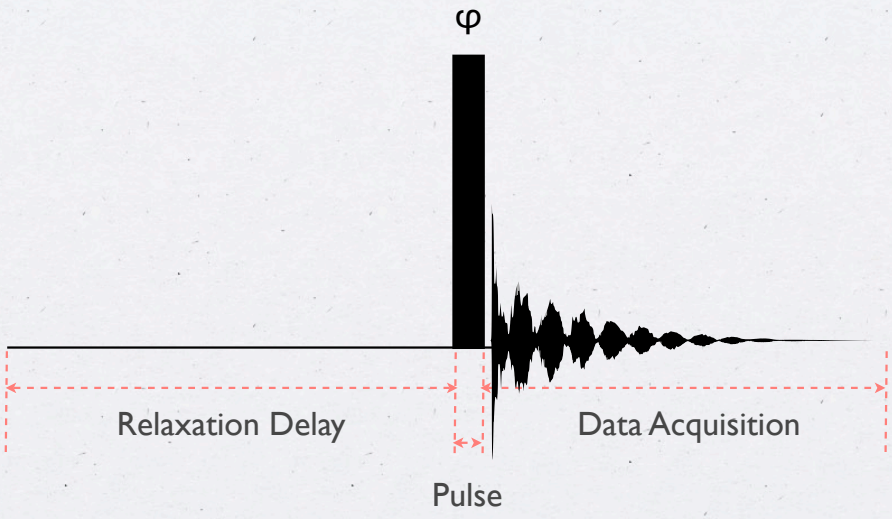
INTRODUCTORY NMR TRAINING

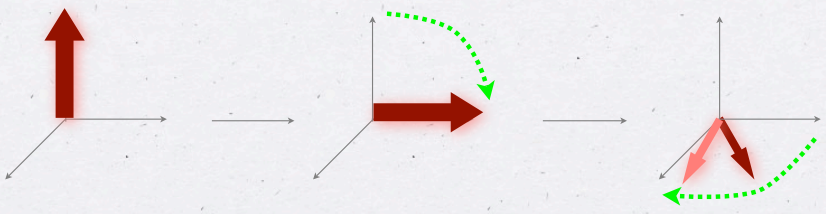
NMR Lab Safety

- ☑ No medical implants.
- ☑ No magnetic objects near the magnets.
- ☑ Empty your pockets and take off your watch.
- ☑ No sample preparations in the NMR lab.

The Simplest NMR Experiment



The Simplest NMR Experiment



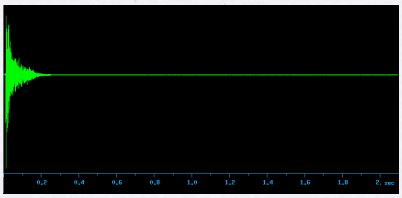
equilibrium

flip

chemical shift

and J evolution

The Simplest NMR Experiment

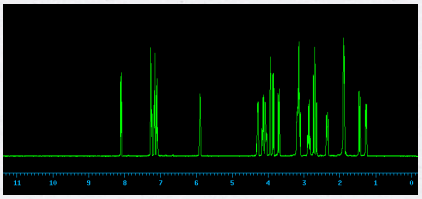


FID = Fourier Induction Decay

Fourier Transform

Inverse Fourier Transform

NMR Spectrum

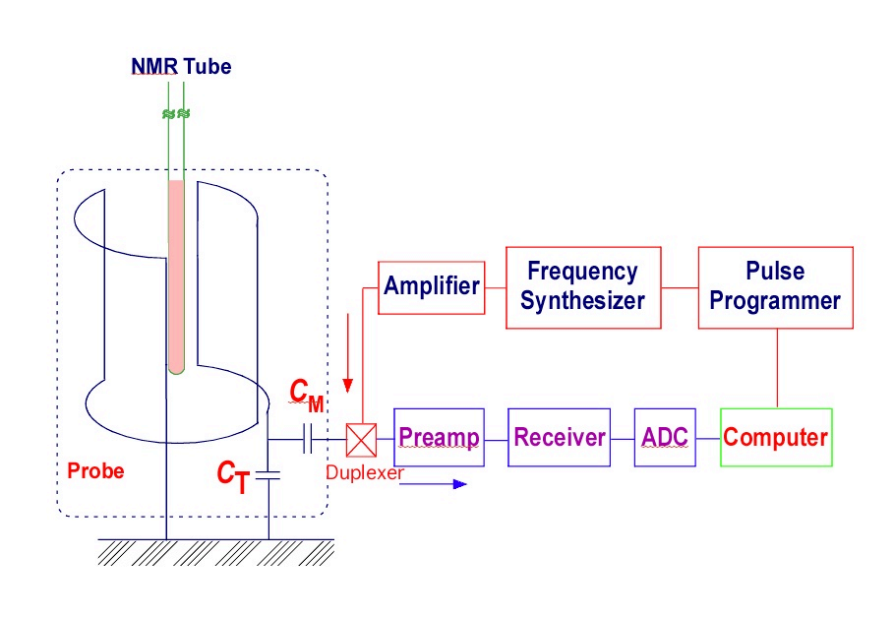


NMR Spectrometers

- Superconducting Magnet
- Probe
- Console
- Computer

(www.bruker.com)

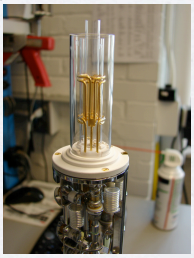
Block Diagram of A Single Channel Spectrometer



What We Have

	Bruker AV300	Bruker AV400inv	Bruker AV400dir	Bruker AV600
Probe	Auto Switchable QNP	BBI	BBO	Cryoprobe
Observable Nuclei	H1, F19, C13, P31	H1/F19, P31-Ag109	P31-Ag109, H1/F19	H1, C13, N15
Channels	Two	Two	Two	Three
VT Range	-150°-180°C	-150°-180°C	-150°-180°C	10°-60°C
Gradient	Yes	Yes	Yes	Yes
Shaped Pulses	Yes	Yes	Yes	Yes
Software	xwinnmr 3.5	xwinnmr 3.5	xwinnmr 3.5	xwinnmr 3.5
OS	Windows XP	Windows XP	Redhat Linux	Redhat Linux

Direct *vs* Inverse Detection

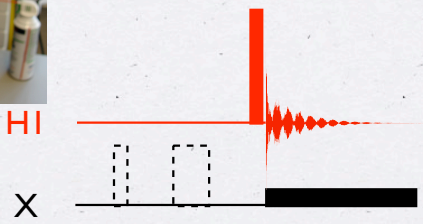


Direct Detection

(X is the inner coil)

(1D C13, DEPT, APT, ...)

