

Step-by-step procedure for NMR data acquisition

Spectrometers

The UTHSCSA 500, 600, and 700 MHz spectrometers are each equipped with 4 independent RF channels and are each operated by a Red Hat Linux workstation running the spectrometer control program, TopSpin. There is, however, one IMPORTANT difference of which you should be aware, which is that the 500 and 700 MHz spectrometers have newer consoles (so called Avance I) compared to the 600 MHz spectrometer (so called DRX console). The newer consoles allow one to run the latest version of the spectrometer control software (currently TopSpin 2.11), whereas the older console is only capable of being operated by an older version, TopSpin 1.38. Thus, although many things are the same between the two versions of TopSpin, there are some important differences. The ones relevant to this data acquisition guide are indicated below.

Note also that some of the commands and procedures will depend on the type of probe installed in the spectrometer, for example, whether the probe is equipped with single (Z) or triple-axis (X, Y ,Z) gradients, whether automatic tuning and matching is available, and whether it's a high-sensitivity cryoprobe or not. A summary of all probes available for the UTHSCSA spectrometers are listed below:

Spectrometer	ProbeName	RF Coils	Gradient Coils	ATM	Cryo
500	TXI	1H, 13C, 15N	X,Y,Z	Yes	No
500	QXI	1H, 13C, 15N, 31P	X, Y, Z	No	No
600	TXI-Cryo	1H, 13C, 15N	Z	No	Yes
600	QXI	1H, 13C, 15N, 31P	X,Y,Z	No	No
700	TCI-Cryo	1H, 13C, 15N	Z	Yes	Yes
700	TXI	1H, 13C, 15N	X, Y, Z	Yes	No
700	QXI	1H, 13C, 15N, 31P	X,Y,Z	No	No

Starting TopSpin

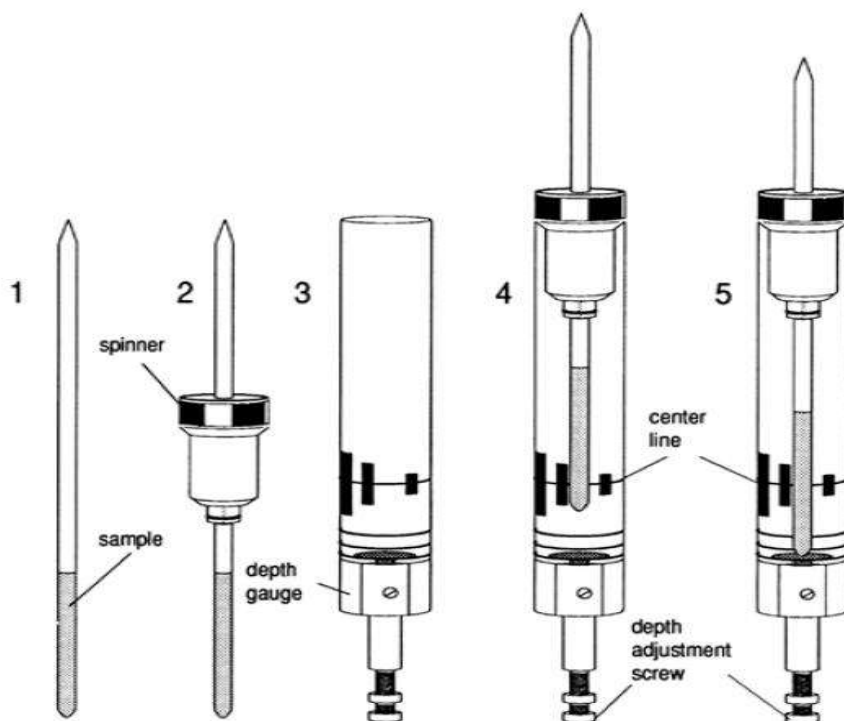
1. First login as a user in the linux workstation. If you don't have an account please contact us to setup one for you.
2. Click the redhat icon, then **BRUKER** menu, and finally topspin2.1 (Av500 & Av700) or topspin1.3 (Av600). TopSpin window will appear.

Preparation for acquisition

1. Since some of the commands of TopSpin require a dataset, open a dataset first. You can do this by clicking the **Browser** menu from the left-hand side panel and choosing appropriate data set. Currently, all the users' datasets are stored in /avance5004, /avance6003 and /avance7002 disks on the 500, 600 and 700 MHz spectrometers respectively. If these data directories are not displayed (as will be the case for a first time user), you can add them by right clicking and choosing “Add new data dir...” menu. To open a data set listed in the browser double click or right click and choose “display” (drag and drop has now been disabled).
2. Now the desired sample temperature can be set by typing **edte** at the command line. Wait for the sample temperature to reach the desired value before proceeding to the next step.

