Operating Instructions for Varian NMR Spectrometers

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I. Preparatory Steps

1. Log in at the RESLOG terminal by entering your code and select the instrument you want to use.

2. Clean your fingers and the outside wall of your NMR sample tubes with Kimwipes.

3. Move the mouse to turn on the screen, which should display the four windows of the VNMR program: Acquisition Status (Acqstat), Master, Spectral, and Text.

   Acqstat - displays the status of the spectrometer
   Master - displays menu buttons and accepts keyboard commands
   Spectral - displays spectrum, FID and other graphics
   Text - displays parameters and other text messages

4. Insert your own sample tube into the spinner by pushing it straight down through the o-ring without twisting or rotating the tube and adjust the tube depth with the sample depth gauge. In the Master window, type e [ret] to eject the reference sample from inside the magnet and remove it. Put the spinner with your sample in the magnet bore on top of the magnet, and type i [ret] to lower the sample into the probe. (Touch the magnet with your free hand before
you take/put the sample from/onto the top of the magnet. This will discharge any static electricity you might pick up from rubbing against the chair, especially when the room air is dry.)

5. Recall the default shim set by typing \texttt{rts [ret]}, and enter the shim filename (usually \texttt{cdcl3}), then type \texttt{load='y' su [ret]} to load the new shim values to the shim coils.

6. Click on the \texttt{Acqi} button to open a new window and click the \texttt{LOCK} button in this new window to display the lock signal. Turn the lock off by clicking on the \texttt{lock OFF} button. Adjust Z0 to set the field on resonance (indicated by a cosine wave with 0 frequency, i.e. a straight step-up line). Turn the lock on by clicking on the \texttt{lock ON} button (refer to the table in the red binder to make sure the lock power is not too high to saturate the lock signal).

7. Shim the field by clicking on the \texttt{SHIM} button, and use the left and right mouse buttons to adjust z, z2 to maximize the lock level (no need to adjust other shims like z3, z4, x, y, xz, yz, xy, etc.). Click on the \texttt{CLOSE} button when done.

Deuterium gradient shimming can also be used to shim the field. To do this, just click on the \texttt{Setup, Shim, Gradient Autoshim on z} buttons in sequence. It should take less than 3 minutes to finish the task. If it takes longer, you can abort it by clicking on the \texttt{Abort Acquisition} button, and adjust the shims manually.

\textbf{II. Simple 1D 1H NMR experiments}

\textbf{Setting up Acquisition}

1. In the Master window, select the experiment number by \texttt{jexpn}, where \textit{n} is the experiment number. (Note that the data currently in this experiment number will be overwritten by the new experiment.) Click on the following buttons in sequence: \texttt{Main Menu; Setup; H1,CDCL3} (if the solvent is CDCL3. Otherwise, click on \texttt{Nuc, Solv; H1}; then the \texttt{solvent_name} buttons).

2. Type \texttt{su [ret].} (optional)

3. Adjust \texttt{nt} (number of transients), \texttt{ss} (steady state scans), \texttt{sw} (spectral width) etc. if needed.

4. Type \texttt{ga [ret ]} to start the acquisition (or \texttt{go [ret]}, which will not do wft after the data acquisition is done.).

5. When the message \textit{BS 1 Completed} is displayed, type \texttt{wft} to see the spectrum after \textit{bs} scans of the signal are collected. Repeat this every time the message \textit{BS n Completed} is shown to check the progress of the signal accumulation. When you see the spectrum is good
enough, type **sa** to stop the data acquisition. (Command **df** displays the accumulated FID. Command **ds** displays the processed spectrum.)

**Processing Data**

6. Use the command **dg** to display the acquisition and processing parameters and make necessary changes.

7. Type **wft** [ret] to weight the FID and transform it into spectrum.

8. Type **aph** (or **aphx**) [ret] to phase the spectrum. If the phase is still not right, use manual phasing: click on the **Phase** button; left click on the upfield side of the spectrum; hold the left or the right mouse button and move the mouse up and down to phase the selected peaks (left for coarse and right for fine adjustment); release the mouse button; left click on the downfield side of the spectrum; hold the left or the right mouse button and move the mouse up and down to phase these peaks. After finishing these manual phase adjustments, type **ds** [ret].

