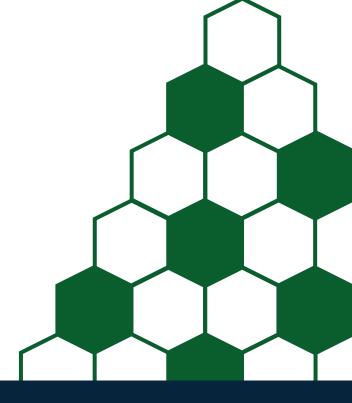
# Using Nuclear Magnetic Resonance (NMR) Spectroscopy

Proton (<sup>1</sup>H) and Carbon-13 (<sup>13</sup>C) NMR Training Manual





# **Table of Contents**

Preface	iii
Warnings, cautions, and notes	iii
Introduction	1
NMR Facility Setup	3
TopSpin User Interface Overview	5
Toolbar icons	6
Menu bar tabs	9
NMR Safety Guidelines	11
General information	11
Magnet quench	12
Medical implants	13
Magnetic objects and electronic devices	14
Summary	16
Task 1: Preparing an NMR Sample	17
Step 1: Supply your sample	18
Step 2: Transfer your sample to an NMR tube	19
Task 2: Acquiring Raw Data	21
Step 1: Create a new experiment	21
Step 2: Prepare your sample for placement	23
Step 3: Place your sample in the NMR spectrometer	25
Step 4: Acquire raw data from your sample	28
Task 3: Processing an NMR Spectrum	31
Step 1: Compute your raw data	31
Step 2: Expand and examine the peaks	32
Step 3: Calibrate the axis	32
Step 4: Identify the peaks	33
Step 5: Integrate the peaks	34
Task 4: Changing Workstations	35
Step 1: Remove your sample	35
Step 2: Transfer your raw data	36

Task 5: Producing an NMR Spectrum	37
Step 1: Plot your NMR spectrum	37
Step 2: Printing and exporting your NMR spectrum	39
Task 6: Completing the NMR session	41
Appendix	43
Glossary	43
Useful links	44
Analyzing 2D NMR spectra	45
List of figures and tables	
Figures	
Figure 1. The NMR facility's equipment.	3
Figure 2. The NMR facility's computer workstations.	4
Figure 3. TopSpin's user interface elements.	5
Figure 4. NMR facility signs prohibiting electronic and metallic medical implants.	13
<b>Figure 5.</b> NMR facility signs prohibiting magnetic objects and electronic devices.	14
Tables	
<b>Table 1.</b> Descriptions of this training manual's warnings, cautions, and notes.	iii
Table 2. Descriptions of the NMR facility's equipment.	3
Table 3. Descriptions of the NMR facility's computer workstations.	4
Table 4. Descriptions of TopSpin's user interface elements.	5
<b>Table 5.</b> Descriptions of toolbar icons for data handling, vertical scaling (intensity and horizontal scaling (zooming).	), 6
<b>Table 6.</b> Descriptions of toolbar icons for horizontal shifting, vertical shifting, and display options.	7
<b>Table 7.</b> Descriptions of toolbar icons for acquisition functions and toolbar icon display.	8
Table 8. Descriptions of Start tab buttons.	9
Table 9. Descriptions of Acquire tab buttons.	9
Table 10. Descriptions of Process tab buttons.	10
Table 11. Descriptions of Publish tab buttons.	10
<b>Table 12.</b> Prohibited and permitted objects inside the NMR facility.	16
<b>Table 13.</b> Safety protocols for NMR facility incidents.	16

### **Preface**

This training manual is intended for new NMR spectrometer users operating NMR equipment at the University of Winnipeg's NMR facility. It includes safety guidelines for operating a 400 MHz Bruker NMR spectrometer and procedures for using TopSpin 3.2 NMR software to complete **proton** (<sup>1</sup>H) and **carbon-13** (<sup>13</sup>C) NMR experiments.

The contents describe the 6 tasks involved in analyzing an NMR sample:

- 1. Preparing an NMR sample.
- 2. Acquiring raw data.
- 3. Processing an NMR sample.
- 4. Changing workstations.
- 5. Producing an NMR spectrum.
- 6. Completing the NMR session.

To schedule an NMR session, visit faces.ccrc.uga.edu. For more information, contact NMR facility staff at nmr@uwinnipeg.ca.

# Warnings, cautions, and notes

This training manual includes important safety and equipment operation information. Table 1 shows the warning, caution, and note icons and their descriptions.

**Table 1.** Descriptions of this training manual's warnings, cautions, and notes.

	Warnings, cautions, and notes			
$\triangle$	Warning: Indicates the risk of serious injury or death.			
<u>^</u>	Caution: Indicates the possibility of breaking equipment or losing data.			
<b>=</b>	Note: Indicates important information or useful tips.			

# Introduction

**Nuclear magnetic resonance (NMR) spectroscopy** is an analytical technique used to analyze the molecular structure of compounds. To analyze a compound, scientists prepare an **NMR sample**, typically a solution that contains a deuterated solvent and a compound under investigation. Next, the NMR sample is placed inside an NMR tube, which is then placed into an **NMR spectrometer**, an instrument that contains a powerful superconducting magnet, for analysis.

During an NMR spectroscopic analysis, the NMR spectrometer's strong magnetic field manipulates the nuclear spin of certain atoms inside the sample, causing a difference in energy levels between spin states. The difference in energy levels is specific to the nucleus and its environment, and it can be probed with radio frequency electromagnetic radiation to produce an **NMR spectrum**. The location and appearance of the spectrum's peaks result in structural information on the analyte compound.

#### Real-world applications

NMR spectroscopy is used in various fields and industries, such as:

- Environmental science: Pollution remediation and organic matter analysis
- Food science: Food testing and quality control
- Medicine and pharmaceuticals: Disease profiling and drug development
- Petrochemicals: Petroleum refining and reaction monitoring
- Materials science: Materials design and synthesis

# **NMR Facility Setup**

Before you get started, you should familiarize yourself with the NMR facility's equipment and computer workstations. Figure 1 and Table 2 show the NMR facility's equipment, and Figure 2 and Table 3 on page 4 show the NMR facility's computer workstations.

