Guide to Varian Spectrometers running VnmrJ

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Last updated : September 26th 2007

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1. THE NMR FACILITY

1.1 INTRODUCTION

The NMR Facility at the UCHSC comprises Varian Inova 800, 600 MHz and 500 MHz spectrometers and also 900 MHz spectrometer as part of the Rocky Mountain Regional NMR Facility. This handout is intended to serve as a practical guide to running experiments and to help diagnose and solve any problems that you may encounter.

Our spectrometers use Sun workstations to run the instrument and consequently the use of these machines requires a basic knowledge of UNIX. It will help you greatly if you have a basic knowledge of UNIX, and how to move, copy and delete files. You should know the difference between absolute and relative pathnames, and you should be familiar with a UNIX editor such as vi or emacs.

1.2 BOOKING NMR TIME

NMR time is allocated in 24 hour blocks. If you want to use the spectrometer you should send an e-mail stating what experiment(s) you want to do and the amount of time these will require. A schedule for NMR time is posted at http://biomol.uchsc.edu/researchFacilities/nmr/schedule.html. You can see who is booked on the machine and also submit a request for machine time from this page. If you just want to send a request for time, email me at David.Jones@uchsc.edu.

1.3 USING NMR TIME

The NMR Facility operates under a charge-back scheme. The current rates are posted at http://biomol.uchsc.edu/researchFacilities/nmr/rates.html . Please note that these rates are subject to review at least annually, Every effort is made to keep this information up-to-date, but you should contact David Jones to make sure that these rates are current at the time that you use the machines.

Users are charged for any NMR time they use. The daily allocation period runs from 10 am-10 pm. You should vacate the spectrometer by 10 am of the day that you are scheduled to finish, making sure that your experiment has stopped and the data has been stored. Users will be billed according to the amount of time that they are logged onto the computer. So please make sure that you log out after you have finished.

If you do not, or cannot use the allocated time, please let us know in advance, as there are lots of people who always want access to these machines.

1.4 STORING AND ARCHIVING YOUR DATA

Data that is collected on any of the NMR spectrometers may be stored temporarily on the external drives provided. Any data that is more than one week old may be deleted if there is insufficient space on these drives. Unfortunately, we do not have the financial or manpower resources to continually back up users data and so users are responsible for ensuring that their data is backed up before it is deleted. The NMR facility cannot be responsible for loss of data.

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2. LAYOUT OF THE SPECTROMETER

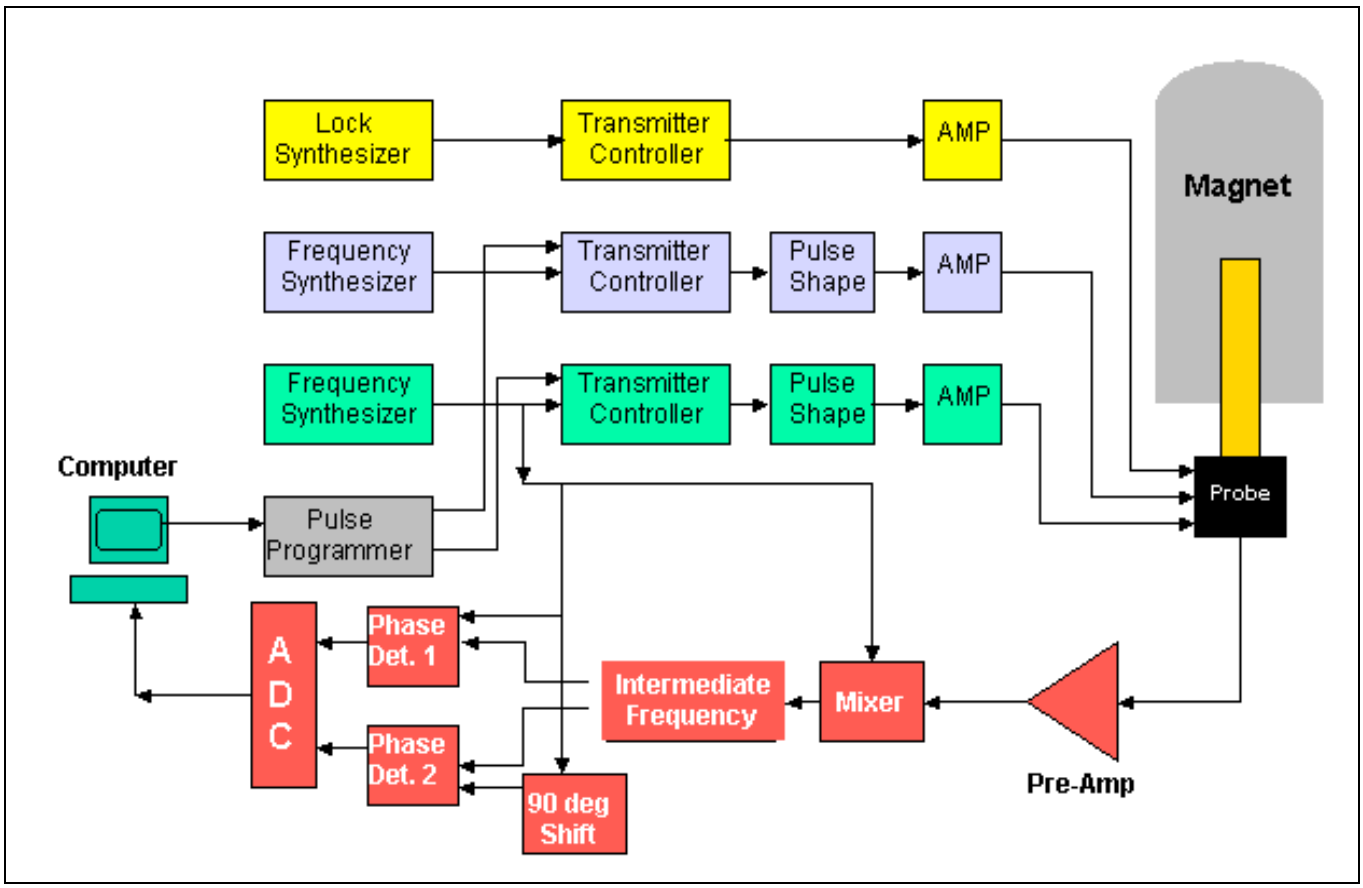

Figure 2.1 Schematic Layout of an NMR Spectrometer

An NMR spectrometer consists of several separate components that are linked together to excite the sample and then receive the NMR signal, these are represented diagrammatically in Figure 2.1. The main parts of the spectrometer are:

1. MAGNET

The magnetic field is providing an ultra high filed super-conducting magnet.

WARNING these magnets are extremely powerful, and will easily pull magnetic objects, such as pocket knives, pens, key chains, ipods, cell-pones etc out of your grasp. They will wipe credit cards, and destroy watches that use springs in the movement. They will interfere with the operation of pace makers and other automated medical devices

The magnet is maintained as a super-conductor by the use of liquid Helium. This is surrounded by a vacuum chamber which in turn is surrounded by a dewar of liquid Nitrogen. The central bore of the magnet contains shim coils that are used to adjust the field homogeneity in the vicinity of the sample

2. PROBE

The NMR sample is placed in a probe inside the magnet which is arranged so that the sample sits at the center of the magnetic field. The probe contains a set of coils that are used both to generate the RF pulses and to detect the signal induced by the effects of the pulse on the sample. In addition, the probe contains a heating element for temperature stability and may also have a Gradient Coil for

applying Magnetic Field Gradients.

3. Lock Circuit (Yellow)

A stable deuterium frequency is generated by a ***Frequency Synthesizer***. This signal is passed through a ***Transmitter*** which controls the input of the deuterium signal to the probe. The signal is passed through an ***Amplifier*** before going to the probe. The lock circuitry is connected to a feed back loop mechanism. This detects changes in the resonant frequency of the deuterium signal and adjusts the magnetic field to compensate for these changes by changing the current in a solenoid which forms part of the shim system.

4. Transmitter Circuit (green/lavendar)

The frequency for the Observe Channel is generated by a Frequency Synthesizer. The timing of the pulses in the NMR experiment is controlled by the Transmitter under the influence of the Pulse Programmer. The output from the transmitter is fed through an optional Pulse Shape Unit and then amplified before passing into the Probe.

5. Detector Circuit (red)

The signal detected in the coil at the Observe Frequency is passed through a ***Preamplifier*** to increase the signal. This is then combined with a second frequency generated by the ***Frequency Synthesizer*** in a ***Mixer***. The resulting signal has a frequency of ~10-20 MHz. This ***Intermediate Frequency*** is then amplified and split into two parts which pass into the ***Phase Sensitive Detectors.*** The detectors are 90º out of phase with respect to each other. The detectors also remove the ***Intermediate Frequency*** generated by the ***Frequency Synthesizer***, which results in an output with a frequency range of a few kilohertz. This signal is passed into the ***Analogue to Digital Converter*** which converts the analogue signal into a digital form that can be stored in the computer.

6. Decoupler Circuit (purple)

In principle there can be as many decoupler circuits as you can afford. They all have essentially the same design, but it is more usual to pack the first and second decoupler channels with the many features and to use more basic designs for any additional channels. The Decoupler circuit again consists of a very stable ***Frequency Synthesizer*** coupled to a ***Transmitter*** which are controlled by the ***Pulse Programmer***. The input signal passes through a ***Pulse Shaping*** unit before being amplified and sent to the probe. Often the Decoupler signal is passed through a filter to remove any unwanted frequency spikes e.g. Deuterium that may have been introduced along the way.

7. Gradient Amplifier (Not Shown)

Most modern NMR spectrometers now include Gradient Amplifiers. These are acoustic amplifiers that can be used to apply a magnetic field gradient across the sample. That means the effective magnetic field in one part of the sample is different from another. These units also come under the control of the pulse programmer.

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3. STARTING THE NMR PROGRAM

3.1 STARTING A NEW SESSION

Each research group that uses the NMR facility is given an account on the spectrometers. Individual users from these groups share the same account and this should be borne in mind when using these machines so that your actions cause the minimum of inconvenience to the next user. You should always start each session by logging out from any previous session and logging back in to the spectrometer.

When you first arrive to use the machine:

- Check for any messages from the previous user
  they may have left a message asking you to save their experiment
- If necessary log out from the previous user's session.
- At the login prompt type the name of your group.
- At the prompt type in the password that you were given
- If you are requested to choose a Desktop, please select CDE Environment
    - The OpenWindows environment is no longer supported.

After the login process has finished, you will see a window that prompts you for some information. It is important that you fill out this screen and then hit OK, as it is used to track the spectrometer usage that we use to justify ongoing support from the University. If you do not see this screen, please let me know

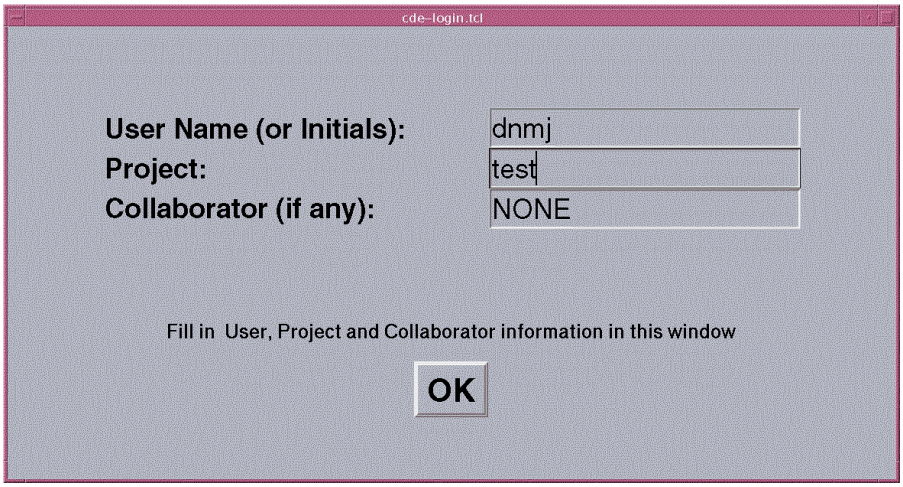

Figure 3.1 Facility User Entry Screen

NOTE If your project is a collaboration with some other group, please enter the last name of the collaborator in the box provided. Again this is important in tracking the total number of groups that use the machine, and justifying institutional support

3.2 STARTING THE NMR PROGRAM

Once you have successfully logged on, you can start VnmrJ by either opening a Unix shell and entering the command vnmrj, or click on the VNMR-J icon on the CDE bar at the bottom of the screen. (See Figure 3.2)

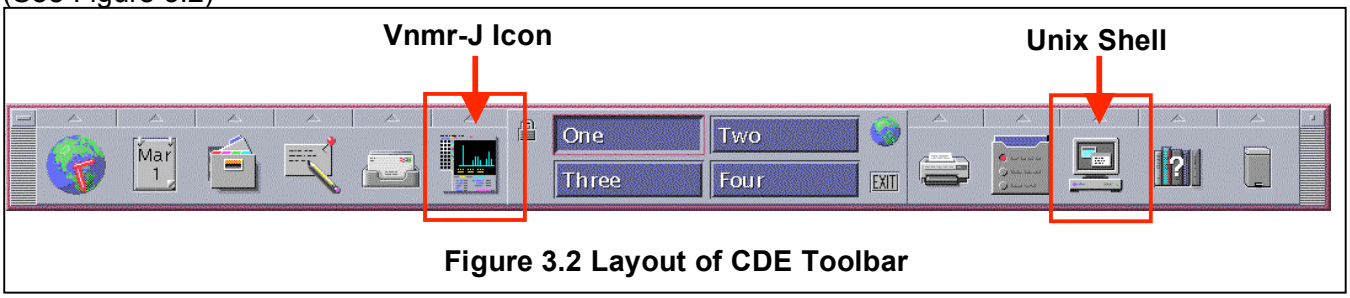

Figure 3.2 Layout of CDE Toolbar

You should now see the VnmrJ window, that looks something like Figure 3.3

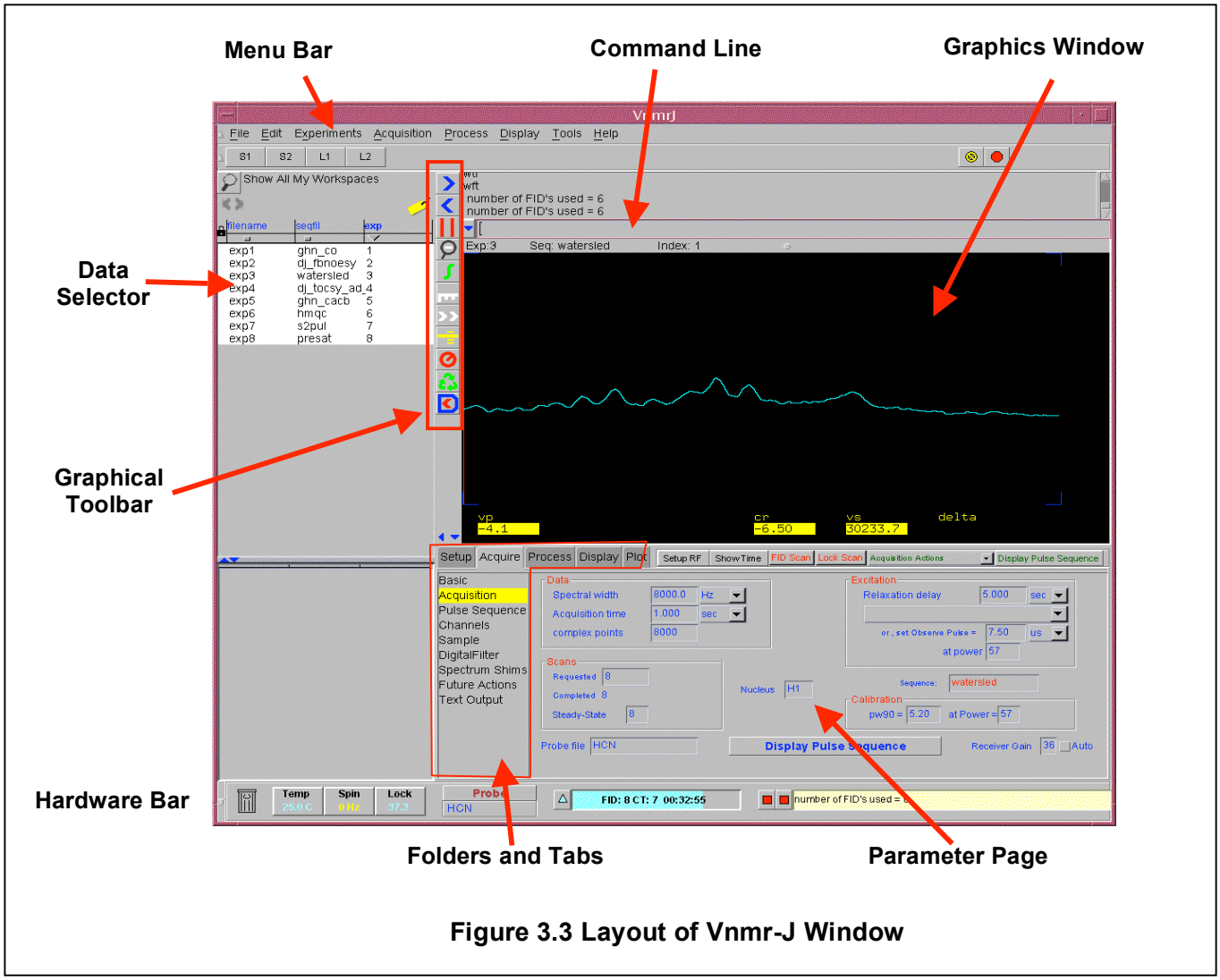

Figure 3.3 Layout of Vnmr-J Window

Menu Bar:
This line is used to open files, save files, setup experiments, setup preferences etc.

Command Line:
This window is used to enter commands for VnmrJ that cannot be accessed via the Graphical User Interface. All commands from previous versions of Vnmr can be entered on this line the same way as before. However, many of the command commands are now available as buttons in the other parts of the window.

Graphics Window:
The raw data and pulse sequences are displayed in this window.

Data Selector:
This window is used to select data sets and to switch between experiments. It is highly customizable by clicking on the Magnifying Glass Icon at the top left. See the Vnmr Manuals for full details on how you can set this up.

Graphical Toolbar:
This tool bar contains icons for many command commands used in viewing and manipulating your NMR data.

