NMR Spectroscopy with VNMRJ & EZ NMR

Locally developed **EZ NMR** for VNMRJ versions 2.2D/3.2A

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1. EZ NMR to Simplify NMR!

- **routine 1D *and* 2D NMR** techniques accessible to students/PDFs that may have minimal experience with NMR and a minimum desire to learn (**minimum ≠ zero!**)
- from acquisition to processing, plotting and saving of data **without commands** for 7 common solvents and 13 less common ones
- **buttons/icons** instead of commands (ca. 400 macros, parameter sets and menus implemented)
- **VNMRJ** commands and macros and the ability for users to write and use their own macros, parameter sets etc. is retained
- 8 NMR spectrometers: 300, 3x400 (1 with an autosampler), 2x500 (1 with an autosampler), 600 and 700 MHz spectrometers with **identical operation** (VNMRJ and EZ NMR)
- **central data storage** (data available on all NMR-related computers) and **central data storage** for undergraduate NMR (data available in W1-50 computer lab)
- **quota system** for data storage: no overuse of disk space by other user(s)
- 4.7 Gbyte **DVD-Writer,** 700 MByte **CD-Writer**, and **USB device support** on data stations for data archiving and transport
- extensive use of local network to make centrally stored **data available on work stations throughout the Department** (Linux, Mac OS-X and Windows)
- **booking** of spectrometer time **via HTML interface**

This manual is also available at: http://nmr.chem.ualberta.ca (HTML and pdf documents) and is intended for general usage on all instruments within the Department of Chemistry except for the instruments referred to as the m400 and u500. The m400 and u500 spectrometers have an auto sampler which dictates different operation. Documentation for usage of the m400 and u500 can also be found at: http://nmr.chem.ualberta.ca.

2. Introduction

This document deals exclusively with high-resolution (i.e. liquid *not* solid state) **Linux-based Agilent NMR spectrometers** which are all linked through the local network.

The main objective of this manuscript is to provide information on how to run these spectrometers in a safe and efficient way by using specific locally developed **menus and panels** referred to as “EZ NMR”. With the help of these panels, 1D and 2D data can be acquired, processed and plotted without explicitly entering commands. The explanation of general VNMRJ commands is kept to a minimum as there are excellent manuals located on each spectrometer and data station (on-line pdf manuals).

Spectrometer host computers and data stations are equipped with the following manuals:

- **VNMRJ Command and Parameter Reference** (very useful)
- **NMR spectroscopy** (useful to learn about the system in general)
- **User Programming** (useful only if you want to write your own macros and pulse sequences)

which are instructive for those wishing to learn more about general VNMRJ commands.

Except for the field strength, associated spectral dispersion and sensitivity, the operation of all instruments and data stations is essentially identical as they are all equipped with similar hardware, use the **same version of the Linux operating system and use similar versions of VNMRJ software**:

- Linux: Red Hat Enterprise Linux 5 (RHEL 5)
- VNMRJ: 2.2D (Mercury) or 3.2A (Inova, VNMRS, and Data Stations)

2.1 The NMR Staff and Spectrometers

The Department of Chemistry has 8 high-resolution NMR spectrometers and a small staff of three personnel dedicated to the care and maintenance of these instruments as well as aiding users with anything liquid NMR related. The NMR staff are also available to measure samples for users that require special techniques, long experimental times (dilute samples), require immediate attention (unstable compounds), training new users, plus many other items.

2.1.1 The NMR Staff

The **NMR staff** can be found in on the third floor of East wing, room E3-17A and room WB-13 in the basement of the West wing within the Department of Chemistry.

3rd Floor East Wing:
The NMR Laboratory Supervisor, **Dr. Ryan McKay** (email: ryan.mckay@ualberta.ca), room E3-17A (phone: (780) 492-9950).

Basement West Wing:
The NMR service staff **Mark Miskolzie** (email: mark.miskolzie@ualberta.ca) and **Nupur Dabral** (email: nupur.dabral@ualberta.ca), and one 400 MHz Inova, referred to as **s400**: basement West wing, room WB-13 (phone: (780) 492-2573).

For concerns with the instruments or data stations please notify the NMR staff immediately in person, by phone, and/or by email.

2.1.2 The Spectrometers

The rest of the NMR instruments are located throughout the Department and can be found:

Sub-Basement:
- one 300 MHz Inova, referred to as **i300**: sub-basement East wing, room SB-3E (492-8619)
- one 400 MHz Inova, referred to as **i400**: sub-basement East wing, room SB-3E (492-8619)
- one 600 MHz VNMRS, referred to as **i600**: sub-basement East wing, room SB-3F (492-8588)

Basement:
- one 500 MHz Inova, referred to as **ibd5**: basement East wing, room EB-44 (492-3507)
- one 500 MHz VNMRS (auto sampler), referred to as **u500**: basement East wing, room EB-44 (492-3507)
- one 700 MHz VNMRS, referred to as **v700**: basement East wing, room EB-44 (492-2801)

First Floor:
- one 400 MHz Mercury+ (auto sampler), referred to as **m400**: main floor West wing, room W1-19 (492-4472)

2.2 Data Stations and Remote Access Points

All general access data stations and remote access points behave the same as the spectrometers.

Data stations support: archiving data to CD, DVD, USB devices, and remote login via ssh within the Department of Chemistry. Please see the Facility Supervisor for permission to access a data station remotely.

Remote access points DO NOT support: archiving data to CD, DVD, USB devices, or remote login via ssh. These systems are for processing and printing data ONLY.

Seven general access data stations are available for data processing and archiving data:

- **W1-19:** d401; **EB-44:** ibdw and d300; **WB-13:** d500**; SB-3G:** d601and d602; **CCIS 4-280:** d501

One remote access point is available for data processing and printing data only:

- **CCIS 4-280:** d501 Remote

3. NMR system overview

Each spectrometer consists of **three main components**:

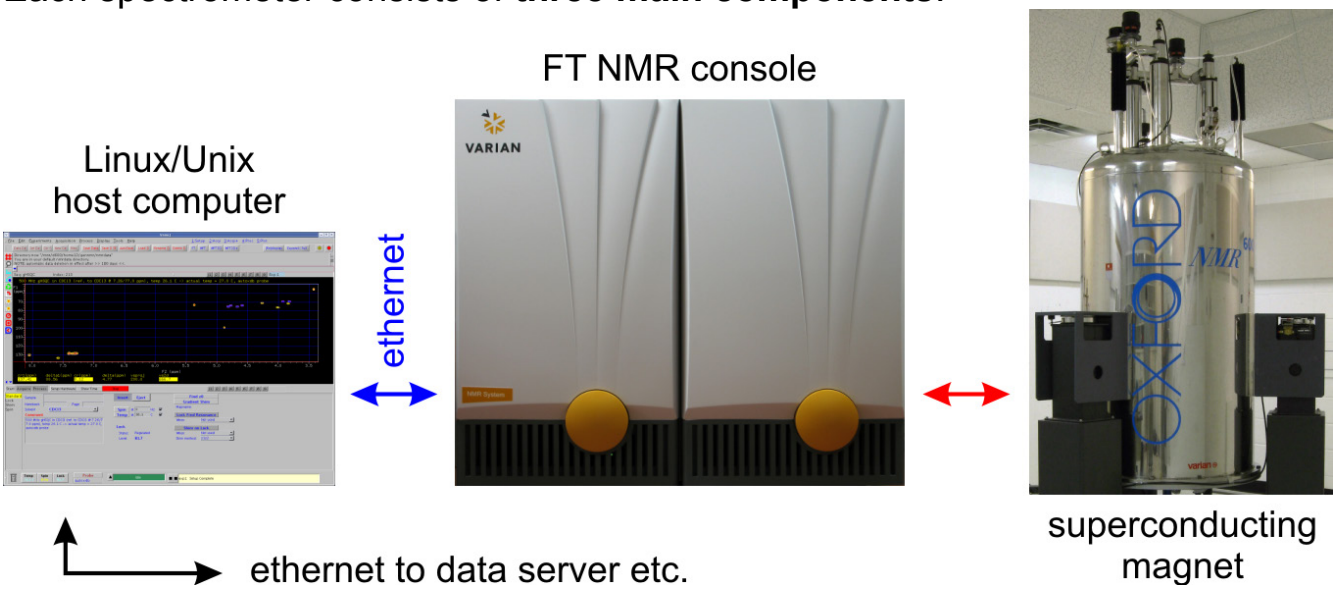


Figure 3.1: The main components of an NMR spectrometer

Aside from inserting and removing your sample the entire system is controlled nearly 100% from the spectrometer host computer.

3.1 Consoles, probes and other hardware

Spectrometer	**i300**	**m400**	**i400**	**ibd5**	**u500**	**i600**	**v700**
Console	Inova	Mercury	Inova	Inova	VNMRS	VNMRS	VNMRS
No. Channels	2	2	2	4	2	4	4
Probe	id	4 nuc	autoxdb	autoxdb	dual cold probe	autoxid	HCN cold probe
Tuning	Manual	**No Tuning**	Protune	Protune	Protune	Protune	Protune
Pulsed Field Gradient	Y	Y	Y	Y	Y	Y	Y
H1 S/N	180:1	150:1	280:1	350:1	2200:1	1200:1	7000:1
C13 S/N	**N/A**	150:1	180:1	250:1	1500:1	165:1	900:1
F19	N	Y	Y	Y	N	N	N
P31	N	Y	Y	Y	N	N	N
Li7, B11, Si29, Sn119, Plus Other Heteronuclei	N	N	Y	Y	N	N	N
2D	Y	Y	Y	Y	Y	Y	Y
3D	N	N	N	Y	N	Y	Y
Temp. Control	Y	Y	Y	Y	Y	Y	Y

3.2 Sensitivity and spectral dispersion

H1 sensitivity and resulting experimental times or sample concentration, respectively, to reach the **same signal-to-noise** is compared below:

	i300	**m400**	**i400**	**ibd5**	**u500**	**i600**	**v700**
H1 sensitivity	240:1	150:1	250:1	350:1	2200:1	1200:1	7000:1
expt. **time** to reach H1 s/n at a given sample concentration [**min**]	67	96	35	11	0.44	1.5	0.04
sample **concentration** required to reach H1 s/n in a given time [**mM**]	44	64	23	7	0.3	1	0.03
sweep width for 10 ppm H1 [Hz]	3000	4000	4000	5000	5000	6000	7000

Spectral dispersion is proportional to the field strength hence a 600 is twice as good as a 300. The probability for spectral overlap in *a two-dimensional* experiment decreases significantly with increasing field strength:

300: 1.0 400: 0.56 500: 0.36 600: 0.25 700: 0.18

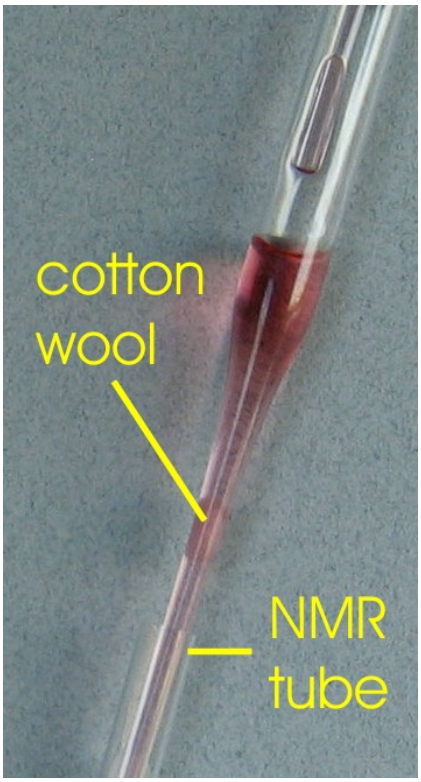
4. Sample preparation and pre-acquisition activities

4.1 Sample preparation

Filtering a sample prior to acquisition can greatly improve the quality of NMR spectra by removing contaminants that will adversely affect the spectral quality.

Contaminants that affect spectral quality in *descending* order of severity:

problem	what to do
paramagnetic substances, e.g. Ni, Mn	**filtration** through Chelex column (**Sep-Pak** for carbohydrates *)
solid or foreign material, e.g. septa pieces	**filtration** through cotton wool or 0.22 μm microfilter
high salt content	**filtration** through **Sep-Pak** for carbohydrates *
precipitate of dissolved compound	**filtration** through cotton wool or microfilter; change solvent



Filtration through cotton wool

Place a small amount of cotton wool (not glass wool) inside a Pasteur pipette. Medical cotton is best; it is said to not contain soluble organic components.

