

# **AECOM NMR COURSE DRX300 REFERENCE** **GUIDE**

## **Summary of steps involved in obtaining routine spectra**

1. reserve time on the nmr instrument (*ref. guide section 1*)
2. prepare nmr sample (*ref. guide section 2*)
3. login to computer and run nmr software (*ref. guide section 3*)
4. put sample into magnet and adjust temperature if needed (*ref. guide section 4*)
5. tune probe for nuclei to be used (*ref. guide section 5*)
6. lock and shim sample (*ref. guide section 6*)
7. create a new dataset and collect data (*ref. guide section 7*)
8. process data (*ref. guide section 8*)
9. plot data (*ref. guide section 9*)
10. repeat steps 7-9 to run additional experiments on same sample
11. replace your sample with reference sample and reset temperature to 25-26 °C if you have changed the temperature (*ref. guide section 10*)
12. exit software and logout (*ref. guide section 10*)

## Reference guide for obtaining spectra on the DRX300

### Table of Contents:

1. Facility Policies and Rules
  2. NMR Sample Preparation
  3. Logging In and Running XWINNMR
  4. Putting the Sample in the Magnet
  5. Tuning the Probe
  6. Locking and Shimming the Sample
  7. Running 1D Spectra
    - Macros for 1D Spectra
  8. Processing Data
    - Integration
  9. Plotting Data
  10. Exiting and Logging Out
  11. Calibrations
    - Calibrating the  $^1\text{H}$  90 Degree Pulse
    - Calibrating the  $^1\text{H}$  Carrier Frequency
- Appendix A: Probes Available for DRX300

## **1. Facility Policies and Rules**

**NMR Usage:** All users should reserve time on the DRX300 using the sign-up sheet outside the lab. If you are not signed up for it, you risk being displaced by someone who signs up while you are using the instrument – signing up is the only way to be guaranteed access to the instrument when you need it. Please see the Usage Policy attached to the sign-up sheet for rules regarding sign-up. There is currently a \$5/hour charge for the instrument.

**NMR Samples:** Under no circumstances are radioactive, chemically active (strong acid/base/oxidizers) or biohazardous samples permitted in the magnet. If a tube containing such a sample breaks in the probe of the magnet, the probe, which costs > \$20,000, could be rendered unusable. If you need to run an NMR spectrum on such a sample, please see the staff for special handling instructions.

**If a user does not abide by these policies, they will lose the privilege of using the instrument.**

## **2. NMR Sample Preparation**

**NMR Tubes:** The probes on spectrometer are machined to fairly fine tolerances - this means that the tolerances on the NMR sample tubes must be correspondingly high. It is recommended that samples be put in a high quality NMR tube: the Wilmad 528PP 5mm NMR tube or its equivalent should be used for samples. Order info:

Wilmad Glass  
Route 40 & Oak Rd., Buena, NJ, 08310  
phone# (800) 220-5171  
internet: [www.wilmad.com](http://www.wilmad.com)

High quality NMR tubes can be reused and it is recommended that you clean the tubes soon after you remove the sample from the tube. There is an NMR tube cleaner available in the NMR facility – in general, removal of stubborn residues can be achieved by soaking the tube in a detergent like “Hellmanex” then rinsing thoroughly with water and then air dry - tubes should NOT be dried in hot (200 degree) ovens - even good tubes will warp and give problems.

**Sample Volume:** For sample preparation it is HIGHLY RECOMMENDED that the sample volume in a 5mm tube be AT LEAST 0.55 mL. It would be ideal if everyone would make every sample the same **0.6 mL** volume for quick and good shimming. The sample should be free of precipitate and particulate matter – filter or spin-down if necessary.

If you are sample limited, a special nmr tube (Shigemi) is available that will allow you to run with sample volumes as low as 0.3ml – please see staff for details.

### **3. Logging In and Running XWINNMR**

- Log into DRX300
- Open shell by clicking on terminal icon (a graphic of shell on terminal) on left side of lower bar
- In shell, type "xwinnmr"

#### 4. Putting the Sample in the Magnet

- Check temperature of probe by typing “edte” in xwinmr window (should see temperature reading of 25 or 26 degrees Celcius). Click on **File -> Exit** in menu bar to close window.

note: to change the temperature, use the command “edte” and then click on Change button. Type in the desired temperature in degrees Centigrade in the **Sample Target Temp** window and click on the **Apply** button. See Appendix A for temperature ranges for each probe. Any temperature setting outside the range of 5 C – 50 C requires the assistance of the staff. Please reset the temperature back to 25-26 °C when you are finished with your experiment.