Inserting Sample

1. NMR tube containing sample should be held in a plastic spinner (use the blue color spinner in 500 and 600 MHz spectrometers; use the white color spinner in 700 MHz spectrometer). Hold the sample by the top, place sample tube in the spinner and the spinner in the sample depth gauge. Push or pull the sample tube so that the depth of the sample above and below the center line of the sample depth gauge is equal. However, never exceed the lower limit (position of the adjustable white platform,



which should be at the line marked 5 mm - 15mm) as this can damage the probe as well as the sample, as the sample will rest on the tip of the tube, not the spinner. This is important for all probes, but especially the cryoprobes installed on the 600 and 700MHz spectrometers.

2. Remove the black cap from the top of the magnet bore. Next, press the LIFT button in upper left portion of the BSMS keyboard. Wait for the airflow (hissing sound that can be heard). Then remove the depth gauge before inserting the sample and spinner into the magnet. Pressing the LIFT button will toggle the air flow off and will drop the sample tube gently to the magnet bore where it will be positioned at the top of the probe.

Locking

3. Lock signal can be seen in the lock display window that can be opened by typing **lockdisp** at the command line.
4. Enter **lock** at the command line and select appropriate solvent from the pop up solvent table window (H₂O+D₂O or CDCl₃ etc) and wait for the “lock finished” message to appear at the bottom of the topspin window. After that Lock On/Off LED will light on the BSMS keyboard. **DO NOT LOCK THE SPECTROMETER USING ANY OTHER METHOD.** If the spectrometer fails to lock, this may indicate that your sample does not include any deuterated solvent.

Tuning and Matching the Probe

The resonance frequency of the RF coils will vary depending on the content (particularly, the salt content) of the individual NMR sample. Thus, it's necessary to “Tune” the RF coil to the correct value to yield the correct resonant frequency for the magnet field strength you are using. This is done by inserting the sample in the probe and then by adjusting two variable capacitors, called “tune” and “match” on the probe such that the reflected radio frequency power is minimized at the proper frequency.

Importantly, the higher frequency, the greater the degree to which the “tuning” of the RF coil will depend on the content of the particular sample. This in practical terms means that it is essentially always necessary to tune the RF coil for ¹H, whereas it is less important for lower frequency nuclei, such as ¹³C and ¹⁵N. Thus, in terms of standard operating procedures, we recommend that you ALWAYS adjust the ¹H tuning, whereas adjustment of ¹³C and ¹⁵N tuning should be considered optional (and in most cases, not necessary).

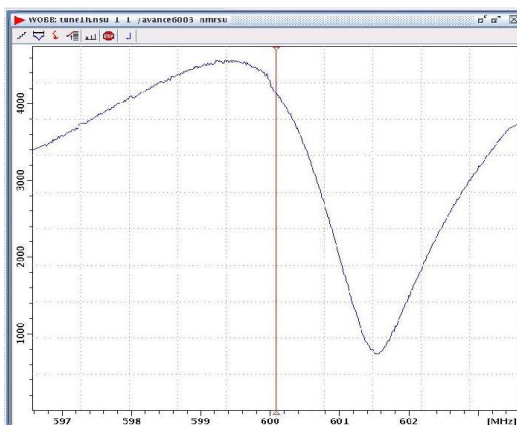
5. On probes equipped with automatic tuning and matching (ATM), this can be done

with the “**atmm**” command. Currently, the conventional TXI probe on the 500 and conventional TXI and TCI cryoprobe on the 700 include ATM. Open an existing dataset and then use the “**edc**” command to save it to a new name (such as tune1h). Now type **rpar Tuneh** and press enter. In the acquisition window type **atmm** and press enter. Click “optimize” at the top of the atmm menu bar and choose start. This will enable tuning and matching of the probe for the selected channel (1H in this case). Alternatively, do not click optimize and manually adjust with the displayed tuning and matching buttons. Repeat this process for the all the desired channels (You will have to setup separate experiments for this, for example using **rpar Tunec and rpar Tunen** to tune either the 13C or 15N channels, respectively).