Inverse Detection



(Proton is the inner coil)

(1D NOE, COSY, NOESY, TOCSY, HMQC, HMBC, ...)

Cryoprobe

- Radio frequency electronics will generate a higher signal and less noise at lower temperatures.
- Cryoprobe uses cold helium gas (11 K) in a closed loop cooling system to cool down the coil assembly and preamp.
- Around 4-6 times better in sensitivity compared to a normal probe.

Sample Preparation

- The optimum filling hight is 4 cm, corresponding to about 600 ul volume for a 5 mm tube. A minimum of 450 ul is required.
- The solvent must be deuterated.
- The solution must be clear and homogeneous.
- For a medium-sized organic compound, a few mg is sufficient for proton spectra, and a minimum of 10 mg for carbon spectra.

NMR Tubes

- Only use 5 mm 7 inch NMR tubes. Talk to NMR staff if you have to use a different tube.
- Always use clean and dry NMR tubes.
- Use appropriate grade for different field strength. (camber and concentricity)
- Never use hard brushes to wash nmr tubes to avoid scratching the surface of the NMR tubes.
- Avoid using strong acid to soak nmr tubes, avoid chromic acid at all costs.
- Never stand your NMR tube in a hot oven to dry it. High quality tubes should be washed properly and laid flat to air dry. Severely crooked NMR tubes will damage probe.

Note:

- ☑ ONLY HIGH QUALITY NMR TUBES ARE PERMITTED AT AV600.
- ☑ SAMPLE SPINNING IS DISCOURAGED ON ALL SPECTROMETERS.

Sample Loading

- Clean NMR tubes with methanol and Kimwipes.
- Position NMR tubes with depth gauge.
- Loading your sample to the magnet.

Positioning NMR Tubes

- Make sure the spinner grabs your tube well.
- If you have 600 ul or more, place the spinner on top of the depth gauge and push the NMR tube down to the bottom.
- If you have less, adjust your sample in a way that solution is centered around the 0 line of the depth gauge. The minimum volume requirement is 450 ul.

Note:

☑ Before positioning your NMR tube with depth gauge, make sure the top of the teflon stopper sits on the 2 centimeter line.

Loading Your Sample to The Magnet

- Turn on the air lift with the LIFT button on the BSMS panel, wait until there is maximum air flow, place your sample with the spinner on the air cushion, and press the LIFT button again to turn off the air flow. Your tube will be smoothly loaded into the probe.
- Alternatively, on xwinnmr, type ej to turn on the air and ij to insert the sample.

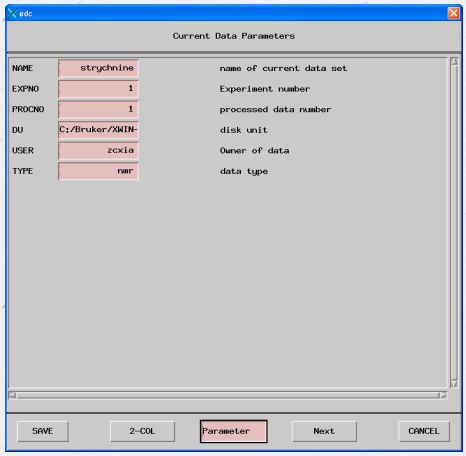
BSMS Panel

Note:

- ☑ Never drop an NMR tube to the magnet without air flow.
- ☑ For J Young tube, see NMR facility staff for help.

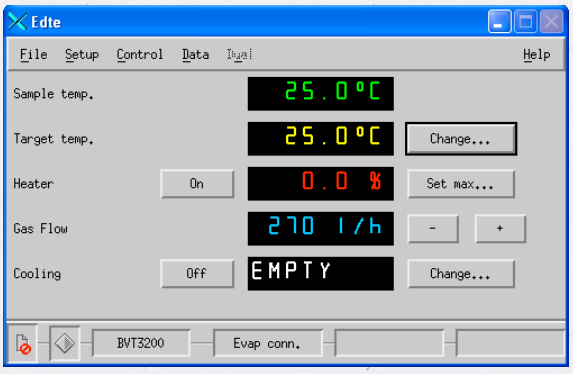
Create a New Experiment

- type edc,
- Next to name, enter a meaningful name for the dataset.
- Next to expno, enter a numerical number.
- Next to procno, enter a numerical number. The default is 1.
- Next to user, type your user name
- Click on SAVE button



Setup Your Temperature - Optional

- type edte to bring up the VT control window.
- Input your target temp, set your max power to 5% and leave the heater on, wait for about 10 minutes for temperature equilibration.
- PLEASE DO NOT PLAY WITH THE GAS FLOW!
- If your desired temperature falls outside the range of 25°C-50°C, please see facility staff for VT training.

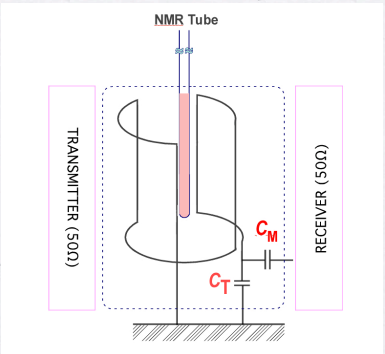


NMR Procedures

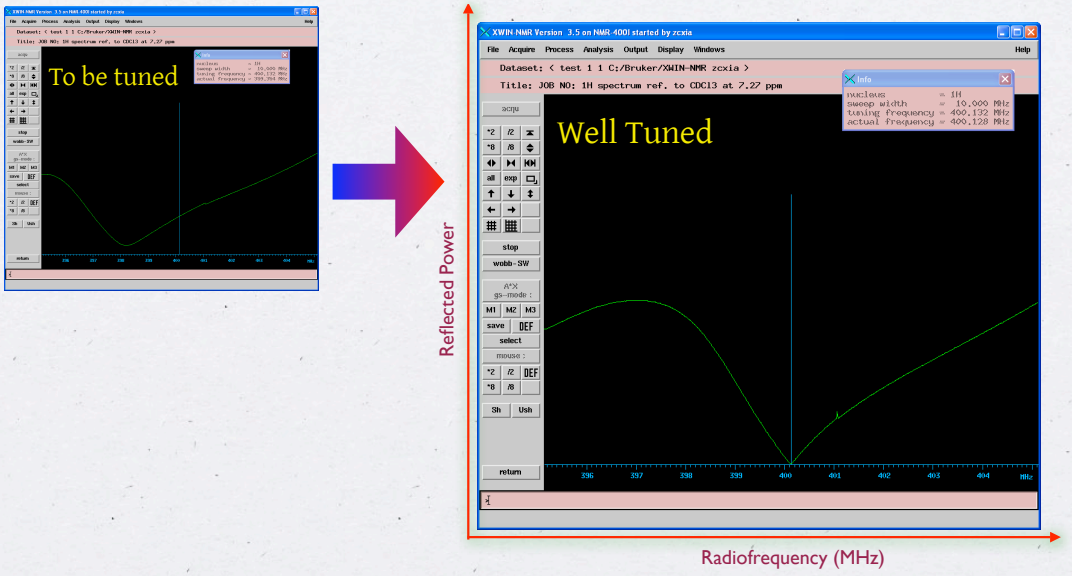
- Probe Tuning and Matching
- Lock/Shimming
- Data Acquisition
- Data Processing

What is Tuning/Matching

- Tuning is a process to adjust the resonance circuit of the probehead so that it coincides with the frequency of the transmitted pulse.
- Matching is a process to adjust the impedance of the resonance circuit until it matches the impedance of the transmitter/receiver lines (50Ω).



