University of Wisconsin–Madison
Chemistry Department

Varian NMR User's Guide

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Note: This guide provides an introduction to use of Varian equipment at the UWChem*MRF*. ***This guide is not intended in any way to be a replacement to the excellent Varian documentation!*** All students should refer regularly to the Varian VNMR Liquids Users Guide for learning and the Varian VNMR Command Reference Guide for specific guidance. All the Varian documentation is available in both hardcopy and on-line.

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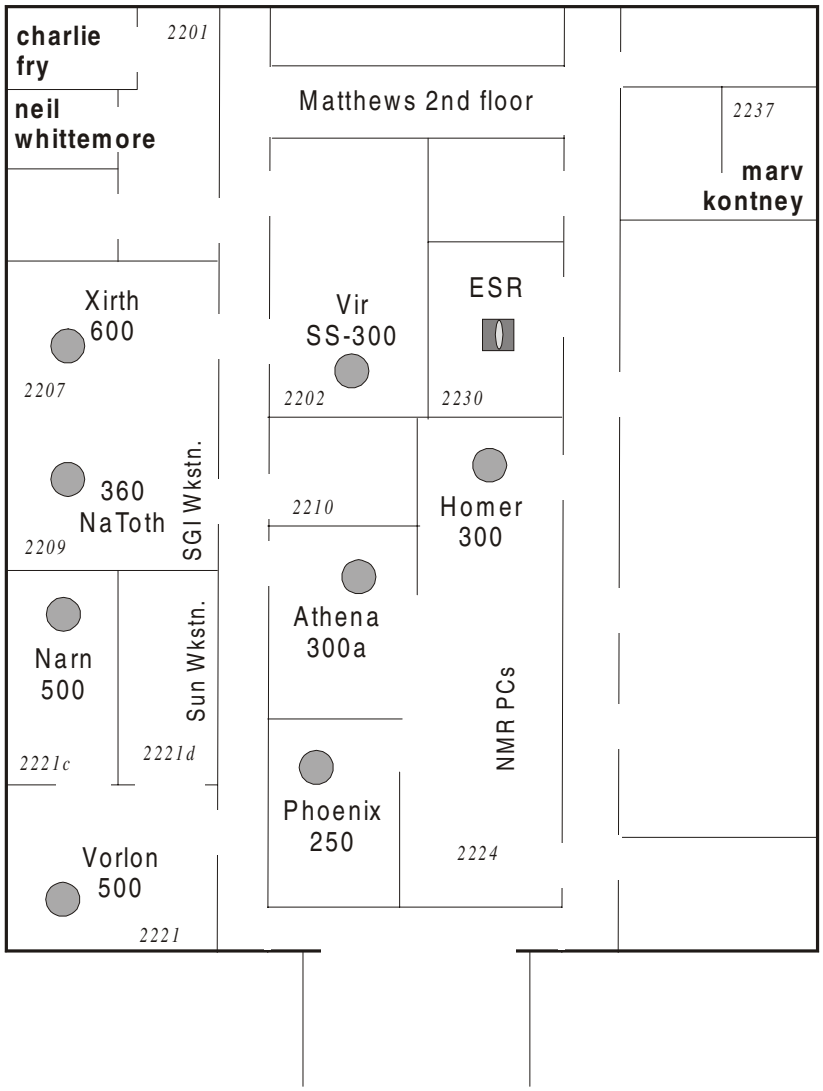
UW Chemistry Magnetic Resonance Facility (UWChem*MRF*)

I. Facility Layout (2nd floor Matthews)



As of July, 2001

ATHENA	–	AC+ 300	routine $^{1}H/^{19}F/^{31}P/^{13}C$	–	auto-sample changer, quad-nucleus probe
HOMER	–	AC+ 300	routine $^{1}H/^{13}C$	–	$^{1}H/^{13}C$ dedicated
PHOENIX	–	AC+ 250	routine BB VT	–	routine BB ($^{29}Si/^{11}B/^{2}H/^{199}Hg$/etc.), variable temperature
VIR	–	UNITY-300	solid-state NMR	–	conformational, motions, solid-state packing, catalysts, amorphous and glassy compounds

NATOTH	–	Avance-360	non-routine BB VT	–	long-term VT, kinetics, concentration limited samples; 5 and 10 mm BB probes, 5 mm inverse probe
NARN	–	UNITY-500	non-routine ^{1}H/BB VT	–	high-sensitivity, sample-limited (^{1}H < 5 mg, ^{13}C < 15 mg), short-run, sophisticated experiments (e.g., HMQC, DQCOSY, gCOSY, gNOESY); limited access
VORLON	–	INOVA-500	inverse exps, 2D studies	–	long-term, sophisticated, gradient-enhanced experiments; combi-chem MAS probe; limited access
XIRTH	–	INOVA-600	long-term 2D studies	–	long-term, most sophisticated, gradient-enhanced experiments (e.g., NOESY/ ROESY, HMQC, DQCOSY); limited access
ESR	–	ESP-300	electron spin resonance	–	paramagnetism, free-radical chemistry

PC's – DEATHSTAR, TERMINUS, MORDOR, VULCAN surround the main printer GKAR
– KOSH has special software; BABYLON (128.104.70.61) is the Win-NT server

Sun's – for data workup, UltraSparc 1 (M170)'s CENTAURI and ZHADUM are located in rm 2010, VORLON is in rm 2201a
– NARN and SHADOW (Sparc 5's) are hosts for the respective instruments

SGIs – NATOTH (Avance host computer) and GQUAN (for off-line data workup)

II. Facility Personnel

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Quick Guide for New Users

created 12/01/97 – updated 9/27/99

I. Login

- Username: practice Password: *[do not use 9th floor password; nothing from a dictionary; at least one number; case sensitive]*
- data to → /zhadum/practice [other partitions: europa, ganymede, starbase]

  UNIX: **mkdir** ***name***
  VNMR: **FILE** ***left-click on name*** **CHANGE** (will place data in ~practice/*name*)
- shims, macros, etc. to /export/home/practice/vnmrsys/shims or ~/vnmrsys/maclib, etc.
- exit VNMR before logging out!!

II. Setup

A. 1st Time:

- **MAIN MENU SETUP 1H,CDCL3** ; this will put reasonable parameters in
- **phasing=100** ; shows complete spectrum while phasing
- **MAIN MENU MORE CONFIG SELECT PLOTTER ngbar_plot** or **shadowp_plot**
  SELECT PRINTER ngbar_print or **shadowp_print**

B. Parameters:

- Setup probe and pulsed-field gradient parameters using macro with probe name:

  e.g., type **bbswg** produces **probe= 'bbswg' pfgon='nny'**
  bbold → probe='bbold' pfgon='nnn'
  hcx → probe='hcx' pfgon='nny'

  It is *critical* the probe parameter is set for correct parameters to be setup.

- **MAIN MENU SETUP Nuc,Solv**

  ^{1}H should be ok; check **nt** and **gain**

  ^{13}C – check decoupler settings, e.g., **probe='bbswg'** (make sure this is the probe installed)
  dmm='w'
  dmf=10000
  dpwr=40

  dm='yyy' su turns decoupler on (see also **UWMACROS DECOUPLER ON**)

C. Variable Temperature

- switch to N_2 gas for 20°C > temp > 40°C
- ***turn VT flow up to eject samples*** (do not switch back to air unless close to ambient)
- Use **UWMACROS SET TEMP** to change temps (or macro **settemp** or similar)

UWMACROS SET TEMP should be used instead of manually setting temperature; this macro avoids inadvertant temperature changes that can otherwise occur.

- ±20° changes take 15 mins or so before probe tuning and shims will be stable.
  ±50° changes take ~30 mins.
  ±100° changes may take 1 h (should be done in steps no bigger than ±50°).
  - I***t is the student's responsibility to finish early enough that their VT work does not affect the next user!!***
- ***hcx probe -80 to +60°C***
  bbswg probe -150°C to +80°C
  bbold probe -150 to +150°C
  h1f19 probe -150 to +150°C

III. Locking and Shimming

A. Sample Prep:

- Need ≥ 0.6ml (4 cm height) solvent for Varian probes to attain good ^1H shims (without extraordinary shimming).
- Set sample to **67 mm** below bottom of spinner (use ruler), or
  center in rf coil region (use Varian depth gauge) if solvent < ~5 cm high.
- On a 500, it is critical that the sample be clear (no particulate floating if possible), and the tube be of high quality (Wilmad 506 minimum, 528 better) with no nicks or scratches. Keeping within these standards will allow excellent quality shims to be attained in ≤ 5 min shimming. Reasonable shims can be attained in other conditions, but with longer shimming sessions, and no guarantee that good quality lineshapes can ever be achieved.

B. Locking:

- use **UWMACROS LOADSHIMS** or manually use **rts**↵ or

- **rts('hcx.shim') loadshims** (loadshims → load='y' su load='n' su)
  rts('bbswg.shim') loadshims
  rts('*your-shims*') loadshims
  [**FILES SAVE SHIMS** or **svs**↵ will save in /export/home/practice/vnmrsys/shims]

- in ACQI window: set **FIELD** → until fid on-resonance (no oscillations) and positive
  LOCK POWER (see START suggestion, but be aggressively higher if needed)
  LOCK GAIN

  NOTE: IF ACQI WINDOW DOES NOT APPEAR, USE 'ACQI' TO CALL IT UP.

- should lock up now
- turn **SPIN ON** if routine 1D (<u>not</u> if 2D or selective 1D experiments)

C. Shimming:

- shim: **LOCK PHASE** → **Z1** → **Z2** to achieve maximum lock signal
- lower LOCK POWER (approaching FINAL suggestion; but keep lock level > 15)

- spinning: **LOCK PHASE** → **Z1** → **Z2** (2^{nd} order) → **Z3** (usually not necessary)
  non-spinning **X** → **Y** → **XZ** (2^{nd} order) → **YZ** → **XY** → **X2-Y2** , then back to above (2D do all without spinning)

- check shims using standard ^1H setup with **nt=1**
  set cursor on solvent singlet, **nl dres** ; should be ≤ 1 Hz in most cases; highly dependent on tube; 507's (stockroom) will sometimes need Z3+Z4 adjustments; 528's typically should not)

IV. Probe Tuning and ^1H Calibration

- Use Hewlett-Packard scope to tune probes:
  - disconnect ^1H cable (with small silver barrel filter attached) from probe, and hook HP scope into probe
  - push H1 PROBE on the scope
  - tune in TUNE capacitor (bottom) to center dip

    tune in MATCH capacitor (upper) to get dip down to bottom 2-3 squares

    readjust both to center and bottom dip appropriately
  - disconnect scope cable and reconnect ^1H
  - tune ^{13}C or other X nucleus if needed at this time
- check ^1H pulsewidth using **array** command: use **pw 30 3 3** to set up first array; check about 360° (going negative to postive as **pw** increases); **pw90 = pw(@ 360°) / 4**
- pw90 check is required for checkout; also necessary before querying facility staff about probe

V. Acquisition

- Check that there is no external attenuation in-line for ^{13}C or other nuclei runs.
- Set **gain=40** and listen/watch for an ADC OVERFLOW beep [there is one beep for completion of the acquisition, and a second beep if there is an ADC overflow]; turn gain down in 10 dB steps until ok (this is recommended setup; computer sees clipping better than you will in next example!).
- Can also perform a **nt=1** acquisition, then **df**; if fid looks normal, type **gf** (wait > 2s!!!), then can go into **ACQI FID** and observe fid directly while changing gain.
- **go** or
  ga (will automatically wft) or
  au (will perform additional commands useful for 2D or auto-saving data)

- ^1H acquisition usually needs only a check of **nt**.
  – **movesw** similar to **^O** in EP on AM/AC's
  – **movetof** similar to changing **O1** on AM/AC's

- ^{13}C, turn on decoupler: **dm='yyy' su**

  - remember to turn off when done!
  - set **nt=1e6** if don't know needed number of scans
    can **wft** after each **bs** scans (**lb=2-4** needed for ^{13}C)
    use **sa** to stop acquisition once S/N is good enough

- cursor close to peak, **nl rl(77p)** will correctly reference $CDCl_3$ peak; **rl1(77p)** for *f1* in 2d's
- **dsx** → wft dscale(-3)
- **ppa pscale pl page** typical plot

VI. Saving and Deleting Data, and Backups

- **FILES SAVE FID** or **svf('*data-name*')** ..... ; saves only raw fid but with *all* parameter's intact
- **MAIN MENU FILE SAVE FID** type in name without quotes
- **MAIN MENU FILE** ***left-click on data-name*** **DELETE** will delete data
- use FTP program on PC's and connect to ZIP's to backup

VII. Logging Out

- exit VNMR first
- *right-click* on background, exit

VIII. Don'ts

- use Unity without VT regulation (run at 26°C if just want ambient)
- use too little solvent; Varian probes require more solvent than Bruker probes; too little solvent will just give you a terrible shimming session
- run VT outside 20 to 40°C without switching over to N_2 gas
- run VT below 10°C without Variac on
- run VT outside -80 to +60°C on hcx probe, -120 to +80°C on bbswg probe

Introduction to Unix and VNMR

created 3/5/95 – updated 12/19/01

I. Common Unix Commands

cd ~ – change directory to home (dir you first go to on login)
cd ~/vnmrsys – change to your vnmr directory
pwd – show current location (path)
df -k – display file system (will show free space)

☞ ***paths*** – see section III on directory structures

mkdir – make directory
rmdir – remove directory

man ***cmnd*** – show manual pages for *cmnd*

ls – list files
ls -la – list files with options lga
alias dir 'ls -la' – in your .cshrc file
cp -r ***name*** – copy recursively from *name* down path (i.e., including all subdirectories)
mv ***fname tname*** – move *fname* to *tname* (i.e., a rename unless a change in path is specified)
rm -r ***name*** – remove from *name* down path (including all subdirectories)
use care; this is a dangerous command!

