Instructions for 2D and Advanced 1D on the DRX

I. Data Acquisition

A. Getting Started.

1. Check Probe. Make sure that the gradient probe (Nalorac Triple-Resonance 3-axis Gradient Probe) is installed. This probe has a rectangular metal box at the bottom labeled "Z · SPEC" and has cables connected for LOCK, H, and X1 (Carbon-13). The X2 connector (Nitrogen-15) is usually not hooked up. The heavy cable attached at the right is the gradient cable. If this is not the current probe, get the NMR facility personnel to change the probe. DO NOT ATTEMPT TO CHANGE PROBES YOURSELF. The procedure is complicated and damage to a gradient probe could cost you $27,000!

2. Insert Sample. Insert your sample as you would for a 1D experiment. Set up a 1D proton experiment with your sample ID and experiment number 1. Lock and roughly shim, optimizing Z^1, Z^2 and the Lock Phase. Do not spin the sample.

3. Set Temperature. Decide what temperature you want to use for your experiment. For routine work we use 298K (25° C), but you can set any temperature between 278K (5° C) and 310K (37^oC). Higher temperatures are possible, but you should consult with NMR facility personnel before doing any high-temperature work. With non-aqueous solvents you can also go below 273K (0° C) but this requires using a liquid-nitrogen dewar and assistance from facility personnel. Gradient probes have a smaller range of temperatures allowed: -20° C to $+60^o$ C. If you need temperatures outside this range you will have to use the old non-gradient probes.

Temperature is always regulated, using a dessicant column air dryer, an air chiller, and a heater within the probe to bring air temperature to the correct value. The probe temperature is monitored by a thermocouple wire placed very close to the sample. To see the sample temperature enter **temon** at the XWinNMR prompt. This opens a window which displays the current probe temperature; you can select either ^{o}K or ^{o}C for the display. To set the desired temperature, enter **te** at the XwinNMR prompt and adjust the value (in ^{o}K) as you would any other parameter. The new value will not take effect until you enter the command **teset**. Be sure to re-enter this temperature (**te**) value for each experiment you perform on this sample before you start acquisition, so that the parameter set will record the actual temperature setting.

When the temperature has reached the set value and stabilized, carefully adjust the Z^1, Z^2 and Z^3 shims. After each pass through these shims re-optimize the Lock Phase as if it were a shim. It may take many cycles through Z^1, Z^2 and Z^3 to achieve the optimal shim. If you think the shims are bad, you can recall previous shim sets using the command **rsh**. An alphabetical list of all shim files is displayed, and you may have to move the slider at the right side to get to the shim files which begin with **n3** (for Nalorac triple-resonance probe). The system for naming shim files is "n3yymmdds_###" where yy is the year (e.g., 99), mm is the month (01-12), dd is the day (01-31), s is the solvent (w for water, c for chloroform, etc.) and ### (optional) is the temperature in ^{o}K. We also

maintain a general shim file which should be the most recent "best" shims; it is simply called "**current-n3**". This should give you a good starting point from which you will only have to adjust Z^1, Z^2 and Z^3. If you solvent is 90% H_2O / 10% D_2O, you will want to use the automated gradient shimming feature. You cannot use this feature for any other solvent.

      4. Tune the Probe. Tuning the probe assures that the resonant frequency of the probe coil is the same as the RF frequency you will be using, and matches to the coil as a load to the impedance of the amplifiers. This gives maximum efficiency of transfer of RF power from the amplifiers to your sample nuclei, and maximum sensitivity in detecting the FID. Each sample modifies the resonant frequency and matching of the probe, so these have to be re-optimized with each new sample. If the probe is tuned correctly, we can use standard values for pulse widths without having to re-calibrate for each sample. Note that the probe must be tuned before you do gradient shimming (H_2O samples only). Tuning is very easy to do now with the "wobble" feature, but you have to be careful not to apply excessive force to the tuning rods or you can seriously damage the probe, requiring repairs costing many thousands of dollars and weeks of down-time. Each tuning rod has a "travel" of a number of turns and at the end of this travel will not longer turn. Forcing the rod at this point will break the variable capacitor in the probe.

      In your 1D proton experiment enter "**acqu**" (or "**a**" for short) to go to the acquisition (FID) window, and then "**wobb**" to start the probe tuning process. After about 30 seconds the tuning profile will be displayed on the screen, along with a vertical line marking the spectrometer frequency (500.03 MHz). The tuning profile is just a graph of probe response as a function of frequency, showing a "dip" in the graph at the resonant frequency of the probe. You are now ready to tune the proton coil of the probe. The Nalorac triple-resonance gradient probe has six knurled knobs extending out of the bottom of the probehead. From left to right they are:

      Silver (short): Attachment to magnet - DO NOT TOUCH!!
      Red (short): ^{1}H Tuning
      Blue (long): ^{1}H Matching
      Gold (long): ^{13}C Tuning
      Purple (short): ^{15}N Tuning
      Silver (short): Attachment to magnet - DO NOT TOUCH!!

Turn the ^{1}H tuning knob (short Red knob) gently and watch the pattern on the screen. The "dip" will move left or right on the screen. Adjust the tuning to center the bottom of the dip on the vertical line. This can be very sensitive so often you have to go back and forth around the center line until you "land" on it. You can also use the LED lights on the preamp box next to the magnet to center the tuning. Turn the ^{1}H matching knob (long Blue knob) to maximize the depth of the dip. The dip should come close to or touch the bottom of the screen. Sometimes the dip moves away from the center line horizontally as you optimize the match, so you will have to re-adjust the tune knob to center it again. If you are using the LED display on the preamp box, adjust the tune knob to center the lights on the horizontal bar, and adjust the match knob to minimize the number of lights

on the vertical bar. When you are finished tuning the probe enter **halt** to exit the wobble mode.

If you are doing a heteronuclear experiment (HSQC, HMQC, HMBC, HSQC-TOCSY, etc.) you will also need to tune the "other" nucleus (usually ^{13}C). This can be done either by setting up a ^{13}C observe experiment and repeating the procedure above, or by waiting until you set up your 2D experiment and tuning ("wobbing") both channels. In the latter case, when you enter "**wobb**" you will get the heteronucleus (e.g., ^{13}C) first. After tuning this nucleus, press the *CHANNEL SELECTOR* button on the preamplifier box (the box next to the magnet) to change to ^{1}H. After about 30 seconds the ^{1}H tuning curve will appear on the screen and you can tune and match ^{1}H. In either case, enter **halt** when you are finished tuning the probe. Note that the ^{13}C channel is tuned with the long "gold" knob; there is no ^{13}C match adjustment.

Sometimes you can get so far off in tuning that the "dip" in the wobble signal is not on the screen anymore. In this case you can widen the "window" of the RF sweep by clicking on **wobb-SW**. You will be prompted:

Enter the new center frequency in MHz!   [accept the current value]
Enter the new wobble sweep width in MHz! [enter the new value]

To widen the window you might want to use a wobble sweep width five times as large. After about 30 seconds the wobble curve will re-appear on the screen with a wider range of frequencies. When you get the "dip" centered using the tune knob at the probe, change the wobble sweep width back to the original value and re-adjust the probe tuning.

5. Adjust the Gradient Amplifier Balance. The gradient amplifiers should put out zero current when there are not gradient pulses being applied. For really demanding gradient experiments the results can be improved dramatically by adjusting this current to zero before starting the experiment. This is done by disabling the gradient amplifiers and observing the lock level. If the lock level goes up, it means that the gradient current was not zero and was "twisting" the ^{2}H signal through the sample slightly, reducing the net signal observed. If the lock signal goes down, it means that the shims have been adjusted to compensate for the slight gradient (the Z^1 shim is just a z-gradient that you use to maximize field homogeneity) so that removing the gradient puts the shims out of adjustment. In any case, you want to disable the gradient, adjust the shim to maximize lock signal, and then enable the gradient amplifiers and adjust the gradient balance to again maximize the lock signal (zero gradient current).

