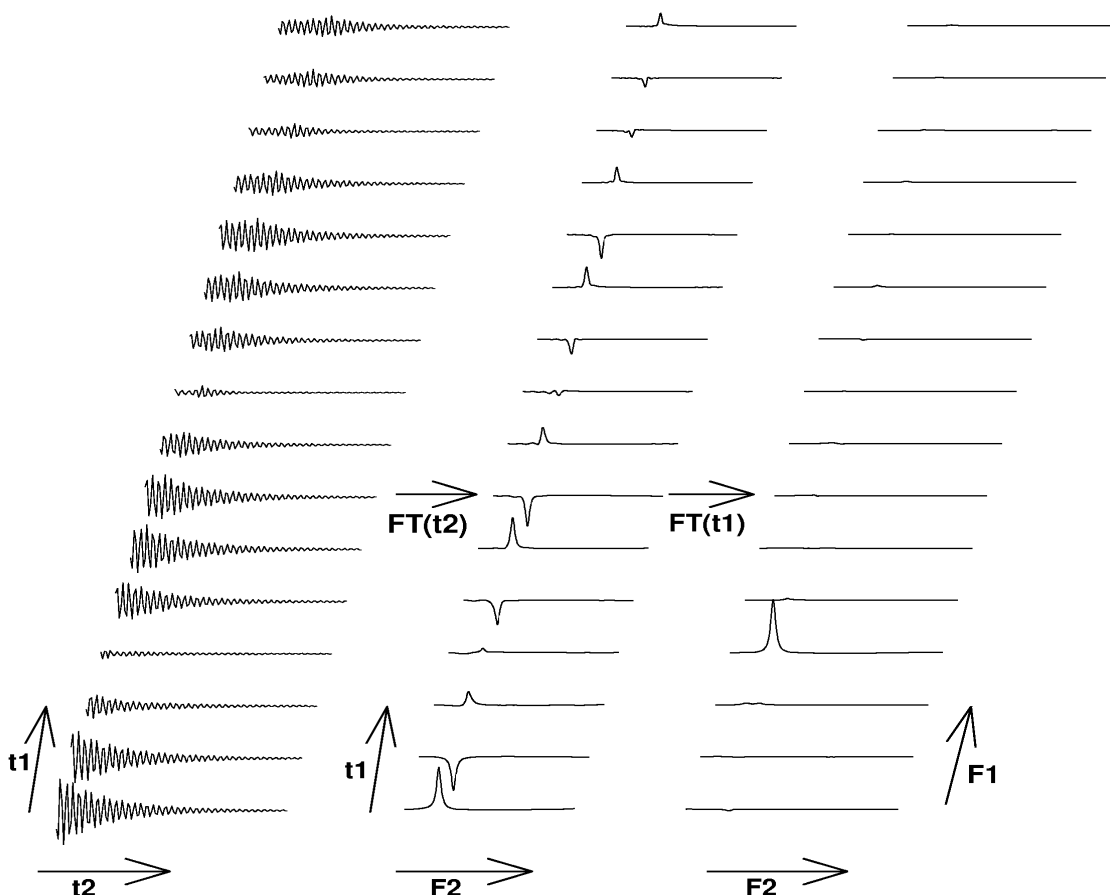


ACQUISITION AND PROCESSING OF 2-DIMENSIONAL NMR DATA ON ARX SPECTROMETERS

I. Overview

- A. This write-up assumes that you are reasonably familiar with everything that is discussed in the write-ups entitled "Acquisition of Routine 1-Dimensional NMR Data - ARX Spectrometers" and "Processing of Routine 1-Dimensional NMR Data - ARX Spectrometers".
- B. All 2-dimensional NMR experiments consist of > 1 pulse with ≥ 1 delay. After the pulses and delay(s), the free induction decay (FID) is sampled. One of the delays will be incremented from the first to the last FID. The incrementable delay constitutes the second time domain. The precise detail of the pulses and delays and the relative phases of the pulses from one scan to the next determine the information that is obtained from the particular 2-D experiment.
- C. It is helpful to bear in mind that you acquire a series of FID's, each with **ns** scans, as shown below.



F2	F1
Intervals used in the digital data sampling are called t_2 . When this dimension is Fourier transformed it is called F2.	Intervals used for evolution of the delay time are called t_1 . When Fourier transformed, this is the F1 dimension.
The center of the F2 dimension is the frequency of the transmitter of the directly observed nucleus.	For <u>homonuclear</u> correlation experiments, the center of the F1 dimension is always the same as for the F2 dimension.
	For <u>heteronuclear</u> correlation experiments, the center of the F1 dimension is always the frequency of the transmitter pulses applied to the nucleus not directly observed.
All hardware must be turned on and the frequencies, power levels, and durations of all pulses must be set in the F2 dimension even if their occurrence is to produce the second dimension. (The F1 dimension is based only on the incrementable delay.)	The only parameters in the F1 dimension that MUST be set properly are the size of the increment to the delay time, the number of different values of the delay, and the parameter ND0, which "informs" the data system whether the incrementable delay is split up into more than one delay.

