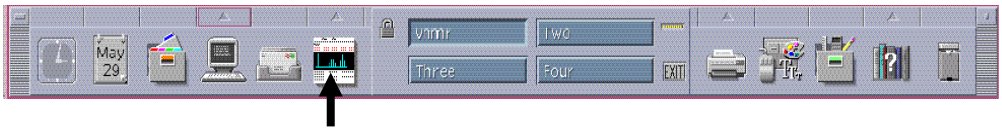
Practical Guide on Varian NMR Systems:

Locking, Shimming, Acquiring, and Processing
(January'09 revision)

- Mouse usage: SUN computers use a three-button mouse. Therefore, terms such as *left-click, right-click, **center**-hold/click* will be used throughout this guide. **This document describes operation of the three hands-on Varian NMR spectrometers (I-500, I-400, and M-300).**
  - The standard probe for the I-500 is a **Broadband PFG probe** that is set up to observe ^{1}H, ^{13}C;
    *Contact the managers for support on how to tune the probe for other nuclei.
  - The standard probe for the I-400 is a **4-nucleus PFG probe** equipped to observe ^{1}H, ^{13}C, ^{19}F, and ^{31}P nuclei.;
  - The standard probe for the M-300 is a **ATB PFG probe** equipped to observe ^{1}H, ^{19}F, and ^{31}P nuclei.

All probes have VT capability (temperature control). *Contact the managers for support on VT expts.

(1) **Login**	At the Sun workstation, inside the login window, type: **username** *<enter>* **password** *<enter>* a) Both **username** and **password** are case sensitive entries; check the *Caps Lock* key if you do not succeed to log onto the UNIX session; b) Wait for the start of the UNIX session; c) Launch the VNMR program by *left-clicking* once on the VNMR icon at the CDE bar (see fig below, **black arrow pointing to the VNMR icon**).  Obs: All NMR commands and parameters are typed at the input window in the VNMR command line (see fig. next page)
(2) **Inserting the sample**	✓ Type **e** <enter> , to eject the standard sample (most frequently $CDCl_3$, D2O), which is always kept inside the magnet; ✓ Replace the standard sample for your sample into the spinner; ✓ Adjust sample's position with the gauge; ✓ Insert the spinner with your sample into the top of the magnet; ✓ Type **i** <enter>, to descend your sample into the probe, inside the magnet.
(2a)	Type ***callshim*** <enter> at the VNMR command line to retrieve the best shim set for the spectrometer

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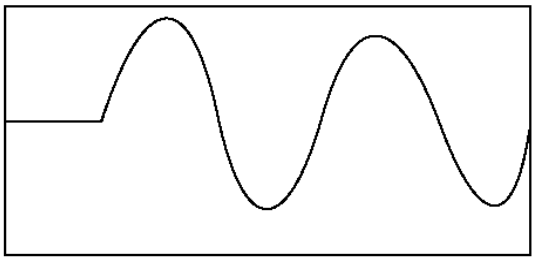
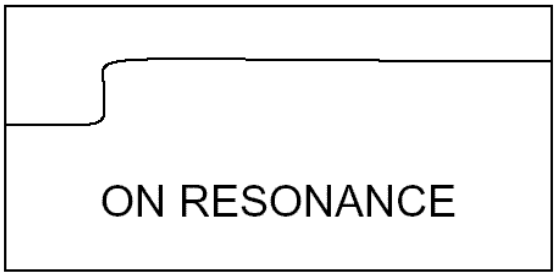
(3) **Locking on deuterated solvent**	**Manual locking** ✓ Keep the sample static (spin = 0). ✓ Click on the **Acqi** button. ✓ Click on Lock OFF. ✓ Increase ***lockpower*** **(+4, +8 units)**. ✓ Increase ***lockgain*** **(e.g., +8, +12 units)**. ✓ Change Z0 in ±4 (or ±1, ±16, ±64) steps until a flat signal is reached [see fig **(b)** on the right side]. [**During this process** you should see a sinusoidal wave - fig **(a)** -, whose frequency becomes progressively smaller indicating that you are getting closer to *on-resonance* position = 0 Hz; if, **however**, the wave starts to quickly bounce up and down, it is a classical symptom of lock *saturation,* i.e., the **lockpower** is too high; in this case, reduce the **lockpower** by ± 4, ±8 units, whichever turns the signal stable again (with no jumping). Proceed changing Z0 until you see a "plateau" on the lock display – see figs **(a)** and **(b)**] ✓ **Decrease** ***lockpower*** (+4, +8 units) and ***lockgain*** (e.g., +4, +8 units); keep the lock level in ~50% mark.	**(a)**  **(b)** 

