

BRUKER NMR TRAINING

Introduction

The goal of this guide is to enable a relatively inexperienced user to carry out a series of basic 1-D High Resolution (HR) NMR experiments. Both Proton observe and Carbon observe will be described.

While every effort has been made to genuinely provide a step by step description, new users will invariably have some questions, and as such will require occasional assistance from NMR staff. The goal of this manual is, where practicable, to enable users to work independently and acquire a basic understanding of how to operate the system.

Safety

For such a sophisticated system there are surprisingly few opportunities for inexperienced users to damage the equipment. New users are advised to acquaint themselves with these potential hazards before commencing.

People fitted with cardiac pacemakers or metallic implants should never be allowed near the magnet!

The magnet is potentially hazardous due to:

- The large attractive force it exerts on ferromagnetic objects.
- The large content of liquid nitrogen and helium.

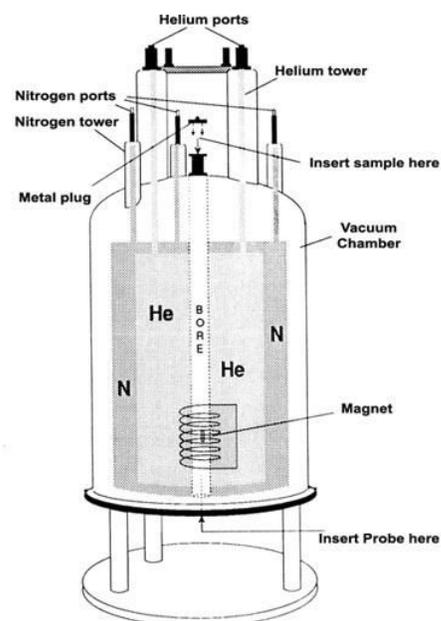
Magnetic Safety

A magnetic field surrounds the magnet in all directions. This field (known as the stray field) is invisible. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way!

Do not allow small steel objects (screwdrivers, bolts etc.) near the magnet. These could cause serious damage if drawn into the magnet bore.

Cryogenic Safety

The magnet contains relatively large quantities of liquid helium and nitrogen. These liquids, referred to as cryogenes, serve to keep the magnet core at a very low temperature. Direct contact with these liquids can cause frostbite. **Exit the space immediately in the event of a magnet quench, whereupon the room may suddenly fill with evaporated gases reducing the level of oxygen needed to sustain life.**



Cryogenic Safety cont.

Some users may need to use a portable liquid nitrogen dewar for variable temperature experiments. Always handle cryogenic liquids carefully. Their extremely low temperatures can produce cryogenic burns of the skin and freeze underlying tissue. The recommended personal protective equipment (PPE) for handling cryogenics includes a full face shield over safety glasses, thermal insulated gloves, long-sleeved shirts, and trousers without cuffs.

A special note on insulated gloves: Gloves should be loose-fitting so they are able to be quickly removed if cryogenic liquid is spilled on them. Insulated gloves are not made to permit the hands to be put into a cryogenic liquid. They will only provide short-term protection from accidental contact with the liquid.

Chemical Safety

Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc.

Users must follow all regulations found in the Notre Dame Laboratory Safety Manual and Chemical Hygiene Plan. This can be found at:

<https://riskmanagement.nd.edu/safety/laboratory-safety/chemical-safety/>

NMR Tube Breakages

We are all human, and sometimes things go wrong. If you break a tube in an NMR lab, please try and clean up as much as possible, collecting the broken glass and disposing of it in a safe manner. We have provided brooms, paper towels and glass disposal boxes for you in each lab. Normally, these items will be found by the door. If a tube breaks inside the magnet, you should stop work immediately and inform NMR facility personnel. If this occurs outside normal working hours, then you should leave a large note on the instrument to warn others not to use it. Please email nmr@nd.edu or leave a note for us.

