

How to perform NMR experiments on the Varian/Agilent Instruments

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Use enough solvent to bring the height of liquid to at least 4 cm. It DOES NOT HELP IN ANY WAY to use less solvent! **Do NOT have particles** of any kind floating in the tube.

Use a pen to label your NMR tube on the top of the tube, or on the cap. Do NOT attach any kind of label to the upper part of the tube.

Always report broken NMR tubes to me. You are responsible for cleaning up glass fragments and spilled chemicals outside the magnet, but I must be informed so I can check that there has been no damage to the probe.

1. Login and double click on the VnmrJ icon.
2. To get your sample into the magnet:
 - If not using the autosampler, on the **Start tab / Standard page** use the **Eject** button to eject the previous sample, adjust your NMR tube in the spinner to the correct height with the depth gauge and insert your sample using the **Insert** button.
 - If using the autosampler, adjust your NMR tube in a spinner to the correct height with the depth gauge and place your sample in an available location in the tray (see page 5).
3. If running a **1D ¹³C spectrum** on the **500**, tune the probe for **both ¹³C and ¹H !!!** (see separate instructions). **NEVER** tune any probe on any other instrument.
4. Click on **New Study** (bottom left corner of the VnmrJ screen).
5. **Enter a Sample Name.** Your data will be stored as
 /home/*yourloginname*/vnmrsys/data/samplename_experiment_version, e.g.
 /home/morin/vnmrsys/data/ethylindanone_PROTON_01.
6. **Select the solvent** from the drop down Solvent menu (or click on the supplied buttons).
7. **Select the experiments** you wish to perform by selecting them from the panels to the left.
8. **To change any parameters** from their default values double click on the time field (or “Node”, in the Study queue see next page for the location of that). Change, e.g., the number of scans to fit in the time available then click on **Save**.

Hint: in the command line enter **time(x,y)** where x= number of hours and y= the number of minutes you want the acquisition to take. E.g. the command **time(2,25)** will automatically adjust the number of scans to fit into 2 hours and 25 minutes.

To modify a Study **BEFORE** it has been submitted:

The order may be changed by dragging the experiments to rearrange them. Experiments can be deleted by dragging to the Trash icon. To delete all requested experiments and start over, Click on “Clear Pending Exp. From Queue”.

9. If using the autosampler **Click on the tray location** in which you placed your tube.
10. Click on **Submit**.

To leave the instrument:

1. **Click on Logout on the Start tab / Standard page.** Answer **OK** if prompted.
2. In the **System menu** select **Logout...** (Remember however, **never logout** of the 300 with the autosampler.)

Location of the "Time Field" (or "node")

The screenshot displays the Bruker TopSpin software interface. The 'New Sample' dialog box is open, showing a list of samples in the 'Study Queue' section. The 'Submit Queue' button is highlighted with a red box and labeled 'Step 8'. The 'Submit Queue' button is located in the bottom left corner of the 'New Sample' dialog box. The 'Study Queue' section shows a list of samples with columns for 'stdID', 'Dose2D', 'Dose3D', and 'Crisis2'. The samples listed are 'PROTON_001 [0:24]' and 'CARBON_001 [8:40]'. The 'Submit Queue' button is located below the 'Study Queue' section. The 'Submit Queue' button is located in the bottom left corner of the 'New Sample' dialog box. The 'Submit Queue' button is located in the bottom left corner of the 'New Sample' dialog box.

Step 8

1D Processing

To retrieve your data at any time:

In the VnmrJ menu click on **File → Open**

Click on the **Process tab** then Click on the **Defaults page**

Click on **Autophase** then **Autoscale**

If it is necessary to phase manually, click on Phase mode button in the Graphics Toolbar, click and hold down the left mouse button on the right side of the spectrum and phase that region, then click and hold down the left mouse button on the left side of the spectrum and phase that side. Click on **Display spectrum** to exit the phasing routine.

Expand **horizontally** as desired using the **Graphics Controls toolbar**. Expand **vertically** with the **middle mouse button** (the **integrals** must not be displayed to adjust the vertical scale of the spectrum.)

To peak pick:

To see the chemical shifts on the screen, go to the **Defaults** page and click on **Peak Threshold**

Adjust the threshold level

Click on **Find Peaks**. [This will freeze the display. To unfreeze it click on the **Display Spectrum button** on the **Process tab**.]

To integrate:

Click on the **Process tab** then Click on the **Integration page**

Click on the **Partial** radio button

Click on **Clear Integrals**

Click on **Interactive Resets** and cut the integral

When done, click on **Scale display to fit**

Enter **dc** if necessary to level the integrals

To normalize, place cursor on an integral, enter a number in *Integral Area* and click on **Set Integral value** (below it)

To plot:

Go to the Process tab/Plot page and click on e.g. Plot Spectrum, Plot Scale, Plot Integrals, Plot Peaks, Plot Parameters (your choice) followed by **Plot Page**.