- dissolve solute of interest in a separate glass vial with **deuterated** solvent
- transfer solution directly to the NMR tube as shown in the figure on the left
- add remaining solvent to reach correct sample volume; if sample availability is low, the additional solvent can be passed through the cotton filter to rinse out remaining sample
- shake sample vigorously to obtain a homo-geneous (well mixed) solution
- see section 4.2 regarding correct sample volume

* For carbohydrate Sep-Pak filtration see the Appendix at the end of this document

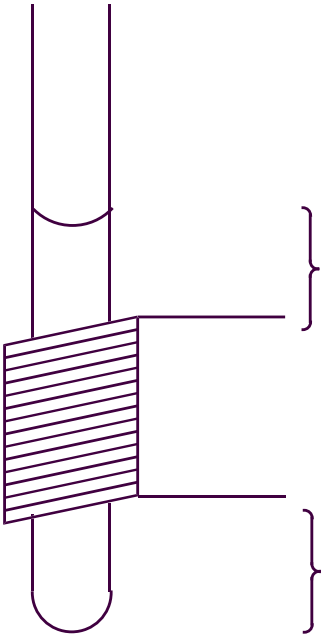
Figure 4.2: Filtration through cotton wool

4.2 Sample volume and sample concentration

Correct sample volume is critical for good results.

- Too low sample volume can make it impossible to obtain high quality spectra; the sample can be impossible to shim; poor magnetic field homogeneity
- The time required for shimming increases dramatically or may fail with incorrect sample volume (especially when sample volume is **too low**)
- Too high sample volume unnecessarily dilutes the sample and may consume potentially costly deuterated solvents (i.e. THF-d8 ~$80/5g)

Experimental time to obtain a certain signal-to-noise ratio is inversely proportional to (sample concentration)^2



Agilent probes are **0.7 mL probes**, hence the concentration of the sample **in mM** is given by:

$$\text{conc.[mM]} = \frac{\text{mg x 1000}}{\text{m.w. x 0.7}}$$

Ideal sample height is indicated by the bar in the figure on the right: it is **55 mm** for 0.7 mL.

Figure 4.3: NMR tube and sample volume

4.3 Spectral References

Parameter sets use automatic referencing as shown below (strictly speaking, only valid **at 27.0°C**):

solvent	**^{1}H** (number of lines)	**^{13}C** (number of lines)
$CDCl_3$	7.26 ppm (1)	77.0 ppm (3)
C_6D_6	7.15 ppm (1)	128.0 ppm (3)
CD_2Cl_2	5.32 ppm (3)	53.8 ppm (5)
CD_3OD	3.30 ppm (5)	49.0 ppm (7)
DMSO	2.49 ppm (5)	39.5 ppm (7)
D_2O	0.1% ext. acetone @ 2.225 ppm (1)	1% ext. acetone @ 31.07 ppm (1)
Ac-D_6	2.04 ppm (5)	29.8 ppm (7)

H1 and C13 spectra of all 7 solvents (incl. common impurities) can be found at http://nmr.chem.ualberta.ca → FAQ → solvents: spectra of supported solvents.

Referencing to *external* acetone puts HDO at 4.75 ppm (27.0°C). The HDO chemical shift changes **+/- 0.008 ppm for every -/+ 1.0°C** (note +/- signs!).
If changes are made by the user, correct referencing can be restored by using the EZ NMR P+P panel *Referencing: Adjust to Default* whereby the referencing is restored in all supported solvents and experiments, 1D or 2D, homo- or heteronuclear.

Alternatively, if different referencing is desired and **in unsupported solvents**, the EZ NMR P+P panel *Referencing: Adj to Line @ Cursor* allows placement of the cursor on a spectral line and entering the desired value in ppm (1D and 2D).

4.4 Access to spectrometers, online reservations, etc.

v700: regular access to Carbohydrate Center only, others by special request

i600: regular access to Carbohydrate Center and the research group of Dr. Vederas

i300, i400, m400, ibd5, u500: **General access** via self-registration and on-line reservation system at http://nmr.chem.ualberta.ca which enforces the reservation rules. Reservation rules are spectrometer specific but do change periodically, see nmr.chem.ualberta.ca for instrument specific details. The reservation rules for the **ibd5** are given below as an example:

ibd5 reservation rules, every day (365 days a year):

- **time slots are 15 minutes from 08:00 (8 a.m.) to 20:00 (8 p.m.)**
- no booking restrictions from 20:00 (8 p.m.) until the next morning 08:00 (8 a.m.) time slot
- **no reservations are allowed more than 24 hours in advance** with the exception of tomorrow's overnight period which can be booked after 16:00 (4 p.m.)
- **the 5 minute rule**: if the spectrometer is booked but not used for more than **5 minutes** of the starting time, the reservation is forfeit
- **Monday - Friday:** no more than *four* consecutive time slots per day between 08:00 (8 a.m.) and 20:00 (8 p.m.)
- **Saturday and Sunday:** *up to **twelve** consecutive time slots (3 hours)* but only once a day for any user between 08:00 (8 a.m.) and 20:00 (8 p.m.)
- **Holidays and U of A closure days:** reservation rules as **Saturday and Sunday**

Time pooling or combining your NMR time with another user is strictly prohibited and will result in withdrawal of your access to the NMR spectrometers.

4.5 Logging into the system and starting VNMRJ

Linux computers are **multi-user machines** that protect data for each user with a password.

It is *critical* that the correct user account is used for the proper function of the system and for saving and locating data.

Logging on to the system requires a username and password, these are group specific and case sensitive.

Figure 4.4: Linux login screen, enter username.

Figure 4.5: Linux login screen, enter password.

Usernames and passwords can be obtained from your research group or from the Facility Supervisor.

If the previous user forgot to log out, please log out of the account and log in using your group specific user account.

The entire NMR system has approximately 3 Terabytes (3000 Gbytes!) of disk space, and over a million FID's. If you saved your data in the wrong place:

- a search of immense proportions for the data can result, especially if you don't remember the exact filename, requiring orders of magnitude more time than a quick log out/log in

- the **ownership** of the file(s), if ever found, is **wrong** and requires intervention by the system administrator to allow you to do anything with the file(s)
- some macros act differently depending on the username

Using the correct username prevents writing the data in the wrong place and automatically provides the correct ownership of the data.

After logging in, one arrives at the Linux desktop, shown below. In fact, there are **two desktops** available to use, both are identical but it is recommended that the first desk top, or default, is used for VNMRJ and the second for data manipulations.

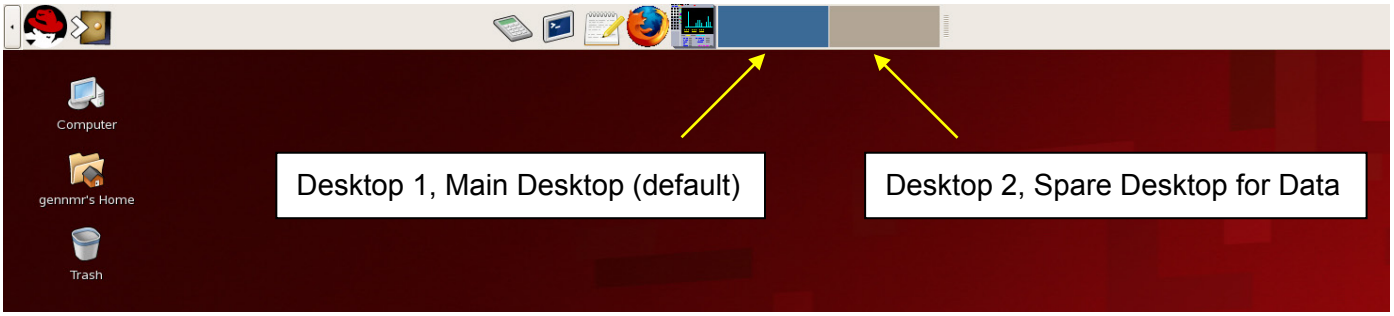


Figure 4.6: Linux main (default) desktop

4.6 The VNMRJ interface

Start VNMRJ with a **single** click on the icon indicated below (**DO NOT DOUBLE CLICK!**):

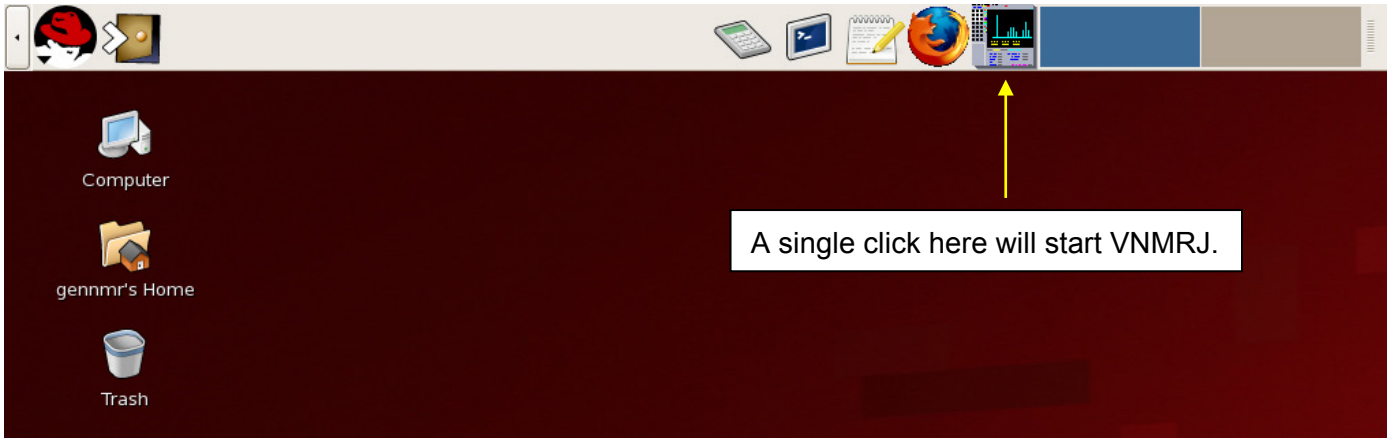


Figure 4.7: Starting VNMRJ

Research accounts require a valid online user reservation name to use VNMRJ:

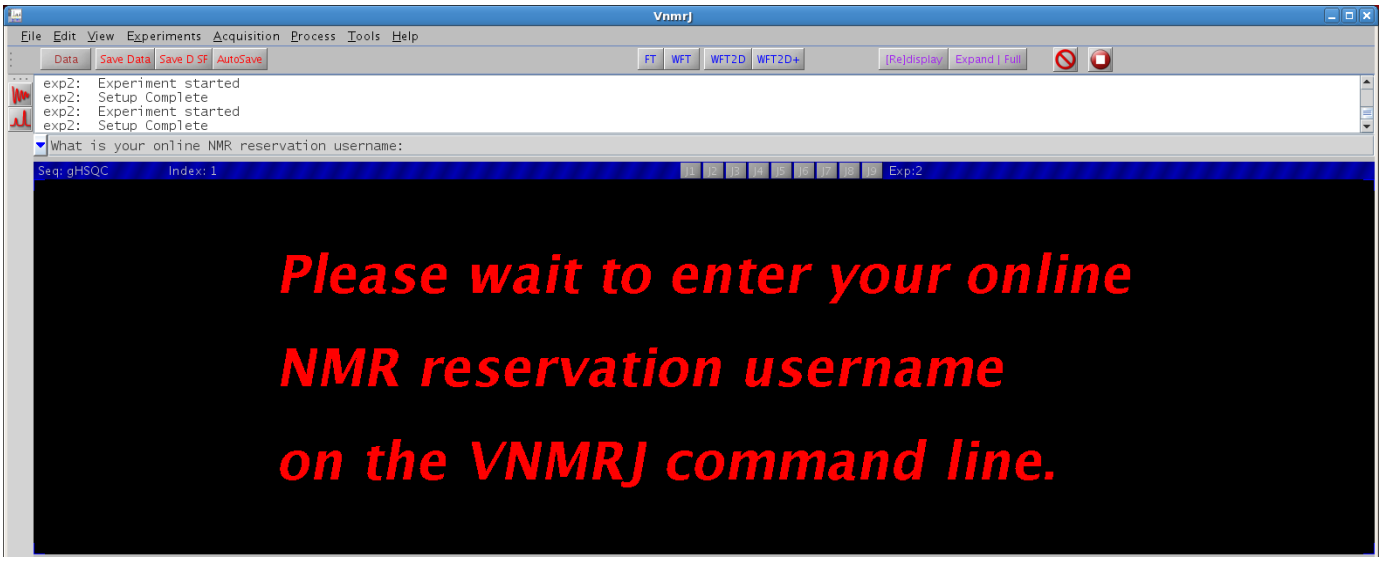


Figure 4.8: User identification

Invalid input will close VNMRJ, a valid online reservation username provides the following information and makes the program available to use:

```
********************************************************************** disk use gennmr: 642.9 MB | 0.6 GB
 No manual tuning - use ProTune for tuning on this spectrometer!  2.2 percent of quota (2007.04.24-08:55)
**********************************************************************
    solvent    lockPOWER lockGAIN    z0 (drift down to less positive/more negative values)
    CDCl3          35        42      4137
    C6D6           31        31      4042          For solvents not listed here: select values
    CD2Cl2         23        40      1405          for a solvent with similar chemical shift, then
    D2O            32        32      723           open the Lock Panel, increase lockPower|Gain
    CD3OD (CD3)    12        40      -360          and search for the lock with the z0 button,
    acetone-D6      8        42      -1724         e.g. for acetonitrile select acetone.
    DMSO           31        31      -1737
  LockPOWER/GAIN are for a shimmed magnet; higher initial values might be needed.
  automatic update of z0, timebase: 2007-02-13-10:30

  NMR questions?                ---> try http://nmr.chem.ualberta.ca
```

Figure 4.9: z0 and disk usage information

Tuning information, white lettering shown at the top of the message above, is spectrometer dependent, e.g. no tuning on m400, ProTune on i400, ibd5, u500, i600 and v700, manual tuning on i300.