- Remove dust cap from magnet if needed.

- Press **LIFT ON/OFF** button of keypad. Remove reference sample (D2O) from magnet then remove reference sample from spinner. Insert your sample into spinner using depth gauge, wipe nmr tube with kimwipe then place sample/spinner on top of magnet.

- Press **LIFT ON/OFF** button of keypad and wait for sample to be loaded into magnet.

## 5. Tuning the Probe

- check tuning of probe for each nucleus you plan to use for your experiments using one of the following macros - if you are running only a proton spectrum on your sample, use **step A** but if you are running a carbon spectrum with proton decoupling, skip step a and use **step B**. NOTE: when the active nucleus is tuned and matched correctly, you should see a “dip” on the display that is close to center of screen and that it goes down to bottom of window.

A. “h1tune” checks for proton tuning only - adjust using yellow rods on probe labeled “T” and “M”. When you have finished tuning, click on “stop” then click on “return”.

B. “c13tune” checks for <sup>13</sup>C and proton tuning

first tune for <sup>13</sup>C:

on the BBI probe, set the tune number to “7843” and the match number to “981” and then do fine adjustment using the last digit in both tune and match.

then click on “wobb-SW” to switch to proton tuning and tune according to step a. When you have finished tuning, click on “stop” then click on “return”.

C. "p31tune" checks for 31p and proton tuning

first tune for 31p:

on the BBI probe, set the tune number to "7971" and the match number to "995" and then do fine adjustment using the last digit in both tune and match.

then click on "wobb-SW" to switch to proton tuning and tune according to step a. When you have finished tuning, click on "stop" then click on "return".

D. "n15tune" checks for 15n and proton tuning

first tune for 15n:

on the BBI probe, set the tune number to "6284" and the match number to "705" and then do fine adjustment using the last digit in both tune and match.

then click on "wobb-SW" to switch to proton tuning and tune according to step a. When you have finished tuning, click on "stop" then click on "return".

## 6. Locking and Shimming the Sample

There are several ways to shim the sample – a couple of them are described here. Bring up lock display by typing “lockdisp” in xwinnmr window.

### 1. Computer locking and shimming on samples:

Type “**lockshim**” which automatically reads in the default shimfile, locks onto the deuterated solvent you choose from the menu and then does computer shimming. After ~5 minutes, the following message should appear “nonmatching END” indicating that shimming is complete. Click on “OK”. Note: If the “lockshim” command takes considerably longer than 5 minutes to complete, then your nmr sample is probably less than ideal (not enough volume, bad tube, particulate matter in sample, etc.).

### 2. Manual locking and shimming on samples:

Type the command “**locklineshape**” which will automatically read in the default shimfile and lock onto the deuterated solvent you choose from the menu. You can then manually shim as described in Lab 1 – it is suggested that you optimize the following shims in an iterative fashion: z1, z2, z3, x, y, xz and yz by pressing the appropriate button on the BSMS keypad and changing the shim value until the lock signal is maximized. If the lock signal becomes too large, it may disappear from the lockdisp display – if this happens, hit the **LOCK GAIN** button on the BSMS keypad and use the knob to lower its setting until the lock signal becomes visible again.

### 3. Locking and shimming on samples dissolved in water with a small amount of D<sub>2</sub>O:

Use the command “**lockshimwater**” which automatically reads in the default shimfile, locks onto the D<sub>2</sub>O and then starts the gradient shimming routine – to run gradient shimming, click on the “RUN” button of the “shim sample” window. Wait for a new window to appear that will ultimately contain several profiles. Make sure that the profile from the last iteration is relatively flat indicating the sample is properly shimmed. Click on “OK” in the profile window. Use File > Exit in “shim sample” window to exit from gradshim. If needed, click on return to get back to 1D menu.

The instrument is now ready for data collection.

## **7. Collecting Data**

- Create new dataset by typing “edc” in xwinmr window.

Type in a unique dataset name in “NAME” field of edc window and type in a number in “EXPNO” field of edc window (in general, for a given sample or project, it is best to start off at expno 1 and increment expno with each new experiment)

- For routine 1D experiments in organic solvents or D<sub>2</sub>O, type in a name of macro corresponding to experiment you wish to run (refer to chart named Macros for 1D Spectra).

- Most 1D macros are setup to collect a minimum number of scans (usually 8). If the spectrum that appears at the end of the macro does not have sufficient signal-to-noise, collect a spectrum with more scans as follows:

Type “ns” in xwinmr window then type in number of scans you want into window that appears followed by a return.