Folders and Tabs:
This is the most important part of the VnmrJ interface. Many of the commands to run the spectrometer are accessed from these panels, and parameters can be entered into text boxes. in the parameter pages The tabs will change depending on the Folder that is selected on the Left of this page.
This panel provides access to Unix terminals and a way to start the NMR program, and access to the multiple desktop feature. The user can open additional windows for editing files and other X/UNIX facilities from these windows. Additional important options in the pop-up menu are Vnmr Help and Vnmrx. The latter can be used to restart Vnmr if it crashes for any reason.

Hardware Bar:
Displays the status of any acquisition that is in progress. It includes the number of scans, time remaining, sample temperature and if the sample is spinning. Messages, including Reponses to querying parameters values, and error messages are also listed here. These boxes can be expanded by clicking on the squares immediately adjacent to them.

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4. ORGANIZATION OF VNMR SOFTWARE

4.1 INTRODUCTION

The Vnmr software contains the graphical interface and also a large number of underlying files that are used to control the spectrometer and set up experiments. It is not a single file rather it is composed of a series of files and sub-programs that are executed in response to input from the user. The following section tries to provide a brief guide to the organization of the software and tries to explain how these files interact.

4.2 VNMR SYSTEM FILES

The main files used in running the Vnmr program are located in the directory ***/vnmr.*** Files in this directory contain important system configuration parameters that are essential to run the machine. They also contain all of the parameter sets and pulse-sequences for standard experiments. This include all the "BioPack" sequences for proteins and RNA. We try very hard to maintain these up-to-date, s users do not need to keep their own versions.

The important files in the directory */vnmr* are (note these pathnames are relative to */vnmr*)

/bin Contains executable commands that are used in running the program for example, *vnmrx*. Most of these commands in this file are not entered directly by the user, more often they are invoked from files in the macro directory.

/**maclib** The macro directory contains system-wide macros. Macros are files that execute a series of commands in a specified order. For example, when a user types "go" in the Command window this executes a macro called "go". The "go" macro performs several tasks before starting acquisition which includes checking the temperature setting and the power levels of the transmitters to make sure the probes do not get burned out. Many of the macros are used to set up a new experiment e.g. the ***presat*** macro will set up a 1D presaturation experiment using the default parameters.

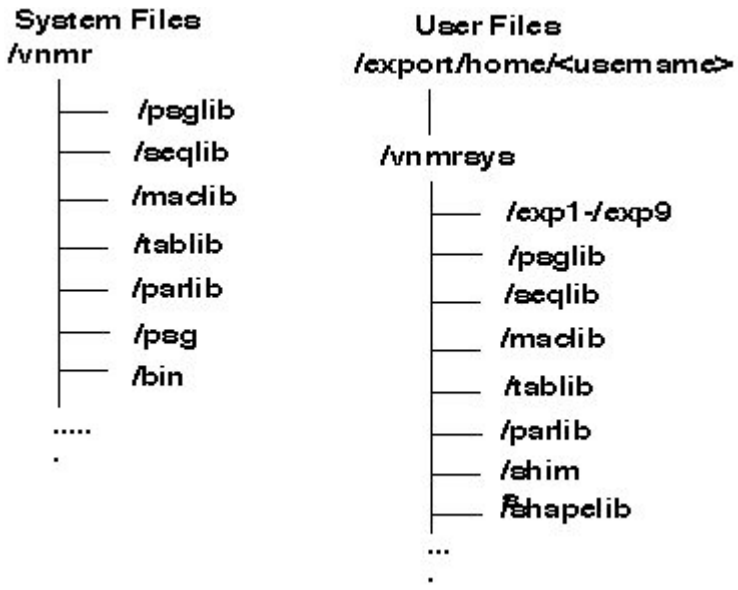

Figure 4.1 File Structure of the Vnmr Program

/parlib This directory contains sets of standard parameters that are used to set up the standard NMR

experiments.

/psglib Every NMR experiment, no matter how simple is controlled by a "pulse-sequence". This is a command file that consists of a series of delays and pulses which are sent to the acquisition computer. The commonly used/standard pulse sequences provided by Varian are included in this directory. All the files in this directory are text files and they do not control the spectrometer directly. This is done by the compiled versions.

/seqlib This directory contains the compiled versions of the programs found in the psglib directory.

/tablib The RF pulses of an NMR experiment are not always applied in the same direction. They often differ from one scan to the next in order to select the desired magnetization and suppress unwanted artifacts. This procedure is known as "phase cycling". The *tablib* directory contains tables of the different phases to be used during the experiment. There is generally, but not always, a phase table for each NMR experiment and it has the same name as the associated pulse sequence found in the *psglib* directory.

/shapelib Many pulses in NMR experiments are "hard" pulses. That is they excite the complete region of interest. Many experiments require the selective excitation of limited regions of the spectrum. This is achieved by using "shaped" pulses. A shaped pulse is a hard pulse that has had its amplitude and/or phase modulated in some way.

/psg The pulse sequences in the *psglib* directory are actually made up from a series of C-macro statements. Each of these macros must be turned into some binary instructions that can be interpreted by the acquisition computer. This directory contains all the so called "library" files which are used to convert the macro statements into a binary executable file that controls the spectrometer.

4.3 USERS FILES

In addition to the system wide files located in */vnmr* each user has their own set of Vnmr related files. These are found in the */export/home/<username>/vnmrsys* directory. Many of the filenames are the same as those found in the */vnmr* directory and contain parameters and experiments the user set up in the course of his/her work:

/psglib }
/seqlib } These files have the same function as those found in */vnmr* except they
/tablib } contain information specific for each user.
/maclib }
/parlib }

In addition to these directories, the users vnmrsys directory contains some unique files:

shims
This directory is used for storing shim files. The shims are the adjustments that are made to the magnetic field to produce a homogeneous magnetic field at the center of the sample. On a routine basis the shims must be adjusted for different sample depths and different temperatures. On a less frequent basis each probe requires its own unique set of shim values.

exp1- exp999
Vnmr allows you to set up many different "jobs" or experiments in which you can acquire or manipulate data. There is no real limit on how many different "experiments you can have. Each experiment has an associated directory which contain the parameters and data:

acqfil Contains the data that is being acquired
datfil Contains the processed data

procpar The parameters of the processed data
curpar The parameters that are to be used in the next acquisition

4.4 COMMAND PRIORITIES,

The organization of the Vnmr program has some important consequences for the contents of the directories and files that were outlined above. Whenever a user types a command or a macro at the command line, the program searches for the file, be it a command, a macro, or a parameter set in a predetermined order. First, it looks in the user's own ***vnmrsys*** directory. If it finds the file it needs in one of the subdirectories it will use that file to perform its task. If no file can be found in the user's directories, it then searches for the file in the system wide ***/vnmr*** sub directories and will use it instead. This is advantageous as all the standard pulse sequences, macros and parameters etc. in the ***/vnmr*** directory are available for all users and they do not need to copy them into their own directories for them to work. However, this arrangement can lead to some problems, for example if the parameter sets have been updated in the system files, but not in the users files. In which, case sometimes experiments do not work which can be a waste of precious samples, time and ultimately money. As a result, we strongly recommend that users

- Never copy sequences or macros etc. from the ***/vnmr*** directory into your own ***vnmrsys*** directory.
- Make sure that your files and pulse sequences always have different names from those found in the ***/vnmr*** directories. A good idea is to put your initials at the beginning of each file name.

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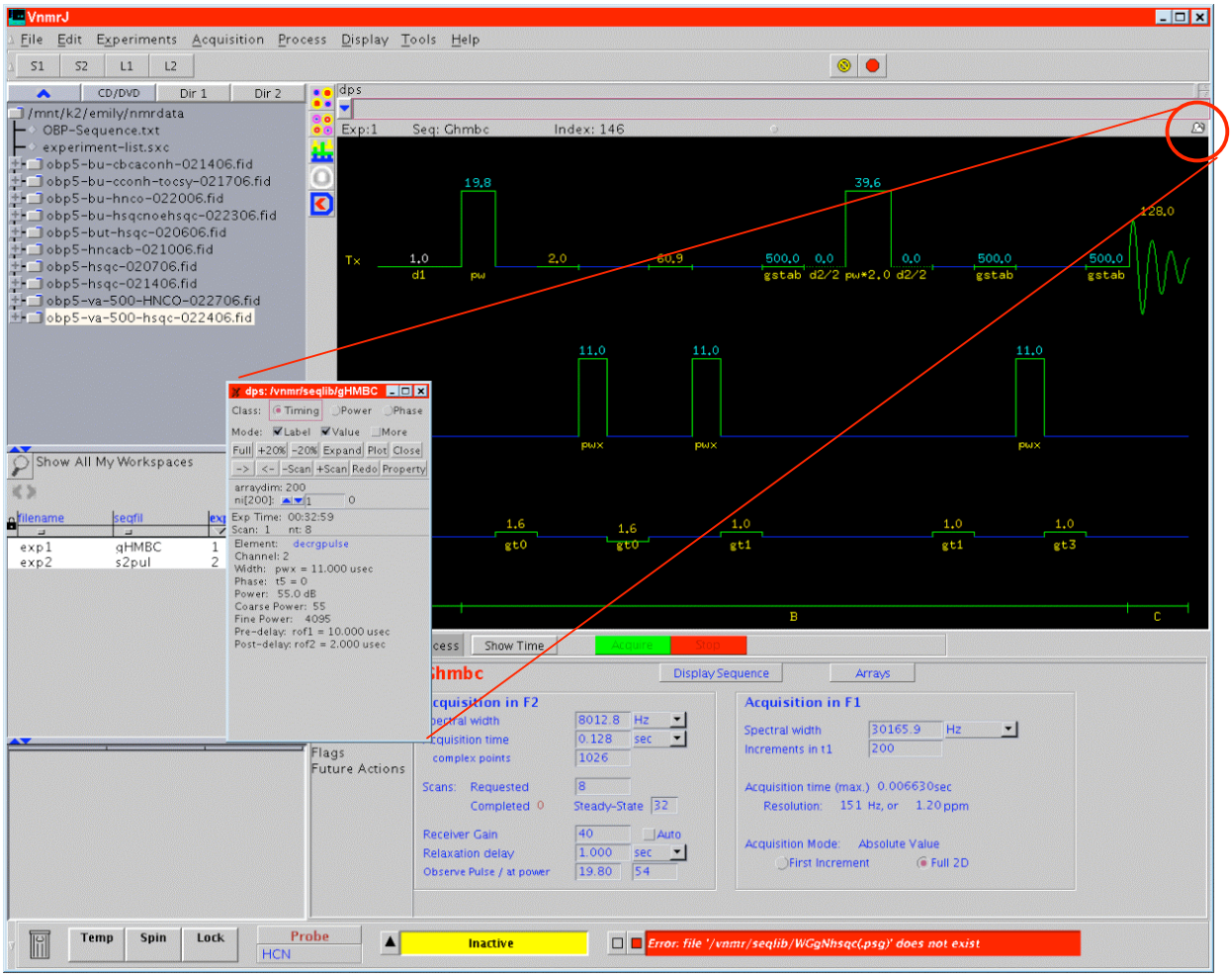
5. PARAMETERS IN NMR EXPERIMENTS

This section is designed to introduce you to some of the most important parameters that you will come across in NMR experiments. Not all experiments are available or used in each experiment and so it is important to know which ones need to be changed. This section will introduce common parameter sets, specific parameters are discussed in later sections.

5.1 PULSE SEQUENCES (DPS)

All NMR experiments, irrespective of how simple or complicated they are, are controlled by a set of commands known as a "pulse sequence". A pulse sequence is very simply a lost of pulses, delays and magnetic field gradients, that tells the spectrometer when to apply the pulse, how long to apply a pulse for, how long to wait until the next pulse, what frequency to apply the pulse at, along which axis to apply the pulse, and other varied instructions. The current pulse sequence for the experiment can be seen by given the command **dps**, or selecting the "**Display Pulse Sequence Button**" on the Tab line.



This display shows all the pulses and delays for the experiment. You can find out more about each parameter by clicking on the little mouse icon in the top right corner of the graphics window. This will open a pop-up window. When you click on any element within the pulse-sequence window with the middle mouse button that element turns grey and the parameters associated with that element and then displayed in the pop-up window (See Figure above).

There are typically between 1-4 lines in the pulse sequence window, each line corresponds to the commands for a different nucleus, so for example, if you are running a 3D H/C/N experiment, there will be 4 lines, the top one is for ^{1}H, the second for ^{13}C, the third for ^{15}N, and the bottom for the **"Pulsed Field Gradients"**. We have to define the parameters for each of these nuclei and the gradients

5.2 PARAMETER SETS

Each NMR experiment uses a different set of parameters to run correctly. Many of the parameters can be shared between experiments, e.g. the length of the ^{1}H pulse, whilst others will be unique to a particular experiment. A short description of how these parameters are laid out will help you to rapidly become familiar with the parameters and which ones are the most important for you to adjust.

Each NMR spectrometer has 4 RF Channels, and one Gradient Channel. The gradient channel is unique, but as you might expect the four RF channels are very similar. The first RF channel is special, as it is also the channel that is used for detecting the final NMR signal; consequently it is sometimes called the transmitter. Channels 2-4 are called decoupler channels, this is an historical hangover, because they were used to decouple, i.e. remove the effects of the signals on these channels, from the signal being observed by the transmitter. The parameters for each channel are controlled independently. Each channel has its own parameters for Nucleus, frequency setting, power level settings, pulse widths and decoupler settings. These parameters are summarized in the table below and described in more detail in the following sections. We will discuss the parameters for the transmitter and the first decoupler channel. The parameters for the second and third decouplers are accessed in the same way as the first decoupler channel by simply putting a 2 or 3 after the name of each parameter.

Parameter	**Transmitter (Channel 1)**	**Decoupler (Channel 2)**	**Decoupler2 (Channel 3)**	**Decoupler3 (Channel 4)**
Nucleus	tn	dn	dn2	dn3
Frequency	sfrq	dfrq	dfrq2	dfrq3
Offset	tof	dof	dof2	dof3
Spectral Width	sw	sw1	sw2	(sw3)
Power	tpwr	dpwr	dpwr2	dpwr3
Decoupler Status	-	dm	dm2	dm3
Decoupler Mode	-	dmm	dmm2	dmm3
Decoupler Frequency	-	dmf	dmf2	dmf3
Pulse Width	pw	pwX	pwY	pwZ
Pulse Power		pwXlvl	pwYlvl	pwZlvl
Number of Data Points	np	ni	ni2	
Acquisition time	at			
Number of scans	nt			

5.2.1 Selecting the Nucleus for Each Channel (tn, dn, dn2, dn3, sfrq, dfrq, dfrq2, dfrq3)

The most important parameter that defines how the spectrometer runs is the name of the nucleus that will be used on each channel. The most important nucleus is the nucleus that will be directly observed in the NMR experiment, typically this is ^{1}H, but can also be ^{13}C. The nucleus for each channel is selected with the ***tn*** or ***dn*** command, e.g. tn='H1' or dn='C13'. After being selected this automatically sets the appropriate absolute NMR frequency for each channel and this is set in the parameter ***sfrq*** or ***dfrq*** etc. These values are in MHz. When you load a standard experiment, see below, almost all these parameters will automatically be set for you, but you should check that these parameters are set correctly just in case.

5.2.2 Setting the Exact Frequency for each Channel (tof, dof, dof2, dof3 and satfrq)

The exact frequency used for transmitting the NMR signal on each channel is fine-tuned with the values of ***tof*** and ***dof*** etc. which are given in Hz. The exact values define the middle of the spectrum and are critical for recording the proper range of signals.

For most protein and RNA experiments that use 90% H_2O in the buffer, the value of ***tof*** has to be carefully optimized to produce proper suppression of the water signal, so that the signals from the sample can be observed. In some experiments, the parameter ***satfrq*** is used as well as ***tof*** . This parameter is the frequency of the water, and it is usual to adjust it at the same time as ***tof***.

The exact setting for ***tof***, ***dof*** and ***dof2*** will depend on the machine you are using and the experiment being performed. Some standard values are given below. From these you should be able to calculate the required offsets for your particular experiment.

Typical Frequency Offsets

Nucleus	Parameter	600 MHz	500 MHz
^{1}H @ 4.73 ppm	tof	-223	-173
^{13}C @ 174 ppm	dof	12504	9635
^{15}N @ 120 ppm	dof2	1700	1100
^{31}P @ 0 ppm	dof	0	0

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5.2.3 Spectral Widths (sw, sw1, sw2)

The spectral width is the range of frequencies that are recorded in the spectrum. The three parameters ***sw***, ***sw1*** and ***sw2*** refer to the settings for the spectral widths of the first, second and third dimensions respectively, and this value is in Hertz. This value is relative to the position of the transmitter, with the center of the spectrum at the transmitter you will collect +/- sw*0.5 Hz on either side of the transmitter frequency To convert between ppm and Hz, multiply the ppm range you want to record by the nuclear frequency. For example, for 10 ppm in the ^{1}H, dimension is 10*600 on the 600 MHz spectrometer.

The value of the spectral width used depends on the specific information provided by each experiment. For example, an HNCO experiment that detect ^{13}CO frequencies will be about 10-20 ppm in the ^{13}C dimension, centered around 174 ppm, but for an HNCACB you will collect 75-80 ppm centered at ~43 ppm.

For most experiments that detect ^{1}H, the spectral width will be 12-14 ppm, but this will depend on your sample and may be more, but may also be less.

1H 12 ppm for Proteins 20 ppm for DNA/RNA
C13 20 ppm for CO, 70-80ppm for others.
N15 40 ppm for Proteins
P31 10-20 ppm depending on structure

5.3 Pulse Lengths And Field Strengths

WARNING The units of power are in decibels (dB). The dB scale is a log scale which means that increasing the value of a power parameter by 20 dB results in a 100-fold increase in the power output to the probe. If too much power is applied for long periods of time, and in NMR we mean 10-100 milliseconds, severe damage can occur both to the equipment, but also to your samples. For proteins, it literally means you can cook your sample. These dangers most often come from the power levels

used for decoupling. As a rule **NEVER** set decoupling powers above 45 dB.

5.3.1 Pulse Lengths (pw, pwC, pwN or pwx, pwx2)

NMR experiments use the magnetic component of a short pulse of Radio Frequency (RF) fields to manipulate nuclear spins. The RF field rotates the spins away from their equilibrium positions and the angle of this rotation depends on both the duration of the pulse and its magnetic field strength (Eqn 1).