- D. While there are only three parameters in the F1 dimension that must be set properly, there are others that make the settings easier and will make processing much easier if they are set prior to the start of data acquisition. It is necessary that they be set in the order given below in order to take advantage of these additional parameters.

II. Acquisition

- A. Acquire a 1-D spectrum of the nucleus of the sample to be directly observed in the 2-D experiment.
1. Fourier transform the data. From this spectrum determine the value of spectral width and center frequency that will include all the peaks with the minimum acceptable spectral width. (Leave about a 1 ppm region on each end that does not contain peaks of interest.)
 - a. This is most easily done by expanding the display to the desired region then selecting the "button" labeled **sw-sfo1** to limit data acquisition to the region currently displayed.
 - b. Do not exclude any real peaks unless you deliberately intend to alias some peaks into a region that will not overlap peaks of interest. If you do not understand aliasing, please ask the staff for assistance.
 - c. In some cases, in order to get the receiver gain (rg) set correctly, you need to use a pulse program with a 90 degree pulse, e.g. **zg** or **zgd**. (Check the specific example experiment to determine whether this is necessary.) If you change to a 90 degree pulse, be sure to repeat the **rga** command to determine the correct value for the receiver gain.

2. Acquire a 1-D spectrum based on these new values. Fourier transform and phase the data and set the reference. This file will contain the data just acquired plus all 1-dimensional acquisition and processing parameters.
- B. If the 2-D experiment to be performed is a heteronuclear correlation, you can obtain a 1-D spectrum of the other nucleus in the usual way. (Don't forget to use the **new** command to create a new data set so you don't overwrite the spectrum you just acquired in **A.** above.) This spectrum can be used to determine the center frequency and spectral width for the F1 dimension and you will be able to use this as the F1 "projection" on the contour plot. This step is not essential if you do not have enough material to obtain a directly observed spectrum of the other nucleus. In that case, you can use an internal projection of the F1 data for the contour plot. The projection is essentially a low resolution spectrum of the other nucleus and can be used to determine chemical shifts with fewer significant figures than usual.
 - C. It is strongly advised that you keep a hard copy of the pulse program for the 2D experiment. See the specific example experiment to determine the pulse program name. Type **edpul** *<pulse program name>*, select **Print**, then **OK**. Guidance for setting many parameters is given in the comments of the pulse programs.
 - D. If you acquired a spectrum in step **B.**, be sure to read in the spectrum you obtained in step **A.** The rest of the 2-D set-up will continue from the spectrum acquired in step **A.** Many parameters will already be set appropriately from that spectrum, e.g. **o1** and **sw**. Create a new data set with the **new** command so you don't overwrite the spectrum you just acquired in **A.** above. Type **eda** to bring up the acquisition parameter menu. Select **PARMODE**, then select **2D**. Then **SAVE** the menu. Reply **OK** to the message "Delete 'meta.ext' files?". This will change the look of the processing window. Type **eda**. There are now 2 columns of parameters, one for the **F2** dimension (the data acquisition dimension) on the left and one for the **F1** dimension (the incrementable delay dimension) on the right.
 - E. Set the following in the **eda** menu. It is imperative that you do the following steps in the order given! Anytime you need specific information, refer to the specific example experiment for reasonable values. If at this point, you go off and just start plugging in values from the example without referring to this section on setting parameters in **eda** in the right order, don't be surprised if you have problems later.
 1. In the F2 dimension:
 - a) Set **PULPROG** to the name of the pulse program for the 2-D experiment. (See parameters for specific example experiment).
 - b) Reduce **TD** in the **F2** dimension to obtain as crude a digital resolution as will be acceptable to obtain the information you need. (See parameters for specific example experiment). Using minimal spectral width will allow TD to be relatively small without unnecessarily sacrificing digital resolution. (Never use default values for the center frequency and spectral width unless you have examined a 1-D spectrum and determined them to be appropriate for your sample!!!)
 - c) For a heteronuclear 2-D experiment:

- 1) Select the "button" labeled **edit** for the parameter called **NUCLEI** in the **F2** column. Then select **DEC**. Select the appropriate nucleus for the **F1** dimension from the displayed list, then select **SAVE**. Although this nucleus is in principle relevant to the **F1** dimension, all acquisition hardware must be set up in the **F2** dimension.
- 2) **O2**, which is the center frequency for the 2nd channel, must be set to the value for **O1** used to obtain a 1-D spectrum of the nucleus of the **F1** dimension. If you did not minimize the spectral width in step **B.** above, look over that spectrum carefully to determine the value for the center frequency and the spectral width (in ppm) that could be used for a minimum spectral width. If you have not acquired a spectrum for the nucleus of the **F1** dimension, you will have to guess. You can set the value for the center frequency in ppm by setting **O2P** (which will change **O2** accordingly since they are not independent). Since you are using ppm units, this will only work properly if the previous step has been done first!!
- 3) If decoupling is to be used, set the parameter CPDPRG (near the bottom of the eda menu). Select the small "button" to the right of the parameter window. This will bring up a menu of choices to select from. (See parameters for specific example experiment).