Type “expt” to get experiment time then click on “OK”.

- Type “azg” to start experiment – you should be able to monitor the data collection in the xwinmr info window.

If you would like to see the spectrum while it is still being acquired, type “tr” to transfer data to computer and then type “trf” to process it. Type “acqu” to return to the acquisition window.

**NOTE:** if a macro does not exist for an experiment you wish to run, please ask us to help you set-up the experiment.

## **Macros for 1D Spectra**

### **<sup>1</sup>H proton spectra**

*h1zg*                    1D proton spectrum

### **<sup>13</sup>C carbon spectra**

*c13zgd*                    1D carbon spectrum w/ proton decoupling

### **<sup>31</sup>P phosphorus spectra**

*p31zgd*                    1D phosphorus spectrum w/ proton decoupling (317ppm spectral width with center at 127ppm)

*p31zg*                    1D proton-coupled phosphorus spectrum (317ppm spectral width with center at 127ppm)

*phoszgd*                    1D phosphorus spectrum w/ proton decoupling optimized for phosphate esters (50ppm spectral width with carrier at 0ppm)

*phoszg*                    1D proton-coupled phosphorus spectrum optimized for phosphate esters (50ppm spectral width with carrier at 0ppm)

### **<sup>15</sup>N nitrogen spectra**

*n15zgd*                    1D nitrogen spectrum w/ proton decoupling (250ppm spectral width with carrier at 120ppm)

## **8. Processing Data**

- type "trf" to process data – make sure you see spectrum at this stage (may have to scale the spectrum on screen using \*8, /8, etc. buttons).

- type "apk" to automatically phase the spectrum if needed. If the spectrum does not appear to be phased, follow the instructions for manual phasing in BAUG, pp. 23-24.

- type "edp" to display and edit the processing parameters. The parameters that are typically used to change the appearance of the spectrum are "si" and "lb". The parameter "si" (size) determines the number of points used to form the resulting spectrum – in 1D spectroscopy, it is often recommended to zero fill one time so set "si" to be the same value as "td". The parameter "lb" (line broadening) determines the amount of line broadening to apply in the window function before Fourier transformation – a smaller "lb" will result in better resolution but poorer signal-to-noise; a larger "lb" will result in better signal-to-noise but poorer resolution.

**Integration:** type "abs" in xwinnmr window to have computer automatically integrate spectrum. To integrate spectrum manually, click on "integrate" button. Expand region you want to integrate then use left hand mouse button to define positions of integrals. To calibrate a specific integral, use left hand mouse button to define active integral (indicated by a "\*" next to integral) then click on "calibrate" and enter in number of protons the signal corresponds to. Click on "return" then "save and return".

## 9. Plotting Data

### **Using the plot command (will plot spectrum and integrals by default):**

- Display region of spectrum you would like plotted out using the “DP1” button.
- Use “utilities” menu to define largest peak for plot. Click on “CY”, adjust line above largest peak using mouse, click on left mouse button then type height in cm of that position on plot (ie. 10 cm).  
NOTE: if Y-axis is not in units of cm, click on “YU” button.
- Click on “MI”, adjust line to smallest peak for peak picking using mouse then click on left mouse button.
- Click on “return”
- Make plot of spectrum by typing “plot” (ignore any error messages, it will likely make a plot anyway)

Note: to generate a plot with peak labels, type “edo” and choose the LAYOUT “1D\_H+pp.xwp” and then type “plot”

### **Using the XWINPLOT:**

- type “xwinplot” and a new window will appear. Refer to XWINPLOT manual or see staff for instructions.
- make plot by clicking on **File** -> **Print** then clicking on the **Print** button.

## **10. Exiting and Logging Out:**

- If you have changed the sample temperature from the normal value of 25 or 26°C, use the command “edte” command to set the temperature (see reference section 4).
- Replace your sample with the reference sample: Press **LIFT ON/OFF** button of keypad. Remove your sample from magnet then remove sample from spinner. Insert reference sample into spinner using depth gauge, wipe nmr tube with kimwipe then place sample/spinner on top of magnet.
- Press **LIFT ON/OFF** button of keypad and to load sample into magnet.
- Type “exit” in xwinnmr window to exit from software.
- Click on KDE menu pulldown then click on **Logout** in menu to logout from computer