How to Tune



Procedures for AV400dir

For Proton:

- Make sure there is no acquisition
- Type: tuneH1
- Type atma
- Wait for "atma finished" at the bottom of the xwinnmr window.

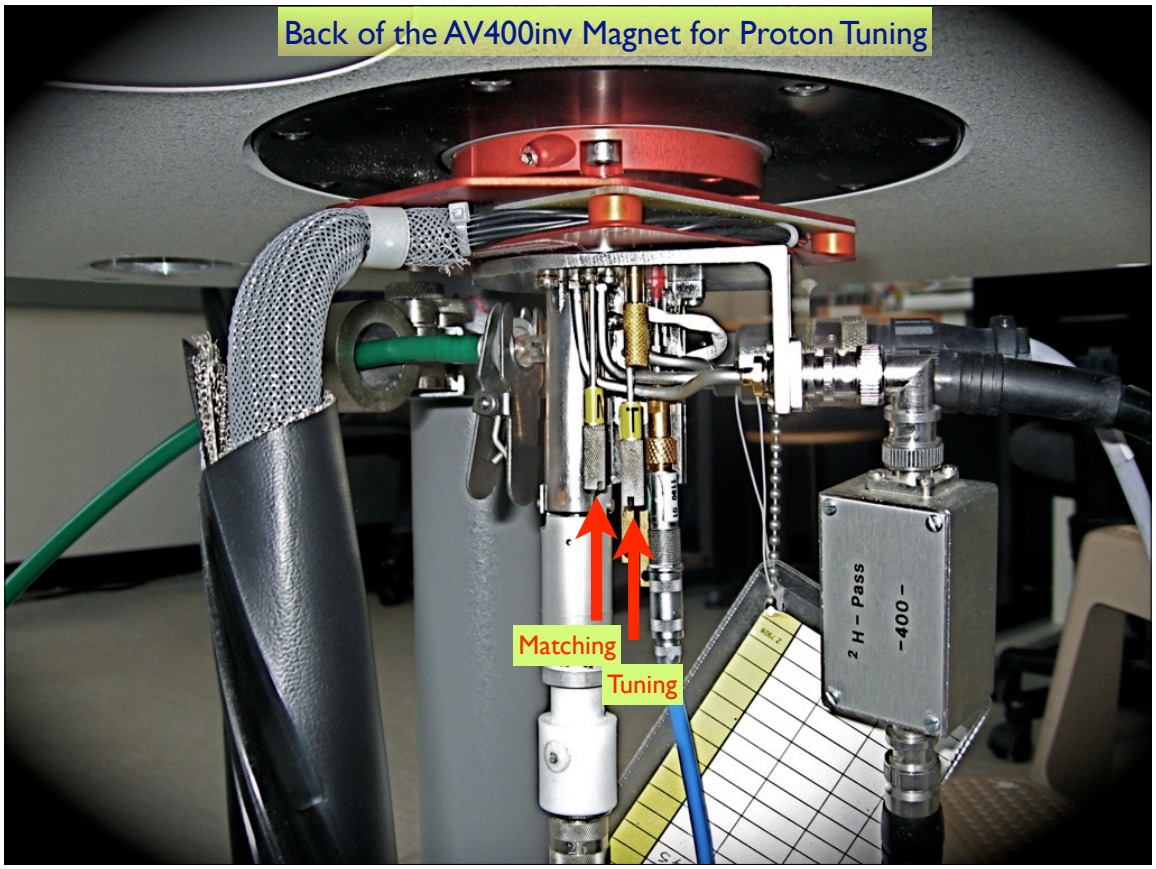
For Carbon:

- Make sure there is no acquisition
- Type: tuneC13
- Type atma
- Wait for "atma finished" at the bottom of the xwinnmr window.

Procedures for 400inv

For Proton:

- Make sure there is no acquisition
- Type: tuneH1
- Locate tuning and matching capacitors under the probe, adjust it carefully to obtain the desired frequency and impedance
- Type: stop



Note:

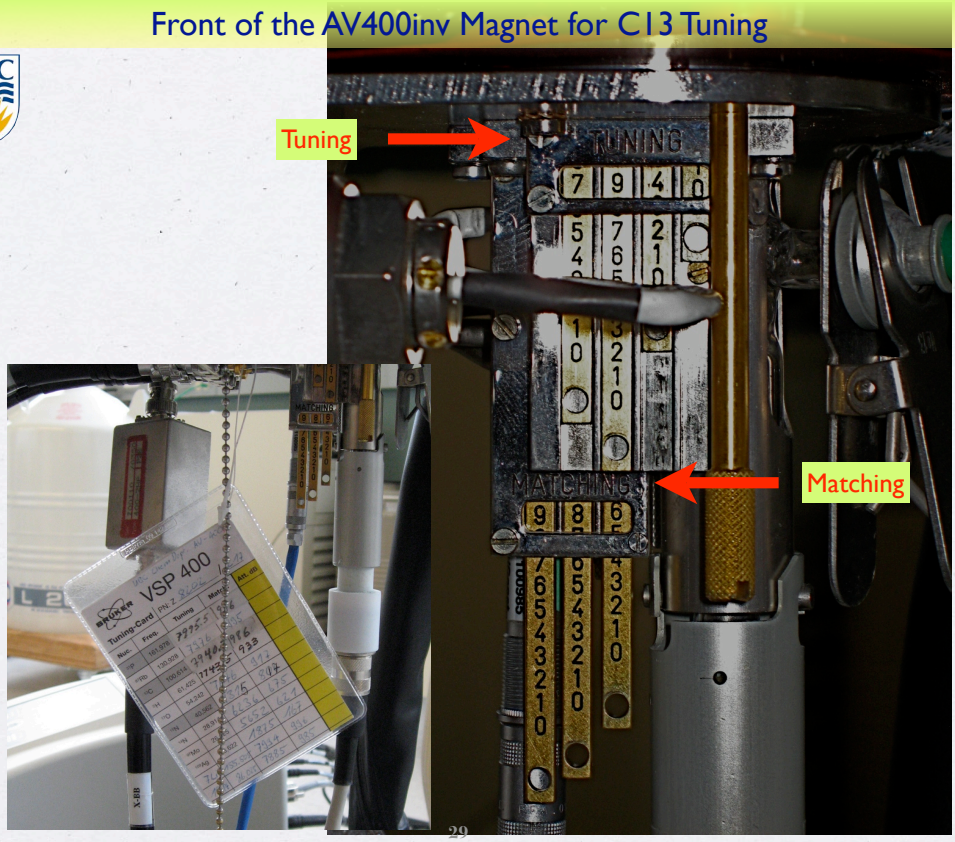
☑ The tuning/matching screws have limited ranges. Attempting to go beyond them will damage the probehead.