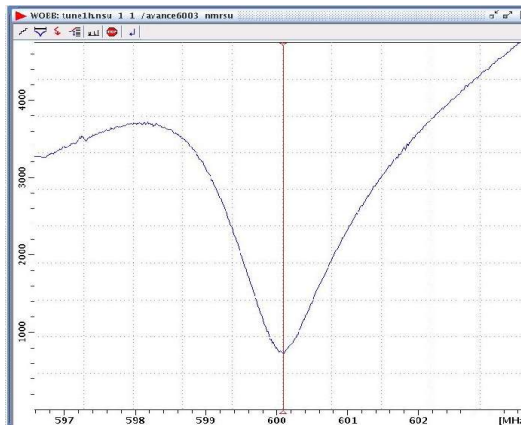
6. On probes without ATM, open an existing dataset and then use the “**edc**” command to save to a new name (such as tuneh). Now, type **rpar Tuneh** and press enter. Then go to the acquisition window by clicking acqu in the current dataset menu bar or simply type **a** at the command line. Now type **wobb** at the command line. Go to the magnet and look for various screws underneath. These are color coded such that they match the color on the plate of the probe where RF cables attach. On all probes, yellow corresponds to 1H, red corresponds to 15N, and blue corresponds to 13C. Adjust those screws using the tool (either attached to the probe by a flexible chain or a separate tool, which we usually keep next to the magnet on the top of the preamp) until you get good tuning and matching. After this type **stop**. To tune other nuclei, such as 13C and 15N open an existing dataset and then use the “**edc**” command to save to a new name (such as tunec or tunen). Now, type **rpar Tunec** or **rpar Tunen** and press enter. Then go to the acquisition window and type **wobb** at the command line and repeat the process as described above.

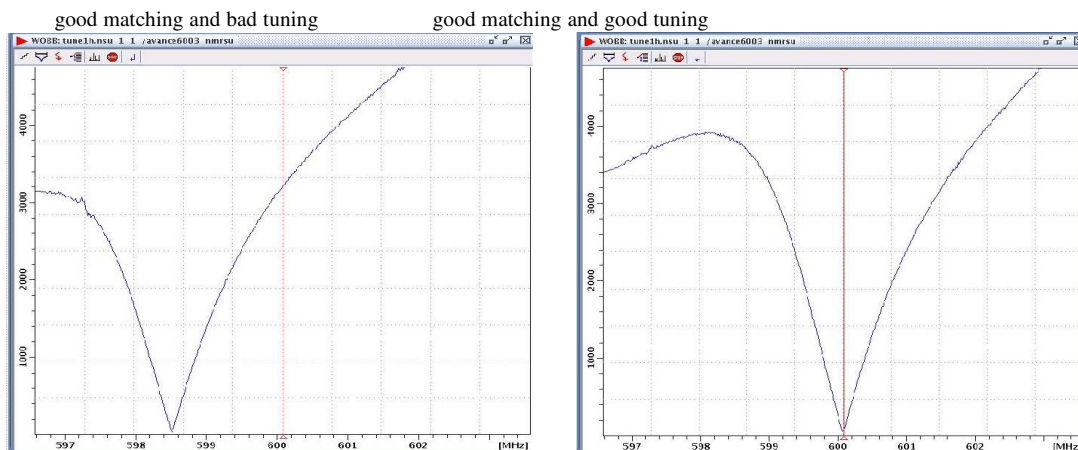
Examples of Wobble Curves with Different Tuning and Matching

bad matching and bad tuning



bad matching and good tuning





Importantly, when manually tuning the cryoprobe on the 600 (which will always be the case since it does not have an ATM device), you will need to type the command **crpoff** prior to initiating the tuning procedure; once the tuning procedure is done, you will need to type the command **crpon** (these commands switch the probe from the normal situation where it uses internal cold preamplifiers to the standard warm preamplifiers (which are needed for tuning) and then back again).

Shimming

Shimming is a process in which minor adjustments are made to the magnetic field until uniform magnetic field is achieved around the sample. This is difficult to do manually, but can be done using automatic procedures. Before shimming any sample, read a recent stored shimfile for the current probe by typing **rsh** and choosing an appropriate shim file from the list.

On the 500 and 700 MHz spectrometers there are two automated procedures possible, named **gradshim** and **topshim**. **Topshim** is strongly recommended over **Gradshim** as it's a newer procedure with a number of improvements relative to **Gradshim**. On the 600 MHz spectrometer, only **Gradshim** is available and therefore will have to be used.

- To run **Topshim**, type **topshim 1h 1d** (or, if you have a Shigemi tube, **topshim 1h 1d shigemi**). If your sample is 100% D₂O or CDCl₃ or any other deuterated solvent, then use **topshim 2h 1d**. This procedure is entirely automated and will adjust the shims along the Z-direction (these are the ones that vary most from sample to sample). Typically, **TopShim** is complete within a minute, although it can be shorter or longer than this depending on the initial homogeneity (generally, starting with poor shims will cause **TopShim** to take longer to converge). Once **Topshim** is complete, click on the **autoshim** button on the **BSMS** keypad, which will automatically optimize the most sensitive Z- and X/Y shims in a continuous manner. Users may wish to save the

current shim set for future use; this may be done by typing `wsh` and by supplying a descriptive filename.