Use the text editor in the CDE windowed environment if possible (much easier than vi), but always finish with a carriage return at the end of the file when creating macros. In addition, you can use *textedit filename* at a unix prompt to bring up the more-usable textedit editor.

vi ***filename*** – edit *filename* using vi editor (*cgf* has couple pages of Vi Quick Reference)
i – insert (must use **ESC** or double up/down arrow to exit insert mode)
a – append; insert *after* cursor position (use at end of line)
x – delete single character (**10x** will delete 10 characters)
dd – delete single line
^ – move to beginning of line (then use **i** to insert at beginning of line)
$ – move to end of line (then use **a** to insert at end of line)
:wq – write and end vi session
:q! – quit, discard changes
/text**<ret>** – go to next occurrance of *text*
:set number – gives line numbers, help with debugging

II. Common VNMR Commands, Parameters and Flags

(☞ particularly useful commands)

☞ **dg** – display group (normal parameters)
dg1 – display second (processing, plot... parameters)
dgs – display shim group

☞ **dps** – display pulse sequence
da – display array
☞ **array** – powerful array command, for stacked acquisition (kinetic exps, pw checks)

☞ **full** – uses full screen; needed after **dssh** and other commands (is NOT the same as the DISPLAY INTERACTIVE FULL button)
wysiwyg='n' – sets display so scaling does NOT match printer

svf('*filename*') – save file (1d or 2d) with associated data, phasing, etc. (FILE SAVEFID)
rtf('*filename*') – reads file (menu alternative MAIN MENU FILE)
svp('*filename*') – save only parameters (1d or 2d); significant savings in disk space!!
rtp('*filename*') – reads parameters
svs('*filename*') – save shim settings to user directory ~/vnmrsys/shims (FILE SAVESHIM)
rts('*filename*') – reads shims (follow with **loadshims** macro; UWMACROS LOADSHIMS)

wexp='svf(\'*filename*\')' – saves data to *filename* at completion of experiment (\' required for quotes within the main quotes
– requires **au** used to start acquisition

explib – lists all experiment areas (menu MAIN WORKSPACE LIBRARY)
cexp(#) – creates experiment area number # (see also WORKSPACE)
delexp(#) – deletes experiment area number # (save disk space; see also WORKSPACE)

temp24 – UW macro to set temp to 24°C (cp into your ~\vnmrsys\maclib, rename and edit for other temperatures); (UWMACROS SET TEMP)
vttype=0 or **2** – variable temp controller not present (=0) or present (=2)
temp='n' – no temp specificied for control
vttype=0 temp='n' su – command preceeding probe change (see macro *tempoff*)!!
vttype=2 temp=24 su – command to re-establish temp control at 24°C *after* probe change (see macro *temp24*)

tn='Si29' – set transmitter to ^{29}Si frequency (uses look-up table; see macro *tuneh*)
d# (e.g., d1=1) – set delay time in seconds (use dps to make sure about timing)
pw=8 – common pulse width in µsec (use dps)
p# (e.g., p2=4000) – pulse width in µsec (use dps)
tpwr – observe transmitter power set in dB, from 0 to 63, larger is more power
tpwrf – observe transmitter fine power control, from 0 to 4095, ~3db spread

nl – first set cursor close to peak, then finds nearest line
☞ **rl(-9.86p)** – first use nl to set cursor exactly on, then sets reference
rl1(-9.86p) – expand about correct reference on diagonal peak, use to ref *f1* axis
lb=5 – gives exponential broadening of 5 hertz with **wft** or **ga**

wft – weighted (depending on parameters set) FT
☞ **aph** – good automatic phase to at least start phasing

np=4k – often set by *sw*, this directly sets to 4096 points acquired, changes *aq*
fn=16k – number FT to, with previous command does two zero fills
lsfid=2 – left shift two points before FT (increase if getting baseline rolling)

☞ **movesw** – macro sets sweep width *sw* to right and left cursor positions

cz – zero all integral resets (use menu button **reset** and mouse for resets)
bc – baseline correction with default spline fit; depends on integral resets
bc(5) – baseline correction using 5th order polynomial fit

pl – plot spectra(um) as displayed
text('*text goes here*') – text header plotted on top of page
atext('*more text*') – adds lines to text header
pap – plot all parameters
ppa – plot only a few parameters
axis='p' – set axis to ppm ('h' for hertz, 'k' for kilohertz)
pscale(-3) – plot scale (in current units)
axish pscale(-6) – macro to set Hz scale and plot axis 6 cm below spectrum
page – go to next page for plotting

clradd spadd – clear exp5 and place current spectrum in exp5 (setup for addi)
addi – add/sub (dual display-like) routine
s1 – save display parameters (can use s1 through s9)
r1 – recall display 1 and display as previously saved with **s1**
md(3,4) – move saved display regions (s1 to s9) from exp3 to exp4

dssh – display stack horizontally
full – return display of one spectrum to full width
dss – display stack using **v0** and **h0** as vertical and horizontal offsets

☞ UWChem*MRF* *macros*

tuneh – tn='H1' gain=0 su ..... (use UWMACROS TUNE H1)
tunesi – tn='Si29' gain=0 su..... (use UWMACROS TUNE SI29)
tunec – tn='C13' gain=0 su

loadshims – load='y' su load='n' ..... (UWMACROS LOADSHIMS)

axisp – axis='p'
axish – axis='h'
dsx – wft dscale(-3)
invert – rp=rp+180 inverts the 0-order phase of a spectrum

tempoff – vttype=0 temp='n' su .....crucial command before probe change

temp24 – vttype=2 temp=24 su vttype=0 su..... model temp macro
settemp – vttype=2 temp=x su [wait] vttype=0 su

☞ **intnorm** – normalizes peak to user specified value (e.g., methyl peak to 3)

☞ **disp2d** – sets up **wp sc** nicely for 2d plots
pconpos – plots contours of 2D spectra with positive peaks getting 10 contours, negative peaks getting just 1
pconneg – opposite of pconpos

lpforward – sets up for forward linear prediction

III. VNMR Directory Structure

There is a user *vnmrsys* area and a system *vnmr* area. All files are looked for in user area first; if not found there, then the system area is checked.

cd ~ move to home account for computer working at /export/home/*username*

cd /vorlon/fry moves to /export/home/fry on vorlon workstation

rtf('*datafile*') reads data (fid) file; *datafile* name must have .fid as suffix (vnmr always appends as such with svf command)
all *datafile*.fid files are actually directories containing:

fid ..... actual fid data
log ..... log file shows times for acquisitions
procpar ..... processing parameters
text ..... a text file created by command text('...')

rtp('*parfile*') reads parameter file; *parfile* name must have .par as suffix (vnmr always appends as such with svp command)
all *parfile*.par files have only procpar and text files

~/vnmrsys

/exp1	data acquisition area; can go up to exp9, but can take significant disk space
/maclib	user macro library
/manual	user manual (help for pulse sequences) directory
/menulib	user menu definitions directory
/psglib	user created or modified pulse sequences
/seqlib	user compiled pulse sequences
/shapelib	user created RF and gradient shapes (e.g., for NOESY1D)
/shims	user stored shim files

/vnmr

/maclib	system macro library
/manual	system manual (help for pulse sequences) directory
/menulib	system menu definitions directory
/psglib	system pulse sequences
/seqlib	system compiled pulse sequences
/shapelib	system stored RF and gradient shapes (e.g., for NOESY1D)
/shims	system (facility) stored shim files

IV. Common Pulse Sequence Setup in VNMR

[this section is directed at students wanting to know details of the vnmr psg setups]

1. Always start by reading in standard parameters using MAINMENU SETUP

2. Then continue by running a macro (e.g., **cosy** runs the cosy macro in /vnmr/maclib) or use SETUP SEQUENCE COSY (reads in the same macro).

3. The better macros read in *only* the parameters specified in **psgset** macro statements within the primary macro routine (only ProteinPack macro do not, and use **rtp** instead). Typically the parameter file found in /vnmr/parlib has the same name as the macro, but not always:

   e.g. macro **hmqc** has statement **psgset('hmqc13c',dg,dg1,pwx,pwxlvl,dpwr,...)**

   which reads the specified parameters in from /vnmr/parlib/hmqc13c. Reading in **dg** and **dg1** setup the appearance of the parameter display in the **dg** and **dg1** screens.

4. The macros for 2d sequences call another macro **set2d** that sets up **np fn ni fn1** based on the desired digital resolution: e.g., **set2d('dqcosy',3,6)** sets up 3 Hz/pt in F2 and 6 Hz/pt in F1.

5. Usually, but not always **set2d** sets the actual pulse sequence used: e.g., inside **set2d** is a statment **seqfil=$1** where $1 is the first parameter passed into the macro (i.e. in this case, the sequence name). **seqfil** is the compiled pulse sequence (psg) residing in /vnmr/seqlib.

6. The pulse sequence source C code resides in /vnmr/psglib. The source code is compiled with the statement **seqgen** (e.g., for cosy **seqgen relayh.c**). Only users and vnmr1 can compile source code, so common code must be placed in the user's vnmrsys/psglib and compiled. The compiled code is automatically placed into the user's vnmrsys/seqlib which must then be copied into /vnmr/seqlib and have the owner set to vnmr1:nmr (e.g., **chown vnmr1:nmr /vnmr/seqlib/relayh** ; root permission is required for the copy and chown unless the user is vnmr1.

7. When writing or editing a pulse sequence, it is easiest to begin with a premade parameter set. New parameters must be created with the **create(parameter<,type<,tree>>)** command. See section 5.2 in the Pulse Programming manual. It is important to correct define the parameter when creating it: e.g., when making a new variable **dpw2d** for a decoupler power setting that should not go above 35 to prevent probe damage, use the command **create('dpw2d','integer')** and follow that with **setlimit('dpw2d',35,0,1)**. Note that pulse length parameters should be defined with **create('pwsl','pulse')** to insure the units for the pulse are in µs. See the **create** command information in the vnmr documentation for other parameter types.

8. Some newer sequences being written at Varian usually now use a phase table named the same as the sequence and located in /vnmr/tablib, although the newest sequences avoid the use of tablib by specifying the table within the pulse sequence itself (rather than externally).

9. Typically the macro finishes by setting some parameters directly, and then displaying the help file for the sequence that resides in /vnmr/manual via the command **man('relayh')** as and example for cosy.

10. Keep in mind that all vnmr usage looks for macros, parameters, pulse sequences, etc. in the userlib (~/vnmrsys) first, and uses that copy if it exists, prior to looking in the system area (/vnmr). Thus, every user can have their own version (not recommended!) of the same sequence. User's are encouraged to copy a sequence into a new name in their own directory. Finally, I am beginning to implement an intermediate area for facility-developed software, e.g., **maclibpath='/vnmr/maclib.path'** . VNMR will look first at ~/vnmrsys, then at /vnmr/maclib.path, and last /vnmr for a macro. I will update documentation here when this implmentation is done.

V. VNMR to Bruker-AM/AC Parameter Conversion Table

[comments apply to VNMR unless specifically mentioned otherwise]

Parameter	AM/AC	VNMR	Comments
Experiments			
standard 1d ^{1}H	**ZG**	**go / s2pul**	go zeros memory, and starts acquisition; seqfil='s2pul'
homonuclear decoupling	**HOMODEC.AU**	**s2pul homo='y'**	set the cursor on the peak and use **sd** to get the decoupler frequency
1d NOE difference	**NOEDIFF.AU**	**s2pul homo='y' dm='yyn'**	array can be used to run multiple decoupler frequencies in one exp.
standard 1d decoupled $X/^{13}C$	**CPD ZG**	**s2pul dm='yyy'**	
NOE-enhanced coupled ^{13}C	**GATEDEC.AU**	**s2pul dm='yyn'**	DEPT is preferred unless you need quat's
quantitative decoupled ^{13}C	**INVGATE.AU**	**s2pul dm='nny'**	*X* nucleus T_1's can be quite long, so this experiment can be arduous
DEPT ^{13}C editing	**DEPT.AU**	**dept**	
homonuclear correlation 2d	**COSY.AU**	**cosy**	sw/(ni*2) gives usable digital resolution; usually need ≤ 6 Hz/pt
long-range cosy	**COSYLR.AU**	**cosy tau≠0**	
double quantum cosy	**DQCOSY.AU**	**dqfcosy**	complete phase cycling is crucial for the dq filter; nt = muliple of 8
phase sensitive noesy	**NOESYPH.AU**	**noesy**	flat baselines are important for observing small noe's; use **calfa**
phase sensitive roesy	**ROESYPH.AU**	**roesy**	flat baselines are important for observing small noe's; use **calfa**
total correlation/WOHAHA	**???**	**tocsy**	useful for mixtures or separated spin systems
heteronuclear correlation	**XHCORR.AU**	**hetcor**	use only if need very high ^{13}C resolution
inverse hetero correlation	**???**	**hmqc**	
multiple bond hetero correl.	**???**	**hmqc bond≠0**	important experiment for observing through linkage bonding
Read and Save Commands			
read data file	**RE** ***filen.ame***	**rt('*filename*')**	in VNMR, use also MAIN MENU FILE click on filename and LOAD
save data file	**WR** ***filen.ame***	**svf('*filename*')**	in VNMR, no menu selections for this
read parameter file	**RJ** ***filen.ame***	**rtp('*filename*')**	
save parameter file	**WJ** ***filen.ame***	**svp('*filename*')**	
read shim file	**RSH** ***filen.ame***	**rts('*filename*')**	in VNMR, will search ~/vnmrsys/shims and /vnmr/shims paths
save shim file	**WSH** ***filen.ame***	**svs('*filename*')**	in VNMR, will save to ~/vnmrsys/shims
load shim file	none needed	**loadshims**	loadshims is UW-written macro having **load='y' su load='n' su**

