# PINMRF

## Varian 300 MHz NMR Spectrometers

## Training Guide for Basic 1D NMR Spectroscopy

## INCLUDING:

## Inova-300-1 w/ 5mm 4-Nucleus Plus probe – 365 WTHR

## Inova-300-2 w/ 5mm Autoswitchable probe – 369 WTHR

## Table of Contents




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11-20-2006: Revision - JSH.

# Basic Spectrometer Operation Guidelines – 1D Spectra
# Varian 300 MHz NMR Spectrometers
# PINMRF

## Please note the following generalities

- Under no circumstances lean against or otherwise move the magnet.
- The Varian VNMR software enables the user to easily access and change functions, options, and their properties. If you are not sure about an option, button or command contact PINMRF staff for assistance.
- If you encounter problems at any time during locking, shimming, acquiring you should halt the experiment and reload the standard parameters for your experiment.
- In order for shimming to work well, samples should be filtered and prepared in good-quality tubes. Sample volume should be at least 0.7 mL. Do not use more than 0.1% TMS.
- The VNMR software works best with the Common Desktop Environment (CDE) graphics on the Sun computer. Please check when you log in that you are using CDE.

## Conventions in this guide

- Buttons are depicted with small caps in bold, e.g. **ABORT ACQ.**
- Commands entered in the command window are described in geneva font, e.g. `h1cdcl ,` and are executed by hitting the return key.

## Important basic functions

- **ABORT ACQ** button -> aborts acquisition (BEWARE this discards data!)
- **RESIZE** button -> enlarges/reduces spectrum window
- **FLIP** button -> flips the spectrum window over the display window and vice versa
- `jexpx` command -> switches to experiment number x (1, 2, 3, 4, etc.)
- `ds` command -> switches back to interactive display mode

## Logging on to the Sun computer and starting VNMR

1. In order to log in enter your login ID, followed by the password into the welcome screens.
2. Click the spectrum icon on the left side of the toolbar to start the VNMR software.

## Sample change

3. e (ejects existing sample)
4. Remove sample from spinner and replace it with your cleaned sample (use Kimwipe and Isopropanol).
5. i (inserts new sample)

## Acquisition of $^{1}$H-NMR Spectra

6. jexp1 (join experiment 1)
7. h1xxxx (macro loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmso, meth; for benzene use xxxx = cdcl) then WAIT for the BEEP!
8. acqi (opens acquisition window)
9. click **LOCK** to open lock sub-window, then set and/or check the following:
    - turn on sample spinning (if not on already) by setting the spin value to 20 (Hz.)
    - the sample should be locked - lock should be established automatically
    - decrease lock power until lock level is stable (not bouncy; 24 for cdcl3)
    - Change lock gain in steps of 4 units until lock level is between 30 - 60%
    - if sample is not locked, increase lock power by 16, wait for lock to be established, then decrease lock power in steps of 4 units until lock level is stable
    - do not change the Z0 and lock phase values
10. click **SHIM** to open shim sub-window
11. adjust Z1C and Z2C (if the lock level increases beyond 100, decrease the lock gain at the bottom of the current window)
12. click **CLOSE**
13. nt=xx (sets number of scans; default is nt=8)
14. ga (starts acquisition) – then WAIT for the BEEP! (spectrum should be displayed)

15. aph (automatic phasing)

for manual phasing:

- click **PHASE**
- click and hold on peak farthest to the right
- while holding the click, drag to correct the phasing for this peak (NOTE: right mouse button allows for fine phase control)
- then click and hold on peak farthest to the left, and drag until correctly phased
- interactively repeat phasing these two peaks until phasing is completed
- ds (switches back to interactive display mode)

16. vsadj (vertical scale adjustment - highest peak automatically adjusted to top of window)

for manual vertical scale adjustment:

- use the center mouse button, a left mouse click above the baseline causes the spectrum to scale up and a left click below the baseline causes the spectrum to scale down, the distance of the cursor from the baseline while clicking determines the multiplication factor

alternative vertical scale adjustment:

- vs=XXX, the current value of the vertical scale (vs) is displayed in the lower portion of the spectrum window

