Pulsed-Field Gradient NMR
Diffusion Measurement Protocol

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## Contents

**Contents**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface</td>
<td>iii</td>
</tr>
<tr>
<td>General Precautions</td>
<td>1</td>
</tr>
<tr>
<td>1. Installation/Removal of the Probe</td>
<td>2</td>
</tr>
<tr>
<td>1.1 Removing the Probe</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Assembling the Probe</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Disassembly of Probe</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Installing the Probe</td>
<td>4</td>
</tr>
<tr>
<td>2. Preliminary Steps</td>
<td>6</td>
</tr>
<tr>
<td>2.1 Starting and Using Topspin</td>
<td>6</td>
</tr>
<tr>
<td>2.2 Selecting Correct Probe Body (Edhead)</td>
<td>8</td>
</tr>
<tr>
<td>2.3 Configuring Gradient Pre-Emphasis Parameters (Setpre)</td>
<td>9</td>
</tr>
<tr>
<td>2.4 Gradient Calibration Constant (Gradpar)</td>
<td>11</td>
</tr>
<tr>
<td>2.5 Field</td>
<td>12</td>
</tr>
<tr>
<td>3. Temperature Control</td>
<td>13</td>
</tr>
<tr>
<td>3.1 Operating Sample Temperature Control</td>
<td>13</td>
</tr>
<tr>
<td>3.2 Temperature Measurement</td>
<td>15</td>
</tr>
<tr>
<td>3.3 Sample Temperature Correction</td>
<td>16</td>
</tr>
<tr>
<td>4. NMR Measurement</td>
<td>17</td>
</tr>
<tr>
<td>4.1 Introducing the Sample</td>
<td>17</td>
</tr>
<tr>
<td>4.1.1 Loading Samples using Lift Air</td>
<td>17</td>
</tr>
<tr>
<td>4.1.2 Loading Samples using Fishing Line</td>
<td>18</td>
</tr>
<tr>
<td>4.1.3 Removing Samples using Fishing Line</td>
<td>20</td>
</tr>
<tr>
<td>4.1.4 Checking Sample Position</td>
<td>20</td>
</tr>
<tr>
<td>4.2 Tuning and Matching</td>
<td>21</td>
</tr>
<tr>
<td>4.3 Shimming</td>
<td>23</td>
</tr>
<tr>
<td>4.4 Copying Datasets (new)</td>
<td>24</td>
</tr>
<tr>
<td>4.5 Common Acquisition Parameters</td>
<td>25</td>
</tr>
</tbody>
</table>
4.6 Processing Parameters........................................................................................................... 28
  4.6.1 Fourier Transform ........................................................................................................... 28
  4.6.2 Phase Correction ............................................................................................................. 28
  4.6.3 Baseline Correction ........................................................................................................ 31
4.7 The ZG Experiment ............................................................................................................. 32
4.8 Relaxation Delay (D1) Estimate ......................................................................................... 34
4.9 P1 Determination ................................................................................................................ 35
4.10 Determination of T1 NMR Relaxation Times .................................................................... 38
4.11 Determination of T2 NMR Relaxation Times .................................................................... 39
5. PFG NMR Diffusion Experiments ....................................................................................... 41
  Performing a PFG NMR diffusion experiment ....................................................................... 48
6 2D NMR Data Analysis ......................................................................................................... 50
  6.1 T1/T2 Relaxation Analysis ................................................................................................. 50
  6.2 Raw data to Excel ............................................................................................................. 52
  6.3 Attenuation Plots in Origin ............................................................................................... 54
Appendix A – Theory of Diffusion ............................................................................................. 56
Appendix B – Theory of Pulsed Field Gradient NMR ................................................................. 57
  B.1 A Brief Overview of Nuclear Magnetic Resonance .......................................................... 57
    Pulsed-Field Gradient NMR: Theory ..................................................................................... 59
Appendix C – Measurement at Low Temperature ...................................................................... 62
Appendix D - Selected Topspin Command Reference ............................................................... 64
Preface

This instructional manuscript is intended as a guide for users at the AMRIS facility who are interested in performing pulsed field gradient (PFG) NMR diffusion experiments using Topspin software on Bruker NMR spectrometers. The text is written such that a minimal background in NMR is required to follow the protocols, while at the same time, the text provides a sufficiently complete description to perform detailed PFG NMR measurements. This protocol is not intended to be an alternative to Bruker instructional material. The reader is referred the reader to Bruker manuals for details and/or protocols not included in this text.

This 1st major revision (2013-2014) has been prepared to reflect changes from the previous Bruker Avance II console running Topspin 2.x software to the Bruker Avance III HD console running Topspin 3.x software. At the time of writing, the 750 MHz wide-bore spectrometer and the 600 MHz standard-bore spectrometers at the AMRIS facility are capable of performing PFG NMR diffusion experiments.

Formatting in this Protocol

This protocol uses the following formatting conventions:

- **Standard Serif Font**
  - body text

- ***Bold Serif font**
  - special notes and/or points of caution

- **Bold Monotype font**
  - commands in the Topspin software (e.g. zg or stop)

- **Normal Monotype font**
  - Parameter names used in Topspin software (e.g. NS or P1)

- **“Monotype italic font”**
  - menus or buttons labels in Topspin software windows and for messages given by the software (e.g. ‘OK’)

- **“UPPER CASE SANS-SERIF FONT”**
  - button labels on the hardware
General Precautions

1. **High magnetic fields are always present in the magnet rooms!** As such, ferrous metal objects can be violently and strongly attracted to the magnet which can cause equipment damage and serious injuries. Other items are sensitive to high magnetic fields such as electronic devices and credit cards and may be damaged in the presence of high magnetic fields. **Do not bring any magnetic or magnetic sensitive items into the magnet room and/or the magnet safety zone** (delineated with yellow tape and barriers) including keys, coins, pens, jewelry, watches, earrings, cell phones and wallets…etc. **It is a good practice to remove all contents of your pockets and all jewelry/watches and verify their removal every time the high magnetic field zone must be entered.** Always consult with AMRIS staff if you have any doubts regarding this warning.

2. NMR magnets contain large amounts of liquid cryogens which may suddenly vaporize in the event of a magnet quench – causing an asphyxiation hazard. **If large plumes of gas are venting from the magnet or if any alarm is sounding leave the building immediately!**

3. The 750 MHz magnet contains exposed cryogenic He piping which should not be disturbed in any way; extreme care must be exercised when working around the magnet near these pipes which run along the west and north walls of the magnet room.

4. Watch your step! Do not disturb (e.g. displace/step on) cables and hoses running along the floor, esp. running between the console and the magnet. Also, be careful not to disturb cryogen-filling equipment stored on top of the magnet platform.

5. **Always wear proper personal protective equipment including a suitable face shield** if sample tubes containing pressurized gas are being handled.

6. **Sample tubes are fragile and sealed glass ends can be sharp!** Avoid applying large forces when handling sample tubes, do not force sample tubes. When working with sample tubes in a spinner, avoid applying force to the sample tube far away from the spinner as this may stress the tube and cause rupture.
1. Installation/Removal of the Probe

In most cases, the technician(s) at the facility will perform the task of installing and/or removing the probe from inside the magnet before and after the user’s assigned measurement time. This procedure requires great attention to detail and caution. Users must have specific permission from AMRIS technicians to perform the tasks described below. However, these instructions are included for completeness and to make users aware of important steps that should be verified for the completion during probe setup/removal.

1.1 Removing the Probe

1. Make sure to stop all running measurements and remove sample. The topspin command `stop` will end all acquisition. The sample lift may be activated/deactivated by toggling the `LIFT` button on the BSMS virtual board (type `bsmsdisp` or click the icon).

2. Turn off the temperature controller – use the `edte` window to turn off temperature control (see the Temperature Control section).

3. Turn gradient amplifiers and master unit off – (At Console) Turn off gradient master unit and all gradient amplifiers and the \( B_0 \) compensation unit. The switches are illuminated toggle switches labeled ‘POWER’.

4. Turn off gradient cooling water chiller – (At Water Chiller) off the gradient water chiller using the power button labeled ‘I/O’.

5. Disconnect and remove gradient cooling lines – Disconnect the inlet and outlet water tubes by simultaneously pushing the connectors in and pulling the tubes out. Use the bucket provided to collect water as the water line is disconnected from probe. Attach the disconnected two water lines (inlet and outlet) to the short coupling tube (green).

6. Remove air hose. *Warning! The retaining clip for the air hose is magnetic.*

7. Disconnect electrical cables for the gradients (lock rotates in place), heater (screwed in) and temperature sensor (pulls strait out) and disconnect the RF coaxial cable (screwed in). Set these cables aside carefully, away from the bore of the magnet.

8. Release the lock and slowly lower the probe out of the magnet bore – Support the probe and release the locking band by rotating clockwise. *Note: There is minimal clearance between the gradient sets and the inner shim. Care should be taken to keep the probe concentric with bore otherwise binding will occur. The probe should not be forced into or out of the bore. Be careful not to jar or drop the probe while transferring it from underneath the magnet. Finally, be aware of your hand placements as pinching points may form when removing/installing the micro-5 gradient set.*
1. Installation/Removal of the Probe

9. Wipe up any spilled water during the removal process.

10. If the probe is removed for a prolonged period (e.g. hours, days) of time, the magnet bore should be plugged to prevent the possibility of attracted small magnetic objects from entering the exposed bore.

1.2 Assembling the Probe

1. Check the RF coil and the gradient set for any dirt residue and/or water on the inner and outer surfaces. Wipe with a clean cloth as necessary.

2. Remove any remaining cooling water from the gradient set – a gentle shaking motion followed with a jet of low-pressure compressed air should be used to clear any water from inside of the gradient. The narrow, closed cooling passages of the gradient cause surface tension effects where the water remains inside the gradient, this means the water clearing process likely needs to be repeated 3-5 times, inverting the gradient between each clearing. *It is very important to remove ALL water from the gradient set and top of the probe body. Free, misplaced water can not only contaminate measurements but also interfere with electrical connections and cause equipment damage.

Figure 1-1: Illustration of the RF coil and gradient set connections on the top of the Micro-5 probe body (left) and photograph depicting the RF coil on the probe body (right)

3. Set the probe body upright on a level, stable surface. Mount the RF coil and gradient set onto the top of the probe body. *Do not tilt the probe body during RF coil coil/gradient set installation.
4. Take notice of the electrical pin alignment on the probe body where these two items should be placed (see Fig 1-1). The RF coil, for example, has five pin sockets – in groups of two and three. The gradient set has its own pin alignment, concentric to the RF coil and also has two sockets for the cooling water supply and return.

5. Inspect the O-rings (4 total) on the cooling water pins on the probe body. If the O-rings are cracked or torn, they should be replaced by an AMRIS technician.

6. Align the RF coil pins and seat the RF coil onto the pins. Align the gradient set electrical pins and cooling water pins and seat the gradient set. It is important when installing the gradient set not to rotate its alignment excessively as it may bind on the RF coil which may become unseated.

7. Ensure that the gradient set is tightly seated on the probe pins. Tighten the retaining screw fitting on the probe to the gradient.

8. (If necessary) It may be necessary at this point to connect the probe body to a network analyzer to verify that the probe is setup correctly and that the tuning/matching is reasonably close to the spectrometer’s operating frequency.

### 1.3 Disassembly of Probe
1. Follow steps 2 – 4 of Section 1.2 ‘Assembling the Probe’ in reverse order to disassemble the probe body.

### 1.4 Installing the Probe
1. Install probe body into the magnet. In order to install the probe body safely it must be oriented exactly upright in the z-direction and remain concentric with the bore. *Tilting the probe body slightly will make it very difficult to insert into the magnet. The probe should not be forced into or out of the bore as this may damage the probe body/gradient set and/or the magnet could result. Be careful not to jar or drop the probe while handling it underneath the magnet.

2. Connect gradient chiller water supply and return lines by pressing the tubes snugly into the quick connect fittings.

3. Connect the air supply hose. The connection between the hose and the probe body should be secured using a retaining clip. The air supply hose should be supported about a foot away from the probe. *Warning! The retaining clip for the air hose is magnetic.

4. Connect gradient and heater power cables by aligning the pins and securing by rotating the lock ring clockwise on the former and screwing in the latter.

5. Connect the temperature sensor cable - The temperature control system of the console requires the use of a interface device (e.g. a small box labeled “TF-2T”) to connect to the probe. Ensure the TF-
1. Installation/Removal of the Probe

2T interface is installed. Connect the lead labeled “Ch. 1” to the probe body temperature sensor port by aligning and pressing the connector in place.

6. Connect the 50 Ω RF coaxial cable to the correct nuclei channel on the front of the probe body and the appropriate pre-amplifier slice.

7. Turn on the gradient chiller – (At Water Chiller) Check the water level in the reservoir; fill the water reservoir with deionized water provided by AMRIS staff if necessary. Start the gradient chiller by pressing the power button labeled ‘I/O’. Shut off low-flow warning alarm if present. For most diffusion measurement applications, a water setpoint temperature of 20°C is suggested. *Check for any water leaks from probe after setup.

8. Turn on gradient amplifier and controller – (At console) switch on the z-gradient amplifier, the B0 compensation unit and finally the gradient master unit. The gradient master unit should be turned on last so that it may properly recognize the amplifiers.

9. It is likely that the console will need to be reconfigured using Topspin to setup and initialize the hardware configuration used for Diffusion NMR. This is a procedure that needs to be performed by the AMRIS staff.

Some known important points regarding this procedure:

- C01 status on the gradient master unit indicates that no gradient safety parameters are stored. The safety parameters are part of the preemphasis parameters and can be loaded with `setpre` and also directly with `gradsafe`. Bruker reports a bug in Topspin 3.2 where the former does not set the hardware parameters but the latter does.