To adjust the gradient balance, first disable the z-gradient by pushing in and holding the Reset button on the Gradient Amplifier under the Z Status column:

GRADIENT AMPLIFIER

X Status	Y Status	Z Status
Power	Power	Power
Run	Run	Run
Coil Overload	Coil Overload	Coil Overload
Amp Overload	Amp Overload	Amp Overload
Offset	Offset	*Offset*
Reset	Reset	*Reset*

While holding this button in, re-optimize the Z^1 shim. Then use the small screwdriver to adjust the Offset screw for the Z axis. This will affect the lock signal, and you want to adjust it for a maximum lock signal, which corresponds to zero gradient current. This requires two people, or you can move the BSMS keyboard temporarily to the top of the console so you can reach the shim knob while holding in the Reset button. Note the lock level with the Z^1 shim optimized and then release the Reset button to enable the Z gradient. If the lock level goes down, the gradient amplifier is not putting out zero current and the Offset needs to be adjusted. Use the small screwdriver to adjust the Offset screw while observing the lock level. Adjust the Offset screw to give maximum lock level, which should be the same as that obtained with the z-gradient disabled.

> On the DRX-600, all three gradients can be disabled by software using the command **setpre**. This brings up a complicated window used for optimizing the gradients, but you only click on **Disable / Amplifier Modules**. With the amplifiers disabled, re-shim the Z^1 and Z^2 shims. These may change if they were previously shimmed with a non-zero current in the Z gradient coil. Remember that the z-gradient is just a very strong and very fast Z^1 coil. With the Z shims optimized, click on **Enable / Amplifier Modules** and if necessary adjust the Offset screw to bring the lock level up to the maximum. Use **File / Exit** to leave the **setpre** window.

Most gradient experiments only use the Z axis gradients. A few (for example, DQF-COSY on 90% H_2O samples) use all three axes. In this case you will need to check the X and Y gradient balance as well as the Z gradient. The X and Y gradients are balanced in the same way, using the Reset button and the Offset screw under the X or Y status column and adjusting the appropriate shim (X or Y).

6. Gradient Shim (90% H_2O samples only). Gradient shimming requires a sample with an extremely strong, single resonance (H_2O). A three-dimensional map is made of your sample using the same techniques used in clinical MRI (Magnetic Resonance Imaging) and the inhomogeneity of the magnetic field is calculated throughout the sample. Then using the known effect of each of the 28 shims on the magnetic field strength at each point in your sample the computer calculates how much each shim needs to be changed to get a perfectly homogeneous field. A graph of field strength vs. position along the z axis in the sample is plotted (turned sideways so that the horizontal axis is field strength deviation in Hz for proton) and an RMS (average) deviation is displayed in Hz. For good samples (no bubbles or suspended solids, 500 μL

or more volume) it should be possible to achieve less than 1 Hz RMS deviation after a few repeats of the gradient shimming routine.

Make sure the temperature is stable at the target value and that you have tuned the proton coil (see above). The sample **MUST NOT BE SPINNING**. The Z^1, Z^2 and Z^3 shims should be optimized as well as the Lock Phase. Adjust the Lock Gain to put the lock level at about 80% of maximum. Enter at the XWinNMR prompt:

> >**wsh   GSHIM**
>   *Override?* **yes**
> >**gradshim**

The gradshim window should have the following entries:

Shimming Method: Diamond "3D" in upper left corner should be selected (yellow)
DISK = u
USER = <Your Login Name>
FILENAME = deflt1step

It is especially important to have your own login ID in the USER field.

Click on "*Start Gradient Shimming*" (blue) to start one cycle of field mapping and shim correction. This will take about 2.5 minutes, during which there are two cycles of mapping. During each cycle the lock signal will fall to a low level and oscillate during the imaging process. When the second cycle is finished, the field map is calculated and the z-axis map is displayed in a new window called "*Shim Results*". This is a graph of field inhomogeneity on the horizontal axis vs. position along the sample tube on the vertical axis; a straight vertical line would be a perfectly homogeneous field. The shim corrections are calculated and applied one at a time to the shim unit (BSMS keyboard). You will see the lock level change (hopefully improve) and you may get some error messages (ignore them). If the lock level goes off scale bring it down to about 80% by decreasing the Lock Gain. When the process is finished, you can move the slider at the right side of the *Shim Results* window to scroll back through the list of shim corrections until you see the heading "*Current Field Map Statistics*". Under this heading will be the RMS deviation of the field in Hz, which is a simple measure of the field inhomogeneity throughout your sample; a value less than 1 Hz is very good. Under this you will find the heading "*Predicted Field Map Statistics*" which tells you what you should expect after the shim corrections are applied. If you do another round of gradient shimming, this is what you should expect for field inhomogeneity in that field map.

If you wish to do another round of gradient shimming, find the *Gradient Shimming* window (it may be hidden behind the XWinNMR window - you can iconify this one) and click on *Start Gradient Shimming* (in blue) again. The whole process will be repeated (about 2.5 minutes) and the new z-axis profile will be plotted on top of the old one in contrasting color in the *Shim Results* window. Repeat the cycle as many times as you like and then exit the *Shim Results* window by clicking the **OK** button in the bottom left corner and exit the *Gradient Shimming* window by clicking the **Exit** button in

the upper left corner. Save the shims with **>wsh   <login_name>yymmdd**, where yymmdd is the date and <login_name> is your login ID.

6. Choosing an Experiment. Under the directory defined by your sample name there can be any number of "experiment" directories, and they must be named 1, 2, 3, 4, etc. Unfortunately, you can't name them COSY, NOESY, TOCSY, etc., but you can give each experiment a title which will appear at the top of the spectrum every time you select that experiment. We have set up a number of 2D experiments as standard methods which you can select.   After loading the parameters for your experiment, you will need to optimize this parameter set for your needs, primarily by setting the spectral window up in both dimensions. Pulse calibration should not be necessary since the most current values should be in the parameter files. Here is a partial list of experiments available:

DQF-COSY	Homonuclear J-coupling correlation
NOESY	Homonuclear NOE correlation (z axis)
ROESY	Homonuclear NOE correlation in x-y plane
TOCSY	Homonuclear Total correlation of spin systems
HSQC	Heteronuclear (^{1}H, ^{13}C) one-bond J-coupling
Edited HSQC	with CH_3, CH pos. and CH_2 negative
Coupled HSQC	without ^{13}C decoupling in observe dimension
HSQC-TOCSY	with TOCSY transfer to ^{1}H spin system
Edited HSQC-TOCSY	with J_{CH} peaks pos., TOCSY neg.
HMBC	Heteronuclear 2,3-bond J-coupling correlation

We also have some 1D experiments available which use selective (shaped) pulses:

Selective 1D-TOCSY	Total Correlation from selected ^{1}H resonance
Biselective 1D-TOCSY	from two ^{1}H peaks, one pos. and one neg.
1D-HMBC	2,3-bond correlation from selected carbon resonance
1D Transient NOE	NOE correlation from selected ^{1}H resonance

Each experiment has some parameters which must be set or optimized by the user. For example, the NOESY, ROESY and TOCSY experiments have a mixing time, the heteronuclear experiments have an optimal J_{CH} value setting, etc. You can either accept the "typical" settings found in the parameter set or you can customize them for your needs.