1d Acquisition Commands			
tune ^{1}H observe	**RJ H1.SET**	**UWmacro TuneH1**	tuneh is a UW-written macro
tune ^{13}C channel	**RJ C13.SET**	**UWmacro TuneC13**	tunec is a UW-written macro
zero and go	**ZG**	**go** or **ga**	**ga** will automatically apply a **wft** after acquisition
automation run	**AU *autom.nam***	**au**	all VNMR programs run from compiled routines
halt acquisition with data	**^H**	**sa**	in VNMR, svf can be issued during acquisition to save data
resume acquisition	**GO**	**ra**	seems to work only for 1d; vnmr's **ra** follows an **sa** that stops acq.
abort acquisition	**^E** or **^K**	**aa**	data is lost
automation setup	**AS *auton.ame***	none (try **dps**)	in VNMR, only parameters used in experiment will be shown
1d Acquisition Parameters			
sweep width	**SW**	**sw**	
center or offset frequency	**O1**	**tof**	
solvent	none (change O1 thru jobfile)	**solvent='cdcl3'**	with solvent set correctly in VNMR, **tof=0** will center spectrum for normal organic compounds
set spectrum window	**EP** set window **^O**	set cursors **movesw**	
set offset frequency	**EP** set cursor **O1**	set cursor **movetof**	
relaxation delay	**RD** or **D1**	**d1**	AM/AC delay differs depending on ZG or AU to run experiment
common pulse width	**PW**	**pw**	90° length fixed by probe on AM/AC's; depends also on **tpwr** on Unity
acquisition time	**AQ** (=TD*DW)	**aq** (=np/sw)	
dwell time	**DW** (=1/(2*SW)	= 1/sw	Bruker acquires complex pairs sequentially, vnmr simultaneously
# of transients to acquire	**NS**	**nt**	Bruker NS -1 which goes continuously → vnmr nt=1e6
receiver gain	**RG** (larger # → larger gain)	**gain** (larger # → larger gain)	on Unity, if gain=0 still clips (get ADC OVERFLOW message), insert attenuator at preamp output
observe transmitter power	none	**tpwr=52** (higher # → higher power)	AM/AC's observe power is fixed; Unity's have linear amplifiers on both observe and decouple
number of points acquired	**TD** (usually = SI)	**np**	
temperature	**TE**	**temp=24**	see **temp24** and similar macros (written at UW)
decoupler transmitter power	**DP<ret>20H** (lower # → higher power)	**dpwr=40** (lower # → lower power)	vnmr parameters are logical

1d Processing Commands and Parameters			
fourier transform	**FT**	**ft**	
number of points FT'd	**SI**	**fn**	zerio-filling occurs here (e.g., np=1024, fn=2048 will zero-fill once)
line broadening parameter	**LB**	**lb**	
interactively set weighting parameters	none	**wtia**	in vnmr, middle button still control intensity in all windows; left button sets parameter
apply exponential line broad.	**EF**	**wft**	in vnmr, **wft** applies whatever weighting function is setup
set reference	**EP** set cursor **G**	set cursor **nl** **rl(0p)**	vnmr gives example for TMS
automatic phasing	**AZPK??**	**aph**	in vnmr, **lp** should ~ 0, otherwise advise **calfa** command and/or back-linear prediction
baseline correction	**EP K**	**bs(5)**	in vnmr, **bs** is *not* recommended (default spline fit)
normalized intensities	**AI<ret>0**	**nm**	in vnmr, vs=100 will fill screen
absolute intensities	**AI<ret>1**	**ai**	
1d Plotting Commands			
plot spectrum	**PX**	**pl**	
plot parameters	in **DPO** setup	**ppa** or **pap**	
plot axis	in **DPO** setup	**pscale**	in vnmr, axis='p' sets axis to ppm
plot coordinates	**X0, Y0**	**sc, vp**	sc is mm from right side, vp is mm vertically up from bottom
plot size	**CX, CY**	**wc, vs**	wc is mm width of chart, vs is vertical scale
plot integrals	**PXD???**	**pirn**	
plot peak picks	in **DPO** setup	**dll** or **ppf**	**printon dll printoff** prints table to separate page (do before any plotting commands); **ppf** plots on spectrum
new page	**NP**	**page**	

2d Acquisition Parameters			
sweep width for F1	**SW1**	**sw1**	AC/AM SW1 is 1/2 of observed sweep width; vnmr sw1 = observed sweep width
# increments/experiments	**IN**	**ni**	in vnmr, phase determines total # experiment = 1× or 2×ni
type of 2d acquisition	**MC2**	**phase**	AC/AM: only absolute value and TPPI are available in software vnmr: absolute value → phase=0 States-Habercorn → phase=1,2 TPPI → phase=3
2d setup	**ST2D**	none	in vnmr, type in sequence macro then **dps**
total time of experiment	none	**time**	
interleaved acquisition	depends on routine	**il='y'**	in vnmr, acquire **bs** scans per increment, loop until **nt** completed
2d Processing Commands and Parameters			
FT size in F1	**SI1**	**fn1**	AC/AM square requires SI1=SI/2=SI2/2
reference in F1	**SR1**	set cursor, **rl1(0p)**	
FT and weight full set	**XFB**	**wft2da**	in vnmr, for absolute value sets use **do2d** or **wft2d**
FT and weight t_2 dimension	**XF1**	**wft1da**	counter-intuitive commands, but mean 1st transform
interactive weighting	none	**wtia**	can be done on t_2 fid, e.g., **wft(1) wtia**, and on t_1 fid, e.g., **wft1da TRACE wtia**
display color map	**EP2D** or **AP2D**	**dconi**	
2d Plotting Commands			
plot contours	**CPL**	**pcon**	in vnmr, see also **pconpos** and **pconneg**, UW written macros
plot size	**CX, CY**	**wc, wc2**	in vnmr, sc still controls distance in mm from right-hand side
Plot 2d using high res 1d		**plot2dhr**	have 1d high res already worked up in separate exp, follow prompts
peak picking and volumes	none	**ll2d**	

VI. Trouble-Shooting

- **wrong parameters** – make sure **probe** parameter matches probe in magnet
- **sample won't spin**
  – if probe has been changed, find TA to try reseating spin collar tube: at top of magnet, push down the aluminum tube guiding sample in
  – check that tube is not inserted too far into spin collar
  – check that VT air is not turned up too high
- **sample won't eject**
  – try turning the VT air up to ~ 80 (turn it back down after inserting)
  – check that VT air is hooked up (rather than N_2 gas which has lower pressure)
  – check that air pressure (gauge in southeast corner) is turned up to mark
- **sample won't shim**
  – read in proper shim file (use UWMACROS LOADSHIMS)
  – check that you have enough solvent (≥0.6 ml) and are 68mm down or centered in rf region
  – check that lock power is not too high, and that lock phase is correctly adjusted
  – let magnet warm for quite a while (up to 1h) after a cold experiment; shims are more sensitive to this than on AM-500;
  – if previous student didn't stop early enough, you will need to adjust especially the lock phase fairly often during the warmup (and wack the previous person as hard as possible with a wet noodle!), and also reshim somewhat over 30 min to 1 hour
- **command doesn't work** – type **return** and try it again; some mistypes carry over to next line
- **S/N seems poor**
  – most likely, an attenuator has been left in line at the output of the preamp leading back to the ADC; if your sample is not very concentrated, remove this attenuator and adjust the **gain** setting
  – check pw90 (at least on the observe side); if unusually long, check with TA or facility staff
- **spectrum on screen is only an inch long or so**
  – type **full** to reset plot window (needed after **dssh** command)
- **says exp locked** – enter the command **unlock(#)** where # is the exp number that's locked
- **won't let jexp#** – probably have not created the experiment (see WORKSPACE)

explib	will list all experiment areas
cexp(#)	will create experiment area #
delexp(#)	will delete experiment area # (saves disk space)

- **cannot get good pw90 calibration**
  – check that probe is properly tuned
  – check that **tpwr** is set correctly (or **pwxlvl** for decoupler calibration)
  – check that external attenuator is *not* placed in ^1H observe position

- **waits a long time before acquisition starts** – have one of the following flags set

  spin ≠ 0 if spin is set to a number, the spectrometer will "regulate" the spinning, taking time before acquisition to make sure the spinning is regulated

  gain='n' for this setting, spectrometer will perform an autogain; recommend setting the gain to a specific value manually and not using autogain

  wshim='a' autoshimming will occur; should not be used except possibly between kinetic runs (simply too inefficient and wastes spectrometer time)

  use the **flagsoff** macro to set all these flags to appropriate values

- **No acqi window** Type **acqi** in vnmr command line

- **Can't Connect to spectrometer**

  Pressing connect button on ***acqi*** doesn't work; check that magnet leg is set to **observe** (not **tune**).

  Try in UNIX terminal window: **su acqproc** twice (once to kill, once to restart); this should re-enable connect to ***acqi***

- **FIFO Underflow Error:** ...Check sweep width; an excessive sweep width (>80,000 Hz) can show this error, try reducing sw and re-acquiring.

Operation of Unity–500

created 11/7/95 – updated 10/16/98

I. Proper Exiting and Logging In and Initial VNMR Setup

- if an experiment is running, and your time is clearly in effect, use:
  svf('*savename*') while experiment is running–will save last **bs** dataset
  use FileManager to check that file wrote correctly (unix command **df** better)
- **exit** vnmrx, right-click-and-hold on background and release on EXIT to exit CDE
- do *not* save the workspace while VNMRX is open

II. Commands for First Time and Novice Users

Some of the following commands/procedures may have to be performed when first starting; many of them will not be needed again (or only occasionally):

- **phasing=100** (or =60 on a Sparc1 if the data is >64k)
- **cexp(2) cexp(3) ...** create additional experiment areas (see also MAIN WORKSPACE CREATE)
- click MAIN MENU MORE CONFIG PRINTER and keep clicking PRINTER until set to Shadowp_LJ (laserjet portrait printing)
  – repeat above except for PLOTTER and click unter set to Shadowp_LJR (landscape plotting)
- **gf** following correct setup to give good fid/spectrum shimming inside ACQI FID window

III. Probe Changes

ONLY FOR TA'S AND FEW STUDENTS OK'ED FOR PROBE CHANGES

- see Table 1 on the next page for a description of probes
- make sure acquisition is complete and data saved by previous user
- stop temperature control by using macro **tempoff** (in /vnmr/maclib)
- physically switch the temp controller off
- eject sample (type **eject** at command prompt, or click **eject** inside shimming/acqi window); type **insert** to turn the air back off
- disconnect rf cabling, VT line, and probe cooling tygon
- disconnect temp/heating cable using blue nonmagnetic screwdriver
- unscrew two probe thumbscrews and guide probe out
- insert correct probe; use care with last 1" — you may have reseat aluminum bore tube by pushing gently downward pressure at top of magnet (necessary if sample won't spin)
- reconnect cables: *keep Nalorac cable and filters separate and use only for that probe*
- power up and restart temp control with UWMACROS SET TEMP or macro similar to **temp24**
- read in new shims and load, e.g.:
  rts('triple') loadshims (better use **UWMACROS LOADSHIMS**)
- change probe and pfg settings appropriate for probe:
  probe='hcx' pfgon='nny'
  probe='bbold' pfgon='nnn'
  probe='1h19f' pfgon='nnn'
  probe='3mm' pfgon='nny' (UNITY) **pfgon='yyy'** (INOVA)

Table 1. Description of Probes on Unity-500 and Inova-500

Use the following general rules for probe selection:

- concentration limited samples: use the largest diameter probe appropriate to the experiment
  - hcx or $^{1}H/^{19}F$ best for 1d or homonuclear ^{1}H experiments
- quantity limited samples: use the smallest diameter probe appropriate to the experiment
  - Nalorac 3mm probe best for all ^{1}H experiments in this case
  - ***strongly* consider using susceptibility-matched inserts for 3mm ^{1}H or 5mm X experiments (~3× saving in amount of material needed to obtain a particular S/N in a fixed amount of time, or ~10× decrease in time for fixed amount of material!!)**

Name	Type	Temp	Description
hcx	5mm 1H {13C, X}	≥ -80°C ≤ +60°C	• inverse triple with PFG • good for ^{1}H and ^{1}H-X heterocorrelation • excellent 1H S/N~800
bbold	5mm broadband	≥ -150°C ≤ 150°C	• standard probe for direct ^{13}C, ^{31}P, ^{29}Si, ... observation • ^{1}H signal-to-noise (S/N) and line shape are poor with this probe
bbswg	5mm broadband switchable (i.e. 1H observe) with pfg	≥ -130°C ≤ +60°C	• standard probe for direct ^{13}C, ^{31}P, ^{29}Si, ... observation • ^{1}H S/N is adequate with this probe, so probe switching for ^{1}H observation is not needed (for best ^{1}H S/N, use inverse or h1f19 probe; bbswg 1H S/N~350)
h1f19	5mm $^{1}H/^{19}F$	≥ -150°C ≤ 150°C	• for best sensitivity ^{1}H work when concentration is limited • ^{1}H S/N is good (540 on EB)
nal3mm	3mm nalorac (INOVA only)	≥ -40°C ≤ 40°C	• for ^{1}H 1D and 2D heterocorrelation ($^{13}C/^{15}N$ only) when sample amount is limited, or need best water suppression • ^{1}H S/N is very good (5mm probes are better for concentration limited samples)
invx	5mm inverse broadband	≥ -150°C ≤ 150°C	• for 2D hetercorrelation work: HMQC, HMBC, HSQC • ^{1}H signal-to-noise is very good with this probe • X S/N is poor with this probe; do not do ^{13}C observe with this probe
triple	5mm triple	≥ -100°C ≤ 100°C	• for 2D ^{1}H–^{13}C–^{15}N work: HMQC, HMBC, HSQC • ^{1}H S/N is very good • ^{13}C and ^{15}N S/N is poor • VT range is limited: -50 to +80°C

Table 2. Calibrations of Probes on Unity-500

Use the following guidelines for probe calibrations:

- short runs use facility numbers (see /vnmr/shims/probes* file for up-to-date numbers)
- ^1H pw90 checks are always recommended time permitting for all experiments
- if probe problems are suspected, check pw90's of *X* and ^1H observe (not decouple)
- always perform calibrations (at minimum ^1H pw90 check) for overnight or longer runs for PT-type experiments;
- for standard decoupling, calibrations are rarely needed even for long runs (although having pw ~< 90° is best)

See file on-line	/narn/vnmr5.3b/shims/probes* ..... if not logged onto narn /export/home/vnmr/shims/probes* ..... if logged onto narn

- (preferable to the following is UWMACROS LOADSHIMS or FILES DATA SHOWSHIMS)

  shim files that are available can be listed by entering the following commands in a UNIX window:

  - facility shim files: type **dir /vnmr/shims**
  - your shim files: type **dir ~/vnmrsys/shims**
  - *user* shim files: type **dir /home/*user*/vnmrsys/shims**

  or simply go to path in the FileManager
- load shims with UWMACROS LOADSHIMS

III. Probe Tuning

- recommended method: **UWMACROS TUNE PROBE ...**

or enter macro similar to **tuneh** (in /vnmr/maclib)

- **gain=0** is necessary for tuning (UWmacros restore original gain setting)
- make sure decoupler is off (**dm='n' su** if necessary)
- move cable (e.g., ^1H) from **obs** or **dec** BNC to **tune** BNC
- switch knob from **obs** to **tune**
- adjust tune and match to achieve 0 on meter (in most cases, getting needle < 10 is sufficient)

On many of the probes, there will be three capacitors:

It is essential that the two similar capacitors stay at nearly the same capacitance (i.e., same number of turns from end), so make sure to move them together

For example, on the bbold probe, the ^1H channel has a gold and two silver rods (all small diameter) connected to capacitors. The silver are both "match" capacitors, and must therefore be turned together: if you move one clockwise by ¼ turn, the other should also be turned clkwise ¼ turn.