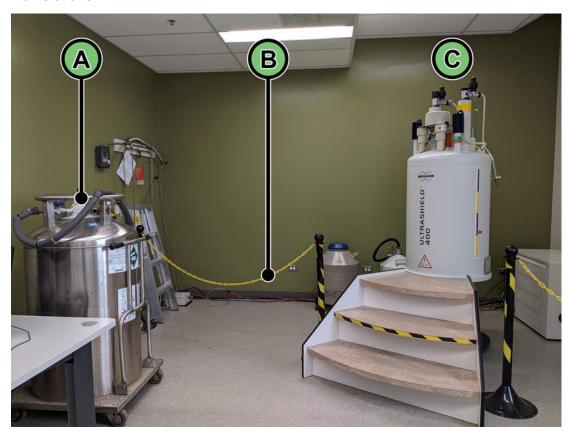


Figure 1. The NMR facility's equipment.

Table 2. Descriptions of the NMR facility's equipment.

	NMR facility equipment			
A	<b>Liquid nitrogen container:</b> Contains liquid nitrogen required to cool and maintain the NMR spectrometer. Only NMR facility staff can handle the liquid nitrogen to refill the NMR spectrometer.			
B	<b>5-gauss line:</b> Indicates the boundary over which you cannot bring magnetic objects or electronic devices. <b>5 gauss</b> refers to the strength of a magnetic force that is strong enough to affect nearby objects.			
©	<b>NMR spectrometer:</b> Analyzes NMR samples by measuring their nuclear spins' interaction with a strong magnetic field. It contains a powerful superconducting magnet that can attract magnetic objects and break electronic devices.			

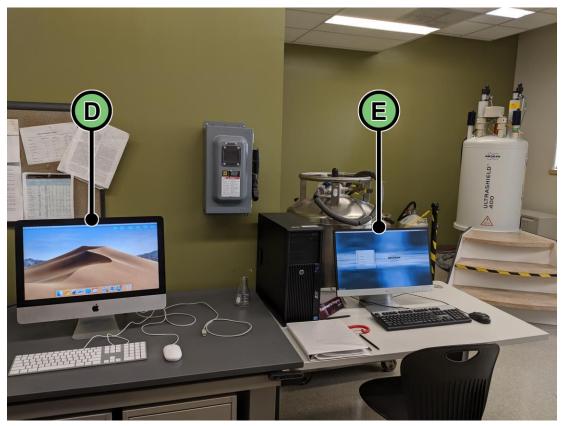


Figure 2. The NMR facility's computer workstations.

**Table 3.** Descriptions of the NMR facility's computer workstations.

# NMR facility computer workstations Mac computer workstation: Processes raw data from the NMR spectrometer. You can use this computer to process and produce your NMR spectrum. Linux computer workstation: Acquires raw data using the NMR spectrometer. Use this computer to measure and acquire raw data from your sample.



Use any computer with TopSpin installed to process your raw data and print your spectrum. Download TopSpin software for free on Bruker's website.

# **TopSpin User Interface Overview**

Figure 3 and Table 4 show TopSpin's user interface elements that you can use to complete the software procedures described in this training manual.

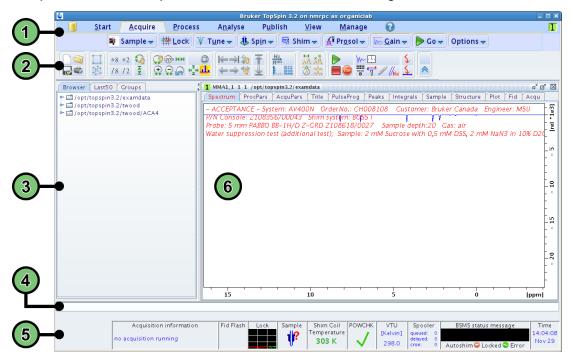


Figure 3. TopSpin's user interface elements.

**Table 4.** Descriptions of TopSpin's user interface elements.

	TopSpin user interface elements
1	<b>Menu bar:</b> Displays tabs and buttons for data acquisition and processing functions. The <b>Main menu</b> icon displays file management options and user preferences.
2	Toolbar: Displays various TopSpin functions and actions.
3	<b>Browser panel:</b> Displays experiment folders and raw data files saved on the computer's hard drive.
4	<b>Command line:</b> Executes text commands for specific TopSpin functions and actions.
5	<b>Acquisition status bar:</b> Displays various data acquisition functions and the NMR sample's acquisition status.
6	<b>Data window area:</b> Displays data acquisition information, processing functions, and the NMR sample's resulting NMR spectrum.

# **Toolbar icons**

Table 5 to Table 7 show the toolbar icons for TopSpin's functions and actions. Text in square brackets are commands that you can enter in the command line.

**Table 5.** Descriptions of toolbar icons for data handling, vertical scaling (intensity), and horizontal scaling (zooming).

Data	Data handling			
	Create a new NMR dataset [new]		Print active window [print]	
	Open an NMR dataset [reb]		Switch to last 2D dataset [.2d]	
	Save an NMR dataset [sav]	电	Switch to last 3D dataset [.3d]	
Verti	cal scaling (intensity)			
*8	Increase intensity by a factor of 8 [*8]	*2	Increase intensity by a factor of 2 [*2]	
/8	Decrease intensity by a factor of 8 [/8]	/2	Decrease intensity by a factor of 2 [/2]	
	Increase or decrease intensity	<b>X</b>	Reset intensity to last saved intensity (contour levels) [.vr]	
Horiz	Horizontal scaling (zooming)			
+	Zoom in [.zi]	MM	Reset zooming (horizontal scaling) to full spectrum [.hr]	
<u>_</u>	Zoom out [.zo]	HTH	Show full spectrum, reset intensity scale [.all]	
	Zoom in or out smoothly		Toggle interactive zoom method [.zoommode]	
ppm	Perform an exact zoom via a dialogue box [.zx]	₫.	Retain expansion and scale when changing dataset [.keep]	
	Show last zoom [.zl]			

**Table 6.** Descriptions of toolbar icons for horizontal shifting, vertical shifting, and display options.

Horiz	ontal shifting		
	Shift to left end of the spectrum [.sl0]	4	Shift spectrum left [.sl]
	Shift to right end of the spectrum [.sr0]		Shift spectrum right [.sr]
<b>₹</b>	Move spectrum left or right		
Vertic	cal shifting		
7	Shift spectrum baseline to the middle of the data field [.su]	100	Move spectrum up or down
<u></u>	Shift spectrum baseline to the bottom of the data field [.sd]		
Display options			
Hz. ppm	Toggle between Hz and ppm axis units [.hz]	<b>****</b>	Toggle display of the spectrum overview [.ov]
<b>L</b>	Toggle units and switch the yaxis on or off [.y]	ΧX	Set up pulse program frequency list [.freqlist]
##	Toggle grid mode (off, axis, fixed) [.gr]	***	Enter multiple display mode
λ	Start distance measurement		