2. In the F1 dimension:

- a) Select the "button" labeled **EDIT** for the parameter called **NUCLEI** in the **F1** column. Select the appropriate nucleus for the **F1** dimension from the displayed list. This step is essential both for proper referencing at the time of data processing and for setting the **F1** spectral width if ppm units are to be input (see below).
- b) Set the parameter **ND0**. (See parameters for specific example experiment).
- c) Set **IN0**, the size of the increment to the delay in the **F1** dimension. This is usually most conveniently set with the parameters **SWH** (spectral width in Hz) or **SW** (spectral width in ppm). **IN0** is the F1 equivalent of **DW** in the F2 dimension. See the 1-D acquisition write-up if you do not understand the relationship between **SW** and **DW**.
 - 1) For homonuclear chemical shift correlation experiments it is usually easiest to set the value of **SWH** in the **F1** dimension equal to the value for **SWH** in the **F2** dimension. (The spectral width in Hz has more significant figures listed than does the value in ppm.)
 - 2) For heteronuclear experiments, it is usually most convenient to set **SW** in the **F1** dimension equal to the ppm range of the spectrum of that nucleus.
- d) Set **TD** in the **F1** dimension to obtain as crude a digital resolution as will be acceptable to obtain the information you need. (See parameters for specific example experiment).
 - 1) **TD** in the **F1** dimension is one of the most important contributors to the total experiment time and should not be made too large.

- 2) In general it is wise use of spectrometer time to have more data points in the **F2** dimension than in the **F1** dimension. Improved resolution can often be obtained through data processing techniques.
 3. The rest of the acquisition parameters are more conveniently set outside the **eda** menu. Be sure to select **SAVE** to retain the changes made and exit the **eda** menu.
- F. Type **ased**. This will bring up a menu that contains only those acquisition parameters that are actually used by the current pulse program. These parameters may be set in any order.
- 1) Make sure that you set all parameters that are mentioned in the specific example experiment that have not already been set up to this point.
 - 2) It is assumed that you know the values for the pulse times and corresponding power levels that you need for the particular pulse program, e.g. 90 degree pulses, 180 degree pulses, etc. Each spectrometer has a notebook that gives pulse times and power levels. Don't forget that they are different for different probes. If the probe is tuned properly for all nuclei that are pulsed in the specific experiment, the values given in the notebook should be close.
- G. Consider the value of **ns**.
- 1) There will be a minimum number of scans that will be required to obtain at least the crudest phase cycling necessary to obtain the 2-D data for a given type of experiment. Unless noted otherwise for the specific example experiment, this minimum number is **8**. Make sure that the value for **ns** that you use is a multiple of this number.
 - 2) The number of scans per FID is one of the most important contributors to the total experiment time and should not be made too large. If more than the minimum number of scans are needed due to the small quantity of sample, you should consider the possibility of making a more concentrated sample. This decision will, of course, be determined by the chemistry of your sample.
 - 3) Set **ds=ns**. This parameter is the number of “dummy scans”, i.e. the number of times the whole pulse sequence will be repeated, but the data will not actually be acquired. This helps to establish a steady state before data are acquired in the event that the total recycle time is too short compared to the T_1 relaxation times. The dummy scans occur only prior to the first FID acquired and thus do not contribute significantly to the total experiment time.
- H. The receiver gain may still need to be set. In many cases, an appropriate value for the receiver gain (**rg**) is the same as the **rg** for a 1-D spectrum acquired with a 90 deg pulse. If you created the current 2-D data set from a 1-D data set of this sample acquired with a 90 deg pulse, **rg** will already be set appropriately. Check the specific example experiment for information on setting **rg**.
- I. Type **as** for a final check of the parameters. This will display the same parameters as **ased**, but will force you to examine them all. When the text of the pulse program is displayed, select **Quit**. It is a good idea to repeat the entire pass through the **as** routine until you have confirmed that all parameters are correct without making any changes.

- J. Turn off the spinner. This will lead to somewhat greater stability of the system and a 2-D experiment is significantly lower resolution than a 1-D experiment anyway.
- K. Type **expt**. A message window will display the following information: 1) the total experiment time; 2) the size of the file that will be created in kbytes and blocks (1 kbyte = 2 blocks); and 3) available disk space in blocks.
- L. Type **ii**. Wait for the "ii finished" message. Type **zg** to start the 2-D acquisition.
- M. Data acquisition may be terminated prematurely (i.e. prior to the completion of TD in the F1 dimension experiments) by typing **halt**.
- N. Alternatively, if you have previously acquired a 2-D data set, you can use it to set up another 2-D data set. Be sure to look at the specific example experiment for relevant parameters.
1. If you plan to run the same experiment on a different sample, acquire a 1-D spectrum and be sure to determine the minimum spectral width appropriate to that sample. Pay attention to whether you need to use a 90 degree pulse for the 1-D experiment (see specific example experiments).
 - a) In the **eda** menu, change the values for **SW** and **O1** (or **O1P**) in the **F2** dimension. For a heteronuclear experiment, also change **O2P** if needed.
 - b) Select **edit** under **NUCLEI** in the **F1** dimension. Re-select the nucleus already shown. This is necessary to make processing easier and will only help if done after step a) above. It can be fixed later if you forget this step.
 - c) Change **SW** in the **F1** dimension if appropriate.
 - d) If you are using a different probe from the one used for the 2-D data set you are starting with, be sure to change the values for the pulse times and their corresponding power levels.
 - e) Set the receiver gain (**rg**) appropriately for the new sample. This is an extremely important step and must not be overlooked!
 2. If you plan to run a different experiment, in addition to the above items, you must:
 - a) Change the name of the pulse program.
 - b) When selecting **edit** under **NUCLEI**, in the **F1** dimension you will not necessarily re-select the nucleus shown.
 - c) Make sure all the pulse times, corresponding power levels, and all other parameters used by the new pulse program get set properly.