9. Spectral Referencing: You do not need to do spectral referencing if you have selected the correct solvent file. However, if you still need to do it, click the left mouse button when the cursor is moved to near the peak to be assigned, type **nl** [ret] to set the red vertical cursor line right on top the peak, click on the **Ref** button and enter the chemical shift for this peak. Command **dscale** or the **dscale** button displays the chemical shift scale for the spectrum.

**Integration**

10. Type **cz** [ret] first. Click on the **Part Integral** button (there are three options on this button - **Full Integral**, **Part integral**, and **No Integral**). You can define new integral regions by first clicking on the **Resets** button, then left clicking at every point of the integral line to reset the baseline (the right mouse button is used to undo the previous reset).

11. To adjust the baselines of the integrals, click the **Lvl/Tlt** button, move the cursor to the up-field region of the spectrum and click the left mouse button, press and hold the left or right mouse button and move the mouse up and down to adjust the level (**Lvl**); then move the cursor to the down-field region, click and hold the left mouse button and move the mouse up and down to adjust the tilt (**Tlt**). Type **isadj** to set the largest integral to full scale. Place the left cursor line onto the integral that you want to define its value, and click the **Set Int** button to enter a proper value representing the number of protons included in this integral region.

12. Command **dpir** (or **dpirn**) displays the integral values on the screen.

**Baseline Correction**

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13. If the spectral baseline shows linear drift, use command `dc` to correct it. To correct more irregularly uneven spectral baseline, command `bc` can be used after the spectral integration reset points are selected (as performed in the following step). You can also try the command string: `region bc cz`.

Plotting Spectrum

14. Use the two red cursor lines (controlled by the left and the right mouse buttons, or the parameters `cr`, for the left cursor, and `delta`, for the distance between the two cursors) to define the plot limits, and click on the `Expand` button. The middle mouse button is used to adjust the amplitude of the spectrum. You can also use parameters `sp` and `wp` to define the plot limits (`sp` is the high-field end, and `wp` is the width of plot, e.g., `sp=-0.5p wp=10p` covers from –0.5 ppm to 9.5 ppm).

15. Type `plot` to plot the spectrum. This is similar to typing a string of commands `pl`, `pscale`, `ppa` (or `pap`), `pir`, `page`). You can also click on the `Plot`, `Plot`, `Scale`, `Params` or `All Params`, `Page` buttons.

Printing Peaks

16. Click on the `Th` button to display a yellow threshold line, use the left mouse button to adjust its position so all the peaks to be printed are above this line.

17. Type `pl` `page` to print the peaks that are higher than the threshold line. You can also plot the chemical shifts of the peaks on the plotted spectrum by including the command `ppf` in the string of the plotting commands (Command `dpf` displays these chemical shifts with the spectrum on the screen.)

Saving Data

18. Since the data in this experiment number will likely be overwritten as soon as the next user uses this instrument, you might need to save your data on the disk. This can be done by first clicking on the `DATA` button, which changes the directory to `/export/home/<machine>/vnmr/sys/data`, in which there are subdirectories with group advisors’ names; selecting your group directory with the left mouse button, clicking the `Set Directory` or the `Change` button to move to the selected subdirectory.

You can create your own directory within your group directory by the command `mkdir`, e.g., `mkdir('lastname')` will create a subdirectory lastname within your group directory.

To save the data, simply type the command `svf` [ret] and enter the filename that you wish to use. Clicking on the `File` button will update the content list in this directory. Note that only the FID and the parameters are stored, and no spectrum is stored.
III. Simple 1D C13 NMR Experiments

1. Click on Main Menu; Setup; C13, CDCL3 (if the solvent is CDCL3; if not, click on Nuc,Solv; C13; solvent_name).

2. Follow steps II.2-II.4 above to start the data acquisition, and the rest of the procedures in II for data processing.

IV. NMR Experiments Using GLIDE

GLIDE is a program that can assist users to run NMR experiments without using the regular commands and parameters. Instead, users only need to answer a series of questions to set up the experiments, and the computer will automatically start the experiments and print out the spectra.

1. Start the GLIDE user interface by clicking on the GLIDE button. A new row of buttons will be shown on top of the screen.