NOTE, it is possible to use TopShim to shim in X, Y, and Z directions simultaneously, using **topshim 3d 1h**. NOTE, however, this will only work on probes with triple-axis gradient coils. which includes all of the UTHSCSA probes, except for the 600 and 700 MHz cyroprobes (which are equipped only with Z-gradient pulsed field gradient coils).

8. To run Gradshim type **gradshim**. The first time **gradshim** is run there is a setup process.
 - Agree with the setup process and click "seen" to ignore all the error messages.
 - For both 3D and 1D buttons (top of window) enter your username in the **User** field and enter `/opt/topspin.x/` in the **disk** field.
 - Create an iteration control file (the following is for 1D shimming).
 - `<edit> <iteration control> <new>`
 - Give the file a name, ie `1D_5steps`
 - Add the following 5 steps:
 - step 1, lowz, size=18
 - step 2, midz, size=18
 - step 3, midz, size=18
 - step 4, highz, size=18
 - step 5, highz, size=18
 - `<save>`
 - The size is the length of the sample chamber. The units don't exactly correspond to mm, but 18 is a good entry for an 18 mm chamber. If these sizes are set too high, the procedure may fail to converge on a homogeneously shimmed field.
 - Thereafter, your iteration control file should be selected by default, but if not, select it from the list. The * button expands the list of available iteration control files.
 - `<Start gradient shimming>`
 - It should say that it will do 5 iterations. But, sometimes program exits after 1 iteration. If so, choosing the iteration control file from the list again will solve this problem.
 - A plot will be displayed of the frequency (as a measure of the field) over vertical coordinates with 0 at the center of the sample. The size you have selected is in a different color. The field is homogeneous when the central

colored part of the plot is vertical.

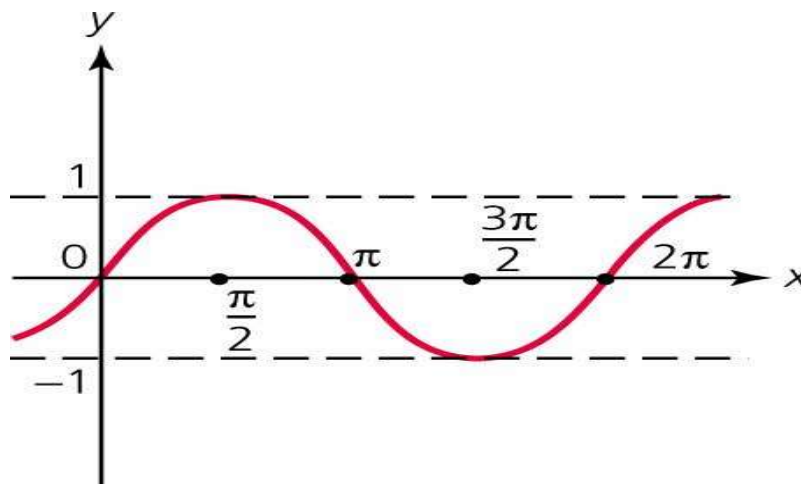
- You may have to repeat gradshim to get the field homogeneous. First delete the previous plot window, else the new lines will be added to the old ones. If the line has a hook on one end that can't be straightened, or appears to be unstable in its effort to converge on a vertical line, your sample chamber may not be centered properly.
9. Wait for the message “setsh finished” to appear at the bottom of the topspin main window. This message indicates that gradshim is finished.

Setting up experiments

1. You need to create a new dataset before starting any experiment. To do this open an existing dataset as described in the Step 1 of “Preparation for acquisition”. Now type **edc** from the command line. In the edc display window which will pop up, type all the necessary parameters like Name, Expno, Dir and User etc and then press OK. This will take you to the new dataset you have created.
2. Next determine the 90 degree proton pulse at high power.
 - ◆ To do this on the 500 and 700 MHz spectrometer (running TopSpin 2.11), issue the command **pulsecal** and wait. Calibrated pulselengths for a hard pulse and a soft pulse will be shown in a new window. Note down these values.
 - ◆ The automated procedure, pulsecal is only available in the newer version of TopSpin. Thus, the following manual procedure will have to be used on the 600. This procedure can also be run on 500 & 700 and may be desirable if one suspects the values determined by the pulsecal procedure are not accurate. To manually calibrate the 1H pulse we use a one pulse experiment. To use this, create a new experiment (calib1h for example) using **edc** command, and then type rpar calib1h on the command line. The intensity of the resulting solvent peak will be maximum when the pulse length is exactly 90 degree and the signal intensity will be minimum (null signal) when the pulse length is equal to multiples of 180 degree (180, 360, 540, 720 etc). But, in practice, due to radiation damping the solvent signal won't be null for 180 degree pulse if your sample is in 90% H₂O/10% D₂O. Only, 360 degree pulse gives the null signal. So, finding out the 360 degree pulse is widely recommended. Open the calib1h dataset and set pl1 (power level for the hard 90 degree) as -3 dB (in 500 MHz & 600 MHz spectrometers) or +2.5 dB (in 700 MHz spectrometer). Set p1 as 1u and then type “**zgef**”. Type “**apk**” (automatic phase correction) to phase the spectrum. Zoom in around the water signal using the mouse and the type **dpl1** at the at the command