III. Basic Processing

- A. Type **edp** to enter the processing menu.

1. Set **SI** in both dimensions. Generally 1K in both dimensions will be appropriate.
 2. Set the window functions (**WDW**) to be used in both dimensions. Depending on the function selected, you will have to set some of the following: **SSB**, **LB**, and **GB**. (See parameters for specific example experiments.)
 3. It is absolutely essential that **MC2** be set properly. (See parameters for specific example experiments.)
 4. Set **PH_mod** in both dimensions.
 - a) If **MC2 = QF**, **PH_mod** must be **no** in the **F2** dimension and **mc** in the **F1** dimension.
 - b) For all other values of **MC2**,
 - 1) Set **PH_mod = no** in both dimensions until you have determined any relevant phase correction constants.
 - 2) When phase correction constants for a particular dimension have been correctly determined and stored for the parameters **PHC0** and **PHC1**, set **PH_mod = pk** in that dimension. With **PH_mod** set to **pk**, the phasing will be carried out automatically by the Fourier transformation command (see below). This is very convenient for later reprocessing of 2-D data.
 5. The parameter **BC_mod** should be set to **no** in both dimensions. (The default in the **F2** dimension will need to be changed since **BC_mod** is used for 1-D spectra.)
 6. Set the reference by setting the parameter **SR** equal to the value for **SR** in a properly referenced 1-D spectrum for each dimension. (In the **F1** dimension, you must type in **SR** even though it may already appear correct to force it to calculate the axes correctly.) Alternatively, after the data have been Fourier transformed, the calibrate "button" on the left will allow you to set the position of the cursor in the **F2** and **F1** dimensions.
- B. Select **Save** to exit the **edp** menu and keep these changes.
- C. Type **xfb**. This will apply the window functions, carry out Fourier transformation in both dimensions, and apply phasing if **PH_mod** is set to **pk**.
- D. When the data are available for display, the threshold may be inappropriate. The buttons used for vertical scaling of 1-D experiments can be used analogously to determine the threshold of the 2-D contour display.
- E. By default, only positive levels are displayed. For phase sensitive experiments, you need to display both positive and negative levels, at least until the phasing is set correctly. Select the "button" labeled +/- two times to select positive and negative levels. (Continuing to select +/- steps through the choices of positive only, negative only, both positive and negative, or positive levels only but using the full range of the color palette.)
- F. Once the display is set up, select **DefPlot** to keep these settings for the threshold and positive and negative levels.

- G. The display can be expanded using the "button" that looks like a box in exactly the same way it is used for 1-D spectra.
- H. For phase-sensitive experiments where the phase constants are not already known, select **phase**. (See the specific example experiment for guidance on phasing.)
1. To determine the phase correction for the F2 dimension (rows):
 - a) Select **row**. Position the horizontal line on the row of interest and press the middle mouse button. If columns also appear to need substantial phasing, be sure to observe carefully that you choose all rows from the same side of the peaks. (Ask the staff for help, if needed.)
 - b) The four "buttons" below the **row** and **col** "buttons" can be used to increase or decrease the amplitude (2* or 2/) of the selected row or increment or decrement the row number (+ or -).
 - c) Once the desired row is displayed, select the "button" to the right of "mov" labeled **1** to move the selected row to window number 1. Select two more rows and move them to windows 2 and 3. Be sure to select rows that have peaks that occur across the spectral width.
 - d) The display of the rows in the three windows can be manipulated somewhat with the "buttons" in the lower left. The buttons will be active for the window whose number is highlighted. To switch to a different window, click on the window you want to select.
 - e) Select which window and mode will be used for determining the cursor for the zero order phase corrections from the "buttons" following "big:" or "cur:". This is analogous to selecting "biggest" or "cursor" for 1-D phasing, but in this case you must specify which window the peak occurs in. For 2-D data, a marker will not be displayed but you will know where it is based on your selection in this step.
 - f) Select **ph0** and hold down the mouse button while you adjust the peak with the marker.
 - g) Select **ph1** and hold down the mouse button while you adjust the other relevant peaks.
 - h) Select **return**, then **Save & return**. This will store the phase constants just determined in the parameters **PHC0** and **PHC1** in the F2 dimension. A message will appear asking whether you want to start **xf2p**. Select **OK** to apply the phase constants just determined. Otherwise, you will have to type **xf2p** to apply the phasing.
 2. To determine the phase correction for the F1 dimension (columns):
 - a) Select **col**. Position the vertical line to select a column with a peak for phasing. The rest of the procedure is entirely analogous to that described for rows above.
 - b) Select **return**, then **Save & return**, then **OK** to start **xf1p**.
 3. Once the phase constants have been stored it is convenient to enter the **edp** menu and set **PH_mod** to **pk**. If you repeat the **xfb** command, the phasing will be included with the

calculation. (If you have not turned on phasing in the **edp** menu, a subsequent **xfb** will not apply the phase constants. You would have to type **xf2p** and **xf1p** to apply the phase constants.)