Disk usage and disk quota information on the central data server is shown in green lettering top right hand corner of the message above. Surpassing the disk quota will prevent saving of data.

LockPower, lockGain and z0 information are spectrometer dependent! z0 slowly changes with time (magnet drift). The z0 information can be used as a guide when automated locking fails.

After starting VNMRJ, and identifying yourself to the system, you should arrive in the last experiment number used before exiting VNMRJ. **VNMRJ has 9 experiments** by default (additional experiments can be created by the user). The dark blue box indicates the **currently joined** experiment.

Use any of the grey buttons to join one of the 9 default experiments.

Figure 4.10: Join experiment buttons and currently joined experiment

The VNMRJ interface is shown below.

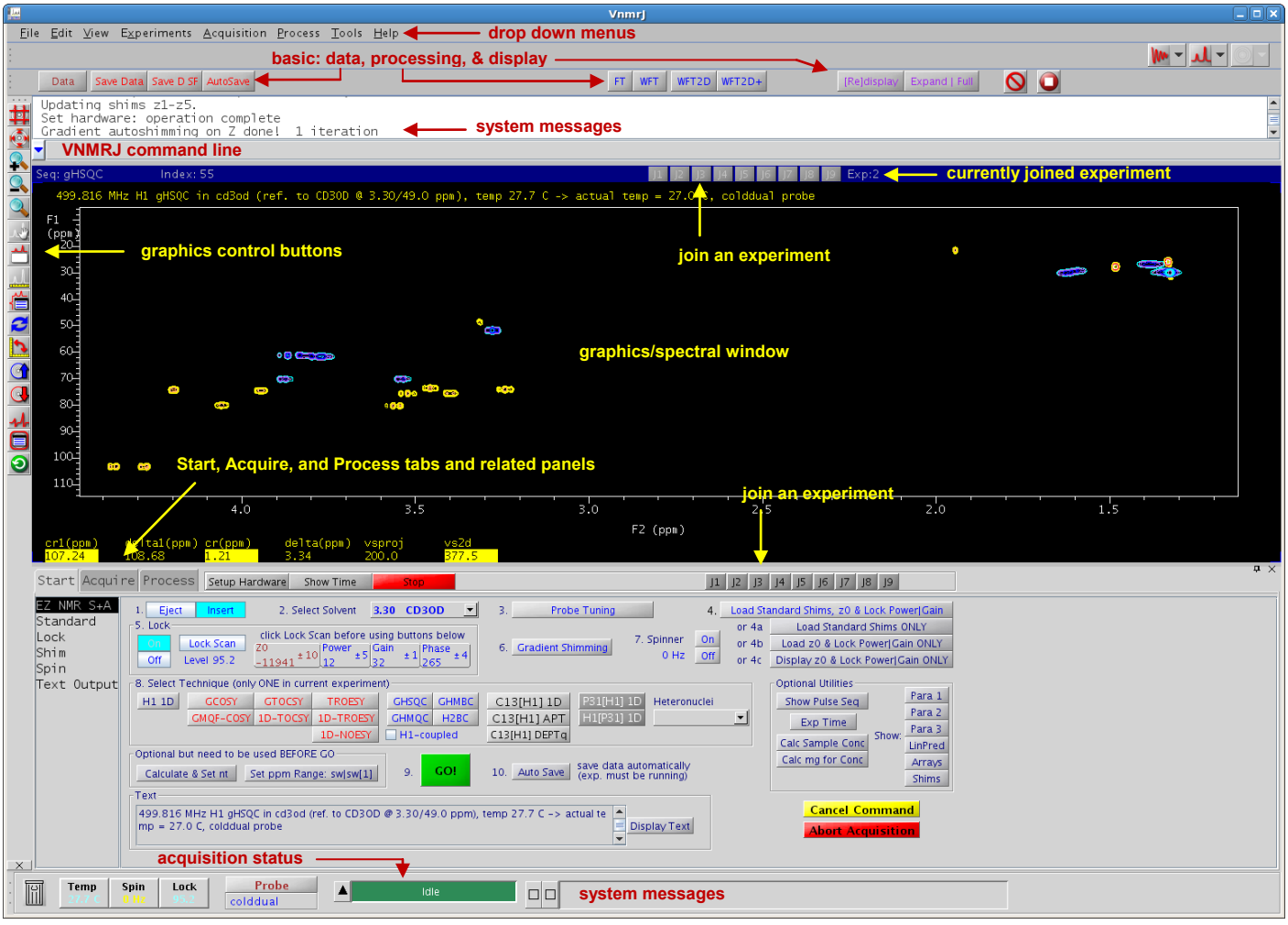


Figure 4.11: The VNMRJ interface

There are two fundamentally different ways to operate VNMRJ:

- changing parameters, entering commands and macros on the VNMRJ command line with the **keyboard**
- using buttons on panels with the **mouse** thereby choosing predefined parameter sets and executing predefined macros

All Linux mice are **3-button** button mice, the scroll wheel that acts as a middle button. The Linux mouse:

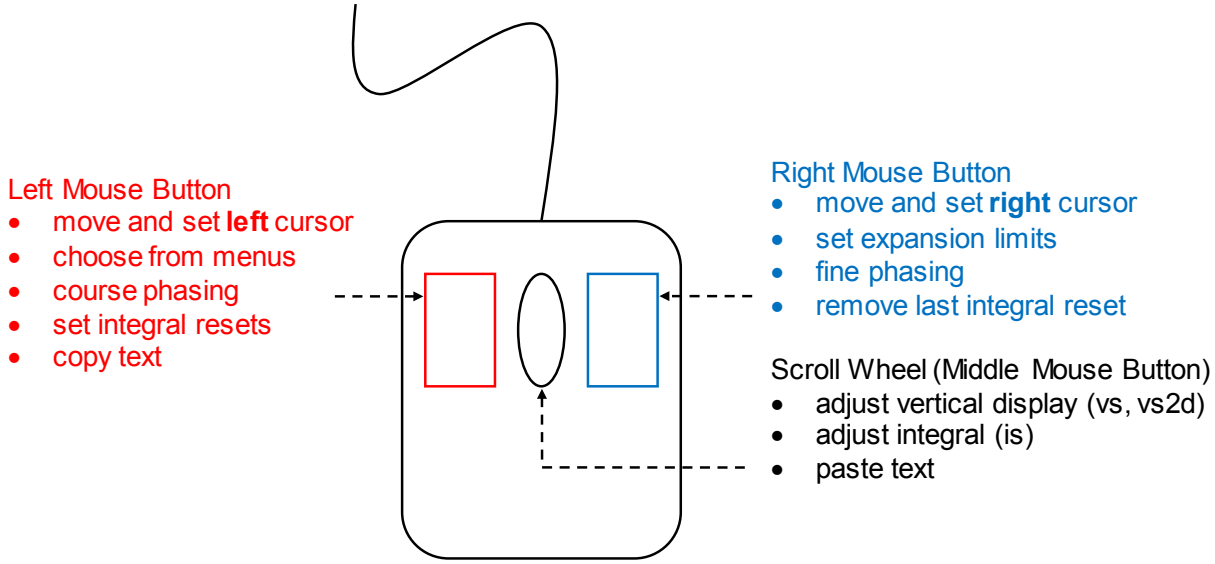


Figure 4.12: The Linux Mouse

Messages coming from VNMRJ and the console (system messages) are limited to one to three lines. The acquisition status, outlined in Fig. 4.11, does not indicate which experiment is being acquired. Click on the buttons adjacent to these information boxes to open information and acquisition status windows. Place these windows on the second monitor and enjoy feedback from the spectrometer.

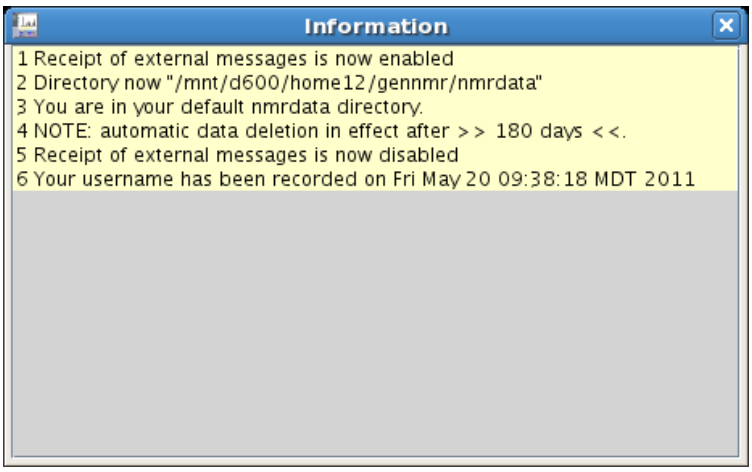


Figure 4.13: The Information window

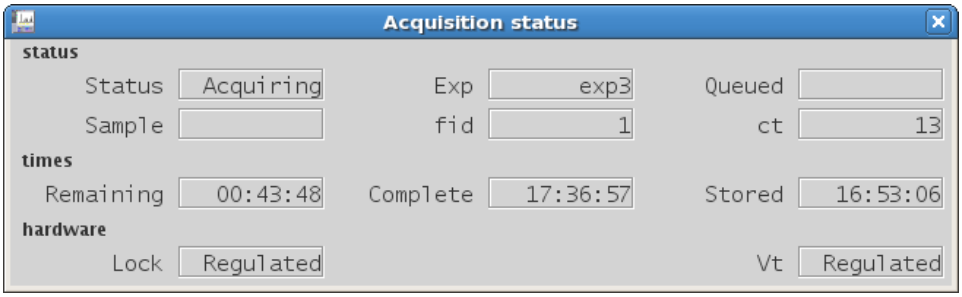


Figure 4.14: The Acquisition status window

5. EZ NMR S+A

The EZ NMR S+A (**S**etup and **A**cquisition) panel is an entirely in-house created panel. This panel provides a simple central interface for acquisition of routine experiments. The panel should be used as a **To Do List** by following the numbered steps **(simply count to ten!).**

Notes:

1. In a multi-user environment it is *unknown* what the last user left behind (z0, shims, probe tuning, solvent, etc.) and may not be suitable for your sample
2. The correct solvent is fundamental to the proper setup of the instrument
3. 20 solvents are available in the drop-down list
4. Allow the spectrometer time to complete probe tuning, loading standard shims, finding the lock, and gradient shimming before proceeding to the next step. Completion messages are provided and failure to wait will hang the system.