(Eqn 1) $pw*\gamma*B_1 = \phi$ Tip angle in degrees

Where pw = duration of pulse, B_1 is the field strength of RF pulse (Tesla), ϕ = tip angle and γ = Gyromagnetic Ratio (rad T^{-1} s^{-1}). The length of the pulse is determined by the power of the amplifiers used to transmit the NMR signal. Changing the power changes the strength of the magnetic field associated with the pulse and so changes how strongly it interacts with the nuclear dipoles and so changes the length of the pulse. For many experiments it is necessary to know the field strength (or pulse length) of the pulse at a given power level. The field strength in Hertz is calculated from the time required to rotate the magnetization through 360°. (Eqn 2).

(Eqn 2) $1/pw_{360} = \gamma*B_1/2\pi$ Field Strength in Hertz

When the field strength is expressed in Hertz it essentially gives the effective excitation range of the RF pulse. It is usually more accurate and easier to determine the value a 360° or a 180° pulse than a 90° pulse but it is not always possible to do so. We will discuss how to calibrate pulses in later sections, for now it is sufficient to know that the field strength of the pulse in Hz, is the inverse of the 360° degree pulse in seconds (Eqn 2).

The parameters that control the length of the pulses usually use a name that begins with **pw**. For the transmitter this is usually just **pw** by itself. For the ^{13}C this is usually **pwC**, and for ^{15}N it is **pwN**. However, you should be aware that other parameters may also be used, including p1, pwx, pwy . It is important that you try to recognize the different names of the pulse by using the **dps** command, and what they may mean.

5.3.2 Pulse Power Levels (tpwr, pwClvl, pwNlvl, pwxlvl, pwxlvl2)

The length of a pulse is determined by the power setting of the amplifier used for the pulse. The higher the power, the shorter the pulse. The power level for pulses on the transmitter channel is controlled with the parameter ***tpwr***. Unfortunately there is little consensus for power levels used for pulses on the decoupler channels. You will see parameters such as **pwClvl**, **pwNlvl**, **dhpwr**, **dhpwr2**, etc. it depends very much on who wrote the experiment. There has been a move to try and make these more consistent across different experiments, but you should use the pop up parameter query box to find out what parameter is used. If in doubt ask. Generally, short 90° pulses are applied at power levels of 60 dB and have pulse widths of 10-40 us.

5.3.3 Calculating Power Levels and Field Strengths

Once you have calibrated the 90°or 360° degree pulse width for each channel, you can calculate what the pulse width will be at a different power level setting from the following relationship (Eqn 3) which is rearranged to give Eqn 4. (You need a calculator to do this!!)

(Eqn 3) $\Delta\ dB = 20\ \log\ (RF_{Measured}/RF_{Required})$

(Eqn 4) $\log(RF_{Required}) = \log(RF_{Measured}) - \Delta dB/20$

Where ΔdB is the change in power level, $RF_{Measured}$ is the field strength of the calibrated pulse in Hz, and $RF_{Required}$ is the field strength of the pulse at the new power level. This may appear complex but it has a very simple result. Each change of 6 dB in power changes the field strength by a factor of 2. So a change in 12 dB will change the field strength by a factor of 4. Some other useful steps to remember are:

dB Change	Factor
3	1.414
6	2
10	3.16
12	4
14	5
20	10

As an example, if the 90° pulse is 31.3 us at 51 dB then the field strength is 8000 Hz. Therefore at 45 dB, the field strength will be 4000 Hz and the 90° pulse will be 62.6 us. And at 42 dB the field strength is 2828 Hz and the 90° pulse is 88.5 us. These simple relationships will prove very useful in the following sections.

5.4 PRESATURATION

Presaturation is a method used to suppress the large water signal so that the signals from the molecule of interest can be observed. Presaturation works by applying a very weak radiofrequency field to the signal from the water for a period of 1 - 2 seconds. This causes the water signal to be effectively to precess around the axis of the pulse .However, because the pulse is very weak, it turns out that it does not affect every part of the sample in the same way and so this effectively scrambles the signal from the water and reduces its intensity.

5.4.1 Status Parameters

The parameters for presaturation will often use "**Status Parameters**". Varian NMR pulses sequences are broken up into different "status" periods, labeled A,B,C etc.... You can find out the values of these parameters by using the **dps** command. The status parameter is indicated as a solid line at the bottom of the pulse sequence and the different status periods and separated by check marks on the line and have the labels A, B and C etc under the line. A parameter that makes use of the status periods has an entry for each status period. For example if satmode='ynn', it means it has a value of "y' in period A and 'n' in periods B and C. If a status parameter has only has one entry, then it takes that value for all the status periods.

An important note, the value of the status parameter is always reset to its initial value at the end of the experiment. For this reason any parameter that controls decoupling(see below) must always be set have "n" as the first value so that it is switched off after the experiment is finished, so that it does not fry your sample or the probe.

5.4.2 Parameters that Control Presaturation (satmode, satfrq, satdly, satpwr)

satmode This parameter controls which period of the pulse is used to apply saturation of the water signal. It is a "Status Parameter" and can have multiple entries. Typically satmode='ynn', however, it is possible to have values such as 'yyn'.

satfrq This parameter control the precise frequency at which the RF pulse is applied to the water

signal. It is typically set to be the same value as **tof** for proteins and nucleic acids. Fine tuning this parameter is essential for god water suppression

satdly This is the time of the presaturation period. Typically this is between 1 1.5s

satpwr This is the power level used for presaturation of the water signal. For samples in 90% H2O based buffers, this is between 6-10 dB, for samples in 100% D2O, this is typically 0 dB.

5.5 DECOUPLING

In many experiments, decoupling is used to remove the effects of the J-coupling interaction which results in a splitting of the lines and a decrease in the overall sensitivity. Typically decoupling is used during the acquisition period for N-15/C-13 labeled protein samples. There are several different decoupling techniques that can be used. Two very popular methods are called GARP and WALTZ-16. WALTZ-16 produces high quality decoupling over a range equal to $2*B_1$ field strength and is used primarily to decouple Protons from C13/N15 and P31. Its limited range makes it unsuitable for decoupling the wide spectral width of C13. In this case we use GARP which provides adequate decoupling over $5*B_1$ Field Strength. However, even GARP sometimes uses too much power that could damage the instrument or the sample. In these cases, it is often common to use techniques called WURST.

5.5.1 Parameters that control decoupling (dm, dmm, dmf, dseq)

dm The "Decoupling Mode" controls whether decoupling is on or off. e.g. dm=`nny' sets the decoupler on during status period C. If RF pulses are to be applied on the decoupler channel during any period, dm must be set to ‘n’ for that period. Also dm MUST be 'n' for the first value (see above)

dmm "Decoupler Modulation Mode" sets the decoupling sequence. e.g. dmm=`ccg' sets GARP decoupling during status C and "continuous" or "pulse" mode at all other times. dmm=`ccw' sets WALTZ decoupling for period c. The first entry in dmm should always be 'c' and must be ‘c’ if pulses are to be applied. For user programmed decoupling sequences, e.g. WURST, a ‘p’ is used to indicate a user defined program.

dpwr The power used for the decoupling period on the first decoupler channel. This is power should be 45 dB or less. It is necessary to know the length of the 90° pulse at this power level, either by directly calibrating the pulse at this power, which is not always possible, or by calculating it from a pulse calibrated at a higher power level. Unlike pulses which only last for a few microseconds, decoupler sequences and solvent saturation can last 0.2-2 seconds. If the decoupler is turned on for this period of time at full power serious damage can result. Consequently decoupler power is limited to a maximum of 45 dB. *dpwr* and *dpwr2* control the power level setting for decoupling on the first and second decoupler channels respectively. The parameter *satpwr* is used for irradiation of solvent and is typically set to 6-10 dB for H_2O and 0-4 dB for D_2O.

dmf "Decoupler modulation frequency" is a measure of the decoupler field strength in Hertz. **dmf** should be set =1/pw90 where pw90 is the 90° pulse calibrated at the power level used for **dpwr**.

dres "Decoupler Resolution". This parameter is an internal value that is used to calculate the length of a 90° pulse on the decoupler for user programmed sequences. For WALTZ set dres=90 for GARP set dres=1.0

dseq "Decoupler Sequence" is the name of the file that contains a user supplied decoupler scheme. This parameter is only used if **dmm** = 'ccp' for example. If a decoupling scheme other than GARP or WALTZ is being used, e.g. WURST, this parameter must be set, along with the correct value of **dres**.

5.6 PULSED FIELD GRADIENTS (PFGON, GT#, GZLVL#)

Pulsed Filed Gradients are important parts of NMR experiments that perform a variety of tasks. They are used in shimming, are used to suppress unwanted signals, such as water or other solvents and also to help select the signals that we are interested in. The gradients are indicated by the bottom line in the graphics window after giving a **dps** command.

Each pulse sequence may contain a number of different gradients. All of which can be optimized if necessary. The parameters that control the gradients are

pfgon This is a "**Flag**" parameter, which is similar to a "**Status**" parameter in that it can have multiple values. On the 500 and 600 is set to "nny", which means that there are no gradients on X or Y, but gradients are on, on the Z axis. On the 800 and 900 this is 'yyy', i.e. gradients are on all axes.

gradtype This is flag parameter and should be 'nnp' on the 500 and 600

gzlvl# This controls the strength of the gradient, where # is a number. Gradient levels can take values from +32768 to - 32768. However, it is recommended that values are not set above 24000.

gt# This is the duration of the gradient, typical values are 400 us to 1 millisecond. When setting gradient times, care should be taken to determine if the parameter has been defined as a pulse or a real number (see 5.10 below). If is a pulse then setting gt1=500 defines a 500 us gradient, if it is real number then the same command would set it to 500 seconds. This is actually easy to figure out using the **dps** command.

5.7 SIGNAL AVERAGING (D1, NT AND SS)

The amount of signal observable in a single scan is often too low to be distinguished from the noise. However, we can increase the total signal by repeating the experiment many times. This is because the signal adds up in a linear fashion but noise adds up according to the square root of the number of scans (***nt***). This has some important consequences: in order to double the signal to noise in the spectrum you have to collect four times the number of scans already acquired, i.e. if I acquire 256 scans in an experiment, to improve the signal to noise ratio by a factor of two I would have to collect an additional 768 scans, making a total of 1024.

Most experiments require that you set the number of transients to a multiple of 16 or 32 which is determined by the phase cycle. After each scan there is a delay (typically **d1**) during which the system is allowed to return to equilibrium. For protein samples, this delay is typically 1-1.5s. However, this delay is a compromise between the full relaxation time and the time constraints placed on the user. As a result, the system does not return to equilibrium after the first scan. This may lead to unwanted artifacts if data collection is started immediately after the first scan. Therefore the system is usually set to perform a number of "steady-state" scans (***ss***). During these scans the spectrometer executes all the pulses and delays in the pulse sequence but does not collect any data. The value of *ss* depends on the sequence. For simple 1D Proton experiments *ss* can be set to 4 or 8. For experiments with decoupling it may be necessary to set *ss* to 64 or 128 or even higher to allow the temperature to reach

equilibrium.

5.8 Acquisition Times (at, np, ni and ni2)

The length of the acquisition time (***at***) used in an experiment depends on several factors. First, NMR theory says that in order to resolve two signals that are Δ Hz apart, the interval between sequential data points (the dwell time) must be $1/\Delta$ seconds. Secondly, we have to sample our data for a sufficient period of time to allow the sine waves arising from the different signals to evolve away from each other. However, in most NMR experiments with biological macromolecules, relaxation destroys most of the signal very quickly. Normally we collect between 2048 or 4096 data points (***np***) using the typical spectral widths given above. This results in acquisition times of between 100-300 ms. For smaller molecules where relaxation is not a limiting factor it is not unusual to collect up to 64000-128000 data points.

As the spectral width, the number of collected data points and acquisition time are not independent, it is usual to set ***sw*** and ***np*** to the desired values and the computer works out the value of ***at***. The number of points collected should be adjusted for each sample. If the T2 relaxation rates are fast the signal will decay very quickly and in such cases there may be no point in collecting 4096 points if all the signal has decayed within the first 1024. All you collect in the other 3000 or so is noise.

For 2D and 3D experiments, we also have to consider how long to acquire the data in the indirect dimensions. Unlike the directly detected dimension, where we can define the acquisition time, in the indirect dimensions we can only define the number of points collected in these dimensions. These are given by the values of **ni** and **ni2**. The exact value of these parameters depends on several factors. For many 3D experiments, these values are limited by the pulse-sequence, and if you try to set them too high you will get an error message when you give the **dps** command, or try to start the experiment. However, if these values are not limited by the pulse sequence, then you have to consider the T2 relaxation times, just as you did in the 1D. It is typical to use values of 128-256 for ni in a 2D experiment, but for 3Ds these values are more often closer to 50 or 60.

5.9 Receiver Gain and ADC Overflow (gain and dsp)

The ***gain*** parameter controls the amount of signal that reaches the NMR receiver. It can be considered a volume control. The receiver is very sensitive so that it can detect very small signals in the presence of very large signals, such as solvent or buffer. However it can also receive too much signal in which cases you will get an “ADC Overflow” warning, i.e. "it is too loud for me to hear anything." You can adjust the gain from 1-60. The gain should be set as high as possible without getting an ADC overflow warning. Typically values in the range 30-40 are acceptable.

You should also make sure that the parameter **dsp=’r’**. This sets some additional hardware that helps to improve the overall sensitivity of the spectrometer. If you continue to get ADC overflow warnings even after changing the gain to below 20 it means that something is wrong with your NMR experiment, i.e. shimming is poor, solvent suppression is not working, or not optimized. You should check how well you set up the experiment.

5.10 How To Set Parameters

As you have learned, the parameters in an NMR experiment can take different forms. In some cases they are numeric values, or other cases they may be words, or just single characters such as y or n. How you enter the value of a parameter depends on the type of parameter that you are setting. In all cases the present setting of any type of parameter can be determined by typing the name of the parameter followed by a question mark? The different types of parameters are as follows

Real

Parameters that are simple numbers are described as “real”. The spectrometer interprets the

meaning of the real number depending on its context. For example, delays such as d1, d2 and mix are always interpreted as having units of seconds whilst power levels such as tpwr, dpwr and pwxlvl are always interpreted in decibels. These real parameters are set by typing:

parameter_name = value. eg. tpwr=57

Pulses

Pulse widths are special types of real parameters and are always interpreted as being in micro-seconds. To set a pulse width to 45 μs you would enter pw=45. If you enter pw=45e-6 this will set a pulse width of 45 picoseconds. Several other parameters may also be defined to have units of micro-seconds. The ones that you may encounter are shaped pulse widths (typically 2000-3000 μs) and gradient durations (500-5000 μs).

Strings

Many parameters have alphanumeric names, e.g. the name of the pulse sequence or the solvent. String variables must have the value enclosed in single quotes. e.g. to set the pulse sequence you may type:

seqfil='dj_fbnoesy'

Flags

Flags are special types of string variables. The most commonly encountered flags are the "**Status Parameters**". Each pulse sequence is normally divided into "status" periods typically labeled A, B and C. The status parameter controls the action of some event during that period. For example in a 1D experiment, you only want to suppress the solvent at the beginning of the experiment and not during the acquisition period. To do this we set the parameter satmode='ynn'. This means set the solvent suppression on off during period A ('y') and off during and B and C. If we set the parameter satmode='n' then it will be off during all three periods.

5.11 COMMANDS AND MACROS

Commands and macros (which are just sets of commands) can be entered by clicking on buttons or by typing at the command line. Many of the commands used in Vnmr may take optional arguments which can be numbers or strings or a mixture of both. The optional arguments are enclosed in parentheses () and any alphanumeric arguments must be entered enclosed in single quotes. The following examples illustrate these points:

- wft - FTs an FID after applying a defined weighting function
- wft(3) - Transforms the third FID in a series, eg during a pulse calibration
- dssh - Display all spectra in a series plotted side-by-side
- dssh(1,11,2) - Displays a series of spectra starting at 1 and ending at 11 with a increment of 2 between the boundary values i.e. It displays Nos. 1, 3, 5, 7, 9 and 11.
- dpcon(6,1.4) - Plots 6 contour levels of a 2D spectrum with each contour at a level 1.4x the level of the previous contour
- dpcon('pos',6,1.4) - This does the same as the previous example but plots only positive contour levels

This section gets ahead of itself but tries to anticipate two VERY important commands that you will use on a regular basis when using the spectrometer.

5.12 SAVING AND RETRIEVING YOUR DATA (TEXT AND SVF)

After running your NMR experiment you must store your data. The first stage to this is to enter a

description of the experiment that you ran. This will help you to identify the data at a later date. To do this enter the command "**text**". You will then be prompted for the text that you wish to enter. The text can be anything that you wish, but good guidelines are to include the sample, the experiment, the temperature, the concentration and the date at the very least. There are no restrictions on the format: e.g. a typical entry would be:

text (` LUSH plus VA, pH 6.5, 25 C, HSQC, 030606')

This line also shows an alternative way to enter the text line. This text can be edited at any time by giving the command "**textvi**". This opens an editing window which you can use to edit the text file.

After you have entered the text. Use the "File" Button from the Main Menu to locate and change to the directory where you are going to store your data. Typically this is located in the */home/<user>* directory. You can save you data using the menus or by giving the command "**svf**". You will now be prompted for a file name to save your data. Please include the date on all files, so that we know which ones not to delete.

You can recall your data for later use by opening it from the "**File**" menu

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6. RUNNING YOUR SAMPLE

OK so here we are, we worked through the hard part and now we can put your sample in and start collecting data on your new protein/DNA/carbohydrate sample. But before we begin lets just outline out plan. There are several things we need to do before we can collect our data. First we must set the temperature, then the probe must be tuned and shimmed to obtain the optimum performance of the spectrometer. Both of these operations are critical for collecting high quality data. Fortunately the developments in technology since 1996 have really made this pretty easy. The following assumes that you have started the NMR program. If not, do so now.

6.1 Setting the Temperature

6.1.1 Setting the FTS Controller

The FTS controller consists of two parts: the main control unit which has the LED display and touch pad for adjusting parameters and the compressor unit which cools the input air into the probe if necessary.