**Table 7.** Descriptions of toolbar icons for acquisition functions and toolbar icon display.

Acqui	Acquisition functions			
	Start acquisition [zg]		Open Lock display window [lockdisp]	
	Halt acquisition [halt]		Open Variable Temperature control window [vtudisp]	
STOP	Stop acquisition [stop]	// <sub>Hz</sub>	Open MAS control panel [masdisp]	
₩~	Open online FID display window [acqu]	\$	Set RF frequency with the cursor [seto123]	
<u></u>	Calculate experiment time [expt]	<u>\$</u>	Set SW to current display region and/or change center frequency [setsw]	
	Open BSMS control panel for manual locking, shimming, and sample handling [bsmsdisp]			
Toolbar icon display				
<b>&gt;</b>	Show more icons [.onerow 1d]	<b>*</b>	Show fewer icons [.onerow 1d]	

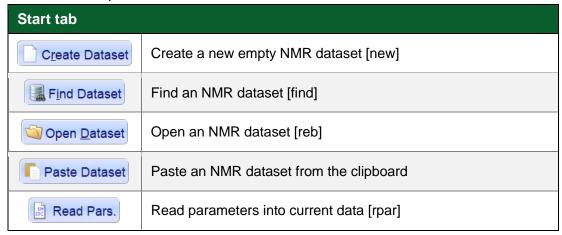


To access TopSpin manuals and a list of commands and keyboard shortcuts, click on the **Help** icon in the menu bar. For detailed descriptions of TopSpin's user interface elements, hover the cursor over the icons and buttons.

### Menu bar tabs

Table 8 to Table 11 show the menu bar buttons for the Start, Acquire, Process, and Publish tabs. Text in square brackets are commands that you can enter in the command line. Clicking on the drop-down arrow inside some of the buttons reveals more options.

**Table 8.** Descriptions of Start tab buttons.



**Table 9.** Descriptions of Acquire tab buttons.

Acquire tab	
<b>Sample</b> ▼	Access sample setup commands
<u>L</u> ock	Lock the magnetic field for the current sample [lock]
<b>V</b> T <u>u</u> ne <b>▼</b>	Tune and match the probehead automatically [atma]
<b>♣</b> Sp <u>i</u> n →	Control the sample rotation
Shim ▼	Autoshim the sample using TopShim [topshim]
<b>≨</b> Pr <u>o</u> sol →	Update the prosol parameters [getprosol]
<u> </u>	Auto-adjust the receiver gain [rga]
<b>©</b> Go ▼	Start acquisition [zg]
Options 🔻	Access acquisition options such as:  Shape tool TopGuide [topguide] Selective 1D experiment setup

Table 10. Descriptions of Process tab buttons.

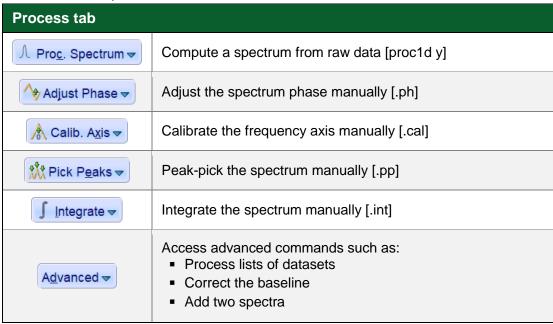
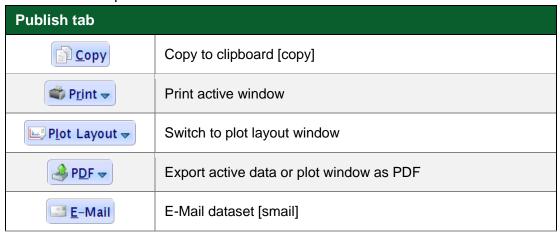


Table 11. Descriptions of Publish tab buttons.



# **NMR Safety Guidelines**

Equipment in the NMR facility is delicate and expensive. Improper use of NMR equipment can expose you and others to serious, and potentially fatal, safety hazards. Additionally, broken equipment can be expensive to repair or replace (the NMR system costs at least \$500,000).

To ensure a safe work environment, each person using NMR equipment must follow the NMR facility's safety guidelines and procedures.



# Caution:

The NMR spectrometer's invisible magnetic field is ALWAYS active. The NMR safety guidelines apply at all times, even when you are not operating any NMR equipment directly.

#### **General information**

When you are in the NMR facility, follow these safety rules:

- Wear proper laboratory attire and safety equipment.
- Do not eat or drink in the NMR facility.
- Do not bring prohibited objects past the 5-gauss line.
- Use only non-magnetic tools past the 5-gauss line.
- Be careful climbing the steps when approaching the NMR spectrometer.
- Clean up after yourself before leaving the room.
- Notify NMR facility staff if an NMR tube breaks.
- Ask NMR facility staff if you have any questions.

If the NMR spectrometer is not working properly:

- 1. Do NOT attempt to repair the NMR spectrometer yourself.
- 2. Report malfunction to NMR facility staff immediately.

# **Magnet quench**

A superconducting magnet generates the NMR spectrometer's strong magnetic field. To maintain the NMR spectrometer, NMR facility staff use cryogens, specifically liquid nitrogen and liquid helium, to cool the magnet. **Cryogens** are substances that produce extremely low temperatures that are cold enough to cause cold burns and frostbite. Regular cryogen refills ensure that the NMR spectrometer maintains its superconductivity.

If the NMR spectrometer loses its superconductivity, its magnet "quenches." A **magnet quench** occurs when the magnet's coil suddenly loses its absolute zero temperature, which causes rapid coil resistivity and heat generation. As a result, the NMR spectrometer loses its magnetic field.

During a magnet quench, the NMR spectrometer releases gaseous cryogens rapidly, resulting in a loud hissing noise and a cloud of vapour moving upwards to the ceiling. A magnet quench triggers the fire alarm and opens the emergency smoke vents to prevent asphyxiation from the displaced air.