2. Click on the Setup button in the GLIDE window, a new Setup window appears. Select the type of experiment to be done (To select different experiment, move the cursor to the upside-down-triangle button next to Experiment, click and hold the right mouse button to reveal a table of experiments available, scroll down to the name of the experiment needed and release the mouse button), the solvent used, eject or insert sample (usually insert), autolock, autoshim on or off. If the sample is already in and the field is already locked and shimmed, select insert, autolock off and autoshim off. You can also enter a filename under which the data will be stored, and any comments about this experiment in the Save As and the Text windows. Click the Setup button, one or more new buttons named Acquire, Process, Plot, and Store will appear under the Custom button.

3. Click on the Acquire button under the Custom button. A new window will show up for you to make more specific selections. If a more specific experiment is selected, another window will show up for selecting parameters, such as the number of scans, (or the number of FIDs), relaxation delay, pulse angle, etc. After all the questions are answered, you can click on the Do button to start the experiment, or on the Close button, if you have not checked the parameters for processing and plotting.

3. Click on the Process button, select Fourier number and Line broadening, then click on the Close button.

4. Click on the Plot button; make your choice among the options for the Spectral Width, Plot Integral, Plot Parameters, and Plot Peaks; click on the Close Plot button.
5. Click on the large **Go** button to start the experiment. The computer will automatically collect the FID, transform it into spectrum, plot the spectrum, and store the data as instructed by you.

6. To exit from GLIDE, click on the **Exit** button in the Glide user interface window.
V. Advanced 1D NMR Experiments

A. Arrayed Experiments

1. Enter more than two values, separated by a comma, for the parameter to be arrayed. E.g. typing \texttt{pad}=0,600,600,600,600,600 will set up five experiments using 0 sec, 600 sec, 600 sec, 600 sec, 600 sec for \texttt{pad}, respectively. This will run five spectra with 10 minutes delay between each spectrum - a convenient way to monitor chemical reactions in the solution.

2. Alternatively, type \texttt{array('parameter',\#steps,starting,increment)} or just type \texttt{array}, and you will be prompted for the four arguments.

3. All FIDs can be transformed into spectra together by \texttt{wft}.

4. Command \texttt{dssa} displays all spectra in stacked form with offsets determined by \texttt{ho} (horizontal offset) and \texttt{vo} (vertical offset).

5. Command \texttt{dssh} displays all spectra horizontally.

6. Use \texttt{pl(start,finish,step)} or \texttt{plarray} to plot the spectra.

B. Proton Homonuclear Decoupling

1. Run a regular proton 1D spectrum.

2. Determine the peak(s) to be decoupled by setting the cursor on the peak(s) and type \texttt{sd}.

3. Type the macro command \texttt{homodec}. Change \texttt{dpwr} if needed.

4. Start the acquisition by \texttt{ga}.

C. APT (Attached Proton Test) Experiment

1. Run a regular C13 spectrum, if time permits.

2. Type \texttt{apt} to load proper parameters (or click on the APT button).

3. Adjust \texttt{d2} (1/JCH for CH, CH3 up and C, CH2 down) and \texttt{nt} (multiple of 4) if needed.

4. Type \texttt{ga} to start. Phase the spectrum so that CHn peaks are up and CHn+1 peaks are down or vice versa.

D. DEPT (Distortionless Enhancement by Polarization Transfer) Experiments

1. Run a regular 1D C13 spectrum if time permits.

2. Type \texttt{dept} or click on the DEPT button to load proper parameters.

3. Change \texttt{j} (averaged CH coupling), \texttt{nt} (multiple of 16), and \texttt{d1} (relaxation delay for protons) if needed.

4. By default, parameter \texttt{mult} is arrayed with four numbers, 0.5,1,1,1.5 in order to obtain 4 subspectra. If only a DEPT135 spectrum (with CH, CH3 up and CH2 down) is needed, change \texttt{mult} to 1.5, which will run only one spectrum.

5. Type \texttt{ga} to start the experiment.
6. Type **adep** **t dssa** to analyze the spectra, and **pldept** to plot them.
   The full analysis of the DEPT result can also be done by clicking on the **Main Menu, Analysis, DEPT, Full Analysis** buttons in sequence.
   (If the DEPT135 is run, only one spectrum is obtained and no need to do step 6.)

