Seton Hall University

500 MHz NMR

SOP manual

NMR Information:

Varian Inova NMR
^{1}H Frequency = 500 MHz
Original purchase date: August 2001
System upgrade: 2008 to three channels

To schedule NMR time on the 500 or to check availablility go to:
http://academic.shu.edu/chemistry/calendars/CIS/

Username: guest
Password: abcd

Version: November 8, 2011

Note 1: the work station was recently changed and the main NMR program was switched to VNMRJ. The instructions in the SOP were developed for the VNMR1 program.

Note 2: the optimized shim file is called bestshims and is current as of 25 September 2011.

Table of Contents:

<u>NMR Cooperative Maintenance and Use Program:</u> ***anyone who wishes to use the either NMR must participate in a training program, contribute to the maintenance of the instruments/facility and contribute to the advancement of the instrument.***

Each Time:

1. **Check when the NMR was last filled. It is generally good to wait several hours after a fill before running high quality spectra.**
2. **Read the N_2 and He boil off and fill levels and record in white binder.**
3. **Walk around the magnet, checking each of the air legs and ensure that the pressure leveling pads are in place, and the legs are inflated.**
4. **Record your experiment in the hardbound notebook**
5. **Run experiment!**
6. **Return Dummy (H_2O in D_2O) sample, lock, shim, take 1H and make sure NMR is left in good working condition.**

Routine problems: consult your faculty advisor or an expert user. If problem persists, contact Prof. Sowa (200) or Profs. Murphy/Sabatino (500).

Emergency problems contact: Prof. Sowa (973)738-2886, and/or, Prof. Murphy (973)714-1715, Prof. Sabatino (973)255-8062.

Safety Precautions

Hazards associated with maintenance and use of an NMR instrument are described in the university's Chemical Hygiene Plan. In summary, there are three main hazards to NMR experimentation:

a) exposure to strong magnetic fields

The NMR is a strong, superconducting magnet. If you have a pacemaker, it is suggested that you stay beyond the 10 gauss line which is at least 10 feet away from the magnet. Generally all objects that are easily attracted to a magnet such as metal tools, keys, chairs should be kept beyond the 5 gauss line which is at least 5 feet away from the magnet. In addition, credit cards with a magnetic strip, computer drives, cell phones, computer disks and flash drives should be kept at least 5 feet away.

b) exposure to cryogenic inert gasses of helium and nitrogen

Cryogenic gasses are hazardous for two reasons. First, the extreme cold is enough to immediate freeze skin tissue and can have the effect of a severe burn. People who routinely fill the NMR with liquid nitrogen should be aware of this hazard and wear safety glasses, gloves and protective clothing. Second, these gasses displace air. In the case of a sudden release and accumulation of these gasses, suffocation can occur. The room is equipped with an oxygen sensor which monitors oxygen levels in the room and beeps if the levels are unsafe. All users should be aware of the potential of a magnet quench. This is a catastrophic event that causes the helium and nitrogen gas to suddenly release from the magnet. It may be preceded by a loud bang and evacuation of N_2 and He gas. If this event occurs, immediately evacuate the room and notify the Lab Safety Officer, David Edwards and any of the faculty in charge of the NMR.

c) NMR tubes

NMR tubes can easily break in one's hands when they are loaded into the spinner. Use proper caution when you place the tube into the spinner. Tubes that are cracked at the rim are especially susceptible to breakage and it is recommended that these be discarded. Tubes may also break due to chemical events inside the tube such as pressure build-up due to gas release or a reaction exotherm. Thus, be aware of the chemical hazards associated with your experiment.

Style and Contribution Comments

This manual is intended to assist all users of the 500 MHz NMR with helpful procedures or routine and advanced experiments. Contributions and updates are welcome!

Style comments:

Underlined commend, e.g., Acquire means that a button needs to be selected in either the upper or lower menu bar.

Italics, nt=4 means that a command needs to be entered in the command line.

Contributions:

Please submit your typed contributions to Wyatt Murphy so that they may be entered into the master manual.

You may also directly write hand-written contributions into the manual and these will be periodically checked and updated.

Reserving NMR Time

NMR time is reserved using the internet based NMR scheduling program found at the following URL:

To schedule NMR time on the 500 or to check availablility go to:
http://academic.shu.edu/chemistry/calendars/CIS/

Username: guest
Password: abcd

Please note that users with reservations supersede walk-up users. If someone has reserved times via the online scheduling program, you are required to stop your experiment and remove your sample. To be sure you have time on the NMR, please use the scheduler to avoid conflicts. The only exception to this is if the NMR needs immediate servicing by Dr. Sabatino or Dr. Murphy. Normal services such as cryogen fills and calibration will be scheduled via the online scheduler.

Sample Preparation

A) NMR tubes:

a) *Caution! Do not use tubes that are cracked or chipped, especially at the top. These have a tendency to break while inserting or ejecting into the NMR probe resulting in contamination of the probe.*
b) Approved NMR tubes are carried by the SHU stockroom. Use a 6" 506 PP or 8" 507 PP brand or equivalent, e.g. a tube that is rated for the spectrometer frequency as follows (http://www.sigmaaldrich.com/analytical-chromatography/spectroscopy/learning-center/nmr-spectroscopy/nmr-tubes.html):

200 MHz: Wilmad (506-PP, 507-PP, 528-PP)
500 MHz: Wilmad (528-PP, 535-PP, 545-PP)

For New ERA NMR Tubes (http://newera-spectro.com/NewEraNMRcatalog.pdf):

MHz	General Application	Sample Tubes
100 - 300	Routine organic chemistry Educational applications	NF-L15-, NF-L7S-
300 - 400	Routine organic chemistry Educational applications Routine synthetic chemistry research High throughput	NL-ML5-, NL-MP5
400 - 500	Routine synthetic chemistry research High throughput	NF-HL5-, NF-HP5-
500 - 700	Organic chemistry research Metabolic mixture analysis (biofluids) High throughput	NF-UL5-, NF-UP5-
700 - 900	Structural biology, Metabolic analysis Multi Purpose research	NL-SL5-, NL-SP5

c) A dry and proton free NMR tube is helpful for ^{1}H NMR analysis. Rinse a clean NMR tube with a few drops of D_2O. This will exchange any surface –OH groups with deuterium. Place tube in an oven set at 120 – 130 °C for at least 1 h. Remove the tube from the oven and immediately cap with a rubber septum or a polyethylene cap until the tube has cooled to room temperature.

B) Sample amounts:

1) You only need 0.75 mL of a suitable deuterated NMR solvent. Don't waste expensive solvent!
2) ~ 5 mg is needed for a routine ^{1}H NMR spectrum.
3) ~ 30 mg is needed to a routine ^{13}C NMR spectrum.

C) Deuterated solvents:

The SHU stockroom stocks most of the common NMR solvents. For organic compounds, $CDCl_3$ and acetone-d_6 are the least expensive solvents. Sometimes, $CDCl_3$ can be difficult to lock. Solvents stored in 0.75 to 1.0 mL ampoules are the most are quite convenient to use as there purity and dryness is very reliable. Solvents stored in larger, screw capped containers should be stored over molecular sieves and housed in a desiccators to maintain dryness. As

counterintuitive as it may seem, this includes D_2O. It is highly recommended that you use TMS as an internal reference. This give you a high quality resonance to shim upon, as the solvent peak may be obscured by your sample resonances.

Logon/logoff

To logon:

1) Enter your name, date and sample information into the 500 NMR notebook.
2) Logon: enter your ID
3) Password: enter your password (**you must have a password on your account!**)
4) In menu bar, click on icon that shows graphic of a VNMRJ spectrum. You are now logged-on!

To logoff:

1) Write any comments/observations about performance of instrument in notebook.
2) In command line type: *exit*
3) In menu bar, log off your account
4) You will be asked a question to save changes and complete exit routine. Click on Yes. You are now logged off.

Screen security lock:

During long-term acquisitions, the computer will go into security lock. To use the software, reenter the password.