7. Reading in a Parameter Set. There are two ways to set up an experiment with the appropriate parameters: you can read the parameters in from a standard parameter file using the command **rpar** (read parameters), or you can "clone" the parameters from another experiment using the command **edc**. Usually you will use **rpar** the first time you do an experiment (e.g., DQF-COSY) but after that it may be easier to use **edc** to copy parameters from your own experiments. That way if you customize the parameter set to meet your typical needs you will always get those parameters instead of some more general parameters.

a. Using rpar. Set up an experiment using **File / New** and filling in the experiment name (sample code name) and experiment number. Enter "**rpar**" at the XWinNMR prompt. You will get a long list of parameter files which all start with upper case letters at the beginning of the list. Use the slider at the right side of the window to scroll down to the bottom of the list where the filenames start with lower case letters. Specifically, look for those files which start with "std". These are the customized parameter files for our DRX-500, as opposed to Bruker's general parameter files. After the prefix "std" the filename describes the experiment (cosydf, roesy, hsqc, hsqct, etc.) and the method of data acquisition (magn, st, tppi, ea, etc. for magnitude, States, TPPI, Echo-Antiecho, etc.). Finally the filename ends with a code for the probe, since parameters have to be optimized for the specific probe used. Thus **std-hsqc-ea-n3** would indicate a parameter set for an HSQC aquired in Echo-Antiecho mode for the Nalorac triple-resonance probe. Click on the desired parameter set and select "Copy All". You may get an error message if you are loading 2D parameters into a 1D dataset, but if you just click OK it will re-configure your new experiment for the 2D parameters. Note that unlike the Varian system, this is a complete parameter set including solvent and spectral window settings. These will have to be re-set for your particular needs after you use **rpar**.

b. Using edc to "clone". Go to the experiment which is most similar to the one you want to carry out. Usually this would be the same experiment but carried out on a different sample. You can use **File / Search** to select the experiment name and experiment number, and "**ppr**" to check the pulse program of this experiment. Then enter **edc** and fill in the new experiment name and experiment number you want to copy ("clone") the parameters to. Click Save to create this new experiment, which will have all the same parameters as the old one but will contain no NMR data. There is one problem with this approach: You must be sure that the new experiment you are creating does not already exist. If it does, **edc** will simply move you to that experiment without actually copying the parameters. This is an easy trap to fall into if you are not careful - the only thing different will be that generally an existing experiment will have data associated with it. If you see a spectrum displayed, or an FID after entering "a", you have not cloned the experiment - you have simply moved to another existing experiment. If you have truly created a new experiment the spectrum area will be blank with the message "NEW 1D EXPERIMENT" or "NEW 2D EXPERIMENT".

B. Optimizing Parameters for Your Sample. There are two ways to examine and edit the acquisition parameters for your experiment. The more general method uses the command **eda** (edit acquisition parameters), which displays in a menu format all of the acquisition parameters - a very long and complicated list. The second method uses the command **ased** (acquisition setup editor) to display in a menu format only those parameters which are relevant to the pulse program (experiment) you will be doing. In either case, you get a menu with a grey background and menu boxes in pink containing the current value of each parameter. A parameter is edited by clicking in the menu box and typing in the new value followed by <enter>. You can also place the cursor within

the text of the menu box and backspace and/or enter text, but you cannot highlight and replace portions of the text in the way most word processing programs operate.

1. Using eda. The menu format of **eda** gives you two columns if you are doing a 2D experiment: the F_2 (observe channel) column is on the left and the F_1 (indirect) column is on the right. This is very convenient and straighforward for setting up 2D experiments. Edit the following parameters:

a. **TD: Total Number of Data Points.**

i. In F_2. In the F_2 dimension, this is the size of the FID in data points. Typical values for 2D work are 4096 (deluxe), 2048 (narrower SW or broad peaks) and 1024 (very narrow SW or very broad peaks). As for 1D experiments, the number of data points acquired in the FID is related to the spectral width in Hz (**SWH**) and the acquisition time (**AQ**):

$$TD = 2 \text{ x SWH x AQ}$$

Note that **TD** represents the total number of data points, both real and imaginary. Some NMR software (e.g., Felix) counts the number of data points in complex pairs (real, imaginary) so the number of pairs is **TD**/2. **TD** is always set to a power of 2 and adjusted so that the acquisition time (**AQ**) is appropriate for the sample and the experiment. There is no point in collecting FID data after the FID has decayed into noise, so fast-relaxing FIDs (i.e. broad peaks in the spectrum) can use a shorter **AQ** and therefore a smaller **TD**. If your spectral width in the observe dimension is narrow (small **SWH**), the equation above tells you that **TD** can be smaller and still allow a reasonable acquistion time. A special consideration comes into play in heteronuclear experiments which use ^{13}C decoupling (e.g., HSQC and HMQC). The ^{13}C decoupler puts out a lot of RF energy during acquisition of the FID, and this can overheat the sample or overload the pulse amplifiers. The way to avoid this is to keep **AQ** short (maximum 220 ms or 0.22 sec.) and **D1** (relaxation delay) long (minimum 1 sec.), so that the "duty cycle" (percent of time in use) of the ^{13}C decoupler is kept to a minimum. To get a short **AQ**, you use a smaller **TD** value. Typical values are 2048 or 1024 for ^{13}C-decoupled experiments, depending on the spectral width.

ii. In F1. In the F1 dimension, this is the number of FIDs which will be acquired in the 2D experiment. Use the following rule of thumb for resolution in the F1 dimension:

td(F_1) =   200 Low   400 Medium   512 High   750 Deluxe

This is referred to in pulse programs as td1 (total data points in the F1 dimension), but you cannot access this as a parameter; it can only be changed in the **eda** menu. The size of the data file and the length of time required for the experiment are both proportional to this number. A larger value means more resolution (better definition of peaks) in the vertical (F1) dimension of your 2D spectrum. Small values lead to fast experiments but

your crosspeaks will be stretched out in the vertical dimension. In gradient experiments, it is usually possible to use **NS**=1 (a single scan for each FID) so that a large number of FIDs, 512 or 750, can be collected in 15-45 minutes of acquisition. Thus a "deluxe" 2D experiment does not have to take a long time to acquire, as long as you have lots of sample (and disk space). If you have a limited amount of sample (less than 10 mg.), then you have to use larger numbers of scans for each FID just to have enough signal-to-noise in your 2D spectrum. In that case the gradient experiments do not offer a big time savings over the non-gradient experiments. Note that **td1** is the number of FIDs acquired, which is not always the same as the number of t1 increments. With TPPI, the number of t1 increments is equal to **td1**, but with States of Echo-Antiecho it is equal to **td1**/2 and t1 data points are arranged in complex pairs (real, imaginary).

b. SW: spectral width in ppm. This is the width of your spectral window in each of the two dimensions of a 2D experiment. If you are doing a homonuclear experiment (^{1}H in both dimensions) you will want SW to be the same in both dimensions. You may want to edit the SWH (spectral width in Hz) field so that the two dimensions are exactly equal. The SW parameter in the F1 (indirect) dimension will not work correctly if the parameter ND0 (number of D-zeros) is not set right; this should come from the standard parameter set so it will be correct. The correct value of ND0 for the experiment you are doing can be found in the pulse program text at the bottom of the pulse program (use command EDCPUL).

c. O1P and O2P: Center of the Spectral Window in ppm. This is the ppm value you want at the center of your 2D spectral window. Scroll down a bit in the **eda** menu until you find the parameter **NUCLEI** with entries of "edit" under the F_2 and F_1 headings. Under the word "edit" you will find the nuclei for each of the two dimensions; for a homonuclear experiment both will be 1H, for a heteronuclear experiment it will normally be 1H for F_2 and 13C for F_1. Immediately under the 1H heading you will find menu boxes for **O1** and **O1P**. Enter the center of the F_2 (observe) spectral window in the O1P box in units of ppm. Further down, for the parameter **NUC2** you will find the nucleus for the second (F_1) dimension. Immediately under this are the menu boxes for **O2** and **O2P**. Enter the center of the F_1 (indirect) spectral window in the **O2P** box in units of ppm. Unfortunately, Bruker seems to have forgotten their lovely format of F_2 in the left column and F_1 in the right column: these values are both listed in the left column.

d. SOLVENT: Lock solvent. This is the deuterated solvent which you have locked on when you first inserted your sample. The possible parameter values are:

Acetic	Aceton	CDCl3	CD2Cl2	CD3CN
C6D6	D2O	H2O	DEE	DME
DMF	DMSO	Dioxan	EtOH	MeOH
THF	Tol	Pyr	ACND2O	MEOHD2O

This parameter is automatically set when you lock using the **lock** command, but unfortunately any time you read in a parameter set using **rpar** you replace this value with

some irrelevant value from the standard parameter set. This is just a bookkeeping parameter, since the *lockshift* parameter (^{1}H chemical shift of the lock solvent) is set with the **lock** command and doesn't change when you read in parameters with **rpar**.

