

# STANDARD OPERATING PROCEDURE FOR NMR EXPERIMENTS

MAGNETIC RESONANCE RESEARCH CENTER AT THE UNIVERSITY OF NOTRE DAME  
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## 1. SAFETY

**If you have a cardiac pacemaker or an insulin pump: STOP!**  
**You may not enter the NMR rooms!**

### 1.1 MAGNETIC SAFETY

- A magnetic field (known as the stray field) extends from the NMR magnet in all directions and will interfere with electronic medical devices. **People with implanted medical devices such as cardiac pacemakers or using insulin pumps are NOT allowed in NMR rooms!** Except for this interference with medical devices, the magnetic field is not harmful for a human body.
- Ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet with surprising force if brought too close. This may damage the magnet and cause personal injury!
- **Do not bring loose small steel objects** (key chains, staples, paper clips, etc.) to the NMR room. These objects can be drawn into the magnet bore and cause serious damage to the NMR instrument or **permanently disable it!**

### 1.2 SUFFOCATION HAZARD

The NMR magnet contains large quantities of liquid helium and nitrogen to keep the superconducting solenoid cold. The cryogenics in the NMR magnet may suddenly evaporate in the event called a “magnet quench”. In addition, compressed nitrogen and helium stored in the NMR rooms may also suddenly leak. Both nitrogen and helium are non-toxic but **the large volume of the released gasses will reduce oxygen level in the room creating a suffocation hazard.** Oxygen sensors installed in NMR rooms continuously monitor oxygen levels and will emit **a loud high-pitched noise** if a dangerous reduction in the oxygen content in the room is detected.

**If you hear the oxygen alarm, you must:**

- 1. Exit the room immediately. If there are others in the room—urge them to do so too.**
- 2. Close the doors and prevent others from entering.**
- 3. Call Public Safety and NMR staff (see phone numbers outside on the door).**

### 1.3 LOW-TEMPERATURE EXPERIMENTS SAFETY

**You must request a special "Variable Temperature training"** should you need to perform NMR experiments at the temperatures different from the room temperature.

For experiments at ultra-low temperatures, you will need to use a portable liquid nitrogen dewar. Liquid nitrogen is extremely cold and will produce cryogenic burns of the skin and freeze underlying tissue upon contact. You must use the **required personal protective equipment (PPE)** for handling cryogenics: **safety glasses, thermal insulated gloves, long-sleeved shirts, trousers without cuffs, and closed shoes.** An optional PPE is a **full face shield** over safety glasses.

A special note on insulated gloves: gloves should be loose-fitting so you are able to quickly remove them if cryogenic liquid is spilled onto 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. The NMR room SCH162 has a pair of cryogenic gloves for you to use.

### 1.4 CHEMICAL SAFETY

**No open chemicals are allowed in NMR rooms.** You must prepare samples in your lab, load in the NMR tubes, **and tightly seal them.** To carry the samples to the NMR room you must use an enclosed sample tube carrier.

**It is your responsibility** to be fully informed of any hazards associated with your samples and know the Standard Operating Procedures (SOP) for removing a spill should your sample tube accidentally break in the NMR room.

All users must follow the standard safety regulations found in the Notre Dame Laboratory Safety Manual and Chemical Hygiene Plan: <https://riskmanagement.nd.edu/laboratory-safety/chemical-safety/>

### 1.5 NMR TUBE BREAKAGES AND SPILLS

If you break a tube in an NMR lab, please, clean up as required by the SOP for your compounds and the NMR solvent. Please, collect the broken glass and dispose of it in a safe manner: to a broken glass collection box in the NMR lab. Each NMR room has a spill kit, a broom, paper towels, and a glass disposal box (a location labeled on the wall with words "Spill Kit").

If a tube breaks **inside the magnet**, you should **stop your work immediately** and **inform NMR facility personnel.** Please, leave a large note on the computer keyboard saying "Out of Order" to warn others not to use it. Please, log out of the NMR workstation and describe the accident in the "Problems with the instrument" section. Please, **give us complete information on the sample content** to allow us to use correct solvents for cleaning the probe!

### 1.6 CONTACT INFO:

NOTE: An email sent to [nmr@nd.edu](mailto:nmr@nd.edu) automatically reaches all NMR facility personnel.