Ejecting and Inserting NMR samples:

1) *Caring for the spinner:* A clean and well preserved spinner will ensure that all NMR tubes spin properly. Make sure your hands are clean and free of dirt and grease (nitrile gloves are recommended). Handle spinner only on the upper black band. After loading a new sample, wipe spinner clean with lint-free paper towel.
2) While in VNMRJ, press EJECT.
3) Carefully remove the DUMMY sample from the spinner. Do not break the dummy sample! The DUMMY sample is a sample of D_2O with a green cap. Put the DUMMY sample in the tube rack on the console.
4) Carefully insert your sample tube into the bore of the spinner. When you do this, do not hold your NMR tube on the far end as this will create too much leverage and may cause the tube to break. Hold your tube in the middle while carefully inserting it into the bore of the spinner. While you're doing so, do not lean against the NMR. This will cause the level regulator to walk out of position and deflate the air leg opposite to the steps. Wipe spinner and tube with a lint-free paper towel.
5) Adjust the height of the sample using the brass sample adjuster device. Note that the sample depth must not be below the indicated level. Also, the entire volume of the sample must be within the shaded region on the brass sample adjuster. Also you should not add more solvent than needed to clear the top level of the dotted box. More sample will yield poorer spectra.
6) While in the EJECT mode (air blowing out), carefully place sample in the bore of the magnet.
7) Press INSERT. You will hear a soft "kerplunck" indicating sample has been properly inserted.
8) Note that the sample will not automatically be spinning as you should shim in nonspinning mode. In fact, most of the techniques for which you will be trained will NOT allow spinning.
9) Check that the sample temperature (VT) is approximately 30 ^{o}C. This is to minimize thermal gradients in high power PFG experiments.
10) When you experiment is complete, return the DUMMY sample to the probe. Lock, shim and take a 1H spectrum. Inspect the spectrum which is a singlet of residual H_2O at 4.79 ppm. Make sure that you are leave the spectrometer in good shape for the next person!

Stuck spinner:

If the spinner has been inserted into the magnet without a sample, it will not eject. You will need to increase pressure at the main line to 50 psi and then eject. Please return the pressure to 40 psi. If you are uncomfortable doing this, please contact Dr. Sabatino or Murphy.

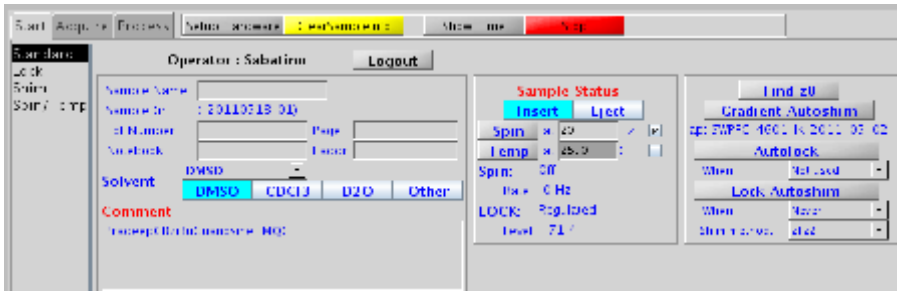
Note: If a sample has been broken in the magnet bore, you must inform Dr. Sowa, Murphy or Sabatino immediately as this problem must be immediately addressed. We will not get angry if you tell us, but we will if you leave the problem for someone else to find!

Acquiring a ^{1}H NMR Spectrum (vnmrj)

1) Enter name, date, sample information and spectrometer condition into the notebook.
2) Logon and insert sample into the magnet.
3) Click on Study and enter sample information.
4) Select proton and solvent from the experimental selections.
5) Read in latest shim file: type *rts*, enter *bestshims*.
6) Type *su* to setup hardware, and then type *gmapsys*. To load a shim map, cd to sysdir
7) Tune probe. ***Caution****, if you are not familiar with the step, in most cases, you can skip it and still obtain a good spectrum. Overturning the capacitors can lead to serious damage of the probe.*

   For tuning ^{1}H on the 500 SW/PFG probe
   - Take proton cable from ^{1}H filter (right side of tune panel box) and place it in tune interface (J5321)
   - Set CHAN = 1
   - ATTEN = 8
   - Minimize proton (red) tune stick to approx. 0

   Note: Turn tune (long) + matching (short) in opposite directions starting with tune)
   - Once optimized, turn CHAN = 0
   - Return cable to 1H filter and begin acquiring 1H expt.



8) Find z0. First try the Find z0 button.
   If the instrument is in good calibration, this will work well.
9) Find z0 manually. Make sure lock is off. Click on LOCK SCAN.
   a) Increase power to 40-45 and gain 40.
   b) Note the initial z0 value. The goal is to Adjust z0 so the wave form decreases in frequency and eventually forms a plateau as shown below:


i) High frequency far from z0

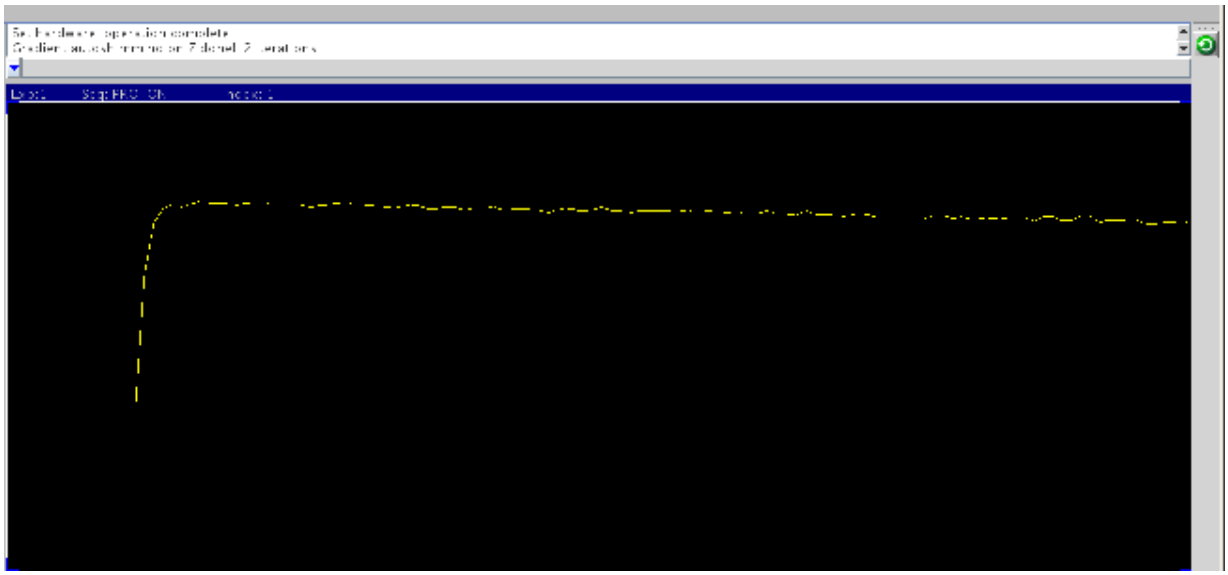

ii) Low frequency, close to z0


iii) plateau, at z0

   c) While adjusting z0, you will need to adjust the signal strength. Do this by increasing the transmitter power (power) and the receiver gain (gain). When the power signal is too high, the lock signal quickly bounce up and down. You will also see the red RCV OVFL light blink or stay lit on the repeater box on the desk next to the monitor. You

need to make sure that the RCV OVFL light is turned off, as no usable data can be obtained under these conditions. When this happens, lower the power. If the signal is very noisy, lower the gain. If the signal reads > 99 %, lower the power and gain to ~50 – 70 %.

Image of locked deuterium signal



d) Typical z0, power and gain values are found in the table below: (please contribute!)