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Getting Started

Login into NMR facility computers

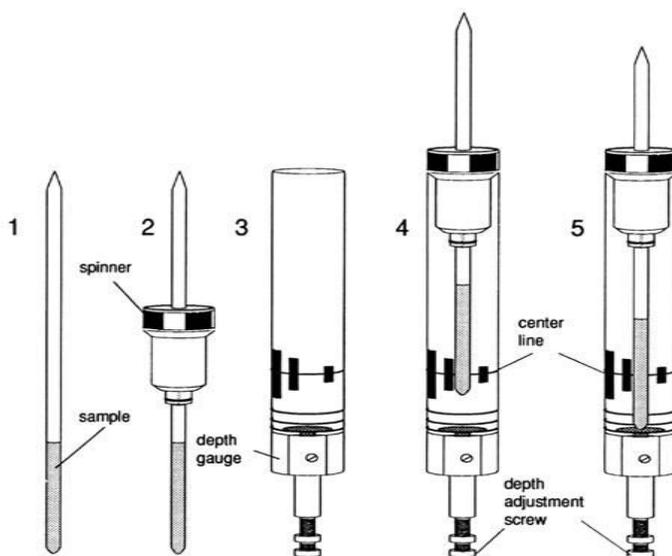
If your NMR account has been created use your university NetID and password to login into any NMR facility computer, otherwise contact NMR facility personnel.

Prepare your sample

Inserting the Sample into the Spinner

The glass tube containing the sample to be analyzed is held in a plastic spinner. A sample depth gauge is provided to control the depth of the sample tube in the spinner. This is to ensure that the sample is correctly aligned with the coils inside the probe. The depth gauge has a graduated scale which can be used to set the depth of the sample.

1. Holding the sample by the top, place the sample tube in the spinner and the spinner in the depth gauge.
2. Gently push the sample tube down so that the depth of the sample above and below the center line is equal. If there is enough sample volume then push the sample down so that it just touches the white plastic base. It is recommended the sample height is 5-6 cm (~2 inches, volume ~0.6 ml).



3. Remove the depth gauge before inserting the sample and spinner into the magnet.

Inserting the Sample into the Magnet

The sample in position 1 is the Standard CDCl₃ sample. It must always remain there.

NEVER REMOVE THE SAMPLE FROM SLOT 1.

To insert the sample with the spinner into the magnet use the following procedure:

1. Place your sample in any free slot. Note what slot it is in.
2. Un-LOCK (lock off) the current sample and turn off spinning (to off).
3. Type the command `sx x`. Where 'x' is the slot your sample is in, i.e., `sx 6`. This will eject the current sample and load the sample in slot 6.
4. The standard CDCl₃ sample must be put back into the magnet when you finish your experiments before you logout. The standard should be locked and not spinning.

Spinning

To start or stop spinning:

Type the command `ro on` to start sample rotation.

Type the command `ro off` to stop sample rotation.

Locking the Sample

1. Type the command `lock`. This will display the solvent selection menu.
2. Select the appropriate solvent from the menu and click OK.
3. The system will begin to auto-lock.