# **WARNING:**

If you notice the signs of a magnet quench, evacuate the room and contact NMR facility staff immediately. Cryogens evaporate into colourless, odourless, and tasteless gases. Asphyxiation from a magnet quench is a fatal safety hazard.

If the NMR spectrometer's magnet quenches:

- 1. Evacuate the room immediately.
- 2. Notify anyone near the NMR facility to evacuate the area.
- 3. Report incident to NMR facility staff immediately.
- 4. Do NOT re-enter the room until further notice from NMR facility staff.
- 5. Seek medical help if anyone is seriously injured.

# **Medical implants**

The strong magnetic field inside the NMR facility is a serious safety hazard for people who have certain types of medical implants (Figure 4). The NMR facility has strict safety rules for who can and cannot enter the room and use the NMR spectrometer.



**Figure 4.** NMR facility signs prohibiting electronic and metallic medical implants.

#### Electronic

Electronic medical implants (or neurostimulators) include pacemakers, cardiac defibrillators, and cochlear implants. Strong magnetic fields can cause medical implants with electronics to malfunction or break. People with electronic medical implants are strictly prohibited from entering the NMR facility.

If you have any electronic medical implants:

- 1. Do NOT enter the NMR facility.
- 2. Notify NMR facility staff if you must use NMR equipment.



# **WARNING:**

Entering the NMR facility with an electronic medical implant that regulates heart function, such as a pacemaker, can be fatal. Always follow the warning signs posted on the NMR facility door.

#### Non-electronic, metallic

Non-electronic, metallic medical implants include pins, surgical clips, and joint prostheses. Although people with non-electronic, metallic medical implants can enter the NMR facility, they must not pass the 5-gauss line.

If you have any non-electronic, metallic medical implants:

- 1. Do NOT pass the 5-gauss line.
- 2. Notify NMR facility staff if you must use the NMR spectrometer.



#### **WARNING:**

Passing the 5-gauss line with a magnetic implant can cause serious injuries. If you are unsure whether your medical implant is magnetic, stay out until you consult NMR facility staff.

# Magnetic objects and electronic devices

The NMR spectrometer's strong magnetic field attracts magnetic objects and damages electronic devices (Figure 5). Magnetic objects pulled towards the NMR spectrometer travel at rapid speeds and strike the magnet, potentially causing a magnet quench and an asphyxiation hazard.





**Figure 5.** NMR facility signs prohibiting magnetic objects and electronic devices.

Do NOT bring magnetic objects or electronic devices past the 5-gauss line, such as:

- Ferrous work tools (e.g. wrenches and screwdrivers)
- Bank and credit cards with magnetic media
- Gas cylinders
- Watches and jewellery

- Keys and spare change
- Pocket knives
- Metal chairs
- Mobile phones
- Tablets and laptops



# **WARNING:**

Magnetic objects, especially heavy and sharp objects, can become dangerous projectiles that can cause serious injury or death. Keep all magnetic objects away from the NMR spectrometer.



# Caution:

The magnetic field can damage watches and erase data from bank and credit cards with magnetic media. Empty your pockets of *any* magnetic objects before approaching the NMR spectrometer.

When in doubt, stay out until you ask NMR facility staff. Assume each metallic object is magnetic until proven otherwise.

If you see a magnetic object strike the magnet:

- 1. Do NOT attempt to pull the object yourself.
- 2. Report incident to NMR facility staff immediately.
- 3. Seek medical help if anyone is seriously injured.

# **Summary**

Table 12 shows the prohibited and permitted objects inside the NMR facility, and Table 13 shows safety protocols for NMR facility incidents.

Table 12. Prohibited and permitted objects inside the NMR facility.

If you have any	Then
Electronic medical implants	Do NOT enter the NMR facility.     Notify NMR facility staff if you must use NMR equipment.
<ul> <li>Non-electronic, metallic medical implants</li> </ul>	<ol> <li>Do NOT pass the 5-gauss line.</li> <li>Notify NMR facility staff if you must use the NMR spectrometer.</li> </ol>
<ul><li>Magnetic objects</li><li>Electronic devices</li></ul>	<ul> <li>Do NOT bring inside the 5-gauss line.</li> <li>Leave beside the computer workstations.</li> </ul>
<ul> <li>Non-magnetic tools</li> </ul>	■ Use inside the 5-gauss line.

**Table 13.** Safety protocols for NMR facility incidents.

Incident	If you	Then	
NMR spectrometer malfunctions	<ul> <li>Notice that the NMR spectrometer is not working properly</li> </ul>	<ol> <li>Do NOT attempt to repair the NMR spectrometer yourself.</li> <li>Report malfunction to NMR facility staff immediately.</li> </ol>	
NMR spectrometer pulls a magnetic object	<ul> <li>See any magnetic object pulled towards and strike the magnet</li> </ul>	<ol> <li>Do NOT attempt to pull the object off yourself.</li> <li>Report incident to NMR facility staff immediately.</li> <li>Seek medical help if anyone is seriously injured.</li> </ol>	
NMR spectrometer magnet quenches	<ul> <li>Hear a loud hissing noise</li> <li>See a vapour cloud moving upwards to the ceiling</li> <li>Experience symptoms of asphyxiation (e.g. weakness, dizziness, nausea, etc.)</li> </ul>	<ol> <li>Evacuate the room immediately.</li> <li>Notify anyone near the NMR facility to evacuate the area.</li> <li>Report incident to NMR facility staff immediately.</li> <li>Do NOT re-enter the room until further notice from NMR facility staff</li> <li>Seek medical help if anyone is seriously injured.</li> </ol>	

# Task 1: Preparing an NMR Sample

Before you can acquire raw data, you need to prepare an NMR sample to place inside the NMR spectrometer.

Preparing an NMR sample involves 2 main steps:

- 1. Supply your sample.
- 2. Transfer your sample to an NMR tube.

To prepare an NMR sample, you use the following materials:

- Goggles (optional)
- Gloves (optional)
- Test tube
- 3 Pasteur pipettes
- NMR sample
- NMR tube with cap

- Deuterated solvent
- Spinner
- Depth gauge
- Kimwipes
- Methanol
- 1% tetramethylsilane (TMS) (optional)

To prepare a solid NMR sample, you also use the following materials:

- Spatula
- Glass wool
- Sonicator (optional)
- Weighing paper
- Weighing scale



To avoid contaminating your NMR sample, ensure that all of your materials are clean and not broken. Do not use an NMR tube if it is damaged or warped. Always inspect your laboratory materials before using them.