Example: Let's say you're setting up an HSQC, which is a 2D experiment correlating ^{1}H peaks in the F2 dimension with the directly bonded ^{13}C peaks in the F1 dimension. You want the following spectral window:

    ^{1}H: 0.5 - 5.5 ppm ^{13}C: 5 - 155 ppm

Enter **eda** to display the acquisition parameters window, and edit the following parameter values:

	F2	F1	
SW	~~10.000~~ **5.0**	~~100.000~~ **150.0**	ppm
NUCLEI	edit	edit	
NUC1	1H	13C	
O1P	~~6.500~~ **3.0**		ppm
NUC2	13C		
O2P	~~50.000~~ **80.0**		ppm

Note that I have only shown the parameters of interest, leaving out many lines in the menu. Because this is a ^{13}C-decoupled experiment, we need to be sure that the acquisition time (**AQ**) is no more than 220 ms to avoid overheating and damage to the amplifiers. With the above value for **SW** in F2 and **TD**=2048, we read in the **AQ** menu box that **AQ**=0.4096 sec. (**AQ** = **TD** / (2***SWH**)) which is too long. Set **TD** to 1024 and note that **AQ** is now 0.1024 sec or 102.4 ms, which is an acceptable value. Check **D1** to make sure it is 1 sec. or longer.

        To edit the **SOLVENT** parameter, you can either scroll down and find it in the menu, or exit **eda** by clicking on *SAVE* and enter **SOLVENT** at the XWinNMR prompt:

        >SOLVENT

The current parameter value will be displayed and you can edit it.

        2. Using ased, the Acquisition Setup Editor. ASED gives you a menu which includes only the parameters which are used in the pulse program you will be running. It is much simpler and shorter, and it prompts you with text from the pulse program explaining each parameter and giving suggested values. You only need to edit a few of these parameters because you read in the correct parameters for your experiment using **rpar**. In fact, the only parameters you will edit for routine experiments are the number of scans (**NS**) and the ^{1}H 90° pulse width, and even the 90° pulse can be left at the standard

value if you have tuned (**wobb**) the probe. If you are running a non-standard experiment or modifying a standard one, **ased** will be extremely useful. In **ased**, some parameters are listed with a menu box so that the parameter value can be edited, while others simply have a fixed value displayed.   These fixed parameters are calculated in the pulse program, usually from other parameters. For example, a 180° pulse is always twice the length of a 90° pulse, so if you specify the exact value of the 90° pulse the program can calculate the 180° pulse from it. You might see:

P1	**9.40**	usec	f1 channel - 90 degree high power pulse
p2	18.80	usec	p2=p1*2

The P1 value can be edited, but the p2 value (lower case) is calculated from P1. Notice also that there are comments to the right of each parameter that tell you what it is and sometimes give hints on how to set it. These comments come from the pulse program text.

> **a. PULPROG: The Pulse Program.** This parameter determines the pulse program which defines the actual sequence of pulses and delays which run the NMR experiment. *Don't change it* - it is set by **rpar** when you decide which experiment you want to do. The names of pulse programs are rather cryptic, but there are some clues:

inv4 - HMQC or HMBC	invi - HSQC	tp - TPPI
st - STATES	ea, et - Echo-Antiecho	pr - Presaturation
gp - Gradient Program	si - Sensitivity enhanced	ed - edited
gs - Gradient	sel - selective	nd - not decoupled
df - double-quantum filter	lp - low-pass filter	19 - watergate
mlev, dipsi, dip - TOCSY	lr - long range	ek - Kati Kover

For example: **ekinvieasidiped** = Kati Kover's HSQC-TOCSY, Echo-antiecho, Sensitivity enhanced, edited. You don't have to know any of this; in fact the only thing you need to know is DON'T CHANGE THIS PARAMETER!! It will be set by **rpar** and it controls everything else. Sometimes you may want to look at the pulse program to see what kind of hints or additional information (even literature references!) it contains. You can do this with the **edcpul** (edit current pulse program) command, which opens a jot window containing the text of the pulse program. Bruker pulse programs are amazingly simple and easy to understand, compared to Varian pulse programs. On the other hand, you can destroy equipment by editing them if you don't know what you're doing! Only facility personnel are allowed to edit pulse programs.

> **b. NS - Number of Scans.** This parameter is usually adjusted to determine the length of time the experiment will take. Depending on the sample concentration, you may need anywhere from a 15 minute acquisition to a 24 hour acquisition. Usually you will be "filling up" the time available by increasing the number of scans. It is best to have an **NS** value which is an integer multiple of the length of the

phase cycle in the pulse program. You can view the phase cycle by displaying the pulse program with the command **edcpul** (edit current pulse program). Near the end of the text file you will see the phase cycle, for example:

        ph1=0
        ph21=0 0 0 0 2 2 2 2
        ph2=0
        ph3=0 2
        ph4=0 0 2 2
        ph32=0 2 2 0 2 0 0 2

Each line represents the phase of a particular pulse in the sequence. For example, the phase represented by ph21 will by on the x axis (0) for the first four scans, then on the -x axis (2) for the next 4 scans. If **NS** > 8 it will start over at the beginning of the list. The longest cycle is 8 scans in length (ph21 and ph32), so you will want to use a multiple of 8 scans for **NS**. To find out how long the experiment will take, enter **expt**. If you want it to run longer, increase the value of **NS** by a multiple of the phase cycle length. If you want it to be shorter, decrease **NS** in the same way.

        c. DS: Number of Dummy Scans. We seldom use a long enough relaxation delay (**D1**) to allow full relaxation of spins to equilibrium before the start of the next scan.   Thus the first few scans of any experiment, which start from an equilibrium state, will give FIDs different from the subsequent scans, which reach a steady state in terms of relaxation. To avoid having these first few scans unbalance the cancellation of artifacts in a phase cycle, a number of dummy scans are performed without actually acquiring any data. When the steady state is reached, the real scans begin with acquisition of FIDs. The pulse program usually has a suggested number of dummy scans, which is either a fixed number (e.g., 16), or a multiple of the **NS** value (e.g., 2***NS**). This suggested value can be viewed by looking at the end of the pulse program (**edcpul**) and finding the DS entry, for example:

        ;DS: = 16

Sometimes **DS** is calculated from **NS** in the pulse program, so that it actually doesn't matter what you set it to. **DS** may seem like an unimportant parameter, but some experiments will actually fail completely if **DS** is set wrong!

        d. P1, P2, P3...: Pulse Widths. Every pulse sequence consists of radio frequency pulses and delays. The pulse width for each pulse is the length of time that the RF power is turned on. Pulse widths are measured in microseconds (us) because they are usually very short in duration - typically around 10 μs. High power pulses put out 50-300 watts of RF power and can fry samples, probes and amplifiers if they are on for milliseconds or seconds. Pulse widths must be calibrated to determine how long it takes to rotate the sample magnetization by an angle of 90°. This 90° pulse width is quite consistent on the DRX spectrometer as long as the probe is re-tuned for each sample (**wobb** command), so we generally use standard values which are retrieved with **rpar** and

do not calibrate them. You can calibrate the 90° proton pulse if you want to be especially accurate, but the 90° carbon pulse is more difficult to calibrate so we always use the standard value. Bruker has a "tradition" for naming pulse widths which makes it a bit easier to know what they stand for:

> P0:   variable pulse width (e.g. in DEPT or COSY-35)
> P1:   90° pulse on observe channel (e.g., ^{1}H)
> P2:   180° pulse on observe channel
> P3:   90° pulse on second channel (e.g., ^{13}C)
> P4:   180° pulse on second channel

All other pulses are for special purposes. The observe channel (F_2 in 2D experiments) is the nucleus for which you are recording the FID; in almost all 2D experiments except for HETCOR this is ^{1}H. The second channel is only used in heteronuclear experiments and is usually set for ^{13}C. A bit farther down in the **ased** menu you will see a division into channels: f1 refers to the observe channel and f2 to the second channel; these are hardware radio frequency channels - do not confuse them with the two frequency domains, F_1 and F_2, of a 2D experiment:

```
============== CHANNEL f1 =================
NUC1        1H                  nucleus for channel f1
P1          9.40    usec        f1 channel - 90 degree high power pulse
p2          18.80   usec        p2=p1*2
PL1         0.00    dB          f1 channel - power level for pulse
SFO1  500.1325006   MHz         transmitter frequency


============== CHANNEL f2 =================
NUC2        13C                 nucleus for channel f2
P3          13.00   usec        f2 channel - 90 degree high power pulse
p4          26.00   usec        p4=p3*2
PL2         -3.00   dB          f2 channel - power level for pulse
SFO2  125.7715724               decoupler frequency
```