Unlocking the Sample

1. Type the command `lock off`.

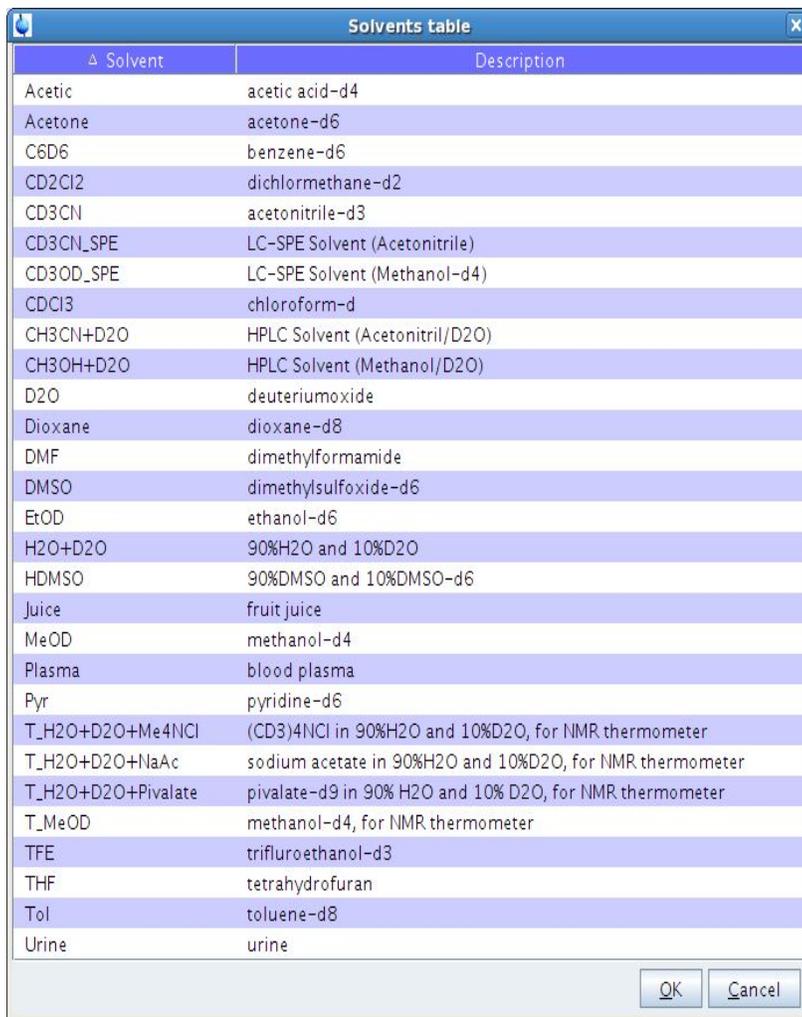
Shimming

Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to re-shim each time a sample is changed.

Routine shimming should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed.

TOPSHIM 1d will shim the sample automatically. Your sample must be spinning for this to work.

1. Type the command `topshim` to start automatic shimming.
2. The duration of 1D shimming will vary from several seconds up to a few minutes depending upon the available signal strength.
3. TopShim will finish after the optimal magnetic field homogeneity has been obtained.



Δ Solvent	Description
Acetic	acetic acid-d4
Acetone	acetone-d6
C6D6	benzene-d6
CD2Cl2	dichloromethane-d2
CD3CN	acetonitrile-d3
CD3CN_SPE	LC-SPE Solvent (Acetonitrile)
CD3OD_SPE	LC-SPE Solvent (Methanol-d4)
CDCl3	chloroform-d
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)
CH3OH+D2O	HPLC Solvent (Methanol/D2O)
D2O	deuteriumoxide
Dioxane	dioxane-d8
DMF	dimethylformamide
DMSO	dimethylsulfoxide-d6
EtOD	ethanol-d6
H2O+D2O	90%H2O and 10%D2O
HDMSO	90%DMSO and 10%DMSO-d6
Juice	fruit juice
MeOD	methanol-d4
Plasma	blood plasma
Pyr	pyridine-d6
T_H2O+D2O+Me4NCl	(CD3)4NCl in 90%H2O and 10%D2O, for NMR thermometer
T_H2O+D2O+NaAc	sodium acetate in 90%H2O and 10%D2O, for NMR thermometer
T_H2O+D2O+Pivalate	pivalate-d9 in 90% H2O and 10% D2O, for NMR thermometer
T_MeOD	methanol-d4, for NMR thermometer
TFE	trifluoroethanol-d3
THF	tetrahydrofuran
Tol	toluene-d8
Urine	urine

Acquiring Data

Any data set acquired with the spectrometer is automatically stored in what is called the “current” data set.

Before you acquire any new data, you should always create a new data set in which it can be stored. This will prevent you from overwriting existing data. The term “creating a new data set” may be a little misleading. Up to this point you have not yet acquired any data, however, by creating a new data set the computer prepares a set of files in which your data will be stored.