- Gradient channel should be properly set using the `edscon` menu. At time of writing, gradient channel 1 is the GREAT/60 gradient rack and channel 2 is the GAB/2 gradient amplifier on the 750 MHz and 600 MHz spectrometers.

- The UXNMR.PAR configuration file must have the following line “##$GRADRES = 8” This can only be verified and changed using a text editor.

- A ‘LVDS buffer full’ error indication in `gradstat` appears to require a reboot of the gradient master unit to resolve.
2. Preliminary Steps

2.1 Starting and Using Topspin

At the time of writing, Bruker Topspin v. 3.2.3 is the software package used to operate the NMR spectrometer for PFG NMR diffusion measurements. This version of Topspin can be launched by typing `/opt/topspin3.2/topspin` in the Linux terminal or via the Linux GUI file explorer to navigate to the `/opt/topspin3.2/` directory and doubling clicking the icon labeled “topspin”. For new users, the data directory may not be setup in the directory explorer panel on the left. In order to setup this directory, right-click inside that window and select "add dir".

Topspin can be operated by selecting menu commands located in the dropdowns on the top menu bar and/or clicking the toolbar icons located just below the menu bar (Fig 2-1). However, Topspin commands may also be typed in and executed using the command line interface. To execute a command, select (e.g. click on) the command line bar (located immediately above the status bar along the bottom), type the command and press enter. In this manuscript, Topspin commands to be executed in this manner will appear in this style. A list and description of commonly used Topspin commands is given in Appendix D for the reader’s convenience.

NMR data in Topspin is organized in a hierarchical manner:

- Experiment Set (known in Topspin as NAME) holds sets of ‘Experiments’ which has an alphanumeric label.

- Experiments (abbrev. EXPID in Topspin) consist of a complete set of acquisition and processing parameters and the actual primary data, the free induction decay signal. Experiments have only numeric non-negative integer labels. Experiments hold ‘Processing’ files.

- The lowest level, are ‘Processing’ files (abbrev. PROCID in Topspin). Processing files contain different processing (e.g. Fourier transforms, FIDs…etc.) of the primary experimental data of the Experiment. Like experiments, processing files are labelled only with numeric non-negative integer labels. However, the processing file with label ‘1’ is reserved for acquisition of the primary data and is the default processing file.

There are many ways to open an experiment or processing file including right-clicking on the file name and selecting “Display” or left-clicking on the filename and dragging the cursor over to the right side of the screen. Basic use of Topspin is beyond the scope of this manual; readers are instead referred to Topspin Beginners Guide which provides a most useful brief explanation of the NMR hardware and the basics of the Topspin interface. The guide is included with Topspin and can be found by selecting the following menu Help > Manuals [docs] and clicking on ‘English’ under ‘Beginner’s Guides’ heading or typing docs into the Topspin command prompt and pressing ENTER.
Figure 2-1: Schematic listing the various components of the Topspin interface (top) and detail of the Topspin data browser and the various components of the Topspin data hierarchy (bottom). These screenshots were taken using Topspin 3.1 running under Linux.
2.2 Selecting Correct Probe Body (Edhead)

1. Enter the topspin command **edhead**. A warning about regarding “No PICS probe is found” usually appears and can be disregarded.

2. Ensure that the appropriate probe body is selected.
   
   a. On the 750 MHz spectrometer, the Micro 5 probe body equipped with Diff60 gradient set corresponds to the probe body configuration labelled ‘10 mm DIFF60 1H Z-GRD Z81112/2’, number 34.

   b. On the 600 MHz spectrometer, the Micro 5 probe body equipped with Diff30 gradient set corresponds to the probe body configuration labelled ‘5 mm MIC 1H XYZ-GRD Z3395/022, number 39.

3. Press either of the ‘Define as Current Probe’ or ‘Exit’ buttons.

4. A RF routing diagram (edasp) appears showing the current probe wiring (Fig. 2-1). In general, this does not need to be reconfigured during routine measurement sessions. Click ‘**close**’.

---

**Figure 2-2:** Edhead probe listing (left) and channel routing (right) in Topspin 3.2 on the AMRIS 750 MHz spectrometer.
2. Preliminary Steps

2.3 Configuring Gradient Pre-Emphasis Parameters (Setpre)

It is important to ensure that the proper sets of gradient pre-emphasis settings are loaded into the console. The following is procedure for loading gradient pre-emphasis settings using the Topspin setpre interface. Note that the setpre interface also provides many other gradient settings not just set pre-emphasis adjustments. *Incorrect pre-emphasis values can lead to poor gradient performance and measurement artifacts. In extreme cases, incorrect pre-emphasis configurations can even damage the gradient hardware.*

1. Enter the topspin command *setpre*. The SetPre window will appear with an additional menu bar item labelled ‘SetPre’ (Fig. 2-2).

2. Select the following menu item *SetPre > File > Read from...*

3. A list of saved pre-emphasis settings appears. Select an appropriate pre-emphasis file. At time of writing, the latest gradient pre-emphasis calibration (from Oct, 2013) for the 750 MHz spectrometer with Diff60 gradient set is recorded in the ‘STD_PREEMP_DIFF60’ and ‘STD_PREEMP_DIFF60_BUP’ files. Click ‘OK’.

4. The values in the file are loaded into Topspin’s memory and transferred to the gradient master unit and the console.

5. It is a good idea to define these values as default - Select the following menu item *SetPre > File > Write to ...* Select the file named ‘default’ in the file selection window and click ‘OK’. Verify that when *SetPre > File > Read default* is selected the values in the SetPre window does not change.

A detailed description of gradient pre-emphasis calibration and B_0 shift compensation can be found in sections 2.6 - 2.9 of the *Bruker Diffusion NMR User Manual* (Ver. 3 2009, pp. 15-26). It is a good practice for each user to keep their own pre-emphasis set for their use. An example set of z-channel gradient pre-emphasis parameters is presented in Table 2-1.
Figure 2-3: SetPre gradient pre-emphasis configuration window (titled ‘SetPre’) and the SetPre menu bar group.

Table 2-1: Example of Z Pre-Emphasis Settings for DIFF60 on AMRIS 750 MHz Spectrometer.

<table>
<thead>
<tr>
<th>Time Base, ms</th>
<th>Time, ms</th>
<th>Gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.</td>
<td>10.</td>
</tr>
<tr>
<td>2</td>
<td>2.</td>
<td>1.</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Max Current Setting 50 A
Coarse Offset 0
Offset % -0.93
Impedance Low
Resistors 21.2%
Capacitors 30.6%
2. Preliminary Steps

2.4 Gradient Calibration Constant (Gradpar)

In Topspin, gradient pulse intensities are expressed in terms of a percent of the maximum gradient current. However in diffusion measurements, it is important to convert these arbitrary values into physical units of the magnetic field gradient, G mm\(^{-1}\). In Topspin, this conversion factor quantity is known as the gradient calibration constant (GCC) and is dependent on the specific gradient amplifier and gradient probe set.

The GCC value is experimentally determined by comparing the apparent diffusion coefficient for a well-known sample(s) under controlled conditions to that found in literature (see Table 2.2 on page 27 of the Bruker Diffusion NMR User Manual, ver. 3). For example, a 1% water in D\(_2\)O is commonly used reference sample which has a self-diffusivity of 1.91 \(\times\) 10\(^{-9}\) m\(^2\) s\(^{-1}\) at 298 K. To calibrate the gradients, the new GCC is computed using the following relation:

\[
GCC_{\text{new}} = \sqrt{\frac{D}{D_{\text{literature}}}} \times GCC_{\text{old}}
\]

where \(GCC_{\text{old}}\) is the “old” value (i.e., the value of GCC applied during calibration experiments), \(D\) is the apparent self-diffusivity of the reference sample obtained using PFG NMR experiments using the \(GCC_{\text{old}}\) calibration, and \(D_{\text{literature}}\) is the known value from literature of self-diffusivity for the reference sample.

For the Diff60 gradient set, the GCC will be around 300 G mm\(^{-1}\)@50 A and for the Diff30 the GCC will be around 180 G mm\(^{-1}\)@60A using Bruker GREAT60 gradient amplifiers. At time of writing, the GCC for the Diff60 on the 750 MHz spectrometer was determined to be 297.35 G mm\(^{-1}\) @ 50 A and for the Diff30 on the 600 MHz spectrometer was determined to be 182.8 @ 60 A.

Before each measurement session, the value of the GCC needs to be checked. It is not expected that this value will change from session to session if the same hardware and software are used. However, if necessary, the GCC can adjusted to an appropriate value using the following procedure.

To check and adjust the gradient calibration values in Topspin 3:

1. Execute the Topspin command gradpar. The GradPar window will appear.
2. The value under ‘Gradient calib. Constant’ contains the current value of the GCC. Change the value to the desired GCC if necessary.
3. The value of the ‘Gradient scaling factor Z’ should be set to 1.0 and values of 1.0 are appropriate for X and Y as well. Note that in general there are 3 gradient channels which may have different effective GCCs and are scaled by the corresponding ‘Gradient scaling factor’ for the X, Y and Z channels. For diffusion measurements, the X and Y channels are not used.
4. Click ‘Apply’.
2.5 Field

The shim coil can produce a 0\textsuperscript{th} order field correction known in Topspin as \textit{Field} and is a value specific to the spectrometer in use and is determined and monitored by the staff at AMRIS. This field correction is designed to counteract the natural drift of the main superconducting magnet field, which is needed for locking and obtaining accurate chemical shifts values. It is good idea to check the value of field at the beginning of each new set of measurements. A good practice is to ask the AMRIS staff what the standard field value of the magnet is and use that value. The current value of the field at time of writing is around -3270.

For proton measurements, there is usually no reason to adjust the field value from the nominal value determined by the AMRIS staff. For some X nuclei samples involving very large (100ppm+) chemical shifts, e.g. $^{13}$C/$^{129}$Xe, the \textit{Field} value can be adjusted to lower the RF and receiver offset frequencies, $01/01\text{p}$.

To check and adjust the \textit{Field} value in Topspin 3:

1. Open the BSMS virtual board (execute the command \texttt{bsmsdisp} or click the \texttt{Não} icon).

2. Select the ‘Lock/Level’ tab at the top of the window. Click on the button labeled ‘Field’, it is located under the ‘LOCK’ heading.

3. The current \textit{Field} value will appear in the bottom display labeled ‘Absolute / Actual’. To change it, type the desired value into the ‘Absolute/Actual’ field and press ENTER. The value can also be changed in many other ways including the ‘Step + / - ’ buttons, the on-screen wheel and using the mouse scroll wheel.

4. Click the ‘STD BY’ button. If the value was changed, it is a good idea to click on ‘Field’ again to verify that the value entered was accepted and then ‘STD BY’ again.

5. Alternatively, the \textit{Field} value may also be set during shimming using the \texttt{setshim} interface (execute the Topspin command \texttt{setshim}). The \textit{Field} value will be towards the bottom of the shim listing.

If the \textit{Field} value is to be changed to lower the receiver/RF offset, the \textit{Field} can be changed while acquiring scans from the spectrometer in \texttt{gs} mode in the same way as optimizing the shims are performed. Please refer to the section on shimming for more information on this procedure.
3. Temperature Control

Sample temperature control is achieved by use of a carrier gas (air or N$_2$) which is heated or cooled (for sub-ambient conditions) in feedback using the temperature sensor located in the probe, near the sample. In Topspin, temperature control setup is performed using the edte interface which provides many features including temperature control, monitoring and temperature sensor correction. To open the interface, execute the topspin command edte. It is a good practice to leave this window open during measurements to monitor sample temperature and heater power during acquisition.

3.1 Operating Sample Temperature Control

In Topspin 3, there are only three routine adjustments in the edte interface: (1) temperature control on / off (2) set point temperature and (3) gas flow rate.

To turn the temperature control on or off:

- Click the ‘On’ or ‘Off’ button near the top of the window in the Temperature tab. The indicated VTU State should change to the correct state.

To change the temperature set point:

- Click the ‘Set’ button in the Target Temperature column of the Channel 1 row (labelled with the probe’s name) in the Temperature tab. A prompt window will appear. Enter the desired set point temperature and click ‘OK’.

- The temperature controller will now adjust the heater power to achieve the set point temperature. The regulation state should read “Transient” during temperature changes and “Steady” at steady state.

To change the gas flow rate:

- Click the ‘Set’ button in the Target Gas Flow column of the Probe Gas row in the Temperature tab. A prompt window will appear. Enter the desired set gas flow rate and click ‘OK’. A normal value for gas flow rate is around 400 – 800 L hr$^{-1}$, a flow rate value of 535 L hr$^{-1}$ is suggested for normal operating conditions.

Some important considerations for temperature control:

- Ensure that the temperature sensor, the heater power connector and the gas supply hose are connected to the probe before enabling temperature control! Damage may occur to the temperature control components if this is not verified.

- The time constant of temperature response for the probe body system is expected about 10 minutes. This means the probe temperature will obtain steady state at about 30-40 minutes after temperature set point changes.
• Tuning/Matching of the probe can be significantly affected due to changes in temperature.

• The gradient cooling water temperature can cause temperature induced convection inside the sample tube. This effect is pronounced in the ASTM $^{13}$C sensitivity standard sample, 40% dioxane in p-benzene-d6, even with a short sample height (< 20 mm). Convection artefacts are observed in PFG NMR experiments where the attenuation curves are diffusion time dependent with an increase in apparent self-diffusivity with increasing diffusion times. It has been observed that convection artefacts can be reduced by increasing the gas flow rate to 1070 – 1400 L hr$^{-1}$. An even more effective way to eliminate convection artefacts for room temperature measurement is to set the gradient chiller set point temperature and the sample temperature to the same value (e.g. 25 °C). For samples that are insensitive to convection effects, a gradient chiller temperature of 20 °C should be used.

