



Chemistry Department

NMR/Instrumentation Facility

Users Guide - VNMRJ

Prepared by Leila Maurmann

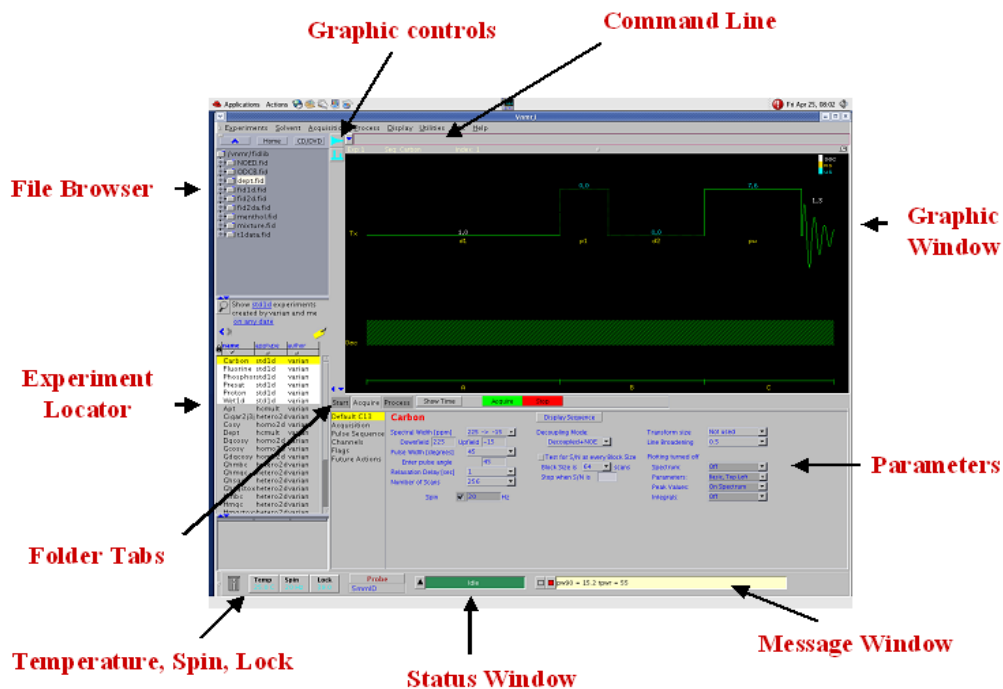
The following procedures should be used to acquire one-dimensional proton and carbon NMR data on the 400MHz NMR instrument.

The Experimental setup should be followed for any experiment.

- Experiment setup
 - insert/eject sample
 - spinning
 - lock
 - shimming
- Proton spectrum
 - integration
 - lineshape
 - digital resolution
 - coupling constant
- Carbon spectrum
 - ^1H -decoupled
 - ^1H -coupled
- General commands
 - display (pulse sequence, acquisition parameters, scale, threshold)
 - saving
 - referencing
 - phasing
 - printing