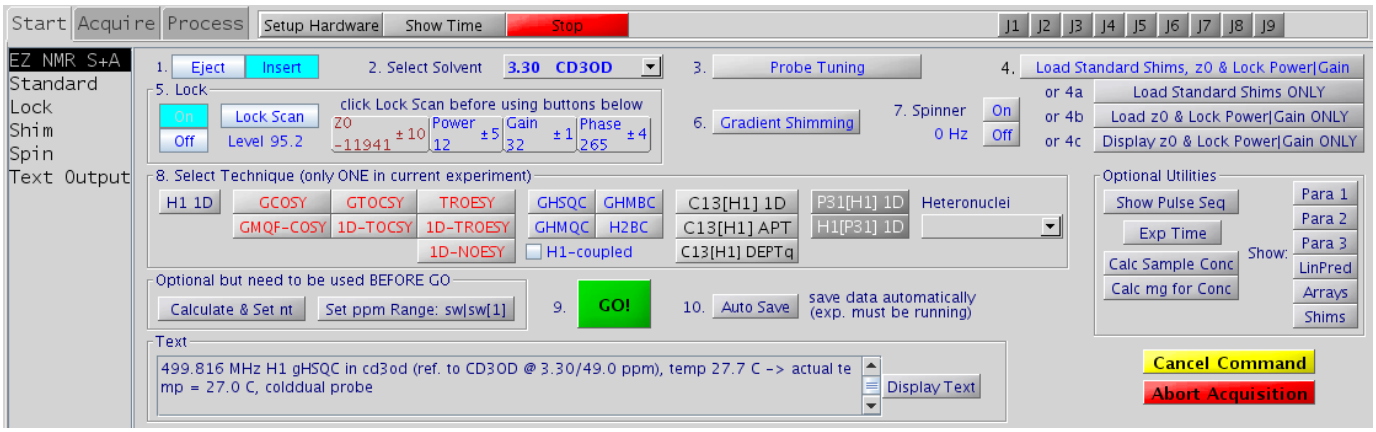
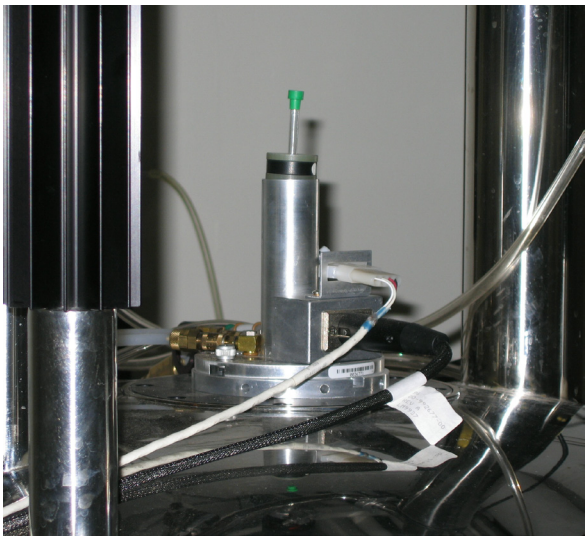
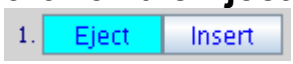


Figure 5.15: EZ NMR S+A panel

5.1 Step 1 - Inserting a sample



- click on the **Eject** button

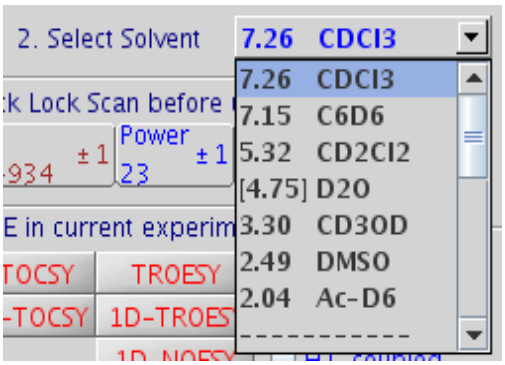


- if a sample is in the magnet it will float on a cushion of air to the top of the magnet
- insert your sample correctly into the spinner (see figure 4.3); **use the depth gauge**
- place sample **with spinner** as shown in the figure to the left; the sample floats on a cushion of air
- click on the **Insert** button

- wait until sample is all the way inside the magnet (the system will turn the air lift off)

Figure 5.16: Inserting the sample

5.2 Step 2 – Select Solvent



- **select the correct solvent from the drop-down menu**
- seven common solvents will provide automatic referencing
- the remaining 13 less common solvents below the dashed line may require manual referencing

Figure 5.17: Solvent drop-down menu

5.3 Step 3 – Probe Tuning

- **ProTune or automated tuning on: i400, u500, ibd5, i600 & v700.**
- **NO tuning on m400.**
- **Manual tuning on i300.**

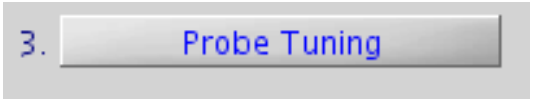
All parameter sets are based on a properly tuned probe. Failure to tune can result in partial or complete loss of NMR signals in your experiment.

Experiments that involve a hetero or X nucleus (where X can be C13, P31, B11, etc.) both the X nucleus and H1 need to be tuned and in that order!

Examples of experiments that need both the X nucleus and H1 tuned: APT, C13[H1] 1D, gHSQC, gHMBC, B11[H1] 1D, H1[P31] 1D, etc. but not F19.

Tuning depends on:

- solvent
- sample volume
- salt content
- NMR tube



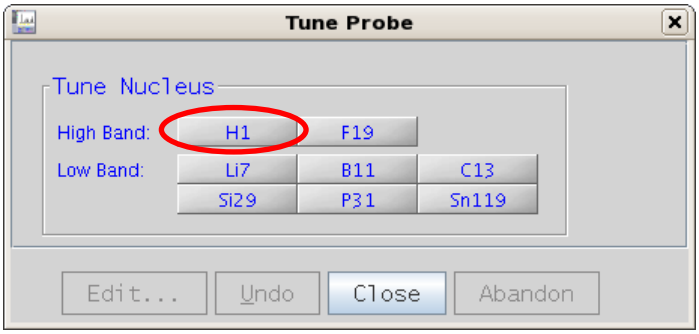


Figure 5.18: Tuning with ProTune

Tune Probe

- click on **3. Probe Tuning**
- in the Tune Probe pop up window, click on the desired nucleus , H1 is selected as an example, to initiate tuning
- the following messages will be displayed, please wait for the completion message before continuing

Automated tuning protocol initiated.
Tuning to 498.124 MHz at 3 percent of optimum.
Please wait for completion message
IN THIS WINDOW before continuing.

Tuning done:
ok - tuned to 498.125 MHz with match at
1.4 percent of optimum.

- repeat for second nucleus if needed (e.g. tune C13 and H1 for HM[Q|B]C, HSQC and APT, etc.)

Manual tuning on the i300:

Please see the NMR Service staff for instruction.

Notes:

Use **no force** when tuning. The tuning rods move freely and do not require force to move them. Appling force may damage the probe. The **repair is costly** and will result in **significant down time** of the spectrometer.

Tune each nucleus in turn. Select channel (Chan) 1 for H1 and channel (Chan) 2 for C13. Set attenuation (Atten) to 9 for maximum tuning sensitivity.

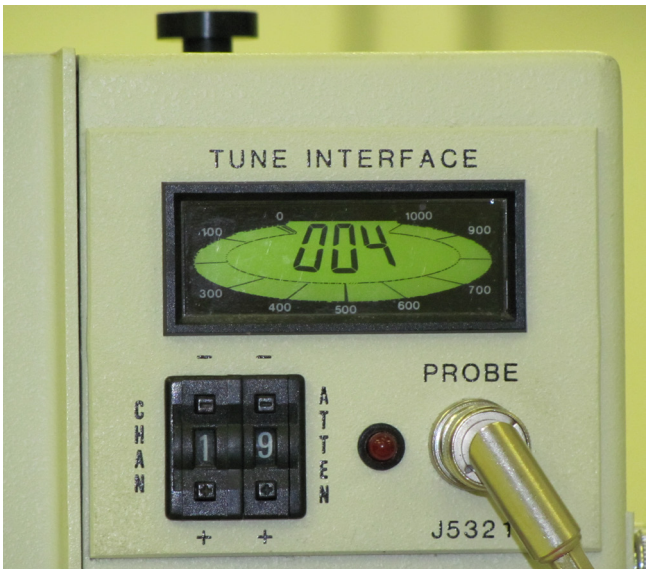


Figure 5.19: i300 Tune Interface

Correct manual tuning is achieved by *minimizing* the reading of the reflection meter (digital meter underneath the Tune Interface label) next to the magnet. A guideline of 10 or less on the reflectance meter is given to indicate good tuning.

When complete set Chan to 0 and Atten to 9.

Do not forget to return the cable(s) to their original connections when done!

5.4 Step 4 – Load Standard Shims, z0, Lock Power|Gain

Why lock? Every magnet slowly drifts (field drift) to lower magnetic field strength, typically 1 to 5 Hz/hour. To achieve frequency stability over the duration of an experiment (16 hours or more for some 2D, days for 3D, 4D, 5D!), FT spectrometers use the **deuterium signal of the solvent as an internal lock**. Drift compensation and stability is achieved through comparison of the spectrometer frequency with the lock signal frequency.

The amount of deuterium is quite different in $CDCl_3$, D_2O, and CD_3OD. Therefore, lock power and lock gain are solvent/spectrometer-dependent (use EZ NMR S+A button **4c: Display z0….** or enter **z0** on the vnmr command line to run the z0 macro).

Some solvents like CD_3OD have *two* deuterium signals. Locking on OD is difficult, therefore locking on CD_3 is recommended, “much more likely to happen” and assumed throughout these notes. If locked on OD, the spectral window and referencing will be off by ca. 1.5 ppm.

Loading Standard Shims and Automated Locking step-by-step

Figure 5.20: Load Standard Shims, z0, Lock Power | Gain

- click on **4. Load Standard Shims, z0, Lock Power | Gain** (note that z0 is the Lock Frequency)
- a standard **shim set is loaded and sent automatically to the magnet**
- the system will find the lock frequency (z0) automatically
- the system automatically turns the lock **on,** and will provide a message when the lock has been found, please wait for the completion message before continuing
- clicking on the **Lock On** button in *5. Lock* panel may also be needed to lock to the solvent
- **click on Lock Scan and wait about 10 seconds**, if the lock level does not go up or the lock does not look like similar to Fig. 5.24 you may need to add lock gain and/or lock power, adjust the lock phase or lock the sample manually (see the next section for manual locking)

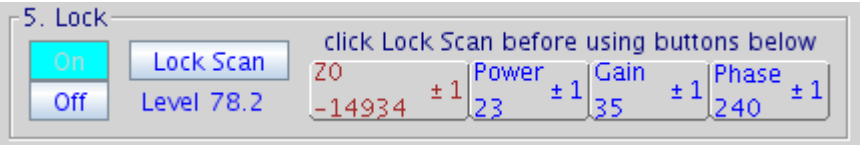


Figure 5.21: Lock Control

5.4.1 Locking manually step-by step

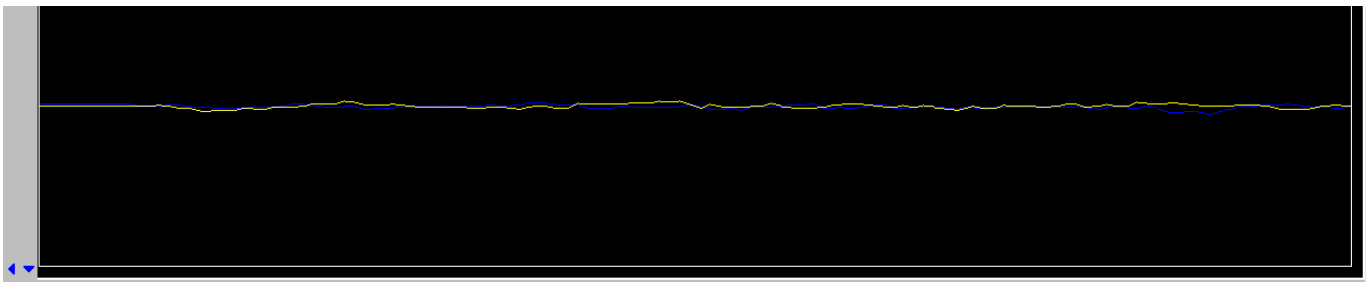


Figure 5.22: Lock Scan (far off resonance)

- change the lock frequency by clicking on the z0 button until a lock signal is observed as a wavy line
- the middle mouse button toggles 3 sensitivity settings of the z0 button
- if necessary increase Lock Gain first, and then if necessary increase Lock Power