The second channel is called the decoupler channel for historical reasons even if there is no decoupling. Note that each channel has at least one power level (**PL1**, **PL2**, ...) which sets the power of the RF pulses. RF power is measured in decibels (dB) of attenuation, with the smallest number of decibels (-6) corresponding to the highest power. The 90° pulse width is different at different power levels: at lower power, it takes longer to rotate the magnetization by 90° so the pulse width is longer. In this example, we use higher power for ^{13}C (-3 dB vs. 0 dB) and a longer pulse width (13 us vs 9.4 us) to get the same rotation (90°). This is because the carbon nucleus is only 1/4 as strong a magnet as the proton, and because the carbon RF coil in the probe is on the outside of the ^{1}H coil and therefore farther from the sample. In fact, the 13C pulse amplifier puts out 300 watts at maximum power and the ^{1}H amplifier puts out only 50 watts. To get an idea of power levels, check out the following suggested levels:

Power	90° pulse	Purpose
-3 dB	13.0 μs	^{13}C hard pulse
14 dB	75 μs	^{13}C decoupling for ^{1}H observe
0 dB	9.4 μs	^{1}H hard pulse
10.7 dB	30 μs	^{1}H pulse for TOCSY mixing
18 dB	66 μs	^{1}H for cw ROESY mixing
20 dB	82 μs	^{1}H decoupling for ^{13}C observe
56 dB	4.0 ms	^{1}H presaturation for 90% H_2O / 10% D_2O
75 dB	31 ms	^{1}H presaturation for D_2O solutions

As a rule of thumb, the pulse width increases by a factor of two for every 6 dB decrease in pulse power (Bruker "power level" = attenuation increases as power decreases). The 90° pulse width depends not only on power level but also on the probe being used and to some extent on the sample. Tuning the probe (wobb command) makes up for most of the sample variability. Most pulse programs depend on accurate pulse width settings for their success.

        e. Gradients. The "gradient channel" refers to the pulsed field gradients. Again, you don't need to change anything if you loaded the parameters using **rpar**. A gradient is a deliberate disruption of the homogeneity of the magnetic field by adding a small field which adds to or subtracts from the main magnetic field. The added field depends in a linear fashion on the position within the sample; for example a z-gradient will depend on the position up or down the sample tube. In mathematical terms:

$$B_{eff} = B_o + G_z * z$$

where B_{eff} is the actual magnetic field experienced by a molecule at a vertical distance z from the center of the sample. G_z is the gradient strength, which is proportional to the amount of electrical current put into the gradient coil which generates this field. Thus a molecule below the center of the sample will experience a slightly lower field and a molecule above the center will experience a slightly higher field when the gradient is turned on. If the probe is equipped with "triple-axis" gradients there is a possibility to independently generate field gradients in the x and y directions, perpendicular to the sample tube axis. Gradients can be turned on and off rapidly, or "pulsed". They take only tens of microseconds to turn on and off and generally are left on for about a millisecond. Gradients can also be turned on and off more gradually, resulting in a smoothly shaped curve of increasing and decreasing gradient strength as a function of time. Gradients are used to destroy artifacts, to select the desired NMR signal and to destroy strong solvent signals such as water.

        Each gradient pulse has a duration, a shape and a gradient strength in percent of maximum gradient current. The duration is typically 1000 us (1.0 ms), the shape is the first 180° of the sine function ("sine.100") and the gradient strength varies from -100 to 100%. Bruker has three ways of defining gradients in a pulse program, so it can be confusing - they seem to be still working out how to interface this new technology with

the user. Sometimes gradients are specifically listed as parameters in **ased** under the heading "Gradient Channel". For example, for Watergate (a water suppression method):

```
                                ============ GRADIENT CHANNEL ===========
GPNAM1      sine.100             SINE.100
GPNAM2      sine.100             SINE.100
GPX1        25.80        %       x-gradient strength 1
GPX2        25.80        %       x-gradient strength 2
GPY1        25.80        %       y-gradient strength 1
GPY2        25.80        %       y-gradient strength 2
GPZ1        20.00        %       20%
GPZ2        20.00        %       20%
P16         1000.0       usec    homospoil / gradient pulse
```

In this case there are two gradient pulses in the pulse program, and each has its shape (**GPNAM** = gradient program name), its intensity in each of the three directions (**GPX, GPY, GPZ**) and its duration (**P16**). There is also a gradient recovery delay after each gradient which allows the gradient field to disappear; it is called **D16** and is usually set to 200 usec. Note that the comments to the right of the menu box often give useful hints as to the correct gradient strength settings.

   Another way to specify gradients is to define a gradient program (**GRDPROG**) which is a text file defining all of the gradients in the pulse sequence with their shapes and strengths. This text file resides somewhere else and only its name appears in the **eda** list.   The gradient program uses a number of constants (e.g**., CNST18, CNST19, CNST20, CNST21**...) to define the strengths (in percent) of each of the gradients. The correct values for these constants can usually be found in the pulse program comments. In this case the gradients are largely invisible in **eda** and **ased**, except for the parameters **GRDPROG**, **P16** and **D16**. This method is primitive and error-prone, but with **rpar** all parameters should be set correctly and there is no need to examine or understand any of this.

C.   Interactive Parameter Optimization (GS).   Some parameters need to be set interactively by observing the effect of parameters on the size and appearance of the FID. The **gs** command starts your pulse program with the current parameters and repeats it indefinitely without adding the FIDs into a sum. In this way you can interactively adjust parameters and watch the effect on the FID in real time. You must be viewing the FID window (enter "**a**" or "**acqu**") to see the FID. Click on **Ush** ("unshuffle") to view the real and imaginary FIDs separately. You can change acquisition parameters manually by entering the parameter name or you can open the *GS* window and adjust parameters with sliders or by clicking with the mouse. To exit *GS* mode, enter the command **halt** or exit the *GS* window; for gradient experiments do not click on the *STOP* button as this can freeze the lock system in "sample and hold" mode.

   1.   Adjusting the Receiver Gain (RG).   Every sample has a different concentration, and different experiments have different sensitivity, so it's important to increase the amplification of the FID as much as possible without overloading the

digitizer (ADC). For simple 1D spectra you can use the automatic **RG** adjust routine (**rga**), but for anything more complicated you need to manually adjust **RG** while pulsing repeatedly and observing the FID. Display the FID window (enter **a** or **acqu**) and enter **gs.** To adjust **RG** you need a vertical scale on the screen in absolute units of integers coming out of the digitizer. If you don't see a vertical scale on the screen at the left of the FID display, or if the units are in centimeters (cm at top of vertical scale) you need to set the vertical scale:

> Go back to the spectrum display (click on Return) and click on *Utilities*. If you see no vertical scale at all, click on *Y* (Toggle Y axis on/off); if you have a vertical scale in units of cm, click on *YU* (Toggle Y axis units between absolute and centimeters). If you can't get a vertical scale no matter what you do, you might have the scale color set to the same color as the background (usually black) so that it's "there" but not visible. In that case enter **setres** to bring up the *User Interface Settings* window, and select *Axis* under the *Colours* heading. Adjust the Red, Green and Blue sliders if necessary (often the color is OK but it needs to be reminded) and then click *Apply*. Click on *File* and *Exit* to leave the *User Interface Settings* window. When you have a visible vertical scale with absolute units (abs at top of scale), return to the FID window by entering **a** or **acqu**.

Adjust the vertical scale (*2 or /2 buttons) so that you can see the 100,000 mark near the top of the screen. Now adjust the RG value to get the maximum signal in the vertical scale without exceeding the limits of 100,000 or -100,000 with either FID at any point. I usually adjust **RG** in powers of 2 (2048, 4096, etc.) but you can enter any integer value.