• Temperatures below ambient cannot be achieved in normal operation. To achieve sample temperatures below ambient, active cooling must be used either through heating liquid nitrogen vapor or using the BCU chiller. Instructions on how to perform sample cooling is provided in Appendix C.

• A loose temperature sensor connection will cause the system to malfunction and display an erroneous sample temperature of 400°C.

• Always consult with the AMRIS staff to operate the sample temperature outside of the ambient temperature range, i.e. 20 – 30 °C. The temperature limits published by Bruker of relevant NMR equipment are provided in Table 3-1.
  
  o The typical operating range of normal (glass) RF coils with the standard plastic spinner is between about 0 to +40 °C.
  
  o The typical operating range of specialized extended-temperature range ceramic RF coils (e.g. Bruker EVT) with ceramic spinner is between about -30°C to +80°C.

• The shim system temperature should remain between 5 and 75 °C (278 and 348 K)! This temperature is usually displayed in the status bar. This temperature should be monitored occasionally and should be close to room temperature ~300 K.
Table 3-1: Temperature limits for spinners and Micro 5 probe body.

<table>
<thead>
<tr>
<th>Component</th>
<th>Temperature Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic Spinner, Blue (Z42516)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0°C to +80 °C</td>
</tr>
<tr>
<td>Kel-F Spinner, Transparent (H00177)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0°C to +120 °C</td>
</tr>
<tr>
<td>Ceramic Spinner (H00804)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-150 °C to +180 °C</td>
</tr>
<tr>
<td>Micro 5 w/ Standard RF coils (5 – 10 mm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-40 °C to +60 °C</td>
</tr>
<tr>
<td>Micro 5 Probe w/ Bruker EVT RF coils (5 mm)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-100 °C to +200 °C</td>
</tr>
</tbody>
</table>


3.2 Temperature Measurement

The indicated sample temperature seen in edte may not be an accurate indication of the sample temperature. Therefore, it is important to measure the actual temperature and calibrate the edte interface to correct the indicated temperatures. The sample temperature can be directly measured using an external temperature sensor or using the chemical shift of a temperature sensitive sample such as ethylene glycol or methanol. The former method is preferred because it represents a more direct measure of the sample temperature.

An external temperature measurement can be performed using by construction of a custom measurement apparatus – it consists of a Pt100 temperature sensor inserted into to a standard 5 mm NMR tube filled containing a sand bath of approximately with a bath height of around 40 mm. This apparatus is fitted into a sample spinner and lowered into magnet by the temperature sensor wire. To insert the apparatus into the spectrometer, follow the procedures outlined in subsection 4.1.2 for loading a sample with a guide wire and also subsection 4.1.4 to verify the proper apparatus positioning.

The NMR chemical shift dependence on temperature of sensitive samples can also be used to measure sample temperature and calibrate the linear temperature correction built into the edte interface. The procedure to conduct this measurement and calibration is provided in the Variable Temperature Control for NMR Probes User Manual, v. 2 on pp. 59.
3.3 Sample Temperature Correction

Topspin 3 can apply a 1st order correction to compensate for the offset of the indicated sample temperature and the actual sample temperature measured by the two methods listed above. When temperature correction is enabled, the sample temperature indication will display both the corrected value and the measured value, labeled ‘Corr.’ and ‘Measured value’, respectively.

To enable/disable temperature correction:

1. Execute the toppspin command edte to open the temperature control window. Switch to the correction tab.

2. Click on a correction parameter set from the list under the ‘Available correction settings’ heading. The row of the parameter set should become highlighted. Click the ‘Set’ button at the bottom of the window.

3. Check ‘Enable temperature correction with these values’. The sample temperature should now indicate the corrected and measured values. Uncheck, to switch off temperature correction.

To create/edit a temperature correction parameter set:

1. Execute the toppspin command edte to open the temperature control window. Switch to the correction tab.

2. For a new parameter set, click the ‘New’ button towards the bottom of the window. To ‘Edit’ and existing parameter set, click on the correction parameter set from the list under the ‘Available correction settings’ heading and click the ‘Edit’ button. The temperature correction dialog will appear.

3. The values of the ‘Slopes’ and ‘Offsets’ can be calculated using linear-regression from measured data points (in Kelvin) (using a spreadsheet for example) or they can be calculated using two data points with Topspin.

4. To let Topspin calculate the offset and slope click the ‘Calculate’ button. Another dialog appears. Enter in two sample and target temperatures in units of K in the correction range. Click OK to close the slope/offset calculation dialog. The values are computed and placed into the Slope and Offset fields.

5. Click OK to save/update the parameter set.

At time of writing, the Diff60 on the 750 MHz spectrometer was calibrated with a slope and offset of 1.200447 and -58.92239, respectively, between a temperature range of 270 and 313 K.
4. NMR Measurement

4.1 Introducing the Sample

There are two methods which are typically used to insert samples into the magnet bore for NMR spectroscopy: (1) using the sample lift air system and (2) using a fishing line attached to the sample. Before samples are lowered into the bore, the samples need to be inserted into a spinner and adjusted to an appropriate height such that the amount of sample is maximized in the narrow RF irradiation region. Figure 4-1 presents many key points about sample positioning using the sample positioning gauges.

*Always use a sample positioning gauge when inserting samples into the magnet! Ask the AMRIS staff to provide one for use during the measurement session if one cannot be located.

4.1.1 Loading Samples using Lift Air

Procedure to load samples into the magnet using the lift air:

1. Stop any running acquisition on the spectrometer (execute the stop command as needed)

2. Check the top of the magnet bore for a cap, remove if in place.

3. Activate the sample lift air by toggling the LIFT button on the BSMS virtual board (type bsmsdisp or click the icon ). Alternatively, the ej command can be used. A loud noise of flowing air should begin.

4. Wait about 30 seconds and check the top of the magnet bore for a sample. *Always check for samples in the bore prior to sample loading; never assume no sample is inserted! If a sample is present it will float to the top of the bore on a cushion of flowing air. Carefully lift the floating sample out of the bore, remove it from its spinner and set aside in an appropriate rack or container. *If the sample contains or is suspected to contain pressurized gas, be sure to wear a face shield!

5. Place the spinner into the sample position gauge (depicted in Fig. 4-1). Carefully, guide the sample tube through the spinner by grasping the sample tube close to the upper surface of the spinner. The fit is very tight and sometimes is helped by wiping the interior O-ring surface and/or providing a small amount of clean water to lubricate the O-ring. In addition, a twisting motion can also aid in moving a stuck sample tube. *Avoid applying large forces, do not force it. Avoid applying force to the sample tube far away from the spinner as this may stress the tube and cause rupture. Be careful when handling the sample tube as the upper end is usually sharp.

6. Align the position of the sample in the spinner such that the NMR sample is maximized in the black shaded region marked '5/8 mm' on the sample positioning gauge (see Fig. 4-1) on the 'Receiving Coils' side. This shaded region corresponds to the approximate region of RF irradiation. It is a good practice to wipe the bottom of the sample tube to help remove finger prints after positioning.
7. With the lift air activated, carefully align and lower the sample into the magnet bore and release it. The sample will float on the air stream and will bob up and down. *Verify that a flow of air is present inside the magnet bore before releasing the sample!

8. Deactivate the sample lift air by toggling the $\text{LIFT}$ button on the BSMS virtual board (type $\text{bsmsdisp}$ or click the icon ). Alternatively, the $\text{ij}$ command can be used. The loud noise of flowing air should cease and the sample will slowly lower itself into the probe body. Usually, an audible click will be heard when the sample lowers into position.

9. Place the cap onto the top of magnet bore (if applicable).

4.1.2 Loading Samples using Fishing Line

Procedure to load samples into the magnet using fishing line:

1. Follow Steps 1 – 4 in the above procedure to load a sample using the air lift. Deactivate the air lift after removing any previous sample in the magnet bore (follow step 8 in above procedure).

2. Tie the fishing line closely around the sample tube and secure using a double knot. *Ensure the fishing line is non-magnetic. The fishing line tie should form a ring which can slide up and down the length of the sample tube with some resistance. The fishing line ring is held in place partially by a sample label which usually consists of paper/tape wrapped around the sample tube near the top. If there is no sample label on the sample, wrap a small length (two or three turns is sufficient) of low-
adhesive tape, such as blue masking tape, around the top of the sample tube, approximately 1 – 2” from the top of the sample tube.

3. Bring the fishing line ring to the bottom surface of the sample label or tape wrap near the top of the sample.

4. Place the spinner into the sample position gauge (see Fig. 4-1). Carefully guide the sample tube through the spinner by grasping the sample tube close to the upper surface of the spinner. The fit is very tight and sometimes is helped by wiping the interior O-ring surface and/or providing a small amount of clean water to lubricate the O-ring. In addition, a twisting motion can also aid in moving a stuck sample tube. *Avoid applying large forces, do not force it. Avoid applying force to the sample tube far away from the spinner as this may stress the tube and cause rupture. Be careful when handling the sample tube as the upper end is usually sharp.

5. Align the position of the sample in the spinner such that the NMR sample is maximized in the black shaded region marked '5/8 mm' on the sample positioning gauge (see Fig. 4-1) on the 'Receiving Coils' side. This shaded region is corresponds to the approximate region of RF irradiation.

6. While the fishing line ring is mated to the bottom surface of the sample label or tape wrap, wrap a small length of low-adhesive tape around the fishing line ring. *Verify the fishing line connection by gently tugging on the fishing line to ensure that the ring does not move.

7. Re-check the sample position with the black shaded region marked '5/8 mm'. It is a good practice to wipe the bottom of the sample tube to help remove finger prints after positioning.

8. The lift air should be deactivated. Carefully align and lower the sample into the magnet bore. Hold the spool just above the magnet bore and slowly unspool it to lower it into the magnet bore until the line becomes slack. It is best to keep the fishing line vertical / parallel with the magnet bore so that the sample lowers straight.

9. When the fishing line becomes slack, raise the fishing line a small distance and release the line two or three times. This helps ensure that the sample is seated properly in the probe. Usually, an audible click will be heard when the sample lowers into position.

10. Follow the procedure in subsection 4.1.4 to check the sample position.

11. Place the cap onto the top of magnet bore (if applicable).
4.1.3 Removing Samples using Fishing Line

To remove samples loaded with the fishing line is straightforward and follows along the same line as loading. Ensure that the sample is removed so that the fishing line is parallel with the magnet bore. *Spool excess fishing line length to prevent the fishing line from becoming a tripping hazard or caught on other surfaces.* The tape that holds the fishing line ring in place can be difficult to remove as it is stuck together. If it is hard to remove, it is best to remove the sample tube from the spinner and carefully work to remove it. In this way, there is no stress being applied to the tube from the O-ring in the spinner.

4.1.4 Checking Sample Position

The sample position can be verified using a custom flexible Teflon rod on the 750 MHz spectrometer; it is usually located on the top of the spectrometer. The Teflon rod contains a red line calibrated to the position of a properly inserted sample. Insert the long end of the rod into the magnet bore and lower as far as possible. The red line will coincide with the top of the magnet bore if the sample is located in the probe properly. If the red line does not coincide with the top of the magnet bore, the rod may be used to gently tap on the top of the sample so that it seats into the magnet. However, it may be necessary to repeat the sample loading procedure.
4. NMR Measurement

4.2 Tuning and Matching

Tuning and matching is the process of optimizing the electrical characteristics of the probe body to (1) operate the NMR resonance frequency of the sample (tuning) and (2) minimize electrical impedance (matching). The result of these two optimizations is that it maximizes the RF absorption of the sample. This is accomplished by changing the capacitance of two adjustable capacitors on the probe body. Tuning and matching should be performed any time the sample is changed and when the temperature is changed.

To tune and match the probe to the sample:

1. Stop any running acquisition using the stop command. Open any experiment that contains the correct spectrometer configuration (e.g. correct nuclei, probe body, spectrometer frequency) and execute the command wobb (short for wobble). The spectrometer should begin acquisition and the wobb window appears. The main plot of the window is RF frequency plotted on the horizontal axis and a measure of impedance (in arbitrary units) on the vertical axis. A vertical red line is also plotted which represents the spectrometer’s operating frequency (SFO1). Note the same display also appears on the pre-amplifier LCD screen during wobb.

2. Turn computer screen and zoom in on the signal (if necessary) so that it can be seen from someone positioned sitting under the magnet. If an minimum is not visible in the wobb window (Fig. 4-2), a larger sweep width may be necessary - click the icon in the toolbar and increase the sweep width value. *It may be necessary to use a network analyzer to tune the magnet to the proper nuclei frequency. In that case, remove the probe and connect the coaxial cable to the network analyzer.

3. The tuning and matching capacitors are located on the underside of the probe body, labeled ‘T’ and ‘M’ respectively. Capacitances are adjusted by rotating inwards and outwards. Since the probe body contains two independent channels, make sure to only adjust the rods that are colored for the correct channel. The $^1$H/$^9$F channel is colored in yellow while the X channel ($^{13}$C, $^{129}$Xe…) is colored in blue.

4. The goal of this process is to obtain the lowest RF absorption peak which is centered around the red line marker (the spectrometer’s frequency). Figure 4-2 presents an example of the wobb window for a well-tuned/matched condition.

5. Tuning and matching may be difficult on the initial probe setup and large changes in capacitor position may be needed. For coarse adjustments, rotate the tuning capacitor to adjust the horizontal position of the RF absorption and rotate the matching capacitor to adjust the vertical position of the RF absorption. For finer adjustments, changing either capacitor will affect both the tuning and the matching. Therefore, the tuning and matching rods need to be changed alternately.

6. Once the probe is tuned and matched, press (or execute) “stop.”
*If necessary, a 50 Ω can be used to account for the resistance in the coaxial cable. When using the 50 Ω resistor, the command `wobb ext50` should be used instead of `wobb`. The cable is first connected to the resistor and the software will measure the affect of the resistance of the cable on tuning and matching. A message will pop up indicating that the reference data has been taken. Press “Ok” and connect the coaxial cable to the probe body. Press “Ok” to indicate that the cable is connected and the `wobb` sequence will initiate. Once the `wobb` sequence is active, the software will then be able to distinguish that effect from the true resistance.