line. Now type “**popt**” at the command line and press enter. A window will pop up. Enter the STARTVAL, ENDVAL and INC parameters for the parameter p1 (90 degree hard proton pulse). For example, enter 30 usec, 36 usec and 0.4 usec for STARTVAL, ENDVAL and INC fields respectively. Then click the start optimize button. After popt is finished you can see that solvent signal intensity will be plotted as a function of p1 value. Find out the p1 value corresponding to 360 degree (p1 value at which you see a null). From that value 90 degree pulse can be calculated. Readjust the STARTVAL, ENDVAL, and INC values if you don't see any NULL.

- ◆ Since all multiples of 360 degree pulses (720, 1080 degrees etc) give you a null signal (see the sine wave diagram), beginners sometimes get confused in choosing the correct null. This is especially true when you have high salt sample. So, use the following tip to avoid this confusion. Calculate the 90 degree pulse from the value you got for 360 degree pulse. In the calib1h experiment set p1 to 90 degree pulse. Acquire the signal (**zg** followed by **efp** or **zgefz**). Type **apk** to phase correct it. The signal should be a maximum. Now set p1 to 360 degree pulse. Acquire the signal (do not phase correct the signal). You should see a null signal. Finally, set p1 to 270 degree pulse. Acquire the signal (Again, do not phase correct it). Now you should see a signal with negative maximum. If you don't see the signal intensities as explained above then it is likely that you are seeing a wrong null and pulse length calibrated for 90 degree is wrong.