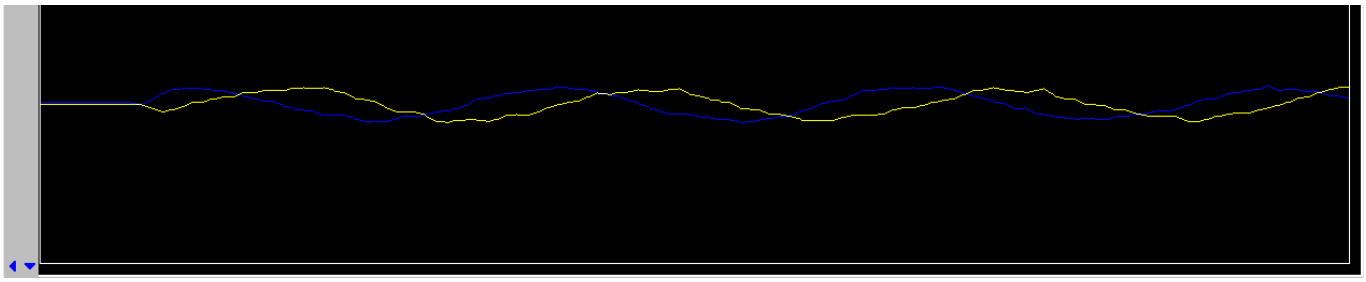


Figure 5.23: Lock Scan (off resonance)

- change z0 to **reduce** the number of “frequency beats” as shown below

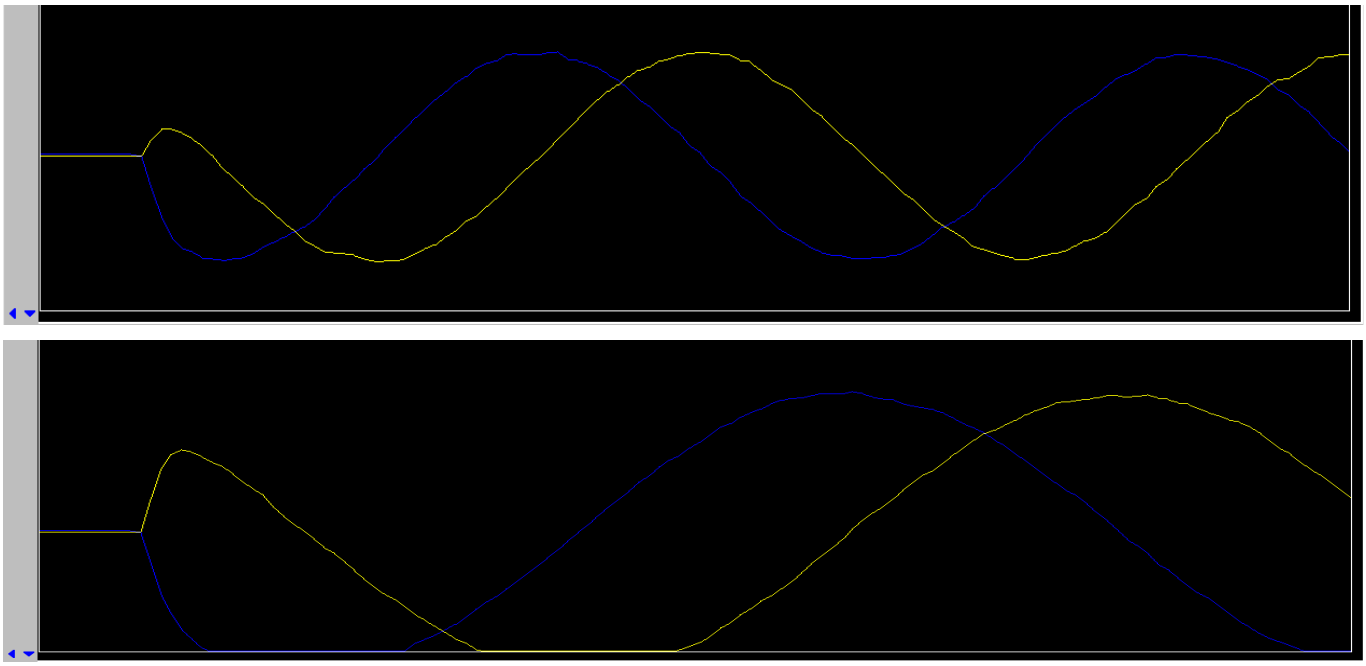


Figure 5.24: Lock Scan (approaching on-resonance)

- change z0 until **on resonance: the lock signal will be similar to that shown in the figure below, a “plateau”**

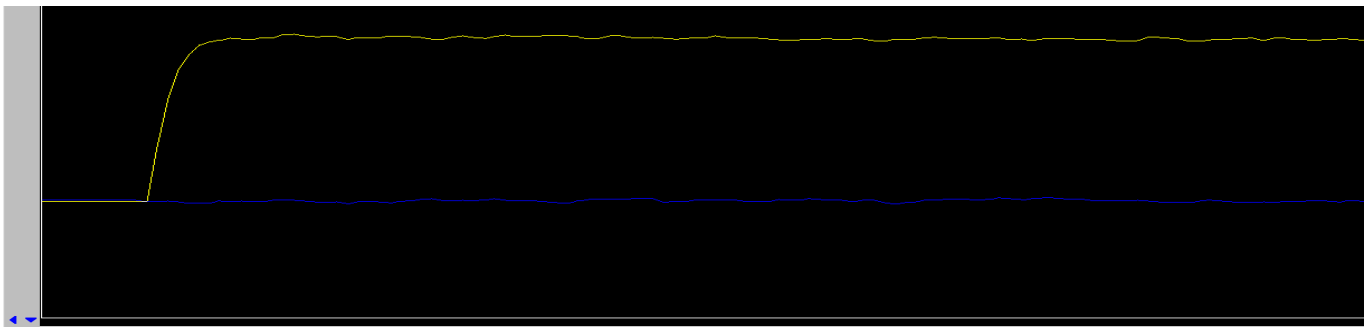


Figure 5.25: Lock Scan (on resonance)

- click on the button Lock On in *5. Lock* panel. Further adjustments to z0 are no longer necessary
- **adjust lock phase** if necessary to maximize Lock Level then **turn off Lock Scan**
  - for systems such as the u500, i600, or v700 (VNMRS console) adjust lock phase with the yellow lock line on top, all other instruments (Inova and Mercury+) only have a single yellow lock line

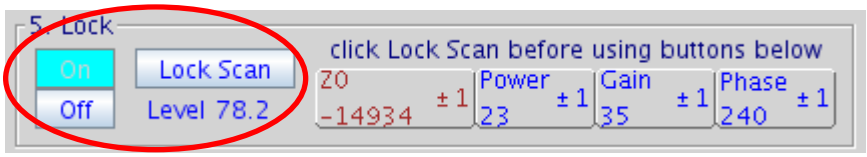


Figure 5.26: Lock Control (on resonance, locked)

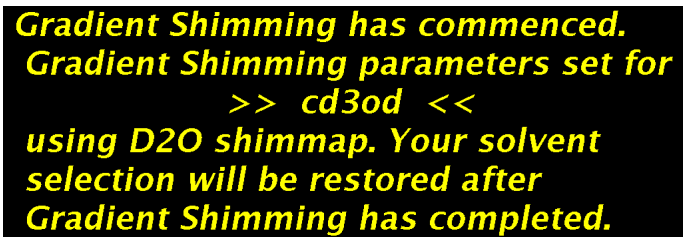
If not successful turn Lock Off and/or continue to search for the z0 frequency. Once locked, avoid high lock power which causes saturation, i.e. more power flows into the sample than can be dissipated through relaxation processes resulting in sample heating, poor quality spectra, and potentially no lock.
Use information from z0 macro to estimate suitable values.

General guidelines:

- If needed, increase lock gain and when necessary increase lock power.
- **The Lock Level should be between 80 and 100 (but *not* over 100) for a shimmed sample otherwise the lock may be lost under the effect of gradients.**

5.5 Step 6 – Gradient Shimming

Purpose of Shimming: optimize the homogeneity of the magnetic field by using shim gradients: Z^1(linear), Z^2 (squared), X, Y and many more
(There are as many as 28 shims available on some instruments!).



- **6. Gradient Shimming**
- message displayed when gradient shimming starts indicating a temporary change of solvent
- wait until complete and then turn the spinner on if desired

Figure 5.27: Gradient shim button and gradient shimming message.

Gradient shimming is an automated shimming protocol that will adjust shim gradients Z^1 to Z^4 in a very short period of time and is the recommended method of shimming a magnet.

Only if gradient shimming fails (i.e. gradient shimming fails to converge, all peaks within the spectrum are split or similar irregularity, etc.), use **manual shimming**:

- change **Z1** to obtain **maximum lock level** on “speedometer”
- change **Z2** to achieve the same, then return to Z1
- repeat until no further improvement can be achieved
- the middle mouse button changes the sensitivity of the shim and lock buttons
- **if shim changes are large, re-optimize lock phase**

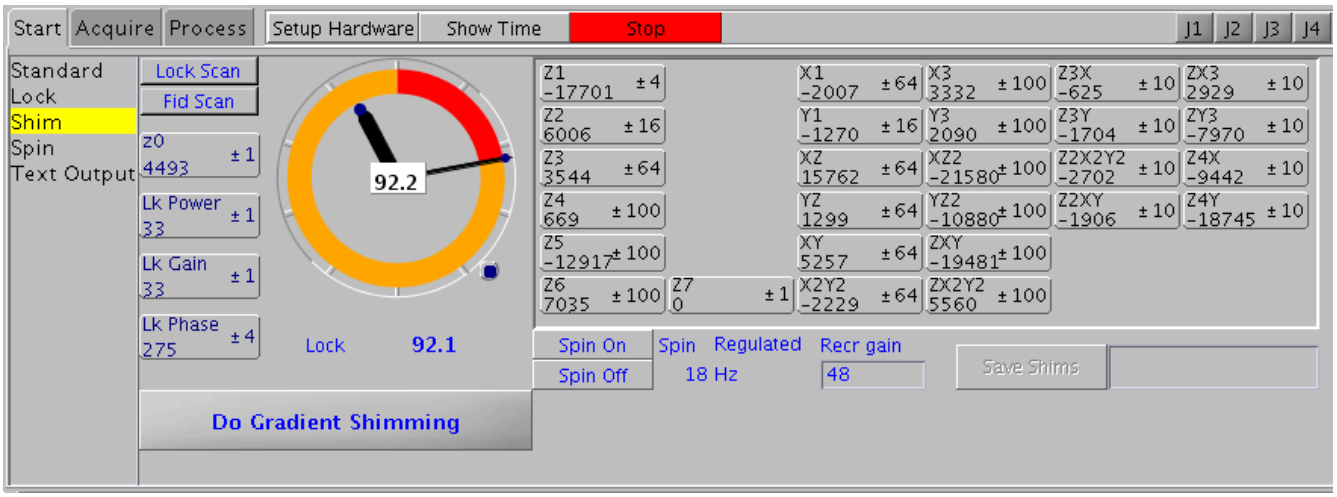


Figure 5.28: Start Tab - Shim panel

5.6 Step 7 – Spinner

Turn spinner on if desired.

Figure 5.29: Spinner button

Notes:

1. On most modern NMR spectrometers the improvement seen in magnetic field homogeneity from spinning a sample is very minor.
2. The system will turn the spinner off for some techniques, i.e. all 2D experiments and selected 1D experiments.

5.7 Step 8 – Select Technique

Common 1D and 2D experiments can be selected as shown in the following Figure:

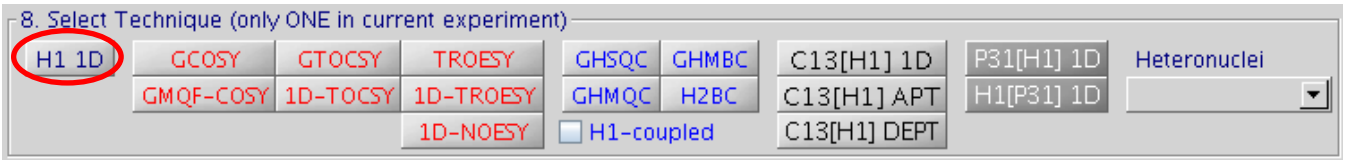


Figure 5.30: EZ NMR techniques

Note: It is recommended to always record a H1 1D before recording any 2D or heteronuclear experiments. The time requirement for a H1 1D is minimal and this allows one to check

- if the sample is really worthwhile (purity, concentration, etc.)
- if the homogeneity of magnetic field (shimming) is what it should be
- if other adjustments are necessary (spectral width, nt, etc.)