Figure 4-2: `wobb` window depicting a well-tuned/matched sample on the AMRIS 750 MHz spectrometer running Topspin 3.2 running under Linux.
4.3 Shimming

In many cases, there is a standard parameter set and experiment used for shimming protocols. The shims are often stored for each user and each type of sample they measure. Shimming procedures can be found in 6.8 of the Avance Beginners Guide manual. Below is a list of useful commands for the shimming process.

- **Rsh** – This command reads shims from a list of saved parameter sets. Type `rsh`, select the desired shim set, and press “Ok.” The message “rsh:finished” will appear at the bottom of the screen when the operation is complete.

- **Wsh** – After shimming is complete, it is necessary to save the new shims. Type `wsh`, give a name for the new shim set, and press “Write.”

- **Setshim** – Sometimes users prefer to type in the values of each shim direction rather than scrolling to it using the knob on the BSMS keyboard. This command recalls a complete list of the current shims and allows one to type in values.
4.4 Copying Datasets (new)
In Topspin, experiments are rarely created from scratch. Instead, experiments are duplicated using the new command and then specific acquisition parameters are customized for the sample/experiment.

Procedure to copy an existing dataset:

1. Open an existing dataset to be duplicated. It is usually best to pick an existing dataset for a sample that is most related to the desired sample of investigation and acquired using the same resonance. If this is a new sample, it is recommended to copy a ZG experiment and optimize the acquisition parameters first.

2. Execute the new command. Topspin displays a dialog (Fig. 4-3) which is used to create a copy of the open dataset.

3. Enter or verify the name of the new experiment set (NAME). Enter a new (unused) experiment number (EXPNO).

4. The processing number (PROCNO) for acquisition should always be set to 1.

5. The large textbox labeled ‘TITLE’ is a convenient space for experimental notes and description. Enter any notes for later review.

6. In Topspin 3, it is important to make sure that the option ‘Use current parameters’ is selected. Ensure all other options in the ‘Options’ heading are unchecked or unexpected parameters may be loaded by default.

7. Click ‘OK’.

Figure 4-3: new window in Topspin 3.2 running under Linux.
4. NMR Measurement

4.5 Common Acquisition Parameters

Acquisition parameters are used to instruct the console to configure the spectrometer to perform an acquisition for the desired pulse program. Common (and important) acquisition parameters and their explanations are presented in this section. Refer to the Topspin Acquisition Commands and Parameters document for more information.

Acquisition parameters are listed in the ‘AcquPars’ tab of the main Topspin experiment window. An abbreviated list of pulse program relevant parameters can be toggled to/from by clicking the \[\boxed{}\] and \[\text{A}\] buttons, respectively. In addition, acquisition parameters can be viewed and changed by typing their name into the Topspin command line and pressing Enter. *Most acquisition parameters are sample-specific and need to be experimentally optimized / determined. These values need to be determined to be experimentally optimized/determined.

1. **PULSEPROG** – the name of the pulse program to perform the experiment.

2. **NS** – the number of scans (i.e., the count of pulse sequence acquisitions run to obtain the NMR signal). Larger numbers of scans contribute to a larger signal-to-noise ratio and larger total experiment times. It is important to ensure that NS is an integer multiple of the length of the phase cycle for the particular sequence. The phase cycle is part of the pulse program and can be viewed in “PulseProg” tab, it appears as a sequence of integers near the bottom of the pulse program file. For example:

   ph1= 0 0 0 2 2 2 2 1 1 1 3 3 3 3 3
   ph2= 1 3 0 2
   ph3= 1 3 0 2
   ph31=0 0 2 2 2 2 0 0 3 3 1 1 1 1 3 3

   is an example of a phase cycle found near the bottom of a pulse program. In the example phase cycle, the phase cycle length is 16 scans and therefore NS for that pulse program must be an integer multiple of 16.

3. **SWH/DW** – the spectral width (SWH) is the width (in Hz) of the spectral window which is determined by the sampling rate. DW is the dwell time which is the time period between two successive data points recorded by the software. For the fixed number of data points (TD) acquired by the spectrometer the following rule applies: the larger is the DW, the smaller is the SW:

   \[ SWH = SW \times SF01 = \frac{1}{2DW} \]
4. AQ – acquisition time of the experiment. AQ should be chosen such that the perceivable NMR signal spans about $\frac{2}{3} - \frac{3}{4}$ of the FID (or baseline noise occupies the rightmost $\frac{1}{4} - \frac{1}{3}$ of the FID) for an optimized spectral signal-to-noise ratio and productivity (see Fig. 4-5). The FID can be viewed after acquisition by selecting the ‘FID’ tab in the main window. Note that if AQ is too short, not all of the FID will be recorded – leading to loss of signal to noise and appearance of signal artefact in the spectrum where the true spectrum is modulated by a sinc function (see Fig. 4-5). If the AQ is too long, the experimental acquisition time will be un-necessarily increased.

5. TD - the number of data points collected during acquisition. It is directly related to AQ and DW and the above remarks also hold for TD:

$$AQ = TD \times DW$$

6. D1 – relaxation delay between scans to allow magnetization to return to equilibrium before performing another scan. For experiments, the delay should be 3-5 times larger than the $T_1$ relaxation time of the sample. A coarse method to determine a reasonable value of D1 is discussed in a later section. In addition, the determination of $T_1$ NMR relaxation times using the inversion-recovery pulse sequence is also described later in the manual which can be used to more precisely set D1.

7. P1/PLW1 – P1 is the RF pulse duration that is required to rotate magnetization 90°. The PLW1 value is the power level for the RF pulse and is usually set to 30-45 W. *Always consult with AMRIS staff regarding power levels before increasing PLW1 from the above values.* PLW1 and P1 are inversely related and it is typically desirable to have a short P1 to produce a broadband of RF excitation frequencies. However, excessively large values of PLW1 can lead to probe arcing (a passage of a spark) which can damage the probe. P1 is experimentally determined by performing an optimization experiment but should be around 10 – 20 µs depending on the power level used and the RF coil in use. The optimization procedure is described in an upcoming section.

8. O1/O1P – offset frequency (for channel 1) that fine-tunes the RF and receiver reference frequencies relative to the Larmor frequency of the main magnetic field (BF1). BF1 is the basic transmitter frequency for channel 1 in Hertz. This frequency is set automatically for each particular nucleus. The offset frequency is specified in units of Hz for O1 and ppm for O1P. O1/O1P allows the RF irradiation to be in or close to resonance of the sample. In Topspin, this is done in the ‘Spectrum’ Tab by selecting the ⇪ button on the main toolbar and then selecting where the RF frequency should be placed.

9. SFO1 – The transmitter frequency. This is the frequency used to excite the observed nucleus in channel 1 (SFO1 = BF1 + O1). SFO1 frequency will be at the center of the spectrum and can be viewed as the central frequency in the window through which the spectrum is observed.
4. NMR Measurement

10. \( \text{RG} \) – the receiver gain used to acquire the signal. \( \text{RG} \) can be automatically determined after all other parameters have been set by typing \texttt{rga}.

![Diagram of NMR Measurement Parameters]

**Figure 4-4:** Schematic illustration depicting relationship of acquisition parameters, \( AQ/TD, DW, SW/SWH, \) and \( SI \).

**Figure 4-5:** Schematic illustration of a FID signal (a) and its corresponding spectrum (b) for a correctly sized acquisition time \( (AQ) \). For comparison, a FID signal (c) and its corresponding spectrum (d) for a truncated FID, an example of an acquisition time \( (AQ) \) that is too short.
4.6 Processing Parameters

For most 1D pulse sequences, processing the data typically consists of three parts (i) converting the FID into a spectrum (ii) phase correcting the spectrum and (iii) baseline correcting the spectrum.

4.6.1 Fourier Transform

A Fourier transform converts the acquired FID into a frequency-domain spectrum consisting of \(SI\) spectral points, which span a frequency range of \(SW/\text{SWH}\). \(SI\) is a processing parameter which can only be set to a power of 2. \(SI\) should typically be set to at least the power of 2 larger than \(TD\); for example, if \(TD = 10,000\) then \(SI = 16,384\).

There are two common Topspin commands used for Fourier transform, \(ft\) and \(ef\). The command \(ft\) performs a fast Fourier transform on the FID data. The command \(ef\) is a compound command which first multiplies the FID data by a decaying exponential function and then performs a fast Fourier transform on the resulting function. The purpose of exponential function is used to reduce the contribution of baseline noise to the spectrum, resulting in a higher effective signal to noise ratio. The decay rate of this decaying exponential function is specified using the processing parameter \(LB\) (short for Lorentzian broadening or Line-broadening factor). Care must be used to determine \(LB\) as it can artificially broaden narrow lines. A good rule of thumb is to measure the half maximum line width of the narrowest line and divide by 5 to 10.

4.6.2 Phase Correction

The receiver and the signal represented by the FID are usually misaligned in phase with each other during an experiment. Phase correction compensates for this shift and rotates magnetization between the real and imaginary channels to produce a Lorentzian, adsorption line shape. There are two phase correction terms: a 0\(^{th}\) order correction used to correct the phase shift of on-resonance peaks and 1\(^{st}\) order correction used to correct the phase shift of off-resonance peaks. These correction terms are stored in the processing parameters as \(PHC0\) and \(PHC1\). The command \(ph\) displays an informative window of many phasing options. The command \(pk\) performs a phase correction using the saved \(PHC0\) and \(PHC1\) parameters.

There is also an alternative to phasing that simply calculates the magnitude of the complex value at each spectral point, removing all phase information. This magnitude spectrum is obtained by running the \(mc\) command after Fourier transform. There is also a phase correction mode processing parameters in Topspin known as \(PH\_mod\) that instructs Topspin on phasing intent. In most cases, the \(PH\_mod\) for the spectral dimension should have a value of \(pk\).
4. NMR Measurement

The commands **apk** and **apk0** perform automatic phase correction using 0\(^{\text{th}}\) and 1\(^{\text{st}}\) order phase terms and 0\(^{\text{th}}\) order only terms, respectively. When **apk/apk0** are run, Topspin automatically attempts to determine the optimum values of PHC0 and PHC1 to produce a pure adsorption spectrum. Any old values of these parameters are overwritten. It should be noted that when baseline distortions are present, **apk** may produce 1\(^{\text{st}}\) order phase corrections that can artificially distort the spectrum. In that case either manual phase correction or apk0 should be used.

Finally, the phase can be adjusted manually using the following procedure:

1. Perform a Fourier transform of the FID, ef or ft.

2. Click on the interactive phase correction button, \(\bigtriangledown\), on the toolbar or run the command .ph.

3. Zoom in on the main peak or on a peak (if there are multiple). Near the center of the selected peak right click and select ‘Set Pivot Point’ from the context menu. A red vertical line should appear near the center of the selected peak.

4. Click and hold the zeroth order button, \(\underline{0}\), and use the mouse to correct the phase (move the mouse forward and backwards) to adjust the phase on the selected peak. A good phase produces a symmetric, smooth peak with an even and flat baseline.
5. If there are more than one peak, 1st order phase correction may be needed. Zoom out to see all the peaks; look at the furthest peak from the red line (it may be helpful to zoom in on the far peak). Like in step 4, click and hold the 1st order button, \( 1 \), and use the mouse to correct the phase (move the mouse forward and backwards) to make the furthest peak from the red line appear symmetric and smooth with an even and flat baseline.

6. Click the save and return button to save the phase. *If doing phase correction on a spectrum which is part of a 2D dataset, click on the button to save these phase correction parameters to the parent 2D file for 2D Fourier transformation and for subsequent slices.*

![Diagram showing phase correction](image.png)
4. NMR Measurement

4.6.3 Baseline Correction

Baseline correction is used to fix minor baseline distortions of the NMR spectrum when it is not flat and not centered at zero. It is important to have reasonably flat and zero centered baselines for accurate integrals. In Topspin, baseline correction is performed by fitting the distorted baseline with a polynomial function and then subtracting the fitted polynomial from the original spectrum, yielding an improved baseline.

The command **abs** performs an automatic baseline correction using the polynomial method. The following three processing parameters are used by **abs**: (i) **ABSF1** the left spectral limit for the baseline correction, (ii) **ABSF2** the right spectral limit for the baseline correction and (iii) **ABSG** is the order of the polynomial to fit to the distorted baseline. **ABSF1** and **ABSF2** can usually be set to 1000 and -1000, respectively, which corrects for the entire spectrum. For uncharacteristic distortion near the edges of the spectrum, **ABSF1** and **ABSF2** should be chosen such that the distortion is not in the baseline range. For spectra with relatively flat baselines, **ABSG** of 1 (or even 0) is usually sufficient and prevents the software from using unnecessary corrections. For noticeably distorted baselines, **ABSG** of 5 should be used as it allows Topspin as many degrees of freedom as possible to correct for a curved baseline.

Baseline correction can also be performed manually using the command **bas** and selecting manual integration. This feature is rarely used and the toolbar short cut appears to have been removed. The interface is similar to interactive phase correction; coefficients of the polynomial function (red line) are selected and changed by clicking the appropriate coefficient (A-E) and moving the mouse forward and backward. The goal is to match the generated polynomial function to the NMR spectrum.
4.7 The ZG Experiment

The command zg is both the name of a pulse sequence and a software command. The command zg instructs the spectrometer to perform acquisition on the current dataset. *Executing zg on a dataset that contains existing NMR data will be overwritten!* The pulse sequence entitled ZG is the one pulse sequence in which the magnetization is excited by a single RF pulse (typically, a 90° pulse) and the signal is acquired. Fig. 4-6 presents a schematic pulse program of the zg pulse sequence.