Solvent	***z0***	***power***	***Gain***	***Phase***
Acetone-d_6				
CD_2Cl_2				
$CDCl_3$	-3580	39	42	202
Methanol-d_4				
D_2O	-7420	27	33	205

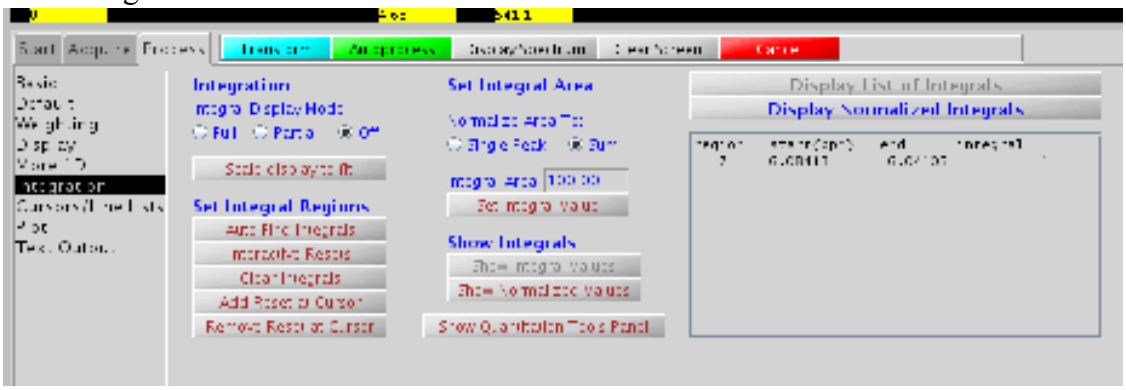
10) Click on Gradient AutoShim (or type *gmapshim*). This shims the sample. You should not adjust individual shim buttons! The program is set to stop optimizing the shim after five cycles. If this occurs, re-optimize the z0, the gain and the power, and Gradient Autoshim again. Occasionally, the AutoShim routine will choose values that lose the lock. Simply start over with *bestshims*. Patience is essential in achieving an optimal shim.
11) Re-optimize z0 and the lockphase while lock is regulated.
12) Click spin ON and regulate speed to 20 Hz
13) Default transients is 8. Type *nt = x* to increase transients. Increasing in factors of 4 is recommended (4, 8, 16, 32, etc.).
14) Default relaxation times d1=1 (set d1=2 for better sensitivity)
15) Type *ga* to start acquisition.
16) Type *aph* to phase spectrum.
17) Set full scale or type, f full

18) vp = 12; adjust vertical phasing
19) expand peak region at 0 ppm (TMS reference peak in $CDCl_3$, reference solvent peak in absence of TMS)
20) nl
21) In default; set Reference Cursor to 0.00 ppm
22) In basic menu; click on display spectrum
23) For integration:

- Expand area of interest
- Click on Integration
  - Partial
  - Interactive reset (and left click on both sides of selected peak)
  - Integration OFF

Note: expand next region and continue as previously described

24) Calibrate integration by selecting and expanding a known peak
25) nl
26) set integral area to:
  - single peak
  - integral area 1.00



  - set integral value
  - display list of integrals
  - remove integrals and full scale integrals

27) click on threshold to set threshold scale
28) text ('name') or type it in the comment box
29) svf ('name') click on save button and enter name for your fid file
30) Optional: dpir – to display integration
31) Optional: dpf – to display chemical shift
32) automatic plot page or pl pscale pir ppf ppa page (to plot integration and chemical shift values, scale, parameters on same page)

Note: expand regions and plot chemical shifts and integration values

  - pl pscale pir ppf ppa page

Helpful Hints for ^{1}H NMR Spectra:

#	Hint	Initial/date
1	To check if z0 stable, while the signal is at a plateau, turn lock ON, then OFF. If signal reads "Regulated" the Z0 is stable.	DS/10/24/11
2	If you've made a mistake with the integration, type cz and start over	DS/10/24/11
3	Type axis='h', to convert chemical shift values to Hertz and axis='p', to return chemical shift values to ppm	DS/10/24/11
4	To set-up new expts (for sequential acquisition of multiple expts) click File, New workspace and take note of location of new expt. Type jexpn, where n=1,2,3,4…..to join the expt	DS/10/24/11
5	For samples that are very concentrated in with protons such as samples intended for 13C analysis, you will often get an ADC overflow error. If this occurs, reduce the pulse width to 1 (type *pw=1*).	DS/10/24/11
6	To abort acquisition type *aa*.	DS/10/24/11

Acquiring a ^{13}C NMR Spectrum – Proton decoupled.

1) jexp2
2) In the experiment panel select Carbon
- Select deuterated solvent (i.e. $CDCl_3$)
- su or click Set up Hardware in the Acquire tab

3) For tuning ^{13}C on the 500 SW/PFG probe. ***Caution****, if you are not familiar with the step, in most cases, you can skip it and still obtain a good spectrum. Overturning the capacitors can lead to serious damage of the probe.*
   - Ensure counter is set to 31 – 32 on the X - channel
   - Take X cable from probe (J5311) and place it in tune interface (J5321)
   - Set CHAN = 1 (decouple 1H in channel 2)
   - ATTEN = 8
   - Minimize X channel (green) tune stick to approx. 0

   Note: Turn tune (long) + matching (short) in opposite directions starting with tune
   - Once optimized, turn CHAN = 0
   - Return cable to filters on Probe (J5311)

4) Lock and shim as previously described

Note: Not necessary to repeat for same sample

5) Modify accordingly no. of scans nt, by increments of 4 (note: nt = 256 for 50 mg / 0.7ml)
6) Default relaxation times d1=1 (set d1=2 for better sensitivity)
7) ga or click Acquire (highlighted green)

Note: wft (to observe spectra after block segments of acquisition are complete)

8) aph (or alternatively click on autophase full)
9) expand $CDCl_3$ solvent triplet signal @ 77ppm or TMS @ 0.00ppm
10) nl
11) reference cursor to 0.00ppm
12) f full
13) adjust threshold – Th
14) text ('name') or type it in the comment box
15) svf ('name') click on save button and enter name for your fid file
16) Optional: dpf, to display frequency on spectrum
17) Automatic plot page
18) Expand
19) pl pscale ppf pap page

Once complete:-

20) Eject sample
21) Replace with dummy sample (green NMR cap ~ D_2O)
22) Turn off the decoupler! The decoupler used in this experiment generates heat and can also burn-out if left on for long periods of time. It is essential to turn it off. To do this, simply run a ^{1}H NMR spectrum.

Acquiring a ^{1}P NMR Experiment

1) jexp3
2) In expt. panel, select phosphorus (in std 1D panel)
3) Select deuterated solvent (i.e. $CDCl_3$)
4) su or click Set up Hardware in the Acquire tab

- For tuning 31P on the 500 SW/PFG probe
  - turn counter to 22 using the X-channel tune stick
  - place capacitor for 31P into the open slot of the probe

Note: In the plastic tube labeled P004601 use capacitor stick with blue label = 13T

- Screw in capacitor stick into empty hole of SW probe (screw in CCW)
- Remove x-channel cable from Probe (J5311) – tune interface (J5321) (loosen CW)
- Replace ¼ wavelength cable with 31P ¼ wavelength cable (~202MHz)
- Set to CHAN = 1(note: decouple 1H in channel 2)
- ATTEN = 8
- Minimize X channel (green) tune stick to approx 0

Note: Turn tune (long) + matching (short) in opposite directions starting with tune)

- Once optimized, turn CHAN = 0
- Return cable from the tune interface (J5321) to filters on Probe (J5311)

5) Lock and shim as previously described

Note: Not necessary to repeat for same sample

6) Modify accordingly no. of scans (nt by increments of 4)
7) ga or click Acquire (highlighted green)
8) f full or click on full spectrum
9) aph, autophase
10) vp = 12, to adjust vertical phasing
11) click on process
12) integration
    - partial
    - interactive resets and click on both sides of the peak to determine ratio
13) th (set threshold to limit)
14) text ('name') or type it in the comment box
15) svf ('name') click on save icon and enter name for your file
16) pl pscale pir ppf ppa page

Once Complete

17) return ¼ W 31P cable for ^{13}C cable (120 – 170MHz)
18) remove ^{31}P capacitor (13T blue lable and return to P004601 plastic tube
19) return X Channel counter to ^{13}C = 32