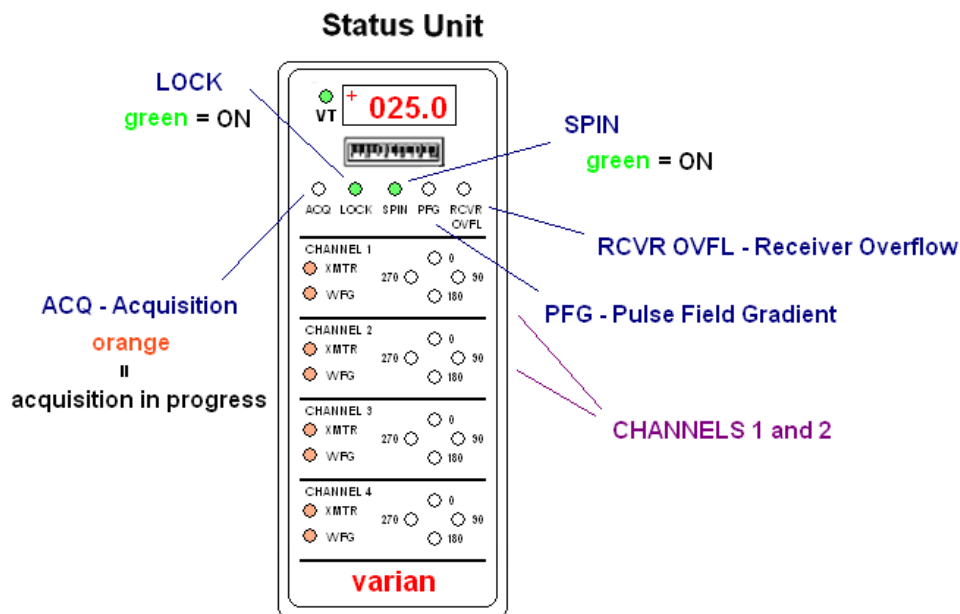
Experiment setup

VNMRJ display



Observe NMR computer and status unit

- The Status Unit next to the computer allows you to check on the Temperature, Spin and Lock of the system.



Important observations:

1. Check temperature (should be 25.0°C), **green light** for VT should be ON
2. LOCK **green light** should be ON
3. SPIN **green light** is ON when sample is spinning. One-dimensional experiments are typically carried out with samples spinning at 20Hz (exceptions: NOE and 2D)
4. ACQ light should be OFF if there is NO acquisition. If the **orange light** is blinking there is an acquisition in progress. Green lights for 0, 90, 180, 270 on the channels 1 and 2 also indicate that an experiment is in progress. Type **aa** on **vnmrj** to stop it
5. PFG a **red light** will blink if there is an experiment running
6. RCVR OVFL a **red light** will blink if there is an overflow. Stop acquisition and reduce gain.

An experiment can be started when the Status Unit lights stop blinking on ACQ and on channels 1 and/or 2.

*The Status Window on **vnmrj** will show the message "Idle" in a green background.*

If you need to stop an experiment type aa

Sign in and open software

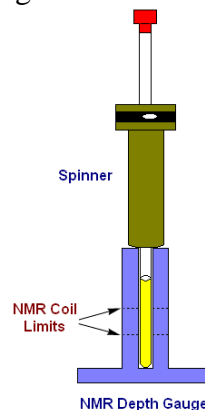
- Sign in the log book
- Log in (using the ID and password that was set up on training)
- Open **vnmrj** (click on icon on top toolbar - one left mouse click)

Remove lock sample and insert your sample

- Remove Lock sample - CDCl_3 - and type e

The Lock sample will come up from the magnet. Remove it carefully!!

- Load your sample on the spinner and adjust the height using the depth gauge:



- Place the sample in the magnet (it should float) and insert the sample by typing **i**
- OR
- Use EJECT and INSERT buttons on [Start] - [Standard] in the **vnmrj**

Check your directory

- Your directory should be visible on the *File Browser* area on the left part of the **vnmrj** display. If not, click on the blue arrow ▼ on the top toolbar to access your directory.

Set up the experiment desired

- On the *Experiment Locator* area click on the experiment you wish to run and drag it to the *Graphic Window*. The display will show the pulse sequence for the experiment you selected.

Selecting sample temperature

- The temperature is kept at 25°C if you need to change it go to [Start] - [Spin/Temp] and adjust the temperature desired. Allow a few minutes for temperature to equilibrate.

Contact the lab manager before setting any temperature.

Careful with the sample sensitivity, solvent properties and PROBE specifications when working with variable temperature experiments.