- switch knob back to **obs**
- move cable back to **obs** or **dec** BNC
- tune other channels as needed

inverse ^1H/X probe: for ^1H channel, tune the gold (match) and silver (tune1) knobs first, then make sure black knob (tune2) is within ¼ turn of silver knob

IV. Lock and shim

Use care when clicking on* CONNECT *on the acquisition window; fast clicking can crash the computer (requiring up to 30 min to correctly reboot!), so use patience when going to acqi

- click into the LOCK panel in the acqi window and turn off the lock
- change Z0 until there is no oscillation in the lock signal: do not hesitate to turn up lock power and lock gain achieve lock, but lower LOCK POWER as soon as possible to avoid lock saturation
- set the LOCK POWER to recommended settings (only go up to potentially safe setting if shims are poor; set back once shims have improved) and use LOCK GAIN thereafter to adjust amplitude
- adjust LOCK PHASE analogous to a shim to get positive going signal
- turn on LOCK
- adjust LOCK PHASE as a shim to maximize lock signal (make sure to return to LOCK PHASE fairly often when shimming, especially after large changes in Z2)
- click into SHIM window and shim normally
  - start by 1st order shimming Z and Z2; when finished take nt=1 acquisition to check line shapes use **nl dres** or if S/N is excellent use **nl res** to get indication of line shape
  - target 50% full linewidth ≤ 1 Hz for most samples, spinning or non-spinning
  - now 2nd order shim Z2 (choose a direction to move Z2; this will decrease lock signal [1st order shim had lock signal maximized at current Z2]; see if Z1 improves; if so continue, if not go other direction in Z2)
  - shim X Y XZ XY XY X2-Y2 all 1st order, then repeat 2nd order Z Z2 shim
  - check line shape; if not at target try spinning sample; if improves considerably turn spin off and work on X Y shims; if did not improve much with spinning then need to target higher order Z's
- Table 4 shows shims dependencies for the Unity-500; 2nd order shimming is required on all 500 MHz instruments (i.e., you simply cannot expect to get a good shim without it)

Table 3. Field and Lock Power Settings for Unity–500

solvent	^1H δ (ppm)	Z0 (field as of 97/12/01)	FINAL lock power	STARTING lock power
acetonitrile-d3	1.93(5)	1350	10	20
acetone-d6	2.04(5)	1200	12	20
dimethysulfoxide-d6	2.49(5)	750	14	24
deuterium oxide-d2	4.63(DSS)	-2000	28	40
methylene chloride-d2	5.32(3)	-2700	15	25
benzene-d6	7.15(br)	-4900	18	30
chloroform-d3	7.24(1)	-5000	25	35

☞ Z0 will change by ~ +100 units each week.

Table 4. Major Shim Interactions on Unity–500

[+ means shim move in same direction—positive change in Z4 results in positive change in Z2] Much of the table is not completed; since new shims installed, most interactions are now much weaker.

Adjusted shim	Stong interaction		Weak interaction		Adjusted shim	Stong interaction	Weak interaction
Z^5	Z^3	?	Z^4	?	XZ	X	Z
Z^4	Z^2	-	Z^3	?	YZ	Y	Z
Z^3	Z	?	Z^2	?	Z^2X	ZX	Z, X^3
Z^2	Z	-			Z^2Y	ZY	Z, Y^3, ZXY
					X^2Y^2	XY	

Table 5. Shim Sensitivities on Unity–500

[number following shim is normal adjustment when shim fairly close to correct]

Sensitive		Moderate				Insensitive	
Z	16 to 4	Z^3	64 to 16	XY	64	X^3	64
Z^2	16 to 4	Z^4	64	Z^2X	64	Y^3	64
Y	16 to 4	X	16	Z^2Y	64	ZX^2Y^2	64
YZ	16	XZ	16	ZXY	64		

Experiments on Unity–500

created 5/04/97 – updated 12/21/97

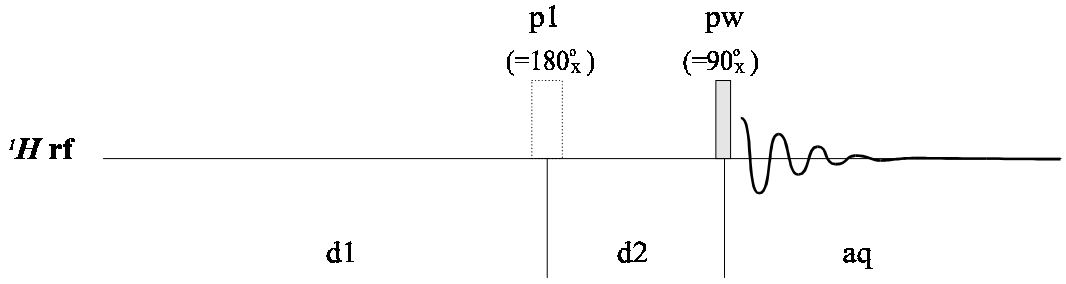
I. Normal 1d ^1H Acquisition

(21-Dec-97)

A. Discussion

- 1d ^1H acquisition on the Unity is simplified by the **solvent='*solventname*'** command. When set appropriately, **tof=0** should center the transmitter correctly.
- With identical transmitter and decouple channels, it is simple to decouple *X* nuclei on the Unity-500 while observing ^1H; e.g., for ^{31}P set **dn='P31'** and other parameters correctly (see later section).
- **calfa** is an important baseline flattening (timing correction) macro on the Unity when setting up 2d experiments. Note the use of this command in the acquisition section.
- Spin-lattice relaxation (T_1) can be measured/estimated with this sequence: set **p1=2*pw90** **pw=pw90** and **d2** appropriately (both p1 and d2 are normally are set =0; see later section for details on T_1 estimates).
- Note that Varian sequences commonly 'hide' some delays. In this sequence, a delay **rof1** prior to, and delays **rof2** and **alfa** following **pw** are not shown. See Varian's documentation on pulse programming for more details.

1d ^1H Acquisition (s2pul)



B. Critical Parameters

d1 – relaxation delay; assuming pw~30° d1>T_1 to obtain quantitative integrations
aq – acquisition time → determines (ignoring sample effects) resolution ~ 1/aq
pw & **twpr** – critical for pw90 calibration and many other experiments

C. 1d 1H Acquisition

- to setup parameters (method **b** is recommended)
  a) either read in a data file (**rt** or FILE *left-click* LOAD) or parameter file (**rtp**) for normal ^1H acquisition, or
☞ b) click on MAIN MENU, SETUP, and select nucleus and solvent

c) alternatively, run s2pul macro (but normally will need to reset **spin='n'**, **gain=0**; check temp and vttype, and check tpwr (=58 typically) and pw (=5-8μs typically).

- use UWMACRO TUNE PROBE and tune probe
- use **go** to start acquisition
  - **ga** will automatically apply a **wft** (weighted Fourier transform) following acquisition
  - use **wft** to manually transform
  - **dscale** will display the axis
  - **dsx** will apply **wft dscale(-3)**
- optimize **sw** by setting *right-click* or *-drag* (right mouse button) and *left-drag* (left mouse button) cursors, and use **movesw**
- set reference by placing cursor close to peak, then type **nl** and **rl** command, e.g., **rl(7.24p)**
- if always getting ADC OVERLOAD beep, then receiver gain is too high; **gain** can be set three ways (number **1** recommended):

☞ 1. – simply lower **gain** until ADC OVERLOAD warning goes away; if warning stays with **gain=0**, insert attenuators into ***bottom*** BNC (preamp output), not into the probe connections
  – make sure **gain** is within 10B of overload warning

2. – reduce **nt=1** and enter **gf** ; make sure to wait a few seconds (menu will flash) before
  – click on acqi and then **FID**; make sure SPECTRUM is not selected and click on **DOWN** until you see the red horizontal lines that indicate the ADC clipping limits; adjust **gain** until the fid fills ~1/2 of the region to the clipping voltage
  – switch **nt** back to original value
3. set **gain='n'** which will implement autogain adjustment (***not*** recommended, especially for 2d where huge artifacts can result)

D. ^{1}H *pw90 Calibration*

- normally can perform calibration on sample; best to *not* perform it on a clean solvent since T_1 for solvents can be very long
- set **tpwr** to desired setting; typically 58 for 5mm probes, 52 for 3mm probes
- set **d1=5-10s** depending on sample T_1 (**d1=5** is usually ok)

☞ recommended: perform a coarse check of pulse widths using:

array<ret>
variable to array: **pw**
number of increments: **20**
starting value: **3**
size of increment: **3**

- set **nt=1** and use **go**; should see a sinusoidal response; if not, usually **d1** needs to be longer
- to obtain an accurate **pw90**, check about the 360° value with an array increment size of 0.5 to 1 μs
- plot arrayed spectra using the following commands:

wft dssh ; transforms all spectra and displays in a horizontal stack
full ; resets plot area to full screen

E. 2nd Order Shimming on a 500 MHz Spectrometer

- Make sure the lock is not saturating. Check by watching that the lock level increases consistently with increasing LOCK POWER. Once the lock level drops or stays steady with increasing power, back off the power by roughly 20%. I've heard some Varian chemists look for a 50% decrease when dropping LOCK POWER by 6 dB when LOCK POWER is ok (i.e. low enough to not be saturating), but I've not seen consistent results doing this. I simply look for a "bounce" in the lock level, and go 20% below the setting at which the bounce is last observed (can be difficult for fast-relaxing solvents like D_2O).
- Adjust LOCK GAIN to give lock level in 25-65 range. Optimize lock level using LOCK PHASE. Optimize lock level with **Z** and **Z²**.
- Change **Z²** in one direction enough to change lock level by 5-10% (since optimized in previous step, level will decrease). Re-optimize lock level again with **Z**. If newly optimized lock level is lower than the previous one, try changing **Z²** in the other direction; otherwise continue changing **Z²** in same direction until re-optimization does not improve the lock level.
- Keep changing **Z²** in the same direction and optimizing lock level with **Z**, until an overall maximum has been found. Set **Z** and **Z²** to this maximum setting.
- Keep lock signal value between 25-65 using the LOCK GAIN; check that LOCK PHASE is set correctly on regular intervals (especially after any large changes in **Z²**).

- Other 2nd order shim combinations that would require a similar shimming iterative scheme such as described above (i.e. find a simple maximum in lock level, change the high order shim in a particular direction—lock level decreases—then re-optimize with lower order shims to see if lock level improves from what started with):
  Z^3: Z^2, Z: will require 2nd order corrections at Z^2 to obtain overall maximum in lock signal
  Z^4: Z^3 2nd order correction involving 2nd order optimization of Z^2 (thus each change in Z^4 can require a significant effort to see if there's any improvement at all).
  XZ: X
  YZ: Y
  XZ^2: XZ(2nd order), X
  YZ^2: YZ(2nd order), Y

F. 1d Data Workup and Plotting

- **dc** will correct any linear baseline shifts
- **bc(5)** will correct fifth-order baseline, assuming **region** command (run automatically on a **bc**) can find peak and baseline regions; **bc** is *not* recommended for 1d, but is ok for 2d workup (default spline fit; see manual for details)
- display the axis using **dscale(-3)** and **axis='p'**, or use the macro **dsx**
- plot spectra using the following commands:

  pl ; plots spectrum with **sc** being cm in from *right* side **wc** being width in cm

  pap ; plots all parameters along left hand side, or **ppa** for the major parameters only; does plot out **text**, which can be entered manually, but easiest way is to use the CDE File Manager to go to ~/vnmrsys/exp1 (assuming working in exp1) and double click on **text** file; simple editing of the file can then be performed

  ppf ; plots peak pick
  th controls threshold height used);
  dpf will show peak picks on screen
  dll lists peak picks with intensities (**printon dll printoff** will print)

pirn ; prints integral values under axis:
– start with **cz** to clear integrals
– click MAIN MENU DISPLAY INTERACTIVE PARTIAL INTEGRAL
– type **region** (if you don't like the regions, start again with **cz** and use the RESETS button to enter regions with the mouse manually)
– type **intnorm** or use UWMACRO MORE NORMINT or DISPLAY NORMINT [**adjism** is Varian's not-so-good command] to adjust the peak amplitude
– enter **vp=12** to get axis out of list area
– integrals will plot with **pl** when on screen; **pirn** is needed to list values below axis

II. *X* Acquisition (e.g., ^{31}P or ^{13}C)

(22-Jun-98)