17. **CURSOR, BOX, EXPAND, FULL**:

- left-click and drag red left cursor to desired left cutoff position (cr position is shown at bottom of spectrum window)
- click **BOX**
- right-click and drag red right cursor to desired right-hand cutoff position (delta = difference in ppm)
- click **EXPAND** (expands spectrum to cursor box limits)
- click **FULL** (returns spectrum to full size)

18. integration:

- NOTE: the button for integration consists of three sequential choices: **PART**(ial) **INTEGRAL**, **FULL INTEGRAL**, **NO INTEGRAL**; the button shows the next mode that can be accessed, not the current integration mode
- click **PART INTEGRAL** (green integral trace appears; see also '**Adjustment of Integral Trace**' at the end of this document)
- cz (clears currently defined integral)

- click **RESETS**
- click the left mouse button closely to the left of the left-most peak
- click the left mouse button closely to the right of the left-most peak
- repeat for each peak or group of peaks across the spectrum (clicking the right mouse button at any time anywhere in the spectrum window causes an undo of the last integral section)
- click **FULL INTEGRAL, NO INTEGRAL, PART INTEGRAL**
- click inside a known integral region to leave the cursor inside the region
- click **SET INT**
- enter desired whole integral for the selected region (the display window generates a list of integrals)
- click **NO INTEGRAL**

19. set peak picking threshold:
    - click **TH** to display the yellow threshold line
    - click and drag the yellow line to the desired threshold
    - click **TH**
20. view chemical shift labels and scale:
    - dpf displays the peak labels
    - dscale displays a scale beneath the spectrum
    - ds clears the labels and scale to redisplay the spectrum alone
21. text('xxxxx') (enters a title to be printed on top of your spectrum)
22. print, printp, printi or printpi (i = with integration, p = with peak labels)

Proceed with additional samples from step 3, continue to step 23 for $^{13}C$ spectrum, or to step 39 'Finishing up Acquisition of Spectra'

## Acquisition of $^{13}$C-NMR Spectra (note: $^{1}$H spectrum must be acquired first)

23. jexp2 (join experiment 2)
24. c13xxxx (macro loads solvent-dependent parameters; xxxx = cdcl, acet, d2o, dmso, meth; for benzene use xxxx = cdcl). WAIT for the BEEP!
25. nt=xx (sets number of scans; default is 256)
26. ga (starts acquisition) - then WAIT for the BEEP! (spectrum should be displayed)
27. wft (check progress before the beep) sa (stops acquisition and retains data)
28. aph (automatic phasing, the software does a good job if the signal-to-noise ratio is good)
    for manual phasing see step 15
29. vsadj (vertical scale adjustment - highest peak automatically adjusted to top of window)
    for other vertical scale adjustment methods see step 16
30. **CURSOR, BOX, EXPAND, FULL**: (same as step 17)
31. set peak picking threshold: (**TH** same as step 19)
32. view chemical shift labels and scale: (dpf, dscale, ds, same as step 20)
33. text('xxxxx') (enters a title to be printed on top of your spectrum)
34. print or printp (p = with peak picking)

Proceed with additional samples from step 3, continue to step 35 for $^{19}$F or $^{31}$P spectrum, or to step 39 'Finishing up Acquisition of Spectra'

## Acquisition of $^{19}$F and $^{31}$P NMR Spectra (note: $^{1}$H-NMR must be acquired first)

35. jexp4 (join experiment 4)
36. f19cdcl or p31cdcl (loads solvent-dependent parameters)
37. nt=xx (sets number of scans)
38. ga (starts acquisition) - then WAIT for the BEEP! (spectrum should be displayed)

Phasing and other processing procedures are exactly as for $^{1}$H spectra; see steps 15 – 21, above.

## Finishing up Acquisition of Spectra and Logging Out

39. e (eject sample)
40. remove your sample and insert standard sample (clean with Kimwipe and Isopropanol)

41. i

42. jexp1 (go back to experiment 1)

43. standard - then WAIT for the BEEP!

44. exit – then WAIT for all 4 VNMR windows to close.