Examples of experimental acquisition parameters of the zg pulse sequence are shown in Table 4-1. Often parameters need to be optimized for the sample such as D1, the delay between scans, and P1 (the duration of RF pulse required to rotate magnetization \( \frac{\pi}{2} \) radians or 90° at a given transmitter power). The optimization of D1 and P1 is discussed in following sections. The signal obtained from a zg is a simple NMR spectrum of the sample, which can be useful to characterize the sample. The zg spectrum also has diagnostic utility; it can be used to check that the hardware/software is functioning properly and consistently, that shimming is optimized, and the quality of the sample is sufficient and unchanged during different measurement sessions. It is customary to do zg experiments on samples with identical acquisition parameters before and throughout each measurement session to check for consistency.

Figure 4-6: Pulse program diagram of the zg pulse program.

**Procedure to acquire a zg spectrum:**

1. Make a copy of an existing ZG sequence by using the new command. (See Section 4.4 for more information).

2. Review the common acquisition parameters outlined in Section 4.5 and modify as needed. Make sure NS is a multiple of 8 (due to the phase cycle of the zg pulse sequence).

3. Obtain an appropriate receiver gain by running the command rga (esp. on \(^1\)H).

4. Begin acquisition by running the command zg. The spectrometer will begin acquisition as indicated by a flashing FID icon in the status bar. The progress of the acquisition can be seen in real time by selecting the ‘Acqu’ tab in the running experiment’s window.
5. When the acquisition is complete, the acquired FID will appear in the ‘FID’ tab and the status bar will display ‘no acquisition running’. The acquired data can now be processed.

**Procedure to process the acquired data:**

1. Fourier Transform - Run the command `ef` to produce a spectrum. The value of LB can be checked if reasonable by observing that a spectrum produced by `ef` and `ft` have similar line widths. (See Section 4.6.1 for more information).

2. Phase Correction – run the command `pk` or `apk` to perform phase correction (See Section 4.6.2 for more information).

3. Baseline Correction – run the command `abs` to perform baseline correction (See Section 4.6.3 for more information).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Example Parameter Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULPROG</td>
<td>Pulse sequence used for experiment</td>
<td>ZG</td>
</tr>
<tr>
<td>NS</td>
<td>Number of scans, a multiple of 8</td>
<td>8</td>
</tr>
<tr>
<td>D1</td>
<td>Relaxation delay</td>
<td>1.00 s</td>
</tr>
<tr>
<td>PLW1</td>
<td>RF power level, in watts</td>
<td>25 W</td>
</tr>
<tr>
<td>P1</td>
<td>90° RF pulse duration</td>
<td>11.25 μs</td>
</tr>
<tr>
<td>AQ</td>
<td>Acquisition time</td>
<td>1.00 s</td>
</tr>
<tr>
<td>DW</td>
<td>Acquisition dwell time</td>
<td>55.5 μs</td>
</tr>
<tr>
<td>TD</td>
<td>Number of time domain points</td>
<td>18026</td>
</tr>
<tr>
<td>SWH</td>
<td>Sweep width</td>
<td>9.01 kHz</td>
</tr>
<tr>
<td>O1</td>
<td>Receiver Offset</td>
<td>4.50 kHz</td>
</tr>
<tr>
<td>RG</td>
<td>Receiver Gain</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 1-1. Example of acquisition parameters for zg pulse sequence.
4.8 Relaxation Delay (D1) Estimate
4. NMR Measurement

4.9 P1 Determination

To determine the duration of RF pulse required to rotate magnetization $\frac{\pi}{2}$ radians or 90° the Topspin command `popt` is used. The `popt` program is a general purpose parameter optimization tool that can optimize a number of acquisition parameters. The program conducts a series of experiments varying the specified parameter(s) in a specified range(s) for a chosen 1-D pulse sequence (typically zg). The spectrum of each experiment of the series is plotted in the same axis, providing a graphical method to observe the influence of the variable parameter(s) on the acquired signal.

In the case of P1 determination, the RF pulse length, $P1$, is the parameter to be varied over a broad range and the resulting signal acquired from zg is plotted. This experiment is phase-sensitive and as a result, care must be taken when setting the phase correction parameters.

Experimental Procedures

1. Tune and match the probe.

2. Open a zg data set with appropriate parameters for the given sample. Make a copy of the data set using the `new` command.

3. If the 90° pulse length is known approximately, set $P1$ to that approximate value. Otherwise, a $P1$ value of 10 µs may be used. *Ensure PLW1 is set to the desired power level. The relaxation delay, $D1$, should be set appropriately; a short $D1$ will cause incorrect apparent pulse behavior.*

4. Run `rga` and acquire the spectrum using zg. Process the data set as normal with phase and baseline correction. Ensure that PH_mod is set to ‘pk’.

5. Select a strong peak (a singlet is desirable) from the spectrum. Place the RF frequency in resonance with the selected peak using the button on the main toolbar. This will set the value of SFO1 and O1.

6. Re-acquire the spectrum using zg. Re-process the data set as normal with phase and baseline correction. Save the phase by clicking the button and then clicking the save and return button.

7. In the spectrum window, select a narrow window of spectrum where the selected strong peak is. Be sure to include baseline on both the left and right sides of the strong peak. Save this window for display by typing the `dp11` command.

8. Run the command `popt` to load the optimization program, a new window appears (Fig. 4-7) which is used to configure a parameter optimization experiment.
9. For this experiment, the RF pulse length, $P_1$ will be varied. Type ‘$P_1$’ into the field labeled ‘PARAMETER’ and press ENTER.

10. The range of $P_1$ values of observation need to be specified in the ‘STARTVAL’ and ‘ENDVAL’ fields. In addition, either the number of observations or the increment size needs to be specified in the ‘NEXP’ and ‘INC’ fields, respectively. A desirable optimization range of $P_1$ is chosen such that the resulting RF rotation at the minimum pulse length is much smaller than 90° and larger than 360° at the maximum pulse length (Fig. 4-8). Typically, a $P_1$ range between 5 µs and 90 µs with an increment of 5 µs produces satisfactory results.

11. Click the ‘Start optimize’ button to begin the $P_1$ optimization experiment sequence. Typically, dialogs specifying the total run time of the experiment and if a protocol should be overwritten or appended are displayed, which should be accepted/confirmed.

12. The popt program will iterate through the specified $P_1$ range, recording the dp11 window of the resulting spectrum in a single PROCID (999 by default). In the process, many windows will appear and disappear automatically. *It is not recommended to try to run any other commands or open other windows while the optimization process is running.

13. Once the iteration sequence is completed, the result (a composite spectrum) can be viewed by selecting the ‘Spectrum’ tab and viewing the extents (click the $\mathbb{Q}$ button). In most cases, the popt program will automatically display the result, but will appear as the processing file 999 in the experiment. *Warning: Do not attempt to run ef or ft on the composite spectrum as this will cause an irreversible loss of data! For small phase problems, manual phase correction can be used.

14. The resulting composite spectrum displays the Fourier transformed and phase corrected NMR signal as a function of the RF pulse length ($P_1$) on the horizontal axis. The signal dependence on $P_1$ should be sinusoidal (see Fig. 4-8). For a sinusoidal dependence, the 90 degree pulse length is taken to be half the location of the first zero or one quarter of the location of the second zero. The latter value is most commonly used; however both methods produce adequate results and should yield the same value. The location of a zero can be linearly interpolated between the closest pair of adjacent positive and negative peaks. *If the dependence is not sinusoidal it is possible that the acquisition parameters (esp. PLW1 and D1) are not appropriate, the tuning/matching is poor or there is a hardware problem.

15. Steps 9 – 14 may be repeated in a region close to the 1st or 2nd zero to try to increase the precision of the determined 90° pulse length. It is a good practice to change (rename) the processing ID from 999 to a new value to save the current optimization result before re-running popt.
4. NMR Measurement

Figure 4-7: Example of the `popt` configuration window running in Topspin 3.1 running under Linux.

![P1 Determination](image)

Figure 4-8: Example of a P1 optimization experiment – a plot of acquired NMR signal as a function of the RF pulse length, shown along the horizontal time scale (in μs). Shown also is the location of the 1st zero, the 2nd zero and the position of the corresponding 90° pulse length. This plot was obtained for a sample contained 40% \( p \)-dioxane in benzene-d\( _6 \) using \( ^{13} \)C resonance on the AMRIS 600 MHz spectrometer.
4.10 Determination of T1 NMR Relaxation Times

The inversion recovery sequence (t1ir) is a commonly used pulse sequence used to observe the $T_1$ NMR relaxation time(s) of a sample. The pulse sequence consists of a 180° RF pulse, a variable delay, and a 90° RF pulse and is presented schematically in Fig. 4-9. The 180° RF pulse inverts populations from equilibrium along the +z to the –z direction. The subsequent delay ($\tau$) allows the magnetization to equilibrate towards the +z direction by $T_1$ NMR relaxation. Finally, the resulting magnetization in the longitudinal axis is rotated into the transverse plane and acquired.

$$t1ir$$

![Diagram of t1ir pulse program](image)

By changing the delay and observing the NMR signal dependence on the delay, the $T_1$ relaxation time(s) can be determined. This experiment is phase-sensitive; therefore, magnetization along the +z and –z will obtain phases that are 180 degrees out of phase with each other. In some samples, there may be a distribution of $T_1$ relaxation times. The dependence of longitudinal magnetization, $M_z(\tau)$, on relaxation delay, $\tau$, can be described (ideally) by the following relation:

$$M_z(\tau) = M_{eq} \left( 1 - 2 \sum_{i=1} p_i \exp \left( -\frac{\tau}{T_{1,i}} \right) \right)$$

where $M_{eq}$ is the magnetization at equilibrium, $i$ is an index of relaxation ensembles, $p_i$ is the ensemble fraction of relaxation ensemble $i$ and $T_{1,i}$ is the $T_1$ NMR relaxation time constant for ensemble $i$. NMR relaxation occurs during the $\tau$ time period which causes attenuation of the signal measured at the end of the sequence.

Experimental Procedures

i. Run a simple “zg” experiment.

ii. Process the FID (Fourier transform, phase correction, baseline correction) Select the line (peak) on interested and expand the window to include only that line.
4.11 Determination of T2 NMR Relaxation Times

iii. Type **dp11** to set the range of frequency for observation.

iv. Go back to the acquisition window.

v. Change the pulse sequence to “t1ir1d.”

vi. Type **popt**.

7.

A window to optimize will open. The PARAMETER of interest to change is D7 which corresponds to τ in the sequence. It is good to know a general range in which you expect to see your signal invert from negative phase to positive phase. If this is not known, select a wide range such as 0.1 to 1 second. Input 0.1 as your STARTVAL and 1.0 as your ENDVAL. NEXP is the number of experiments. INC is the increment of D7 between each experiment. To be sure, it is good practice to set the NEXP value to 2 so that only two experiments run initially to check that the phase of your signal inverts during the time interval that you have selected. After the proper range for signal inversion has been verified, indicate either NEXP or INC to determine how many experiments are performed over that range. If it is important to have a precise value for your $T_1$, narrow the range for D7 a general idea of the real value is obtained. Once all optimization parameters are set, click on “Start Optimize” to begin. The program will run several times based on the number of increments indicated. The value of D7 at which the signal is zero ($t_{inv}$) and the $T_1$ relaxation of the line under observation are related by

$$T_1 = \frac{t_{inv}}{\ln 2}$$

8. $T_1 = \frac{t_{inv}}{\ln 2}$

**4.11 Determination of T2 NMR Relaxation Times**

The CPMG pulse sequence is a 2D pulse sequence used to measure $T_2$ NMR relaxation times. CPMG is short for its original authors: Carr, Purcell, Meiboom and Gill. The pulse sequence consists of a 90° RF pulse followed by a train of 180° RF pulses and then the signal is acquired. The first pulse rotates magnetization from equilibrium along z into the transverse plane. While magnetization is in the transverse plane, it experiences signal decay due to $T_2$ NMR relaxation, and also decays due to small variations in the magnetic field and molecular translation through the non-uniform magnetic field. The latter two signal decay mechanisms can be compensated for in the CPMG pulse sequence through the train of 180° RF pulses – they generate a series of spin echoes.

The CPMG pulse sequence uses a list file known as ‘**VCLIST**’ (variable counter list). The VCLIST consists of a set of integers (one on each line) which determines the number of 180° RF pulses to generate in the pulse train (see Fig. 4). By changing the length of the pulse train, the sequence extends the time period in which $T_2$ NMR relaxation affects the signal while compensating for other decay effects. The reduction of signal due to relaxation can then be measured as a function of $t = 2n\tau$, where $n$
is the number of vc loops. The relationship between this reduction of signal or attenuation (Ψ), τ, and the $T_2$ NMR relaxation time ($T_2$)

$$\Psi \propto \exp\left(-\frac{2n\tau}{T_2}\right),$$

where $n = 2, 4, 6, 8, 10, \ldots, m$. The values of “n” are defined by the “vclist” of the program. An exponential fit of the attenuation data as a function of τ will yield the $T_2$ NMR relaxation time for the sample. The values of D20 should be determined such there is both sufficient attenuation over the course of the 2D experiment. At the same time, D20 should be short enough to prevent all signal from attenuating before the end of the 2D sequence.

**Figure 1. Schematic of cpmg pulse sequence.**

**Experimental Procedures**

i. Create a 2D cpmg dataset with reasonable acquisition parameters for the sample of interest. *In order to use this sequence, you must change the dataset from 1D to 2D.*

ii. Input a reasonable value for D20, check/modify the values of “n” in the “vclist”, insert the value of “m/2” (see above) as TD for “F2”.

iii. Type **zg** to run the experiment.

iv. Processing is done using T1/T2 relaxation software. This process is described later in the manual. As a “fitting function” use “exp dec”. As a “list file name” use “vclist”. Please note that the units on the horizontal axis of the resulting plot are not seconds. The values on this axis are given in terms of “n”.