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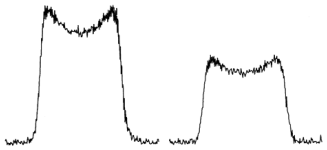
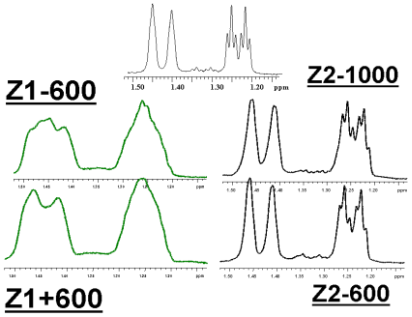
(4) **Shimming**	Automatic ^{2}H shimming : ✓ Type **gmapshim** *<enter>* to carry out gradient shimming automatically using the lock signal from the solvent ▪ During ^{2}H gradient shimming, the sample stops spinning (***gmapshim*** sets spin = 0) and the lock circuitry is DISABLED; ((The information is seen on the ACQSTAT window from within VNMR)) The ^{2}H gradient shimming process provides **two** profiles (see fig **(a)**) and converges after a few iterations. At each iteration, its # and r.m.s. error are displayed in the text window. <u>The overall process takes a few minutes to complete</u>, when the r.m.s. error is ≤ 1.00. ▪ In ^{2}H gradient shimming, only Z1 to Z4 are shimmed. Any Z shim higher than Z4 and non-spin shims (X, Y, XZ, etc) are not changed. Fig **(b)** depicts the Z1 shim when it is OFF by ±600 units and Z2 if off by -600 and -1000 units. Notice that when Z1 if OFF the lines broaden to an extent that no fine structure is seen (see top spectrum) and when Z2 is OFF the lines are skewed, besides broadening.	**(a)**  **(b)** 

(5) **Acquiring**	✓ For <u>PROTON</u>: *left-click* <u>once</u> on **Setup** button → **^{1}H,CDCl$_3$ (see fig nest page, <u>white arrow</u>)** or, for any other solvent, **Setup → H1→ Solvent**; type **su** *<enter>* ✓ For <u>CARBON</u>: *left-click* <u>once</u> on **Setup** button → **^{13}C,CDCl$_3$** or, for any other solvent, **Setup → C13→ Solvent**; type **su** *<enter>* ✓ Type **ga** *<enter>,* to start acquisition. The sample spins at 20-25 Hz. ○ By default, the number of scans (*nt,* **<u>BLACK arrow</u> in the fig next page**) for <u>PROTON</u> is **16** and for <u>CARBON</u> is **1024**. To increase S/N by **a factor of 2**, increase *nt* **by a factor of 4** before acquisition starts ○ For low S/N samples, type *nt* = 1e6 *<enter>* before acquisition, type **ga** <enter> and monitor the spectrum at every <u>4-scan block</u> by typing **wft** *<enter>* For NMR processing, type **wft** *<enter>*

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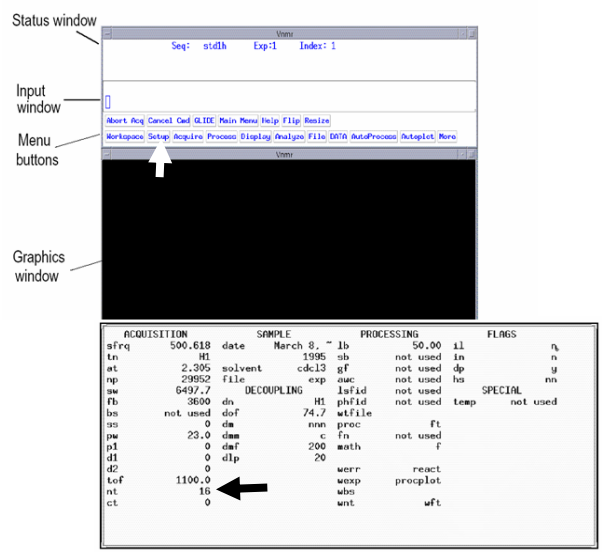