## 11. Calibrations

### Calibrating the $^1\text{H}$ 90 Degree Pulse

#### **- Method 1 for determining high power 90° observe $^1\text{H}$ pulse:**

1. make sure your sample is in, the probe is tuned and your sample is locked and shimmed
2. run the macro "**h190**" which will automatically acquire and process a single scan spectrum
3. run the macro "**p1see**" and input a pulse value expected for 360° pulse (should be about 4\*(default p1 value) ) if signal appears negative, pulse is too short and repeat step 3 with a longer pulse length

if signal appears positive, pulse is too long and repeat step 3 with a shorter pulse length

repeat until null signal is obtained; 90° pulse = (p1/4)

#### **- Method 2 for determining high power 90° observe $^1\text{H}$ pulse:**

1. make sure your sample is in, the probe is tuned and your sample is locked and shimmed
2. run the macro "**h190**" which will automatically acquire and process a single scan spectrum
3. zoom ~0.5 ppm about a signal in spectrum using the **dp1** button

4. run the macro "**paropt**" – answer that p1 is to be varied and input a starting value, increment and # of p1 values to be tried (try to cover a series of p1 values that are close to  $360^\circ$  pulse (should be about  $4 \times$  (default p1 value) ).
5. examine resulting plot to determine where null is achieved and calculate  $90^\circ$  pulse =  $(360^\circ \text{ pulse}/4)$

## Calibrating the $^1\text{H}$ Carrier Frequency

1. make sure your sample is in, the probe is tuned and your sample is locked and shimmed
2. run the macro "**h190**" which will automatically acquire and process a single scan spectrum
3. go into the UTILITIES menu by clicking on the **utilities** button
4. click on **O1** and use the mouse button to position the cursor at the top of the water signal then press the middle mouse button.
5. type "**o1**" and record the o1 value – this is the carrier position of water.
6. click on the **return** button to exit out of the utilities menu

## **APPENDIX A: Probes Available for DRX300**

The following is a list of probes available for use in the DRX300 – if you would like to use a specific probe for your experiments, please see the staff to have it put in the instrument at some convenient time.

- a. **5mm BBI z-gradient probe** is a “multinuclear inverse” probe that has two coils; an inner coil tuned to proton and an outer coil that is capable of being tuned to and observing nuclei ranging from  $^{109}\text{Ag}$  to  $^{31}\text{P}$  (see Table of Nuclei at end of this guide to see what nuclei fall in this range).

$^1\text{H}$  sensitivity ~ 239:1

$^{13}\text{C}$  sensitivity ~ 80:1

the sample concentration should be > 1-5mM for routine 1D proton experiments, >50mM for routine 2D proton experiments including  $^1\text{H}\{^{13}\text{C}\}$ -HMQC/HMBC and >100mM for routine  $^{13}\text{C}$  spectra.

minimum sample volume in normal nmr tube ~ 600 ul

minimum sample volume in Shigemi tube ~ 300 ul

The advantages of this probe are its high proton sensitivity, water suppression performance and range of nuclei that can be studied.

The disadvantages of this probe are that  $^{19}\text{F}$  cannot be observed without recabling (see staff for info) and that the broadband coil has to be retuned for each nucleus.

b. **5mm QNP z-gradient probe** is a “quad” probe that has two coils; an inner coil tuned to  $^{19}\text{F}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$  and an outer coil that is tuned to proton.

$^1\text{H}$  sensitivity ~ 153:1

$^{13}\text{C}$  sensitivity ~ 100:1

the sample concentration should be >1-5mM for routine 1D proton experiments, >50mM for routine 2D proton experiments including  $^1\text{H}\{^{13}\text{C}\}$ -HMQC/HMBC and >100mM for routine  $^{13}\text{C}$  spectra.

minimum sample volume in normal nmr tube ~ 600 ul

minimum sample volume in Shigemitsu tube ~ 300 ul

The advantage of this probe is its ability to switch from  $^{13}\text{C}$  to  $^{31}\text{P}$  to  $^{19}\text{F}$  without retuning.

This probe is recommended for  $^{19}\text{F}\{^1\text{H}\}$  and  $^1\text{H}\{^{19}\text{F}\}$  work and work that may require frequent switching between broadband channels (eg. a kinetic study involving observation of  $^{13}\text{C}$  and  $^{31}\text{P}$  at various timepoints).

c. **10mm BBO probe** is a “multinuclear” probe that has two coils; an inner coil that is capable of being tuned to and observing nuclei ranging from  $^{109}\text{Ag}$  to  $^{31}\text{P}$  (see Table of Nuclei at end of this guide to see what nuclei fall in this range) an outer coil tuned to proton for proton-decoupling.

$^{13}\text{C}$  sensitivity ~ 320:1

minimum sample volume in normal nmr tube ~ 2.5 ml