**E. NOESY1D (Gradient-enhanced 1D NOE Experiment)** (The best choice when using INOVA spectrometers)

1. Take a normal 1D 1H spectrum.
2. Type **NOESY1D** [ret] to load relevant parameters.
3. Use the two cursors to enclose the peak region to be irradiated (inverted), and click on the **select** and **proceed** buttons in sequence.
4. adjust **nt** and **mix** (mixing time, typically 0.5 sec) if needed, and **ga** [ret] to start the experiment.

**F. TOCSY1D**

1. Take a normal 1D 1H spectrum.
2. Type **TOCSY1D** [ret] to load relevant parameters.
3. Use the two cursors to enclose the peak region to be irradiated (inverted), and click on the **select** and **proceed** buttons in sequence.
4. adjust **nt** and **mix** (mixing time, typically 0.05 sec), and **ga** [ret] to start the experiment.

**G. Proton 1D difference NOE using Mercury NMR Spectrometers**

1. Run a regular 1D proton spectrum.
2. Type the command **noedif** to load proper parameters. Adjust the irradiation power **dpwr** if needed.
3. Determine the irradiation points on the multiplets to be saturated by moving the cursor to the points, typing **sd**, and **fn=dof**, where **n** starts from 0 to up to 8 (up to 8 points can be irradiated alternately for a period of **tau** and for d1/tau times).
4. Set the cursor to a location with no peak, type **sd** and **dofoff=dof** (used for the control spectrum).
5. Adjust **ss** (4 or 8) and **nt**; type **ga** to start the experiment.

**H. Proton 1D difference NOE using INOVA Spectrometers** (traditional way)

1. Run a regular proton 1D spectrum.
2. Type the command **cyclenoe** to load proper parameters.
3. Determine the irradiation points by moving the cursor to the center of the multiplet, typing `sd`, and `satfreq=dof`. Adjust spacing (in Hz) and pattern (1 or 2) to cover the whole multiplet to be saturated.

4. Set the irradiation power `satpwr` (-10 to 10) and `sattime` (on the order of T1), and `mix` (optional).

5. Set the cursor to a location with no peak, type `sd` and `control=dof` (used for the control spectrum).

6. Adjust `ss` (4 or 8) and `nt`; type `ga` to start the experiment.
VI. Proton 2D gCOSY experiment

1. In expn \((n = 1-9)\), take a simple 1D H1 spectrum. Use the two cursor lines to select a narrower spectral region with all the peaks included; then, type the command `movesw` to fix the new spectral window. Recollect a 1D H1 spectrum to make sure the new region is properly selected.

2. Move to the next experiment number by `jexp m` \((m = n + 1)\). If `expm` is not existent, type `cexp(m)` to create it.

3. Type `mp(n,m)` \([\text{ret}]\) to copy the parameters in `expn` to `expm`. Type `gCOSY` \([\text{ret}]\) to load all parameters used in the gCOSY experiment. Type `su` \([\text{ret}]\).

4. Check the total time needed for the experiment by typing `time` \([\text{ret}]\). Adjust `nt`, if needed, then start the experiment by typing `go` \([\text{ret}]\).

Processing Data

5. The spectrum can be examined before the data acquisition is actually finished if `proc1` is set to `ft` (if `proc1` is set to `lp`, you can temporarily change it to `ft`, and change it back to `lp` after the data acquisition is finished). To generate the spectrum, type `wft2d` \([\text{ret}]\). The resultant 2D spectrum will be displayed. (Command `dconi` will re-display the 2D spectrum.)

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar by the right hand side of the spectrum and click the middle button. Finer adjustments can be done by clicking on the `+20%` and `-20%` buttons.

7. The expansion of the spectrum can be done by defining the lower left and the upper right corners of the selected region with the left and the right mouse buttons followed by clicking the Expansion button.

Plotting the Spectrum

8. To plot the 2D spectrum displayed, type `plcosy('pos',i,j,k,m)` \([\text{ret}]\), where `i` is the number of contour levels to be plotted (usually >10), `j` is the intensity ratio of every adjacent levels (usually between 1.2 and 1.5), and `k` is the experiment number where the 1D proton NMR spectrum is stored and is to be used as the projection in both F1 and F2 axes, and `m` is a multiplier to adjust the amplitude of the projections.