Procedures for AV400inv

For Carbon:

- Make sure there is no acquisition
- Type: tuneC13
- Locate tuning and matching capacitors under the probe, dial the numbers in, fine tune it to obtain the desired frequency and impedance
- Click wobb-SW
- OK to change nucleus
- Wait until proton wobble curve shows up.
- Tune Proton as described before
- Type: stop

Front of the AV400inv Magnet for C13 Tuning



Note:

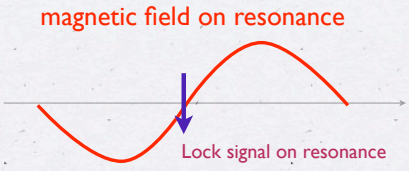
- Tuning AV300 is done only by NMR Facility Staff
- Pulsing at a significantly different frequency from the probe resonance circuit can cause expensive damages to preamp electronics.

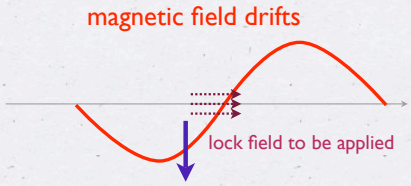
Notes to Tune/Match

- You do not have to tune/match if you just need a simple quick proton spectrum on all spectrometers.
- 400inv is configured to be H1/P31 by default, while 400dir to be H1/C13. You may not need to tune if you have a strong sample to run a quick and dirty 1D spectrum for the default nucleus. If you have to tune X channel to a different nucleus, please make sure to tune it back.
- Your sample, solvent, volume, concentration, temperature, all affect tuning. For nuclei with high gyromagnetic ration (e.g. H1, F19), tuning tend to be more sensitive to sample conditions.

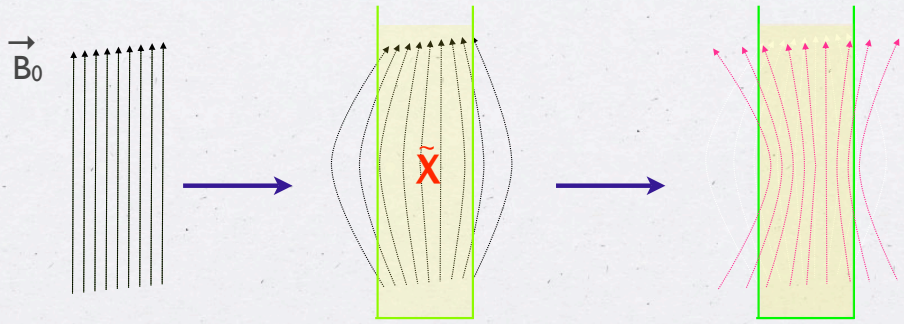
Locking

- The lock channel on a spectrometer is essentially a channel specific to solvent deuterium (lock signal = deuterium signal).
- Locking is to adjust the lock field through a feed back lock circuit to compensate any drifting of magnetic field, such that the magnetic field is always on resonance with a predefined lock frequency.
- If unlocked, high resolution info may be lost due to line broadening. In addition, shimming on lock signal is generally easier than shimming on FIDs.





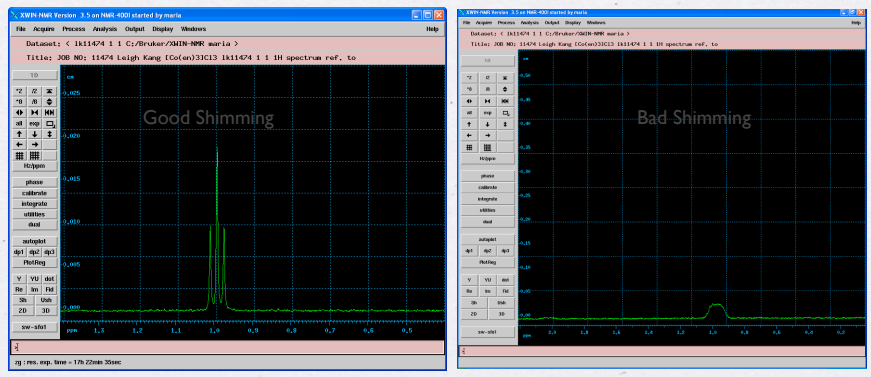
Shimming



your sample introduces magnetic field gradient

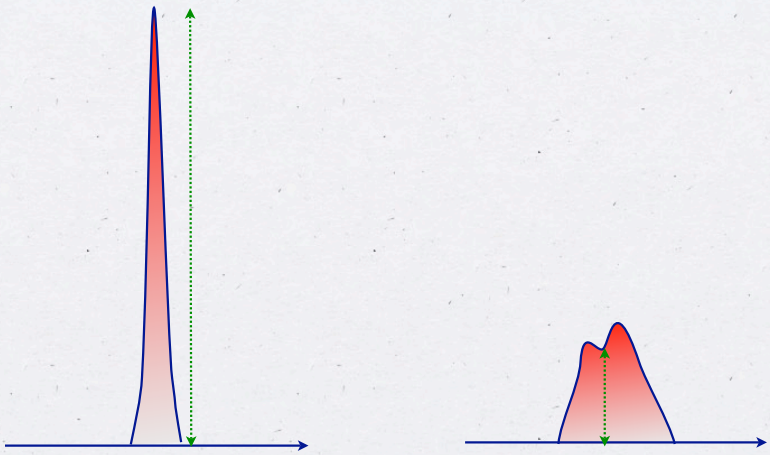
which is to be corrected by field gradients generated by shimming coils in a controlled way

Why Shimming



Poor shimming leads to unacceptable resolution and lost sensitivity

Shimming with Lock Signals



A perfect shimming leads to maximum intensity of solvent lock signal.