45. Log out from the Sun computer by clicking the **EXIT** button in the toolbar at the bottom of desktop.

## Supplementary Instructions

### Miscellaneous Functions

- to change the chemical shift reference, place the cursor on the peak of known shift, then enter rl(x.xx*sfrq). x.xx is the chemical shift value to be entered (e.g. 7.26)
- to observe a spectrum during acquisition enter wft and process the spectrum as described above
- if the number of scans was not satisfactory repeat wft after additional scans
- if the spectrum is satisfactory type sa (stop acquisition) and process the spectrum
- axis='h' changes the axis unit to Hertz; axis='p' changes it to ppm
- if you would like to restart phasing with its initial values type lp=0 rp=0 ds, then repeat the phasing procedure

### Storing and Retrieving NMR Data and Shim sets

- to save NMR data from the current experiment, enter: svf('filename')
  (filenames can include letters, numbers, dash and underscore characters **ONLY**)
- to retrieve stored NMR data into the current experiment, enter: rt('filename')
- to save and retrieve shims files use: svs('filename') and rts('filename')
  (after retrieving shims you must enter su to load the shim values into hardware)

## Adjustment of the integral trace

The **LVL/TLT** button activates interactive zero and first-order baseline correction mode. The zero order correction is represented by the ***LVL*** parameter; the first order correction is represented by the ***TLT*** parameter.
Position the cursor on an integral region of interest, about halfway vertically up the screen, and click the left mouse button. A horizontal line will intersect at the cursor and two vertical lines will be placed on either side of the cursor. Now moving the cursor above or below the horizontal line, but within the two vertical lines, and clicking the left or right mouse button will adjust the zero-order baseline correction parameter ***LVL***. Placing the cursor right on the horizontal line and clicking the mouse button will restore the initial value of ***LVL***.

Now move the cursor to another region of the spectrum, outside the vertical lines, and click the left mouse button again. A new horizontal line and two vertical lines will be displayed again and a single vertical line will be displayed in the middle of the region where ***LVL*** was being updated. The mouse will now control the first-order baseline correction parameter ***TLT***. Clicking the left or right mouse button above or below the horizontal line will now increase or decrease ***TLT***., and will also change ***LVL*** so that the total drift correction at the single vertical cursor in the middle of the previous region will be held constant. This process substantially reduces the necessity to iteratively adjust the two parameters ***LVL*** and ***TLT***. As with the zero-order correction, clicking onto the horizontal baseline will restore the initial value of ***TLT***.

Each time the cursor is moved outside the two vertical lines and the mouse button is clicked, a new vertical and horizontal line is displayed. The parameter adjustment alternates between adjusting ***LVL*** and adjusting ***LVL*** and ***TLT***. The left and the right mouse button both adjust the baseline correction parameters and differ only in their sensitivity; changes with the left mouse button are eight times larger than changes caused with the right mouse button.

The middle mouse button adjusts the integral scale (height of integral trace). To exit the interactive baseline correction mode, type ds .

Using the sample temperature controller for Variable Temperature NMR

If you need to carry out VT experiments, please consult with PINMRF staff for further training. VT operation requires extended instrument time for the probe to return to room temperature prior to the next user starting his or her experiment. The upper limit for sample temperature control is 80° Celcius, or 10°C below the boiling point of your solvent, whichever is lower.

## Varian 300 MHz NMR Spectrometers
## Parameter Files and Macros

Both Inova300-1 and Inova300-2

h1acet h1cdcl h1d2o h1dmso h1meth

f19cdcl

homodecjsh (sets up a homonuclear decoupling experiment from the current $^{1}$H experiment)

noediffjsh (sets up an NOE difference experiment from the current $^{1}$H experiment)

presatjsh (sets up a presaturation experiment from the current $^{1}$H experiment)

Inova300-1 only

b11cdcl

h2cdcl (requires training and recabling)

Inova 300-2 only

c13acet c13cdcl c13d2o c13dmso c13meth

p31cdcl (requires training on retuning the probe)

deptjsh (sets up DEPT-135 experiment from the current $^{13}$C experiment)

cosyjsh (sets up a 2D-COSY experiment from the current $^{1}$H experiment)

hetcorjsh(1) (sets up 2D-HETCOR experiment from the current $^{13}$C experiment with the $^{1}$H spectrum already in experiment 1)