4. After the 2-D spectrum is phased correctly, you may want to display only positive levels for those experiments that produce positive peaks only. (See specific example experiment.)

IV. Plotting

A. Type **edg**.

1. If you plan to include projection plots on the contour plot, you must specify what data are to be used in the **EDPROJ2** and **EDPROJ1** sub-menus.
 - a) To use a 1-D spectrum as the projection, you must select "external (1r)" for the parameters **PF2EXT** and **PF1EXT** for the **F2** and **F1** dimensions, respectively. Then specify the full data set name of the appropriate spectrum, i.e. disk unit, user name, data set name, experiment no., and process no., in each of the two sub-menus.
 - b) The vertical scaling for an external file will be based on the tallest peak in the spectrum. If that is not appropriate, increase the parameter **PF2CY** or **PF1CY** by an appropriate factor.
 - b) To use an internal projection rather than an external file, set **PF2EXT** or **PF1EXT** to positive, negative, or positive/negative, depending on the phase of the peaks for the specific experiment.
2. For heteronuclear experiments, the **F1** axis will certainly need to be changed from the default value.

B. Input a title in the same way as you would for a 1-D spectrum.

C. To plot an expanded region, either select **LIMITS** and input the downfield and upfield limits for the **F2** and **F1** dimensions, or expand the display and select **DefPlot**. The contour levels for the plot will be determined from the last time they were set with **LIMITS** or **DefPlot**, not from the current display if it is different!

D. To preview the plot, type **view** as for 1-D spectra. Type **edo** to select the appropriate printer.

E. Once all the plot parameters are set, type **plot**.

V. Advanced Processing

A. Linear Prediction

1. Provided that a reasonable sampling of the FID has been obtained, further data points can be calculated reasonably well with linear prediction. It is generally most useful in the **F1** dimension which is often severely truncated due to spectrometer time limitations.

2. In the **edp** menu, there are three parameters that must be set in each dimension in which linear prediction is to be implemented.
 - a) Select **ME_mod**. Valid choices are **LPf** and **LPb**, each followed by **r** or **c**. **LPf** refers to a forward prediction (appropriate for 2-D use) and **LPb** refers to a back prediction (appropriate for observation of very broad lines where a significant part of the beginning of the FID is lost due to the necessity of a relatively large time delay to allow the system to recover from the pulse). The choice of whether to append **r** or **c** depends on how the data were acquired. For linear prediction in the **F2** dimension, choose **LPfc**. In the **F1** dimension, the value depends on the value for **MC2**. For States-TPPI or States, use **LPfc**. For TPPI, use **LPfr**. Whenever **ME_mod** is any value other than **no**, linear prediction will be a part of the processing whenever a 2-D Fourier transform command is issued.
 - b) **NCOEF**, the number of coefficients to be used in the calculation, should be set to the value used for **TD** in that dimension divided by about 6 - 8.
 - c) The value of **LPBIN** should be set to the total number of points for the linear predicted FID, i.e. the size of **TD** had the data been acquired that long rather than calculated. In general, this should be 2 times the value you plan to use for **SI** in that dimension. (This factor of 2 arises because for some reason, Bruker chooses to describe TD as the total number of points and SI as the number of complex points.)

B. Extracting rows from the raw data matrix (i.e. the "ser" file):

1. It can be helpful to remove a row from the raw (unprocessed) data matrix to aid in deciding what window function to use prior to the Fourier transformations.
2. To remove a row from the raw data matrix, type **rser row#**. This will extract the row specified, copy it to the "fid" file of the data set ~TEMP 1 1, and switch the display to this data set.
3. To try out various window functions, first type **ft** and phase the spectrum in a way that is appropriate to the experiment. Type **winfunc**. This will put the fid with the selected window function in the top of the display window and the transformed spectrum with the window function that has been selected applied to the data. You may need to select **ph-mod** to actually apply the phasing that you just determined above. You will then be able to easily select different windowing functions and parameters to view their effects on the spectrum. Select **return** to exit.
4. To easily return to the 2-D data from which the row was extracted, select the **2D** button on the bottom row.

C. For homonuclear experiments, the windowing chosen for F2 can be used in F1. However, for heteronuclear experiments, the optimum F1 windowing may be different from F2.

1. To determine F1 windowing, you must extract data from a processed data file in which the transform has been carried out in the F2 dimension only. To accomplish this, use the command **xf2** rather than **xfb**. Extract a column by typing the command **rsc column#**.

2. This command will put the selected column in ~TEMP 1 1 which may be manipulated exactly as described above for a row of data from the raw data matrix.

D. To plot rows or columns from the data matrix that has been transformed in both dimensions, it is convenient to put the processed data in a different process number of the data set name and experiment number of the 2-D data set rather than the scratch file specified with ~TEMP 1 1. To extract a row, type the command **rsr row# proc# (n)** (for example, **rsr 86 86 n**). The **(n)** means that it is optional to append the **n**. If it is not there, the display will be switched to that process number. If it is there, the display will not switch (which can be convenient if you plan to extract several rows). To extract a column, use the equivalent format with the **rsc** command.