# Step 1: Supply your sample

Supplying your sample involves different procedures depending on whether your sample is a liquid or solid.

#### Supplying a liquid sample

To supply a liquid sample:

- 1. Use a Pasteur pipette to draw a small amount of sample.
- 2. Add approximately a ½ drop of sample in a test tube.
- 3. Use a clean Pasteur pipette to draw deuterated solvent.
- 4. Add 0.5–0.7mL of deuterated solvent to the test tube.
- 5. Add 1 drop of 1% TMS to provide a reference point for calibration (optional).

#### Supplying a solid sample

To supply a solid sample:

- Use a spatula to transfer a ½ drop of sample onto a piece of weighing paper.
- 2. Measure the sample on a weighing scale.
  - For <sup>1</sup>H NMR, use at least 20 mg of sample.
  - For <sup>13</sup>C NMR, use up to 50 mg of sample.
- 3. Transfer your sample from the weighing paper to a test tube.
- 4. Use a clean Pasteur pipette to draw deuterated solvent.
- 5. Add 0.5–0.7mL of deuterated solvent to the test tube.
- 6. Swirl the test tube or use a sonicator to dissolve the sample.
- 7. If there are noticeable solid particles, use a clean Pasteur pipette and glass wool to filter the sample.



#### Caution:

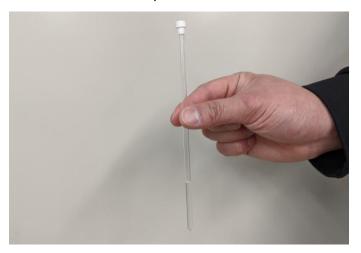
Ensure that your sample does not contain any solid particles. Solid particles negatively affect sample quality and distort the resulting spectrum.

8. Add 1 drop of 1% TMS to provide a reference point for calibration (optional).

# Step 2: Transfer your sample to an NMR tube

To transfer your sample to an NMR tube:

- 1. Use a clean Pasteur pipette to transfer approximately 3 cm of the mixture containing your sample and deuterated solvent to an NMR tube.
- 2. Cover the NMR tube with a cap.



3. Use a permanent marker to write your initials and experiment number on top of the cap.



- 4. Go to the NMR facility.
- 5. Place your NMR sample in the NMR sample tube rack.

# Task 2: Acquiring Raw Data

Next, you need to use the NMR spectrometer to acquire raw data from your sample.

Acquiring raw data involves 4 main steps:

- 1. Create a new experiment.
- 2. Prepare your sample for placement.
- 3. Place your sample in the NMR spectrometer.
- 4. Acquire raw data from your sample.

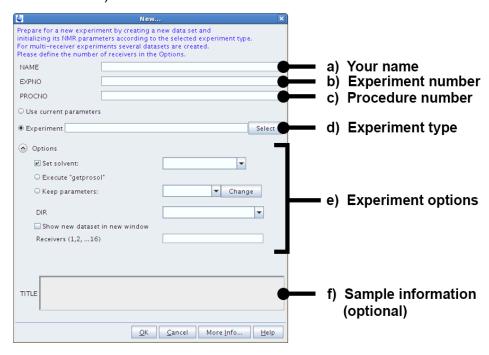
# **Step 1: Create a new experiment**

To create a new experiment:

- 1. Log in to the Linux computer using your course section or principal investigator's (PI) username.
- 2. Double-click on the TopSpin 3.2 icon on the desktop.
- 3. Click on the Start tab in the menu bar.
- 4. Click Create Dataset (or press Ctrl+N).



5. In the dialogue box, enter your dataset information (see page 22 for more information).



- a) Enter your name.
- b) Enter your experiment number.
- c) Enter your procedure number.
- d) Select your experiment type:
  - i. Press Select.
  - ii. In the dialogue box, click on the Source = drop-down menu at the upper-right corner and select /opt/topspin3.2pl7/exp/stan/nmr/par.
  - iii. Select **PROTON** for <sup>1</sup>H or **C13APT** for <sup>13</sup>C.
  - iv. Click Set selected item in editor.
- e) Select your experiment options:
  - i. Select **Keep parameters**.
  - ii. Click on the **DIR** drop-down menu and select a directory path so you can access your raw data on the Mac computer later.
  - iii. Enter your sample information (optional).
- 6. Click OK.
- 7. Click **Read Pars**. to select a parameter set for your current dataset.



- 8. In the dialogue box, select your experiment type.
- 9. Click Read.
- 10. Click OK.

# Step 2: Prepare your sample for placement

To prepare your sample for placement:

1. Insert the NMR tube into a spinner.

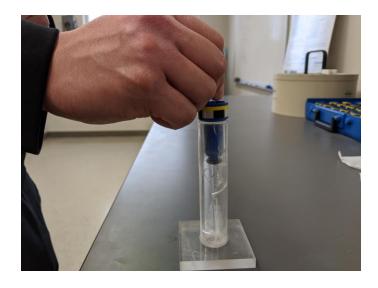


- 2. Place the spinner containing the NMR tube in the depth gauge.
- 3. Insert the NMR tube slowly down the depth gauge until it reaches the bottom.



# Caution:

NMR tubes are fragile. Do not insert the NMR tube at a slanted angle. Gently insert the NMR tube straight down the depth gauge to avoid breaking it and cutting your hand.



- 4. Add a small amount of methanol to a Kimwipe.
- 5. Remove the NMR tube and spinner from the depth gauge.
- 6. Clean the NMR tube and spinner with the Kimwipe to wipe away any fingerprints or solid particles.



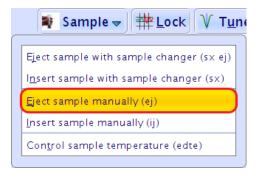
# Caution:

After cleaning, avoid touching the spinner and bottom half of the NMR tube to prevent recontaminating them with fingerprints. Fingerprint oils cause poorer spectrum resolution and transfer contaminants to the NMR spectrometer's probehead. Hold your sample by handling the top part of the NMR tube.

# Step 3: Place your sample in the NMR spectrometer

To place your sample in the NMR spectrometer:

- 1. Click on the Acquire tab in the menu bar.
- 2. Click **Sample > Eject sample manually (ej)** to activate air flow.