2. Adjusting the Transmitter Offset (O1). This is relevant only in experiments with solvent signal (usually water) suppression. With samples in D_2O and especially for samples in 90% H_2O / 10% D_2O, the water signal is eliminated or radically reduced by irradiating it for a period of a second or so before starting the pulse sequence. The irradiation equalizes the populations of the two spin states for the water molecules so that the pulses do not produce any observable signal (FID). This process is called presaturation and is usually indicated by the letters "pr" in the pulse program name. Presaturation will only work if the irradiation occurs exactly at the frequency of the water peak in the spectrum - this means that the center of the spectral window (O1) must be precisely at the water frequency. In GS mode open the GS window and select O1 as the parameter to be optimized. Position the GS window so you can see the real and imaginary FIDs. The sensitivity is initially set to 0.01 Hz per click of the mouse, so you need to click on the *10 button one to increase this to 0.1 Hz. Then click either to the right or left of the slider to move O1 upfield or downfield in 0.1 Hz steps. Note the appearance of the FID. Since the residual water signal (in D_2O) or the water signal (in H_2O) dominates the spectrum, there should be one main frequency observed in the (time domain) FID.

If the spectrometer frequency ("carrier" or center of the spectral window) is placed exactly on the water resonance, you will see no "wiggles" in the FID since you will have zero (audio) frequency. Instead you should see an exponentially decaying signal in each of the components (real and imaginary) of the FID. If you do see wiggles (oscillation), then you know that the carrier is not on the water signal and you need to change O1. Moving O1 will either increase the number of wiggles (higher frequency,

wrong direction to change O1) or will decrease the number of wiggles (lower frequency, keep going!). Generally the nearest 0.1 Hz is adequate for this adjustment. Often the shims are not perfect and you will see some oscillation no matter what value of O1 you use. In this case you try to get the minimum oscillation or minimize this integral (area) of the FID, a value which is displayed in the acquisition window and updated for each successive scan. You might wonder why you would want to mimimize your NMR signal, but keep in mind that this is the water signal. Your desired data usually appears as a "fuzz" growing on the envelope of the water FID.

D. Starting the Experiment (zg).

1. Adjusting the Experiment Time (expt). The final parameter to adjust is the number of scans (**NS**), which will directly affect the amount of time the entire experiment will take. Keep in mind that for 2D experiments, the signal-to-noise ratio depends on the total number of scans acquired, which is the product of **NS** and **td1**, the number of FIDs acquired. For example, if **td1**=750 and **NS** =8, you will acquire 8*750 = 6000 scans in the complete 2D experiment and 750 FIDs will be saved in the data file. If you set **td1** to 375 and **NS** to 16, you will still acquire a total of 6000 scans and the signal-to-noise ratio in the 2D spectrum will be about the same. In general, set **td1** for the kind of resolution you need in the F1 dimension (see discussion of Total Number of Data Points, above) and adjust **NS** for the amount of time you have available. Use the command **expt** to check how long the experiment will take, adjusting **NS** and repeating the command until you are happy with the length of the experiment. How long is long enough? It depends on sample concentration and the type of experiment. Experiments which rely on J coupling for magnetization transfer give much more intense crosspeaks than those which rely on NOE (NOESY, ROESY). Of the J-coupling experiments, the homonuclear experiments are the most sensitive since the natural abundance of ^{1}H is essentially 100%. Heteronuclear experiments (usually ^{13}C, ^{1}H) are about 100 times less sensitive because the ^{13}C nucleus occurs only about 1% of the time in natural abundance molecules. Thus the 2D experiments can be ranked in order of sensitivity:

TOCSY > DQF-COSY > HSQC, HMQC > HMBC, NOESY, ROESY

If you have a 15 mg sample of a molecule with molecular weight 700 (36 mM concentration), you can get an excellent TOCSY or DQF-COSY with NS = 1. The HSQC or HMQC might require NS = 2 and the ROESY or NOESY could take 4 or 5 hours to give good signal-to-noise ratio in the crosspeaks. With 1.5 mg of the same sample (3.6 mM), even the homonuclear J-coupled experiments might take several hours and the heteronuclear and NOE-based experiments might require 24 hours of acquisition time. All of these rules of thumb are very subjective; they depend on what you consider to be an acceptable signal-to-noise ratio in a 2D experiment.

2. Checking the first FID of a 2D experiment (rser). Unless your experiment is really, really routine you might want to see how it performs for a few FIDs. A good rule of thumb is never to walk away from a 2D experiment until it has completed at least one FID. Enter **zg** to start acquisition. After the first FID is saved you will see *experiment*

number advance to 2 in the scan counter window. Then you can process and examine the first FID by entering **rser** (<u>r</u>ead FID from <u>ser</u>ial file) and 1. This loads the first FID into a 1D experiment called *~TEMP*. There you can apply any window function you want manually (I use the **sinm** command with **ssb** set to 0 for COSY and 2 for all other 2D experiments) followed by the **ft** command. The spectrum may be pretty ugly, but it should have some relationship to the 1D (usually ^{1}H) spectrum. Remember that in DQF-COSY the signal starts at zero so you will need to look at a later FID to see any signal in the 1D spectrum. To return to the 2D experiment, enter **a** or **acqu**.

3. Multiple Queued Experiments (multizg). If you are setting up more than one experiment on the same sample, you do not need to be around to start the second and third experiments. Simply set up each experiment in consecutive experiment numbers under the same sample ID (experiment name) and use the **multizg** command to queue them. For example, let's say I have a natural product sample called *JH142A16C* and I set up four experiments:

Name=JH142A16C

       EXPNO 1   1D 1H spectrum (you always need one of these)
       EXPNO 2   DQF-COSY   (1 hour 16 min.)
       EXPNO 3   HSQC   (4 hour 3 min.)
       EXPNO 4   HMBC   (12 hour 42 min.)

In each of the 2D experiments I have checked all of the parameters (**eda** and **ased**) and optimized the **RG** (**gs**). I have adjusted **NS** and checked the experiment time with **expt**. To be especially careful, I ran each experiment (**zg**) for a few FIDs and checked the first FID (**rser**) and halted the experiment (**halt**). Now I would like to start experiments 2-4 so that they run consecutively. I simply go to the first experiment (re 2) and enter multizg. When prompted for the number of experiments, I enter 3 (for expno 2, 3 and 4). A window will appear telling me the total time required (18 hours 1 min.) and the DQF-COSY experiment is started. When that experiment finishes, the HSQC will start and when the HSQC is finished, the HMBC will start. I should be able to come back the next morning and find lots of good data!

There are two things which can spoil this happy scenario: running out of disk space and/or overwrite protection. When you run expt, the window which tells you the length of time for the experiment also tells you the amount of disk space it will require. You can check disk space with the "df -k" command in UNIX; be sure to check the disk where your own data resides. This is the same disk as your login directory, so entering **cd** and then **pwd** in a UNIX window will tell you which disk. The second potential problem is more subtle. If you ran one of the 2D experiments as a dry-run long enough to save at least one FID to the disk, multizg might halt when it tries to overwrite this data. In this case you would come back the next morning to see a window telling you that the file exists and asking you if you want to overwrite. This is not a good way to start the day. The easiest way to avoid this is to turn off the overwrite protection (ZG safety) in XWinNMR. To do this, enter **setres** and set *ZG Safety* to **Off**. Exit the *User Interface Settings* window and then start **multizg**. You will not have to change this again, and you

will never again be prompted with the "Data Set Exists, Overwrite?" question. Be careful!

II. Data Processing

Note: 2D Data should not be processed on the DRX-500 after your acquisition is finished. Data can be processed off-line on the SGI workstation UANMR using either Bruker XWinNMR software or Felix software. This saves you money and frees the instrument for other users to acquire data!

A. Checking the Processing Parameters (edp). Usually if you used rpar to set up your experiment parameters the processing parameters should be ideal for the experiment you are doing, and you can skip this section. When things screw up (like when your 2D spectrum looks like a big "X") you need to pay attention to these parameters. When you enter **edp** (edit processing parameters) you will get a window with two columns: one for the direct (F2) dimension and one for the indirect (F1) dimension.