- Check the temperature setting on the FTS control unit.
- The Set Point (SP) should be adjusted to 10° C below the temperature required for your sample. Adjust the setting by pressing the up and down arrows on the control unit.
- If the temperature setting on the FTS is to be higher than 30° C, switch off the compressor. Save our planet!!
- Wait for a few moments for the temperature to adjust, this should be fairly rapid. If the temperature overshoots and continues to heat or cool in the same direction, press the "Run" button twice.

6.1.2 Setting the Sample Temperature

The sample temperature is set from within the NMR program:

- Select the "Spin and Temp" Folder (See Below)
- Use the Temperature slider to set your desired temperature

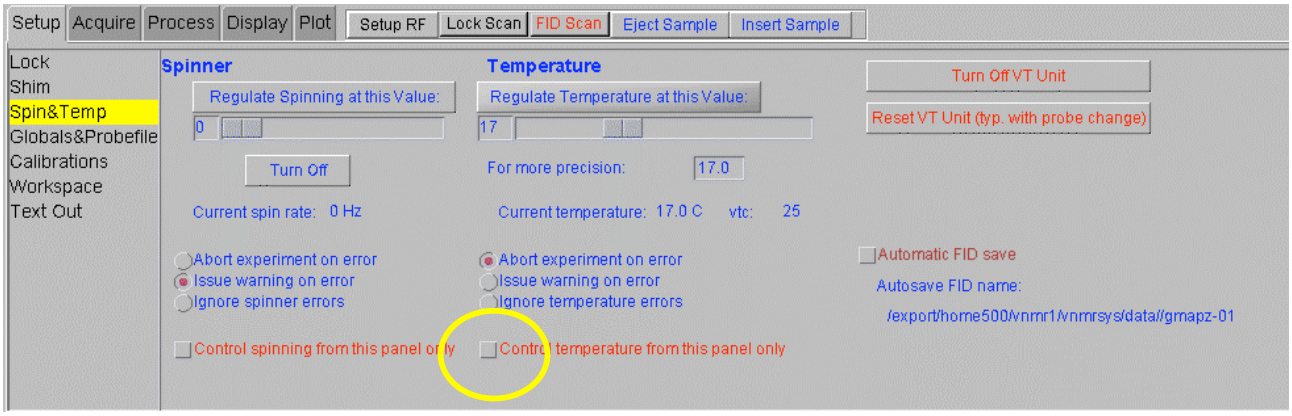


Figure 6.1 The Spin and Temperature Control Panel

- Select the optional button to Control Temperature From within this panel only
  This prevents you from mistakenly mis-setting the temperature with the su or go command at a later time

- If the temperature was different to the previously set value you should see the value on the display module change. As the temperature approaches the required temperature the rate of change slows and it can take several minutes to regulate the last 1-2 degrees, this is not unusual.

- If your sample is sensitive to high temperatures you should wait a few minutes before putting your sample into the magnet. Otherwise you can proceed immediately.

6.2 Sample Volume and Depth

The NMR spectrometers have all been optimized for a sample volume of 600 µl in a 5 mm NMR tube. However, Shigemi tubes can often give superior water suppression for protein samples. However, only 1 out of every 10 Shigemi tubes is suitable for use at 800 or 900 MHz. If you do not have sufficient volume for at least 500 µl of a 0.5-1 mM sample, you should use. These require 320 µl of sample but should only be used when your sample is limited as they can be much harder to set up

- The sample should be in a Wilmad 5 mm NMR tube with the serial no 528 or 535 marked on the outside of the tube.
- The sample tube should be inserted into the correct spinner and the bottom half of the tube should be wiped with a clean tissue and isopropanol to remove dirt and grease.
- Adjust the position of the tube so that the bottom is at the 63 mm mark in the Varian depth gauge. (Shigemi tubes will need to be lower). Do not touch the bottom of the sample tube with your fingers.

NOTE If your sample is running above room temperature for a period of days, some of the solvent will evaporate from the main body and condense in the upper portion of the tube. This will significantly alter the magnetic field homogeneity during the course of the experiment. If this is likely to be the case for your sample you should consider using a Shigemi tube to prevent this from occurring.

6.3 Inserting and Removing Samples

Samples can be ejected by typing "e" on the command line, and then inserted by typing "i". Alternatively, select the "Setup" tab, and click on the "Lock" folder to access the "Eject" and "Insert" buttons. Either way, you should start by ejecting the previous sample. When you do this.....

- You should hear the loud sound of rushing air.
  If you hear a somewhat musical tone that slowly rises in frequency it means a sample is already in the magnet and is being ejected. Wait until the sample appears at the top of the magnet.
- Gently remove the previous sample, making sure you do not catch it on the sides of the upper barrel.
- Place your sample gently into the top of the upper barrel. Make sure that it is free from the sides and that it bobs up and down freely on the air column.
- Click on the "Insert" button or type "i". The sample should now descend into the probe. It will locate with an alarming "Clunk". If it does not then click "eject", wait a few seconds and then click "insert".
- You should now wait 5-20 mins for your sample to equilibrate before you tune and shim the probe. The time you will have to wait will depend on the temperature of your sample before it went into the probe and the temperature you have selected to run the experiments. If you have taken your sample out of the fridge and are running at 40 °C you will have to wait the full 20 mins. (Go and get a coffee!!). If you are running above room temperature it is advisable to let your sample sit at room temperature for several minutes in order that the dissolved air can come out of the sample.
- When your experiments are finished please remove your sample and replace it with one of the standard samples supplied (one of the ones in H2O is best). Follow the same procedure to change the sample.

6.4 TUNING THE PROBE

Different sample conditions such as solvent or ionic strength and salt alter the capacitance of the NMR transmitter/receiver coil. The process of tuning adjusts the probe so that it efficiently transmits the NMR signal, similar in some ways to tuning your radio at home to get your favorite music channel. Figure 6.1 shows a schematic layout of one of the spectrometers and a more detailed diagram of the connections on the preamp unit and the magnet/console interface.

Each channel has to be tuned separately. When tuning, the transmitter corresponds to channel 1, the decoupler to channel 2, decoupler 2 to channel 3 and decoupler 3 to channel 4. This should be familiar from the description of the parameter sets described above. How many channels you need to tune depends on your experiment. If you are only recording 1H NMR spectra on non-labeled samples then you only need to tune the transmitter for 1Hs. However if you want to do N15 or C13 decoupling or you are running a 3D spectrum on a labeled sample you will need to tune these channels as well.

MTUNE or REFLECTED POWER

There are two approaches to monitor how well the probe is tuned. The first, and well established method is to monitor the signal on the LCD display in the tuning interface. This is sometimes referred to as the reflected power display. The other, generally better, approach is to use the program "mtune". This has a separate interface that is displayed on the computer monitor. Instructions on how to use mtune and appended below. Whichever method you chose both methods require that the probe is recabled in exactly the same way before you can tune.

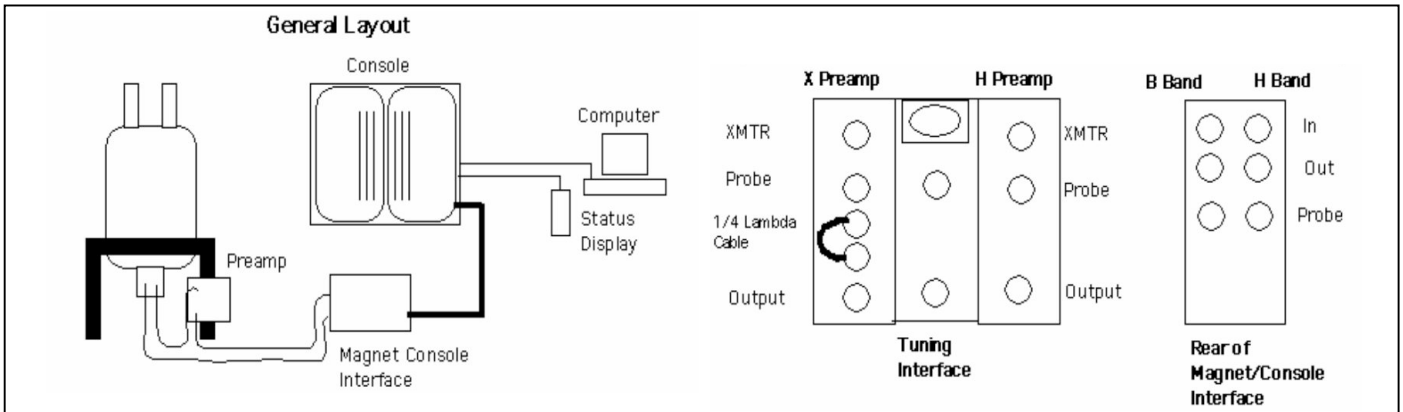


Figure 6.2 Layout of Spectrometer Preamplifiers and Tuning Unit

6.4.1 Tuning Proton Only

- Make sure the temperature is set to the required value.
- Check that the following parameters are set.
- solvent = `D2O'' If you use a different solvent set this parameter accordingly
- tn='H1'
- type "su" in the command window.
- Find the input labeled 1H on the probe
- Follow the cable attached to this connector back to where it connects to the square filter on the 1H Preamp (See Figure 6.2).
- Disconnect the cable from the filter. Leave the filter connected to the Preamp.
- Reconnect the free end of the cable to the Probe socket on the Tune unit (located below the small LCD screen)

- Now take the cable from the Output socket at the bottom of the 1H preamp to the Output socket of the tuning unit.
- If you are going to use "mtune", start the mtune interface (see below).
- DO NOT PRESS ANY BUTTON ON THE CHANNEL SELECTOR ON
- If you are using the LCD Display, press the channel selector button next to the LCD so that it reads 1. The green LCD display will light up
- Set the Attenuation to 8, if the reading is off scale adjust the attenuation to a lower value to get the reading on
  scale.

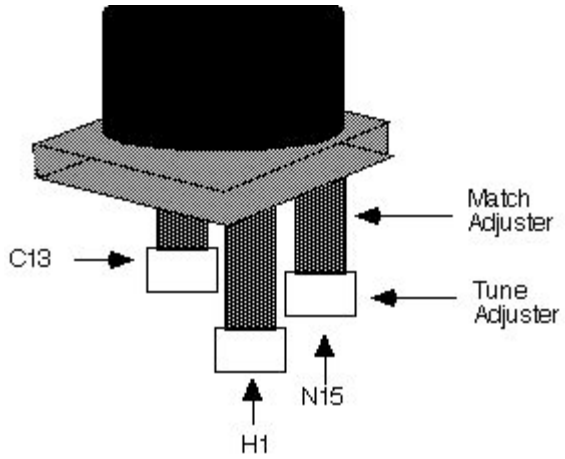


Figure 6.3 Location of the tuning rods on a Triple Resonance HCN Probe

- On the probe find the rod labeled Proton (Figure 6.2), it usually has a red label
- The rod has two movable parts, the lower smooth part adjusts the "Tune" capacitor and the upper knurled part adjusts the "Match" Capacitor.
- Adjust the Tune capacitor so that the number on the display is a minimum
- Now turn the Match Capacitor a little in a clockwise direction (the number may get bigger)
- Now readjust the Tune to minimize the reading.
- If the new reading is smaller than the previous minimum, turn the Match in the same direction as before and repeat the tune adjustment process.
- If the new reading is higher than the original reading, turn the Match back in the opposite direction and readjust the Tune to minimize the display,
- Keep adjusting the Match and Tune in this way until the reading reaches a minimum. It should be possible to get the meter reading down to 1-2 on all probes with the attenuation set to 8.
- When you have finished set the channel selector to 0 and move the cables back to their original positions.

6.4.2 Tuning the Decoupler Channels for C13 and N15

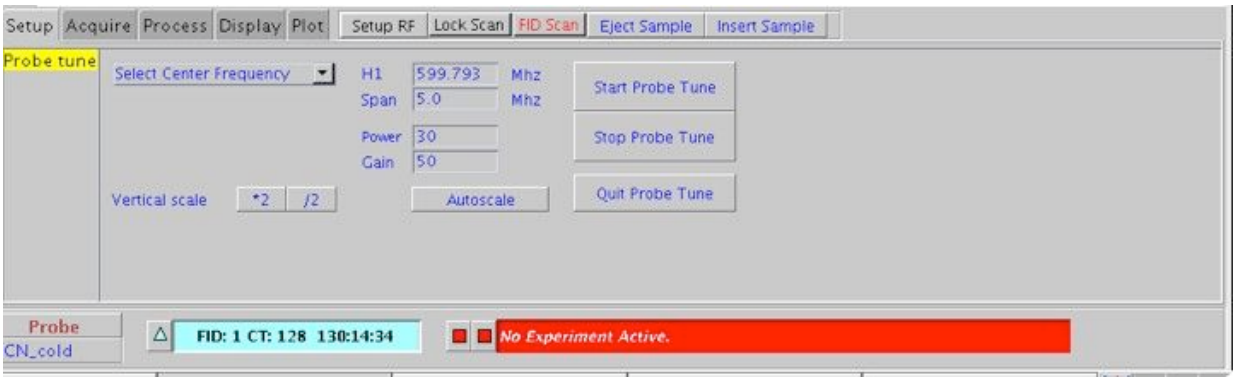
- Load in an experiment that already has the correct channel setup, e.g N15 HSQC or
- Set tn='H1' dn='C13' and dn2 = 'N15'
- type su
- To tune the C13 channel, find the C13 connector at the probe.
- Follow the cable back to where it makes a connection to a large cylindrical filter
- Disconnect the cable at this point
- Connect the free end of the C13 cable to the Probe input of the Tune interface.
- Now move the cable connected to the output at the bottom of Broadband-Preamplifier to the output of the tune unit
- Start the mtune program (see below) or if it is running, select C13.
- Alternatively set the channel selector switch on the tune unit to 2 to use the LCD display

- Adjust the match and tune capacitors of the C13 channel in the same manner as for tuning protons.
- Tuning N15: Use the same procedure for ‘C13’ except locate the ‘N15’ input on the probe, and set the channel selector switch to 3 or set mtune to read N15.
- If you are tuning both C13 and N15 you do not need to move the cable connected to the tune Output back to its original position before you tune N15.
- When you have finished set the channel selector switch back to 0 and return the cables to their original positions.

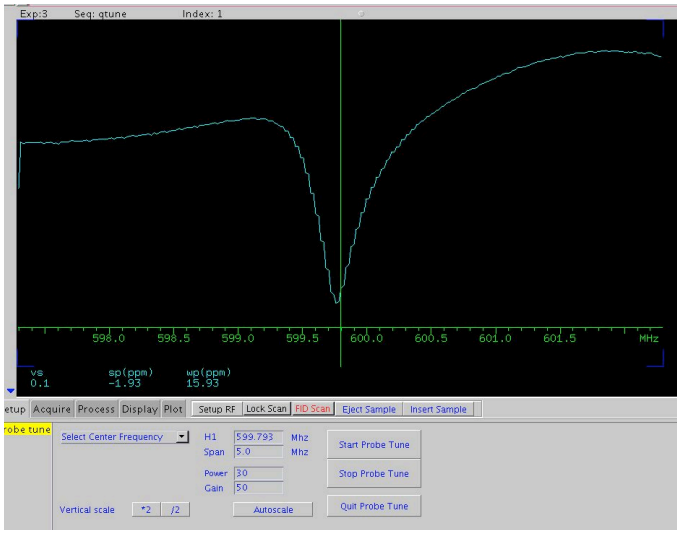
6.5 Using "MTUNE" to Monitor Probe Tuning

Mtune is a program that can be used to monitor probe tuning. It operates in a slightly different way from using the LCD monitor, and is generally more accurate. Use this approach to the probe if you are having difficulties or if you have been instructed to use mtune.

- Load in an experiment that you will be running. eg. N15 HSQC or alternatively set tn='H1', dn='C13' and dn2='N15'
- type the command "mtune"
- The parameter page will now change to show the following screen



- Set the "**Power**" to 30 and the "**Gain**" to 50
- Set the "**Span**" to 5 MHz
- From the drop down menu labeled "**Select Center Frequency**", choose the nucleus you want to tune. Remember, you should have already cabled the probe for tuning.
- Click "**Start Probe Tune**" button and once the screen displays something, click the "**Autoscale**" button and you should see something like this



If not, you may need to increase the "Power", but do so in small steps, or 2 or 3.

The green vertical line marks the frequency of the signal you are tuning.

The blue line shows the frequency response of the RF coil. A well tuned probe has a narrow minimum centered at the selected frequency marker.

- Adjust the computer monitor so that you can see the screen when you are sitting under the magnet.
- Now tune probe.
- Adjusting the "Tune" capacitor will make the minimum move from left to right
- Adjusting the "Match" capacitor will adjust the width and depth of the minimum.
- Start by adjusting the "Match" to get the response to dip to its minimum level
- Then adjust the "Tune" to center the dip on the green frequency marker line
- If necessary you may need to adjust the "Vertical Scale" button
- Once the selected channel is tuned, click the "**Stop Probe Tune**" button
- Re-cable the probe for tuning the next channel e.g. 13C
- Select the new frequency from the drop-down selector
- Click the "**Start Probe Tune**" and tune the probe as before
- Repeat for any additional channels you wish to tune.
- For 13C and 15N on HCN probes, you should go back and re-tune each channel to make sure there is no significant cross-talk between the channels. This can often cause mis-tuning and poor probe performance.
- Once tuning is complete click the "**Quit Probe Tune**" button

6.6 LOCKING

The lock circuit is used to maintain a stable magnetic field. It uses the deuterium signal of the solvent as a fixed reference frequency. The lock circuitry detects changes in the magnetic field and adjusts the field strength accordingly to maintain the deuterium signal at the reference frequency. The exact frequency of the deuterium signal is different for each solvent but the spectrometer uses a list of the deuterium frequencies stored in one of the configuration files to adjust its parameters to accommodate these differences. Initially we adjust the lock circuit so that the Deuterium signal of our sample is “On resonance”.