Acquiring a homonuclear COSY, NOESY, and TOCSY Spectrum

- Acquire ^{1}H NMR in exp 1

Note: ensure to follow procedure for tuning, locking, shimming to obtain a well resolved 1D 1H NMR spectrum

- Save fid file (Leave it open in workspace 1, exp 1)

gCOSY exp – (^{1}H – ^{1}H through bonding coupling – 3 bond length distance)

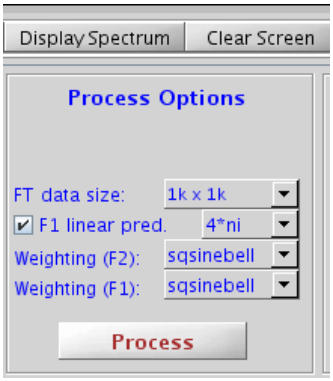
1) jexp4
2) select solvent, click spin off
3) In experiment panel select (HH gCOSY)

Note: Observe pulse sequence for expt and setup expt

4) dps dg, displays pulse sequence and group
5) su, to set-up hardware hardware

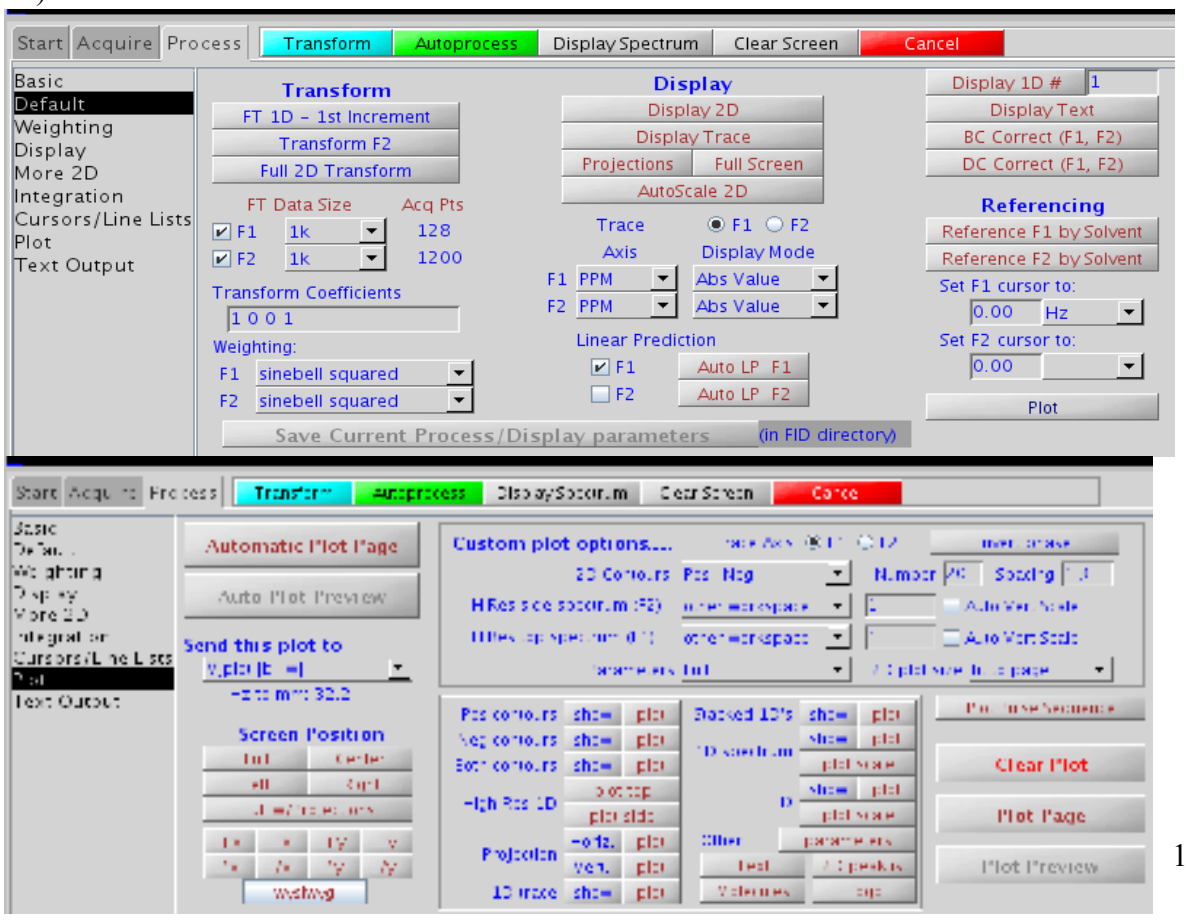
Under Acquire tab

6) d1= 1(ensure delay time = 1)
7) nt = 1 (No. of transients, i.e. scans) Increase nt = 4 for better resolution)
8) ni = 128 (No. of integration, points collected in spectra)
9) go (acquire spectrum, approx. 3 min)



In Process tab

10) ensure F1 linear pred. is on (4 x ni)
11) click on process (Note display of 2D spectrum)
12) set weighting (F2): sqsinebell, set weighting (F1): sqsinebell
13) go to plot – plot option set HiRes side spectrum (F2) = workspace 1
14) text ('name') or enter name in comment area (F1) = workspace 1
15) automatic plot page
16) save fid in folder



TOCSY expt. (^{1}H – ^{1}H same spin system coupling)

1) jexp5

In the expt. Panel (chempak), select TOCSY (in HH Homo 2D)

2) dps dg
3) su

Set in Acquire

4) d1 = 1 (delay time = 1)
5) nt = 2 (No. of transients, i.e. scans) Increase nt = 4 for better resolution
6) ni = 200 (No. of integrations, i.e. points collected in spectra)
7) keep spinlock duration, i.e. mixing time @ 80ms (i.e. between 50 – 100 msec) or mixN = 0.08
8) go (acquire spectrum, typical acquisition time = 17 min, set nt = 1 for shorter expt. i.e. 9 min)

In process box

9) ensure F1 linear pred is ON (2 x ni)
10) set weighting (F2): sqcosine, set weighting (F1): sqcosine
11) click on process (note display of 2D spectrum)
12) go to plot – custom plot options and set HiRes side spectrum F2 = other workspace 1
HiRes side spectrum F1 = other workspace 1
13) text ('name') or enter name in comment area
14) automatic plot page
15) save fid in folder
16) click on box (right side panel), select region and expand
17) automatic plot page

ROESY/ NOESY expt. (^{1}H – ^{1}H transfer magnetization through space coupling)

1) jexp6

In expt. panel, select NOESY or ROESY (in HH Homo 2D)

2) dps dg
3) su

Set in Acquire

4) d1 = 1 (set d1 = 2 for better sensitivity) – relaxation time
5) nt = 2 (No. of transients, i.e. scans) Increase nt = 16 for better resolution
6) ni = 200 (No. of integrations)
7) mixN = 0.2 (NOE mixing time = 200 msec, set anywhere from 0.05 – 0.5 for NOESY expt.)
8) go (data acquisition, approx. expt time 40 min)

In process box

9) ensure F1 linear pred is ON (2 x ni)
10) set weighting (F2): sqcosine (use Gaussian for better resolution at risk of sensitivity)
11) set weighting (F1): sqcosine
12) click on process
13) go to plot – custom plot options and set HiRes side spectrum F2 = other workspace 1
HiRes side spectrum F1 = other workspace 1

2D Contours: Pos Only

Display: F1 phased and F2 phased

14) text ('name') or enter name in comment area
15) automatic plot page
16) save fid in folder
17) click on box (rightside panel), select region and expand
18) automatic plot page

Measuring Pulse Width
(This is important for obtaining NOESY and other advanced spectra)

1) Run a ^{1}H NMR experiment.
2) Locate a well resolved peak.
3) Set *nt=1*.
4) Set relaxation delay *d1=5* (i.e., T1 relaxation time = 5 sec.)
5) Type *gain='y'*.
6) Find current pw90, type *pw90?*. As of 1/15/09, the value is 9.5 μs.
7) Type *array* and submit the following entries: type of array = *pw*, number of steps = *10*; starting with *9.5*; increments *3*. This will enable an array on the pulse width starting at a value of 9.5 μs an increasing in steps of 3 μs for a total of ten steps. Values may be changed as needed.
8) Type *ga ai*.
9) When acquisition is complete type wft dssh dssl to process and display entire series of spectra.
10) The initial spectrum (step 1) should be result in a positive peak and corresponds to pw90. The next spectrum which is closest to an inflection point is the pw180. The pw270 is negative and pw360 is an inflection point.
11) You may wish to narrow in on the exact value of pw180 by running an array with shorter steps around the pw180 step (e.g, start at 16 μs, with 0.5 μs increments). The pw90 is ½ of the pw180 value.
12) Once you have found the pw90, you can run an array based on pw90 with 4 steps and in increments equal to the value of pw90. This should show a nice sequence of the effects of pw90, pw180, pw270, pw360.