#### **WARNING:**

Leave ALL magnetic objects and electronic devices beside the computer workstations before you cross the 5-gauss line. The NMR spectrometer's strong magnetic field damages electronic devices and pulls magnetic objects towards it at speeds fast enough to cause serious injury or death.

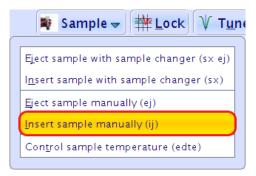


#### Caution:

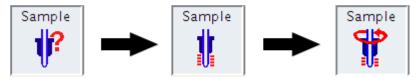
Always activate air flow before placing your sample in the magnet. Placing a sample without activating air flow can break the NMR tube and damage the NMR spectrometer.

- 3. Listen for air flow before placing your sample.
  - If there is a sample above the magnet, remove it before proceeding.
- 4. Place your sample with the spinner on the cushion of flowing air above the magnet.

5. Click **Sample** > **Insert sample manually (ij)** to lower your sample into the magnet.



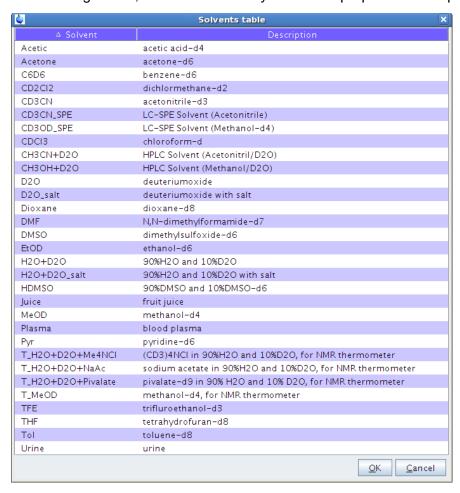
6. Wait for the sample status icon in the acquisition status bar to show that your sample is spinning.



- 7. Click on the **Lock display window** icon in the toolbar or the **Lock display panel** in the acquisition status bar.
- 8. Click **Lock** to control the sample's magnetic field and prevent resonance frequency drift.

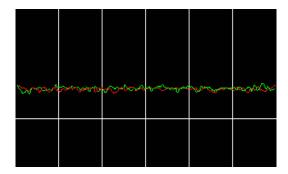


9. In the dialogue box, select the solvent you used to prepare the sample.



#### 10. Click **OK**.

- 11. Wait for the sample to finish locking.
  - When the sample is finished locking, the lock signal in the Lock display menu appears as a straight line.



To manually adjust the lock, click on the BSMS control panel icon in the toolbar (optional).

# Step 4: Acquire raw data from your sample

To acquire raw data from your sample:

1. Click **Tune** to tune and match the NMR spectrometer's probehead.

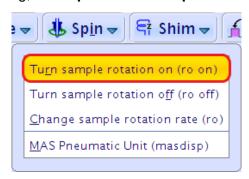


Wait for each data acquisition function to finish processing before moving on to the next step. You can see the current data acquisition status and result written in blue text below the command line.

2. Click on the AcquPars tab in the data window area.



- 3. Adjust your preferred acquisition parameters (optional).
- 4. If the sample status icon in the acquisition status bar does not show that the sample is spinning, click **Spin** > **Turn sample rotation on (ro on)**.



5. Click **Shim** to adjust the magnetic field homogeneity.



To manually adjust the shim, click on the BSMS control panel icon in the toolbar (optional).

6. Click **Prosol** to load the pulse width and power levels for the parameter set.



If you are conducting a 2D experiment, you must perform additional data acquisition steps. Refer to page 30 before proceeding step 4.7.

7. Click Gain to automatically set the receiver gain.



8. Click **Go** to start acquiring data.





The residual time in the acquisition status bar displays the estimated amount of time left for data acquisition.

#### Acquiring data from a 2D experiment

To acquire data from a 2D experiment:

- 1. Click Set Limits.
- 2. Follow the instructions in the dialogue box:
  - a) Find your 1D dataset in the browser panel:
    - i. Press Select.
    - ii. Right-click on your 1D the dataset name.
    - iii. Select Display.
  - b) Zoom into the region of interest:
    - i. Click on the **Show full spectrum** icon in the toolbar.
    - ii. Click and hold the **Zoom in or out smoothly** icon in the toolbar and move the mouse.
    - iii. If necessary, click and hold the **Move spectrum left or right** icon in the toolbar and move the mouse to shift the spectrum.
  - c) Click **OK** to set frequencies and return to the original dataset.
- 3. Proceed to step 4.7 on page 29.

# Task 3: Processing an NMR Spectrum

After you acquire raw data from your sample, you need to process the NMR spectrum for printing.

Processing an NMR spectrum involves 5 main steps:

- 1. Compute your raw data.
- 2. Expand and examine the peaks.
- 3. Calibrate the axis.
- 4. Identify the peaks.
- 5. Integrate the peaks.



If another person needs to use the Linux computer workstation, remove your sample and transfer your raw data before proceeding further (see page 35).

# Step 1: Compute your raw data

To compute your raw data:

- 1. Click on the Process tab on the menu bar.
- 2. Click **Proc. spectrum** to process a **free induction decay (FID)** display of your raw data.



 If you are conducting a 2D experiment, Click Plot spectrum > Symmetrize spectrum.

## Step 2: Expand and examine the peaks

To expand and examine the peaks:

- 1. Click on the **Show full spectrum** icon in the toolbar.
- 2. Click and drag the cursor from the left side to the right side of a peak that you want to expand and examine.
- 3. Repeat steps 2.1–2.2 to expand and examine the remaining peaks.

### **Step 3: Calibrate the axis**

To calibrate the axis:

- 1. Click on the **Show full spectrum** icon in the toolbar.
- 2. Refer to the solvent chemical shift chart to find the solvent peak on the spectrum.
- 3. Expand the solvent peak.
- 4. Click Calib. Axis.



- 5. Click on the middle of the solvent peak.
- 6. In the dialogue box, set the **Spectrum calibration frequency** to your solvent's chemical shift for your experiment type.
- 7. Click OK.

## Step 4: Identify the peaks

To identify the peaks:

- 1. Click on the **Show full spectrum** icon in the toolbar.
- 2. Click Pick Peaks.