<p><b>Evgenii Kovrigin, Ph.D. - NMR Director</b>            email: <a href="mailto:ekovrigu@nd.edu">ekovrigu@nd.edu</a>            280 Stepan Science Hall            phone: (574) 631-8359</p>	<p><b>Justin Pontius - Facility Engineer</b>            email: <a href="mailto:jpontius@nd.edu">jpontius@nd.edu</a>            145A Nieuwland Science Hall            (574) 631-7601 office</p>
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## 2. STANDARD NMR WORKFLOW

The following is a standard protocol for new users to collect basic proton and carbon 1D NMR experiments on Bruker instruments at MRRC.

For detailed discussion of all steps, please, watch the **Notre Dame NMR Operation and Concepts** series: [https://pydio.campus.nd.edu/docs/doku.php?id=nd\\_nmr\\_operation\\_and\\_concepts\\_video\\_series](https://pydio.campus.nd.edu/docs/doku.php?id=nd_nmr_operation_and_concepts_video_series).

### STEP 1. BOOK YOUR NMR TIME IN ADVANCE (IF USING MANUAL NMR INSTRUMENTS)

Prior to using any manual NMR instrument, you must reserve your time slot in **iLab**:

<https://nmr.nd.edu/instrument-locations-and-schedules/>

Automatic 400 in McCourtney does not require a reservation. Instead, you may check its current queue online and head to the spectrometer whenever you see any position "Completed" but have not been "Submitted" yet again: <https://nmr.nd.edu/instrument-locations-and-schedules/automated-400-in-mccourtney-hall/>

### STEP 2. PREPARE YOUR NMR SAMPLE

In your laboratory:

1. dissolve your compound in a deuterated solvent to achieve, optimally, 10-50 mM for proton detection, or 50-200 mM for carbon. Remember a rule of thumb: the concentration twice as low will require four times longer acquisition time.
2. Place your sample solution in a 5 mm NMR tube and tightly close it with a cap. Please, make sure to use a tube that is **no longer than 9"**.
3. **IMPORTANT:** Clean up outside of the NMR tube to remove any residue of the chemicals.
4. **IMPORTANT:** Remove your gloves and **leave them in your lab**. You are **not allowed** to enter the NMR room wearing your gloves. This is a violation of the user policy punishable by revoking NMR access!
5. Place the NMR tube in a secondary container and tightly close it. NMR facility may provide you with a sample tube carrier - just ask us ahead of the time.
6. Remove your goggles and carry the container to the NMR lab.

In the NMR lab:

7. **put on your goggles** to place the NMR tube in a spinner and in the NMR magnet.
8. Once the sample is in a sample changer, you may remove your goggles and proceed with operating the NMR instrument.
9. When you have finished your experiments and ejected your sample from the magnet: **put on your goggles**.
10. Place your NMR tube into a secondary container and tightly close it.
11. Now you may remove your goggles and transport the sample back to your laboratory.

**NOTE:** If you have multiple samples, **every time you intend to hold the NMR tube** in your hand, **your goggles must be on!**

### STEP 3. COME TO NMR ROOM

- **Automatic 400:** see next step
- **Manual NMR instruments:** scan the QR code posted next to the NMR workstation and start your session in **Kiosk**. You are allowed to start you NMR session up to 8 minutes **before** you reserved time (if the instrument is free) or within 8 minutes **after** your time has began.

#### STEP 4. INSERT YOUR SAMPLE INTO A SPINNER AND PLACE IT IN THE SAMPLE CHANGER

1. **Put on your goggles!**
2. Check that your sample height (solution column) is 40 mm or taller.
3. Holding the sample tube by the top, insert the sample tube into the spinner directly in the spinner rack. **Do not touch the spinner!**
4. Place the spinner in the depth gauge.
5. Gently push the sample tube down so it touches the white plastic base.