Notes:

Heteronuclear HMQC / HSQC and HMBC ^{1}H – ^{13}C correlation

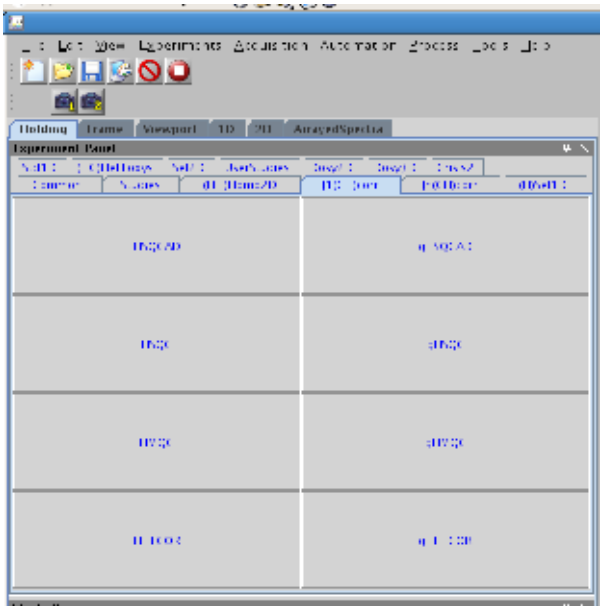
- Acquire ^{1}H NMR in exp 1

Note: ensure to follow procedure for tuning, locking, shimming to obtain a well resolved 1D ^{1}H NMR spectrum

- Save fid file (Leave it open in workspace 1, exp 1)
- Acquire ^{13}C NMR in exp 2

Note: ensure to follow procedure for tuning, locking, shimming to obtain a well resolved 1D ^{13}C NMR spectrum

- Save fid file (Leave it open in workspace 2, exp 2)



HMQC or HSQC expt. (^{1}H – ^{13}C direct bond correlation)

1) jexp7
2) in expt. Panel in Tab J1(CH)corr select gHMQC
3) dps dg, to display pulse sequence and group
4) su, to set-up hardware

Set in acquire

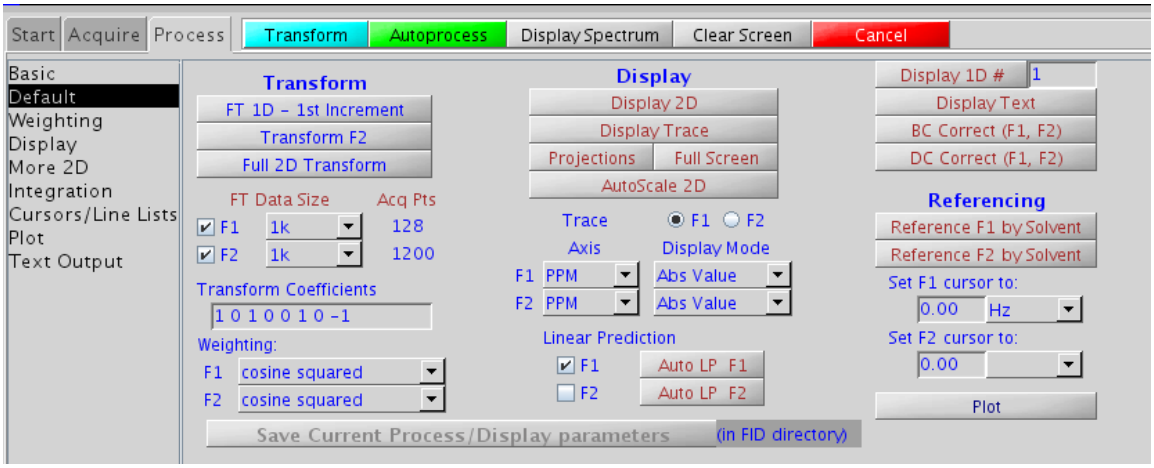
5) C^{13} spectrum -10.0 to 200ppm (alternatively, you can keep track of the spectral width, sw =? in the ^{13}C NMR experiment and use it in the HMQC)
6) nt = 1, increase to 2 or 4 for better sensitivity
7) ni = 128
8) d1 = 1

Note: j1 x h = 140 (hydrocarbons); 155 (aromatics); 160 (carbohydrates)} for coupling ^{1}H-^{13}C

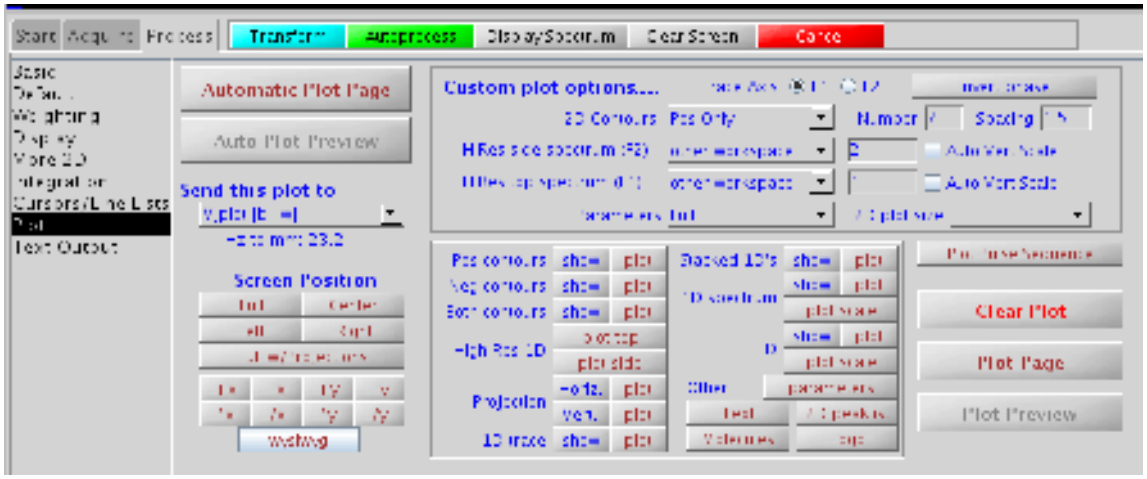
9) su
10) go (acquire spectrum => time of expt ~ 5mins)

In process box

11) ensure F1 linear pred is ON (4 x ni)
12) set weighting (F2): sqcosine => Proton weighting(use Gaussian for better resolution spectra)
13) set weighting (F1): sqcosine => carbon weighting
14) click on process
15) Display F1 and F2 in PPM Phased
16) 2D contours – positive only
17) go to plot –custom plot options and set HiRes side spectrum F2 = other workspace 1 (^{1}H)
    HiRes side spectrum F1 = other workspace 2 (^{13}C)



18) under display set F1 and F2 = Abs Value



19) text ('name') or enter name in comment area
20) automatic plot page
21) save fid in folder
22) click on box (rightside panel), select region and expand
23) automatic plot page

HMBC exp (^{1}H – ^{13}C, long range 3-5 bond coupling correlations)

1) jexp8 or click on File, and click create new workspace, note number of new workspace created

In expt. panel, in tab Jn(CH)corr select gHMBC

2) dps dg
3) su

Set in Acquire

4) nt = 4
5) ni = 200
6) d1 = 1

Note: multiple bond coupling jn x h = 8 (lower number will allow you to see 4 and 5 bond couplings)