Selecting your solvent

- Select your solvent on [Start] - [Standard] pull down menu

Spinning

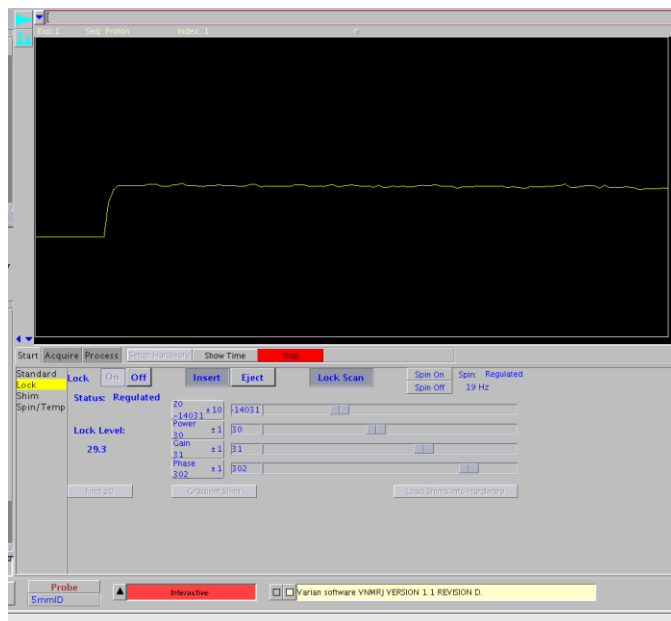
- Check if sample is spinning (**green light** on Status Unit and lower left tab on **vnmrj**)
- Spin rate is set to **20Hz**, set by using the [Start] - [Spin/Temp] tab

Lock your sample

Click on the [Start] - [Lock]

Click on the *Lock Scan* button to activate lock measurement.

You should then see a wave line on the Graphic Window.



Select Lock OFF:

You should see a sine wave if the lock is off resonance.

When the lock approaches resonance, a flat step line will be seen (figure above).

For the initial value, set the **lockgain** at its maximum (30 db)

For the initial value of **lockpower**, set it to ~30. The power you need to lock depends on your solvent and the concentration of the sample. Setting the power value too high will saturate the lock signal, and you will not be able to find the lock. At the same time the **lockpower** will need to be higher than the normal setting for locking.

Change Z0 until the signal changes from a sine wave to a flat line (with some noise).

Lock must be off in order to change Z0.

After establishing the lock, click on Lock **ON**.

If the lock value exceeds 100%, reduce the **lockpower** or **lockgain**; making sure the lock is still on.

Optimize the **lockphase**, until a maximum lock level is reached.

Return the lock level to 40-60 range by decreasing **lockpower** or **lockgain**.

Always minimize **lockpower** – the lock level should not significantly fluctuate if the power is set correctly.

Check if Lock is ON.

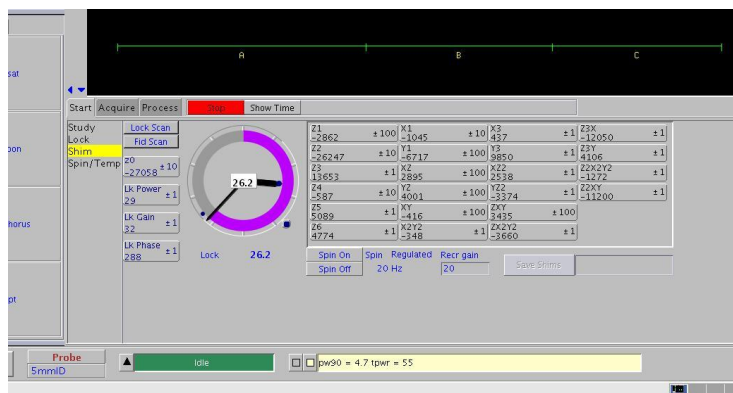
Note:

- you may need to adjust the Z1 shim to increase lock level

Shimming

Click on the [Start] - [Shim]

The shim window should look like the picture below:



Optimize Z1 and Z2 observing the increase in the lock level value. Reduce **lockpower** and **lockgain** if lock level reaches 100.

- Shim (test lineshapes by running a standard 1D ^1H spectrum)

Note:

- If Z1 and Z2 are changed by a large value, readjust lockphase of the lock.