#### AUTOMATIC 400

- Determine the available sample position:

[https://pydio.campus.nd.edu/docs/doku.php?id=required\\_basic\\_nmr\\_training\\_package:required\\_safety\\_and\\_nmr\\_rules\\_course#overview\\_of\\_sample\\_handling](https://pydio.campus.nd.edu/docs/doku.php?id=required_basic_nmr_training_package:required_safety_and_nmr_rules_course#overview_of_sample_handling)

- Transfer the sample into the sample changer carousel holding it by the sample tube.
- Scan the QR code on a wall and set up your experiment through the web interface.
- You may leave the room and monitor your experiment through web interface.
- When your experiment is finished - COME BACK TO PICK UP YOUR TUBE! We dispose of NMR tubes that are NOT picked up after a week or so!
- To remove the tube from the spinner, **use a paper napkin to hold the spinner.**
- Proceed to the **ACCESS TO NMR DATA** and **PROCESSING** sections of this document

#### MANUAL INSTRUMENTS

**NOTE:** You must have started Kiosk when you came into the room (Step 3). If you did not—try now but if your session in Kiosk is not active, your reservation has already expired. This means that it was labeled "no show", charged to your lab for the full time period, and deleted from the schedule since you "did not show up". Anyone can book time now and come to the room to use the instrument. **You must log into iLab as soon as possible, create a new reservation, and start it in Kiosk to continue using the instrument!**

1. Assuming your Kiosk is happily running: log into a Linux workstation with your ND credentials
2. Launch Topspin
3. Transfer the sample into the sample changer carousel holding it by the sample tube. **Do not touch** the samples that are **already** in the carousel. Pick any free position for your sample.
4. When finished: to remove the tube from the spinner, **use a paper napkin to hold the spinner.**

#### STEP 5. INSERT YOUR SAMPLE IN THE MAGNET BORE

5. Unlock spectrometer and stop sample rotation (if it was spinning). Type:
  - `lock off`
  - `ro off`
6. Insert your sample: type `sx N`, where 'N' is the slot number your sample is in, i.e., `sx 6`.

#### STEP 6. TUNE THE PROBE

1. Display the standard experiment that contains nuclei you intend to acquire. If you only need proton, display **Setup\_1H/**. If you will need both proton and carbon: display **Setup\_13C/**.
  - To display an experiment: **right-click** it in File Browser window and choose **Display**

- Follow the tuning protocol as described in **Episode 2. NMR Probe Tuning:**  
[https://pydio.campus.nd.edu/docs/doku.php?id=nd\\_nmr\\_operation\\_and\\_concepts\\_video\\_series#episode\\_2\\_nmr\\_probe\\_tuning](https://pydio.campus.nd.edu/docs/doku.php?id=nd_nmr_operation_and_concepts_video_series#episode_2_nmr_probe_tuning)
  - Display a spooler window and move it to the side
  - `qu ro off`
  - `qu atma`
  - `qu wobb`
  - Inspect tuning result in the WOBB screen and exit it.

## STEP 7. LOAD A STANDARD SHIM SET AND LOCK THE SPECTROMETER

Your sample **must** contain a deuterated solvent!

For details on the locking procedure see **Episode 3. NMR Spectrometer Locking.**

[https://pydio.campus.nd.edu/docs/doku.php?id=nd\\_nmr\\_operation\\_and\\_concepts\\_video\\_series#episode\\_3\\_nmr\\_spectrometer\\_locking](https://pydio.campus.nd.edu/docs/doku.php?id=nd_nmr_operation_and_concepts_video_series#episode_3_nmr_spectrometer_locking)

- To load a standard shimset, type: `rsh bbfo-latest`
- To display the solvent selection menu, type: `lock`
- Select your solvent and click OK.
- Wait for the "lockn done" message in the status line.

## STEP 8. SHIM THE SAMPLE

For details on the shimming protocol see:

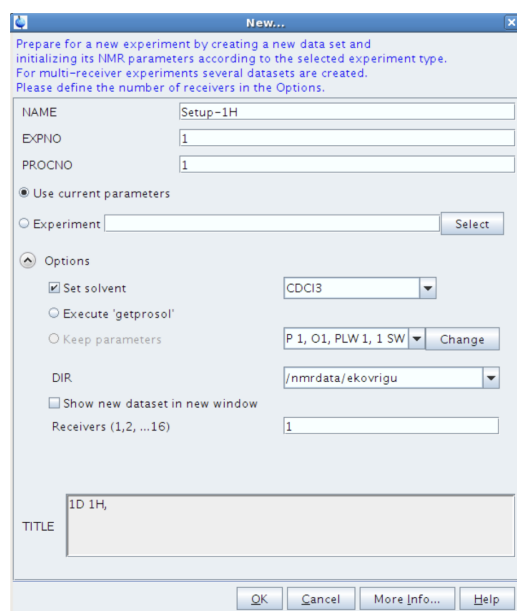
[https://pydio.campus.nd.edu/docs/doku.php?id=nd\\_nmr\\_operation\\_and\\_concepts\\_video\\_series#episode\\_4\\_shim\\_system](https://pydio.campus.nd.edu/docs/doku.php?id=nd_nmr_operation_and_concepts_video_series#episode_4_shim_system)