E. Baseline Corrections

1. If the contour plot appears to have horizontal lines, a baseline correction in the F2 dimension will probably improve it significantly. Type **edp** and page down to the parameters ABSF1, ABSF2, ABSG, and ABSL. Set ABSF1 (downfield limit) and ABSF2 (upfield limit) in ppm to include the region to be corrected. (To correct the entire spectrum, make these larger than the spectral width.) The default values of 5 and 3 for ABSG and ABSL are usually fine. Save the menu. The baseline correction specified by these parameters can be applied with the command **abs2**. (Unlike phase correction, there is no way of turning on the baseline correction such that it will automatically be applied by the **xfb** command. Should you re-do the Fourier transformation of the raw data, you would have to again apply the baseline correction with the **abs2** command.)
2. After baseline correction in F2, you will probably be able to lower the threshold. If vertical lines are visible in the contour plot, a baseline correction in F1 may also be applied. The same parameters as described above must be set in the F1 dimension. Apply the correction with the command **abs1**. (As above, if the data are re-transformed, the baseline correction will have to be re-applied.)

F. Data "Beautification"

1. Homonuclear experiments:
 - a. For experiments that are inherently symmetric, the data can be cleaned up considerably by symmetrization.
 - b. Since symmetrization is a somewhat drastic measure, it is strongly suggested that you first copy the unsymmetrized data to a new process number with the **wrp** command so that both symmetrized and unsymmetrized data are available for comparison. Look carefully at rows or columns of both data sets to make sure that the conclusions will not be changed by symmetrization. The choice of the threshold for a contour plot is somewhat subjective anyway; symmetrization will allow you to choose a lower threshold to display valid cross peaks. Data that have been symmetrized should be described as such when published.
 - c. To apply symmetrization to data acquired in the phase-sensitive mode, type **syma**. (To apply symmetrization to data acquired in the magnitude mode, type **sym**.)
 - d. If the raw data are re-transformed, the symmetrization will not be applied. It is always a separate step.

2. Heteronuclear experiments:

- a. Although symmetrization is not appropriate since the experiment is not inherently symmetric, " T_1 noise" can be reduced by subtracting a "partial projection" calculated from a region that contains no peaks.
- b. As above, this sort of processing is somewhat drastic. The caveat above about comparing the data with and without "beautification" applies equally well here. It is even more important than in the case of symmetrization that data processed in this way be described as such (since symmetrization is usually recognizable and this is not at all recognizable).
- c. To calculate the partial projection, select the "button" labeled **utilities**. Under "f2-axis:" select **calc**. A set of cross hairs will appear. Using the mouse, position the horizontal line at one end of the region with no peaks, then press the middle mouse button. Next position the horizontal line at the other end of the region with no peaks, then press the middle mouse button. A window will appear in which you must specify a process no. where the partial projection will be stored. Click on **Type New Name** and type in an unused process number. The partial projection will be displayed over the contour plot. It will probably appear as a horizontal line until you click several times on the ***2** "button". The "Info" window will identify the range of rows used to produce the partial projection. At any time the partial projection can be displayed by selecting the "button" labeled **part**.
- d. Type **edc2**, enter the process number specified for the partial projection as data set number 2, and save the menu. To subtract the partial projection from the contour plot, type **sub2**. (The **sub2** command always subtracts the data set specified as the 2nd data set from each row of the processed data matrix.)
- e. As is the case for symmetrization, this type of data clean-up will have to be re-applied if the raw data are re-transformed.

VI. Example Experiments

The following examples are suggestions for parameters that should work for each experiment. The parameters given may not be optimum, but rather are intended to get novice users started into the process of running 2-D experiments. These parameters are only useful in conjunction with the directions in the previous sections. Just plugging in these values alone will lead to failure.

A. Double Quantum Filtered Phase-sensitive COSY

ACQUISITION PARAMETERS

<u>F2</u>		<u>F1</u>	
PULPROG:	cosydfst.js	TD:	256
TD:	1K	ND0:	1
P1:	90 deg ¹ H pulse time	SWH:	SWH in F2
L3:	128 (1/2 of TD in F1 dimension)		
RG:	Use the value from a 1-D spectrum acquired with a 90 degree pulse.		

PROCESSING PARAMETERS

WDW:	QSINE	WDW:	QSINE
SSB:	2	SSB:	2
PH_mod:	pk	MC2:	States-TPPI
PHC0:	see below	PH_mod:	no
PHC1:	see below		
SR:	SR of ¹ H 1-D spectrum	SR:	SR of ¹ H 1-D spectrum

PHASING

Acquire a 1-D spectrum using a 90 degree pulse and the same values for **O1**, **SW**, **P1**, and **RG** that you use for the 2-D spectrum. Phase the spectrum and save the phase constants. If you start the 2-D setup from this data set, the correct values for the phase constants, **PHC0** and **PHC1** in the **F2** dimension will already be in the 2-D data processing parameters. Otherwise, write down the values for **PHC0** and **PHC1**, then enter these values for **PHC0** and **PHC1** in the **F2** dimension. Set **PH_mod** = **pk** prior to typing **xfb**. Phasing in the **F1** dimension will not be needed. You will not need to use the phase subroutine to phase this experiment.