- 3. If they are not already selected and highlighted by default, click on the **Define new peak picking range** and **Define peaks manually** icons in the data window area.
- 4. Click and drag the cursor to highlight the top of a peak that you want to identify.
  - The red number indicates the chemical shift for that specific peak.
- 5. Repeat the previous step to identify the remaining peaks.
  - To change the peak picking range (optional):
    - a) Click on the **Change peak picking range** icon in the data window area.
    - b) Click and drag the cursor to move one of the edges of the peak picking range to its new location.
  - To delete the peak picking ranges, click on the **Delete all peak picking**ranges icon in the data window area (optional).
- 6. Click on the **Return**, save changes | icon in the data window area.

# **Step 5: Integrate the peaks**

To integrate the peaks:

1. Click Integrate.



- 2. Click and drag the cursor from the left side to the right side of a peak that you want to integrate.
  - The red number indicates the integration for that specific peak.
- 3. Repeat the previous step to integrate the remaining peaks.
  - To delete the integral regions, click on the **Delete all integral regions**icon in the data window area (optional).
- 4. Click on the **Return**, save changes | icon in the data window area.

# **Task 4: Changing Workstations**

Before you can produce an NMR spectrum, you need to remove your sample and transfer your raw data to a different computer. If you already changed workstations, see Task 5 on page 37.

Changing workstations involves 2 main steps:

- 1. Remove your sample.
- 2. Transfer your raw data.

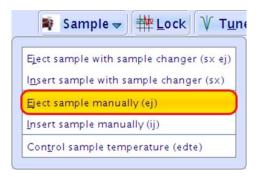


Check your data to ensure they are correct and complete before you remove your sample.

## **Step 1: Remove your sample**

To remove your sample:

- 1. Click on the **Acquire** tab in the menu bar.
- 2. Click Sample > Eject sample manually (ej) to activate air flow.



- 3. Listen for air flow before removing your sample.
- 4. Remove your sample from the NMR spectrometer.
- 5. Remove the NMR tube from the spinner.
- 6. Place the NMR tube in the NMR sample tube rack and the spinner in its container.
- 7. Click on the **BSMS control panel** icon in the toolbar.
- 8. Under **Sample**, click **Lift** to turn off air flow.
- 9. Minimize the BSMS control panel.

## Step 2: Transfer your raw data

To transfer your raw data:

- 1. Close TopSpin 3.2.
- 2. Log out of the Linux computer.
- 3. Log in to the Mac computer.
- 4. Click on the Cyberduck icon on the dock.
- 5. Select your course section or PI's username.
- 6. Search for your name.
- 7. Double-click on your folder.
- 8. Transfer your raw data.
  - If you want to continue using the Mac computer workstation, click and drag your experiment folder (which is named after your experiment number) onto the desktop.
  - If you want to use a different computer with TopSpin installed:
    - a) Insert your flash drive into the USB extension cable.
    - b) Click and drag your experiment folder (which is named after your experiment number) to the flash drive.
    - c) Eject and remove your flash drive.
    - d) See page 41 to complete your scheduled NMR session.
       Proceed to the next step after you log in to the different computer.
- 9. Open TopSpin.
  - If you are using the Mac computer workstation, click on the TopSpin 3.2 icon on the dock.
  - If you are using a Windows computer, double-click on the TopSpin 3.2 icon on the desktop or open TopSpin 3.2 through the Start menu.
- 10. Click and drag your experiment folder onto the TopSpin window.
  - TopSpin automatically opens the file.

# Task 5: Producing an NMR Spectrum

After you finish changing workstations, you can produce an NMR spectrum.

Producing an NMR spectrum involves 2 main steps:

- 1. Plot your NMR spectrum.
- 2. Print your NMR spectrum.

## Step 1: Plot your NMR spectrum

To plot an NMR spectrum:

- 1. Click on the **Publish** tab in the menu bar.
- 2. Click Plot Layout.



- 3. Click on the NMR spectrum in the data window area to expand the plot panel options.
- 4. Adjust the following settings (optional):
  - Integrals

Axis

Peaks

Placement



To toggle the peak and integral labels, click on the **Labels** checkbox.

- 5. Click Axes, Grids, Curve.
- 6. Under **Plot limits**, adjust the axis settings to eliminate any unnecessary white space.
- 7. Click on the **panel arrow** to return to the main plot panel.

- 8. Arrange the spectrum's data and layout for printing.
  - To insert shapes and lines, click on the **Standard element** button.
     To insert NMR data text and graphics, click on the **NMR element** button.



- To move an element, click and drag the element to its new location.
- To resize an element, click and drag one of the green square handles that surround the element.
- To delete an element, right-click on the element and select **Delete**.

### Inserting an inset of an NMR spectrum (optional)

To insert an inset of an NMR spectrum:

- 1. Click on the **NMR element** button.
- 2. Select 1D spectrum.
- 3. Click and drag the cursor to place the insetted NMR spectrum.
- 4. Click on the insetted NMR spectrum.
- 5. Adjust the insetted NMR spectrum's settings in the plot panel.

## Step 2: Printing and exporting your NMR spectrum

When printing an NMR spectrum, you have the options of printing an entire spectrum on one page or detailed parts on multiple pages. You can also export a PDF copy of your NMR spectrum.

#### Printing an entire NMR spectrum on one page

To print an entire NMR spectrum on one page:

- 1. Click on the **Print active window** icon in the toolbar (or press **Ctrl+P**).
- 2. In the dialogue box, select your printing preferences and properties.
- 3. Click Print.

#### Printing a detailed region of an NMR spectrum

To print a detailed region of an NMR spectrum:

1. Click on the **Spectrum** tab in the data window area.



- 2. Adjust the spectrum's vertical (intensity) and horizontal (zooming) scaling.
  - To adjust the spectrum's vertical scaling, click and hold the Increase or decrease intensity icon in the toolbar and move the mouse.
  - To adjust the spectrum's horizontal scaling, click and hold the Zoom in or out smoothly icon in the toolbar and move the mouse.
  - For more scaling functions, refer to Table 5 on page 6.
- 3. Adjust the spectrum's vertical and horizontal shifting.
  - To adjust the spectrum's vertical shifting, click and hold the Move
     spectrum up or down icon in the toolbar and move the mouse.
  - To adjust the spectrum's horizontal shifting, click and hold the Move
     spectrum left or right icon in the toolbar and move the mouse.
  - For more shifting functions, refer to Table 6 on page 7.
- 4. Click on the **Print active window** icon in the toolbar (or press **Ctrl+P**).
- 5. In the dialogue box, select your printing preferences and properties.
- 6. Click Print.