     1. Data Size (SI). The number of data points acquired in each dimension is set by the **TD** parameter in F_2 and F_1 using **eda**. But you may want more data points to define your data matrix (2D spectrum) in each dimension. This is accomplished by zero-filling (adding a bunch of zeros to the acquired data set) before the Fourier transform. For example, you may have acquired 1024 data points in F_2 (**TD**=1024; 512 complex pairs in the FID) and 750 data points in F_1 (**td1**=750; 750 separate FIDs acquired). If you set **SI** to 2048 in F2 and 1024 in F_1, you will be expanding the data size from 1024 to 2048 in F_2 and from 750 to 1024 in F_1, in both cases by adding data values of zero to the acquired data. The data matrix will now be 2048 x 1024 data points, for a total of 2,097,152 data points! As you can see, too much zero-filling can cost you a lot in disk space. For phase sensitive 2D spectra (which is most of the experiments), Bruker keeps four copies of the data matrix (rr, ri, ir, and ii) of which only the rr (real-real) is displayed. Since each data value takes four bytes to represent it, you would have 2,097,152 x 4 x 4 = 33,554,432 bytes (32 MBytes) of disk space to hold one 2D matrix. This is why we run out of disk space very rapidly! This is also why we have a daemon which deletes all of the processed data files (rr, ri, ir and ii) which are older than 3 days.

     SI must be a power of 2, and typical values are 1024, 2048 or 4096 in F_2 and 512 or 1024 in F_1. The F_1 digital resolution is usually set lower (lower **SI**) since the **TD** in that dimension is always much smaller than in F_2.

     2. Spectrometer Frequency (SF). **SF** is the radio frequency value in MHz for a resonance at 0 ppm. **SFO1** is an acquistion parameter since it sets the frequency of the pulses and the center frequency in the spectral window. **SF**, however, is dependcnt on referencing the ppm scale to a reference peak such as TMS or residual solvent so it is a processing parameter. If you process 2D using the Felix software package, you will need the value of **SF** for referencing your 2D spectrum and establishing a ppm scale.

     If you are doing a homonuclear (proton) 2D experiment, **SF** should be around 500.13 MHz in both dimensions. If you are doing a heteronuclear (^{13}C, ^{1}H) 2D experiment, **SF** should be around 500.13 in F_2 and 125.77 in F_1. If it is significantly

different from these values, there is a problem and processed 2D data will have the wrong ppm scale.

3. Data Type (MC2). This rather cryptic parameter tells the software how the 2D data was acquired so it can apply the correct Fourier transform type in each of the two dimensions. There are several ways to encode the phase information for the t_1 / F1 dimension, and the data processing has to be consistent with the way the pulse program does this. If you look at the comments at the end of any Bruker pulse program you will see the correct value for **MC2** listed. Use the XWinNmr command "**edcpul**" (edit current pulse program) to bring up a text window containing the full text of the pulse program, and scroll down to the comments (lines starting with a semicolon) at the end. Possible settings for **MC2** are:

    a. **QF** : magnitude mode, not phase sensitive
    b. **TPPI** : Time Proportional Phase Incrementation method (phase sensitive)
    c. **States**: States-Haberkorn or Hypercomplex method (phase sensitive)
    d. **States-TPPI**: A hybrid of the States and TPPI methods (phase sensitive)
    e. **Echo-Antiecho**: A gradient method of encoding phase in F1 (phase sensitive)

Phase sensitive means that you will have to phase-correct in both dimensions (F_2 and F_1) when you process the 2D data. It has the advantage of sharper peaks and better distinction between peaks and noise, as well as the added information in some experiments from positive and negative crosspeaks. Which method is used in the pulse program (b., c., d. or e.) is largely arbitrary, except that Echo-Antiecho has the advantage for gradient experiments that it "gets back" a factor of 1.41 in sensitivity which is lost as a result of using gradients. For this reasons it is sometimes called "sensitivity enhanced".

As long as **MC2** is set correctly when you execute the 2D Fourier transform (command "**xfb**"), XWinNMR uses the correct processing for that type of data and there is no need to think about it further.

4. Window Function (WDW). You are probably familiar with the exponential multiplier (EM) which is a window function (or weighting function) used to emphasize the first part of an FID in order to increase the signal-to-noise ratio. In 2D NMR there is an additional need to smooth the abrupt transition at the end of the t_1 FID, since sampling is usually cut short in this dimension due to lack of time. The sine-bell family of window functions, based on the trigonometric sine and cosine functions, is a simple and commonly-used weighting function for 2D NMR. There is a wide variety of window functions available, including **EM**, **GM** (Gaussian multiplier), **SINE** (sine-bell) and **QSINE** (sine-squared). Each of these parameters sets only the shape of the window function and various other parameters are used to define the height, width, and other aspects. For example, window function **EM** is associated with the parameter **LB** (line-broadening) which determines how sharply the exponential functions decays.

5. Sine-bell Shift (SSB). If you are using a sine-bell (**SINE**) or sine-bell-squared (**QSINE**) window function, the **SSB** parameter determine the amount of lef-shifting of the sine-bell function. A value of 0 is unshifted (start at 0° in the sine function corresponding to the beginning of the FID and end at 180° corresponding to the end of

the FID). A value of 4 is 45°-shifted (start at $45^o = 180^o/4$, and end at 180°) and a value of 2 is 90°-shifted (start at $90^o = 180^o/2$, and end at 180° - same as "cosine-bell").

6. **Phase Mode (PH_mod).**   Defined for each of the two dimensions, this parameter determines the type of phase correction (if any) to be performed on the spectrum after Fourier transformation. If **PH_mod** is set to **pk**, a phase correction will automatically be applied during processing using the parameters PHC0 and PHC1. If PH_mod is set to **mc** (magnitude calculation), the data is converted to magnitude mode after the Fourier transform. Each type of data acquisition (MC2=QF, TPPI, States, etc.) has a PH_mod setting associated with it in each dimension (F2 and F1); for example **QF** (magnitude mode) requires **PH_mod** = **no** in F_2 and **mc** in F_1. Most phase-sensitive experiments use **pk** in both dimensions. All standard parameter sets (**rpar**) should have the correct values set for processing, consistent with the **MC2** value.

7. Zero-Order Phase Correction (PHC0). For phase-sensitive 2D spectra, this is the phase correction is degrees applied at the pivot point. It is defined for both of the two dimensions.

8. First-Order Phase Correction (PHC1). This is the chemical-shift dependent phase correction, defined for both of the two dimensions.

B. 2D Fourier Transform (xfb). Before you have processed the raw 2D data, the "spectrum" window will display the message*: 2D TIME DOMAIN DATA AVAILABLE, Type 'xfb' to process data.* You will also see this message if your processed data is more than 3 days old on the DRX-500 because we have a "daemon" which checks regularly and deletes processed data to conserve disk space. Once you have optimized all of your processing parameters you can regenerate the 2D spcetrum at any time with the command "**xfb**". For "virgin" data that has never been processed, the command is the same: **xfb**. You don't have to wait until your experiment is finished to process the 2D data - **xfb** will process the data available and the only difference will be poorer signal-to-noise and poorer resolution (broader peaks) in the F_1 (vertical) dimension. In this way you can optimize all of your processing parameters (especially the phasing) while the data is being acquired, and you can also get an idea of whether the experiment is working.

The most common problem in 2D processing is incorrect setting of the MC2 parameter. If, for example, you acquire the data in Echo-Antiecho mode and process it with **MC2** set to TPPI, you will see all of your 2D data reflected across the center in the F_1 dimension, leading to an ugly "X" for a diagonal in homonuclear experiments. If you have problems, display the pulse program (**edcpul**) and scroll down to the bottom where the comment lines (beginning with a semi-colon) appear. Look for the recommended setting for MC2 and then either enter **mc2** at the keyboard or use **edp** to get the full menu of processing parameters and edit **MC2**. The second most common problem is the setting of the PH_mod parameters in F_1 and F_2 (see above). All of these parameters should be set correctly if you are using a standard parameter set (**rpar**).

The raw FID data is always present in the acquisition window. If you change the processing parameters at any time you can repeat the 2D Fourier transform with the command **xfb**, either from the spectrum window or the acquisition window.