40
5. PFG NMR Diffusion Experiments

Diffusion measured can be conducted using two different software packages: dosy and diff. Description of the dosy software will not be discussed in this manual. For information about diffusion experiments using the dosy software, refer to the Bruker dosy manual available online. The diff package includes several sequences commonly used in PFG NMR. In this section, each sequence will be described in brief detail. Then, the procedures will be discussed for conducting diffusion experiments using the diff package and examples of experimental parameters will be provided for each sequence.

The PFG NMR spin echo sequence (Figure 5) was first used to measure self-diffusion. This sequence consists of two rf pulses (\(\frac{\pi}{2}\) and \(\pi\)) each followed by a gradient pulse of amplitude \(g\) and duration \(\delta\) (\(\delta = P17+P18+P17\)). The gradient pulse shape for the sequence shown in trap with a ramp time of P17 and a plateau time of P18. D2 is the gradient stabilization delay. D9 and D10 are the tau remainder. D11 is the gradient amplifier unblank delay. Values for D9, D10, and D11 are determined by the diff software. For the PFG NMR spin echo sequence, the measured signal is reduced by a factor of \(\exp\left(-\frac{2\tau T_2}{T_2}\right)\) due to \(T_2\) NMR relaxation. Under conditions where the NMR relaxation times are sufficiently long, the exponential term that describe relaxation effects for spin echo sequence can be neglected. In some cases however, \(T_2\) NMR relaxation effects are quite strong and cannot be completely ignored. Table 3 gives examples of experimental parameters for various types of samples to be used with this sequence.

![Figure 2. Schematic of the PFG NMR spin echo sequence (diffSe).](image)

<table>
<thead>
<tr>
<th>ACQU</th>
<th>Diffusion time</th>
<th>Glycerin D = 2x10^-12 m^2/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ</td>
<td>ms</td>
<td>12.12.08 #11</td>
</tr>
<tr>
<td>P17</td>
<td>ramp up and down of the</td>
<td>μs</td>
</tr>
</tbody>
</table>


The stimulated echo (STE) sequence (Figure 6) is an alternative to the previous sequence and is useful for NMR diffusion studies of systems in which $T_1 > T_2$. In the stimulated echo sequence, the $\pi$ pulse from the spin echo sequence is split to two $\pi/2$ pulses to reduce the effects of $T_2$ NMR relaxation effects. The signal is reduced by $\exp\left(-\frac{r_1}{T_1}\right)$ and $\exp\left(-\frac{r_2}{T_2}\right)$ due to $T_2$ and $T_1$, respectively. The measured signal is susceptible to the affects of $T_2$ NMR relaxation while the magnetization is in the $xy$-plane. During the time interval between the latter $\pi/2$ pulses of the stimulated echo sequence, the signal is reduced only by $T_1$ NMR relaxation effects. This allows for studies of systems with significantly short $T_2$ times to be performed over a large range of diffusion times including long diffusion times. The PFG NMR stimulated echo sequence is the most common of diffusion pulse sequences which can be used to determine diffusivities of molecules in heterogeneous systems. D5 is the delta
remainder. In the time period $\tau_2$ between the second and third pulses, the application of a sine-shaped spoil gradient of small duration and gradient strength reduces artifacts by destroying any unwanted magnetization. Table 4 gives example of experimental parameters for various samples to be used with the stimulated echo sequence.

Figure 3. Schematic of PFG NMR stimulated echo sequence (diffSte).

Table 4. Examples of experimental parameters for the stimulated echo sequence (diffSte)

<table>
<thead>
<tr>
<th>AC QU</th>
<th>diff</th>
<th>Water $D = 1.8 \times 10^{-9}$ m²/s</th>
<th>Glycerin $D = 2.0 \times 10^{-12}$ m²/s</th>
<th>Raft (short $T_2$) $D = 1 \times 10^{-13}$ m²/s</th>
<th>Raft (short $T_2$) $D = 2.22 \times 10^{-9}$ m²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.22.09</td>
<td>2.22.09</td>
<td>2.22.09</td>
<td>2.22.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>#3</td>
<td>#3</td>
<td>#4 27C</td>
<td>#7 27C</td>
</tr>
<tr>
<td>Δ</td>
<td>Diffusion time</td>
<td>n s</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>P1 7</td>
<td>Gradient ramp</td>
<td>μ s</td>
<td>500</td>
<td>800</td>
<td>750</td>
</tr>
<tr>
<td>(P1 8-P17) (P2 0-P17)</td>
<td>Gradient duration</td>
<td>μ s</td>
<td>100</td>
<td>1200</td>
<td>1645</td>
</tr>
<tr>
<td></td>
<td>Initial gradient</td>
<td>G/cm</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Maximum gradient</td>
<td>G/cm</td>
<td>250</td>
<td>2002</td>
<td>2500</td>
</tr>
<tr>
<td>Pl1</td>
<td>power level d B</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
There are two other sequences which are useful for specific reasons. First, the PFG NMR stimulated echo longitudinal encode-decode sequence (STE LED) is shown in Figure 7. The advantage of this sequence is that it is designed to minimize effects of eddy currents. Eddy currents are currents that are induced by changing magnetic fields. They can have very disturbing effects on diffusion experiments, especially when large magnetic field gradients are switched on or off. Normally, the gradient stabilization delay can be set to a value that is sufficiently long to allow for any eddy currents produced by the gradients to dissipate after the gradient is switched off. However, such delay increases the effect of T2 because the T2 NMR relaxation process takes place during the gradient stabilization delay. The PFG NMR STE LED applies additional rf pulses along with an LED delay (D19) just before acquisition which
allows for eddy currents to die out. Only T1 NMR relaxation occurs during this delay. With this LED delay, the gradient stabilization time can be set to a value much smaller than that required for the standard STE sequence. This sequence is most beneficial for use with samples that are limited by extremely short T2 relaxation times. *To use this sequence, select the option for the LED delay when “diffSte” sequence is active.*  Table 5 gives examples of experimental parameters for this sequence.

![Figure 4. Schematic of the PFG NMR stimulated echo LED sequence(diffSte with LED delay).](image)

### Table 3. Examples of experimental parameters for the stimulated echo LED sequence

<table>
<thead>
<tr>
<th>AC QU window</th>
<th>diff window</th>
<th>Glycerin</th>
<th>Raft (short $T_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC QU window</td>
<td>diff window</td>
<td>Glycerin</td>
<td>Raft (short $T_2$)</td>
</tr>
<tr>
<td>AC QU window</td>
<td>diff window</td>
<td>Glycerin</td>
<td>Raft (short $T_2$)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$</td>
<td>$2 \times 10^{-12}$ m$^2$/s</td>
</tr>
<tr>
<td>$D$</td>
<td>$1 \times 10^{-13}$ m$^2$/s</td>
</tr>
<tr>
<td>$\Delta$ diffusion time</td>
<td>15 μs</td>
</tr>
<tr>
<td>Gradient ramp</td>
<td>800 μs</td>
</tr>
<tr>
<td>Gradient duration</td>
<td>1200 μs</td>
</tr>
<tr>
<td>Initial gradient</td>
<td>33 G/cm</td>
</tr>
</tbody>
</table>

45
Next, the 13-interval sequence is shown in Figure 8. This sequence incorporates alternating (bipolar) gradients where with the previous sequence, the gradients were strictly unipolar. Alternating pulsed-field gradients are used to suppress susceptibility-induced artifacts in diffusion experiments. Magnetic susceptibility is related to the manner in which an object causes distortions in the applied magnetic field due to inherent and/or geometric properties of that object. Samples that are heterogeneous in nature present a specific problem due to the existence of inhomogeneous internal magnetic fields even before the application of an external magnetic field. Susceptibility-induced inhomogeneities in the local field manifest themselves in various manners including phase shifts in the
5. PFG NMR Diffusion Experiments

frequency domain and shifts in the spin echo position in the time domain. The 13-interval sequence is intended to minimize susceptibility distortions of the PFG NMR data. Table 6 gives examples of experimental parameters for various samples to be used with this sequence.

![Schematic of the PFG NMR 13-interval sequence (diffSteBp).](image)

Table 4. Example of experimental parameters for the 13-interval (diffSteBp) sequence.

<table>
<thead>
<tr>
<th>Acquisition Parameter</th>
<th>Corresponding <strong>diff</strong> Parameter</th>
<th>Description</th>
<th>Doped Water 1H</th>
<th>p-Dioxane in Benzene-d6 (40% by vol.)</th>
<th>Porous Membrane (13C2H4) 13C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PULPROG</strong></td>
<td>Pulsprogram Name</td>
<td>Pulse sequence used for experiment</td>
<td>diffSteBp</td>
<td>diffSteBp</td>
<td>diffSteBp</td>
</tr>
<tr>
<td><strong>ZGOPTNS</strong></td>
<td>Use Led sequence</td>
<td>Enables longitudinal eddy-current delay</td>
<td>2 ms</td>
<td>2 ms</td>
<td>5 ms</td>
</tr>
<tr>
<td>- Gradient pulse shape</td>
<td>The type of gradient pulse generated</td>
<td>Sinus</td>
<td>Sinus</td>
<td>Sinus</td>
<td></td>
</tr>
<tr>
<td>- Gradient Pulse duration (delta)</td>
<td>Twice the effective duration of the gradient pulse, δ. For half-sine pulses it is equal to (2<em>D_{18}/1.58). For trapezoidal pulses it is equal to (D_{16} + D_{17} + 2</em>D_{18}).</td>
<td>0.70 ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Diffusion time DELTA</td>
<td>Duration between the 1st and 3rd gradient pulses, Δ.</td>
<td>10 ms</td>
<td>10 ms</td>
<td>12 ms</td>
<td></td>
</tr>
<tr>
<td><strong>D18</strong></td>
<td>Gradient pulse duration in pulse program</td>
<td>The actual duration of a shaped gradient pulse.</td>
<td>0.56 ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Delay between the 2 first rf pulses (tau)</td>
<td>For the BP sequence it is actually the distance between the 1st and 3rd RF pulses, commonly referred to as τ. The check box to the left indicates if a minimum value should be used.</td>
<td>8.18 ms (checked)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Grad list type:</td>
<td>Specifies how the gradient intensity is produced from the minimum and maximum values.</td>
<td>Linear</td>
<td>Linear</td>
<td>Linear</td>
<td></td>
</tr>
<tr>
<td>- Maximum gradient value</td>
<td>The maximum gradient intensity to obtain a PFG NMR attenuation data point for.</td>
<td>250 G/cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gradient start value</td>
<td>The minimum gradient intensity to obtain a PFG NMR attenuation data point for.</td>
<td>30 G/cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TD1</strong></td>
<td>Number of gradient steps</td>
<td>Number of list values generated for the specified gradient intensity range. The number of trials to perform at each gradient value. The value should be a multiple of 16 to run a full phase-cycle.</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NS</strong></td>
<td>Number of scans</td>
<td>Number of scans</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Number of repetitions</td>
<td>The number of times the entire PFG NMR experiment should be repeated. Should be set to 1.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>DS</strong></td>
<td>Number of dummy scans</td>
<td>Number of trial runs to perform before beginning of the acquisition of the signal.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D2</strong></td>
<td>Gradient pulse stabilization time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Performing a PFG NMR diffusion experiment

i. Open or copy an existing 2D data set.

ii. Type **diff** to open the diff dialogue window

iii. Choose the pulse sequence from the list provided next to *Active Method*

iv. Once the pulse sequence is chosen, the window will give a set of parameters important for the diff sequence of choice. Tables 3-6 give examples of parameter values for different sequences and different samples according to their diffusion coefficient(s) and relaxation information. Below is a list of parameters in the diff window along with a short description when necessary.

v. Once the pulse sequence is chosen, the window will give a set of parameters important for the diff sequence of choice. *In order for the software to accept the value that has inputted in this window, it is necessary to type the value, press enter on the keyboard, and essential wave the mouse around in the window to update the window. Below is a list of parameters in the diff window along with a short description when necessary.

a. Gradient pulse shape – for short pulses (< 500 μs) of conservative amplitudes, the Sinus curve is an ideal gradient pulse shape. For longer pulses, use the trap shape which prescribes a trapezoidal shape of a ramp up, plateau, and ramp down. If trap is selected, there will be an option further down in the window to input the gradient ramp time.

b. Effective gradient pulse duration – this is the total time that the gradient is applied. For trapezoidal pulses, this time does account for the ramps.

c. Diffusion time – the delay in which diffusion is observed. *If experiments using different diffusion times are to be compared, the range of gradients for experiments of
higher diffusion times should be scaled down such that each experiment is measuring the same range of attenuation. Diffusion time is proportional to $g^2$ so if diffusion time is increased by a factor of 2, the range of gradient amplitudes should be scaled by a factor of $\sqrt{2}$.

d. Tau – can indicate that the minimum tau should be used for the current dataset or tau can be increased as needed

e. Total experiment time

f. Diffusion grad list type – choose linear to indicate that the amplitude of the gradients should follow a linear progression from minimum (start) to maximum.

g. Max gradient value

h. Gradient start value - *Values of the minimum gradient below 33 G/cm do not usually produced reliable results

i. Gradient stabilization parameters (do not select use default parameters)

j. Spoil duration

k. Spoil amplitude

vi. Press the “exit” button to compile the list of parameters. To check that all inputs were accepted, type **diff** again and review the list.

vii. In the “AcquPars” tab of the dataset, check that the following parameters have appropriate values

a. TD

b. SWH

c. AQ

d. RG

e. P1

f. P11
6 2D NMR Data Analysis

6.1 T1/T2 Relaxation Analysis

The ‘NMR Relaxation Guide’ is a 2D NMR data analysis package software built into Topspin and can be started by selecting the menu ‘Analysis > T1 / T2 Relaxation’ or by executing the tlguide command. Note that the Relaxation Guide should only be used on processed 2D datasets. The relaxation guide produces 2D plots of signal intensity (or attenuation) as a function of the experimental variable(s):

- For T1 NMR relaxation measurement using the t1ir pulse sequence, signal attenuation is plotted as a function of delay times specified in the VDLIST.