# VNMR
# Commonly Used Keyboard Commands

Experiment Setup

**jexpn** - join (go to) experiment #n
**rt('abcd')** - read NMR data file "abcd"
**rtp('abcd')** - read parameter set "abcd"
**rts('abcd')** - read shim set "abcd"
**svf('abcd')** - save NMR data file "abcd"
**svp('abcd')** - save parameter set "abcd"
**svs('abcd')** - save shim set "abcd"
**mf(x,y)** - move NMR data (FID and parameters) from experiment "x" to experiment "y"

Data Acquisition

**h1xxxx** - load acquisition and lock parameters for $^{1}H$ experiment in solvent xxxx
(xxxx = acet, cdcl, d2o, dmso, meth)
**acqi** - open lock / shim window
**su** - set up all parameters in hardware
**gain=xy** - set receiver gain to value "xy" ("xy" = 1 – 60)
**nt=xy** - set number of scans to value "xy"
**bs=xy** - set block size to "xy" scans
**ga** - zero current data, start acquisition, and automatically display spectrum
**go** - zero current data and start acquisition
**aa** - abort data acquisition and discard data
**sa** - stop acquisition and keep data
**standard** - set up spectrometer for $CDCl_3$ standard sample and next user
**f19xxxx** - load acquisition parameters for $^{19}F$ experiment in solvent xxxx
**p31xxxx** - load acquisition parameters for $^{31}P$ experiment in solvent xxxx
**c13xxxx** - load acquisition parameters for $^{13}C$ experiment in solvent xxxx
**deptjsh** - set up DEPT-135 experiment from current $^{13}C$ experiment
**homodecjsh** - sets up a homonuclear decoupling experiment from the current $^{1}H$ experiment
**noediffjsh** - sets up a nOe difference experiment from the current $^{1}H$ experiment
**presatjsh** - sets up a presaturation experiment from the current $^{1}H$ experiment
**cosyjsh** - set up 2D-COSY experiment from current $^{1}H$ experiment
**hetcorjsh** - set up 2D-HETCOR experiment from current $^{13}C$ experiment

Data Processing and Plotting

**text('abcd')** - set title for plot to be the text string "abcd"
**ft** - Fourier transformation
**wft** - exponential multiplication and Fourier transformation (can use after bs scans completed)
**lb=xy** - set amount of exponential multiplication equal to "xy"
**aph** - automatic phase correction

**rl(x.yz*sfrq)** - set chemical shift at location of cursor to x.yz ppm
**th** - set threshold for peak picking using mouse
**dpf** - show peak labels
**ds** - display spectrum with cursor active
**dir** - show a listing of the current directory
**print, printp, printi, printpi** - print spectrum. Include peak labels (**p**) and/or integration (**i**)
**print135, print135p** - print DEPT-135 spectrum with/without peak labels (**p**)

# Practical Demonstration Checkout Requirements
# PINMRF Varian 300 MHz NMR Spectrometers

Prior to performing this practical demonstration you must first take a 30 minute written quiz. The quiz will test you on material from this document and the Facility Overview document, and other NMR-related matters. For both the quiz and checkout, you may use any training handouts and notes.

This is an outline of the tasks you will be required to perform correctly in order to pass the practical demonstration checkout. You will be allowed up to 30 minutes to complete the following tasks. Standard samples will be used for the checkout and will be provided by the NMR lab. PINMRF staff may, at their discretion, observe you performing all or part of the checkout exam.

Test Sample - 10% Ethylbenzene in $CDCl_3$ (or other sample as required)

- login to computer and start VNMR
- remove $CDCl_3$ standard, insert new sample, lock and shim
- run $^{1}H$ spectrum, process, integrate and print
- set up new experiment for $^{13}C$ spectrum
- run $^{13}C$ spectrum, process and print

Standard Sample - 100% $CDCl_3$

- remove test sample and replace with $CDCl_3$ standard sample, lock and shim
- reset spectrometer for next user
- shutdown VNMR and logout from UNIX