Special notes for the Varian/Agilent autosampler

1. Use **ONLY 8"** tubes in the sample changer
2. Use a pen to label your NMR tube on the top of the tube, or on the cap. Do NOT attach any kind of label to the upper part of the tube.
3. Put the spinner(s) with tube into any **GRAY** position in either of the sample trays. **GREEN** positions can also be used. Do not use the position that is directly under the sample changer grip because the sample in that location is in the magnet. The software will colour that location blue.
4. A dummy sample may be in position 100. **DO NOT** remove it. If position 100 is empty **DO NOT** put a sample there.
5. There are several positions that are labelled "**No spinner here**". Do **NOT** put a spinner there.
6. During the week: There is no longer a DayQ and NightQ, only a single queue. The maximum time per protocol (i.e. experiment) is 2 hours. Please do not use more than a total of four hours per day.

During the weekend: Maximum time per sample is 12 hours.

<i>Item</i>	<i>Description</i>
Sample positions. The colors of the sample position indicate the status of that sample position as follows:	
Gray	Empty
Green	Completed
Blue	Active
Yellow	Submitted - Day queue
Purple	Submitted - Night queue
Red	Error
Faded or grayed out colored sample position	Sample submitted by an operator other than the currently logged in operator to the walkup interface.

Common 2D Experiments to use on the Mercury 300 autosampler

COSY Procedure

Select the **Common tab** then **(HH)gCOSY**

- If the sample is dilute increase **Scans per t1 Increment** on the **Acquire tab/Defaults** page. Use a multiple of 1.
- A proton experiment will automatically be submitted also.
- You can use the **Show time** button to see how long the experiment will take.

HSQC procedure (to observe correlations of carbons with their directly attached protons)

Select the **Common tab** then **(HC)gHSQC**

- If the sample is dilute increase **Scans per t1 Increment** on the **Acquire tab/Defaults** page. Use a multiple of 4.
- A proton experiment will automatically be submitted also but you will have to add the ¹³C **experiment yourself**.
- You can use the **Show time** button to see how long the experiment will take.

HMBC procedure (to observe correlations of carbons with protons that are 2 or 3 bonds away allowing you to observe the nonprotonated carbons that can't be observed with an HSQC-type experiment.)

Select the **Common tab** then **(HC)gHMBC**

- If the sample is dilute increase **Scans per t1 Increment** on the **Acquire tab/Defaults** page. Use a multiple of 4.
- A proton experiment will automatically be submitted also but you will have to add the ¹³C **experiment yourself**.
- You can use the **Show time** button to see how long the experiment will take.

NOESY or ROESY procedure

Select the **(HH)Homo2D tab** then **NOESY** or **ROESY**

- If the sample is dilute increase **Scans per t1 Increment** on the **Acquire tab/Defaults** page. Use a multiple of 8.
- A proton experiment will automatically be submitted also.
- You can use the **Show time** button to see how long the experiment will take.
- If you fail to see correlations that you believe should be there: in the case of NOESY, increase the "NOE Mixing time" to 700 (ms) or, in the case of ROESY, increase the "Spinlock Mixing time" to 200 ms.

General notes

You are responsible for backing up your data.

You may transfer your files via an sftp program such as WinSCP from (a) a network hard wired computer if you tell me its IP address, (b) a wireless connection as long as you are on campus or (C) elsewhere through a VPN connection.

You may also simply insert a USB drive into the spectrometer computer and drag files from your data directory to the USB drive window. It is advisable, when finished transferring, to right click on the USB drive icon that appeared on your Desktop when you plugged in the drive and select "Unmount volume" before you remove it from the port.

The autosampler will send you, via an email attachment, your NMR data but in a "tar" format which has also been compressed before sending. Here is the way I extract the NMR data so it can be processed by Mnova, etc. (I downloaded and installed a free program called 7-zip. See <http://7-zip.org/> - version 9.20, 32 or 64 bit version depending on your computer.)

Save the email attachment to your hard drive (something.fid.tgz)

Find it on the hard drive and right click on it.

From the menu select "7-zip", then "Extract here". This creates a file called **something.fid.tar**.

Right click on **something.fid.tar**

From the menu select "7-zip", then "Extract here" again. This creates the folder called something.fid which is your original NMR data.

You can extract the data via other options in 7-zip but I find they create more folders etc and the above way is more straightforward. Of course there are other programs such as WinZip that you can use.