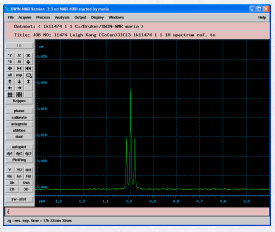
Lock/Shim Procedures

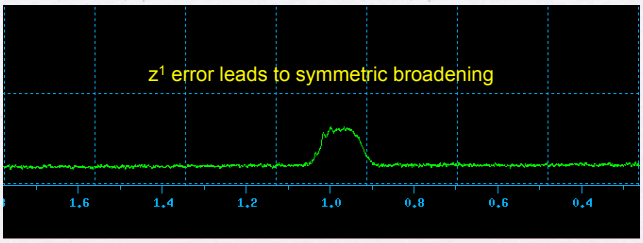
- Type l to open windows to observe lock signal,
- Type rsh shim*, and load the latest system shim files,
- Type lock and select solvent,
- Shim with z1, z2, x and y to obtain maximum lock intensity,
- Press standby button on the BSMS panel to finish it.

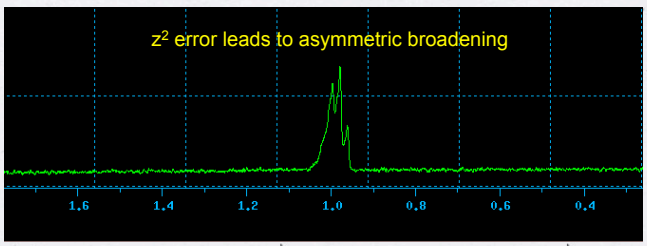
Notes:

- To activate z1/z2 on BSMS panel, toggle on the green light on the onaxis button, press z1/z2; to activate x/y, press x/y, then z0 button.
- Before starting to adjust shim values, make sure the fine button is lit.
- When lock signal goes off scale, lower Lock Gain value.
- No spinning.

When Shimming Is Good Enough



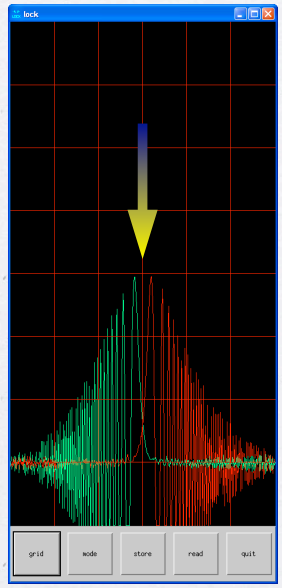




The only way to tell if your shimming is good or not is to take a quick spectrum!

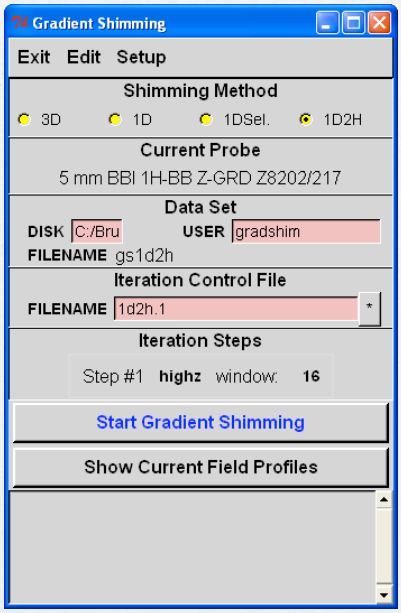
Manual Locking - Optional

- Type l to bring up the lock display,
- Type lock and select solvent as described before,
- Turn the lock off by by pressing Lock On/Off button on the BSMS panel
- Press field button on the BSMS display and turn the knob so that the lock wiggle is positively centered.
- Press Lock Phase button and turn the knob so that the green and red wiggle has the same height, and the base around the central wiggle is flat.
- Turn the lock back on.



Gradient Shimming - Optional

- Type gradshim to bring up the window for gradient shimming,
- DO NOT CHANGE ANY SETTINGS, (it is okay to see some settings are actually different on this window on different spectrometers)
- Click "Start Gradient Shimming" to start automatic gradient shimming,
- Wait for the message: "setsh: finished" at the bottom of the xwinnmr window,
- Click exit on gradshim window to close the window.



Proton Data Acquisition

- Type rpar ubc_1H all (note, it is case sensitive!) to load the default acquisition parameters.
- Set your sweep width. If you wish to cover, for example, 14 ppm from -1 ppm to 13 ppm, Type sw and set it to 14. This defines the whole spectrum width. Type o1p and set it to 6. This sets the center of your spectrum to be 6 ppm.
- Type rga to automatically determine the optimum receiver gain.
- Type ns to set your number of scans. Your number of scans has to be multiples of four.
- Type zg to start your data acquisitions, wait for the message "Checklockshift finished" at the bottom of xwinnmr window.

Carbon Data Acquisition

- Type rpar ubc_13C all (note, it is case sensitive!) to load the default acquisition parameters. This gives a standard broadband proton decoupled carbon spectrum.
- Set your sweep width. If you wish to cover, for example, 220 ppm from 0 ppm to 220 ppm, Type sw and set it to 220. This defines the whole spectrum width. Type o1p and set it to 110. This sets the center of your spectrum to be 110 ppm.
- Type rga to automatically determine the optimum receiver gain.
- Type ns to set your number of scans. Your number of scans has to be multiples of four.
- Type zg to start your data acquisitions, wait for the message "Checklockshift finished" at the bottom of xwinnmr window.

During Acquisition

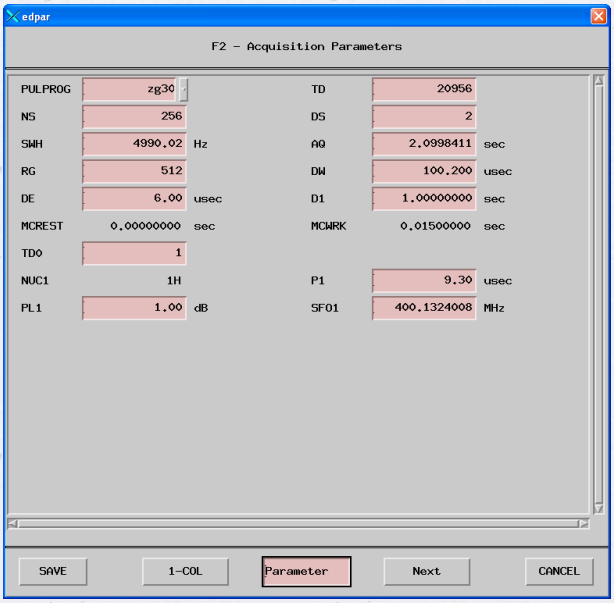
- To check the acquisition status, type a to show status window, that displays how many scans are completed and how much more time is left to complete the data acquisition. Click on return to return to the main display.
- To check the progress of your experiment, type tr at any time during your data acquisition. This transfers the recorded data to computer hard disk for processing.