A. Discussion

- The spectrometer acquisition time of an *X* nucleus experiment should be estimated from similar experiments on the same equipment if possible using eqn (1) or (2) below. ^{13}C and ^{31}P experiment times on the 500's and 360 can be estimated from similar experiments on the AC-300 (Athena) using:

$$S/N \propto cB_o^{3/2}t^{1/2}\xi \qquad (1)$$

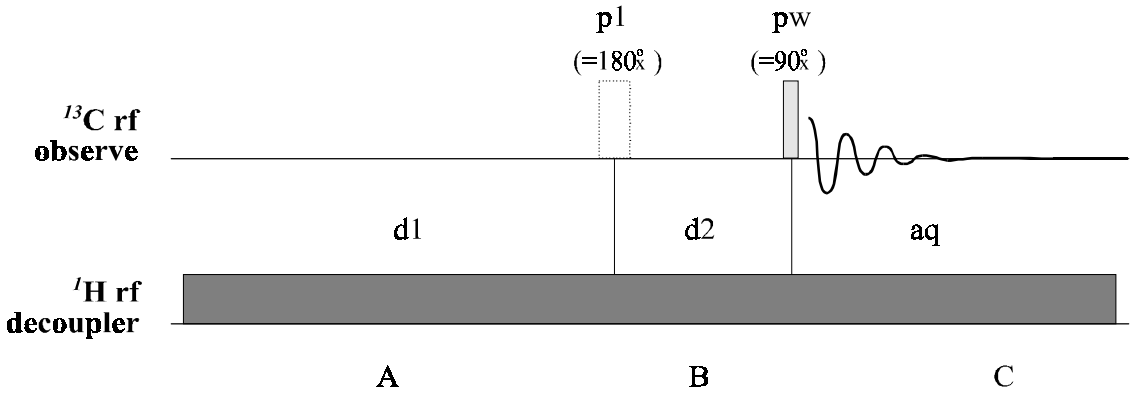
where c = concentration, B_o = magnetic field strength, t = experimental time, and ξ = probe filling factor. To estimate the time under different conditions that would give identical *S/N*, then:

$$t_{new} = t_{old} \cdot \left[\left(\frac{c_{old}}{c_{new}} \right)^2 \left(\frac{B_{o_{old}}}{B_{o_{new}}} \right)^3 \right] . \qquad (2)$$

A reasonable estimate (although direct comparison to experiments on similar compounds is preferred) on the UWChem*MRF* equipment can be made starting with the observation that a 0.1 M solution will typically give publication quality spectra in 20 min on the AC-300 (Athena).

- Standard decoupled (NOE-enhanced), quantitative decoupled (no-NOE; Bruker's INVGATE.AU) and NOE-enhanced coupled spectra (Bruker's GATEDEC.AU) can be obtained through use of the S2PUL sequence.
- Nuclei with negative γ values, such as ^{15}N and ^{29}Si, are best acquired using polarization transfer sequences; DEPT in general is the preferred sequence over INEPT if more than one J_{XH} value is involved; quaternary moieties of negative γ nuclei are best obtained through long-range coupling unless the $T_1(X)$ values are known to be reasonably short.

1d ^{13}C Acquisition (s2pul)



B. Critical Parameters

d1 – relaxation delay; **d1** > $T_1(^1H)$ to obtain optimum NOE
dof – usually =0 when **solvent** set correctly; should be within 5ppm of ^1H coupled to X nucleus of interest
dpwr – decoupler power (larger number is higher power); ***typically never > 46***
dmf – decoupling strength = [pw90(^1H at dpwr)]$^{-1}$
dmm – decoupler modulation mode; either **dmm='ccp' dseq='waltz16' dres=90** or **dmm='ccw'** [both are equivalent] is best for typical compounds
dm – decoupler on/off flag (see Table 6)

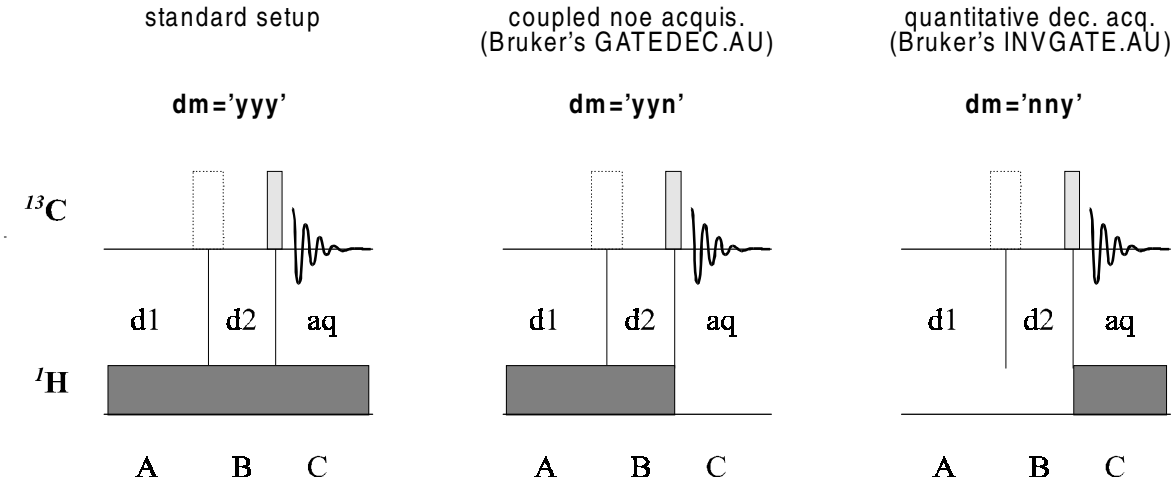
C. 1d X{^{1}H} *Acquisition*

- start by setting the probe parameter appropriately
- to setup parameters (method **b** is recommended)
  a) either read in a data file (rt) or parameter file (rtp) for standard $X\{^1H\}$ acquisition, or
☞ b) click on MAIN MENU SETUP and select nucleus and solvent
  c) alternatively, run s2pul macro (but normally will need to reset **spin='n'**, **gain=0**; check **temp** and **vttype**, and check **tpwr** (=58 typically) and **pw** (=5-8μs typically).
- use UWMACROS TUNE PROBE TUNE C13 [or **tunec** macro] and tune ^{13}C channel; reattach ^{13}C X cable to X Obs BNC
  – make sure ¼ wave cable is correct for ^{13}C
  – make sure low-pass (brown) filter is in-line
  – make sure correct probe cap is inserted (*none* in bbold probe)
  – make sure no external attenuator is in-line
- use UWMACROS TUNE PROBE TUNE H1 [or use **tuneh** macro (will have to reset gain after tunec if UWmacro is not use] and tune decoupler channel; reattach ^1H decoupler cable to decoupler BNC

Table 6. Common Decoupler Parameter Settings

Parameter Settings	Comments
dm='yyy' dpwr=46 su	typical for ^{13}C acquisition; full on decoupler; keep **dpwr ≤ 48**
dm='nnn' su	turns decoupler off; always finish with this command + **su**
dm='nny' su	inverse gated (Bruker's INVGATE.AU) mode; gives quantitative (assuming **d1**>5T_1 !) decoupled spectra
dm='yyn' su	gated spectra (Bruker's GATEDEC.AU); gives coupled spectra but with NOE buildup (DEPT is necessary for ^{29}Si, ^{15}N and other -γ nuclei)
dmm='p'	normal setup for ^{13}C acquisition (8/1/97; **dmm='w'** works fine)
dmm='ccp'	normal setup for DEPT and INEPT most 2d ^{13}C experiments, does hard pulses followed by pulsed decoupling during acquisition
dseq='waltz16' dres=90	normal setup for ^{13}C (and all X) acquisitions; ^1H waltz-16 decoupling
dseq='garp1' dres=1	normal setup for all inverse experiments; X garp-1 decoupling
dmf= [1/pw90]	sets decoupler pulsewidth for composite pulses, where pw90 is the ^1H decoupler 90° pulse width at the **dpwr** setting used for decoupling
dpwr= [typically ~42]	decoupler power in dB; typically want 90° pulsewidth = 100-150μs

1d ^{13}C Variations in VNMR



- use **dm='yyy' su** to turn on decoupler (or UWMACRO DECOUPLER ON; see Table 6 and figure below for more decoupler information)
- use **go** to start acquisition
  - **ga** will automatically apply a **wft** (weighted Fourier transform) following acquisition
  - use **wft** to manually transform
  - **dscale** will display the axis
  - **dsx** will apply wft dscale(-3)
- **gain = 30 to 40** should usually work for ^{13}C acquisition
  - if always getting ADC OVERLOAD beep, then receiver gain is too high
  - it is important the receiver gain be optimized for *X* nucleus experiments
  - see ^{1}H section above for how to set the gain accurately
- optimize **sw** by setting right (right mouse button) and left (left mouse button) cursors, and use **movesw**
- set reference by placing cursor close to peak, then type **nl rl** command, e.g., **rl(77p)**

D. ^{13}C *(X) pw90 and {*^{1}H*} Decoupler Calibrations*

- general rule of thumb for these calibrations is:
  - short runs use facility numbers
  - if probe problems are suspected, check pw90's of *X* and ^{1}H observe (not decouple)
    - if numbers are close to facility values, probe is likely OK
    - if pw90 is much less than facility value, you are doing something wrong (figure it out! : -)
    - if pw90 is much longer (>1μs) than facility value, find TA or facility staff
  - always perform calibrations for overnight or longer runs for PT-type experiments; for standard decoupled experiments, calibrations are rarely needed
- ^{13}C and other *X* nuclei pw90 calibrations require concentrated or labeled samples
  - for ^{13}C, use 50% benzene in acetone-d_6; do not degas these samples
  - addition of $GdCl_2$ or Cr-acac can improve T_1's dramatically

  - set **tpwr** to desired setting; see Table 2 (on lab wall for most current!!) for calibrations for probes in UWChem*MRF* facility
  - make sure you are on resonance (set cursor on multiplet, then **movetof**)
  - set **d1=20s** or longer depending on sample T_1
  - use 360° pulse for final checks always!!
- once the observe pw90 is obtained, assuming in exp1 then **jexp2**
  - move ^1H cable to observe port, and find resonance for benzene doublet (take one scan, move cursor to middle of doublet and use **movetof**); write this value of tof down as ^1H dof
- assuming ^{13}C still in exp1 and ^1H in exp2, do **jexp1 mp(3) jexp3**
  - put ^1H on resonance by setting **dof** = ^1H dof from above
  - setup decoupler calibration experiment with UW macro **pwxdec90**
  - set **pwxlvl = 60** and find **pwx** where antiphase doublet nulls; this is decoupler hard 90
  - set **pplvl = pwxlvl** and **pp = pwx** for **hetcor** and **dept** experiments
  - set **pwxlvl** appropriately for decoupling (probe dependent; see Table 2 on wall) and find **pwx** where antiphase doublet nulls; set **dpwr = pwxlvl** and **dmf = 1/(pwx*1e-6)**

E. 1d X{^{1}H} *Data Workup and Plotting*

- typically will want **lb = 2** or **3** for ^{13}C experiments
- see 1d ^1H section E for plotting description

III. DEPT – Distortionless Enhancement by Polarization Transfer

(22-Jun-98)

A. Discussion of PT versus NOE experiments, and DEPT versus INEPT

Summarizing Derome (see Chap. 6 for very good discussion, p. 129*ff*): **P**olarization **t**ransfer experiments can offer sensitivity enhancements of:

Polarization Transfer – $\left|\frac{\gamma_I}{\gamma_X}\right|$ (1)

NOE – $1+\frac{\gamma_I}{2\gamma_X}$ (2)

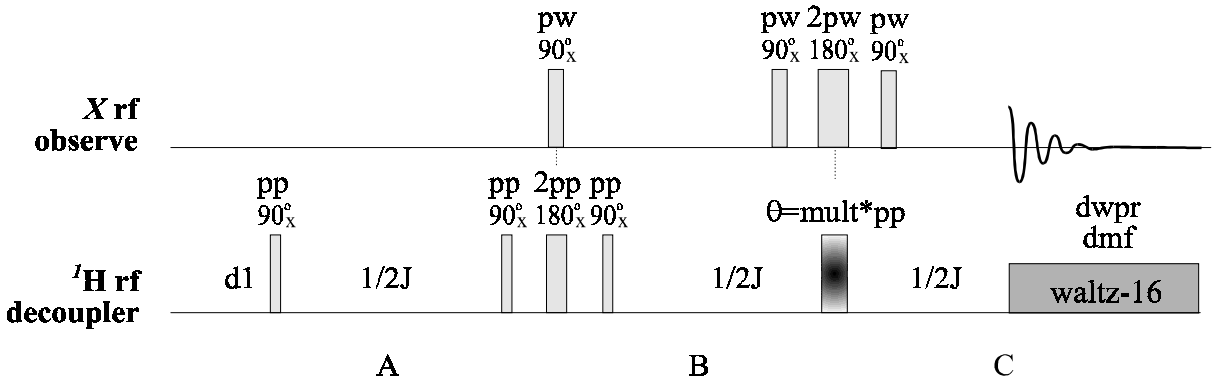
where *X* is the nucleus being observed (e.g. ^{13}C or ^{29}Si), and *I* is the enhancing nucleus (usually ^{1}H, but could also be ^{19}F or ^{31}P). The following generalizations can be followed:

- Polarization transfer is always recommended for nuclei having negative γ values, ^{29}Si, ^{15}N, and ^{103}Rh being three examples. From eq (2) above, the NOE enhancement for these nuclei could result in 0 signal. PT is also always recommended for low-γ nuclei (e.g., starting ^{15}N and lower in frequency).
- DEPT is the best method for obtaining ^{13}C spectra, as well as spectra of other spin-1/2 *X* nuclei, of typically protonated compounds. DEPT is definitely preferred over INEPT if more than one J_{XI} value is involved for the nuclei you want to observe.
- DEPT should be used to obtain coupled spectra (turn the decoupler off during the acquisition: **dm='yyn'**); in general, DEPT will give better S/N than coupled NOE experiments.
- DEPT should be used even if no 1-bond coupling to protons are present for low-γ nuclei if long-range couplings can be used.
- INEPT should be used only if one *J* value is involved and it's size is known.
- typically, only a DEPT-135 is needed (**mult=1.5**), but vnmr makes fully edited spectra easy to obtain; accurate pulse widths are required for good methyl/methine differentiation
- For all these experiments, delays will be dependent on J_{XI}. The better the coupling is known, the better the experiment will work. Make every attempt to measure the couplings from the isotope splittings in the ^{1}H spectrum, or obtain good literature values. Lacking both, be prepared to run a series of experiments using different J_{XI} values to find the optimum parameters.
- For small J_{XI} couplings, a compromise between signal loss from T_2 (inverse natural line width)—especially for low-temp or high MW samples—and PT must be made. In some cases (mainly when $(T_1)_X$ is not too large), the non-NOE decoupled (Bruker's INVGATE) may be the preferred experiment.

