BARE BONES GUIDE to the Operation of the
Varian Inova-400 & Inova-500
NMR Instruments using VNMRj 3.2

This guide is intended to accompany proper training and formal check-out by the NMR Facility Director, or the Director's Designee. You must be OK'd by Facility staff prior to using the instrument. This guide covers the most basic operational details for acquiringand storing routine 1-dimensional NMR spectra for the most common (routine) nuclei: ^{1}H, ^{13}C, ^{31}P, and ^{19}F.

Other guides are (or will be) available for processing/plotting, performing more advanced experiments or for observation of other nuclei.

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Conventions in this manual:

Boldface text indicates commands to be typed on the Command Line[*]

<angle brackets> are used to designate a key to be pressed (i.e. **<Ret>** for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.
    LMB will be used to designate the Left Mouse Button
    MMB will be used to designate the Middle Mouse Button
    RMB will be used to designate the Right Mouse Button
    Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

[*] Always *click* on the command line to *focus* the cursor on the command line before typing commands.

1) SIGN IN the Log-Computer (Excel Spreadsheet) immediately before you start… follow instructions provided.
2) *Click* [ClearSampleInfo] (yellow button) to clear the previous user's sample information.

3) Remove the Reference sample, INSERT your sample and LOCK.
    a) Type **e <Ret>**[**] to eject the sample (or *click* [Eject]), and carefully remove the sample from the top of the magnet, remove the reference tube from the spinner turbine.
    b) WIPE your NMR tube thoroughly. with a Kim-Wipe, slightly wet the Kim-wipe with alcohol if necessary. Be sure the NMR tube is clean.
    c) Put your sample in a spinner-turbine, and then wipe the tube again! (before setting the sample depth)
    d) Set the proper sample depth.
        i) Use the depth gauge by placing the tube in the turbine, place the turbine on the gauge, and push the sample down until it touches bottom.
        ii) If the sample is "shorter" than normal, be sure that the sample is centered above/below the observe coil limits (use the dashed-rectangle on the gauge, or the decal on the wall to check).
    e) Double-check the sample depth, then carefully place the sample in the top of the magnet.
    f) Type **i <Ret>**[**] to insert your sample (or *click* [Insert]).
    g) Under [Start][Standard] → Select your Solvent for locking, then *click* [Find Z0]… the instrument should lock automatically.
        i) If the lock level is not stable (i.e. drifting up/down, lower the lock power using the left/right mouse button).
        ii) You can adjust the Lock power, gain, & phase from either the [Lock] or [Shim] sub-panels, if necessary.

4) SHIM the magnetic field (if you are on the 400 or 500 you will usually only do Z1 & Z2, then gradient shim):
   a) *Click* on **[Shim]** to enter the shim window, and you can Left/Right Click on Z1 & Z2 to maximize the lock level displayed as a rotating dial. {**Note: on the 400, use Z1C & Z2C with a sensitivity of +/- 1**}. You can change the sensitivity by *clicking* the MMB (the scroll-wheel).
   b) The objective is to maximize the lock level, but the actual lock value isn't really important. If the Lock Level goes near 100% , lower the *lock gain* because you can't shim if the lock-level is off-scale.
   c) If shimming isn't very good, try *clicking* on [Read Default Shims] to load standard shims.

5) Gradient Shimming:
   a) Most solvents work well, but solvents with more than 1 peak in the 1H/2H spectrum can yield less than optimal results: Toluene, Methanol-d4 or THF-d8, Pyridine-d5 are examples of these solvents. Try manual shimming, with these **multi-frequency solvents**, or just type "shim<Ret>" after manually shimming, and wait for it to finish shimming before continuing.
   b) *Click* **[Gradient Shim]**. to begin Gradient Shimming.
   c) Wait until gradient shimming is completed… A message will appear in the Message Bar indicating that Gradient Shimming is completed.
   d) Turn the Spinner back ON: *Click* [Standard] →[Spin/Temp]→[Regulate Speed]… be sure the speed = 16. {After gradient shimming, spinner won't restart automatically}.