Loading data

- Your directory should be visible on the top left of **vnmrj**
- Select the experiment (*.fid* extension) you wish to load, and drag it into the **vnmrj** display
- Type **wft aph** to process and display the spectrum

Exiting software

- Click on
 - [Utilities] - [Exit Vnmrj]

OR

- Type **exit** on command line

Log-out Red Hat

- On desktop click on
 - [Actions] - [Log Out]

Proton spectrum

The proton experiment is used for solving simple structural problems, to check the progress of synthetic work and for setting up more sophisticated experiments. The chemical shifts (δ) and coupling constants (J) may be estimated; and the integration of proton resonances gives the number of corresponding protons. This information is useful for peak assignments and quantitative analysis.

The basic one-dimensional proton NMR experiment consists of a very simple pulse sequence, **s2pul** available in Varian systems. The first pulse is when the radio frequency is turned on for a few μ s and then turned off. The first delay (relaxation delay) allows the spin systems to relax back to equilibrium. This is important for achieving the maximum signal-to-noise ratio. If the spin system doesn't relax sufficiently the relative intensity of the observed peaks will be affected. Generally **d1** is set to 1-3 times the longest T1 in the molecule (0.1-10s).

For quantitative measurements the 90° pulse should be used with an appropriate **d1** value.

PROCEDURE

- insert sample, lock and shim

Word in **bold** are to be typed on the **vnmrj** command line

Word/phrase in square brackets [] are icons that can be accessed by a left mouse button click

Acquisition

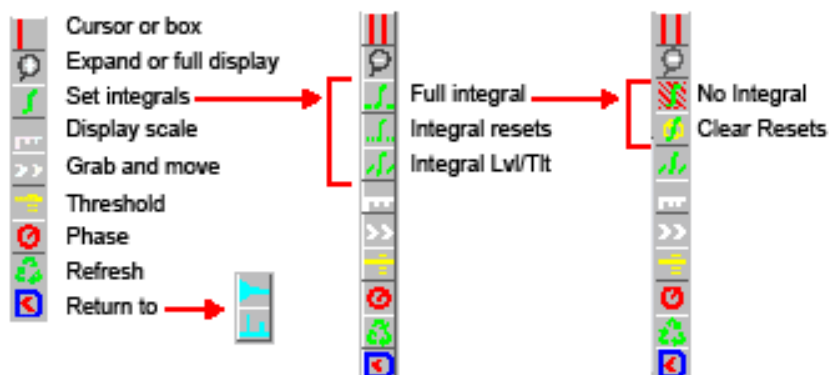
- select [Proton] on the experiment list (click-and-drag to graphical display)
- click on [Acquire] to check acquisition parameters
- parameters that can be changed by user:
 - [Acquire] - [Default H1] and [Acquire] - [Acquisition]
 - **nt** = number of scans/transients (multiple of 4)
 - **ss** = steady state scans (dummy scans) (necessary for NOE experiments)
 - **np** = number of data points acquired (at least 2***sw**, best resolution with 4***sw**)
 - **sw** = sweep width of spectrum (to change use **movesw**) (**tof** and **np** will be changed)
 - **d1** = relaxation time (1-3*T1) If integrals don't make sense, increase **d1** and re-run experiment
 - **gain** - depends on sample concentration (**gain='n'** - ensures that a suitable gain is used, auto-gain)
- **go** or **ga**

Processing

- **wft aph** (weighted Fourier transform with autophasing)
- parameters such as **fn** (at least 2***np**) and **lb** can be changed
- see general notes on phasing, reference, saving and printing a spectrum




NOTES

Integration



- select [Process] - [Cursor/Integration] - [Clear Integrals]

OR

- **cz**
- click on  (Set integrals icon) to get into integral mode
- click on the  (Integral resets icon) and use it to cut the line around each peak to be integrated
- return to the spectrum by typing **ds** on the command line or click on the  (Integral Lvl/Tlt icon) to set the integrals
- to set a specific peak integration value:
 - click on Process - Cursors/Integration
 - place cursor on desired peak and type the desired number of protons in the *normalization value* box
 - save changes by clicking on the *set integral value* icon
 - view integrals with *integral value* icon