B. Critical Parameters

d1	–	relaxation delay; typically = 1-2s
j	=	140Hz; *change if you want to observe* X *with* J_{XH}*>180 Hz or <110 Hz*
pw, tpwr	–	observe *X* 90° pulse width **pw** at power level **tpwr**
pp, pplvl	–	high power ^{1}H 90° pulse width **pp** at power level **pplvl**
mult	–	determine type of DEPT; 0.5 = dept-45, 1.0 = dept-90, 1.5 = dept-135; see discussion below

1d DEPT (dept)



C. DEPT Acquisition

- for short runs, use facility calibrations for **pw, tpwr, pp, pplvl, dpwr (≤46)** and **dmf**
- FILE SETUP SEQUENCES DEPT will setup Dept correctly, including for non-^{13}C acquisitions
  - **mult** is set by the number of coupled protons
  - the interpulse delay is set according to **j**
- for overnights or longer runs, recalibrate (at least) observe and (best) decoupler pulse widths
- set **mult** as needed; dept-135 has **mult=1.5**; for full editing use array **mult=0.5,1.0,1.0,1.5**
- use **au** to acquire for full editing; **ga** is ok for dept-135

D. Calibration

- see ^{13}C section for nominal ^{13}C (X) and ^{1}H decoupler calibrations
- Often, the best way to calibrate the decoupler is to run a DEPT-90 on a compound having a known methylene; this carbon should be nulled in a DEPT-90. Change **pp** (can use an array) to obtain minimum signal at the methylene, and use on unknown or less concentrated sample.
- The delay **d2** and the final decoupler pulsewidth **mult*pp** are calculated by the DEPT macro as follows:

$$\text{D2} = \frac{1}{2J} \quad [\text{ use } J_{C\text{-}H} = 150 \text{ if olefinic present, } 130 \text{ otherwise}]$$

$$\theta \text{ pulse} \equiv \textbf{mult*pp} = \sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{in radians}) = \frac{\textbf{pp}}{90^{\circ}}\left[\sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{in deg})\right]$$

$$\text{Thus} \qquad \textbf{mult} = \frac{2}{\pi}\left[\sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{in radians})\right] = \frac{1}{90^{\circ}}\left[\sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{in deg})\right]$$

For trimethyl-silyl ($J_{Si\text{-}H}$ = 2 Hz),

d2 = 250 msec,

$$\textbf{mult} = \frac{19.47^{o}}{90^{o}} = 0.216$$

These values are calculated internal in the /vnmr/psglib/dept.c pulse sequence code.

E. Data Workup and Plotting

- for full editing, try **autodept** or **padept** macro;
- for dept-135, use **wft** and phase
- **s1** (s#) and **r1** (r#) are enormously useful for comparing data in different workspaces; use these in combination with **md(1,2)** for example to move the save regions in exp1 to exp2
- use **clradd spadd** to move a spectrum into exp5;

  then **addi** to compare that spectrum with currently displayed spectrum
- use **dss** with proper **vo** and **ho** to give a stack
- **pl('all')** to plot the stack

IV. INEPT – Insensitive Nuclei Enhanced by Polarization Transfer

(22-Jun-98)

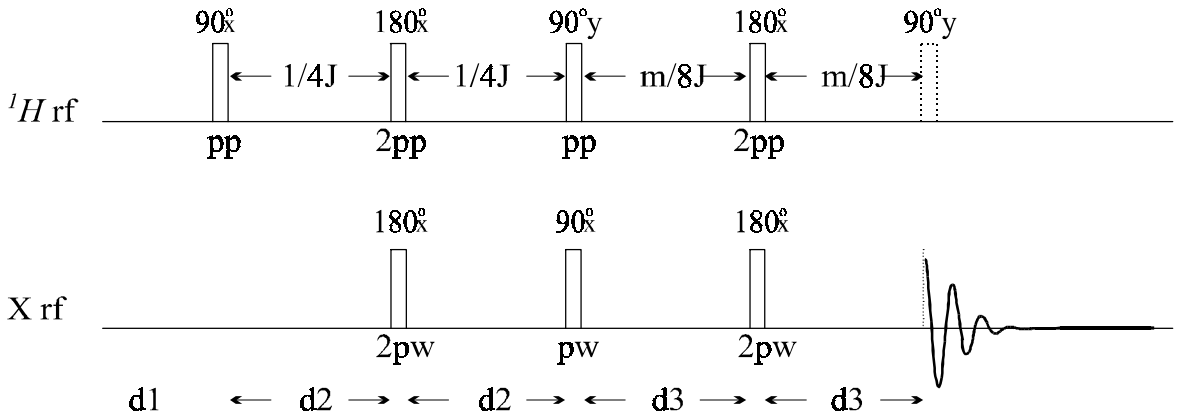
A. Discussion of INEPT

See the discussion section in the previous section on DEPT.

- INEPT should be used if one *J* value is involved and it's size is. Otherwise use of DEPT is recommended.
- The previous statement is not intended to dissuade students from experimenting with the different version of polarization transfer. (After all, the fearless facility manager has been known to give not-such-good advice on occasion. Please do let me know your observation/preferences if you try both INEPT and DEPT on same/similar compounds.)

Refocused Decoupled INEPT

(ineptrd.c [written by cgfry] from Bruker's INEPTRD.AU)



B. Critical Parameters

d1	–	relaxation delay; typically = 1-2s
j	=	140Hz; *change if you want to observe* X *with* J_{XH}*>180 Hz or <110 Hz*
	–	INEPT is sensitive to accurate *J* coupling being entered; use DEPT unless you are fairly certain you know *J* reasonably accurately
pw, tpwr	–	observe *X* 90° pulse width **pw** at power level **tpwr**
pp, pplvl	–	high power ^1H 90° pulse width **pp** at power level **pplvl**
mult	–	multipler for **d3** delay, where **mult= 1** all positive, **2** -CH2- nulled, **3** -CH2- inverted from -CH3 and -CH<

C. INEPT Acquisition

- the INEPTRD macro (run by the menu SETUP SEQUENCES INEPT) is ***not*** the standard Varian sequence, but in my opinion is superior; it incorporates all aspects of Bruker's INEPTRD and INEPTP sequences for decoupled and coupled acquisition, respectively
- for short runs, use facility calibrations for **pw, tpwr, pp, pplvl, dpwr (≤46)** and **dmf**

- FILE SETUP SEQUENCES INEPT will setup Inept correctly, including for non-^{13}C acquisitions
  - **mult** is set by the number of coupled protons and **j** ; **d3 = mult/4j**
  - the **d2** interpulse delay is set according to **j**
- for overnights or longer runs, recalibrate (at least) observe and (best) decoupler pulse widths
- set **mult** as needed; see below

D. Calibration

- see ^{13}C section for nominal ^{13}C (X) and ^{1}H decoupler calibrations
- The delay **d2** and **mult*d3** are calculated by the DEPT macro as follows:

d2 = $\frac{1}{4J}$ echo period involving two **d2** delays creates ^{1}H antiphase state

d3 = $\frac{\text{mult}}{4J} = \frac{1}{2\pi J}\left[\sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{rad})\right] = \frac{1}{360^{\circ} J}\left[\sin^{-1}\left(\frac{1}{\sqrt{n}}\right)(\text{deg})\right]$

where n = # I nuclei coupled with J_{XH}

example: suppose have trimethylsilyl, and want to detect ^{29}Si, and $J_{Si\text{-}H}$ = 2 Hz,

then **d2** = 1/(4×2 Hz) = 125 msec

and **d3** = $\frac{1}{360^{\circ} \times 2\text{Hz}} \sin^{-1}\left(\frac{1}{\sqrt{9}}\right) = \frac{19.47^{\circ}}{360^{\circ} \times 2\text{Hz}} = 27\,\text{msec}$

or **mult** = $\frac{2}{180^{\circ}} \sin^{-1}\left(\frac{1}{\sqrt{9}}\right) = \frac{2 \times 19.47^{\circ}}{180^{\circ}} = 0.216$ (same as in DEPT setup)

These values are calculated internal in the /vnmr/psglib/ineptrd.c pulse sequence code.

E. Data Workup and Plotting

- workup is similar (identical) to ^{13}C 1d and DEPT

V. 1d NOE-Difference Spectroscopy

(24-Dec-97)

A. Discussion

- See the Bruker User's Guide introduction for discussion on this non-trivial, but powerful experiment.
- Sanders & Hunter also contain excellent discussions on NOE experiments (highly recommended)
- Look for instructions on use of GOESY, which should be significant improvement over NOEdiff.

B. Critical Parameters

d1	–	relaxation delay; needs to be ≥ 3 T_1 (see later section for measuring T_1)
dpwr	–	must **not** be set above 14; typically 1-4 works well for saturating multiplets
dm='yyn'	–	typical, but **dm='nyn'** and **d2** can be used to vary saturation within fixed **d1+d2** delays
dof	–	set by command **sd** (preceded by placing cursor on desired multiplet)

C. NOE-Diff Acquisition

- read in a normal ^1H setup: MAINMENU SETUP NUCLEUS/SOLVENT
- SETUP SEQUENCE NOEDIFF will setup the following parameters:

  dn='H1' homo='y' dmm='c' dpwr=1 dm='yyn'

- place the cursor in the middle of the multiplet to be saturated and enter **sd**
- obtain enough data to determine
  i) if multiplet is being sufficiently saturated
  ii) if any other close-by multiplets are being effected by the saturation pulse; if any are, **dpwr** must be decreased until the close-by multiplets are all completely uneffected; this condition is essential–interpretation of the data becomes much more difficult if the chosen multiplet cannot be saturated independent of the other protons (if this happens, consider performing a NOESY or ROESY experiment instead of the NOE-diff)
  iii) currently, I do not know how to plot or save difference spectra if **dof** is arrayed (although this would seem to be the best way to acquire the data)
  iii) see cgfry about implementation of GOESY, which should be a superior experiment to NOEdiff; GOESY info should be available very soon

D. Calibration

- set **dpwr** low, and raise by 3 dB to no more than dpwr=14; use the lowest power that saturates the selected multiplet

E. Data Workup and Plotting

- bring in reference spectrum (with decoupler placed on baseline); make sure expansion and vertical scale are what you want
- make sure exp5 is saved and not in current use, then
- use **clradd spadd** to clear exp5 and transfer the current spectrum to it
- bring in next spectrum (in another exp if desired, but must have same expansion and vs; more precisely, **sp wp vs** must all be identical between spectra)
- use **addi** to compare and subtract spectra; see vnmr manuals for more info

VI. Homonuclear 1d Decoupling

(1-Jul-98)

A. Discussion

- HOMODEC has for the most part been replaced by COSY and cosy-variants, but still has utility for multiplets that are well resolved. HOMODEC can provide actual *J* couplings, whereas fast-COSY usually does not.

B. Critical Parameters

d1	–	relaxation delay; needs to be ≥ 3 T_1 (see later section for measuring T_1)
dpwr	–	must **not** be set above 24; typically 1-14 works well for saturating multiplets
dm='yyy'	–	correct for homodecoupling
dof	–	set by command **sd** (preceded by placing cursor on desired multiplet); can array this variable to obtain all homodec's in same experiment

C. Acquisition

- setup using SETUP SEQUENCES HOMODEC (or just type in **homodec** to run the macro)

  dn='H1' homo='y' dmm='c' dpwr=1 dm='yyy'

- array the **dof** values to acquire all in one experiment
- once decoupler is on, simply use **go** or **ga**

D. Calibration

- adjust **dpwr** (≤14) to reduce multiplet without spilling into close-by multiplets

E. Data Workup and Plotting

- workup as a standard ^1H
- **dssa** should be useful for plotting; see vnmr documentation

VII. COSY – 2d Homonuclear Correlation

(21-Jul-01)

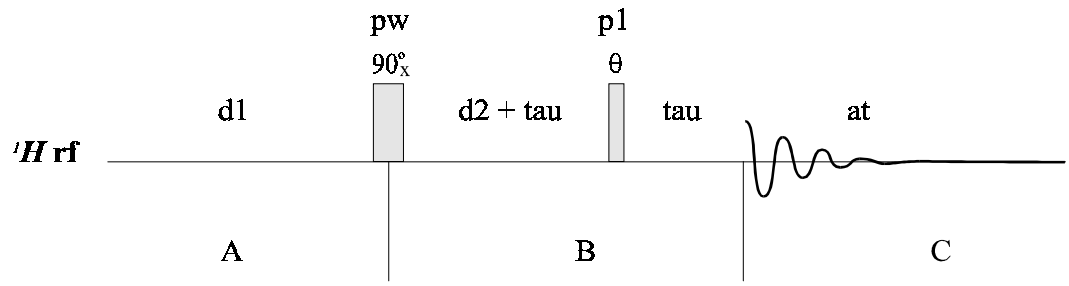
A. Discussion

- Absolute value mode COSY often provides sufficient homonuclear correlation data in a very efficient, robust experiment.
- $J_{HH} \geq 4$ Hz can typically be observed, but this is dependent on **sw** and **ni**

$$digital\ resolution\ in\ F2 = \frac{sw}{np} \qquad digital\ resolution\ in\ F1 = \frac{sw1}{2ni}$$

  assuming zero-filling only in F1 (not common in F2). For very large ni, very small couplings can be observed—often well below the linewidth of a high-resolution 1D experiment.
- A common rule-of-thumb is that $J_{HH} \geq dres1/3$ will be observed in a COSY spectrum; this estimate is definitely a guideline only. *dres1* ≡ *digital resolution in F1* defined above.
- The most efficient method of observing small J_{HH} is with long-range COSY, which involves this sequence and **tau** > 0 (typically **tau** = 50-200 ms).
- **cosy** loses sensitivity from the strong resolution enhancing sinebell (and sinebell-squared) apodization functions used to overcome the absolute value processing. Even so, minimum phase cycling (**nt**=4) usually gives sufficient sensitivity. GCOSY is even faster (**nt**=1, **nt**=2 is better).
- DQF-COSY (next section) removes all singlets, and gives much cleaner diagonals. Use this sequence after a COSY if crosspeaks close to the diagonal or on a strong singlet region are needed.
- **gcosy** is preferred if a PFG (e.g., hcx or bbswg) probe is installed. **nt=1** can be used with this sequence, reducing total acquisition time by 4.