- Check the setting of the "solvent" parameter. This is normally set to D2O, however on some occasions other users will use different solvents e.g. DMSO.
- Check that the temperature is set correctly in this experiment.
- Enter the command "su"
- Select the "**Setup**" tab and the "**Lock**" Folder

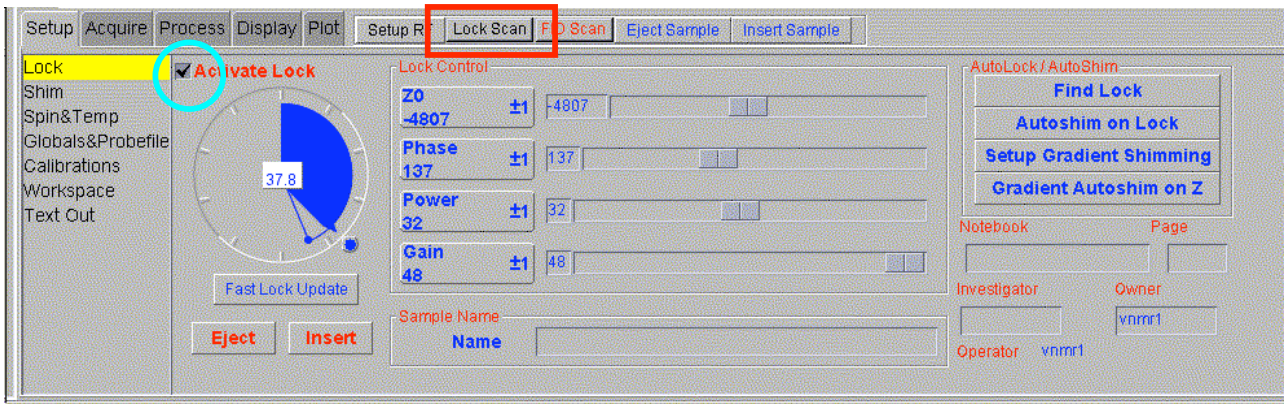


Figure 6.4 Layout of LOCK Control Panel

- Make sure that the "**Activate Lock**" button is NOT checked
- Click the "**Lock Scan**" button (red square above)
  The lock signal should appear in the Graphics Window,
- Change the "**Lock Power**" with the slider:
  For D2O set the Lock Power to 25, for H2O set the lock power to 32 (On the 600 this may

need to be as high as 42)

- Adjust the "Lock Gain" so that you can see the lock signal, initially you may have to set this very high (maximum of 48)
- The signal you see depends on the position of the Deuterium frequency

  If you see a flat line with a step down at the left edge this means the lock frequency is very close to the correct value. Slowly adjust the Z0 value to get the maximum intensity of the lock signal

  If you see a sinusoidal ripple the lock frequency is offset and you must adjust Z0 until you see get the flat line with the step at the left edge.

  if you adjust Z0 and the numbers of sine waves gets smaller in the display then you are getting closer to the correct frequency. If the number of peaks increases you are moving Z0 in the wrong direction.

- Adjust the value of Z0 to get maximum intensity. The final signal should have no sinusoidal modulation and only a small decay
- Click the lock "**Activate Lock**" check box and click on the "**Lock Scan**" button to close the graphics display.

Select the "**Shim**" Folder (see below), and adjust the lock phase to get the maximum Lock amplitude.

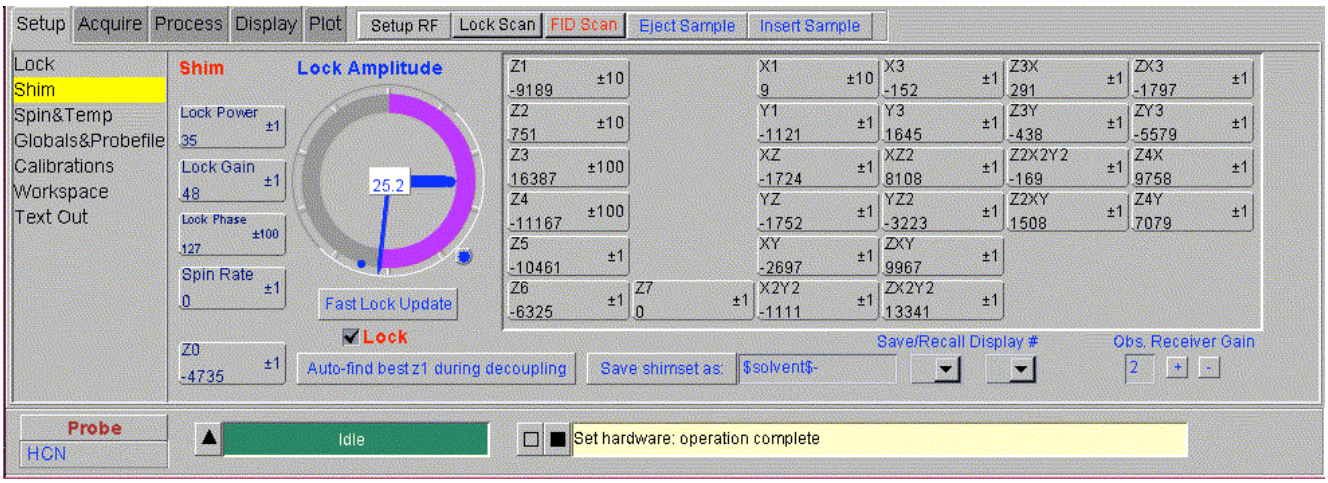


Figure 6.5 Layout of Shim Panel

6.7 SHIMMING

6.7.1 Introduction

Shimming is the process of adjusting the magnetic field to produce a homogeneous field throughout the sample volume. Good shimming is essential for all experiments, particularly those that use selective pulses and gradients for suppression of strong unwanted solvent resonances. A shim is basically a coil of wire wrapped around the sample. Current flowing in the coil produces a magnetic field that depends on the geometry of the coil. The number of shims on each magnet varies from 13 up to 40. They can be classified into two major groups: The Axial shims (also called the Z shims) are those that contain only Z components, i.e. Z1, Z2, Z3, Z4 etc.. The Radial shims (also known as non-spinning shims) are those that contain X and/or Y components, they may also contain Z components e.g. X, Y,

XZ, YZ, XZ2, YZ2 etc.

The Z shims are the most sensitive with Z1 being the most sensitive, but Z4 and Z5 have the most complex effects on the sample. The higher order shims are normally adjusted using a sample of Chloroform in Acetone which has a line width of less than 0.2 Hz. Protein samples typically have line widths 100 times greater than this and it is difficult to observe any effects on the overall line shape. Where you really notice it is with Water Suppression!!

There are two methods for shimming your sample, by hand or to use *Gradient Shimming*. This latter method uses gradients to generate maps of the effects of each shim on your sample and applies a mathematical correction to the shims. It is a very powerful and rapid technique that can achieve amazing results very quickly. The process for Gradient Shimming is discussed in detail below.

6.7 GRADIENT SHIMMING

Gradient shimming is the best and fastest to achieve good line shape. Gradient shimming optimizes only the Z1-Z6 shims it does not optimize the radial shims. Therefore before you start gradient shimming you must have a good set of radial shim values, i.e. values for X, Y, XY X-Y, XZ YZ etc... If you do not have these optimized then Gradient shimming will not produce good results.

There are two distinct stages to the gradient shimming process the first is to generate a *Shim Map* for your sample. This generates a calibration map of how each of the Z shims (Z1-Z6) affects the sample. The second step is to perform the actual gradient shimming.

6.7.1 Generating Shim Maps

The first stage to generating a map is to optimize Z1, Z2, X,Y XZ and YZ shims manually. This is critical if you want to produce a good map on the first pass.

- Open the "Setup" - "Shim" Tab as above. (Figure 6.5)
- Make sure the "Lock" button is checked
- Adjust the "Lock Phase" on the left to maximize your Lock signal

WARNING Holding down the left or right button will cause the shim value to scroll rapidly. However, this is not actually applied to the magnet shims until you release the mouse button.

STEP 1
Adjust Z1 and Z2 by clicking on the respective buttons and maximizing the lock signal. If you need to reduce the lock gain/power to get your signal below 100 %. Clicking on any of the Shim buttons with the left and right mouse button will adjust the shim down or up respectively, by the amount shown on the right hand side of each button. Clicking with the Middle mouse button, will change the step size for that particular shim, so you can adjust the rate at which you change the shim. These values are typically 1, 10 or 100. .

STEP 2
Adjust, X, Y , XZ, YZ in that order, again aim to get maximum lock signal

STEP 3
Go back and adjust Z1 and Z2.

Having made an initial adjustment to the shims we can now set up the mapping parameters.

Select the "**Setup**" - "**Lock**" tab, and click on the "**Setup Gradient Shimming**" button (See Figure 6.4 Above). This will bring up the Gradient Control Panel as shown below (Fig 6.5)

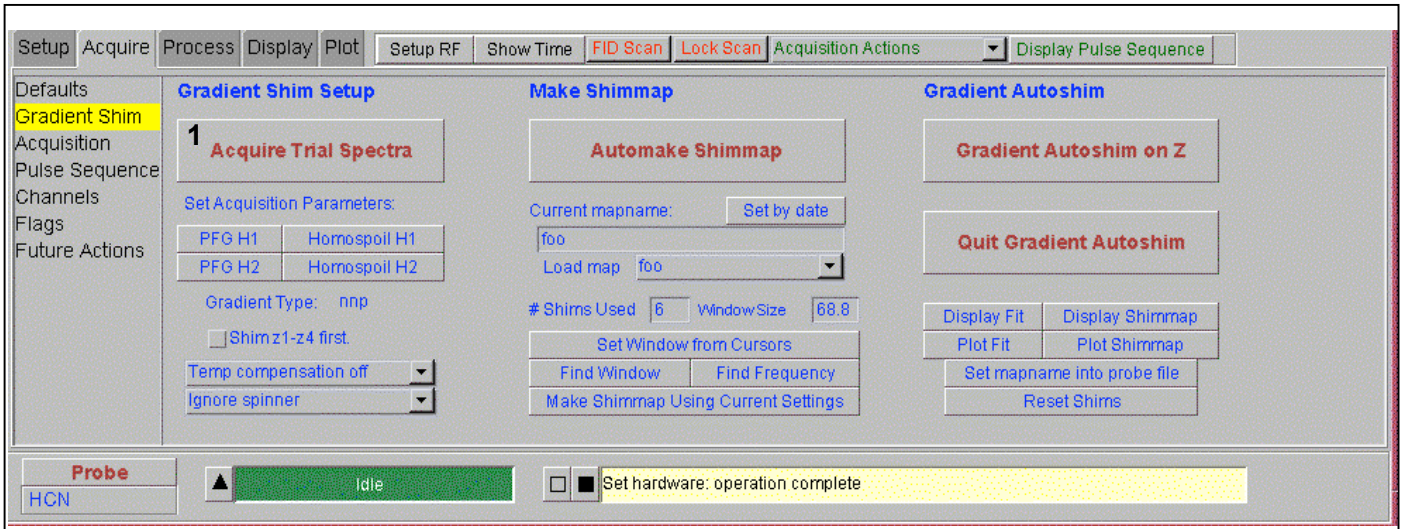


Figure 6.5 Gradient Shimming Control Panel

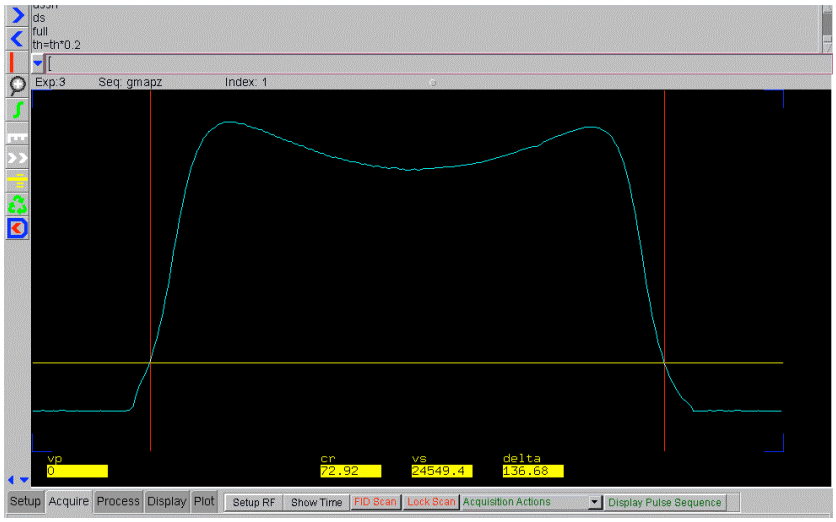
Most of the parameters for gradient shimming are automatically set. However some parameters may need adjusting. I recommend that you check and set the following parameters. (These work on both the 600 and the 500)

gzlvl=4500
pw =1
gain=2
nt=2
d1=5
gzsize=6
pfgon='nny'

- Click the "**Acquire Trial Spectra**" Button
  This will run the experiment and a spectrum should appear as shown below,

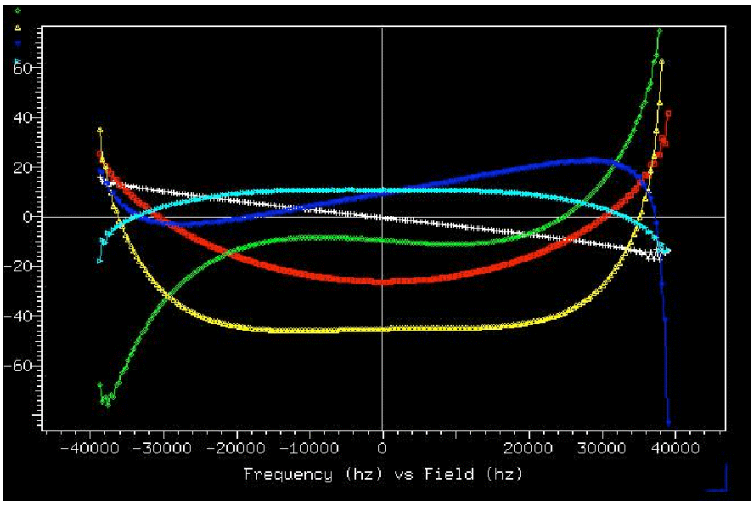
If you see a narrow line at the center of the screen, this indicate the gradients are not switched on, Check that pfgon='nny'!

- Display the second spectrum (command ds(2)), and click on the Threshold button  on the Graphical Tools Panel
- Place the threshold so that it is on the top of the gradient profile.
- Now enter the command "th=th*0.2" on the command line
- This will change the height of the threshold button so that it appears as below
- Now place your left and right cursors on the point where the threshold line cross the gradient profile
- Click the "Set Window from Cursors": button
- Click the "**Make Shimmap Using Current Settings**" button
  You will be prompted for a file name to store the new shim map in the command line



Once you enter the name and hit return the mapping process will begin.

This will take a few minutes, there are 16 spectra to collect! You will see each spectrum plotted as the mapping proceeds. When the process is complete you can display the results by clicking the "**Display Shimmap**" button.
You should see something like this



If you see something that is significantly different, something is wrong. Check that the gradients are on. If you are using a Shigemi tube, make sure that the sample is properly centered

6.7.3 Gradient Autoshimming

- To Start the autoshimming process click the "**Gradient Autoshim on Z**" button
  The output from the autoshim is a little bit different. Initially you will see the spectrum displayed as during the gradient mapping process. Next you will see a graph with a set of white data points and an overlaid red set of points.
- At this point you should select the "**Setup**" - "**Text**" tab, where you will also see some text output about the shims, errors and fits. The program will run the mapping process up to 10 times before it exits. Generally though it only takes 2-4 iterations to produce a good fit. Watch how the output graphs change as the fitting process proceeds.
- If the shim map does not converge correctly, or produces error messages, check the values of the fits in the "**Setup**" - "**Text**" window. Make sure that none of the shims is at a maximum of +/- 32,768. If so, then there is something wrong with the position of your sample, or it is too short. You may need to change the number of shims you are using for the mapping. For shigmei tubes you often can only use 4 shims.
- Also, if you see that on sequential fits, Z6 is alternating between being large and positive on one scan and large and negative on the next, then you may need to exclude it from the fit.
  - Reset the value of Z6 to its initial value. You can find this in the "**Text**" Window
  - In the Box labeled "**# Shims Used**" in the "Gradient Shim Window" enter 5 (See figure 6.5)
  - Check the box next to "**Shim Z1-Z4 first**" button
  - Restart the auto shimming process

When the autoshim process is complete click the "**Quit Gradient Shim**" button. This will return you to your experiment

As good measure you should now go back and check the values of the X Y, XZ and YZ shims, and also the value of "**Lock Phase**". Sometimes these can change a lot during the autoshimming process.

6.7.4 Gradient Shimming With Deuterium

The procedure outlined above assumes that you are using a sample with 90%H2O/10%D2O, in which case we are observing the H2O signal in the mapping process. On some occasions you will run samples that are in 100% D2O. In these cases we need to set up Gradient Shimming for Deuterium. The process is very similar but first we need to move some cables around.

- Locate the cable going into the probe labeled "Lock"
- Follow this cable back until you reach the large cylindrical "Lock" filter.
- The other end of the filter connects to a cable that goes through the hole in the side of the gray magnet/interface box.
- Disconnect this cable from the top of the filter
- Now find another spare cable, there is usually one on the floor near the interface for this very purpose.
- Connect this new cable to the top of the "Lock Filter".
- Connect the other end of this new cable to the Probe Socket of the X-preamplifier.(You may have to remove a cable from this connection, if so, remember which one it is as you will need to put it back later).
- Below the probe-socket, where you just attached the cable, there is a short cable that forms a loop between two connectors on the X-Preamplifier. This is called the "Quarter Lambda" or "Quarter Wavelength" cable.
- On this cable there should be a label that lists a frequency and below that a frequency range. e.g. Unity 130 MHz
  60-190 MHz
- Check that this frequency range covers the frequency of the Deuterium Lock signal
  On the 500 this is 75 MHZ, on the 600 this is 92 MHz. If it does not you will need to

replace it with a cable with the appropriate frequency range. Again it will probably be on the floor near the spectrometer.

- Now back at the spectrometer go the "**Gradient Shim**"
- Click the "**Pfg H2**"" button
- Now set the following parameters as indicated

gzlvl=4000
pw =120
gain=20
nt=4
d1=5
gzsize=6
pfgon='nny'

Notice pw and nt are very different from the values used in gradient shimming of 1H.
You can now proceed as for 1H shimming, the big difference is that it will take a lot more time to run each experiment (it is a good idea to go get coffee or do whatever else you need to do.)

When you have finished the gradient mapping and gradient shimming process, you need to replace all the cables back to their original positions. Work backwards through the instructions if you are unsure about what to do.