6) Setup your experiment (i.e. [PROTON], [CARBON], …etc.) by clicking the button, or select from the [Experments] Pull-down menu. Pay attention to the screen, making sure the blue-bar at the top should shows "busy" after clicking… otherwise, you might have to *click again*. Using the [Experiments] Pull-Down is generally more trustworthy than the buttons on the left…. Just sayin'…

7) The "Comment" box contains descriptive "text" that can be anything you wish to describe your sample. This text can be displayed/printed when you process the data later. You can enter the information in this box now, or while your data is acquiring. HINT: If your *comment* is also to be your *filename*, you can select it, <Ctrl><C> to copy, and later paste it into the "Save As.." filename bar (<Ctrl><V>).

8) Set Parameters, before acquiring the spectrum: {these can also be set using the [Acquire] tab options}
   a) Type **nt = # <Ret>**[**], where "#" is the number of scans (multiple of 4).
      i) 16 to 32 for routine ^{1}H, 512 to infinity for ^{13}C.
      ii) You can specifically set the spectral-window (upper/lower ppm limits) using the [Acquire][Default] panel, or just use the default window.
      iii) Other parameters can be changed under the [Acquire] tab, such as relaxation delay [d1], pulse angle, block size (bs), acquisition time, …etc. If you don't know what these are, consider staying with the default values.
      iv) *{Optional}* You can explicitly set your Spectral Window under **[Acquire][Default]**, by entering the desired limits in the boxes. Or you can select from several "defaults" in the pull-down menu.

9) Begin the Acquisition:
   a) *Type* **ga<Ret>**[**] to begin data acquisition.
      i) After you see "BS # Completed", you can type **wft<Ret>**[**] to "weight and FFT the data"
   b) The spectrum will appear automatically after "nt" scans.
      i) If you selected a large number for nt, but you want to stop it early, you may type: **sa('bs')<Ret>**[**]. This stops the acquisition at the next multiple of "bs". However, ;NEVER use this command when the acquisition is almost done!! *If you use this command and the acquisition completes "nt" scans first, you will crash the instrument.*
      ii) After the acquisition stops, type **wftf <Ret>**[**]. This is a macro that does "wft" and scales the spectrum to fill the screen, and displays the scale.

10) Phase the Spectrum: (*optional*)
    a) Type **aph<Ret>**[**] to autophase the spectrum. If this fails, try **aph0<Ret>**[**].

11) Save your spectrum (fid) to your directory in *rgroups*.
    a) Select "Save As.." (click the blue "floppy disk" icon), or <Ctrl><S>. **Navigate to your data directory**, and type your filename in the dialagon box, then *click* [Save].

** click on command-line first to focus typing!   2

12) If you want to do another experiment (i.e. ^{13}C/CARBON) go <u>back up to Step 6</u> and continue from there.

13) <u>Type **e <Ret>**</u>^{**} (or *click* [Eject]) to eject your sample if you are done with this sample.
   a) If you are done, insert the reference sample (wipe the tube with a KimWipe, and check the sample depth before inserting.
   b) If you have another sample, you can remove your sample from the spinner turbine (be careful not to break the tube), and insert your next sample into the turbine (wipe the tube, check sample depth).

14) <u>Type **i <Ret>**</u>^{**} (or *click* [Insert]) insert the next sample, or the reference sample.

15) <u>Quitting:</u>
   a) When done, the reference sample should be inserted.
   b) <u>Lock and Shim</u> on the reference – (<u>Type **reset<Ret>**</u>^{**} to reset all parameters for the reference sample, and <u>WAIT for the "Setup Complete"</u> message before doing anything else!).

   c) Make sure to click [Timestamp] to register the {End Time} in the Logbook spreadsheet, indicating the time used. If you fail to do this, you will cost your research advisor money… that wouldn't make her/him happy.

16) <u>Data Processing:</u> Instructions for processing, integrating, peak-picking, and plotting are in a separate document. All data processing should be done on your own computer using MestReNova, or on one of the workstation in the NMR lab (not on the NMR spectrometer).

17) <u>Retrieving Data via the **Diskstation – Web Service**</u>:
   a) From outside the NMR lab, you can go to: http://chemnmr.colorado.edu:5000 (port# 5000). This will connect you to the DiskStation file-server.
   b) Login using the public username and password:

   c) The data from the Varian 400/500 can be found under the "Vdata" folder. Navigate to your data directory under your group directory.
      i) Right-click and select "download", and the .fid folder will be "zipped" and saved in your default download location on your computer.
      ii) You can drag the .zip file directly into the MestReNova software for processing.