2d Absolute Value COSY (relayh)



B. Critical Parameters

p1	=	90° for maximum sensitivity; 45° (typical) to reduce intermultiplet crosspeaks
ni	=	usually set satisfactorily by cosy macro; sw1/2ni gives usable resolution; must have ≤ 12 Hz, but want ~ 6 Hz if time allows for best results
nt	=	4 is minimum phase cycle
sw	=	set using **movesw** macro (preceeded by boxing selected area of spectrum)
sw1	=	must equal **sw** for **foldt** macro (commonly used; recommended for fast-COSY)

tau = set 50-150 ms typical for long -range COSY
d1 = best set ≥ $2\times T_1$, but often run with **d1** ~ T_1

C. COSY 2d Acquisition

- for COSY, setup as in ^1H 1d and optimize **sw** with the **movesw** macro, then retake the data to ensure **sw** is set correctly
  - move the parameters: assuming you ran the ^1H in exp1 and have nothing important in exp2, the command **mp(2) jexp2** entered from exp1 will work
  - from exp2 then use SETUP SEQUENCES COSY or enter the macro **cosy** or **gcosy**
  - check that **ni** (sw1/2ni ≤ 12Hz/pt, ~6Hz/pt if time allows) **d1** and **np** are correct (check **time**), then enter **au** to run cosy

D. Calibrations

- can use facility pulse width calibrations; COSY is very forgiving with pulse widths
- even so, performing a **pw90** calibration is always recommended for all 2d experiments

E. 2d Data Workup and Plotting

- COSY data can be processed with the **do2d** macro, or use the **wft2d** command (see also **man('cosy')** or **man('gcosy')**
  - **do2d** applies symmetrization with the **foldt** macro

- general display and plot commands

dconi ; displays 2d's with color map (faster than contours)

plot projections before issuing the following command using menus (use the PLOT button)

pcon ; plots contours
pconpos ; plots phase sensitive contours with positive peaks having 10 contours and neg peaks having 1 contour
pconneg ; opposite of pconpos

plot2dhr ; plots with high-resolution traces (must have 1d in another experiment)

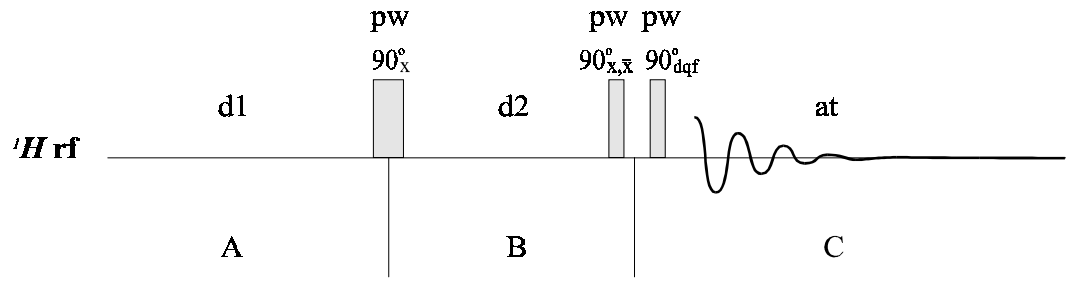
VIII. DQ-COSY – Double-Quantum Filtered COSY (Phase-Sensitive)

(17-Jul-00)

A. Discussion

- DQCOSY is currently accepted by most in the NMR community as the most powerful form of COSY; it is recommended for any experiment beyond the FAST-COSY or GCOSY
- removes singlets (important for strong solvent peaks)
- diagonal in pure adsorption mode with crosspeaks (cleans diagonal for close-in crosspeaks)
- direct coupling (for crosspeak at ν_1,ν_2 is $J_{1,2}$) is anti-phase, whereas indirect coupling (at ν_1,ν_2 is $J_{1,3}$ or $J_{1,4}$, for example) is in-phase, allowing for J assignments in complex molecules
- alternative experiment is E. COSY (see section X)

2d Double-Quantum Filtered COSY (dqfcosy)



B. Critical Parameters

pw, tpwr	= 90° pulse width at power **tpwr**; recalibrate this parameter for all dqcosy experiments
d1	= relaxation delay; set 2-3*T_1 (artifacts will result if **d1** is too small)
ni	= number experiments, or number of points in t_1 ; should be set ok by macro, time allowing; want F1 digital resolution ≤ 6 Hz/pt = sw1/(2ni)
np	= number of points in t_2, usually want ≥ 2048 since costs nothing but disk space and gives better resolution in F2
fn fn1	= usual to not zerofill in F2 (**fn=np**), and give one zerofill in F1 (**fn1=2ni**); **fn1≠fn** is ok for DQCOSY, but to baseline correct in both dimensions set **fn1=fn**
nt	= multiple of 8
sspul	= 'y' gives homospoil-90-homospoil preceding **d1**

C. dqcosy Acquisition

- DQCOSY is setup essentially the same as COSY (use **dqcosy** macro) except want **fn=np**≥2048 and typically **ni** ≥ 350 (**ni** is set up by macro; check that F1 dig resol ≤ 6 Hz/pt = **sw1**/(2**ni**))

D. Calibration

- always recalibrate **pw90** (90° pulse) for dqcosy (experiment is sensitive to quality of this easily-calibrated parameter—see ^1H section for instructions); macro uses **pw90** to set **pw**
- with high-res 1d, or perhaps better **ni**=2 run, carefully baseline correct, then run **calfa**

E. Phase-Sensitive 2d Data Workup and Plotting

- often should not need to phase DQCOSY data at all
- ***phase sensitive*** data should be processed something like the following:
  - set **pmode='full'** ; allows phasing along F2 in 2d spectrum
  - **wft(1)** ; transform just first spectrum
  - **wtia** ; interactive phasing; middle button scales, left sets **lb**
  - **wft1da** ; perform first transform (on t_2 dimension)
  - if integrals have been setup (best on high-res 1d done prior to setting up dqcosy), then **bc('f2')** can sometimes work wonders here
  - click on **TRACE** and select strong intensity trace
    ; trace='f1' changes columns→rows, trace='f2' goes back
  - **wtia** ; interactive phasing on t_1 trace, left button sets **lb/gf**
  - **wft2da** ; performs second (or both) transform(s)
  - pick off two traces that have crosspeaks ; downfield trace save number as **r1**
    ; upfield trace save number as **r2**
  - **ds(r1)** do 0-order phase only
  - **ds(r2)** do 1st-order phase only (click left mouse button on downfield position sets toggle pt)
  - iterate between **ds(r1)** and **ds(r2)** to get good phase
  - **dconi** ; should now have good phasing

    → **trace='f2' dconi** allows phasing along F2 (similar to above) if needed
  - if integrals have been setup (as above), and only if **fn1=fn**, then **bc('f1')** can sometimes work wonders here
  - to plot, **plot2dhr** is a new macro that works quite well; if you want 1d projections, load the high-resolution 1d into a separate experiment before issuing the macro command. Otherwise, I like parameters: **wc=130 wc2=wc sc=0** ; this leaves room for a vertical projection or to print parameters on the page (use **disp2d** to set these)
  - maximum printable parameters on 8.5×11 paper are **wc=230 wc2=150**
  - **rl(..p)** gives F2 referencing, **rl1(..p)** gives F1 reference
  - use **plot2dhr** (preferred) or **pconpos** or **pconneg** to plot phase sensitive spectra

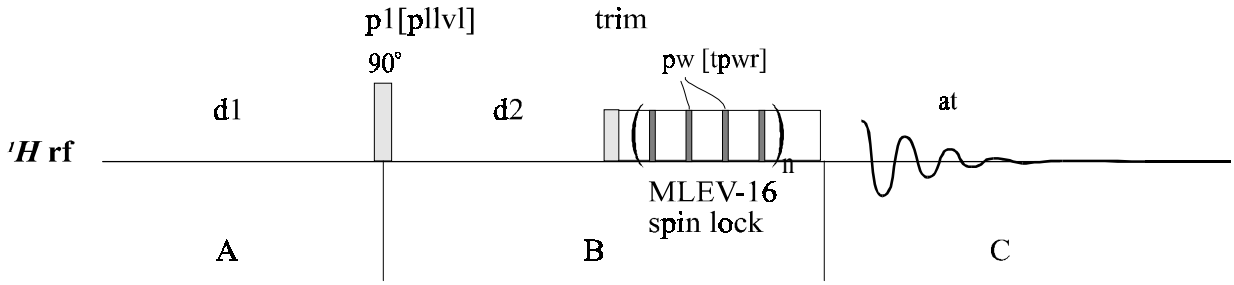
IX. TOCSY (or HOHAHA) – Total Correlation Spectroscopy

(17-Jul-00)

A. Discussion

- TOCSY provides COSY-like spectra, but with all ^1H connected appropriate with the mix time. Thus, mixtures and small molecular fragments can often be more easily identified with TOCSY.
- TOCSY spin-lock mixing provides Hartman-Hahn matching for coherence transfer. Strong coupling is required, and off-resonance effects can be troublesome. Use long-range COSY or DQCOSY to observe correlations involving small *J*-couplings.
- ROE's can be a problem with TOCSY spectra, especially as mix gets large. Clean-TOCSY, a variant not implemented in Varian's standard sequence, can offset ROE's by providing a similar NOE build-up, but this variation will only work for large MW where NOE's are negative.
- Watch the manual page [man('tocsy')] for changes in the TOCSY sequence(s). Implementation of DISPSI-2 spin-lock is underway, as well as gradient homospoils.
- Multiple mix times are often useful. These cannot be run arrayed with phase=1,2; phase=3 (TPPI) must be selected if arraying **mix** is desired. Queuing multiple experiments with different mix times and phase=1,2 is another method for this type of data collection, and is recommended.

2d TOCSY Spectroscopy (MLEV-16 Spin Lock)



B. Critical Parameters

p1, p1lvl = 90° pulse width at high power **p1lvl** (typically 52 to 60); recalibrate this parameter for all tocsy experiments (uses **pw, tpwr** from 1d to set **p1**, **p1lvl**)

pw, tpwr = 90° pulse width (~20 to 40 μs) at power **tpwr** (typically 46 to 52) used for MLEV spin lock; calibration of this parameter is also recommended

ni = number experiments, or number of points in t_1 ; should be set ok by macro, time allowing; must have F1 digital resolution ≡ sw1/(2ni) ≤ 12 Hz/pt but ~ 6 Hz/pt if time allows

mix = mixing time (length of spin lock), typically 30 to 100 ms

nt = multiple of 2 minimum, multiple of 4 if time allows

d1 = relaxation delay; set 1-2*T_1

np = number of points in t_2, usually want ≥ 1024 since costs nothing but disk space and gives better resolution in F2

phase = 1,2 is recommended (phase-sensitive acquisition)

trim = optimize by minimizing signal when **d2=0** and **phase=2**

C. TOCSY Acquisition

- prior to final setup, run **ni**=2 and carefully phase t_2 spectrum
  - then enter **calfa** and reacquire; observe baseline flattening
  - perform integrations on 1st increment if baseline correction is desired
- check that **nt**=multiple of 4 (2 can be used if time is critical)
- check that F1 dig resol ≡ **sw1**/2**ni** ≤ 12 Hz/pt, desire ~6 Hz/pt if time allows
- check that **p1,p1lvl** are set appropriately (should equal pw, tpwr from 1d ^1H experiment—I will likely change this confusing definition during the DIPSI implementation), and **pw,tpwr** are set to give **pw90** ≥ 20μs
- set **window = 2pw** (clean-tocsy only), and optimize **trim** by **mp**(current exp#+1), **phase=2** and **d2=0**, then choose **trim** giving minimized signal

D. Calibration

- recalibrate 90° pulses for **p1,p1lvl** and **pw,tpwr** (see ^1H section for instructions) for longer experiments; short/quick experiments can be run with *probe* file calibrations and macro setup

E. Data Workup and Plotting

- same as **dqcosy** (see DQCOSY section for phase-sensitive workup)

X. Other COSY Variants

(21-Jul-98)

A. Discussion

- E-COSY can provide a good alternative to dqcosy for determining coupling constants
  - E-COSY has the advantage that fewer crosspeaks are present, but all coupling information is still present
  - E-COSY has the disadvantage that the minimum phase cycle is very large (32), so the experiment can take much longer that necessary from S/N considerations
  - see van de Ven for an excellent discussion on E-COSY and other variants
  - gradient E-COSY may provide a usable form of E-COSY; look for more details