Stop An Ongoing Acquisition

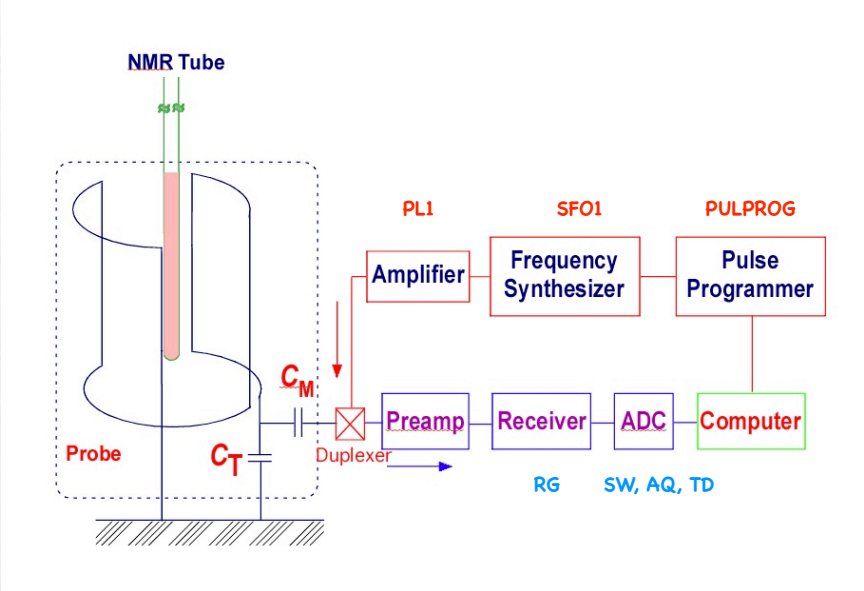
- Type halt to terminate an acquisition with your data saved on the hard drive for processing.
- Type stop to terminate an acquisition without saving your data.
- Type kill to terminate any processes without saving.

Tweaking Acquisition Parameters - Optional

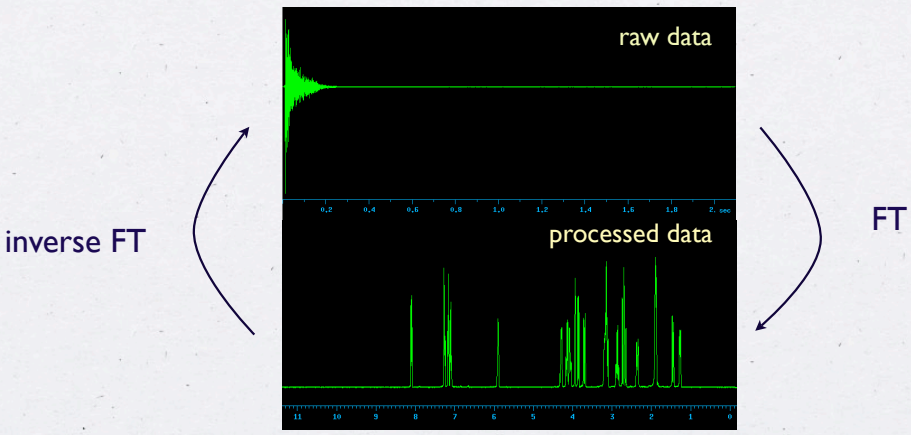
- Type ased to bring up the parameter window.
- Click on SAVE button when you are done with tweaking.
- Most often you only need to change relaxation delay (d1).
- Avoid playing with power level (pl1).



How Parameters Are Controlled - Optional



Data Processing



Note: NMR data are complex numbers, only real parts are shown here.