Heteronuclear techniques (Li7[H1] 1D, B11[H1] 1D, F19 1D, Si29[H1] 1D, Si29 gHSQC, Si29 1D, Sn119[H1] 1D, etc.) can be selected from the Heteronuclei drop down menu.

Optionally one can make adjustments to the selected technique prior to starting the experiment. For example a H1 1D:

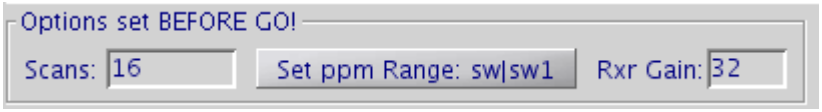


Figure 5.31: Optional adjustments for selected technique

Scans:

- Adjust the number of scans or transients (nt) directly for an experiment.
- Signal to noise improvements scale as a square root. For example if one would like to double the signal to noise obtained for an experiment acquired with 16 scans one would need to acquire 4 times as many scans or 64 scans.
- Experimental time scales directly with the number of scans. For example the total experimental time will increase by a factor of 4 if the number of scans are changed from 16 to 64.

Set ppm Range: sw|sw1

- Calls a macro to calculate the spectral width (sw and/or sw1) based on user input.
- For example setting the sweep width for H1 1D from 8.4 ppm to 1.2 ppm.

The system will prompt:

Enter desired low field (highest ppm) limit: → 8.4

Enter desired high field (lowest ppm) limit: → 1.2

Rxr Gain:

- Adjust the receiver gain (gain) directly.
- If during an acquisition a Receiver Over Flow or ADC Over Flow error occurs, stop the experiment and reduce Rxr Gain until no further errors occur.

5.8 Step 9 – GO!

Figure 5.32: Start the NMR experiment!

Press the very large GO! button to start the experiment. Sit back, relax, your experiment is in progress.

As data becomes available the user can start processing the data. See section 6 for details.

5.9 Step 10 – Auto Save

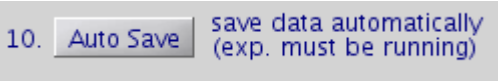


Figure 5.33: Auto Save

Included in the EZ NMR S+A to do list, but optional, is Auto Save.

Auto Save will:

- save the data for the user in their absence
- save the data in the current working directory using a standard naming format (see section 7.2 for details on the standard naming format)
- save the data as long as the experiment is running
- save the data as long as the instrument does not encounter an error
- save the data when the experiment finishes

6. Processing with EZ NMR

Ultimately all acquired data will need to be processed to turn the raw data (FID) into an NMR spectrum. The same philosophies that lead to the creation of the EZNMR S+A panel also lead to the creation of the EZ NMR P+P panel. There are two dynamic panels for processing 1D and 2D data sets.

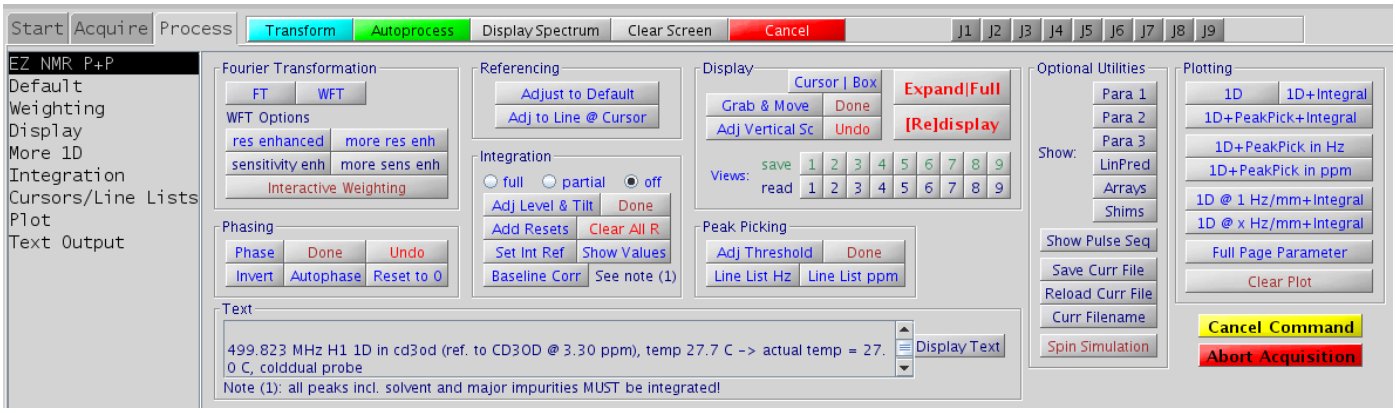


Figure 6.34: EZ NMR P+P panel for 1D data

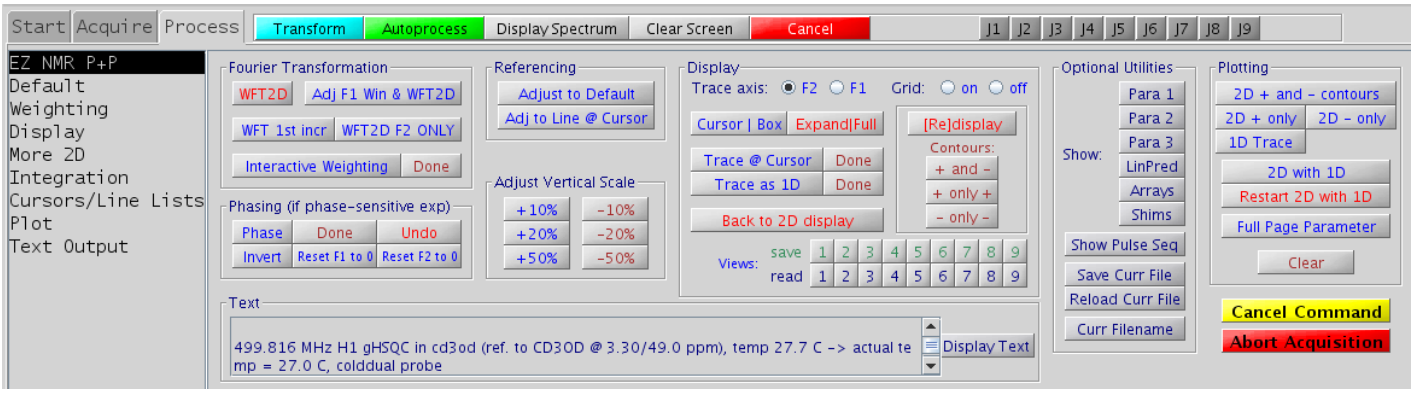
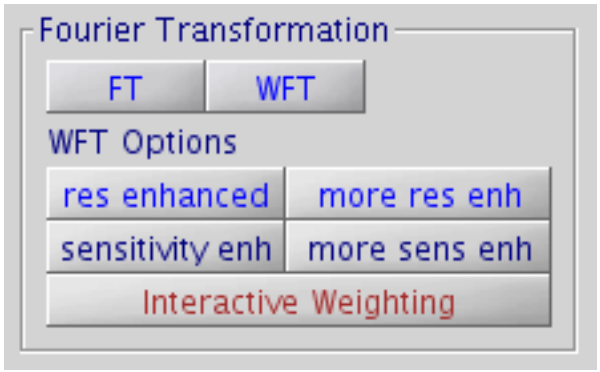
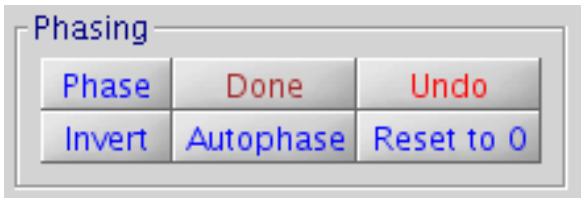
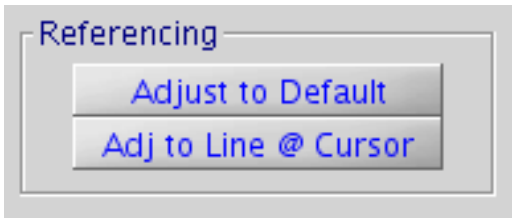
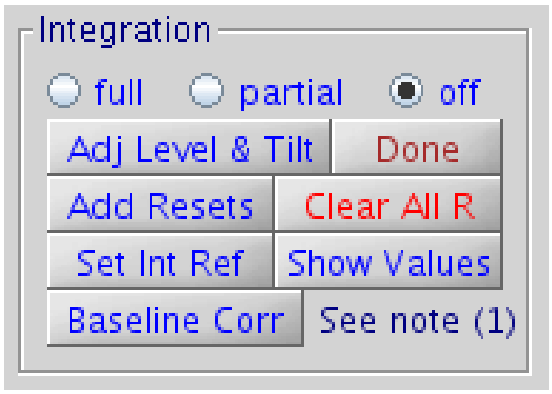
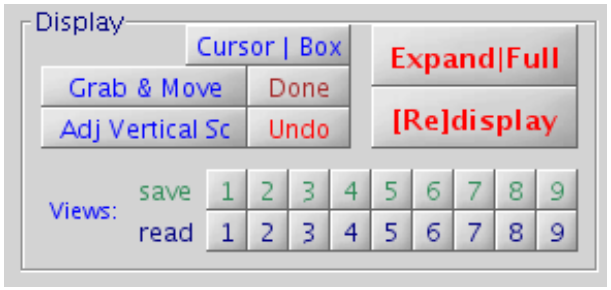


Figure 6.35: EZ NMR P+P panel for 2D data

Experiments can be processed while an acquisition is active. Data is sent from the console to the spectrometer host computer for processing when a block is complete (1D and 2D) and/or when an increment is complete (2D only). What follows below is applicable for an experiment that is running or has completed.

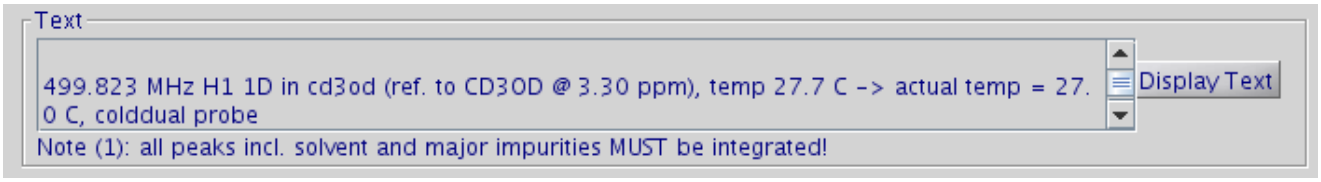
6.1 Processing a 1D data set, a H1 1D

	select from: • plain FT (best for integrations) • WFT (apply default window/weighting/apodization function) • two levels of **resolution** enhancement • two levels of **sensitivity** enhancement • adjust window functions interactively
	• Use the Phase button and click on Done when finished. Use other buttons as needed. • Parameters rp and lp should be reasonable: **360 > rp,lp > -360.** • Reset to zero if needed and start again
	• Put cursor on any desired peak and assign a reference chemical shift or use default referencing
	• start with full or partial • adjust Level and Tilt of integral if needed, finish with Done button • set Integral Resets with left clicks (right click: undo last set point) • clear all resets if needed • put cursor on desired integral region and set integral reference • Baseline correction: all peaks including solvent and major impurities MUST be integrated.
	• adjust Threshold for peak pick, finish with Done button • display peaks in Hz or ppm • plotting has its own selection of Hz *vs.* ppm



- Grab and Move: move the spectrum with the left mouse button, must be finished with Done
- vertical scale adjusts so largest peak fits; can be undone
- up to 9 individual views can be saved and retrieved

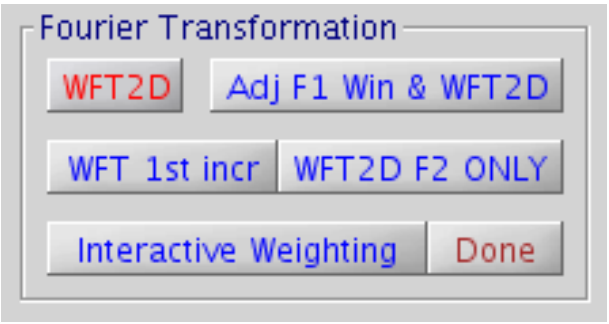
Text can be added, modified, deleted directly in the text box shown below:

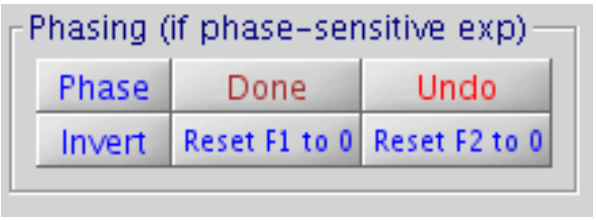


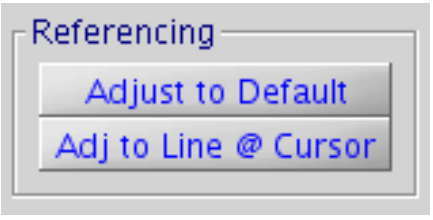
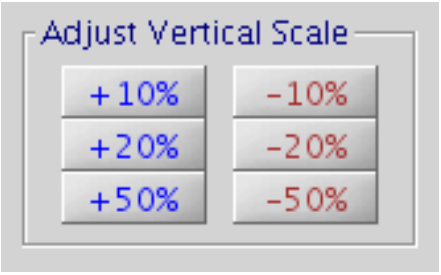
- parameters can be displayed in a separate window (equivalent to the dg, dg2, dg1, dglp, da, and dgs commands)
- Save/Reload for previously saved data only, NOT FOR INITIAL SAVING OF DATA (see 7.1)
- the current filename can be shown
- Spin Simulation can be entered here which leads to a new window

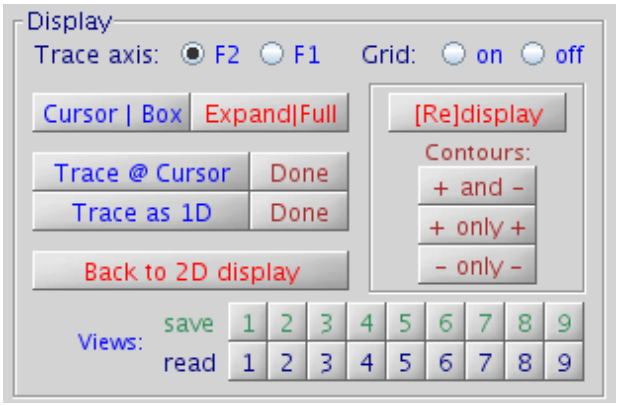
- Select from a variety of the plotting styles. All include a scale in ppm and text.

6.2 Processing a 2D data set, a GCOSY

	• WFT2D: process spectrum in both dimensions (F2 and F1) • Adj F1 Win & WFT2D: use if experiment is still running; F1 window function will be adjusted dynamically • WFT 1st incr: use for some phase sensitive expts. (i.e TROESY), process and then phase 1st increment

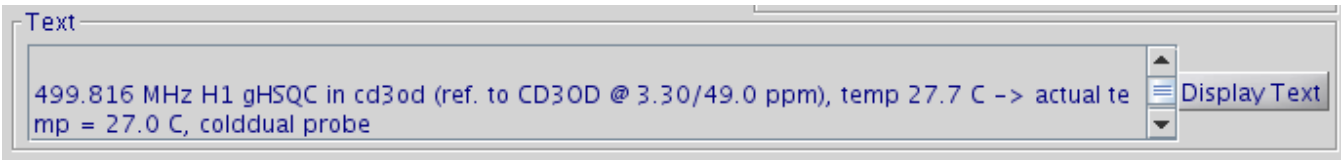
	• For phase-sensitive expt's such as TROESY and GHSQC.
	  • Phase 1st increment
	  • Phase both F1 and F2 dimensions of 2D map
	  • Parameters rp, lp, rp1, and lp1 should be reasonable:
	  360 > rp, lp, rp1, lp1 > -360
	• Click on Done when finished. Use other buttons as needed.

	• Put cursor on desired peak and assign reference chemical shift (F2 and F1) or use Default referencing
	• Increase and decrease vertical scale by step size indicated.

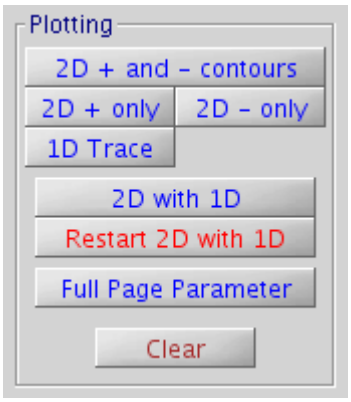


- select display with or without grid
- default axis (F2) normally does not need changing; F2 and F1 radio buttons result in 90° rotation of spectrum
- contours can be displayed:
  - positive and negative (+ and -)
  - positive only (+ only +)
  - negative only (- only -)
- Trace @ Cursor: 1D trace at the top of the 2D
- Trace as 1D: selected trace as 1D without the 2D
- up to 9 individual views can be saved and retrieved

Text can be added, modified, deleted directly in the text box shown below:



- parameters can be displayed in a separate window (equivalent to the dg, dg2, dg1, dglp, da, and dgs commands)
- Save/Reload for previously saved data only, NOT FOR INITIAL SAVING OF DATA (see section 7.1)
- the current filename can be shown



- Select from any of the plotting styles as desired. All include scales in ppm and text.

7. Data and Data Management

Data from the acquisition computer are stored on the disk of the host computer every time a block (bs) is complete.

This file is of temporary nature.

If not saved by the user, the data are lost for good when another acquisition is started in the same experiment. There is no undo, undelete, or trash for data lost in this manner.

ALWAYS SAVE YOUR DATA. Data sets are cheap, in fact data are free and use recycled electrons for storage. Printed spectra are easily lost or damaged and re-synthesizing a compound can be expensive in both time and chemicals.

Always try to be explicit with experimental text, file names, directories. Finding data at the end of a degree will be much easier.

VNMRJ automatically creates a **directory** for every saved experiment:

- *filename* can use almost any characters and be up to 256 characters long
- special characters are not allowed, i.e. will create an error message when saving or loading: ~ ! @ # $ % ^ & * ( ) / [ ] { } ; “ ‘ **white space**

The extension *.fid* is added automatically.

All file names are **case sensitive**: d2o ≠ D2O !

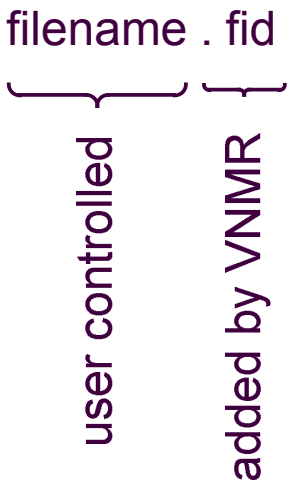


Figure 7.36: VNMRJ file format

Contents of filename.fid directory:

fid	the actual data from the acquisition (FID), a binary file
log	a log file from the acquisition, may contain error messages, a text file
procpar	all acquisition, processing and display parameters, a text file
text	user entered description(s) of the sample and experiment, i.e. sample name, sample concentration, etc., a text file

The file called **procpar** contains all acquisition, processing and display parameters. This means that whenever and wherever you access (load) the data again, they will look **exactly** as saved. It is advisable to adjust a spectrum in a way that is useful for future use. This can be done **before saving and resaving** the data.

Only data that are **saved to disk *and* then read back have the correct** file name on printouts (otherwise *file name* will be *exp*). This procedure serves as a test whether the data are really saved properly.

The Linux user account, **gennmr**, is cleaned out by the computer nightly, all files older than **180 days** (gennmr) are automatically deleted. Users of the gennmr Linux user account are encouraged to archive their data regularly.

7.1 The Data Manager window

The Data Manager is an entirely in-house created window.

Many actions performed in the Data Manager window are based on Linux copy and paste functionality. Text that is highlighted (left click and drag) in Linux is automatically copied to the clip board and is available for pasting. Text can be pasted to the location of choice by simply clicking on the middle mouse button or scroll wheel.

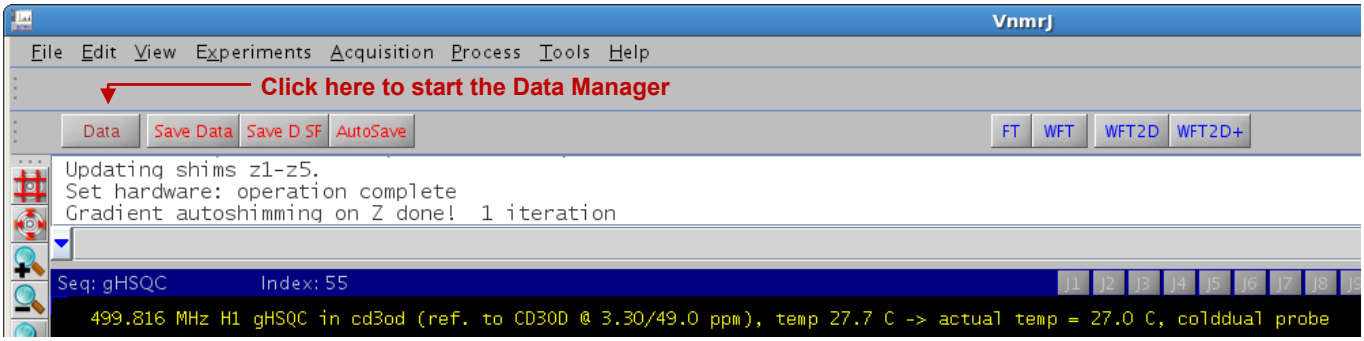


Figure 7.37: Starting the Data Manager

Figure 7.38: The Data Manager window

When opened the Data Manager starts in the default data directory for your Linux user account. Shown above, the Data Manager opened in the gennmr user account starting in the default data directory for gennmr.

The usage of the Data Manager is best shown with an example, navigating to a user's directory within the WEST directory of the gennmr user account.

To navigate to the WEST directory, highlight the WEST directory in the Data Manager window, as shown below.

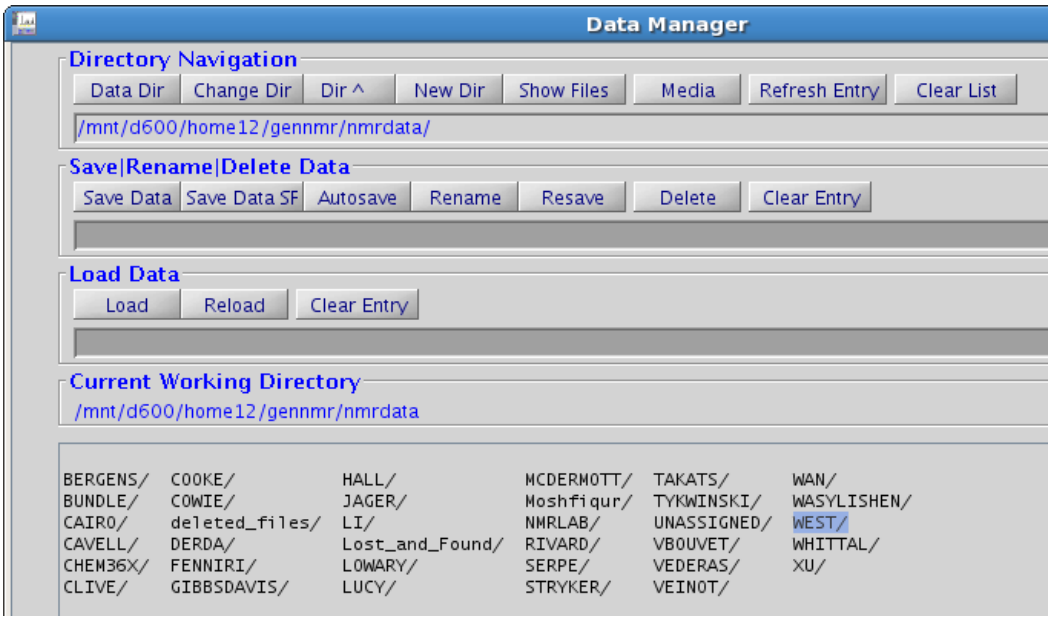


Figure 7.39: Copy directory information for navigation

Paste the directory WEST into the Directory Navigation text box, middle click the mouse at the end of the Directory Navigation text box, as shown below. Names can also be entered directly into the Data Manager's various text boxes using the keyboard.