(5a) **Data processing and analysis**	➢ **<u>From this point on</u>, if you plan to run <u>other experiments</u> on the Varian NMRs (e.g. ^{13}C spectrum, 2D spectrum, etc) you may continue on the following steps. The online data processing is plausible only if you plan to acquire <u>new NMR data</u> on another workspace, and in the meantime process the already acquired data using VNMR (magnet time is charged). Otherwise proceed to step 9.**
	- <u>**For off-line data processing and analysis**</u>, the NMR lab at Princeton University supports **MestRe Nova**, the multi-platform (Windows, Mac, and Linux) NMR processing software being developed by Mestrelab Research SL. • Princeton University has campus-wide license. - Download the most recent version from www.mestrec.com. (You may also download the manual). To activate your license (which will be received after the training), you will need to be hard wired on Ethernet within the Chemistry domain.
	- <u>**NMR Spectra Prediction tools**</u>: a) the ACD software, b) a single copy of the full version of ModGraph's *NMRPredict* software is installed on an PC at room 24, and c) the KnowItAll® (from Bio-Rad Laboratories, Inc.) package.

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(6) **Phasing**	**Automatically** ✓ Type **aph** <*enter*> Alternatively, type **aph0** <*enter*> to adjust only the *zeroth order* phase parameter. **see step 11: Printing**	**OR -- Manually** ✓ *Left-click* on *Phase* button (below the permanent Menu); ✓ *Left-click* once at the rightmost side of the spectrum; ✓ *Left-hold* to phase all peaks up and symmetrically **within boxed region**; ✓ Finish this first step *Left-click*ing on *Phase* button; ✓ Start ***first-order phasing*** by *left-clicking* once at the rightmost side of the spectrum; ✓ Then *left-click* once **at the leftmost side of the spectrum**; ✓ *Right-hold* to phase all peaks up and symmetrically **within and outside the boxed region**; ✓ Finish the second step by *Left-click* on *Phase* button.
(7) **Peak picking**	✓ *Left-click* once on the **Th** button and adjust its vertical position to pick the peaks of interest. (*Left-hold* and point to the **Th**'s horizontal **yellow line**. Adjust it up or do down accordingly); ✓ You may also type **th** = value <*enter*> at the command line, where value is the vertical position in mm; ✓ Peaks can be displayed by typing **dpf** <*enter*>; Sensitivity can be increased with **dpf(2)** or **dpf(1)**. Default is 3. The sensitivity may be increased, if a shoulder could not be picked with dpf only. **See step 11a: Printing**	
(8) **Integrating**	✓ *Left-click* on **Partial Integral** button and then *Left-click* on **Resets**; ✓ A **green integral** curve will appear on the spectrum; ✓ Integrate each peak *Left-click*ing once the **red vertical** cursor downfield to the peak (to the left of the screen) and then *Left-click*ing once upfield to it (to the right). Repeat procedure for all peaks; ✓ After integration is completed, type **bc** <*enter*> for baseline correction; ✓ To clear all resets points, type **cz** <*enter*>. **See step 11a: Printing**	

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(9) **Saving FIDs**	Type ***svf*** *<enter>* and, at the question mark, **type** the name of the spectrum (the VNMR software will append **.fid/** as a suffix to the FID filename); VNMR saves each spectrum as a directory, comprising 4 files: 1) **fid:** the raw FID data; 2) **procpar:** contains all acquisition, processing, display parameters; 3) **log:** text file recording the history of the acquisition; 4) **text:** text file containing the text name associated with the spectrum ➢ Inputted via **text**('...') or **gettext** window, **see Table**.

(10) **Exiting VNMR** **and** **UNIX (LOGOUT)**	✓ Eject your sample from the magnet (type **e** *<enter>*) and put the standard sample ($CDCl_3$ or D_2O) *back into* the magnet; ✓ *Lock on* the standard sample; ✓ Type ***exit*** *<enter>* at the **VNMR command line** and answer the **y/n** questions **before exiting**; ✓ After exiting the VNMR program, *right-click once* anywhere on the BACKDROP: in the window that is opened, select the **last option** at the last line: **LOGOUT -** to finalize the **UNIX** session (*)

(*) It is very important NOT to forget to exit the UNIX Session:

➔ spectrometer charges are based on the length of this Session

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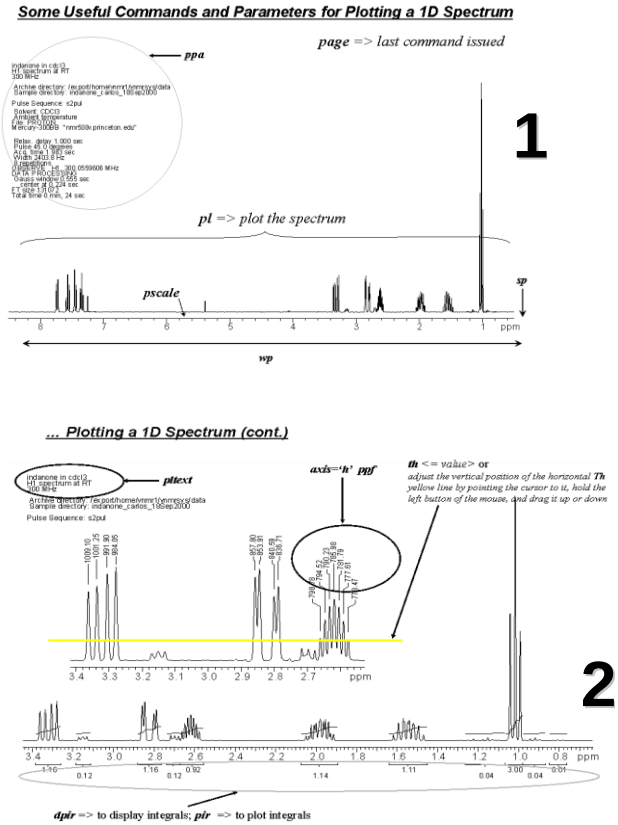
11) Printing	✓ Type ***pl pscale ppa page*** *<enter>* *Where:* ***pl*** *(plot spectrum)* ***pscale*** *(plot scale)* ***ppa*** *(plot parameters)* ***page*** *(clear buffer)* – see fig *The above commands send plot objects to the printer buffer.*
11a) Printing	*Other commands:* ***ppf*** *(plot peaks),* ***pir*** *(plot the integral values, needs vp = 12, minimum) - see fig*
11b) Printing	✓ Anything written, by default, in the *text window* **can be redirected to the printer** *For example,* ***printon*** *dg* ***printoff*** *<enter>* *Where:* ***printon*** *(turn print buffer* ***on****)* ***dg*** *(****d****isplay main* ***g****roup of parameters)* ***printoff*** *(clear printer buffer)* *Other commands:* ***dg1*** *(display group from the 2nd page of the main parameters),* ***dgs*** *(display group shims),* ***dll*** *(display line list)*



OBS:

1) For poor **lock signal** and/or **lineshape**, do the following:
   a. Load spectrometer shim settings by typing ***callshim*** *<enter>* at the **VNMR console line**, which is a macro command that will load the *stdshims* file, and issue **su** *<enter>*.
   b. *Left-click* once on the ***Acqi*** button to shim the sample manually (mostly Z1 and Z2).

2) ***gmapshim*** *<enter>* does ^{2}H gradient shimming.

Manual Referencing – Find the solvent (or TMS peak); click on the peak, and type in **the VNMR command line**:
nl rl(xx<p>) *<enter>.*
Example: ***nl rl***(7.27p) *for* $CDCl_3$

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Some Useful VNMR Commands and Parameters (Some UNIX Commands function within VNMR environment, as seen below)	
xx ?	✓ *Parameters can be **checked** at the VNMR command line by typing the parameter name followed by a question mark.* *They can be **set** by typing the parameter name followed by an equal sign followed by the value, e.g. vs? , nt? , fn?*
vs, vsadj	***Vertical scaling**: can be set via middle button of the mouse. Position the mouse on the peak and increase its vertical scale **by holding the middle mouse's button**.Parameter can also be changed at the keyboard, i. e. **vs=300.** **vsadj** automatically adjusts **vs** to fit the screen with the tallest peak of the spectrum.*
vp	*Vertical position, in **mm** from bottom of page (or screen).*
tar cvf/xvf	*Create a **.tar** file for **ftp** transfer. Ftp sessions only transfer files; FIDs are directories and need to be tar'ed before being transferred to another computer. For example: tar cvf* **myFIDtoday**.tar **myFIDtoday** To "untar" (or unzip) the tar file, type tar xvf **myFIDtoday**.tar
scale, pscale	*Display scale below spectrum on screen (**pscale** to plot the scale).*
text('....') *or **gettext** <Enter>*	*Provide a way to comment the FID for archiving and plotting purposes*
cd<('path')>	*Change directory. Without an argument it sets to the home directory*
pwd	*Print working directory, show the current default directory*
ls	*Directory listing in text window*
cexp(#)	*Create experiment job #, where # is any number between 1 and 999*
jexp#	*Join experiment job #.* ***Obs: all experiment jobs but* exp1 *are deleted upon logout***