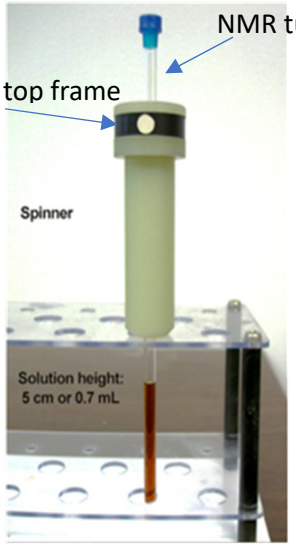
STEP-BY-STEP INSTRUCTIONS for performing ^{1}H and ^{13}C NMR experiments on 400/500 MHz Varian VNMRS and 600 MHz Varian INOVA NMR instruments

The appropriate height of the NMR tube is 6 -7 inches (or 16 to 18 cm)
The height of the NMR solution is 5 cm, the volume is 0.7 ml



1. **Login:** Select your name and enter password.
2. On the Desktop screen, double click on the **OpenVNMRJ** icon to run NMR experiment.
3. To eject previous sample (D_2O) from the magnet click **Eject** button on the Action bar or type **e** in the VNMRJ input window and hit Enter button.
4. Carefully remove ejected sample (D_2O sample) from the top of magnet. Hold the spinner from the top frame, but not from the NMR tube.
5. Remove the NMR tube from the spinner by slowly rotating it (back and forth) while simultaneously moving the NMR tube upwards until it exits the top of the spinner.
6. Wipe the NMR tube and spinner with a Kimwipe before inserting it into the spinner. Carefully insert the NMR tube into the spinner hole and push down until it exits the spinner while rotating the NMR tube. Note: *Do not force the NMR tube into the spinner hole.*

Sample position using a depth gauge prior to placement on the probe
For shorter samples the solution height must be centered on the middle dotted line (**coil area**) of the depth gauge

.

7. Hold the Depth Gauge and insert the spinner with the NMR sample into the top of the Depth Gauge. Then gently push the NMR tube down until the bottom of the NMR tube touches the white top moving part of the depth gauge.
8. Remove the spinner with NMR tube (hold the spinner from the top frame, but not from the NMR tube!) from the Depth Gauge and place it carefully on the upper barrel of the magnet. Before placing the sample in the upper barrel of magnet be sure that air is coming out from the upper barrel of the magnet.
9. Click the Insert button on the action bar to insert the NMR sample into the magnet; or type i and click Enter button to insert the NMR tube into the magnet.

Selecting Proton parameters and performing three important procedures: LOCKING, SHIMMING, and TUNIUNG

10. To acquire the ^{1}H NMR spectrum, type **jexp1** and hit Enter button to join Experiment 1 (workplace). On the left corner (top) of OpenVNMRJ window, click **PROTON** tab to select proton parameters, then type **su** and hit Enter button.

12. Click the Start Tab, select a **solvent from the solvent list**.

13. Under the Start Tab click **Shim** and click **Read Defaults Shims**.

14. Under the Start Tab, click the **Lock** and then click **Find Z0**

15. On the action bar click **Gradient Shim**. Wait until the gradient shimming is complete. A message indicating this (**Idle**) will appear in the lower middle of the OpenVNMRJ message window. Depending on the nature of the solvent used and the volume of the solution, the Gradient shimming will take approximately 1-2 minutes.

16. Click the **Autotune** button on the action bar Tab; the probe will be tuned to the ^{1}H frequency. *Note: Auto tuning is only valid for the 400 MHz Varian VNMRS NMR instrument and 400/500 MHz JEOL NMR instruments; For 500 MHz Varian VNMRS/600 MHz Varian INOVA NMR instruments, tuning is required manually. Do not click the auto tuning button when using these NMRs.*

17. To record other nuclei such as ^{19}F, ^{11}B, ^{31}P, ^{2}H (Deuterium), ^{6}Li, ^{133}Cs, etc NMR spectrum etc user should send email me in advance (Novruz.Akhmedov@ou.edu) for separate training.

Note: Probe tuning is required when there is a significant change in the polarity of the solvent. Changing from a non- polar organic solvent to a polar organic solvent or aqueous solvent generally requires re-tuning the probe. Changes in the ionic strength of the solution (for example, low salt to high salt) also require the retuning of the probe.

To run the ^{1}H NMR spectrum for the five NMR samples in $CDCl_3$, you need to tune the Probe for sample 1 only. For the remaining 4 samples, after placing the 2nd sample on the magnet, there is no need to find lock and tune the Probe, you only need one operation - click **Gradient shimming**, the same should be done for samples 3, 4, and 5. However, for running 2D NMR experiments, it is necessary to find the exact lock value and fine-tune of the Probe for each individual sample.

If there is one sample in $CDCl_3$ and another in DMSO-d_6 (or C_6D_6, THF-d_8, D_2O, DMF-d_7, etc.), all three procedures must be performed for each sample: reading default shims (click **Default Shims** button), locking (click **Find z0**), tuning (click **Autotune**), final step is clicking the **Gradient Shim** button.

Setting acquisition parameters for ^{1}H and ^{13}C NMR spectrum in different workplaces (experiments); Use **exp1** for ^{1}H NMR and **exp2** for ^{13}C NMR spectrum.