- Episode 4. Shim System,**
  - Episode 5. Shimming Workflow,** and
  - Episode 6. Shimming problems caused by the samples.**
- Start sample rotation: `ro on`
  - Launch Topshim interface: `topshim gui`
  - Select your detection nucleus in "Optimize for" field
  - Click **Start** button and open the **Report** tab
  - Monitor the shimming performance and repeat shimming as needed.


## STEP 9. CREATE A NEW DATA SET FOR ACQUISITION

In a home directory of each user, we set up two standard experiments for acquisition of 1D 1H and 13C spectra, respectively. Use them to create new folders for your measurements. As a rule of thumb, you should **always** record a proton 1D even if you only intend to record a carbon on this sample. Proton 1D will serve as a quality control for your sample and the spectrometer performance.

- Display *Setup\_1H* experiment
- Click **Start, Create Dataset** (or type `new`)
- Specify the new dataset **NAME**. Set **EXPNO** to 1
- Check the **Use current parameters** radio button
- Type your sample information in the **TITLE** box.
- Click **OK**. The dataset is created and now displayed.
- On the command line, type `getprosol`.



## STEP 10. ACQUIRE NMR DATA

1. To display acquisition parameters: click **AcquPars** tab, then click the blue “square wave” button  in its toolbar.
2. Set the desired number of scans, **NS**. The value of NS should be **even** or divisible by **4** or **8** (better). Note, that dilute samples will require quadratically more scans. For example, if your new sample concentration is 1/2 of the concentration of a previous one, you have to set NS to 4x larger number of scans to obtain the same sensitivity (signal-to-noise ratio).
3. Set the relaxation delay, **D1**, in seconds. The value of 1-2 seconds is appropriate for normal proton acquisition. To obtain a **quantitative** spectrum, that is with integrals proportional to the number of protons, set D1 to 8-10 seconds.
4. Check how long the experiment will take by typing **expt**
5. Type **rga** to set receiver gain
6. Type **zg** to start the acquisition
7. To check on your data while the experiment is still running: type **tr**, wait till you see a message in the status bar “[long-name ...] saved” and then type **efp; apk**
8. Wait for the experiment to complete or type **halt** if data looks good enough. Proceed to **PROCESSING** section.



## STEP 13. ADDITIONAL NMR EXPERIMENTS

- If you need to record a **carbon 1D on the same sample**: go to **Step 9** to create a new experiment starting with **Setup-13C**. Remember: carbon gives very weak signal. You may need to use large NS of 256, or 1024 or else to obtain a usable spectrum.
- If you need to record additional spectra on a **different sample**: go to **Step 5**

## ACQUISITION RULES OF THUMB

1. If you do additional **experiments on the same sample without taking it out**: no need to repeat locking, and shimming.
2. If you continue using **the same nuclei you originally tuned for**: no need to repeat tuning either.
3. If you keep **the sample in the probe and switch to a new nucleus**, you must re-tune the probe. **NOTE**: If you did a carbon experiment and go to proton, no need to re-tune for proton because the carbon experiment tunes both proton and carbon channels.
4. If you inserted another sample, **you must tune, lock, and shim again!**

## FINISHING YOUR WORK

1. Insert the standard  $\text{CDCl}_3$  sample and lock it. Type the following commands for the spooler queue:
  - `qu lock off`
  - `qu ro off`
  - `qu sx 1`
  - `qu rsh bbfo-latest`
  - `qu lock cdcl3`
2. Wait till spectrometer finishes them and completes locking on  $\text{CDCl}_3$
3. Quit Topspin.
 

**NOTE:** Upon exiting, the Topspin has to release its license. If you rush to log out before it happens, next user will not be able to launch Topspin! Please, give it a few seconds before proceeding to log out.
4. Log out of NMR work station (icon in the middle of the top toolbar)
5. Answer all question on the exit survey.
 