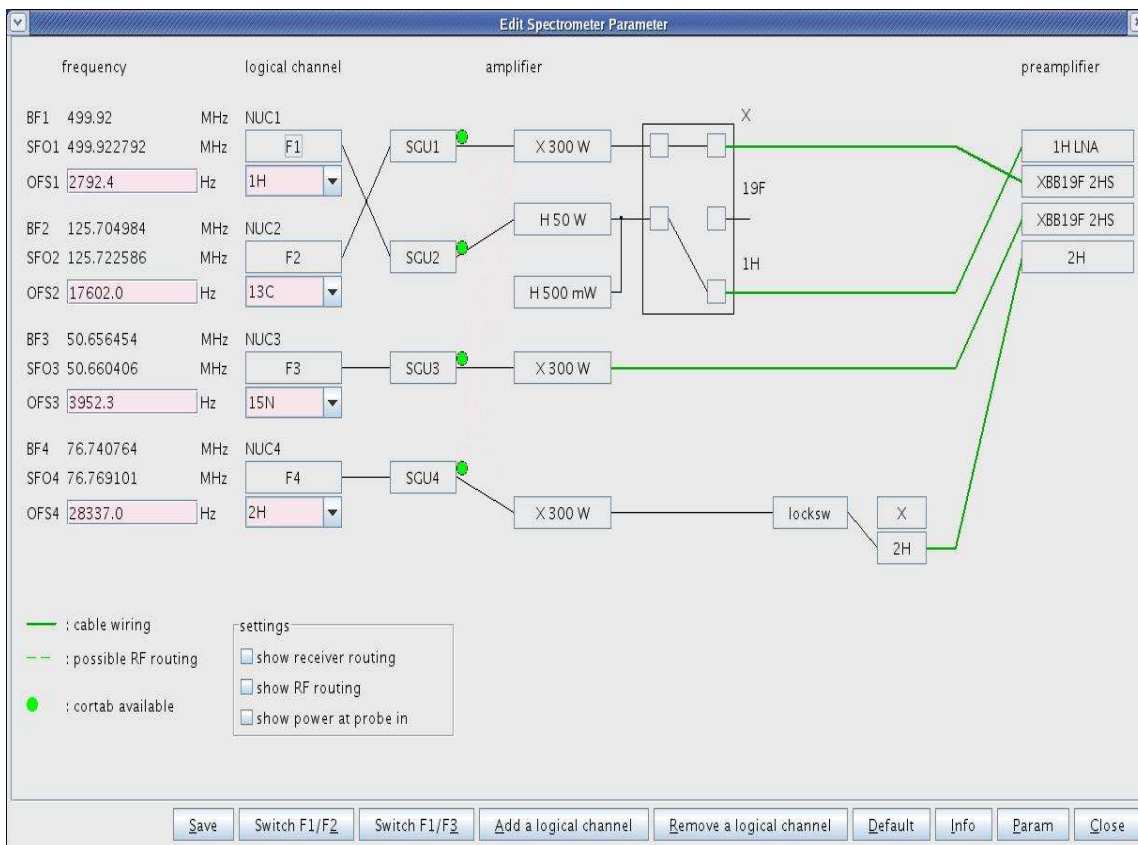


Academy Artworks

3. To setup your experiment of interest, use **edc** to create a new data set and then use **rpar** to read a standard parameter set for the experiment. If you wish to run 1D proton spectrum then choose PROTON or any other suitable standard parameter set from the list and click OK. Similarly, if you wish to run a 2D heteronuclear experiment like HSQC, then choose a parameter set like HSQCETF3GPSI from the list and click OK. This step will ensure that all the necessary standard parameters are copied for the

experiment. Please note that the standard parameter sets are named according to a standard coding method (see the Pulprog.info in the /opt/topspinX.x/exp/stan/nmr/lists /pp/ directory for a detailed explanation of the coding system).

4. Type **edasp** and check that the routings are OK. A typical routing setup for the UTHSCSA spectrometers is shown below. Loading standard parameter sets will ensure the correct edasp settings for you. Nevertheless, it is still a good idea to check the edasp settings before you start any NMR experiment.



5. Issue the command **“getprosol 1H <pulselength> <power>”**. Substitute the values of the proton hard pulse length and power level you got from step 2 above (for example, **getprosol 1H 8.0 2.0**) here. All the other 1H pulses and their power levels (as well as pulse times and power levels for other nuclei) will be calculated and stored in the current dataset.
6. Now you have to set the carrier positions for each of the dimensions of your experiment. These parameters determine the center of the spectrum in ppm and are set by typing the commands o1p, o2p, and o3p. Common center positions in ppm for the different nuclei are listed in the table below:

1H H2O	4.706
1H amide	8.50
13C Calpha	53.5
13C Ca/Cb Center	46.0
13C Carbonyl	173.5
15N amide	118.0

As an example, if you wanted to setup a 3D HNCA experiment, you would type o1p 4.706, o2p 53.5, and o3p 118.0. Although these values should work for most proteins, it may be desirable to optimize them (particularly, 15N carrier position for example) for your individual protein.

- After setting the center, you have to set the desired spectral width in units of ppm. You can set these values by issuing **eda** command or clicking “AcquPars” menu. In the eda window look for SW[PPM] parameter. If you do not see that then click the width menu in the left hand side panel. Set the correct values for ALL dimensions . Typical values for common experiments are listed in the table below:

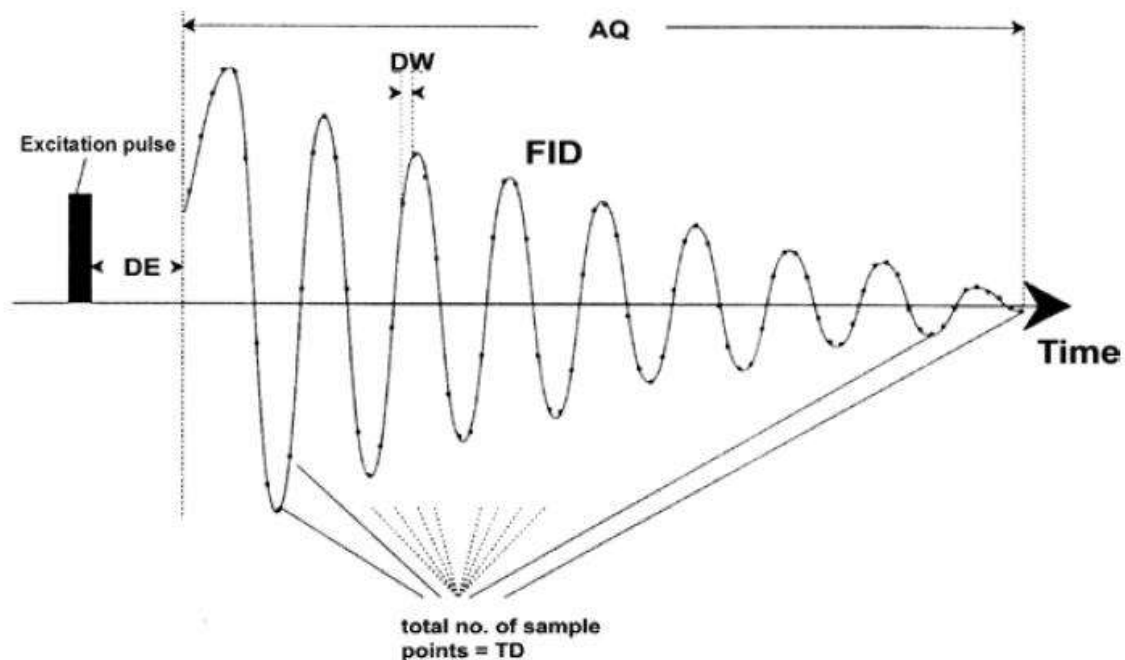
<u>Experiment</u>	<u>Sweep Width/Spectral Width</u>
1D 1H:	16ppm
1D 13C:	200ppm
2D H-N HSQC:	1H – 16ppm, 15N – 28 ppm
3D HNCA:	1H – 16ppm, 15N – 28 ppm, 13C – 24 ppm
3D HNCO:	1H – 16ppm, 15N – 28 ppm, 13C – 12 ppm
3D CBCA(CO)NH:	1H – 16ppm, 15N – 28 ppm, 13C – 60 ppm
3D HNCACB:	1H – 16ppm, 15N – 28 ppm, 13C – 60 ppm
etc	

- Change the number of sample points (TD) in each dimension if you are not satisfied with the default values set by **rpar** command. Typically, TD is set to 16, 32 or 64k for standard 1D experiments. In the multidimensional experiments TD for direct dimension (usually proton) is set for 1k or 2k. Follow the table to get some idea about the TD set for indirectly detected dimension.

<u>Experiment</u>	<u>Indirect dimension</u>	<u>TD</u>
2D-15N HSQC	15N	360–400
2D-13C HSQC	13C	240-320
2D-13C HSQC (Constant time version)	13C	determined by the decremental delay
3D HNCA	15N	64

3D HNCA	¹³ C	120-130
3D CBCA(CO)NH	¹⁵ N	64
3D CBCA(CO)NH	¹³ C	120-130
3D HCCH-TOCSY	¹ H	130-150
3D HCCH-TOCSY	¹³ C	64-80
3D ¹⁵ N NOESY	¹ H	220-280
3D ¹⁵ N NOESY	¹⁵ N	64-96
3D ¹³ C NOESY	¹ H	220-280
3D ¹³ C NOESY	¹³ C	64-96