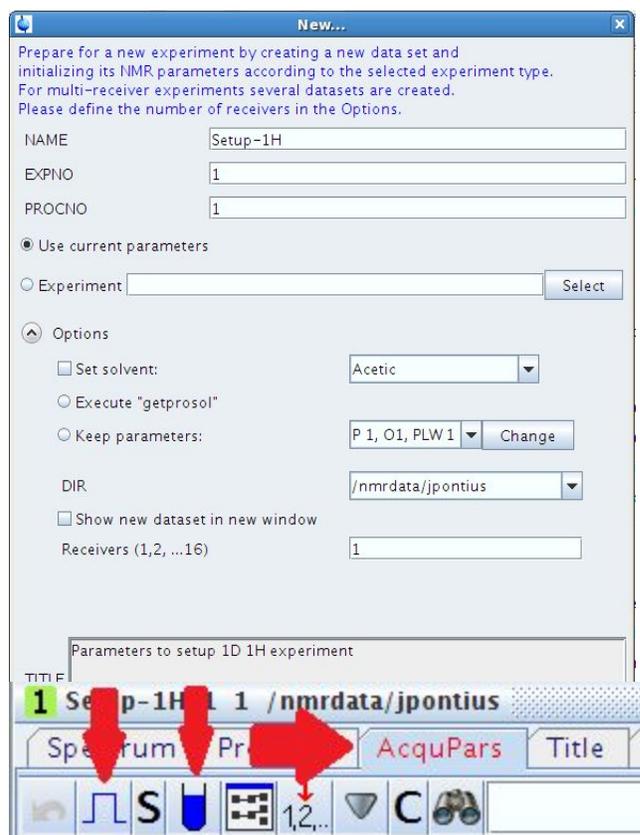
Create a New Data Set

Each user has in his/her personal account, home directory `/nmrdata/user's netID`, two files *Setup-1H* and *Setup-13C* to set up measurements of 1D 1H and 13C spectra, respectively. Those files can be used to create a new data set for measurement of respective spectra. For example, to create a new data set for measurement of 1H spectrum drag the *Setup_H1* file from your folder into the spectrum display area. Then do the following;

Click Start → Create Dataset

1. Specify the dataset **name**, **expno**, and **procno** in the appearing dialog box. If one or more datasets are open, the fields are initialized with the current dataset. There should be no spaces between letters/numbers in the name.
2. If you wish, type a comment in the **TITLE** box.
3. Click **OK**.
4. Once a new data set is created LEFT click on the button  .
5. The only parameter that must be set is the number of scans, **ns**, for example, `ns 128`.

It is possible to check all acquisition and processing parameters by opening the Acquisition Parameters and Processing Parameters tabs, respectively. To view the acquisition parameters you can either type ‘*eda*’ or click on the tab that says **AcquPars**. To display the processing parameters you can either type ‘*edp*’ or click on the **ProcPars** tab. Also, clicking on the pulse icon will show acquisition parameters only applicable to the created experiment. This greatly simplifies experiment setup options.



Measurement of Proton spectra

This section describes the procedure to acquire a proton spectrum. The principal steps are:

1. Create a new data set, e.g. hydrogen-11.
2. LEFT click the set probe/solvent dependent parameters button. 
3. Insert the sample and start spinning, `sx 8, ro on`
4. Lock the sample, `lock cdcl3`
5. Run Topshim, `topshim`
6. Set number of scans, `ns`. How long the experiment will take can be checked by clicking the TIME button.
7. Set the receiver gain with `rga`
8. Enter `zg` to start the acquisition.
9. While experiment is running you may enter `tr` and to view your data type `ft;apk`
10. Enter `halt` if data is good.

13C spectrum with broadband Proton decoupling

This section describes the procedure to acquire a carbon spectrum. The principal steps are:

1. Establish a new data set, e.g. "C13_my-latest.reaction".
2. LEFT click the set probe/solvent dependent parameters button. 
3. Insert the sample and start spinning if not already done.
4. Lock the sample if not already done.
5. Run Topshim if not already done.
6. Set number of scans, `ns`. How long the experiment will take can be checked by clicking the TIME button.
7. Tune the probe; enter the command `atma`
8. Set the receiver gain with `rga`
9. Enter `zg` to start the acquisition.
10. While experiment is running you may enter `tr` and to view your data type `em;ft;apk`
11. Enter `halt` if data is good.

Processing

Fourier Transformation

A Fourier Transformation is used to convert the FID to a frequency spectrum. Before performing Fourier transformation one can also apply various weighting functions to the FID either to enhance resolution or S/N (signal to noise ratio) in the spectra using the '*em*' command. Fourier transformation is implemented using the '*ft*' command. Upon entering this command the display automatically returns to the main window containing the corresponding spectrum. If you wish to reenter the acquisition window, enter '*acqu*'. The spectrum may appear distorted after the '*ft*' command, but this can be overcome using phase correction techniques described below.

Phase Correction

Phase shifts of either the transmitted or received signal within the spectrometer hardware are inevitable and must be initially corrected for. The simplest procedure is automatic phase correction which is implemented using the command *apk*.