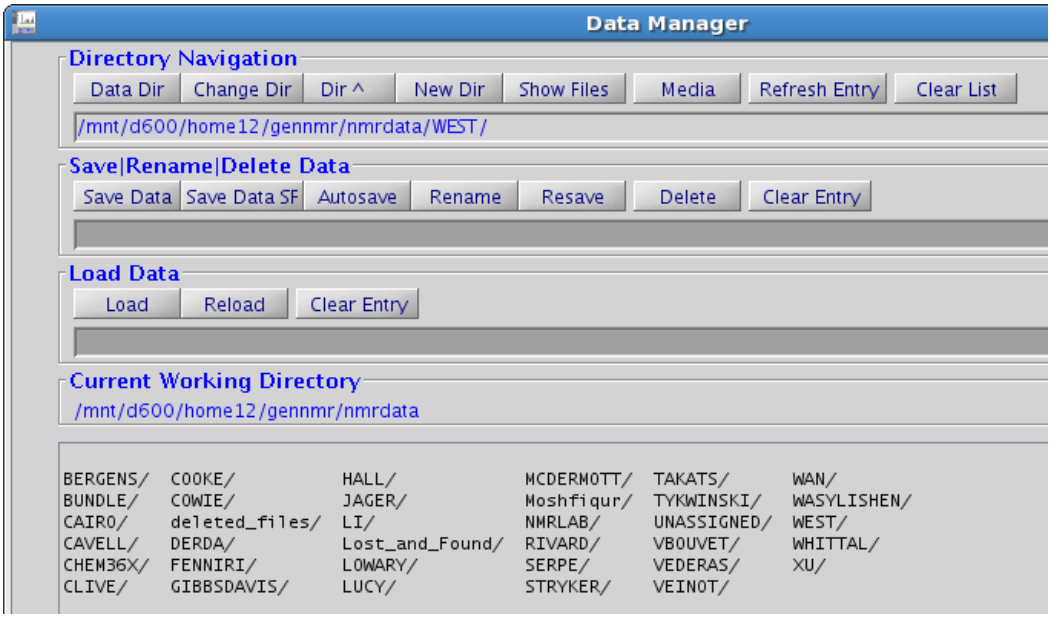


Figure 7.40: Paste directory information at the end of the Data Navigation text box for navigation

Click on the button **Change Dir** to enter the WEST directory. The results are shown below.

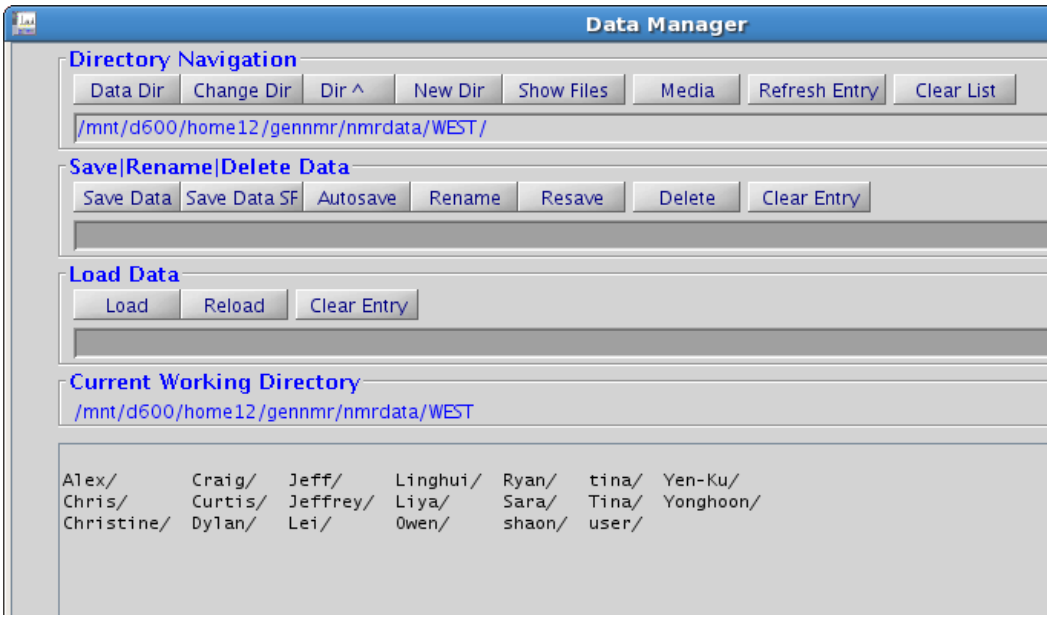


Figure 7.41: Navigating into the WEST directory

The contents of the directory are shown after successfully changing directories. Repeat the steps outlined above until the desired directory is reached.

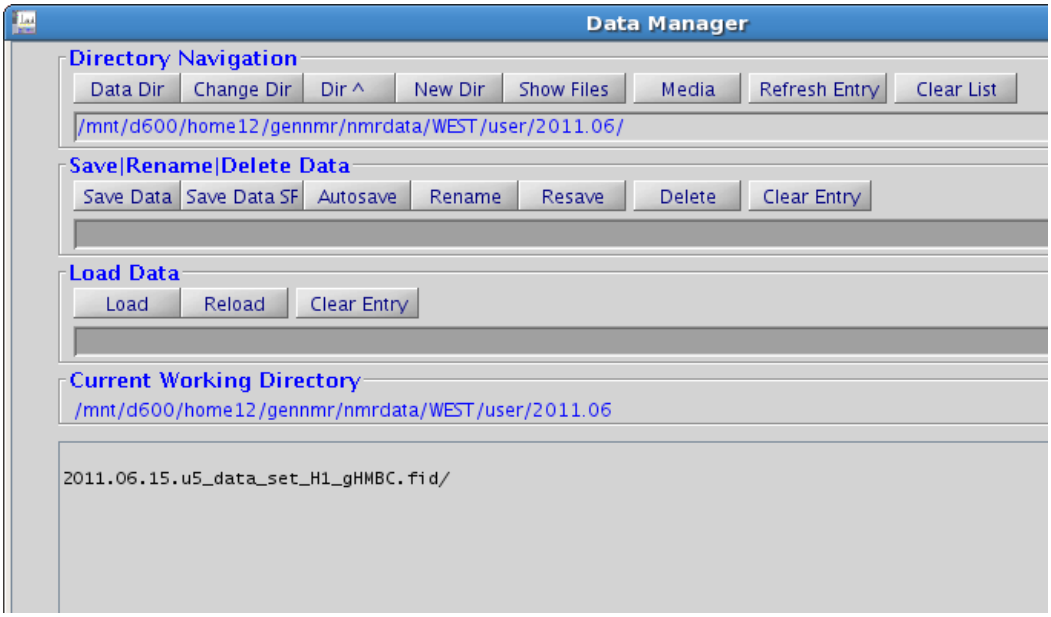


Figure 7.42: The final directory is chosen

Data can be loaded from the directory shown above by highlighting the desired file and pasting it in the text box **Load Data** and then clicking on the button **Load**.

Data can be saved to the location shown above by entering information in the text box **Save|Rename|Delete Data** and then clicking on either **Save Data** or **Save Data SF** buttons.

7.2 The Data Manager buttons

Directory Navigation

Data Dir: Go to default data directory for the user account.

Change Dir: Change to directory indicated in the text box Directory Navigation.

Dir ^: Go up one directory.

New Dir: Create directory indicated in the text box Directory Navigation.

Show Files: Show contents of current working directory.

Media: Go to media directory if CD, DVD, or USB key has been inserted in to the computer.

Refresh Entry: Refresh the Data Navigation text box.

Clear List: Clears the Data Manager window.

Save|Rename|Delete Data

Save Data: Save data in user defined format, free form. All spaces and special characters are replaced with an underscore _.

Data saved over many years of acquiring NMR and creating thousands of files, has led to the following recommended naming scheme:

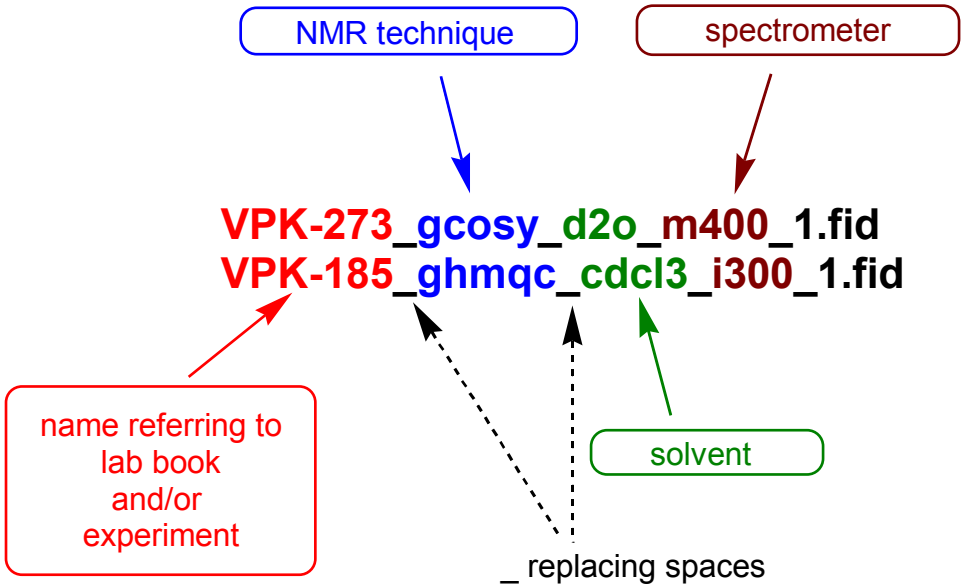


Figure 7.43: Suggested file name structure

Save Data SF: Data are saved in a **predefined format**. The system will append a date stamp, machine stamp, transmitter nucleus, and pulse sequence to the sample name. Files saved in this format will lead to chronological directory content. All spaces and special characters are replaced by an underscore, _.

For example, **Save Data SF** will create the file name:

2011.06.15.u5_data_set_H1_gHMBC.fid

for a gHMBC recorded on June 15, 2011 on the u500 for the sample named data set. The text in red was entered in the **Save|Rename|Delete Data** text box by the user.

Autosave: automatically saves a running experiment in the currently joined experiment. Data are saved in the same manner as the button **Save Data SF**.

Rename: prompts user for new name on the VNMRJ command line for the file name entered in Save|Rename|Delete Data text box.

Resave: no entry required, resaves data loaded from disk in its original location and original file name.

Delete: deletes file(s) entered in Save|Rename|Delete Data field. More than one filename can be entered. All files must be separated by a space. Deleted data end up in the **Linux user account's trash** for recycling when needed.

Clear Entry: Clear all entries in Save|Rename|Delete Data text box.

Load Data

Load: load data from filename entered in **Load Data** text box.

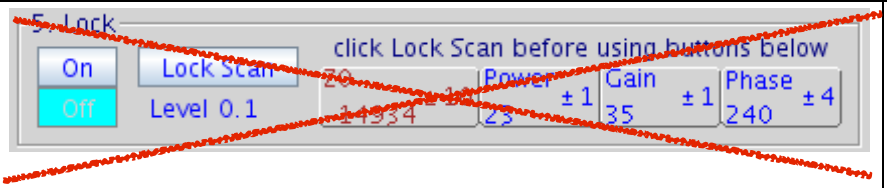
Reload: no entry required, data are reloaded from current file previously loaded from disk.

Clear Entry: Clear all entries in Load Data text box.

8. Exit VNMRJ and log out

When you are finished with your reserved time and/or NMR experiment(s):

8.1 **exit** from VNMRJ

	• Turn the **Spinner Off**
	• ~~Turn the **Lock Off**~~ **Leave lock on**
	• **Eject** the sample and then remove the sample from the top of the magnet
	• Click on the **Insert** button to turn the air lift off
 or 	• Exit VNMRJ by entering exit on the VNMRJ command line followed by the enter key or by clicking on the x in the upper right hand corner of the VNMRJ window

8.2 **log out** of the Linux user account

DO **NOT** log out of your Linux user account without exiting VNMRJ first.

	• click on the small exit door in the top left corner of the Linux desktop and confirm exit when prompted

This procedure will log you out correctly and prompt the next user to log in with the proper Linux username and online reservation name.

9. Appendix

9.1 Sep-Pak filtration for carbohydrates (courtesy Dr. Gordon Alton)

For oligosaccharides with *hydrophobic aglycons* the following conditions are recommended for C18 Sep-Pak cartridges:

1. Wash with 5 mL of methanol then 10 mL of water
2. Load oligosaccharide in water or buffer, the more dilute the better, use a flow rate of less than 10 mL/min (**slower is better**)
3. Wash cartridge with 10 mL of water (flow rate is not important), **keep all solutions until you know where the carbohydrate is!**
4. Elute oligosaccharide with 5 or 10 mL of methanol.

One C18 Sep-Pak can hold up to 10 mg of an octyl glycoside trisaccharide, but is different for each sugar, aglycon, flow rate and load buffer situation. Determine experimentally what works best for your compound.