Graphical Representation of Acquisition Parameters

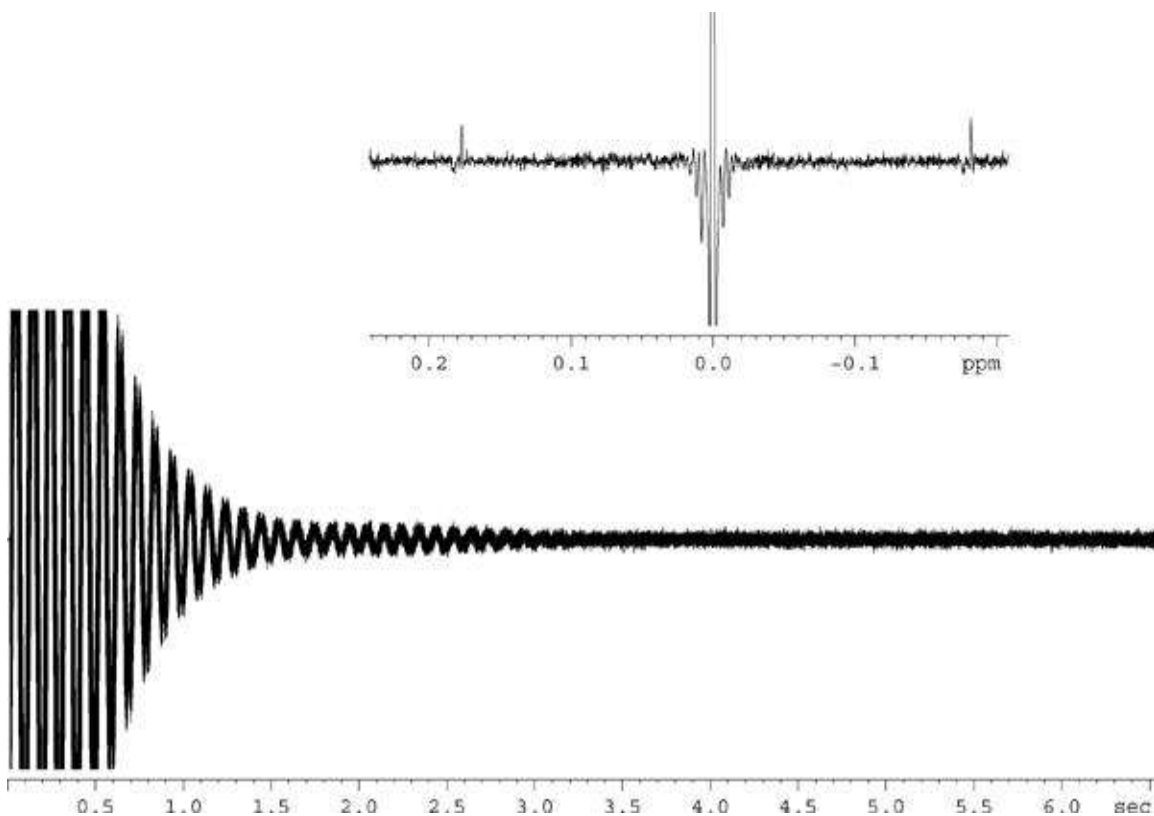


9. Change the number of scans (NS) & dummy scans (DS) if you need to. Increasing the NS will improve the quality (Signal-to-Noise ratio) of the spectrum . But, the total experiment time will also be increased. Typical 1D experiments can have NS of 1k or 2k. But, typical 2D experiments will be completed in an hour if you set the NS to 8 and TD to 360. But if you set NS to 32 then the same experiment will take 4 hrs to complete. So, increase the number of scans only if needed. Also, note that NS should ideally be an integral number of the longest phase cycle in the pulse program. For example, if the longest phase cycle of the pulseprogram is 8 then you should set NS to integral multiples 8. Use the command **expt** to determine the total time needed to

complete the experiment. During dummy scans, the actual experiment will be run but no data will be acquired. This will help sample to reach a steady state or equilibrium. For DS default value set by the **rpar** command should be sufficient.

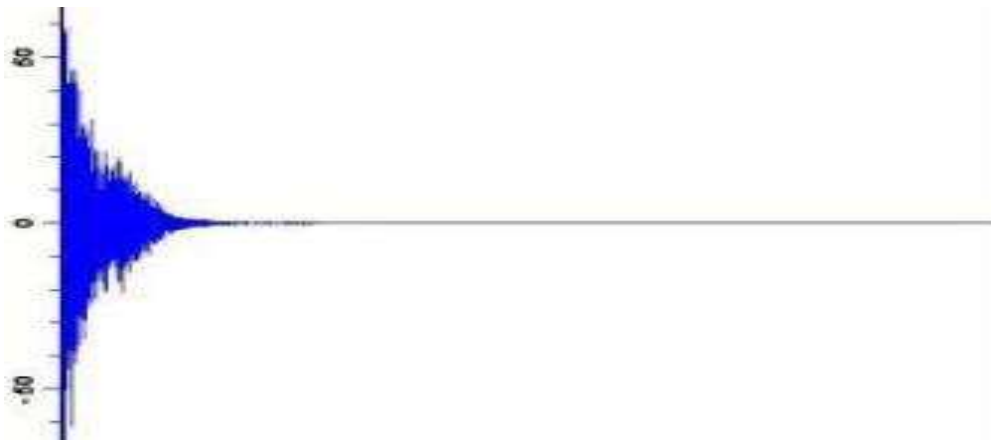
10. The receiver gain is set by the parameter **rg**. Receiver gain is a very important parameter to match the amplitude of the fid to the dynamic range of the digitizer. If the FID is clipped at the top or bottom of the display then **rg** should be reduced. If the gain is too low and not utilizing a suitable (25-40 %) part of the dynamic range of the digitizer, it should be increased. Typically, the receiver gain is set automatically by the **rga** command.

Effect of clipped fid as a result of RG set too high



11. Type **zg** to start the acquisition. You should see an FID in the acquisition window as shown below

FID (time domain signal)



If you have acquired an `fid` (time domain data) then you type `efp` to see the the frequency domain spectrum. The `efp` applies exponential window function to the time domain data and then Fourier transforms the data followed by phase correction using the values `PHC0` (zero order) and `PHC1`(first order). If the spectrum is not phased correctly then you can give `apk` command to do the automatic phase correction.