If you had questions or concerns, click Yes for "Problems with the instrument" and type in that window. The NMR staff will respond to you shortly by email.

## ACCESS TO NMR DATA

Your data is automatically saved to our NMR Data server at [nmr.nd.edu/nmr-data-server/](http://nmr.nd.edu/nmr-data-server/).

**NOTE:** Only **newly created experiments** are copied to the data server. If you decided to re-run acquisition in the **existing** experimental folder, the data will **not** be updated on the server.

## PROCESSING

We advise everyone to perform final processing of your data on your own computers. You may download Topspin on your own workstation free of charge for academic use from our homepage. Additionally, MNova Lite CDE is also available free to all users on ND campus. For all details see [nmr.nd.edu/software/](http://nmr.nd.edu/software/). Below are brief instructions on processing in Topspin. For MNova data processing, see our video series **Data Processing in MNova NMR**: [https://pydio.campus.nd.edu/docs/doku.php?id=mnova\\_data\\_processing](https://pydio.campus.nd.edu/docs/doku.php?id=mnova_data_processing)

### BASIC PROCESSING

To perform automatic processing of your data, type:

- `ef` (this performs a Fourier transform)
- `apk` (automatic phasing)
- `abs` (automatic baseline correction; also attempts to integrate automatically—see below)

### REFERENCING: PPM SCALE CALIBRATION

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 found in the standard solvent tables.

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.
4. Open the **Processing** tab and click on **Calib Axis**. The cursor will be automatically tied to the spectrum. Move the mouse until the cursor is positioned on the TMS or the residual protonated solvent peak.
5. Click the left mouse button when the cursor is at the peak center.
6. Enter desired cursor frequency in ppm: zero for TMS or the solvent chemical shift value for the solvent.





## INTEGRATION

### INTEGRATING THE SPECTRUM


NOTE: The previous processing step (**abs**) tried to find peaks to integrate and, likely, you will want to remove them as described below.

1. Click on the **Process** tab, then **Integrate** button in the TopSpin Menu bar to enter Integration mode:



2. Next, get rid of automatically picked regions:
  - click "Select all regions" 
  - click "Delete selected regions" 
3. Make sure the "Define new integral range" button is highlighted: 
4. Place the cursor to the left of the first peak of interest.
5. Press the left mouse button and drag the mouse to the right of the peak of interest (leave some baseline on each side of the peak!)
6. Release the left mouse button to integrate the peak.
7. Repeat for each additional peak/region of interest.
8. Unhappy with some integral? Click it to select, click delete button  and reintegrate.

### NORMALIZING INTEGRALS

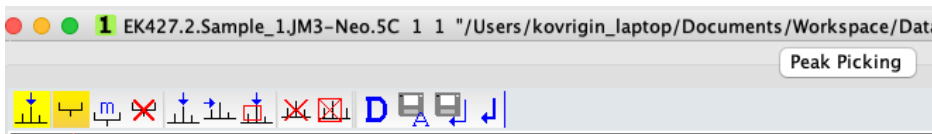
1. Click the integral label of a peak you wish to calibrate to a known number of protons to select it
2. Press the right mouse button.
3. Select **Calibrate Current Integral**.
4. Enter the desired value of the selected integral. All integrals are now normalized to this one.
5. Click on the floppy disk icon to save the integrals to exit the integration mode. 
6. You find the integrals list in the **Integrals** tab of the Topspin of the spectrum window.



## 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 left and above the peaks of interest. Drag to the right and down: a green highlight will form. Peak tops inside the green box will be picked.
4. Release the left mouse button.
5. Click on the floppy disk icon to save the peak list.
6. You find the peak list in the **Peaks** tab of the Topspin of the spectrum window.

## EXPORT OF GRAPHICS

To save the spectrum view as an image file (for use in presentations and documents):

1. select **Publish** tab, click "arrow down" next to **PDF** and select **Save as PNG ... (Data Directory)**. This choice guarantees that your image will accompany your dataset wherever it is transferred to.
2. Give the image a name and hit **Save**. The image file is created in **the current dataset folder** in a subfolder **pdata/1**. You may drag and drop this file into your presentation or report.
3. To save another spectral view: change the view and repeat Publish/Save steps above giving it a different file name.