Acquisition of the 1H NMR spectrum in workplace titled as **exp1**

18. Click **Acquire** button on the main menu Tabs; change the **number of scans** (nt), **relaxation delay** (d1) and **spectral width** (sw) when the expected chemical shift is outside the standard spectral range **(-2 ppm to +14 ppm).** Normally you just need to change the number of scans (nt) to nt = 16, 32 etc. Set **bs** (block size) to 4 for 1H and 16 or 32 for ^{13}C to monitor acquisition.

Setting of block size (**bs**) permits processing of NMR data obtained for every 4, 8, 12, 16 etc. scans before completing the desired number of scans (nt, for example 16) while acquiring the NMR spectrum in progress.

The NMR data (FID) can be processed by typing **ft** at every **bs** value (4, 8, 12, 16 etc) to display the NMR spectrum.

Acquiring of NMR data

To start acquisition of the ^{1}H NMR experiment, type **ga** and hit Enter button. Command **ga** acquires the ^{1}H NMR spectrum (data) and automatically processes it. In the VNMRJ graphic window, you will see the ^{1}H NMR spectrum when the experiment is completed at the desired number of scans.

If you start an acquisition with a command, **go** and the data won't be automatically processed when the acquisition is complete; in other words, you will not see the ^{1}H NMR spectrum in the VNMRJ graphics window, you must process it yourself by typing **ft** and clicking the Enter button.

After processing the NMR data (FID), you will sometimes see the spectrum in phase or out of phase with the peaks. If the peaks are out of phase, type **aph** (auto-phase) and click Enter button to correct the phase of the spectrum. If the **apt** command is not phased correctly, then you must phase the spectrum manually.

After completing the ^{1}H NMR spectrum, type jexp2 and hit the Enter button to acquire the ^{13}C NMR spectrum, if necessary.

Acquisition of ^{13}C NMR spectrum

To acquire the ^{13}C NMR spectrum, join experiment 2, type jexp(2) and click Enter button. In the experiment selector tree (top left corner of the OpenVNMRJ window), click **CARBON** default settings. Carbon parameters will be loaded in exp(2). Then click the **Autotune** button in the action Tabs menu; the probe will be tuned to the ^{13}C frequency.

Before acquiring the ^{13}C NMR spectrum

Click the **Acquire** tab and make sure the used **solvent** is correct and only change the **number of scans** (nt). Note that ^{13}C NMR spectrum has a lower signal-to-noise ratio than ^{1}H NMR spectrum, Because the sensitivity of the ^{13}C NMR spectrum is about 5800 times less than the sensitivity of the ^{1}H NMR spectrum due to its very low natural abundance (1.1%).

Therefore, ^{13}C NMR experiment versus ^{1}H NMR requires more time for the carbon chemical shifts (peaks) to appear in the ^{13}C NMR spectrum. Note that signal-to-noise increases with the square root of the number of scans -- to double the signal-to-noise, you need to collect 4 times as many scans. Therefore, to obtain a ^{13}C NMR spectrum with a sufficient signal-to-noise ratio (S/N), many scans (nt), eg n t =1024 (or 2048, 4096, 8192, 16384, etc.). Set bs (block size) to 16 or 32 to control periodically acquisition.

Starting of ^{13}C NMR acquisition

To monitor the 13C NMR spectrum periodically for every, 16, 32, or 48 scans etc set **bs** (block size) to 16

Type **ga** or click **Go** button on the action bar to start the standard carbon experiment.

When the first block is completed (16) type **lb =1 wft** to see ^{13}C NMR spectrum for 16 scans and wait until the spectrum displays good enough signal to noise ratio (S/N), and then stop the acquisition. The correct value of the applied exponential function (line broadening function, **lb**) is

inversely proportional to the acquisition time (at): **lb =1/at**, therefore, the calculated **lb** value should be applied to the processing of the FIDs of the ^{13}C NMR spectrum.

Stopping an Experiment

There are two ways to stop an NMR experiment:

In the action bar click the **Stop** button.

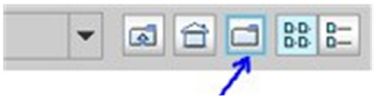
Or type **aa** on the command line and hit the Enter button.

Saving file

(a) On the left-top corner of VNMRJ window click **File** and then click **Save**

(b) Find your supervisor's name (for example, Singh, IS etc), click on it to open

(c) Click the icon (shown by blue arrow) to create folder under your name.



(d) Save your spectrum in a digital form (FID-Free Induction Decay, raw data

(e) Click **File-Save as**-type file name, for example: **128A-fr1-H or 128-fr1-C**

Data Processing using VNMR software is optional to learn.

All data processing, referencing, integration, pick picking, and base line corrections should be done on your own computer (Windows or Mac) using MNOVA software.

After completing the experiments, you should exit VNMRJ program and then log out of your Ubuntu account.

Make sure experiments are stopped before exiting the VNMRJ program. In the upper left corner of the VNMRJ window, click "**File**", and in the scrolling menu, click "**Exit OpenVNMRJ**". Or type **exit** at the command line and click the Enter button.

Log out of your Ubuntu account

On the top of screen (right side) open scroll down menu and then click **Log out** and to confirm click **Log out**.

.