### Exporting an NMR spectrum as a PDF file (optional)

To export an NMR spectrum as a PDF file:

- 1. Click on the Publish tab in the menu bar.
- 2. Click PDF.



- 3. In the dialogue box, select the desktop as your directory path.
- 4. Enter the file name and extension (e.g. NMRSpectrum.pdf).
- 5. Click **Export**.

# Task 6: Completing the NMR session

When you have finished your work, complete your scheduled NMR session before you leave the NMR facility.

To complete the NMR session:

- 1. Close TopSpin 3.2.
- 2. Double-click on your PI's desktop folder.
- 3. Right-click and create your own sub-folder in your PI's desktop folder.
- 4. Click and drag your experiment folder to your sub-folder.
- 5. Transfer any exported PDF files to your e-mail or flash drive.
  - After you finish transferring your PDF files, click and drag them to the Trash Bin.
- 6. Log out of the Mac computer (or Linux computer if you plan to use a different computer to process and print your data).
- 7. Record your session time and information in the log book.



Always record your session time and information in the log book whenever you use the NMR facility's equipment and computer workstations.

- 8. Clean up your materials and supplies before leaving.
- 9. Close the door after you leave the NMR facility.

# **Appendix**

## **Glossary**

**2D (two-dimensional) NMR:** A set of spectroscopic methods that presents NMR data in a plot of two frequency axes rather than just one.

**5 gauss:** The strength of a magnetic force that is strong enough to affect nearby magnetic objects and electronic devices.

**5-gauss line:** A boundary surrounding the NMR spectrometer that indicates where magnetic objects and electronic devices are prohibited.

**Carbon-13 (13C) NMR:** A spectroscopic method that examines the nuclear magnetic properties of <sup>13</sup>C atoms in a molecule to analyze its structure.

**Cryogen:** A substance that produces extremely low temperatures that are cold enough to cause cold burns and frostbite. In NMR spectroscopy, it is used to cool an NMR spectrometer's magnet and maintain its superconductivity.

**Fourier transform:** A mathematical function that can convert a time-domain signal to a frequency domain signal. In NMR spectroscopy, it is used to convert an FID signal into a practical display that conveys more useful information from an NMR sample analysis.

**Free induction decay (FID):** A magnetic resonance signal that is detected by an NMR spectrometer and contains all of the information inside an NMR spectrum. It is generated from the decay of transverse magnetization.

**Magnet quench:** The sudden loss of a superconducting magnet's superconductivity. It results in rapid coil resistivity and heat generation, which is caused when the magnet's coil suddenly loses its absolute zero temperature.

**NMR sample:** A sample used in NMR spectroscopy that typically contains a deuterated solvent and a compound under investigation.

**NMR spectrometer:** An instrument that contains a powerful superconducting magnet and is used to analyze the molecular structure of compounds.

**NMR spectroscopy:** An analytical technique used to analyze the molecular structure of compounds.

**NMR spectrum:** A range of signals produced from an NMR spectroscopy analysis. It contains peaks that represent specific atoms inside the sample.

**Proton (¹H) NMR:** A spectroscopic method that examines the nuclear magnetic properties of ¹H atoms in a molecule to analyze its structure.

#### **Useful links**

#### NMR facility information

- UWinnipeg NMR facility website: whmis.uwinnipeg.ca/nmr
- UWinnipeg NMR facility instrument booking: faces.ccrc.uga.edu

### TopSpin software

- Bruker Software downloads: bruker.com/service/supportupgrades/software-downloads/nmr.html
- Bruker TopSpin 3.X user manual: bruker.com/fileadmin/user\_upload/8-PDF-

Docs/MagneticResonance/Service\_NMR/General/user\_manual\_topspin\_ts35.pdf

Bruker – TopSpin control and function keys:

bruker.com/fileadmin/user\_upload/8-PDF-Docs/MagneticResonance/Service\_NMR/General/control\_and\_function\_k eys.pdf

#### NMR spectroscopy references

- The Center for Imaging Science The basics of NMR: cis.rit.edu/htbooks/nmr/inside.htm
- The Resonance NMR 101: theresonance.com/nmr-101
- The Resonance NMR applications and techniques: theresonance.com/applications-techniques
- SpectroscopyNOW NMR knowledge base: spectroscopynow.com/nmr
- Sigma-Aldrich NMR deuterated solvent properties reference chart: https://www.sigmaaldrich.com/technical-documents/articles/stable-isotopes/nmr-deuterated-solvent-properties-reference.html
- The National Institute of Advanced Industrial Science and Technology (AIST)
  - Spectral Database for Organic Compounds (SDBS):

https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre\_index.cgi?lang=eng

## **Analyzing 2D NMR spectra**

#### <sup>1</sup>H-<sup>1</sup>H COSY

To analyze a <sup>1</sup>H-<sup>1</sup>H COSY spectrum:

- 1. Draw a diagonal line through the area of the spectrum where the hydrogens correlate with themselves.
  - Note the mirror image between the correlations above and below the diagonal line. Due to the spectrum's symmetrical nature, you only need to analyze one of the mirrored correlation sets (either above or below the diagonal line).
- 2. Choose a peak on the left axis.
- 3. Draw a horizontal line to the right until you reach a contour.
- 4. Draw a vertical line to the top axis.
  - The horizontal and vertical lines are connected to 2 peaks in both spectra. This indicates that these 2 protons are coupled to each other and are likely 3 bonds or less away from each other.
- 5. Write down which protons are coupled to each other (e.g. " $H_1$  is coupled to  $H_3$ ").
  - Generally, you see protons that are within 3 bond lengths away from each other. However, you can sometimes see a weak 4-bond coupling in COSY experiments.
- 6. Repeat steps 2–5 to correlate and analyze the remaining signals.

#### <sup>13</sup>C-<sup>1</sup>H HSQC

To analyze a <sup>13</sup>C-<sup>1</sup>H HSQC spectrum:

- 1. Choose a peak on the top axis.
- 2. Draw a vertical line to the bottom until you reach a contour.
- 3. Draw a horizontal line to the left axis.
  - The horizontal and vertical lines are connected to 2 peaks that are coupled to each other.
- 4. Repeat steps 1–3 to correlate and analyze the remaining signals.

