a worry.

ds (number of dummy scans)

It is best to stay with the default value of ds (usually 8 or 16) for reaching the steady state.

Once the parameters sw, o1, rg and ns are set beyond the default one can enter *expt* and thus know how long the experiment time will take. You can adjust TD1 to give greater or lesser resolution in f1 and trade off between resolution TD1 and signal to noise, NS and total experiment time as you choose.

Once all parameters are set you simply start acquisition with *zg*. You do not need to run to the full TD1 of slices if the resolution is sufficient before that (see below).

While the experiment is running, if you want to look at a particular slice (the 1D fid) say of the 123rd slice you can enter the command *rser* 123. (read serial file) This transfers the 123rd FID/slice from the 2D file to a file called ~TEMP 1 1 where you can process it like a 1D spectrum. This will give you some idea of resolution and signal-to-noise which is good to know early on especially if you are running a long 2D experiment. This also will give you some idea what the default processing will do to the fid…see below. [You can return from the ~TEMP file back to the 2D file with a click on the 2D button.]

Processing a 2D Data Set

The processing of a 2D data set in many ways is more important than acquiring the data. There are many ways to obscure good data by bad processing and many ways to enhance data from a weak sample. The latter is the real challenge. The default processing parameters are not necessarily the best for all parts of the spectrum. There are a lot of parameters to tweak and one can take hours and hours optimizing for a given display.

Processing is done by the command *xfb.* This will transform your raw signal using the default processing settings. You might want to play with the following parameters to improve the spectrum quality.

window functions

Just as in 1D, one massages the 2D fids post-acquisition to enhance sensitivity with an exponential window function (LB) or resolution with Gaussian broadening (GB with '–'LB) or sine bells or sine-squared bells (with shift SSB). One does the same for each dimension in 2D. The default values in HMQC and COSY are for 0 shifted sine bells in both dimensions. This costs in intensity but gives good resolution and eliminates the sin(x)/x wiggles of poorly processed data.

Optimal processing for the best signal-to-noise or best resolution can take quite a bit of time. You have to try different window functions and different values, for example of GB and or LB *in each dimension*. For example you could process first only in the 2nd dimension with *xf2* and then select a given slice: *rsr 85* would put the 85th row (the spectrum from the 85th fid) into the file ~TEMP 1 1. It can

then be *trial* processed with various window functions and values. The tool *wm* (select Manual Window Adjustment) allows this to be done easily. One can observe changes in linewidth, intensity, noise and processing wiggles with window function parameters. This is often a major optimization step.

Once you settle on the optimal processing of the f2 dimension you then issue the command xf1 and then do the same processing of the f1 dimension. In order to get one of the columns of your 2D spectrum into the 1D ~TEMP file use the command rsc # (read serial column) where # is the number of the column you want to see.

magnitude vs. phase sensitive 2D acquisition and processing

There are two modes of data acquisition, governed in acquisition by the details of pulse program (indicated by the presence of *ip*n commands or nd0=2, etc) and in processing by the parameter MC2 . (Phase sensitive pulse programs usually have ph or tp or st in their title.)

The first mode, designated in processing by MC2=QF, does not distinguish phase in the 2nd dimension and deals only with the magnitude part of the transform. It is processed in such a way that all peaks in the 2D are positive. In this case the parameter PH_mod which governs the phasing of the spectra in each dimension is set to 'no' in the f2 dimension and to 'mc' in the f1 dimension. 'mc' means that the value of $[|Real|^{**}2 + |Imag|^{**}2]^{1/2}$ is taken at each point in the 2D plane. This is generally sufficient for routine COSY and HMQC where one has a high signal- to-noise and is not looking for subtle resolution or complexities of multiple J couplings. The lines in this acquisition/processing mode are broadened, usually because of the dispersive imaginary component figures into the intensity.

The second mode of 2D acquisition is 'phase sensitive' meaning not only that the pulse program runs in a different 2D quadrature detection way but that the processing is also phased---often resulting in positive ***and*** negative parts of lineshapes. The increased complexity of processing is generally offset by improved resolution and information. Depending on the pulse program, MC2 is set to TPPI, States or States-TPPI.

phasing 2D spectra

The phasing of phase sensitive 2D spectra is done by clicking the phase button. First you will need to pick several prominent peaks in different areas of the spectrum. Right click on each peak and select "add". Then click "R" and phase rows, then click "C" and phase columns.

A properly phased spectrum will have clean looking positive (and negative) features with minimal dispersion wings…..an improperly phased spectrum will have wide negative and positive intensity streaming out from the bases of the stronger peaks.

SI: number of data points in each dimension

There are of course two SIs: SI2 and SI1. Both have to be 2^n for the Cooley-Tukey fast FT algorithm but they also determine the size of the processed 2D file SI1* SI2 which can be important for available disk space and for transfer times for data so they should be no bigger than necessary. SI2 is easy, almost always SI2=TD2 so that the observed data is zero-filling once on the fids. Generally TD1 is a smaller number, say 128 or 256. If you zero fill once making SI1 256 or 512 respectively you get the minimum sized file. For SI2=1k and SI1=512 one has 512k Real point and 512k Imaginary points, or a 1Mbyte processed file in addition to the TD1*TD2 acquired fid points in the ser file. [For phase sensitive processed data one has in addition the the Re*Re file, the Re*Im and Im*Re and Im*Im files, so if SI1*SI2=1Mbytes, the total processed data is 4Mbytes!

Tdeff: limiting number of acquired scans (f1) or points used in FT (f2 or f1)

For a variety of reasons one may wish to limit the number of points used in f2. For example if you have selected 2k points in t2 and it turns out that there is very little fid intensity in the last half of the time t2, it is a waste of size and disk space to process the full 2k points. The data is already acquired and it would be wasteful to reacquire so one adjusts the parameter TDeff. Its normal value of 0 means that all acquired points are taken. If, in this case TDeff (in f2 column) is set to1k only the first half of the fid is used in the processing and allowing also for a smaller SI of 1k to be used.

Similarly in the f1 dimension if one wants not to have the longer t1 fids processed one limits TDeff(in f1 column) to <TD1. This might for instance be useful if the instrument drifted off lock in the course of an overnight experiment and the last 52 spectra were no good…the first 204 spectra might be fine, thus TDeff =204 in the f1 dimension would fix the problem.

>>>>>*TDeff HAZARD*: if you edc copy a set of parameters in which TDeff is not 0 then in the new parameter set you may ***inadvertantly*** not be processing all the data you acquire. That is, forgetting that you have changed TDeff in a data set can cause problems later on…such as truncated fids giving rise to puzzling sinx/x wiggles!

removing vertical stripes…T1 noise.

These peaks may be removed by improving acquisition parameter (often increasing d1 will help) or by processing: make a projection of them in a region where there are no diagonal or off diagonal peaks from your sample, then subtract it from each row of the spectrum:

First select a region in the f2 (horizontal) dimension that does not have any peaks in it. Record the beginning and ending row numbers.

Then enter command *proj*. A screen appears that lets you enter the row numbers and choose a destination PROCNO to that projection (under same filename and EXPNO). Usually I assign that to '2'. The projection will appear on window as a 1D spectrum. Click on "2D" button to get back to 2D display.

Then enter *edc2* and define the second data set as the just assigned file name: usually filename 1 2, where the 2 is the PROCNO just assigned with the command *edc2.* Exit with SAVE.

Then issue the command *sub2* which subtracts the maximum value in the projection from each column of the 2D plot. This will remove most of the striping.