OTHER COMMENTS

Since the diagonal peaks and the cross-peaks will be anti-phase absorption, you need to display both positive and negative levels in the contour plot.

To perform this experiment with presaturation, run the 1-D experiment with presaturation, make the 2-D experiment from that file, and use the pulse program **cosydfprst.js** instead of **cosydfst.js**.

B. Phase-sensitive TOCSY

ACQUISITION PARAMETERS

F2

PULPROG: tocsyst.js
TD: 1K
P1: 90 deg ¹H pulse time
TL3: transmitter power level for "TOCSY" pulse
P6: 90 deg "TOCSY" pulse time
P17: 2.5 msec
L3: 128 (1/2 of TD in F1 dimension)
L1: number of times the MLEV-17 composite pulse sequence must be repeated to yield the total mixing time (~70 msec) - Calculate: $L1 = [70 \text{ msec} - 2 * P17(\text{in msec})] / [64 * P6(\text{in msec}) + P5(\text{in msec})]$ See comments for example.
RG: Use the value from a 1-D spectrum acquired with a 90 degree pulse.

F1

TD: 256
ND0: 1
SWH: SWH in F2

PROCESSING PARAMETERS

WDW:	QSINE	WDW:	QSINE
SSB:	2	SSB:	2
PH_mod:	no (until phase constants are determined)	MC2:	States-TPPI
SR:	SR of ¹ H 1-D spectrum	PH_mod:	no (until phase constants are determined)
		SR:	SR of ¹ H 1-D spectrum

PHASING

Use the phase routine to phase all diagonal peaks positive absorption. Phase rows, then columns. All TOCSY cross-peaks will be positive absorption.

OTHER COMMENTS

Since all peaks of interest are positive, you can display just the positive levels for the contour plot.

Example calculation for L1: For the ARX500 5mm inverse broadband probe, the 90° "TOCSY" pulse is 26 μs at a power level of 11.00 dB. The ased menu will calculate that P5 is 17.3 μs (2/3 of 26). P5 is always 2.5 ms. Convert all times to ms so the ratio is unitless.

$L1 = [70 - 2(2.5)] / [64(.026) + .0173] = 38.7$; The closest integer is 39. Use $L1 = 39$.

To perform this experiment with presaturation, run the 1-D experiment with presaturation, make the 2-D experiment from that file, and use the pulse program **tocsyprst.js** instead of tocsyst.js.

C. Phase-sensitive NOESY

ACQUISITION PARAMETERS

F2

PULPROG: noesyst.js
TD: 1K
P1: 90 deg ¹H pulse time
L3: 128 (1/2 of TD in F1 dimension)
D8: mixing time (approx. T₁ for small molecules;
50 - 250 msec for macromolecules)
RG: Use the value from a 1-D
spectrum acquired with a
90 degree pulse.

F1

TD: 256
ND0: 1
SWH: SWH in F2

PROCESSING PARAMETERS

WDW:	QSINE	WDW:	QSINE
SSB:	2	SSB:	2
PH_mod:	no (until phase constants are determined)	PH_mod:	no (until phase constants are determined)
SR:	SR of ¹ H 1-D spectrum	SR:	SR of ¹ H 1-D spectrum

PHASING

Use the phase routine to phase all diagonal peaks absorption. Ignore cross peaks for phasing. Phase rows, then columns. If the diagonal is phased negative absorption, then positive NOE cross peaks will be positive absorption. Negative NOE cross peaks will be negative absorption. Any anti-phase peaks are COSY cross artifacts, not NOE cross peaks. It is usually a good idea to look at rows to be sure whether cross peaks are NOE peaks or COSY artifacts. The **scan** button in the utilities subroutine is quite useful for this. Follow the online helps to learn to use **scan**.

OTHER COMMENTS

Sample preparation is very important. The sample should be de-gassed to remove O₂ which is paramagnetic and can prevent the observation of NOE's. The best method is a freeze-thaw method. It is simpler to flush the sample with Ar, but it is generally less effective.

To perform this experiment with presaturation, run the 1-D experiment with presaturation, make the 2-D experiment from that file, and use the pulse program **noesyprst.js** instead of noesyst.js.

Noesy spectra usually need baseline corrections in F2 and sometimes in F1. See **Advanced Processing** (above) for how to apply baseline corrections to 2-D spectra.

Since you will probably have both positive and negative peaks, you need to display both positive and negative levels in the contour plot.

D. Phase-sensitive TROESY (ROESY without TOCSY)

ACQUISITION PARAMETERS

<u>F2</u>		<u>F1</u>	
PULPROG:	troesyst.js		
TD:	1K	TD:	256
P1:	90 deg ¹ H pulse time	ND0:	1
TL3:	transmitter power level for "TOCSY" pulse	SWH:	SWH in F2
P3=P4:	180 deg "TOCSY" pulse time		
NS=DS:	8		
L3:	128 (1/2 of TD in F1 dimension)		
L5:	number of times the 180 deg composite pulse sequence must be repeated to yield the total mixing time (same mixing time that would be used for a NOESY) - Calculate: L5=mixing time (in msec)/(P3+P4)(in msec) See comments for example.		
RG:	Use the value from a 1-D spectrum acquired with a 90 degree pulse.		