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7. RECORDING A SPECTRUM

You are now ready to collect your first spectrum. Before we do, it should be noted that the spectrometer has not been completely optimized yet, but we will get to that in the next stage. First we need to set up the experiment. There are two ways to do \ this. The first is to locate a previous 1D experiment and load it, or we can set it up from scratch. We will start by setting up the 1D presat experiment, which uses presaturation of the solvent. This will allow us to discuss some of the other aspects of data collection and data processing.

7.1 SETTING UP FOR 1D DATA COLLECTION

- Set up the PRESAT experiment
  - Select "**Presat**" from the experiment window
  - Make sure that tn='H1'
  - Set transmitter offset (tof), spectral width (sw) and number of points (np) as outlined above
  - set tpwr=57 and pw=4. These are initial values and we will optimize them later.

- Unset any unwanted parameters:
  - Set hs='n', sspul='n'

- Set up presaturation parameters:
  - Set satmode='ynn', satpwr=10, satfrq=tof, satdly=1.5, d1=0.
  - set ss=4 and nt = 8

- Run the experiment by typing "go" in the command line
- When the experiment is compete (10-20 seconds) you can display the resulting with the command "**df**"

The result you see is the Free Induction Decay (FID). This is the NMR signal measured in the "time domain". It doesn't look much like the spectra you are used to seeing. We now need to process this data so that we can make some sense of it.

NOTE : If you get warning messages about "**Receiver overflow**" or "**ADC overflow**", then you will have to optimize the water suppression as discussed below in Section 8.

7.2 OPTIMIZING WATER SUPPRESSION IN A PRESAT EXPERIMENT

Optimizing water suppression is one of the most important things that you can do to get good spectra. Exactly what you do to optimize water suppression depends on the experiment that you are recording. So optimizing water suppression for a presat experiment does not give you optimal suppression in an HSQC experiment for example. So there is no golden set of guidelines to get good water suppression. However, there are some things that should be common in all water suppression.

First, always use nt = 1. If you get the best water suppression in a single scan, then it generally gets better with more scans. Second, if possible, look at the FID when trying to decide what is best. Use the command "dfsh" to look at the FID array. Third, make sure that **ssfilter** = 'n' and **ssntaps**='n', these parameters digitally remove the water signal from the spectrum, and can mask the effects of your optimization.

A note of caution, it takes some practice looking at water suppression to really know what is the best. Only time and experience can really teach you this.

Ok, having said that, lets optimize the water suppression in a presat experiment.

- Set nt=1, ss=4, ssfilter='n' ssntaps='n'

- Set up an array for the transmitter offset (tof) over a range of ±5 Hz around the suggested value
- Set satfrq=tof, this makes satfrq have the same values as tof, which means we now have 2 parameters that are arrayed. If you type the command "**da**" and look at the output in the "**Text**" window you will see that arraydim = 121. That means it is going to do 121 experiments.
- Now type array = '(satfrq, tof)'
  Now when you give the "da" command you will see that it is doing only 11 experiments and tof is being arrayed in step with satfrq.
- Start the experiment
- Display the FIDs using the "**dfsh**" command
- Choose the value of tof that corresponds to the spectrum with the lowest intensity of the water signal.

Once you have the water suppression reasonably optimized you can set nt=16 and collect your spectrum.

7.2 PROCESSING YOUR DATA

7.2.1 Fourier Transforms (ft, df, ds)

The FID is converted into the typically NMR spectrum with the command "**ft**". If you give this command the NMR spectrum should now be displayed. If it is not, enter the command "**ds**" (Display spectrum). At this stage do not worry if it looks a bit screwy!

7.2.2 Adjusting Weighting Functions (wti, wft, lb, gf, gfs, sb, sbs)

An FID which decays exponentially to zero contains most of its signal intensity in its first part. The first part of the FID also contains the broader signals which arise from the rapidly decaying components of the sample. Signals from the narrower components extend into the later parts of the FID. It is these narrower components which allow us to resolve signals that have similar frequencies. Sometimes it is desirable to study only these narrow components and so obtain well resolved spectra. Unfortunately the intensity of these narrow components is very small and often cannot be distinguished from the noise.

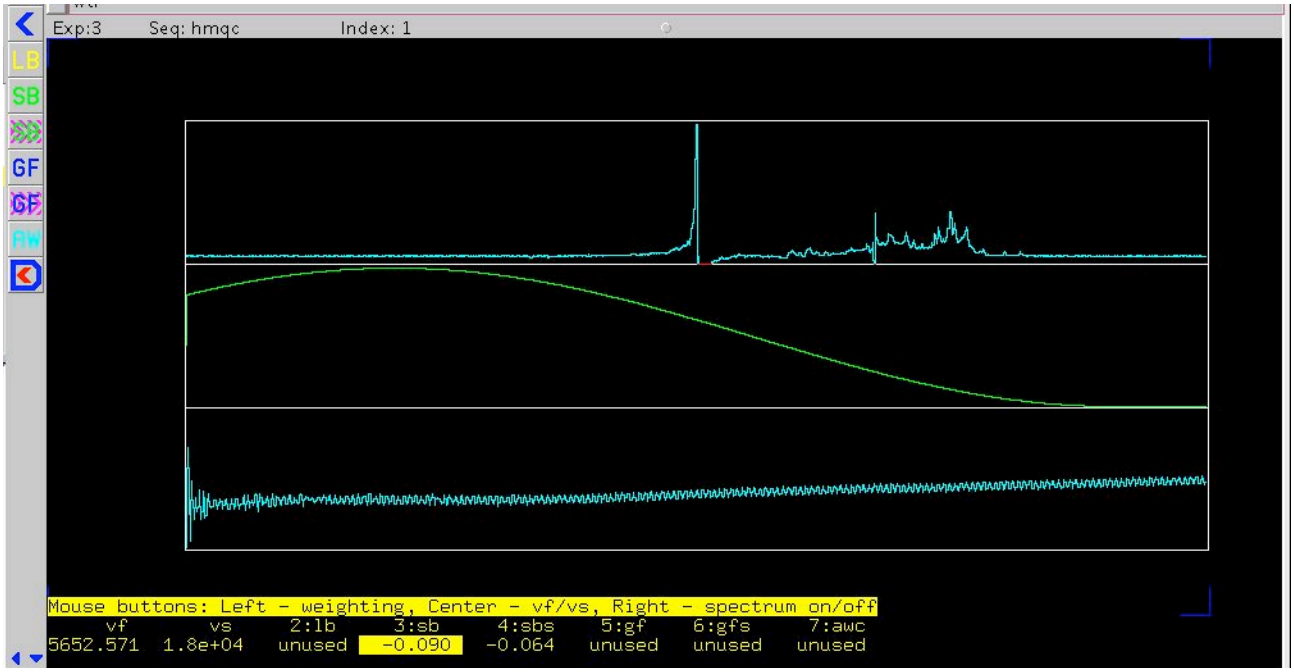
Weighting functions are used to manipulate the raw FID data in order to enhance the information content. They can be used to enhance narrow components e.g. to resolve J-couplings, reduce the contribution from broader lines, correct artifacts e.g. if the acquisition time was too short, or to reduce noise e.g. when the acquisition time was too long.

There are many different weighting functions that can be applied to an FID and nearly every user has their own favorite. The following guidelines set up a weighting function that removes any truncation artifacts and provides a reasonable resolution enhancement without throwing out too much signal.

- Start the interactive weighting adjustment by entering the command "**wti"**.
- The Interactive display has three panels;
  - the lower one shows the FID,
  - the middle one, the weighting function and
  - the upper panel shows the spectrum
  - (the spectrum is turned on by clicking the right mouse button in this panel).

- The bottom line of the graphics display shows the current settings of the weighting functions parameters. These are **lb**, **gb**, **gbs**, **sb**, **sbs**, **awc** etc.
- The Tool bar on the left edge contains a set of buttons that are used to select the specific weighting function used.

- In order to adjust a parameter, you must first click on the parameter button in the left hand toolbar to select it and then click the mouse in the middle panel of the graphics window to change the setting. The current value is shown at the bottom of the graphics window.
- To turn a parameter off, click on the parameter button twice so that the setting at the bottom of the graphics window reads "unused"



- Start by turning all functions off by clicking on the buttons on the Graphical Tool Bar
- Click on the "**SB**" tool button (the one with no hash marks on it) and then click in the function window
- Adjust the appearance of the function so it has a maximum just before or at the **end** of the FID on the right hand side of the window
- Now click on the "SB shift "button (The SB button with red arrows in the background)
- Adjust the function so that the maximum is now near the beginning of the FID and it reaches zero at or just before the end of the FID
- Now activate "**GF"** and click towards the right edge of the function window.
- Activate "**GF Shift**" (GF with red arrows in background) and adjust the position of the maximum so that it is about 1/4 to 1/3 in from the beginning of the FID. You may have to readjust "**GF**" to achieve this.
- You can now display the spectrum full size by typing "**wft**".
- This applies the function that you set up and FTs the data. This command will apply the weighting function to all subsequent FIDs in the same job until you change it.

Weighting functions must always be used with caution. The inappropriate usage of weighting functions will introduce artifacts into the processed spectrum. Therefore you should always be fully aware of the consequences of applying a particular function. For example, if the first point of the FID is made zero by application of an unshifted sine-bell function it is a mathematical consequence that the total signal intensity in the spectrum is also zero. This means that for every positive intensity there must be an equal and opposite negative intensity. This may lead to the cancellation of smaller signals by the artifacts associated with stronger signals. In addition some weighting functions can introduce artifacts that may appear like real signals. These effects may lead to the mis-interpretation of your data.

7.2.3 Phasing a Spectrum (rp and lp)

A newly transformed spectrum very rarely looks right. The peaks will have different amplitudes

relative to each other and may appear to be twisted. A distortion arises when the phase of the transmitter signal is shifted relative to the receiver signal. This occurs in almost every spectrum because of the construction of the electronic circuitry. This shift is constant over the whole spectrum and can be corrected by applying a "Zero order phase correction" (parameter = ***rp***). An additional phase correction must be applied to each signal which depends on its chemical shift. This is because each signal in the NMR spectrum starts to precess at a different frequency in the XY plane as soon as the magnetization has been displaced from the Z axis. Consequently after a pulse and prior to data acquisition the relative phase of each signal (i.e. its position in the XY plane) depends on the chemical shift difference from the reference frequency. This correction is called the First order phase correction (***lp***). The parameter ***rp*** and ***lp*** stand for "right phase" and "left phase" respectively. This is just an historical name, and really they should be called zero order and first order phase corrections. These correspond to P0 and P1 respectively in programs like nmrPipe

- Enter the command "**ft**"
- Click the "**Phase**" button on the side bar 
- Place the cursor above a peak on the right hand side of the spectrum and click the left mouse button. Red cursors should appear on the screen.
- Hold down the left mouse button and move the mouse up or down to adjust the appearance of this peak . The selected peak should be all positive and the baseline around the peak should be almost flat.
- Release the cursor
- Move the cursor to the left side of the spectrum and click it on an isolated peak
- Hold down the left mouse button and adjust the appearance of the spectrum so that the remaining peaks appear all positive

- If there is a large roll in the baseline or you get into trouble you can reset everything by setting rp=0 and lp=0 and starting the phase process again.

It may be necessary to repeat the phasing process to get the best phasing. If the baseline is not flat you may need to adjust the pre-acquisition delay: see next section.

7.2.4 Adjusting for Flat Baselines

Following the last pulse of an experiment there is a series of small delays to allow various parts of the hardware to settle prior to acquisition. These delays are imposed by the computer and some of these cannot be changed. The receiver is then gated on and the data is collected. During these delays the magnetization will dephase. A parameter, ***alfa***, has been provided to allow the user to adjust the total time after the last pulse to correct this dephasing. The delays are adjusted by use of the macro "**calfa**". This macro calculates the value of ***alfa*** based on the phase corrections that must be applied to the spectrum:

- Record and phase a spectrum making sure the baselines on either side of the spectrum are as flat as possible.
- Enter the command "**calfa**"
- Re-record the spectrum
- You should only need to adjust the zero order correction (rp) to obtain a properly phased spectrum.
- If you need to adjust lp to correct the phasing enter the **calfa** command again and repeat the process

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8. CALIBRATIONS AND OPTIMIZATONS

The following section will continue to describe the optimization process. Many parameters in an NMR experiment need to be carefully optimized in order to obtain a high quality spectrum. This includes pulse widths and power levels for maximizing sensitivity, solvent suppression and minimizing artifacts. Fortunately we can use the array feature to collect several different spectra each with a different value of the parameter we want to optimize and then compare them side by side.

This section and those that follow deal with setting up NMR experiments. Wherever possible the guidelines tell you how to set up the experiment from scratch. As an alternative you can load a pre-existing parameter set for that experiment if one exists. In this latter case you will only need to adjust a limited subset of parameters.

8.1 Calibrating a Pulse on the Transmitter Channel

The strategy is to acquire a 1D spectrum using a pulse length less than a 90° pulse. The resulting spectrum is phased and acts as a reference for the calibration. In any subsequent spectra pulses than produce a net rotation less than 180° or more than 360° will have positive intensities whilst those than produce rotations between 180° and 360°will have negative intensity. An exact 180° or 360° pulse produces no net signal intensity. We will try to calibrate a 360° pulse and then calculate the length of the 90° pulse from that value.

8.1.1 Setting Up the Calibration Experiment

- Set up the PRESAT experiment
  - Select the "**Presat**" experiment from the "**Experiments**" Menu
  - Set tn=`H1'
  - Set transmitter offset (tof), spectral width (sw) and number of points (np)
  - set tpwr=57 and pw=4.
- Remove the 2D and unwanted parameters if they exist:
  - Set ni=1 phase=1
  - Set hs='n', sspul='n'

- Set up presaturation parameters:
  - Set satmode='ynn', satfrq=tof, satdly=1.5, d1=0.
  - set ss=4 and nt = 1
  - If your sample is in 90% H_2O set satpwr = 10
  - If your sample is in 100% D_2O set satpwr = -16 (yes that is "minus" sign)

- Run the experiment by typing "go" in the command line
- Transform and phase the resulting spectrum.

8.1.2 Setting up the Pulse Width Array

Typical 90° pulse widths are 5-10μs for 1H and 10-15μs for C13. Consequently we will se up a series of experiments with pulse widths in the region of 20-40us for 1H that will bracket the length of the 360° pulse.

Type in the command "array" and answer the questions in the following fashion:

```
Parameter to be arrayed?   pw
Number of steps?           11
Starting Value?            20
Enter array increment?      2
Then type "go"
```

When the experiment has finished type “**ft dssh**"

You should see a series of spectra display horizontally across the screen

If you need to adjust the vertical scale and displayed region, display a single spectrum with the “**ds**” command. And adjust the displayed region as required and then type “**dssh**”.
The display should look like Figure 8.1:

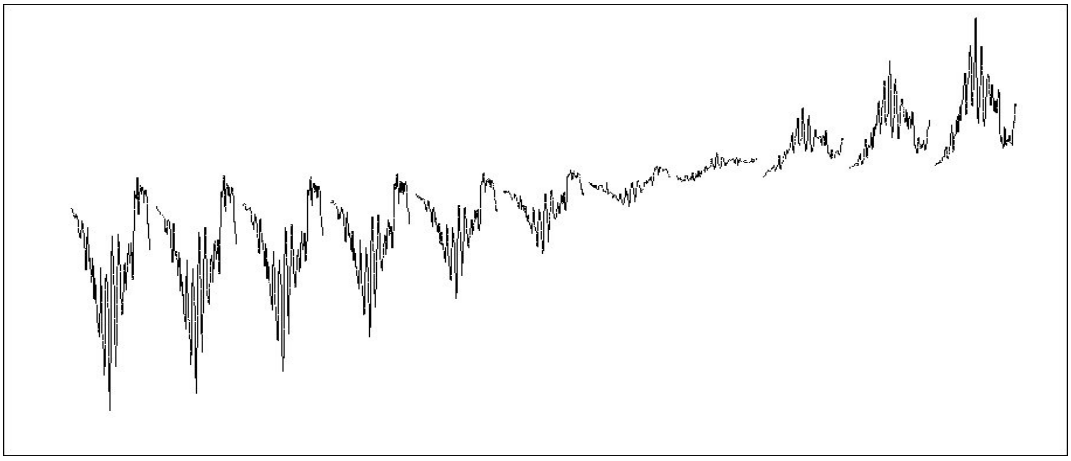


Figure 8.1 Typical output from a pulse array

The 360° pulse is the spectrum with the minimum intensity. In this example this is somewhere between points 7 and 8 (i.e. between 32 and 34 µs). You can display the number of the array with the command “**dssl**” or by clicking on the number key. 1 The values of the elements in the array can be displayed using the "**da**" (display array) command. The values appear in the “**Text**” pane.

If necessary adjust the range of the array to obtain a final result similar to the one above. You can also cut down the range of the array to get a more accurate measurement of the 360° pulse width When you have finished calibrating your pulse width, you can set pw to a 90° pulse.

8.2 Calibrating X-Nucleus Pulses on the Decoupler Channels

Many modern NMR experiments use samples that have been labeled with C13/N15. These experiments involve applying X pulses and X decoupling in addition to the pulses on 1H. In these cases you need to calibrate the X nucleus pulse using the same configuration that you will use for the experiment. In these cases the calibration is most readily achieved using the HMQC pulse sequence. The experiment is initially set up as a 2D experiment but a simple set of commands changes this into a 1D that we can use to calibrate the X nucleus pulses:

8.2.1 Calibrating the First Decoupler Channel for C13

- Calibrate the 1H pulse as described above.
- Select the "**hmqc**" experiment from the “Experiments” menu
- Set up the 1H Parameters that you calibrated above
  Set tn='H1' and set pw, tof, sw, and np as outlined above.
  Set ss=4 and nt=16
- Change unwanted parameters (Removes 2D setup):
  Set ni=1, phase=1, PFGflg='n', null=0, sspul='n', hs=’n’
- Set up Presaturation Parameters for samples in H_2O:
  Set satflg=’ynn’, satdly=1.5, satpwr=10, satfrq=tof

- Set up C13 parameters :
  set dn and dof to relevant values (see chapter 5).
  Typically dof should be used so that it corresponds to about 35 ppm for proteins
  set dm='n'
  set homo='n'
  set pwxlvl=60 (on the 600 this is 63)
  set j=140 for C13
  set pwx=12 for C13 (These are initial estimates of the 90º pulse)
- Enter the go command
- Transform and phase the spectrum
- Now set up an array for the C13 pulse pwx:
  array pwx from 7-17 us in 1 us steps.
- Run the experiment.
  It will take several minutes. If necessary increase the number of scans to obtain good S/N ratios
- Transform the spectra and display the results using the command "**dssh**"
- The spectrum with the maximum intensity corresponds to the 90° pulse on the C13 nucleus.