Lineshapes and shimming

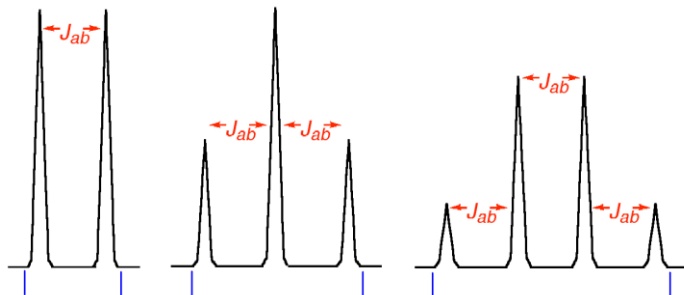
- inspect your spectrum and observe the lineshapes - this will give you indication on the shimming: if there are any humps or unusual splitting on **all** peaks it means you need to shim again.

Digital Resolution

- place cursor on a peak and on the command line type **dres**
- a value for linewidth and resolution will be shown on the Message window (bottom right of **vnmrj**)
- typically proton NMR will show resolution below 0.2Hz/pt
- optimal linewidth will vary with molecular weight of compound and solvent
- for small molecules in non-viscous solvents, linewidth should be less than 1Hz.

Measuring coupling constants and integration

- coupling patterns: doublet, triplet, quartet, etc - measure the distance between the peaks to get **J** values
- when selecting the area of the peak to be integrated you should select the point at the base line, before and after the peak (marked below)



Carbon spectrum

The naturally abundant carbon-12 does not have a nuclear spin, and therefore no NMR. When performing a carbon NMR, only the 1% naturally occurring carbon-13 is being acquired. The sensitivity is thus much lower than proton NMR and the spectrum takes more time for acquisition.

Protons attached to a carbon atom will cause splitting of the carbon signal, lowering the signal-to-noise ratio. The routine ^{13}C experiments are performed using proton decoupling and NOE conditions (see **Acquiring ^1H -decoupled ^{13}C spectrum**). The carbon spectrum of a compound will display a single sharp signal for each structurally distinct carbon atom in a molecule.

In order to produce a spectrum where carbons are coupled to adjacent protons the proton-decoupler is turned off (see **Acquiring ^1H -coupled ^{13}C spectrum**).

Carbon atoms with long T1 experience saturation and result in reduced intensity, which is the case of quaternary carbons, *i.e.* carbons with no directly bonded protons. A long **d1** can be helpful when trying to observe quaternary carbons.

Acquiring ^1H -decoupled ^{13}C spectrum (**dm='yyy'** - decouple + NOE)

PROCEDURE

- insert sample, lock and shim

Word in **bold** are to be typed on the **vnmrj** command line

Word/phrase in square brackets [] are icons that can be accessed by a left mouse button click

Acquisition

- run a proton spectrum
- select [Carbon] on the experiment list (click-and-drag to graphical display)
- click on [Acquire] - [Default C13] to check acquisition parameters
 - check if [Decouple+NOE] is selected or **dm='yyy'**
- parameters that can be changed by user:
 - [Acquire] - [Default C13] and [Acquire] - [Acquisition]
 - **nt** = number of scans/transients (multiple of 4)
 - **ss** = steady state scans (dummy scans) (necessary for NOE experiments)
 - **np** = number of data points acquired (at least $2 \cdot \text{sw}$, best resolution with $4 \cdot \text{sw}$)
 - **sw** = sweep width of spectrum (to change use **movesw**) (**tof** and **np** will be changed)
 - **d1** = relaxation time (1s, no integration is performed)
 - **gain** - depends on sample concentration (**gain='n'** - ensures that a suitable gain is used, auto-gain)
- **go** or **ga**

Processing

- **wft aph** (weighted Fourier transform with autophasing)
- parameters such as **fn** (at least **2*np**) and **lb** can be changed
- see general notes on phasing, reference, saving and printing a spectrum

NOTES

If incomplete decoupling is observed:

- acquire a proton spectrum of the sample
- place cursor in the center of the proton region and type **movetof**
- **tof?** - a message on the bottom right of **vnmrj** will give an approximate **tof** value
- on the carbon acquisition type **dof=#** (# obtained for **tof**)