VII. Other 2D NMR Experiments
In general, if there is a macro file for the experiment to be performed, you can simply start by typing the name of that macro (e.g. gHSQC, gHMQC, gHMBC, gHSQCAD, gHMBCAD, NOESY, TOCSY, ROESY, etc.) after running a regular 1D spectrum with desired parameters. To display the pulse sequence of this experiment, type \texttt{dps} [ret]. To see if the manual file exists, in the unix terminal window, check the entries in the directories \texttt{/vnmr/maclib/} and \texttt{~/vnmrsys/maclib/}.

By default, \texttt{gHMQC} and \texttt{gHSQC} will set up H/C hmqc and hsqc experiments with \texttt{sw1} set to cover about 0-160 ppm; and \texttt{gHMBC} will set \texttt{sw1} to 0-220 ppm.

By default, \texttt{NOESY} will set up the experiment with mixing time \texttt{mix}=0.5, which can be changed according to the molecular size to optimize the NOE cross peak intensity.

Some 2D experiments (e.g. gHSQC, gHMQC, gHSQCAD, gHMQCAD, NOESY, TOCSY, ROESY) generate phase-sensitive spectra (you can tell from the parameter \texttt{phase}: if \texttt{phase} is \texttt{arrayed}, it is phase sensitive, if \texttt{phase} is \texttt{1}, it is magnitude mode), which need \texttt{wft2da} (instead of \texttt{wft2d}) command to transform into spectra, and these spectra often require phase corrections. The following is one convenient way to do it:

**Phase Correction of Phase-sensitive 2D Spectra (e.g. NOESY, gHSQC, gHMQC, gHSQCAD, TOCSY, ROESY)**

1. Display the 2D spectrum with command \texttt{dconi}.
2. Use the left mouse button to select a row across a large peak on the upper right corner.
3. Type \texttt{ds} to display this selected row and phase the peak with only the 0\textsuperscript{th} order phasing parameter.
4. Type \texttt{dconi}, and select another trace across a peak on the lower left corner of the 2D spectrum.
5. Type \texttt{ds} to display the second row selected. Phase this new peak with only the 1\textsuperscript{st} order phase correction (do not change the 0\textsuperscript{th} order phase). – This is done by clicking on the \texttt{Phase} button, left-clicking on the peak region selected in step 3 above immediately followed by left-clicking on the region containing the new peak and phase this new peak.)
6. Type \texttt{dconi} and see if all the rows are phased.
7. To phase columns, rotate the 2D matrix by clicking on the \texttt{Return}, \texttt{More}, and \texttt{F2 Mode} (or \texttt{F1 Mode}) buttons; then follow the above steps to phase the new rows.

If you cannot perform phase correction, check the parameter \texttt{pmode}. It should be set to \texttt{full}.

For NOESY spectrum of small molecules, always phase the diagonal peaks to negative so that the NOE cross peaks are positive.

**Reference Assignment of Heteronuclear 2D spectra (e.g. gHSQC, gHSQCAD, gHMQC, gHMBC, gHMBCAD)**
1. Select a peak whose chemical shifts on both axes are known by using the left mouse cursor.

2. Type $\text{rl}(mp)$ $\text{rl}(nd)$, where $m$ is the chemical shift of the peak on the F2 axis and $n$ is that on the F1 axis. Note that $d$ has to be typed after $n$.

To plot the heteronuclear 2D spectrum, use the command $\text{plhxcor}$ instead of $\text{plicosy}$:

$$\text{plhxcor}(\text{pos|neg'},i,j,k,l,m,n)$$

where the first argument is used to select either positive or negative peaks (or both if ignored), $i$ is the number of contour levels (e.g. 20), $j$ is the intensity ratio of adjacent levels (e.g. 1.3), $k$ is the exp # with the H1 spectrum for plotting the F2 projection, and $l$ is the exp # with the X spectrum for plotting the F1 projection (setting $k$ or $l$ to -1 suppresses the projection). $m$ is the multiplier to adjust the H1 projection and $n$ is that for the X projection.

To plot the actual projections of the 2D spectrum, use buttons $\text{Proj}$, $\text{Hproj(Max)}$ or $\text{Hproj(sum)}$, $\text{Vproj(max)}$ or $\text{Vproj(sum)}$, and $\text{Plot}$ to plot the projections first (the amplitude of the projections can be adjusted by using the middle mouse buttons before plotting), then use the command $\text{pcon(\text{pos|neg'},i,j)}$ and $\text{page}$ to plot the 2D spectrum.