C. The 2D Display. Click on **intensities** to get an 2D intensity plot. A color bar to the right of the 2D matrix display shows the colors which correspond to various intensity levels. Click on the **+/-** button until you see both positive and negative intensities. The color code is based on the colors of the visible spectrum: Red / yellow / green in order of decreasing positive intensities and violet / blue / light blue in order of decreasing absolute value of negative intensities. Click on the **=** button and hold down the left mouse button; sliding the mouse up and down will adjust the intensity scale. Move the mouse up until you just begin to see the background of noise around the crosspeaks.

To expand a rectangular region of the 2D matrix, click on the [   ] button. This gives a **+** cursor controlled by the mouse. Position the **+** at the lower left corner of the desired region, click and hold the left mouse button, and drag up and to the right to enclose the region. Release the left mouse button and click the right mouse button to expand this region to fill the graphics display. To return to the full 2D matrix display, click on "all".

D. Phase Correction in Two Dimensions.   You will see phase errors as a streak extending right and left (F_2 phase error) or up and down (F_1 phase error) from a crosspeak. One side of the streak will have the color corresponding to positive intensities and the other side will be colored for negative intensities. To correct the phase, click on the Phase button. The 2D matrix will be reduced to a small square in the upper left corner of the screen, and three 1D windows will be stacked vertically on the right half of the screen. These 1D windows are labelled 1, 2 and 3. Use the **=** button to re-adjust the vertical scale so you can see the crosspeaks and a small amount of noise. The strategy is to select a row (horizontal slice) containing a crosspeak (or diagonal peak in a homonuclear spectrum) in the downfield (left) region of the spectrum and move this to 1D window #1.   Then another row is selected which has a peak in the upfield (right) region of the spectrum and this is moved to 1D window #2. A third window is available if you want to put a 1D slice there with a peak near the center of the spectrum. Then a pivot peak is selected in one of the 1D windows and the phase parameters **ph0** (zero-order phase) and **ph1** (first-order phase) are adjusted so that both (or all three) peaks have absorptive phase. The process is interactive and all three 1D windows show the effect simultaneously of the **ph0** and **ph1** corrections. The same process is used for the F_1 phase correction, except that columns (vertical slices) are selected instead of rows.

1. F_2 phase correction. Click on **row** and move the horizontal line cursor onto a crosspeak (or diagonal peak) which appears as far as possible to the left side of the 2D matrix.   Click the middle mouse button to select this row and then click on the **mov 1** button (in a row of three buttons: mov **1 2 3**) to move this 1D slice to 1D window # 1. With the **1** in the upper left corner of this window highlighted, you can click on the **/2** or ***2** buttons in the lower group of display buttons to adjust the vertical scale of the 1D display. Now click on **row**, move the horizontal line cursor onto another crosspeak or diagonal peak which appears as far as possible to the right side of the 2D matrix, select the row with the middle mouse button and then click on the **mov 2** button. If you want to you can select another row with a peak near the middle of the spectral window and place that 1D slice in window #3. Now select the pivot peak - as with phase correction of 1D

spectra, you can select either the biggest peak or a peak chosen with the cursor. For example, to select the biggest peak in window #1 you would click big 1 (from the row of three buttons: big **1 2 3**). Alternatively, to select a particular peak in window #2 which is not the largest peak, click cur **1** (from the row of buttons: cur **1 2 3**). An arrow will appear in window #2 and you can use the mouse to position this arrow on top of the desired peak in the 1D slice. Click the middle mouse button to select the peak.

Once you have selected a pivot peak, use the **ph0** button (hold down the left mouse button and drag up and down) to phase-correct this peak in the appropriate 1D window. Then use the **ph1** button to phase-correct another peak at the other end of the 1D spectrum, observing this peak in another 1D window. With these two corrections all peaks in all windows should now be absorptive. You may need to adjust the vertical scale to exaggerate the phase errors - this is done by first highlighting the window number (click on the box in the upper left corner of the desired 1D window) and using the ***2** and **/2** buttons in the lower group of display buttons (the upper group is linked to the 2D matrix display). When you are satisfied with the phase correction for F_2, click on **Return** and **Save & Return**. Confirm the **xf2p** option to begin the process of applying this phase correction to all of the rows of the 2D matrix.

2. F_1 Phase Correction. To phase correct in the F_1 (vertical) dimension, repeat the above process selecting columns (col) instead of rows. Try to find one column with a peak near the bottom of the 2D matrix and another with a peak near the top. Either one of these peaks can be used as the pivot peak in the 1D window. After clicking on **Return** and **Save & Return**, confirm the **xf1p** option to apply this phase correction to all of the columns of the 2D matrix. These phase correction parameters are saved as the **PHC0** and **PHC1** parameters for F_2 and F_1, which can be viewed in the edp menu. You can also write down these parameters and use them for data processing using the Felix software.

E. Displaying a Contour Map. Contour plots (topographic map type plots) are much easier to view and convey much more detail than intensity (color-coded) plots. To view the data in this format you have to define the intensity level (in absolute integer units) of each contour. Click on **intensities** to display an intensity plot and use the **+/-** button to toggle until you have both positive and negative intensities displayed (check the color bar at the right to see which intensities are displayed). Enter the command **levcalc** to calculate the intensity levels of the contours, and then enter **edlev** to edit the spacing between levels. Edlev displays a menu showing all of the contour levels. Place the cursor at the bottom after the word **FACT:** and enter 1.25 and click **Save**. This sets the ratio of successive contours to 1.25, resulting in closely spaced contour lines. If necessary, use the **+/-** button to make sure you have positive and negative intensities displayed and then click on **contours** to calculate a contour map display. The levels will be shown in the upper right of the graphics display with color coding and actual intensity levels. Use the **/2** and ***2** buttons to adjust the contour threshold so that the noise is just barely showing up in the contour plot.

F. Plotting the 2D matrix on Paper. Start with a contour plot rather than an intensity plot. Select **Windows** and **Plot-editor** to start the XWinPlot program. A parameter list and title can be generated just like you do for 1D plots. To create the 2D matrix "object"

just click on the 2D spectrum icon (looks kind of like a Rorschach ink-blot test) and click and drag from upper left to lower right to place the 2D plot within the margins of the paper. Highlight the 2D plot object by clicking first on the "select object" icon (nine green squares around a sort of zigzag line) and then on the 2D plot. Green squares will light up around the plot to show that it has been selected. Then click on **1D/2D-Edit** and use the ***2** and **/2** buttons to adjust the contour threshold. Click on **Close** to exit this menu. You can expand a region by clicking on the Expand button and then clicking and dragging the mouse from lower left to upper right of the desired region. To return to the full 2D spectrum click **Edit** and **Undo**. When you like what you see on the screen, select File / Print. Make sure the printer is set up correctly: click Setup and scroll down in the Printer Type window to make sure that **HP LaserJet 5L** is selected, then click **OK**. Click on **Print** to send the plot to the printer and select **File / Close** and confirm when you are finished using XWinPlot.

You can make color plots if you wish - these are useful for COSY spectra or for edited HSQC where the sign (positive or negative) of a crosspeak carries useful information. We normally use the HP LaserJet (black and white) printer (Position A on printer switchbox) but we also have an HP DeskJet (color) printer (Position B on printer switchbox). In XWinPlot, highlight the 2D contour plot and click on **1D/2D-Edit**. The contour levels are listed in the lower left of this window and a color chart is displayed on the right side. Each contour can be given a different color, but it's usually most informative to have the negative contours red and the positive ones black. Since black is the default, you only need to edit the negative contours. Highlight the highest (absolute value) negative contour by clicking on it. Then hold down the Shift key on the keyboard and click on the lowest (absolute value) negative contour. This will highlight all of the negative contours. Click on the red square in the color chart, which will cause that square to be larger than the others. Click on **Apply** and **Close**. Then select from the main XWinPlot menu **Options / Printer Setup**. Under *Printer Type* scroll through the list and **select HP DeskJet 660C**, and for *Format* select **Color**. If you are processing data on the UANMR satellite station, the default printer is **HP DeskJet 1120C**, so you will always be connected to a color printer. On the DRX-500, if a color printer is available set the printer switchbox (to the left of the SGI computer) to position B and print as you normally would from XWinPlot. The 2D color contour plot should come out of the DeskJet. **Be sure to set the switchbox back to position A when you are finished!!!**