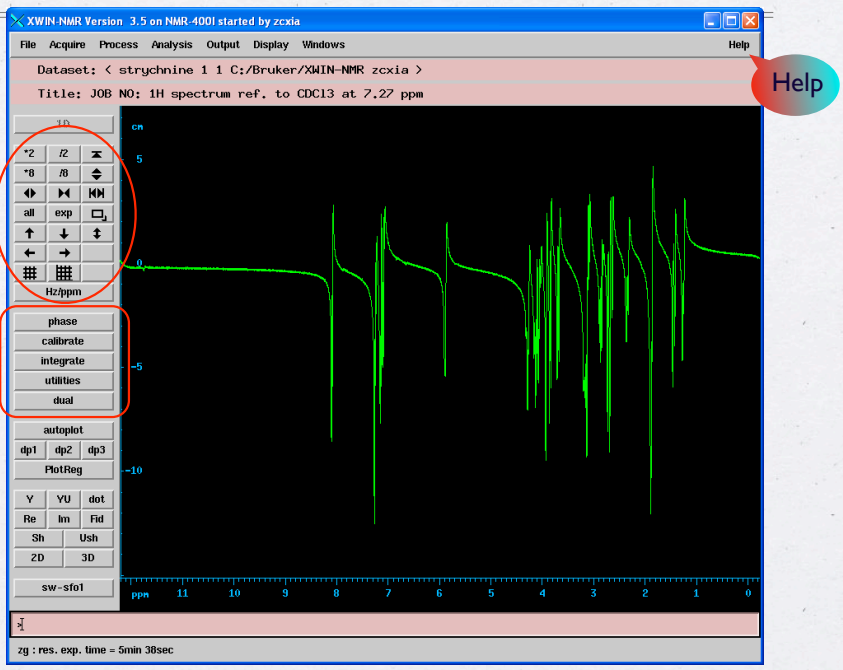
Getting Around with XWINNMR 3.5

X,Y

manipulation

Post-processing,

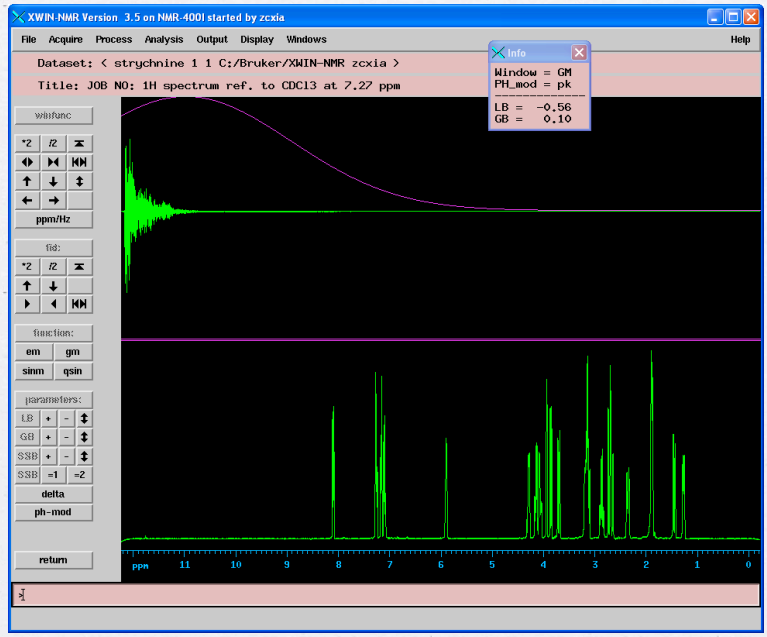
each has a submenu



Weighted Fourier Transform

FID can be mathematically manipulated to improve sensitivity or resolution. (apodization).

Type winfunc to interactively adjust the apodizaton function.

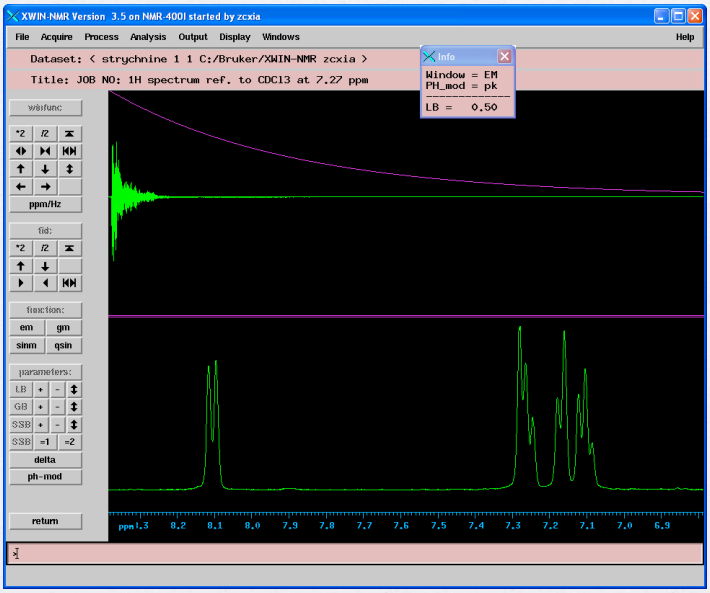


If you want sensitivity

Typical parameter for small molecule:

type lb and set it to 0.5Hz,

type eft.



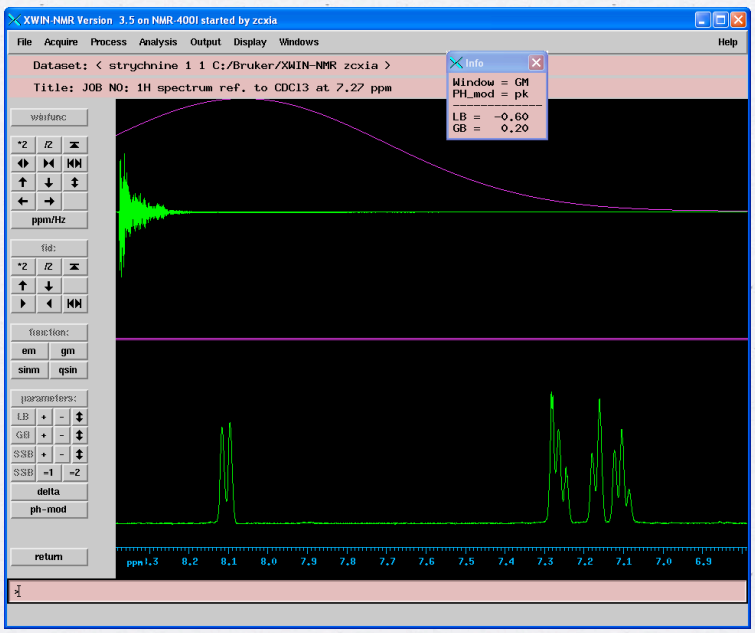
If you want resolution

Typical parameter for small molecule:

type lb and set it to -0.5Hz,

type gb and set it to 0.2.

type gfp.



Phase Correction

- Click on **phase** to enter phase correction submenu.
- Click on **cursor** and set the cursor position to the signal at the edges of the spectrum.
- Place the cursor on **PH0** and hold down the left mouse button, move the mouse until the base line of selected signal is the same on both sides.
- Place the cursor on **PH1** and hold down the left mouse button, move the mouse until the signals furtherest away from the selected one is also in-phase.
- Click on **return** and save it to exit.
- Phasing can be done automatically by typing apk.

Baseline Correction

- Type abs to automatically correct the baseline.
- You can also correct baseline in a specific region of your spectrum. Type absf1 and absf2 to specify the limits of the desired region and do absf to automatically correct the baseline only in this region.

Integration

- Make sure the baseline is flat- if not, baseline correct it.
- Click on integrate to enter the integration submenu.
- Move the cursor into the spectral window, click on the left mouse button.
- Click the middle mouse button once at each end of the signal, integration appears automatically.
- For calibration, double click to select an integral, click on calibrate and enter a value (i.e. number of protons).
- Select "Save and store 'intrng' " to return to 1D main window.

Chemical Shift Referencing

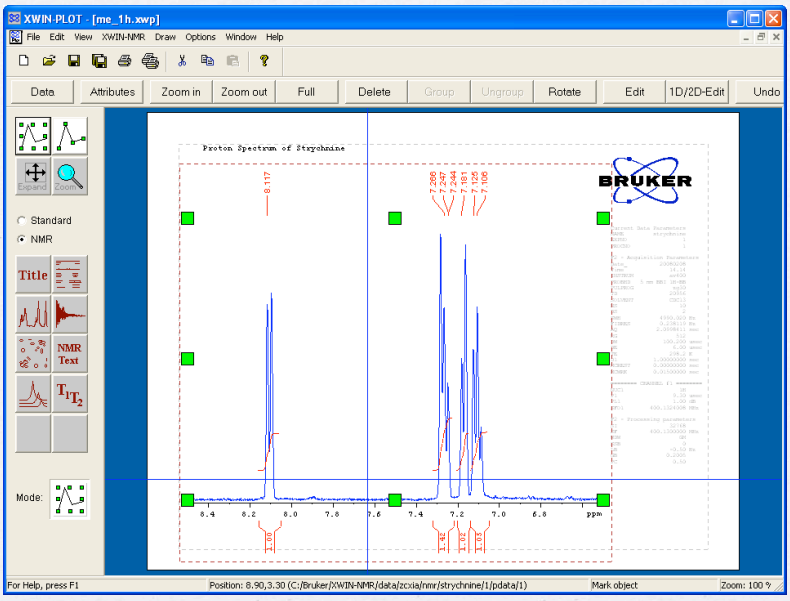
- Click on calibrate,
- Move the cursor to a signal with a known chemical shift (typically, this can be TMS or your solvent signal) and click on the left mouse button. Type in the chemical shift value.
- If your solvent signal is not resolved, and you do not have a reference compound, type sref to automatically calibrate your chemical shift using lock signal.