7) su
8) go (acquire spectrum => time of expt. 30 mins)

In process Box

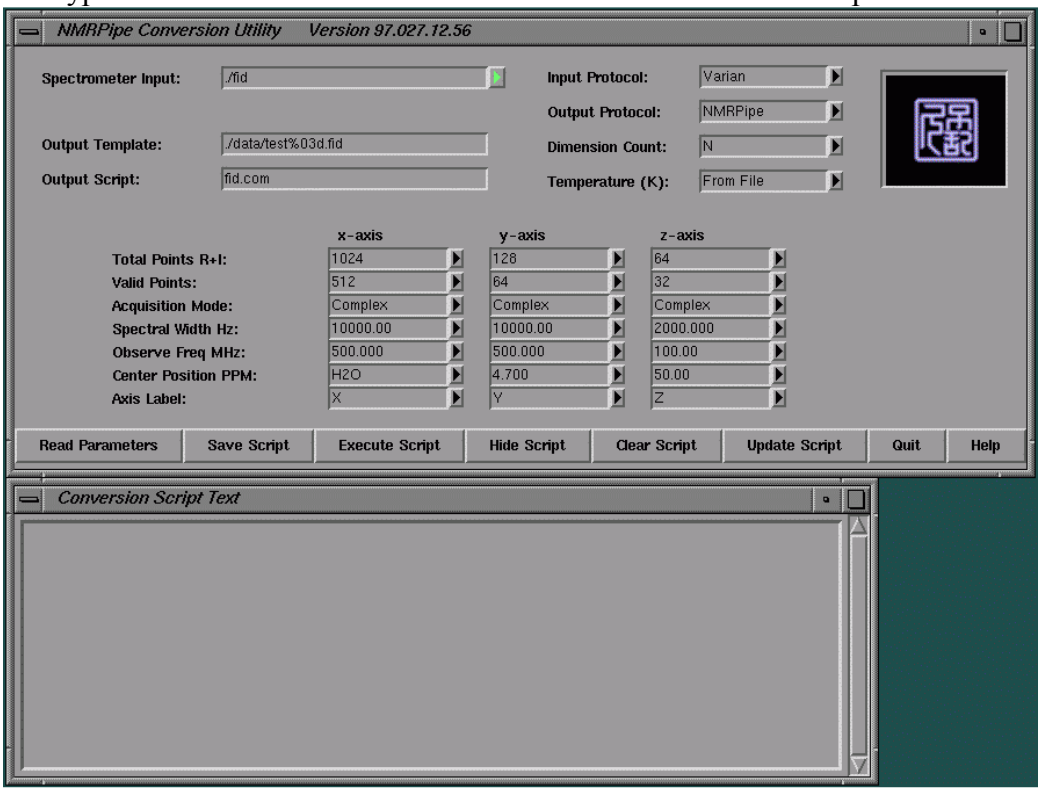
9) ensure F1 linear pred is ON (4 x ni)
10) set weighting (F2): sinebell => proton weighting function
11) set weighting (F1): sqcosine => carbon weighting function
12) click on process

Note: use Gaussian for better resolution spectra at risk of sensitivity

13) 2D contours – positive only
14) go to plot – custom plot options and set HiRes side spectrum F2 = other workspace 1 (^{1}H)
set HiRes side spectrum F2 = other workspace 2 (^{13}C)
15) text ('name') or enter name in comment area
16) automatic plot page
17) save fid in folder
18) click on box (right side panel), select region and expand
19) automatic plot page

Data Processing using NMRPipe (offline)

1. NMRPipe is a UNIX-based(Linux, MacOS, etc.) NMR spectral processing and analysis system. Installations on Windows systems are not supported. For detailed information, please check NMRPipe website at http://spin.niddk.nih.gov/NMRPipe/.
2. Transfer data: using a secure FTP client (WinSCP, Fetch, sftp, etc.) to connect to the workstation (IP: 149.150.200.1) using the PC desktop in the NMR room. Change to the data directory: /export/home/*username* (*username* is your login name).
3. Get the whole directory of the dataset to a USB flash drive, which in turn will be loaded to NMRPipe for processing.
4. Data conversion: the Varian data should be converted into NMRPipe format before it can be processed. A program facilitates the conversion process.
   a) Type "varian" in a terminal window. A window like this will open:



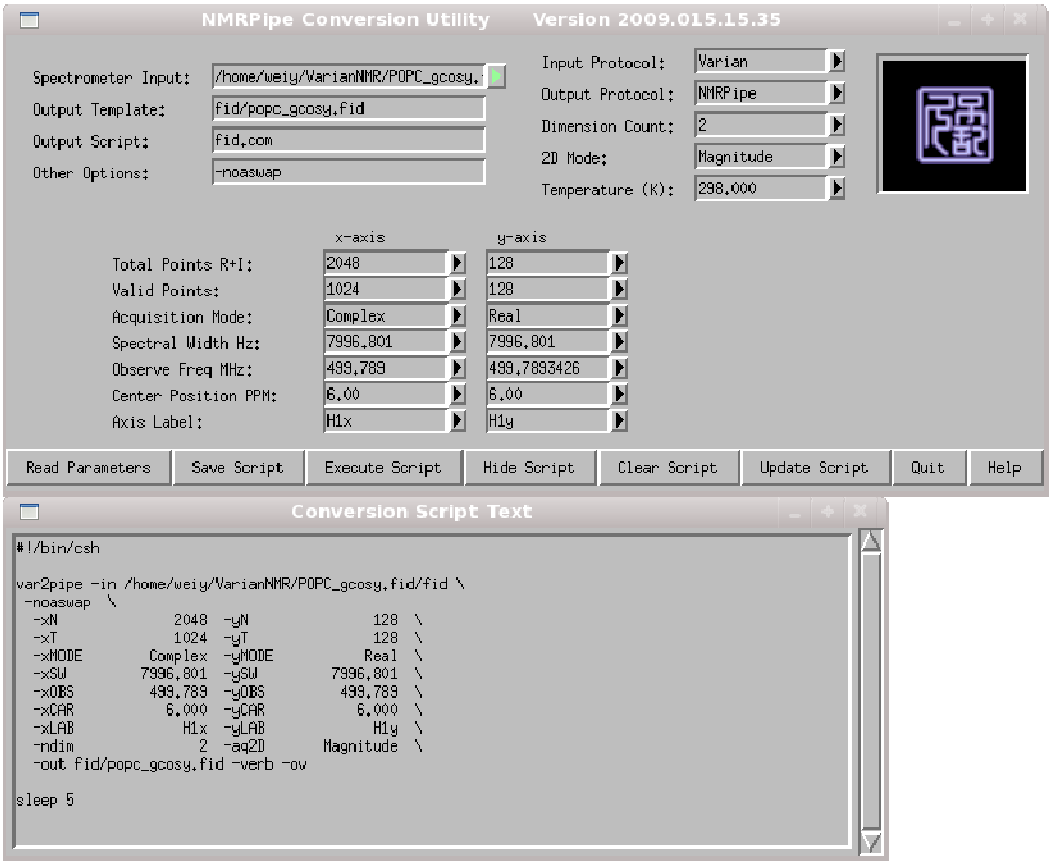
   b) In "Spectrometer Input", navigate to the data directory, and select the "fid" file.
   c) In "Output template", enter where the converted file to be saved, and filename should be appended with ".fid".
   d) Click on "Read Parameters", the program will try to locate and read automatically the raw data file, and the acquisition specific parameters will be displayed in grey selection boxes. The parameters that need user inspection and eventual re-selection or manual input are highlighted in yellow. The following parameters often require manual input
      - Dimension Count: for GCOSY, NOESY, and TOCSY, select "2D"
      - 2D Mode: for GCOSY, select "Magnitude"; for NOESY and TOCSY, select "States"
      - Observe Freq MHz: for y-axis, change to H1

- Center Position PPM: 6.00 (or other number if you have changed the spectral width in the experiments) for both x- and y-axes
- For GCOSY, “Total Points R+I” should also be changed to be the same as “Valid Points”, and “Acquisition Mode” should be “Real”