NOTE For C13 calibration you must judge the maximum from the signals at the high field end of the spectrum. i.e. 0-3 ppm. Do not judge the intensity from the signals in the center of the spectrum

8.2.2 Calibrating the Second Decoupler Channel for N15

The procedure to calibrate the N15 pulses is very similar for calibrating C13 pulses. However, in this case we need to select the correct experiment from the following Menus "**Experiments**" -> "**Protein HSQC Experiments**" -> "**N15 2D**" -> "**N15 HMQC**". For this to work you need to have "Biopack" Activated. If you do not see this, then please come and get help.

- Set up the 1H Parameters that you calibrated above
  Set tn='H1' and set pw, tof, sw, and np as outlined above.
  Set ss=4 and nt=16
- Change unwanted parameters (Removes 2D setup):
  Set ni=0, phase=1,hs='n'
- Set up Presaturation Parameters for samples in H_2O:
  Set satmode='ynn', satdly=1.5, satpwr=10

- Set up N15 parameters :
  set dn2='N15' and dof to relevant values
  set dm2='n'
  set homo='n'
  set pwNlvl=58 on the 500 and 63 on the 600
  set JNH=95 for N15
  set pwN=41 for N15

- Enter the go command
- Transform and phase the spectrum
- Now set up an array for the N15 pulse pwN:
  array pwN from 20-60 us in 2 us steps.
- Run the experiment.
  It will take several minutes. If necessary increase the number of scans to obtain good S/N ratios
- Transform the spectra and display the results using the command "dssh"
- The spectrum with the maximum intensity corresponds to the 90° pulse on the N15 nucleus.

8.3 Calibrating a Selective Pulse on Water

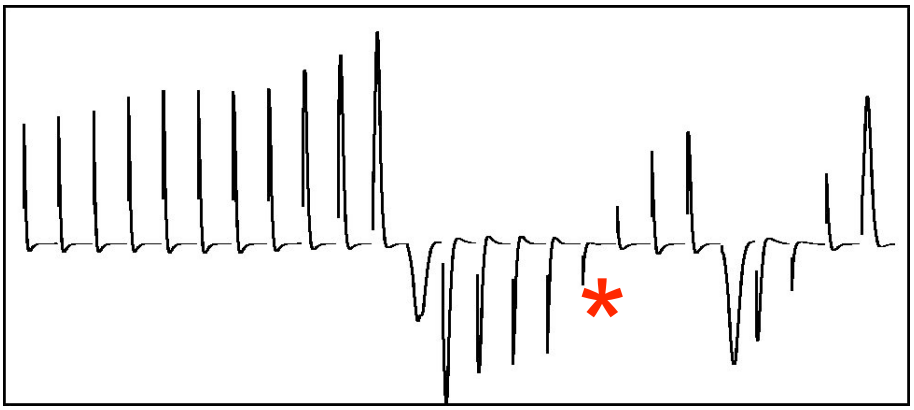
IN some experiments that involve the technique of “Water Flip Back”, a selective pulse is used to return the water signal to the Z axis immediately prior to acquiring the data. Calibrating these selective pulses is complicated by a process known as "radiation damping". In H_2O samples, because of the high concentration of the solvent (100 M) if the water is not aligned along the +Z axis it precesses and induces a voltage in the receiver coil. This induced voltage in turn generates a magnetic field in the sample which acts like a selective pulse on the water signal which in turn generates a larger off Z component of the water which generates an even larger voltage in the coil which....well you get the idea ...

The length of a selective pulse is normally limited by the constraints within the pulse sequence. Consequently we set the length of the selective pulse to a fixed value and adjust the power of the transmitter to obtain the 360° pulse. Subsequently we adjust the POWER level to obtain the 90° pulse. For Proton only spectra the selective pulse can be 3000-5000 µs duration. For 1H/15N and 1H/C13 spectra the pulse is often restricted be less than 2000 µs :

When calibrating a selective pulse on the water the radiation damping effect makes it difficult to determine the length of the 360° pulse from the appearance of the spectrum. It is much easier to determine the 360° pulse from the appearance of the FID.

1. Set up the Selective Pulse Experiment
   - Enter the command "sh2pul"
   - Set pw to the required value (see above)
   - set p1=0 d2=0
   - Set pwpat=’gauss', or whatever pulse you will be using. NOTE sometimes this can be a square pulse.
   - Set d1=1.5, ss=8, nt=1
   - Set gain=2

2. Set up an array for tpwr from 10-35 dB
3. Run the experiment
4. Display the resulting FIDs with the command “dfsh” (display FID shifted horizontally)
   - The resulting series should look something like this:



The second "null", indicated with the red star, is the 360°pulse. The 90° pulse has the same duration as the 360° pulse but the power level is four times lower. i.e. from the relationships in chapter 5 this is the value of tpwr -12 dB. Typical power levels for a 360° pulse are 26-30 dB and so typically the power for the 90° pulse is 14-18 dB. If the array does not give a perfect 360° pulse choose the power level that gives a pulse that is slightly longer than the 360° pulse. If you want to you can now set the power level to this value and find the exact length of the pulse by arraying “**pw**”. Ultimately you will optimize it more accurately in the experiment itself.

8.4 Validity of Pulse Calibrations

The 90° pulse width is a direct function of the strength of the RF. If you change the power of the RF by changing the value of **tpwr,** then the length of the 90° pulse will also change. Therefore it is important that you always use the same transmitter power in your experiments that you used to calibrate your pulse lengths. The Proton pulse widths are the most sensitive on the probes that we have available and may change dramatically depending on the sample, salt concentration and temperature. Consequently the Proton pulse widths should be recalibrated for every new sample as a matter of routine. Generally we have found that the pulse widths for other nuclei are relatively insensitive to large differences in sample conditions. Therefore after calibrating these pulse widths for the first time you can use this value for most other samples.

8.5 Pulse Calibrations on the 500

A word of caution, the second radio frequency channel on the 500 can only generate 1H frequencies. (Do not confuse this with the decoupler channel or channel 2 that you think about within the NMR program). The spectrometer automatically adjusts to this if you set tn='H1' and dn='C13' for example to produce the output along the correct RF path. However if you set tn='H1 and dn='' or dn='H1' then the proton output is produced on the first RF channel. The pulse lengths on the first and second RF channels are different. So make sure that you always calibrate the pulse length for H1 with the same setting of tn and dn as you will use in your final experiment.

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9. 2D EXPERIMENTS

9.1 INTRODUCTION

This section provides a brief introduction to 2D experiments and the additional parameters that are required to set up such experiments. A pulse sequence for a typical 2D experiment is shown in Figure 9.1

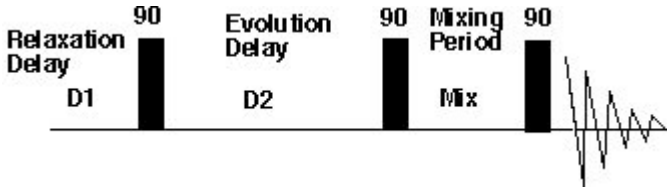


Figure 9.1 The Pulse Sequence for a 2D NOESY Experiment

In the above NOESY experiment the first pulse rotates the equilibrium magnetization into the XY plane. This is followed by the evolution delay (*d2*) during which time the magnetization precesses in the XY plane according to its chemical shift. The second pulse converts XY magnetization into Z magnetization. This pulse is followed by a period called the mixing time. The composition of the mixing period differs for each type of experiment. In some experiment it can be a short delay in others it can be a series of pulses. For the NOESY experiment the mixing period is a delay of 50-200 ms during which time the nuclear spins undergo cross-relaxation. The third pulse converts the Z magnetization into observable XY magnetization. If d2 is set to 0 then all the signal at the start of the experiment is converted into observable magnetization by the 2nd and 3rd pulses. However if d2 is not 0, then free precession occurs in the XY plane after the first pulse and the intensity of the observable magnetization depends on the angle through which the magnetization precesses during this period. If we now repeat the experiment with a larger value of d2 the magnetization will precesses through a different angle and this will change the final observed intensity. This is the basis of most 2D and 3D experiments. We repeat the experiment many times with increasing values of d2 and store each experiment separately. The signals in the resulting spectra will have their amplitudes modulated as a function of the precession frequency during the evolution period. This modulation can be used to recreate the frequency of the signal during the evolution delay by a second Fourier Transformation applied in an orthogonal direction to the first.

9.2 GENERAL PARAMETERS FOR 2D EXPERIMENTS

The indirectly detected dimension (t1 or F1) of a 2D experiment has acquisition parameters which are exactly analogous to those that control acquisition in a 1D experiment. The four main parameters are discussed below:

sw1 The spectral width in the F1 dimension (1D equivalent **sw**). The value of the spectral width determines the amount by which the evolution time is increased in successive FIDs. For most experiments the increment for each successive value of the evolution time =1/**sw1**. This is analogous to the dwell time in the directly detected dimension.

ni Number of increments (Equivalent to np in 1D). **ni** is the number of times the evolution time is incremented. For 1H experiments **ni**=256 for TOCSY and NOESY experiments and may be up to 400 for COSY experiments. For N15 HMQC it is not necessary to use more than 128 increments. However, this should be determined for each new sample. For example a spectral width of 3000 Hz in F1 which is typical for heteronuclear experiments means that after 256 increments the evolution time is 85 ms. Often for large molecules T2 relaxation means that all the signal has decayed in a much shorter period and the latter increments contain only noise. In these cases it is better to acquire fewer increments with more scans for each increment and so

use the available spectrometer time more efficiently.

phase Phase is a special parameter that is used to obtain quadrature detection in the F1 dimension. (i.e. whether the signal is moving faster or slower than the reference frequency). For most 2D experiments set phase=1,2. In the F2 dimension quadrature detection is achieved by having two receivers that are 90° out of phase with respect to each other. The concept of two receivers in the F1 dimension is meaningless. Instead in 2D experiments we acquire 2 spectra for each value of the evolution delay. In the first experiment the value of phase=1 whilst in the second experiment phase=2. When the value of phase = 2 the phase of the pulse before the evolution delay and the phase of the receiver is shifted by 90° relative to phase =1. This effectively simulates having two phase shifted receivers in the F1 dimension.

d2/f1180

d2 is the initial value of the incremented evolution time. In theory this delay is set to zero. In practice as soon as a pulse creates any XY magnetization it starts to precess under the influence of the B_0 field. In addition there are other delays that are required for the hardware and software to switch on and off. Consequently we correct the evolution time for these delays and evolution during pulses. If d2 =0 then the interval between the 1st and Second increments is not a complete dwell time. This can introduce artifacts into the baseline. In most experiments we recommend that you set d2=(1/2*sw1). In some pulse sequences there is a special flag e.g. f1180 which if set to 'y' automatically implements this feature, and in these cases d2 is set to 0. A nice feature of setting d2 (or f1180) is that you can automatically set your phase corrections in the indirect dimension to -90, 180 (see below) and any peaks that are folded with have opposite phase to the non-folded peaks.

9.3 2D HETERONUCLEAR EXPERIMENTS

The HSQC experiment is used to record a ^{1}H-^{15}N correlation experiments on a protein. There are two basic HSQC experiments. The first is a so-called "gradient-enhanced" experiment that uses gradients to simultaneously select the signals of interest and suppress the water. This is a good general purpose experiment. The second experiment is a so-called "flip-back" hsqc. This does not use gradients to select the water signal, but it has a distinct advantage for protein samples that are at pH >7.0. Which one you use really will depend on the experimental conditions.

9.3.1. Sensitivity Enhanced HSQC

Select "**Experiments**" –> "**Protein HSQC Experiments**" –> "**N15 2D**" –> "**N15 HSQC**"

1. Set up the 1H Parameters
   - Set tof, tpwr and pw appropriately
   - Set d1, sw and np appropriately
   - Set nt to a multiple of 8

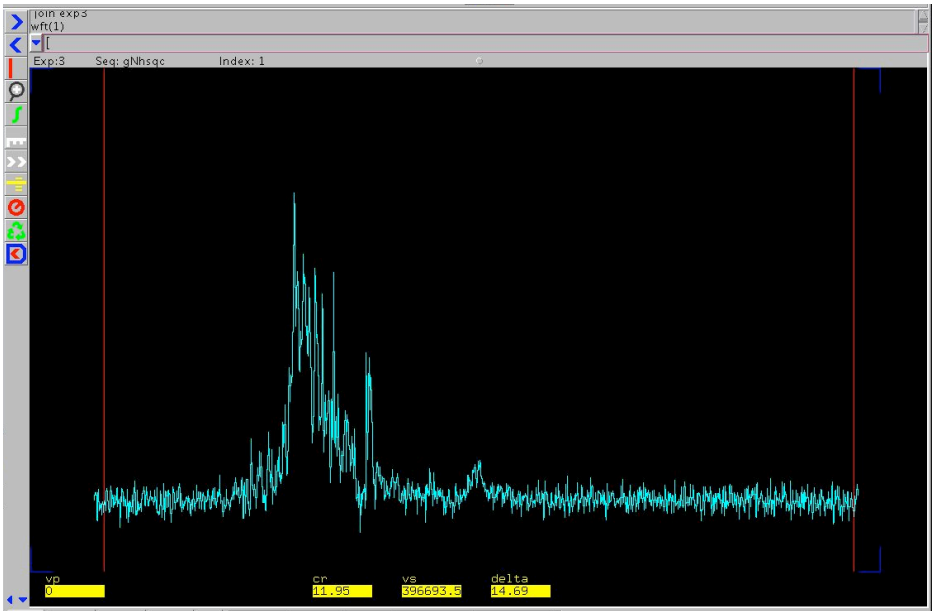
2. Setup ^{15}N parameters
   - Check pwN and pwNlvl
   - Check dof2, dpwr2, dmf2, dm2 and dmm2

3. If the sample is ^{13}C labeled
   - Set C13refoc='y'
   - Check pwC and pwClvl
   - Check dof2, dpwr2, dmf2, dm2 and dmm2

4. Record an experiment and Fourier Transform it.

   If this experiment is working then you should see signal on the left hand side of the spectrum, at

a higher frequency than the water, as shown in the picture below. The water is the small bump in the middle.



5. Optimize your signal

Your signal intensity depends on accurate calibration of gzlvl2. The strength of gzlvl2 is dependent on gzlvl1, and should be similar but a little smaller than gzlvl1. Theoretically this value should be 0.987*gzlvl1, but it is better to array it. Typically if gzlvl1=12000, gzlvl2 will be in the range of 11850-11950.

6. Optimize the water suppression

You will need to array tof, pwHs, gzlvl3 and gzlvl5 and/or gzlvl6. gzlvl3, gzlvl5 and gzlvl6 are gradient strengths. These typically will be around 11000, 2000 and 2600 respectively, but they will change depending on your sample. gzlvl6 may be very sensitive to small changes in the gradient strength or 10 or 20 units. To get optimal water suppression can take some careful optimizations with these pulses

7. Setup the 2D parameters

set ni=128 or 256
phase=1,2
nt=8 or 16
f1180='y
Check sw1 and dof2 are reasonable

8. Start your experiment, once it finishes, save it.

9.3.2 HSQC with Flip-Back Water Suppression

First, let me say that there is a Varian NMR experiment that will perform a so-called "Watergate/FlipBack HSQC". In my opinion there are some serious flaws in the design of this experiment that significantly detract from its usefulness. Therefore the recipe that I am giving you here, is for a version that I wrote. The best way to set up this experiment is to find an existing parameter set and use that. If you want to use this experiment then I can provide you with the parameter set.

1. First calibrate a selective Gaussian pulse on the water of ~2.1ms in duration(see Section 7.3)

2. Load a setup/parameter file for **dj_fbhsqc**

3. Set up the 1H Parameters
   - Set tn, tof, tpwr and pw appropriately
   - Set d1, sw and np appropriately
   - Set nt to a multiple of 8

4. Set the N15 Parameters
   - Set dn2=`N15' and dof2
   - Set pwN and pwNlvl to calibrated values
   - Set dm2=`nny', dmm2=`ccg'
   - Set dpwr2 and dmf2 to calibrated values (see Section 5.3)
     Remember GARP gives decoupling over $5xB_1$
   - Set j=90
   - Set ni=1, phase=1

5. Set the Selective Pulse Parameters
   - Set shape =`gauss'
   - Set shpwr to calibrated value (See 1)
   - Set sh1pw, sh2pw,to the calibrated value

6. If this is a C13 labeled sample, set C13refoc='y'

7. Optimize Water suppression
   - Set up arrays for, sh2pw sh1pw, from 1.7-2.1 ms
   - In each case choose the value that gives the best water suppression. It is not as easy to judge this as in other experiments. Check the appearance of the FIDs with dfsh and dfsh(`imag')
   - Array tof
   - You can also array several of the gradients, particularly gzlvl2, however the most important parameters are shpwr, sh1pw and sh2pw.

8. Set up 2D Parameters
   - Set ni=128 and phase=1,2
   - Set nt=a multiple of 16.
   - Set f1180='y'

9. Start the experiment, once it is complete save the experiment

9.4 PROCESSING 2D SPECTRA

Processing 2D spectra is very similar to processing 1D spectra with a few additions. The following scheme shows one way to process 2D data. The overall goal is to optimize the processing in the directly detected dimension, transform all the FIDs, perform a baseline correction in the directly detected dimension. Optimize the weighting functions for the indirectly detected dimensions and then perform the second FT. The following additional parameters are used in processing 2D spectra:

fn1	Size of the final matrix in the F1 dimension
rp1, lp1	Zero order and first order phase corrections for the F1 dimension
lb1, sb1, sbs1, gf1, gfs1	The weighting functions for the F1 dimension

9.4.1 Processing the First Dimension of a 2D Experiment

1. Read in your stored 2D data file

2. Set up the zero filling and phase parameters
   Set fn =2*np and fn1=2*ni or 4*ni
   This zero-fills the data, i.e. adds additional data points between the real ones.
   There is no point in zero filling more than this except under special circumstances.
   After the first zero fill there is no increase in resolution in subsequent zero fills.