- For T2 NMR relaxation measurement using the CPMG sequence, attenuation is plotted as a function of the values specified VCLIST. To convert to time units from VCLIST units, multiply the VCLIST value by twice the value of D20, viz. the value of the echo delay.

- For diffusion measurements, the signal attenuation is plotted as a function of applied gradient intensities stored in the file named DIFFLIST.

The T1/T2 relaxation analysis package can be used to fit the data and calculate diffusion coefficients. This is convenient and sufficient for quick diffusivity results during measurement sessions. However, better fits and flexibility of experimental attenuation data are obtained by manipulating the data obtained with the T1/T2 analysis package with other software packages such as Excel and Origin, for which the next section is dedicated.

Processing 2D time-domain data

1. The command xf2 performs exponential multiplication and Fourier transform and phase correction on each spectrum in the data set. Phase correction will be performed according to data stored in the “ProcPars” tab (i.e. pk or mc, PHC0 and PHC1). If manual phase correction need be used, a single slice must be extracted using the ef command and manually phase corrected. In order to use the same phase corrections for all spectra within the dataset, the button must be selected within the manual phase correction window.

2. Automatic baseline correction for all spectra within the dataset is performed using the command abs2.

Processing 2D frequency-domain data

See Figure 9A and B for a schematic of the processing windows in T1/T2 relaxation that can be found in “Analysis”.

50
1. Click “Extract Slice” to choose the slice for integration. Select “spectrum” to be extracted from 2D dataset. Input the slice number which should be extracted. Close the window by pressing “Ok.”

2. Click on “Peaks/Ranges”. Choose the option “Manual Integration” to define the peaks. Click to highlight the button to indicate that range selection is activated. Use the mouse to define a region of interest which displays the peak of interest. *Analysis of several peaks can be conducted by selected more than one range.* From the toolbar at the top of the window, select the button and from the pulldown menu, select “Export regions to relaxation module and .ret”. Close this window.

3. Click the “Relaxation” button to display a graphical representation of attenuation data. On the left of this window, there is an option to select peak or area. Depending on the selection, the software will use the amplitude of or area under the peak(s) of interest. In the case of multiple peaks, the button at the top of the window which resembles an arrow can be used to change between different attenuation plots of different peaks.

4. Click the “Fitting function” button to display a window of relaxation parameters which will be used for fitting. The most important parameters include
   a. **DRIFT POINT** – if signal to noise is low and/or there is some scatter in the data, it may be helpful to increase the number of drift points in order to see all the data.
   b. **FUNCTION TYPE** – indicates the type of function which is used for fitting. Choose “vargrad” for diffusion experiments and “uxnmrt2” for the cpmg sequence.
   c. **LIST OF FILE NAME** – indicates the list used for fitting. Choose “difflist” for diffusion measurements and “vclist” for the cpmg sequence.
   d. **GAMMA, LITDEL, BIGDEL** – these values correspond to the gyromagnetic ratio, pulse duration, and diffusion time used for attenuation calculations.

5. Press “Ok” to get out of the fitting function window.

6. Press “Start Calculation” to fit the data. If more than one peak is being analyzed, the button in the top of the window should be used to fit each set of data simultaneously.

7. Press “Display Report” to see the raw attenuation data and save it as a text file for later data analysis using Excel and Origin softwares. (see Figure 10)
6.2 Raw data to Excel

This section describes one way of taking raw data and creating attenuation plots to obtain diffusion coefficient(s) and associated weight factor(s). Many other methods

Excel offers a convenient way to import the data obtained from T1/T2 relaxation analysis and calculate the terms needed for the attenuation plot. First, import the data file to a worksheet in an Excel workbook (see Figure 11). The only two columns of interest are entitled “Gradient” and “Expt.” These values are the amplitude of the magnetic field gradient applied (in G/cm) and the normalized signal, respectively, for each slice. The raw data often appears busy and so it is useful to link the two columns of interest to another worksheet used for calculation (see Figure 12). The highlighted columns entitled “Gradient (G/cm)” and “Expt” are obtained directly from the same columns in Figure 11 by linking the two worksheets. The values in the middle column entitled “q2teff” are obtained by using excel to calculate the q2teff term in the attenuation equation (see Appendix B – Eqn. R) which is equal to

\[(\gamma \delta g)^2 t_{eff}\]  

where \(\gamma\) should be in Hz/G, \(\delta\) should be in s, and \(g\) should be converted to G/m. These values are stored in the cells which are highlighted in red. The effective diffusion time (teff) is equal to

\[(A - \frac{\delta}{3})\]  

for the spin echo, stimulated echo sequence, and stimulated echo LED sequences and
(Δ - τ/2 - δ/6) for the 13-interval sequence.98,99 Also, if the GCC wasn’t changed to reflect calibration using the diffusion coefficient of water, calibration of the gradient amplitudes can be done in excel as well.

Figure 7. Examples of the raw data text file obtained from T1/T2 relaxation analysis software.
6.3 Attenuation Plots in Origin

Origin is used in this data analysis protocol to plot the data and use nonlinear fitting methods to obtain the diffusion coefficient(s). The two columns of interest in Fig. 12 are entitled “$q^2t_{\text{eff}}$” and “expt” (surrounding by a black square). Data in these columns should be imported into an Origin worksheet so that the attenuation (“expt” column) can be plotted on a semi-logarithmic plot as a function of “$q^2t_{\text{eff}}$” (See Fig. 13). The diffusion coefficient(s) of molecules contributing to the NMR signal is obtained through fitting by the attenuation equation (see Appendix B for details). This equation takes the form

$$
\Psi = \sum_{i=1}^{n} p_i \exp\left(-q^2t_{\text{eff}}D_i^m\right)
$$

where $D_i^m$ is the measured diffusion coefficient of species $i$ and $p_i$ is the associated weighting factor. This equation is a sum of exponential terms describing the possible behavior of several different ensembles of molecules. For a homogeneous sample in which there is a single ensemble of molecules diffusing, a monoexponential form of the attenuation should be used to fit the data. This form of
exponential function is standard to the Origin software’s fitting function. When there is more than one ensemble of molecules with distinct diffusion coefficients, it is necessary to create biexponential, triexponential, etc. forms of the exponential equation for the fitting function to use. Refer to the help guide available within the Origin software for help with the creation of non-standard nonlinear fitting equations. An example of such an equation in the biexponential form is

\[ y = A \times \exp(-Bx) + C \times \exp(-Dx) \]

where \( y \) is the attenuation, \( x \) is the \( q^2 t_{eff} \) term, \( B \) and \( D \) are diffusing coefficients for the two species, and \( A \) and \( C \) are the associated weighting fractions. The weighting fractions represent the fraction of total molecules which are contributing to the signal which has a distinct diffusion coefficient of \( B \) (for the \( A \) fraction) or \( D \) (for the \( C \) fraction). A constraint should be implemented in the fitting function that the sum of all weighting factors should be equal to 1.

![Figure 10. Example of an attenuation plot for a homogeneous sample. The black line indicates a fit of the data points which was used with a monoexponential form of the attenuation equation. The slope of the line obtained from the fit yields the diffusion coefficient for this sample.](image)

6 2D NMR Data Analysis
Appendix A – Theory of Diffusion

Diffusion is the process of stochastic thermal motion of molecules which can be differentiated into two basic types: transport diffusion and self-diffusion. Transport diffusion involves the movement of molecules in order to reduce macroscopic concentration gradients (or gradients of chemical potential) while self-diffusion occurs under conditions of the absence of macroscopic concentration (or chemical potential) gradients. The transport diffusion coefficient describes the relationship between the flux of molecules and a concentration or chemical potential gradient under non-equilibrium conditions. In self-diffusion, molecular transport is characterized by the random translational motion of molecules driven by thermal energy. Self-diffusion occurs in liquid-like systems such as lipid bilayers under the conditions of the absence of macroscopic gradient of chemical potential. Self-diffusion can be described and treated similarly to transport diffusion if one considers an uneven distribution of labeled and unlabeled molecules under conditions when overall molecular concentration remains the same in each point of the considered volume. Many experimental techniques suitable for studies of self-diffusion involve the observation of labeled molecules in a sea of unlabeled but otherwise identical molecules. In these cases, the self-diffusion coefficient can be derived from the Fick’s First Law considering a concentration gradient of labeled molecules. The diffusive flux of labeled molecules is as follows

\[ J(r,t) = -D \nabla c(r,t) \quad (A) \]

where \( D \) is the self-diffusion coefficient and \( c(r,t) \) is the concentration of (labeled) molecules moving in one-, two-, or three-dimensional space. By applying the principle of conservation of mass, the change in concentration with time at a certain position in space \( r \) can be described as

\[ \frac{\partial c(r,t)}{\partial t} = -\nabla \cdot J(r,t) \quad (B) \]

The combination of Eq.’s (A) and (B) give Fick’s Second Law,

\[ \frac{\partial c(r,t)}{\partial t} = \nabla \cdot (D \nabla c(r,t)) \quad (C) \]

Thermal stochastic motion of molecules will cause the concentration gradient to approach the zero value with increasing diffusion time. The self-diffusion coefficient in the right-hand part of Eq. 3 can be placed before the first gradient operator because this diffusivity is expected to be independent of position (since overall molecular concentration remains the same for all positions). Eq. C can be solved by replacing the spatially-dependent concentration term with a term describing the probability that a molecule will diffuse from its initial position \( r_0 \) to the position \( r \) after time \( t \). The solution gives a Gaussian distribution that is also known as diffusion propagator.

\[ P(r_0,r,t) = (4\pi Dt)^{3/2} \exp \left\{ -\frac{(r-r_0)^2}{4Dt} \right\} \quad (D) \]

where \( P(r_0,r,t) \) is the probability that a molecule with an initial position, \( r_0 \), will migrate to a final
position, \( r \), after time \( t \). This probability function is a function of the displacement \((r-r_0)\) rather than the initial position. The Einstein relation can be easily derived from the diffusion propagator (Eq. 4). This relation is written as
\[
\langle r^2 \rangle = nDt
\]  
where \( n \) is the dimension \((n = 1\) for 1D diffusion\) and \( \langle r^2 \rangle \) is the mean square displacement (MSD).

Appendix B – Theory of Pulsed Field Gradient NMR

B.1 A Brief Overview of Nuclear Magnetic Resonance

Nuclear magnetic resonance spectroscopy is a technique based upon the inherent magnetic properties of certain atomic nuclei in order to obtain detailed information about the structure and dynamics of molecules. In the framework of classical theory, NMR spectroscopy can be understood by first considering that certain nuclei possess an intrinsic source of angular momentum known as nuclear spin angular momentum. For a macroscopic system consisting of many nuclei, the total angular momentum is a vector that represents the direction and magnitude of the sum of individual spin angular momenta. Nuclei with non-zero spin angular momentum also have an intrinsic magnetic moment. In a macroscopic system, alignment of magnetic spins of individual nuclei along the direction of the external magnetic field leads to a non-zero net magnetization. The static magnetic field \( B_0 \) exerts a torque on the net magnetization which causes a precessive motion about the direction of the external magnetic field at a frequency known as the Larmor frequency, \( \omega_0 \):

\[
\omega_0 = -\gamma B_0
\]  

where \( \gamma \) is the gyromagnetic ratio of the nucleus under observation and \( B_0 \) is the amplitude of the static magnetic field. In NMR, the direction of the \( B_0 \) field is usually assumed to coincide with the \( z \)-axis of the laboratory (viz. non-rotating) coordinate system. Magnetic resonance can be achieved by applying an additional magnetic field \( B_I \) that oscillates in the transverse \((x,y)\) plane with the Larmor frequency. In NMR spectrometers, the application of a current through the RF coil at the Larmor frequency will rotate the net magnetization vector from the direction of the external magnetic field \((z\text{-axis})\) to the transverse plane. The angle that the magnetization is rotated, or the tip angle, is given by:

\[
\Phi = \gamma B_I \tau^*
\]  

where \( \Phi \) is the tip angle and \( \tau^* \) is the duration of the rf pulse or \( B_I \) field. A tip angle of 90° will tip the net magnetization vector completely in the transverse \( xy \)-plane, where the signal is detected.
The excitation of magnetization by a transverse RF pulse produces a non-equilibrium state. The process by which the excited net magnetization vector returns to its equilibrium position with time is known as NMR relaxation. There are two measures of NMR relaxation: spin-lattice or longitudinal NMR relaxation $T_1$ and spin-spin or transverse NMR relaxation known as $T_2$. At thermal equilibrium (i.e. before the RF pulse), there is a net magnetization oriented along the $z$-direction and no net transverse magnetization (magnetization in the $xy$-plane). $T_1$ relaxation can be approximately viewed as an energy exchange process in which the system returns from a high-energy state to a low-energy state in which energy gained from the RF pulses is transferred from the excited nucleus (spin) to its surroundings (the lattice) in order to reach thermal equilibrium. This causes the $z$-magnetization to return to its equilibrium value. The effect of $T_1$ relaxation on $z$-magnetization, which is initially oriented into the $–z$ direction, as a function of time can be described by:

$$M_z(t) = M_0 \left(1 - e^{-t/T_1}\right)$$  \hspace{1cm} (B-3)

where $M_z(t)$ describes the magnetization in the $z$-axis as a function of time, $t$, $M_0$ is the equilibrium magnetization value in the $z$-direction and $T_1$ is the spin-lattice relaxation time. The value of $T_1$ is dependent on a number of macroscopic parameters including the magnitude of $B_0$ and sample temperature.