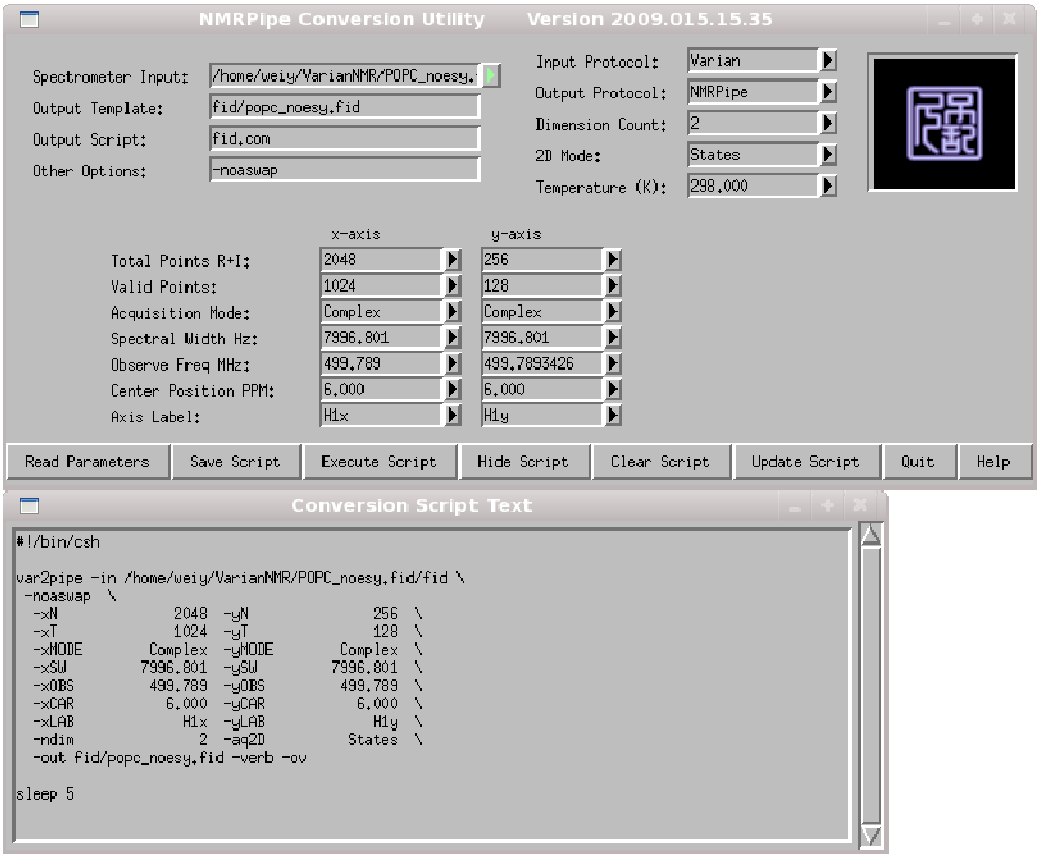
e) Now the program window should be similar to the following. Note the second window shows the conversion script. Click on “Save Script” will save the conversion script to disk, which can be run from the command line; or click on “Execute Script” to run the script immediately. For examples showing here, the converted data will be saved in the “fid” directory with name “popc_gcosy.fid” or “popc_noesy.fid”, respectively.

f) Click on “Quit” when finish.

GCOSY:



NOESY and TOCSY:



5. Data processing: NMRPipe processing is done by scripts with visual aid from the program NMRDraw.
    a) NMRDraw is a user-oriented X11 graphical interface for inspecting and processing multidimensional NMR data. Type “nmrDraw” in a terminal window to start the program. For detailed description and tutorial for NMRDraw, please refer to the NMRPipe Introductory Turotial at http://spin.niddk.nih.gov/NMRPipe/doc1/.
    b) GCOSY – magnitude processing
        - The following script (proc2dgcosy.com) will process the magnitude GCOSY data. No phasing is required in this script.

```
#!/bin/csh

nmrPipe -in fid/$1.fid \
| nmrPipe  -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -size 2048                          \
| nmrPipe  -fn FT -auto                               \
| nmrPipe  -fn POLY -auto -verb                       \
| nmrPipe  -fn TP                                     \
| nmrPipe  -fn LP -ord 8 -pred 128 \
| nmrPipe  -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -size 2048                          \
| nmrPipe  -fn FT -auto \
| nmrPipe  -fn MC \
| nmrPipe  -fn REV -di -verb \
| nmrPipe  -fn POLY -auto \
| nmrPipe  -fn TP \
```

```
| nmrPipe  -fn POLY -auto \
   -ov -out ft/$1.ft2
```

- Run the script by typing "proc2dgcosy.com POPC_gcosy". The script will read "fid/POPC_gcosy.fid" and write the processed spectrum to "ft/POPC_gcosy.ft2".
- In "NMRDraw" open "POPC_gcosy.ft2" to inspect the spectrum.

c) NOESY and TOCSY – phase sensitive processing

- The following script (proc2d.com) will process phase sensitive NOESY and TOCSY data. The x-axis (direct dimension) phase should be obtained by processing the first increment in NMRDraw, and the y-axis (indirect dimension) phase is set by the pulse program (or pull out a vertical slice and determine the phase and enter into the script)

```
#!/bin/csh

nmrPipe -in fid/$1.fid \
| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -size 2048                          \
| nmrPipe  -fn FT -auto                               \
| nmrPipe  -fn PS -p0  287.2 -p1 -245.0 -di -verb     \
| nmrPipe  -fn POLY -auto -verb                       \
| nmrPipe  -fn TP                                     \
| nmrPipe  -fn LP -ord 5 -pred 128  \
| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -size 2048                          \
| nmrPipe  -fn FT -auto                               \
| nmrPipe  -fn PS -p0    0.0 -p1    0.0 -di -verb     \
| nmrPipe  -fn POLY -auto \
| nmrPipe  -fn TP \
| nmrPipe  -fn POLY -auto \
   -ov -out ft/$1.ft2
```

- Run the script by typing "proc2d.com POPC_noesy". The script will read "fid/POPC_noesy.fid" and write the processed spectrum to "ft/POPC_noesy.ft2". Adjust the phase in NMRDraw if necessary and rerun the script.

6. Converting to "NMRView" format (optional): NMRViewJ is a java-based NMR spectral analyzing program. Its cross-platform (Linux, MacOS, Windows) feature allow all users to analyze multidimensional NMR spectra on any computer. For information and download of NMRViewJ, go to http://www.onemoonscientific.com/nmrview/summary.html. The following script (p2view.com) will convert NMRPipe format spectra to NMRView format.

```
#!/bin/csh

nmrPipe -in  ft/$1.ft2 \
|pipe2xyz -nv -out nmrview/$1.nv \
 -ov -verb
```

Data Processing Using Mnova NMR v7

- Software made available by Mestrelab Research
- Free trial version downloadable at: www.mestrelab.com

****Authorized users only****

Computer and Console Emergency Shutdown Procedure

Warning: the 500 NMR is design to run permanently. Problems have occurred when attempting to turn the instrument back on. Thus, perform this procedure only when necessary (e.g., a scheduled power outage).

To turn off:

1) If you are running an experiment, type *aa* (to abort acquisition) then type *exit* to exit the vnmr or vnmrj program.
2) On the Solaris menu bar, select EXIT.
3) On the keyboard press the cresent moon key. This will turn off the computer.
4) …
5) The computer and console is now off.

To turn on:

1) …
2) Turn on computer by pressing ON button on computer console.
3) Log into the vnmr or vnmrjprogram and test to see if computer and console are communicating.

To Reset: Authorized users only.

1) Turn off computer as above.
2) Open door on the console and find RST button. Press this button once.
3) Wait 2 minutes, then, turn on computer.