PROCESSING PARAMETERS

WDW:	QSINE	WDW:	QSINE
SSB:	2	SSB:	2
PH_mod:	no (until phase constants are determined)	MC2:	States-TPPI
SR:	SR of ¹ H 1-D spectrum	PH_mod:	no (until phase constants are determined)
		SR:	SR of ¹ H 1-D spectrum

PHASING

Use the phase routine to phase all diagonal peaks absorption. Phase rows, then columns. If the diagonal is phased negative absorption, then all ROESY cross peaks will be positive absorption.

OTHER COMMENTS

Sample preparation is very important. The sample should be de-gassed to remove O₂ which is paramagnetic and can prevent the observation of ROE's. The best method is a freeze-thaw method. It is simpler to flush the sample with Ar, but it is generally less effective.

Example calculation for L1: For the ARX500 5mm inverse broadband probe, the 180° "TOCSY" pulse is 52 μs at a power level of 11.00 dB. Convert all times to ms so the ratio is unitless. For a mixing time of 0.5 sec, L5=500/(.052+.052)=4807.6 The closest integer is 4808. Use L5=4808.

To perform this experiment with presaturation, run the 1-D experiment with presaturation, make the 2-D experiment from that file, and use the pulse program **troesyprst.js** instead of troesyst.js.

Roesy spectra usually need baseline corrections in F2 and sometimes in F1. See **Advanced Processing** (above) for how to apply baseline corrections to 2-D spectra.

Since you will probably have both positive and negative peaks, you need to display both positive and negative levels in the contour plot.

E. Phase-sensitive HMQC (with BIRD pulse)

ACQUISITION PARAMETERS

F2

PULPROG: hmqcbirdst.js
TD: 1K
TL0: high power proton pulse power level
P1: 90 degree pulse time for proton at power level TL0
P2: 180 degree pulse time for proton at power level TL0
DL0: high power X-nucleus pulse power level
P3: 90 degree pulse time for X-nucleus at power level DL0
P4: 180 degree pulse time for X-nucleus at power level DL0
CPDPRG: program for X-nucleus composite pulse decoupling (garp)
DL5: power level for X-nucleus decoupling (garp)
P31: 90 degree pulse time for X-nucleus at power level DL5
NS=DS: 16
L3: 128 (1/2 of TD in F1 dimension)
CNST2: one-bond J value for X-H bonds - This is used by the pulse program to calculate the delay D2.
D7: delay for inversion recovery - Set this to approximately $0.35 * 1.3 * T_1$ of the fastest relaxing protons.
D1: set such that the total time from start of data acquisition of one scan until the end of the preparation period of the next scan is $1.3 * T_1$ of the fastest relaxing protons. Calculate:
 $D1 = 1.3 * T_1 - AQ - D7 - 2 * D2 - 2 * P1 - P2 - P3$
RG: After all parameters are set, type **rga**.

F1

TD: 256
ND0: 2
SW: SW for X-nucleus spectrum

PROCESSING PARAMETERS

WDW:	QSINE	WDW:	QSINE
SSB:	2	SSB:	2
PH_mod:	no	MC2:	States-TPPI
SR:	SR of ^1H 1-D spectrum	PH_mod:	no
		SR:	SR of X-nucleus 1-D spectrum

PHASING

Use the phase routine to phase all peaks positive absorption. Phase rows, then columns.

OTHER COMMENTS

On the ARX500 you must insert a filter for ^{13}C HMQC. It is labeled "Needed for ^{13}C HMQC". Attach it to the BB coil of the probe and connect the X-BB cable to the other end of the filter.

Since all peaks of interest are positive, you can display just the positive levels for the contour plot.

F. HMBC (A gradient version of HMBC is recommended as an alternative whenever use of the microprobe is acceptable. See the NMR lab staff to run the gradient HMBC.)

ACQUISITION PARAMETERS

F2

PULPROG: hmbc.js
TD: 1K
TL0: high power proton pulse power level
P1: 90 degree pulse time for proton at power level TL0
P2: 180 degree pulse time for proton at power level TL0
DL0: high power X-nucleus pulse power level
P3: 90 degree pulse time for X-nucleus at power level DL0
CNST2: one-bond J value for X-H bonds - This is used by the pulse program to calculate the delay D2.
D1: relaxation delay
D6: delay for evolution of long range couplings (60 - 70 msec)
NS=DS: 16
RG: After all parameters are set, type **rga**.

F1

TD: 512
ND0: 2
SW: SW for X-nucleus spectrum

PROCESSING PARAMETERS

WDW: QSINE
SSB: 2
PH_mod: no
SR: SR of ¹H 1-D spectrum

WDW: QSINE
SSB: 2
MC2: QF
PH_mod: mc
SR: SR of X-nucleus 1-D spectrum

PHASING

No phasing is needed since the experiment is not acquired in the phase-sensitive mode.

OTHER COMMENTS

Display only positive levels for the contour plot since there are no negative data.