$T_2$ relaxation is defined as the relaxation (loss of signal) that occurs when magnetization is oriented in the $x$-$y$ plane. In most cases, this relaxation is dominated by interactions between the nuclei (spins). Energy is exchanged between the spins which can induce slight changes in their individual local magnetic fields causing a slight change in frequency. Immediately after excitation by the RF pulse, all spins are in phase coherence with each other (i.e., they precess exactly at the same frequency and the accumulated phase is 0). After some time when $T_2$ relaxation effects are significant, the spins lose coherence, which is reflected by the existence of phase differences for different nuclei. As a result, the transverse magnetization decays. The time dependence of transverse magnetization under the influence of $T_2$ NMR relaxation can be described by

$$M_{xy}(t) = M_{xy,0} e^{-t/T_2}$$  \hspace{1cm} (B-4)

where $M_{xy}(t)$ is the transverse magnetization in time, $M_{xy,0}$ is the initial transverse magnetization after excitation, and $T_2$ is the spin-spin relaxation time. This magnetization eventually decays to its equilibrium value of zero because of $T_2$ relaxation.

As mention briefly above, the frequency of the signal is detected in the transverse plane. Immediately after the magnetization tipped into the transverse plane by the rf pulse, the signal starts to decay due to $T_2$. The decaying transverse magnetization that is recorded is referred to as a free induction decay (FID) which is converted from time domain signal to frequency domain signal using Fourier transformation. The frequency of signal that is detected varies somewhat from the Larmor frequency.
These slight variations are due to a phenomenon known as chemical shift. Electrons in orbital clouds exert currents which produce magnetic fields ($B_e$). The existence of this additional field leads to a slight deviation of the local magnetic field from the $B_0$ field. Nuclei with different local environments will experience different effects due to differences in electron distribution, (i.e. magnetic fields of $B_0 - B_e$). Chemical shifts are typically expressed as a ratio of the measured frequency shift to the operating frequency of the magnet to provide a value that is universal among all magnetic field strengths using the following relation:

$$\delta^*(\text{ppm}) = \frac{\omega - \omega_{\text{ref}}}{\omega_{\text{op}}}$$ (B-5)

where $\delta^*$ is the chemical shift in ppm, $\omega$ is the measured frequency, $\omega_{\text{ref}}$ is a reference frequency, and $\omega_{\text{op}}$ is the operating frequency of the magnet. The reference frequency is usually taken as the frequency of a compound known as TMS. Because there is a standard reference used for chemical shift as well as a lack of dependence on magnetic field strength in ppm, chemical shift can be used universally to give important information about the composition of the molecule under observation. There are countless applications of nuclear magnetic resonance which include ways to determine physical, chemical, electronic, structural, and dynamic properties of molecules. This brief overview is intended to set up basic theory behind application of interest used to study translational dynamics of molecules, pulsed-field gradient NMR.

**Pulsed-Field Gradient NMR: Theory**

Pulsed-field gradient NMR spectroscopy allows for direct measurements of mean square displacement (MSD) as well as of the related diffusivity and the diffusion propagator. The technique is based on the application of an external magnetic field with amplitude $B_{\text{ext}}$ which is superimposed on the $B_0$ field and which is heterogeneous along the z-axis ($B_{\text{ext}} = gz$, where $g$ is the magnetic field gradient and $z$ is the coordinate along the z-axis). This magnetic field gradient $g$ leads to a dependence of the Larmor frequency on the position of the nucleus. This frequency is described as

$$\omega = \gamma (B_0 + gz).$$ (K)

The application of magnetic field gradients allows for the detection of a change in nuclear position in the sample along the z-direction. The magnetization phase angle accumulated by each nuclear spin during the application of a gradient pulse, $\varphi(t)$, is described as

$$\varphi(t) = -\gamma \int_0^t (B_0 + gz) dt$$ (L)

assuming that their positions, $z$, do not change during the time in which the gradients are applied. The two gradient pulses in a spin echo or stimulated echo are intended to dephase and rephase the
magnetization as a means to keep track of the displacement of molecules over time. The change in phase accumulation between the application of the first and second gradient pulse goes as

\[ \Delta \varphi_{z_i} = -\gamma \left\{ \int_0^z (B_0 + g z_1) dt - \int_0^z (B_0 + g z_2) dt \right\} = \gamma g \delta \Delta z \]  

where \( z_1 \) is the position of the nucleus when the first gradient is applied, \( z_2 \) is its position when the second gradient is applied and \( \Delta z = z_2 - z_1 \). If the nuclei do not change their positions during the diffusion time (\( \Delta z = 0 \)), the change in phase accumulation due to the applied gradient \( g \) would be zero, and the space dependent term of the frequency would disappear. In this case the measured NMR signal would not be reduced due to application of the gradients. For the case when \( \Delta z \) is not equal to zero, a non-zero value of \( \Delta \varphi_{z_i} \) leads to attenuation of the measured NMR signal. The spin echo attenuation for all spins starting out at \( z_i \) can be written as

\[ \Psi = \int \cos(\Delta \varphi_{z_i}) P(\Delta \varphi_{z_i}) d\Delta \varphi_{z_i} \]  

where \( P(\Delta \varphi_{z_i}) \) the phase distribution for spins with an initial position \( z_i \). This distribution term is determined by the distribution of \( \Delta z \) (Eq. D) and is described by a Gaussian function. Equation 14 can then be written as

\[ \Psi_{z_i} = \exp\left(-\left\langle \Delta \varphi_{z_i}^2 \right\rangle \right) / 2 \]  

where \( \left\langle \Delta \varphi_{z_i}^2 \right\rangle \) is the average of \( \Delta \varphi_{z_i}^2 \) over the displacements \( \Delta z \). This average is written in terms of mean square displacement \( \left\langle z^2 \right\rangle \) (Eq. E) as

\[ \left\langle \Delta \varphi_{z_i}^2 \right\rangle = \left( \gamma \delta g \right)^2 \left\langle z^2 \right\rangle \]  

Eq.16 is expected to hold for any initial position. Hence, it describes the overall attenuation in a macroscopic sample. The total spin echo attenuation for all spins \( \Psi \) due to the application of the gradient pulses is then

\[ \Psi = \exp\left(-\left( \gamma \delta g \right)^2 t_{\text{eff}} D \right) \]  

where \( t_{\text{eff}} \) is the effective diffusion time. For cases such that the duration is much smaller than the time between the application of the first gradient and that of the second (i.e., \( \delta \ll \Delta \)), the effective diffusion time can be taken as \( \Delta \). This equation can be amended to take into account the presence of multiple ensembles of molecules diffusing within the sample at different rates. Attenuation is then described by a sum of exponential terms of ensembles \( i \) with distinct measured diffusion coefficients \( D_i \) and associated phase fractions, \( p_i \).
\[
\Psi = \sum_{i=1}^{n+2} \rho_i \exp \left( -q^2 t_{\text{eff}} D_i^m \right), \tag{R}
\]

where \( q \) is equivalent to \( (\gamma \delta g)^2 \). In a typical PFG NMR diffusion experiment, the attenuation of signal is measured as a function of one of the experimental parameters such as \( g \) or \( \tau \) for the purpose of finding the experimental diffusion coefficient(s).
Appendix C – Measurement at Low Temperature

Notice: The following instructions were written for the AMRIS 750 MHz spectrometer equipped with Avance II hardware running Topspin 2. The chilling unit and variable temperature control units have been since upgraded. These instructions may not reflect best practice for the modern Avance III / Topspin 3 setup. Consult with AMRIS staff and review Variable Temperature Control for NMR Probes, v. 2 before operating the spectrometer temperature control at sub-ambient temperatures.

Brief Guideline for low temperature setup using the Liquid N₂ Evaporator with a Liquid N₂ Dewar:

1. Enter the desired sample temperature in the edte window. There may be an offset between the temperature read by the temperature sensor and the real temperature of the sample. Note that the PID controller parameters which are used by the controller at normal temperatures may not be appropriate at low temperatures. An self-tune may need to be performed in the Topspin 3 edte window. *Always consult the AMRIS staff when performing measurements at or near these temperature extremes.

2. Fill the supplied dewar with liquid N₂. *Do not fill to the brim; leave the narrow neck of the vessel empty. The space is needed for working of the safety valve on the setup.

3. Make sure the supplied Nitrogen heater assembly is free of moisture. If water has condensed on its surface, use of a heat gun is recommended to remove any visible traces of water. Heat gun should be set to moderate temperature setting. The Nitrogen heater assembly contains delicate electronic and electrical components and so use of extreme heat might damage the equipment.

4. Insert the moisture/water free Nitrogen heater assembly into the liquid N₂ dewar filled in step 2, gradually with care. Once fully inserted lock the assembly in place using the supplied o-ring and clamp. *First time users should note that as the heater is lowered into the dewar, some nitrogen spills out from the dewar and if the o-ring is left on the brim of the dewar, it might get exposed to liquid nitrogen. Care should be taken to prevent direct exposure of the o-ring to liquid N₂ as it can crack the materials of the o-ring. A good way is to hold the o-ring with the heater and let it touch the dewar only, when the heater is completely inserted into the dewar.

5. Once the heater is safely locked into place, the outlet hose of the heater assembly should be connected to the probe body without delay. Extremely cool nitrogen gas coming out from the hose hardens the hose material and it loses its flexibility. As a result the hose may not align properly with the inlet on the probe body and the joint will leak.

6. Connect the electric cord on the heater assembly to the right connector on the main NMR console. This connector is located on the BVT unit in the console and is labeled as N₂. *Always consult AMRIS staff, if unfamiliar with the NMR console.
7. At this point a new option for cooling power should become available in the edte window, right below the heating power display/control. The cooling power controls the heat supplied to the heater assembly that is immersed in the liquid N\textsubscript{2} dewar and thus controls the rate of boil off. This controls the flow rate of the cooling N\textsubscript{2} gas delivered to the probe. No separate control for the flow rate of the cooling gas is available/needed. Typically 35\% cooling power is enough to cool to -50 °C. For higher temperature (+10 to -20) typically 15\% cooling power is enough. Intermediate powers should be used to attain temperatures between -50 °C and -15 °C.

8. The rate at which the nitrogen boils off and hence the dewar needs to be refilled are controlled by the cooling power. With 15\% power the dewar, which was initially full (as in step 2) is expected to last for up to 16 hrs. With 35\% heater power, the dewar will empty in as little as 8 hrs.

9. Use of high cooling power ≥40\% should be avoided as it may cause high pressures due to rapid boil off in the dewar which will triggers the relief valve. This valve tends to stick from icing and may not close completely once triggered. Such a leak will dramatically affect the performance of the cooling setup and will result in temperature fluctuations.

10. An experienced user may however turn the cooling power up to 40\% for the initial 20 minutes or so, when the probe is being cooled down from the room temperature for first time to expedite the cooling process. When cooling for the first time waiting times of up to 50 minutes for the entire probe assembly to cool down and attain a steady state are typical.

11. Before disconnecting the setup for refilling of the dewar or to end the experiments, bring the temperature back to room temperature. This step is important because if the cooled probe is directly exposed to air, the icing/ and build up of moisture might happen inside the probe and can damage the electronic or compromise the probe performance. When refilling the dewar, it is advisable to raise the set point to 40°C and pass air at high flow rates through the probe. This will prevent icing/buildup of moisture inside the probe. Dry the heater assembly completely as discussed in step 3, before re-immersion in liquid nitrogen.
Appendix D - Selected Topspin Command Reference

File Handling

**new** - copy the parameters from currently open experiment into a new experiment.
**close** - closes the current window
**closeall** – closes all open windows
**tlguide** – opens the T1/T2 relaxation guide
**re** - show spectrum (real part)
**fid** - show FID

**Acquisition Parameters:**

**TD** - time domain, number of sampling points
**AQ** - acquisition time, duration of the acquisition = DW * TD
**DW** - dwell time, time between samples
**SW** - acquisition spectral width, the
**O1** - receiver offset frequency (for channel 1)
**RG** - receiver gain

**P**L**W1** - RF power level (in W, for channel 1)
**P** - RF pulse lengths
**D** - pulse sequence delays/durations
**P**1 - Pi / 2 RF Pulse duration
**P**2 - Pi RF Pulse duration

**VCL**I**ST** – Variable Counter list (used in CPMG..etc)
**V**D**L**I**ST** – Variable Delay list (used in t1ir..etc)
**D**I**F**FL**I**ST** – listing of diffusion gradients used in PFG NMR experiments

**Acquisition Commands/Macros:**

**zg** - zero data file and acquire
**gs** - "go scan"
**wobb** - perform tuning / matching of probehead
**wobb ext50** - calibrate tuning / matching with external 50 ohm resistor.
**popt** - parameter optimization
**diff** - configure diffusion pulse sequence acquisition parameters.
**edte** - configures temperature control unit
**rga** - perform automatic receiver gain adjustment
**rsh** - read shims from file
wsh - write shims to file
setshim - set shimming values
setpre - set B0 compensation unit pre-emphasis parameters
edhead - configure current probe body
gradpar - gradient calibration constant

Processing Parameters:

SI - number of spectral points
LB - line broadening, exponential window function
PHC0 - 0th order phase correction
PHC1 - 1st order phase correction
ABSF1 - left ppm value for baseline correction
ABSF2 - right ppm value for baseline correction
ABSG - degree of polynomial to fit baseline (0-5 order)

Processing Commands/Macros:

ft - perform Fourier transform (1D)
ef - exponential multiplication + Fourier transform (1D)
pk - apply phase correction from stored values
apk - automatic phase correction
apk0 - automatic phase correction (0th order only)
apk1 - automatic phase correction (1st order only)
xf2 - perform 2D FT in the 2nd (spectral) dimension
abs - automatic base line correction (1D)
abs2 - automatic base line correction (2D)