Troubleshooting

To clear out red "fail" light on Magnet/Sample Regulation board

1. Log into vnmr1, but do not launch the vnmr graphics
2. Open a shell, type "cd /vnmr/bin"
3. Type "./setacq"
4. Follow the setacq instructions: press "RST" button on the console CPU board, answer "N" to the firewall, and "2" for the Ethernet questions. It should re-download the software that will enable the console computer to boot. The CPU LEDs should flash and eventually the MSR should go green.
5. A long serial cable can be connected from the console diag port (25 pin) to the A or B serial port on the Sun. Go to /etc/remote and set hardwire to either a or b depending where you plugged it in. Open a shell and type "tip hardwire". This will display the console CPU bootup in the shell window.

dn error, anytime you get the *dn* error in an account, it means it is still locked from a previous experiment (i.e. the user didn't properly and completely log out). To fix this, log into the account but don't start VNMRJ.

1. Open a terminal shell, then enter
2. \cd vnmrsys
3. Ls
4. delete any files that say lock_#_primary
5. The account should work again.

More Experiments:

The following standard experiments are available but have not been set-up. Any volunteers?

^{1}H NMR

- T1/T2 measurements
- Variable temperature experiments
- Solvent suppression
- Saturation experiments
- 1D NOE
- 1D TOCSY

^{13}C NMR

- APT
- DEPT
- INEPT

Other nuclei

- $^{14/15}$N NMR
- ^{19}F NMR
- ^{29}Si NMR

2D NMR

-DOSY
-2D ADEQUATE and INADEQUATE
-CIGAR
-HETCOR

Liquid Nitrogen Fills

This is a very critical aspect of NMR maintenance. If the liquid nitrogen levels get too low, the liquid He will boil off rapidly and magnet will quench. This error costs many thousands of dollars to repair and months of valuable experimental time.

Checking liquid N_2 level: This must be done every Friday or Monday. Procedure: remove the cap labeled "Nitrogen Service" on the top of the magnet. Insert brown stick into the liquid nitrogen reservoir and count 30 seconds. This will cool the stick. Remove the stick and return the cap. Measure the frost level on the stick and write this into the notebook. It is considered an emergency if the N2 level is 2" or less.

Checking He and N_2 boil-off rate. This must be done once a day. Look on the left leg of the 200 magnet and locate the He and N_2 gauges. Record the level on the gauge as indicated by the floating metal ball. Write the levels into the notebook. Generally, the readings are 2 cc/h for He and 50 cc/h for N_2. If these are much higher, please contact Prof. Sowa or Prof. Murphy.

Liquid Nitrogen Fill:

Stop all acquisition experiments. Carefully roll the liquid nitrogen tank close to the 200 NMR. Attach ¾" rubber tubing to the "liquid" outlet on the tank. **Caution: the wrench you use for this is magnetic and may suddenly be attracted by the magnet. Please avoid this.** Attach the end of the tubing to the specially designed "T" metal tube and insert metal tube into the "Nitrogen Service" port. Slowly open the valve on the nitrogen tank and carefully increase flow until you can hear liquid nitrogen being dispensed. Maintain flow at reasonable rate until the nitrogen dewar in the magnet is filled, then, close the valve on the tank. Allow the rubber tubing to warm so that the rubber is flexible (you may need to carefully warm the tubing with a heat gun.) Detach the metal tube and place it back on the storage rack.

NMR Training Program

Contact Prof. Murphy to obtain initial training on the 500. This will include background information on the spectrometer and its maintenance prior to running 1D NMR experiments. Following the completion of exercises described in section A, contact Prof. Sabatino to obtain training on 2D NMR experiments and a separate set of exercises described in section B. Once students have successfully completed this training program, they may begin running experiments on the 500.

A) Complete the following exercises and submit work to Prof. Murphy for evaluation. Please print and scan each spectrum and submit report electronically.

1) Lock and shim a 1% menthol sample in $CDCl_3$. Report Z0, lockpower, lockgain, lockphase, Z1c, Z2c.
2) Take ^{1}H NMR. Report chemical shifts and any coupling constants that can be resolved. Integrate the spectrum and obtain integrations within 20 % of the expected value. Save spectrum in test folder using your name, name of experiment and date of acquisition, print and scan spectrum.
3) Take $^{13}C\{1H\}$ nmr (proton decoupled) and report chemical shifts (note; for the 500, ppm values are good to 2 decimal points). Save spectrum in test folder using your name, name of experiment and date of acquisition, print and scan spectrum.
4) Return Dummy sample, lock, shim and run ^{1}H NMR.

B) Complete the following exercises and submit work to Prof. Sabatino for evaluation. Please print and scan each spectrum and submit report electronically.

5) Tune, lock and shim a 1% menthol sample in $CDCl_3$ and take ^{1}H NMR spectrum in experiment 1. Report chemical shifts and any coupling constants that can be resolved. Integrate the spectrum and obtain integrations within 20 % of the expected value.
6) Take $^{13}C\{1H\}$ NMR (proton decoupled) and report chemical shifts (note; for the 500, ppm values are good to 2 decimal points) in experiment 2.
7) Take ^{1}H-^{1}H gCOSY in experiment 3. Process the spectrum, provide 1D NMR projections on the x and y-scale. Save spectrum in test folder using your name, name of experiment and date of acquisition, print and scan spectrum. Complete ^{1}H assignment for menthol from the COSY data.
8) Take ^{1}H-^{13}C gHMQC in experiment 4. Process the spectrum, provide 1D NMR projections on the x and y-scale. Save spectrum in test folder using your name, name of experiment and date of acquisition, print and scan spectrum. Complete ^{13}C assignment for menthol from the HMQC data. Note: Quaternary Cs are not detected in the ^{1}H-^{13}C gHMQC/HSQC

9) (Optional) Take ^{1}H-^{1}H NOESY in experiment 5. Process the spectrum, provide 1D NMR projections on the x and y-scale. Save spectrum in test folder using your name, name of experiment and date of acquisition, print and scan spectrum. Confirm stereochemistry for menthol from the NOESY data.

References

These excellent books for performing NMR experiments are available in the SHU Library:

1. *Modern NMR Techniques for Chemistry Research*, Andrew E. Derome, Pergamon Press: New York, 1987.

2. *200 and more NMR experiments : a practical course*, Stefan Berger, Siegmar Braun, Wiley-VCH: Weinheim, 2004.

3. *Modern NMR spectroscopy : a guide for chemists*, Jeremy K.M. Sanders, Brian K. Hunter, Oxford University Press: New York, 1993.

These are excellent on-line references:

1. G. A. Pearson on How to Shim an NMR Magnet (http://nmr.chem.uiowa.edu/manuals/Shimming-GAP-NMR-magnet.pdf, accessed 9/30/2011).
2. Spectral Database for Organic Compounds, http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi, accessed 9/30/2011.
3. University of Indiana NMR Facility, Training and Guides (see links): http://nmr.chem.indiana.edu/nmrblog/?page_id=2, accessed 9/30/11.

Useful articles:

1. "NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities," Hugo E. Gottlieb, Vadim Kotlyar, Abraham Nudelman, *J. Org. Chem.* **1997,** *62,* 7512-7515.

2. "NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist," Gregory R. Fulmer, Alexander J. M. Miller, Nathaniel H. Sherden, Hugo E. Gottlieb, Abraham Nudelman, Brian M. Stoltz, John E. Bercaw, Karen I. Goldberg, *Organometallics* **2010**, *29*, 2176–2179.

http://dx.doi.org.10.1021/om100106e

NMR Duties

General NMR Maintenance:

1. Room maintenance (printers, sweep floor, log books, book shelves, white board)
2. Left over NMR samples….
3. Cryogen flow meter readings (N_2(l) and He(l))
4. Cryogen level readings (200: N_2 level; 500 N_2, He level)
5. Liquid nitrogen fills
6. 200 Spectrometer Calibration
7. 500 Spectrometer Calibration (Update shim maps, Line shape test , Tune ^{1}H and ^{13}C frequencies)

8. Other_____

NMR Contributions Wish List

9. Contribute an update or correction to the NMR SOP manuals.
10. Export data from 200.
11. Set-up routines for APT, DEPT, INEPT, COSY, HETCOR for 200.
12. For 500: develop routines for multinuclear NMR (2H, 19F, 31P, …).
13. For 500: develop advanced 2-D, 3-D experiments.

14. Other_____