Peak Picking

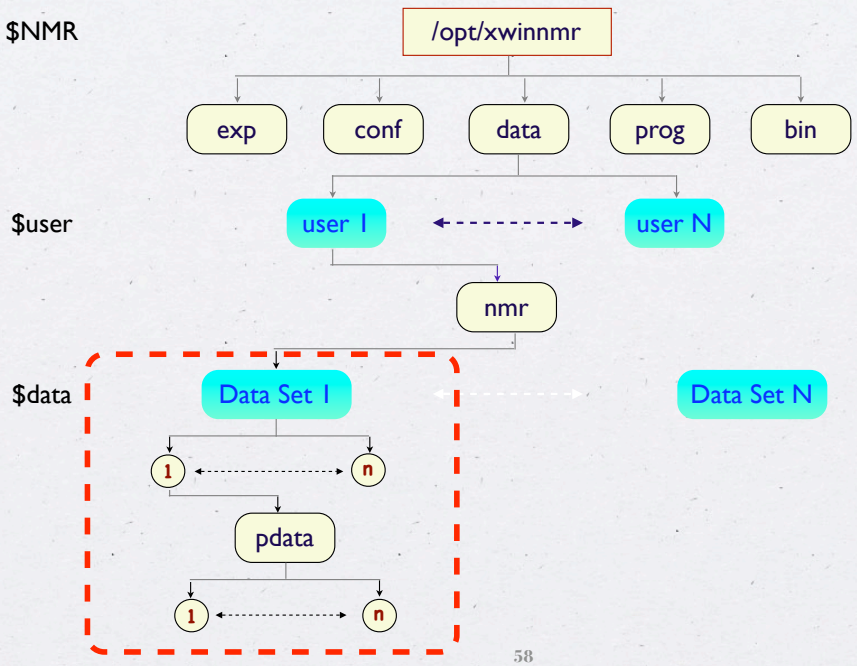
- Click on utilities,
- Place the cursor on MI and move it to define the threshold,
- Enter pps to see a list of the picked signals.
- Click on return.

Plotting

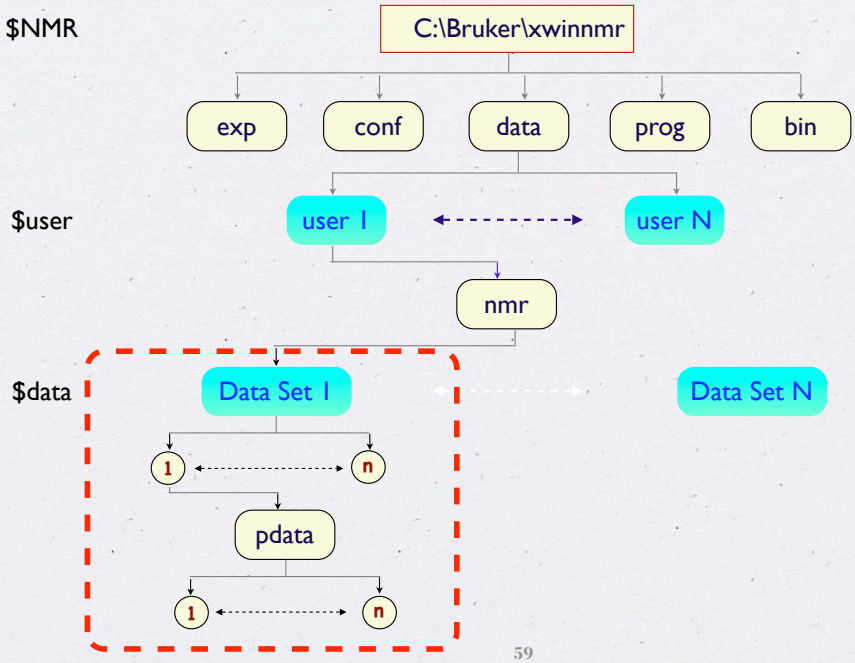
type xwp to
use xwinplot



File Structure for AV400dir and AV600



File Structure for AV300 and AV400I



Spectrometer FTP Access

Instrument Name	Host name for FTP
AV300	av300.chem.ubc.ca
AV400 (inv)	av400inv.chem.ubc.ca
AV400 (dir)	av400dir.chem.ubc.ca
AV600	av600.chem.ubc.ca

Off-line Data Processing

- ACDlabs (http://www.acdlabs.com)
- Mnova (http://www.mestrec.com)
- Spinworks - free - (http://www.umanitoba.ca/chemistry/nmr/spinworks/)
- Topspin (latest version of Bruker xwinnmr)
- Xwinnmr

Access to NMR Facility

- ☑ ACCESS TO NMR FACILITY IS GRANTED ONLY AFTER PASSING CHECK-OUT PROCEDURES BY NMR STAFF!
- ☑ PLEASE PRACTICE WITH YOUR SENIOR GROUP MEMBER BEFORE APPLYING FOR CHECK-OUT.

Criteria for Access (AV300)

- Sample Loading
  - position NMR tubes with depth gauge
  - load NMR samples to the magnet
- Experiment setting up
  - shimming
  - load parameter files for proton data acquisition
  - adjust SW, O1 and number of scans
  - start and stop an experiment

Complete the list on the left within 15 minutes without notes and errors

Criteria for Access (AV400s)

- Sample Loading
  - position NMR tubes with depth gauge
  - load NMR samples to the magnet
- Experiment setting up
  - proton tuning and C13 (AV400dir) or P31 (AV400inv) tuning
  - shimming
  - load parameter files for proton data acquisition
  - adjust SW, O1 and number of scans
  - start and stop an experiment
- Data processing
  - exponential apodization + Fourier Transform
  - integration
  - baseline correction
  - peak picking

Complete the list on the left within 15 minutes without notes and errors

Where to Seek Help

- Paul @7 3548 Room B460A
- Maria @2 6787 Room B460
- Zorana @2 6787 Room B460