Acquiring ¹H-coupled ¹³C spectrum (**dm='yyn'** - couple + NOE)

PROCEDURE

- insert sample, lock and shim

Word in **bold** are to be typed on the **vnmrj** command line

Word/phrase in square brackets [] are icons that can be accessed by a left mouse button click

Acquisition

- run a ¹H-decoupled ¹³C spectrum
- change decoupler parameters:
 - **dm='yyn'**

OR

- click on Acquire] - [Default C13] to check acquisition parameters
 - check if [Couple+NOE] is selected
- **go** or **ga**

Processing

- **wft aph** (weighted Fourier transform with autophasing)
- parameters such as **fn** (at least **2*np**) and **lb** can be changed
- see general notes on phasing, reference, saving and printing a spectrum

“Quantitative” ¹³C spectra

The normal carbon spectra are qualitative and integrations do not give the corresponding number of carbon present in each peak. The main reasons are due to NOE and the different relaxation times of carbon atoms (T1). If you wish to take a quantitative spectrum, NOE and decoupling need to be removed from the pulse sequence (**dm='nny'**; assuming **d1=5*T1**). A very long relaxation delay needs to be used as well. *The integration will still have large errors.*

GENERAL COMMANDS

Create and join experiments

VNMR allows you to acquire data in several workplaces

- **cexp(#)** - # is an integer number, creates an experiment
- **jexp(#)** - # is an integer number, joins an experiment

If the message “*Experiment locked*” appears on the Message window:

- **unlock(#)** - # is the experiment number

Acquisition parameters

- click on [Acquire] - [Defaults] / [Acquisition] / [Pulse Sequence] on Folder tabs

Processing parameters

- click on [Process] - [Process] / [Linear Prediction] on Folder tabs

View and change parameters

- type the name followed by a question mark, e.g. **np?**
- the **np** value would be shown in the Message window
- to change parameters use **name=**

OR

- locate parameters in Folder tabs and change them directly on window

Spectral width

- the spectral width can be changed by selecting the regions where the peaks are located (with ~1ppm margins) and typing **movesw**
- all peaks should be included to avoid folded peaks in your NMR spectrum
- **vnmrj** has set scale ranges you can use that will change **sw** and **tof** (offset frequency), this is located on [Acquire] - [Default] - this is important in the setup of 2D experiments

To stop acquisition

- **aa**

Saving a spectrum

- **svf**
- when prompted type the name desired on the command line

Scale in ppm or Hz

To view scale:

- **dscale**

OR

- click on  icon

Change scale unit:

- unit can be changed on [Process] - [Display] - Axis: select Hz, ppm or kHz

OR

- or by typing: **axis='h'** for Hz or **axis='p'** for ppm

Reference

Place cursor on peak to be set as reference:

- **nl rl(##.p)**

OR

- on [Process] - [Cursors/Integration] - [Reference: by Solvent, by TMS, by Cursor and on Reference] choose ppm using ▼ and type number in box.

Threshold

- use **th**

OR

-  using the yellow line to set height


Peak picking

- **vsadj** - automatic vertical adjustment
- **dll** - display peaks on text window
- **dpf** - display frequencies on peaks

Phasing

- reset phasing by using **rp=0** and **lp=0**
- **aph** for auto phasing

OR

-  to manually phase the spectrum using the left mouse button
- after manual phasing type **ds**

Printing

printing can be set on [Process] - [Plot] - click on boxes to be printed

- Plot Spectrum
- Plot Scale
- Plot Text
- Plot Integrals
- Plot Scaled
- Plot Normalized
- Select Plot Parameter Template and Plot Frequencies options

Click on [Clear Plot] to clear options and reselect

When done click on [Plot Page]

OR

- **pll pscale pir page**

- | | | |
|---------------|---|-----------------------------------|
| pl | - | plots spectrum |
| pscale | - | plots scale |
| pir | - | plots integral areas |
| pirn | - | plots normalized integral regions |
| ppf | - | plots peak frequencies |
| pap | - | plot all parameters |
| ptext | - | plots text label |
| page | - | send to printer |