3. If d2 was set to a non zero value or f1180='y'
   set rp1=90 and lp1= -180

4. Set Weighting functions for the F2 dimension
   Enter the command wft(1) followed by wti(1), this sets up the interactive window
   Adjust the weighting function as described in section 5.7.2

5. Set Baseline Correction Points
   - Enter the command "cz" to clear the baseline correction routine
   - Enter the command **wft(1)**
     Make sure the full spectrum is displayed at this point
   -
   - Click on the "**Integral**" Button 
     A green integral line will appear. It should be a solid line.
     If not re-enter the command "cz"

   - Click on "**Integral Resets**" Button 

   - Starting on the left hand side of the spectrum find the first region of baseline without any peaks.
   - Click the left mouse button at the left most edge of this region
   - Move the cursor towards the right of the spectrum until you reach the first peak.
   - Click the left mouse button on the baseline just before the peak.
     The integral line above the baseline that contains no peaks should appear as a dotted line. If it does not, move the mouse under the line and press the right button. Repeat the selection process.
   - Repeat the baseline selection process for all those regions where there are no peaks.
   - If you are in doubt leave the region out. (i.e. make sure the line above it is a solid line)
   - When you have finished those regions which contains peaks should have a solid line above them and the baseline should have a dotted line above it.

5. Transform the First Dimension
   Enter the commands: **wft1da bc('f2')**
   This transforms the F2 dimension
   The command bc('f2') performs a baseline correction on the partly transformed data.
   The spectrum is displayed as a series of interferograms

9.4.2 Processing the Second Dimension of a 2D Experiment

After processing the first dimension, the spectrum should be displayed as an interferogram with the axes being labeled with the ^{1}H chemical shift along one dimension and time along the other (vertical) dimension. We need to transpose these axes so that the axis labeled with time is horizontal

- Click the "**Transpose Axes**" button 
- Now click the "**Trace**" button 
  This should give you a horizontal line, and as you move the cursor over the spectrum, a spectrum, in this case the FID, should appear at the top of the spectrum. If the spectrum is off the top of the screen set wc2=130 and redraw the spectrum.

- Select an FID that contains a reasonable amount of signal. Make sure you do not select the solvent or impurity peaks. These will generally have a lot more signal than the sample
- In the command window type wti. This transfers the selected FID into the interactive window
- Adjust the weighting functions as for a 1D spectrum. Make sure that the weighting function is set so that it is zero at the end of the FID. If the weighting function extends beyond the FID it will introduce severe errors into the final spectrum

- Enter the command wft2da to complete the Fourier transform.
- The final spectrum will be display as an intensity plot

9.4.3 Phasing a 2D Spectrum

Phasing a 2D spectrum may appear a little daunting at first but with a little practice it becomes very easy. The principle behind the process for each dimension is to find two peaks that are well separated in the relevant dimension and to store these into separate 1D files. The zero and first order phase corrections are applied in a separate but at the same time a correlated way to these separate spectra.

- Display your 2D spectrum

- Click the "Trace" button 

- Now choose a trace which has an intense peak on the right hand side of the spectrum.
  As you move the cursor the row number is display at the top of the command window

- Once you have found a suitable row, type r1=NUM where NUM is the row number selected

- Repeat the process to find a row with a peak on the left hand side of the spectrum
  In this case type r2=NUM

- Now Enter the command ds(r1) to display the first selected row
  This displays the first selected row

- Click on the "Phase" button 

- Place the cursor above the selected peak and adjust the phase with the LEFT or RIGHT mouse buttons (coarse and fine respectively)

- Now Enter the command ds(r2) to display the second selected row

- Click on the "Phase" button 

- Now click **once** on the right hand end of the spectrum, in approximately the same place as the previous peak,

- Now click on the peak on the left edge of the spectrum.
- Adjust the phase of this peak as before.
- Once you are happy with the appearance of this spectrum redisplay the 2D spectrum. This will automatically apply the phase correction to the 2D spectrum
- To Phase the Other Dimension click on the "**Transpose Axes**" button  and repeat the process for this second dimension
- **NOTE** If additional phase corrections are required in the F2 dimension. The whole transformation process must be repeated because bc('f2') was applied to an incorrectly phased spectrum.

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10. PROTEIN 3D EXPERIMENTS IN BIOPACK

Many standard experiments on small-medium sized proteins can be effectively run on the Varian spectrometers using BioPack. It is not possible to describe all the available experiments here. But rather give an introduction to the philosophy behind Biopack and pointers to things to look out for when setting up an experiment.

10.1 THE PHILOSOPHY OF BIOPACK

BioPack operates on the basis that pulse lengths and pulse shapes can be accurately calculated for all experiments if we know the length of a 90 º pulse for each nucleus, and the power level for that pulse. The relationships for power and pulse length given in chapter 5 can be used to calculate all the other pulses that you need. This makes the assumption that there is a linear relationship between power and pulse length. This is shown as the dotted line in Figure 11.1. This is true for power levels below about 54 dB on Varian spectrometers. Above 54 dB, we typically find that the RF field strength no longer increases linearly with an increase in RF power, but drops off. This is known as “compression”, and is observed on ^{13}C and ^{15}N channels more so than on ^{1}H channels because the power required to excite the ^{13}C and ^{15}N is much higher than that required to excite ^{1}H. BioPack accounts for this by including a special parameter for each channel that describes the degree of compression for each channel.

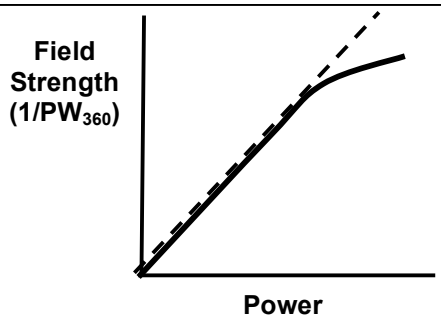

Figure 11. 1 Relationship between pulse power and RF field strength

10.2 PROBE FILE AND BIOPACK CALIBRATION

Almost all the required calibrations for using BioPack have already been set up. These are stored in a special file known as the "Probe File".

The probe file is a directory stored in the users "vnmrsys/probes" directory. The probe has a name such as "HCN". There may be several different probe files, for example HCN for the room temperature probe, or HCN_cryo from the cryo probe. The current Probe File in use is shown at the bottom of the vnmrj window underneath the button labeled "Probe".

When starting out, you need to make sure that the probe file in use is current for the probe in the magnet. These calibrations will change for RT vs CRYO probe, or if there has been a repair to the probe, or amplifiers have been swapped. These all change the power levels and lengths of pulses.

The latest system probefile containing the most recent calibration is always found in /vnmr/probes/HCN. If you are in any doubt about your probefile, copy this file to your directory as follows

- Exit vnmrj
- Open a UNIX terminal
- cd vnmrsys/probes
- cp -r HCN HCN-bup-<date> # where date is todays date just in case.
- cp -r /vnmr/probes/HCN ~/vnmrsys/probes

IMPORTANT NOTE

After copying the updated probe file, the calibrations stored in this file are only used for new experiments that are set up anew. If you have an existing experimental parameter set and switch to

that experiment, it will use the OLD calibrations. In order to apply the calibrations from the new probe file, you must re-load the experiment by selecting it from the "Experiment" menu. An even safer approach is to change to a different vnmr workspace containing an unrelated experiment and then load the desired experiment.

10.3 SETTING UP YOUR OWN CALIBRATIONS

10.3.1 Making a New User Defined Probe File

Each user can select to use a different set of calibrations based on their particular sample. This may happen because you have a sample with high salt concentrations.
If you cant to do this, first copy the existing HCN probefile to another name e.g.

- cd vnmrsys/probes
- cp -r HCN HCN_dnmj
- cd HCN_dnmj
- mv HCN HCN_dnmj
- Now in VNMRJ click on the "Probe Button" at the bottom of the vnmrj window, and select the new probe file from the drop down list at the top.

10.3.2 Required Calibrations for BioPack

There are several parameters that you need in order to setup BioPack to run all the NMR experiments. The basic calibrations you need for BioPack are as follows:

- ^{1}H 90º pulse at **high** power – typically 60 dB [this gives you **pw** and **tpwr**]
- ^{1}H 90º pulse at 6dB lower power (i.e. 54 dB in this example)

- ^{15}N 90º pulse at **high** power – typically 60-63 dB [this gives you **pwN** and **pwNlvl**]
- ^{15}N 90º pulse at 6dB lower power (i.e. 54 dB in this example)

- ^{13}C 90º pulse at **high** power – typically 60-63 dB [this gives you **pwC** and **pwClvl**]
- ^{13}C 90º pulse at 6dB lower power (i.e. 54 dB in this example)

^{15}N Decoupling Power	dpwr2	typically 41-43 dB
^{15}N Decoupling Frequency	dmf2	1/90 pulse at 41-43 dB

You can use command attval(<pw>,<power>) where power is the lower power Nitrogen power level used above and pw is the 90 pulse at that lower power level. This produces a list of pulse widths and power levels in the "Text" window. Look for a power level that will generate a pulse width of ~250us. These values can be used for dpwr2 and dmf2 above.

Based on these calibrations you should calculate a compression factor for each channel. These are given the symbol **compH**, **compC** and **compN.** Typical values are 0.95-1.0 for ^{1}H, and 0.8-0.9 for C and N. However we will optimize this value for each experiment.

- The compression factor is calculated as (pw at lower power) / (2* pw at high power).

10.3.3 Updating the Probe File With Your Calibrations

- Once you have this information on hand, select "**Experiments**" -> "**Protein Triple Resonance**" -> "**NH Detected**" -> "**HNCO**". This will load the HNCO experiment, which is used as the basis for setting the calibrations for all other experiments. If this is the first time you have loaded this experiments, then you will see a splash screen that tells you to update the

parameters.

- Enter in the values of **pw, tpwr, pwN, pwNlvl, pwC, pwClvl, compH, compC**, **compN**, **dmf2**, and **dpwr2** that you determined above. And on the 500 and 600 set **dmm2='ccg'** and **dm2='nny'**
-

- You also need to set gradient levels for coherence selection gradients. We met these in the HSQC experiment described above. In most BioPack experiments these are **gzlvl1** and **gzlvl2**. If you set these to 12000 and 11950 respectively it should give you a good starting point,

- Set **nt=16**, **ss=8**, **ni=0**. **ni2=0**, **phase=1**, **phase2=1**

- Collect a spectrum, and Fourier Transform it. You will have to use a strong weighting function

- You should see signals above the water, just as you did in the HSQC, although these will be significantly weaker.

- To optimize the signal intensity you should start by optimizing the value of **gzlvl2** in an array.

- Then optimize the values of **compC** and **compN**, and to some extent **compH** to find the maximum signal intensity.

- Optimize the water suppression
  The most important parameters are usually **tof**, **gzlvl5**, **gzlvl6** and **gzlvl3**. However, all other gradient levels will have an impact on the water suppression. Do not adjust **gzlvl1** or **gzlvl2**, as this will destroy your signal.

- Once you are satisfied with the optimization, select the "**Globals and Probefile**" Tab, and click on the "**Update Probefile with these Parameters**" button. This will transfer all your calibrations to the so called probefile, and then perform some calibrations and display a message saying "**All Parameters Updated**"

10.4 Running Triple Resonance Experiments with BioPack

Now that you have all the calibrations in place. You can run any of the experiments in BioPack, by simply selecting that experiment from the pull down menus. A list of available experiments and the pulse sequence used in the experiment is given below.

When you load a new experiment, the help file will printout a list of parameters that you will need to adjust for that experiment in the "Text" pane. You should read these and check for any specific instructions that are particular to that experiment. If you miss the instructions, you can type man(' SEQNAME') where SEQNAME is the name of the pulse sequence used for the experiment. A list of the experiments and their corresponding pulse sequences are given below.

For each experiment, it is good idea to check the values of **gzlvl2**, **compH**, **compN** and **compC** to maximize signal intensity as in the HNCO experiment above. Water suppression must also be optimized, and again this should be done on a single scan, and modifying gradient levels, and **tof**. Also if there are selective pulses on water, then these should also be optimized wherever possible.

10. 5 Experiment and Pulse Sequences Available in BioPack

COMMON NAME	SEQUENCE NAME(S)
PRESAT - includes PRESAT, WET,	
shaped-pulse PRESAT,	jump-return
watergate (soft, 3919, W5)	water
watergate NOESY	wgnoesy
watergate ROESY	wroesy
watergate TOCSY	wgtocsy
wet NOESY	wnoesy
wet ROESY	wroesy
"quiet" NOESY	qwnoesy
CPMG-NOESY	CPMGnoesy
SS-NOESY	SSnoesy
magic-angle DQFCOSY	gmacosy
z-filtered DIPSI-TOCSY	zdipsitocsy
CLEANEX N15-HSQC	gCLNfhsqc
Fast N15-HSQC	gNfhsqc
Fast N15-HSQC with Homodecoupling	gNfhsqcHD
Fast N15-IPAP-HSQC with Homodec.	gNfhsqc_IPAPHD
N15-HMQC	gNhmqc
N15-HSQC	gNhsqc
N15-HSQC with Homodecoupling	gNhsqcHD
N15-HSQC(IPAP)	gNhsqc_IPAP
CPMG-N15-HSQC	CPMGgNhsqc
watergate N15-HSQC	WGgNhsqc
N15-T1 (TROSY)	gNT1
N15-T2 (TROSY)	gNT2
N15-NOE (TROSY)	gNNOE
N15-NOE	gNnoe
N15-TOCSYHSQC	gtocsyNhsqc
N15-HSQCTOCSY	gNhsqctocsy
N15-NOESYHSQC	gnoesyNhsqc
N15-HSQCNOESY	gNhsqcnoesy
C13-HMQCNOESYHSQC(4D)	gChmqcnoesyNhsqc
N15-HMQCNOESYHSQC(4D)	gNhmqcnoesyNhsqc
N15-HSQCNOESYHSQC(4D)	gNhsqcnoesyNhsqc
N15-HSQCTOCSYNOESYHSQC(4D)	gNhsqctocsynoesyNhsqc
C13-NOESYHSQC	gnoesyChsqc
C13-HSQCNOESY	gChsqcnoesy
C13-TOCSYHSQC	gtocsyChsqc
C13-HSQCTOCSY	gChsqctocsy
N15,C13-NOESYHSQC	gnoesyCNhsqc
C13-HMQC	gChmqc
CT-C13-HMQC	CTgChmqc
Fast 13C-HSQC	gCfhsqc
C13-HSQC	gChsqc
2H pw90 calib	ddec_pwxcal
2H decoupling	ddec_s2pul
C(CO)NH (or C(CC-TOCSY-CO)N-NH)	gc_co_nh
H(CCO)NH (or H(CC-TOCSY-CO)N-NH)	ghc_co_nh
CBCA(CO)NH	gcbca_co_nh
CBCANH	gcbca_nh
HCACO	ghca_co
HCACON	ghca_co_n
HCACOCANH	ghca_co_canh

HNCO	ghn_co
HNCO_JNH	ghn_co_JNH
HNCO_NOE	ghn_co_noe
HNCOCO	ghn_coco
HNHA	ghnha
HNHB	ghnhb
HNN	ghnn,
HNCN	ghcn,
LR-JCH	gLRCH
LR-JCC	gLRCC
HN(CO)HB	ghn_co_hb
HNCA	ghn_ca
CT-HNCA	ghn_ca_CT
HNCACB	ghn_cacb
CT-HNCACB	ghn_cacbCTP
HN(CO)CA	ghn_co_ca
HN(CA)CO	ghn_ca_co
HN(COCA)CB	ghn_coca_cb
HCCH-TOCSY	hcch_tocsy
HCCH-COSY	hcch_cosy
DE-H(C)CH-TOCSY	ghcch_tocsy

12. TROUBLE SHOOTING

Obviously things are not always going to go smoothly and there will be times when the computers hang up and/or the pulse sequences don't work. So what do you do at such times: There are some things that you can do before you call for help that may help to diagnose or solve the problem.

12.1 No Lock Signal

- Check the Lock Power and Lock Gain are high enough
- Check that the sample is in the probe. Occasionally the spinner will catch on the upper barrel. Make sure you click on the "Eject" button before trying to remove it. This will prevent the sample falling into the probe and causing serious damage.
- Eject the sample tube and check both the spinner and tube for any obstructions
- Check the sample depth
- Check that the cable to the lock channel on the probe is connected to the lock filter on the side of the magnet interface box. Sometimes people switch this cable in order to perform gradient shimming with Deuterium.
- Still no lock? Did you put D2O in the sample?

12.2 The Pulse Sequence Doesn't Work

- Check that the spectrometer is cabled correctly. It is a common mistake to leave the spectrometer cabled for tuning.
- Check that the Gain is set correctly
- Check that the Probe is tuned correctly
- Check your pulse width calibrations
- READ any Error Messages carefully, there may not be a fatal error, it may just be a warning.
- Check for Error messages in the Console Window. You may need to reboot the Acquisition Controller

12.3 Restarting the Acquisition Controller

- At times it may be necessary to restart the Acquisition Controller.
- Check the Acquisition Status Window
    - If the Status is set to **Idle** everything should be OK
    - If Status is **Inactive** then restart the Acquisition Process
        - In a UNIX window type **su acqpro**c
        - You will see the message "Killing Expproc"
        - When the command prompt returns
        - type **su acqproc**

    If the acquisition Status returns to Idle everything should be OK.

12.4 Rebooting The Console

On occasions restarting the Controller Does not Work and the Console must be rebooted.

- Start by killing the acquisition process by giving the command "su acqproc" as above
- Open the Left hand Door of the NMR Console
- Towards the bottom of the unit you will see a box with a large number of cables coming from it

- Near the top left corner of this box you will see a Green LED,
- Below the LED is a gray box with an ethernet cable attached to it
- Above the LED is a small Red button
- Locate the Red button and press it once.
- The Green LED should go off and a red LED should now be showing.
- Close the door to the NMR console
- Watch the remote status unit (The display with the temperature reading on it)
- After about 30-45 seconds each of the LEDs on the Remote Status unit will blink once in turn.
- When this is complete enter the command "**su acqproc**" again
- This should restart the acquisition process and you should be able to restart your experiment

12.5 More Help?

Before you contact the system manager:

- Note down the exact symptoms of your problem and check if it is reproducible.
- Check **ALL** windows for error messages and make sure you write down the message as completely as possible
- The numbers to contact are:

- (303) 724 3600 (Office)
  (303) 724 3601 (Lab)
  (303) 916 7246 (Cell)
  (303) 221 7430 (Home)

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