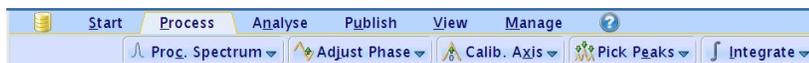
Calibration of Spectrum (Referencing)

It is conventional in NMR spectroscopy to calibrate the spectrum by setting the TMS peak to 0 ppm. Prior to calibration it may be useful to expand the spectrum horizontally in the region on either side of the TMS signal itself, as this will aid in pinpointing the exact position of the peak. **If the sample does not contain TMS use a residual solvent signal for referencing. Chemical shift values for common solvents are shown in the table that is attached to the lab entry door.**

Expand the Spectrum Horizontally

1. Using the mouse position the cursor within the spectral window and click the left mouse button. It will automatically be tied to the spectrum.
2. Using the mouse position the cursor on one side of the region of interest and click and hold the left mouse button.
3. Using the mouse position the cursor on the other side of the region of interest and release the left mouse button. The defined region will be automatically expanded to fill the entire screen.

Calibration Procedure

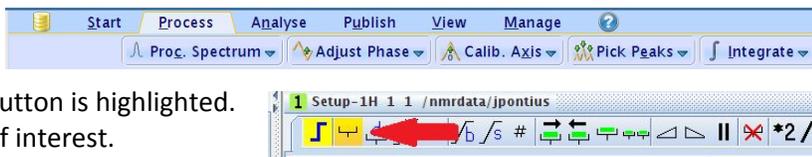


1. Open the "Processing" tab.
2. Click on "calibrate". The cursor will be automatically tied to the spectrum. Move the mouse until the cursor is positioned on the TMS **or solvent** peak.
3. Click the left mouse button, and when prompted for the cursor frequency in ppm, enter zero **for TMS or solvent chemical shift value for the solvent.**

An automatic calibration of the spectrum may also be achieved by entering the command *sref* if your sample has TMS. This procedure searches for a single peak around 0ppm, which may cause an error if your sample doesn't contain TMS. This starts a procedure in which the software searches for a signal in the region of 0 ppm and automatically sets its value to exactly 0 ppm. For the *sref* procedure to work, the appropriate solvent must be chosen in the lock routine.

Integrating the spectrum

1. Click on the 'Process' tab, the 'Integrate' button in the TopSpin Menu bar.
2. Make sure the "Define new integral range" button is highlighted.
3. Place the cursor downfield of the first peak of interest.
4. Press the left mouse button and drag the mouse up-field of the peak of interest.
5. Release the left mouse button to return.
6. Repeat for each additional peak/region of interest.



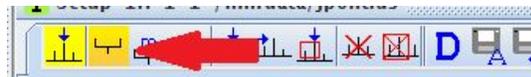
1. Place the cursor within the integral label of any peak you wish to calibrate and press the right mouse button.
2. Select 'calibrate'.
3. Enter the desired value of the selected integral.
4. Click on the floppy disk icon to save the integrals.



Peak Picking

Peak picking can be performed interactively in the peak picking mode.

1. Click on the 'Process' tab, the 'Peak Pick' button in the TopSpin Menu bar. This puts you in a "Interactive Peak Pick" mode.
2. Make sure the "Define new peak picking range" button is highlighted.
3. Press and hold the left mouse button over the peaks of interest. A green highlight will form.
4. Release the left mouse button.
5. Click on the floppy disk icon to save the peak pick.



Plotting

To Plot, or Print your spectra, enter the command **prnt**. This will automatically print your spectra as displayed (what you see is what you get).

You may also use the commands below if you want to change the look of your plot.

- **plot** : will start the Plot Editor, the interactive program for viewing or designing plot layouts.
- **autoplot** : will plot data according to Plot Editor layout (1D,2D), with the current limits and scaling.
- **prnt** : will plot exactly what is in the spectra window of TopSpin (what you see is what you get).

Archiving Data

1. Data is automatically saved to our NMR Data server. Follow the link on our homepage nmr.nd.edu to login to the NMR Data server.

You may use NMR Facility workstations in each of our labs to process your data free of charge using the same version of Topspin as it is on the spectrometer host computers. Alternatively you may download Topspin on your own workstation free of charge for academic use from our homepage nmr.nd.edu.