- see COSY for long-range and relayed experiments

B. Critical Parameters

- section not ready yet

C. Acquisition

- section not ready yet

D. Calibration

- section not ready yet

E. Data Workup and Plotting

- section not ready yet

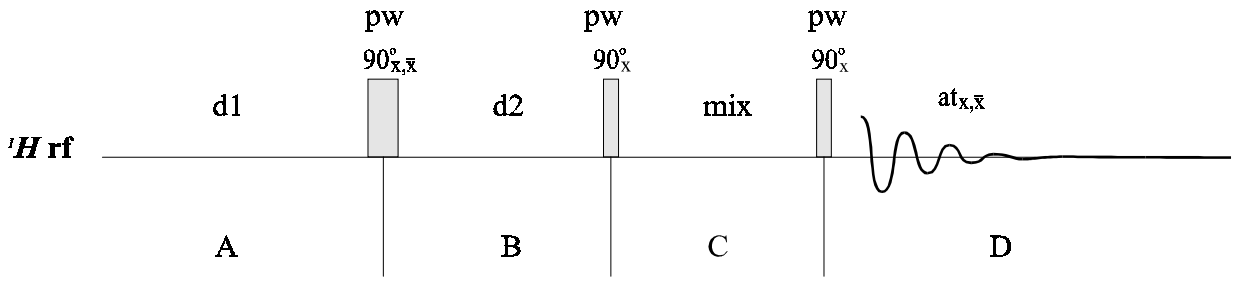
XI. NOESY – 2d NOE and Exchange Spectroscopy

(17-Jul-00)

A. Discussion

- NOESY can be a powerful experiment for the correct types of compounds, and with proper care during acquisition and analysis of the data. **Care is recommended during interpretations!** See Sanders&Hunter Chap. 6 for an excellent introduction to NOE's; see the Bruker User's Guide for more details about NOE experiment particulars.
- Often, combinations of MW, solvent, and temperature conspire to make NOESY crosspeaks non-realizable during nominal mix time in this transient experiment. ROESY will often be better alternatives for small (≤2000) MW.
- Performing at the least an inversion-recovery null-estimate of ^1H T_1 values is highly recommended prior to acquiring NOESY spectra. This method of obtaining ^1H T_1's is quick, and simple to setup. Perform full T_1 analysis for best results (see **dot1** macro).
- Degassing leads to longer T_1 values for smaller MW compounds, and is recommended for 1D variants. The longer T_1 values, which can lead to greatly enhanced NOE build-ups, sometimes cannot be maintained for 2D experiments, due to limitations in time for the experiment.
- NOESY crosspeaks for small MW will be positive (opposite to the inverted diagonals) as will exchange crosspeaks (use temp variation to differentiate exchange from NOE crosspeaks). For larger MW, crosspeaks will be negative (same sign as diagonals).
- Multiple mix times ($\leq T_1$) will be required for quantitative work in using NOESY build-up curves for obtaining distance information.
- **ulnoesy** is recommended (but currently unavailable) over the vnmr standard sequence **noesy**. For now, use **tnnoesy** if needing presaturation, otherwise **noesy** (or equivalently SETUP SEQUENCES NOESY).

2d NOESY and EXSY Spectroscopy (ulnoesy)



B. Critical Parameters

pw, tpwr = 90° pulse width at power **tpwr**; recalibrate this parameter for noesy experiments
ni = number experiments, or number of points in t_1 ; should be set ok by macro, time allowing; want F1 digital resolution ≤ 6 Hz/pt = sw1/(2ni)
nt = multiple of 8
sspul = 'y' gives homospoil-90-homospoil preceding **d1**
d1 = relaxation delay; set 2-4*T_1 (do not set too small, or will get very bad t_1 noise)
np = number of points in t_2, usually want ≥ 2048 since costs nothing but disk space and gives better resolution in F2
mix = mixing time; often is varied to provide build-up curves. Set close to T_1 should provide maximized crosspeaks; for high MW (>2000) ~0.3s should work.
mixvar = (ulnoesy only) variation in **mix** in percent; 10 works reasonably well to remove cosy/tocsy type crosspeaks; will increase t_1 noise, so not recommended unless know is needed

C. NOESY Acquisition

- set-up similar to DQCOSY
- make certain to optimize the **gain**
- make certain to optimize baseline flatness for the particular value of **sw** you are working at:
  - acquire spectra with **ni = 2**, and phase carefully
  - run **calfa** which uses the **lp** value from the phasing
  - reacquire the **ni** = 2 data and rephase; you should get a good phase with **lp=0**; if you do not, rerun **calfa** and require again
  - if the baseline still has some curvature (either convex or concave), then you might want to:
    - note the values of **rof2** and **alfa**
    - keeping the sum **rof2+alfa** constant, change **rof2** and **alfa**
    - reacquire and note the effect of the change with **rof2** and **alfa**; one direction should move the baseline towards convex, the other towards concave; some combination should give optimal baseline flatness
    - write down the values of **sw rof2** and **alfa**; these should always work for this **sw**
- as a minimum, estimate T_1 using inversion-recovery null method to provide setting for **d1**

D. Calibration

- always recalibrate 90° pulses for **pw,tpwr** with NOESY spectra (see ^1H section for instructions)
- always work from a measured T_1 estimate

E. NOESY Data Workup and Plotting

- same as **dqcosy** (see DQCOSY section for phase-sensitive workup)
- baseline flatten/fitting routines can be particularly useful for NOESY spectra workup; it is recommended that the baseline is made as flat as possible prior to acquisition (see section C), but **wft1da bc('f2') wft2da bc('f1')** can be particularly useful here; **fn1 = fn** is required for the integration regions in F2 to work for the F1 baseline correction

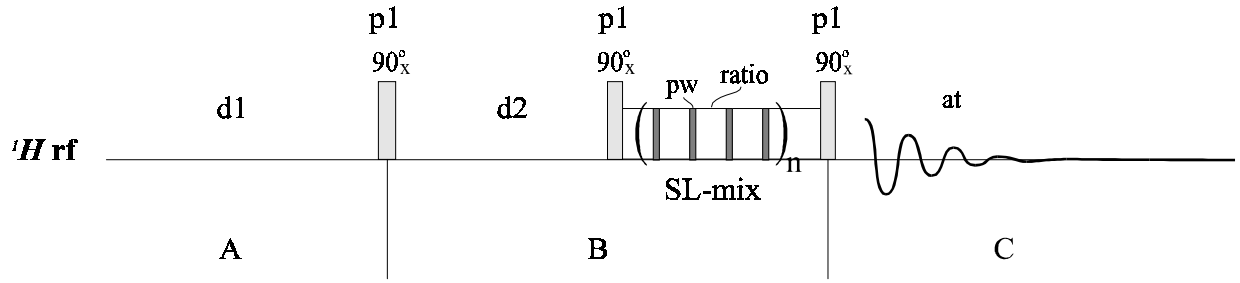
XII. ROESY – 2d NOE Spec.; Mixing via a Spinlock in the Rotating Frame

(17-Jul-00)

A. Discussion

- ROESY spectra will produce a maximum +0.5 enhancement for ^1H independent of MW. The experiment is still transient (as in NOESY), however, so enhancements are always smaller than 0.5. As always with NOE/ROE experiments, absence of a crosspeak does *not* confirm lack of proximity (see Sanders & Hunter for a good introduction on this subject).
- An ROE is produced by inverted magnetization created via chemical shift during t_1 (d2) and locked to the *y'* axis by the pulsatile spin-lock during the mix time. **pw** should be set at 30°, and **ratio** set to ~6 for **tpwr** ~ 52 on bbswg probe.

2d ROESY Spectroscopy (ulroesy)



B. Critical Parameters

p1, p1lvl = 90° pulse width at power **p1lvl**; recalibrate this parameter for roesy experiments
pw, tpwr = 30° pulse width at power **tpwr**; recalibrate this parameter for roesy experiments
ratio = number of **pw** with no rf between actual **pw** pulses; usually set ~6 (should be ≥5)
The spinlock field strength used in this type of experiment (Kessler spinlock) is:

$$B_{1sl} = \frac{1}{pw_{360}(\text{ratio}+1)}$$

Type **slroesy** to get the spinlock power for a particular **ratio** and **pw**.

ni = number experiments, or number of points in t_1 ; should be set ok by macro, time allowing; want F1 digital resolution **sw1/(2ni)** = 6 Hz/pt
nt = multiple of 2, multiple of 8 preferred
sspul = 'y' gives homopoil90-homospoil preceding **d1**
d1 = relaxation delay; set 2-3*T_1 (do not set too short, or will get very bad t_1 noise)
np = number of points in t_2, usually want ≥ 2048 since costs nothing but disk space and gives better resolution in F2
mix = mixing time; often is varied to provide build-up curves. Set close to T_1 should provide maximized crosspeaks; for high MW (>2000) NOESY is preferred.

C. ROESY Acquisition

- see NOESY section; adjust baseline similarly, and make certain to use actual ROESY sequence (do this by setting **ni=2**) when performing the **calfa** correction

D. Calibration

- calibrate **pw, tpwr, p1, p1lvl** for all ROESY experiments
- watch out for coupling partners that are centered in the spectrum; these will give TOCSY crosspeaks in a ROESY; in this case, change **tof** to get the coupling pair off-center

E. Data Workup and Plotting

- same as dqcosy; see DQCOSY section for phase-sensitive work-ups

XIII. HETCOR – 2d Heterocorrelation Spec. ("normal" *X* nucleus detection)

(27-Dec-97)

A. Discussion

- typically want to run HMQC (see next section) or HSQC, as dramatic improvement in S/N is obtained in the inverse experiments
- HETCOR is still run when high digital resolution is needed in the *X* dimension; a common example would be ^{13}C-^{1}H correlation spectroscopy where resolved closely grouped aromatic ^{13}C are needed to improve assignments of the aromatic protons

B. Critical Parameters

- section not ready yet

C. HETCOR Acquisition

- section not ready yet

D. Calibration

- section not ready yet

E. Data Workup and Plotting

- section not ready yet

XIV.HMQC and HSQC – 2d Heteronuclear Spectroscopy ("inverse" experiment with ^1H detection)

(20-Jul-01)

A. Discussion

- Two sequences for measuring ^1H-*X* chemical shift correlations, gHSQC and gHMQC are very similar, and both will work fine for most organic compounds.
- HSQC will give sharper lines, especially for $-CH_2-$ since ^1H–^1H homonuclear coupling in the F1 axis is not suppressed in HMQC. HSQC is more sensitive to pulse width calibrations (has more π-pulses), but HMQC can suffer T_2 sensitivity losses in viscous and high-MW compounds.
- Gradient sequences are typically superior in S/N, especially for long-range HMBC.

B. Critical Parameters

- **pw** – ^1H 90° pulse width at power **tpwr**
- **pwx** – *X* 90° pulse width at power **pwxlvl**
- **j1xh** – J-coupling for correlations of interest, set to 140Hz for 1-bond HSQC and HMQC experiments (see HMBC for long-range experiments)
- **ni** – fixes the *digital resolution in F1, dres1 = sw1/(2ni)*; because of the large J-coupling in 1-bond experiments, *dres1* need only provide sufficient resolution for ^{13}C chem shift, and is therefore set larger than typical in a COSY experiment. For COSY, *dres1* = 4 Hz/pt, where in HSQC/HMQC *dres1* ≥ 30 Hz/pt is usually sufficient.
- **d1** – best set ≥ $2\times T_1$, but not uncommon to run at ~$1.5\times T_1$
- **sw1, dof** – sets the ^{13}C F1 spectral axis; set the next section as to how to set

C. HMQC Acquisition

- for long HMQC runs, or lossy samples, calibrate the ^{13}C decoupler using a ^{13}C-enriched sample and PWXDEC90 sequence (see section D below)
- tune the probe at ^{13}C then ^1H for your sample
- acquire a standard ^1H spectrum and optimize **sw** using **movesw**
- calibrate **pw90** by arraying **pw**; make certain **d1** is not too small (~$1.5\times T_1$)
- optimize the **gain** for **pw=pw90** and acquire a ^1H high resolution spectrum at the optimized sw
- **mp(3)** ; assuming started in exp1
- **jexp2** and obtain just enough ^{13}C (*X* cable to observe port) to see the solvent peaks (nt=1 usually)
  - if in D2O, you can rely on **setref** for approximate referencing, or use **decref** if the ^1H spectrum has a good reference
- optimize sw of ^{13}C by estimating where protonated carbons will show for your compound and using **movesw ga**
- **jexp3** and type **gHMQC** or **gHSQC**; enter **2** when asked where ^{13}C parameters are
- check that **pwx, pwxlvl, dmf** and **dpwr** are set correctly
- check **j** and **d1**
- check **ni** and **nt**
- if running **hmqc** instead of gradient sequences, optimize **null** (set **ni** = 1 and array **null ~ T_1**) or use **null=0.7*T1** if you know T1 (especially important for solvent if working in non-deuterated solvent)
- **au**

D. Calibration

- general rule of thumb for these calibrations is:
  - short runs, check proton **pw90**, for **pwx,pwxlvl** use facility numbers
  - if probe problems are suspected, check pw90's of *X* and ^{1}H observe (not decouple)
    - if numbers are close to facility values, probe is likely OK
    - if pw90 is much less than facility value, you are doing something wrong (figure it out! : -)
    - if pw90 is much longer (>1μs) than facility value, find TA or facility staff
  - perform calibrations for overnight or longer runs for HMQC/HSQC experiments
- tune probe at ^{13}C then ^{1}H for ^{13}C enriched sample ($^{13}CH_3I$—Indirect 1—or NaOAc-^{13}C)
- calibrate **pw90** for ^{1}H as normal
  - set **tpwr** to desired setting; see /vnmr/probes/*probe*/*probe* for most current
  - make sure you are on resonance: set cursor on $^{12}CH_3{}^{*}I$ singlet (surrounded by $^{13}CH_3{}^{*}I$, so looks somewhat like a triplet) , then **movetof**
  - set **d1=1 at=1** for Indirect 1 samplpe
  - use 360° pulse for final checks always!!
- once observe **pw90** is obtained, assuming in exp1 then **jexp2**
- obtain ^{13}C in exp2 (move *X* cable to observe port) and place $^{13}C^{*}H_3I$ quartet on resonance (place cursor in middle or quartet and **movetof**)
- **jexp3 mf(1,3) wft;** put ^{13}C on resonance by setting **dof** = ^{13}C tof from above
  - setup decoupler calibration experiment with UW macro **pwxdec90** ; check **tof** and **dof**
  - check **pwxlvl = 58** and find **pwx** where antiphase doublet nulls; this is decoupler hard 90
  - set **pwxlvl** (~46) appropriately for decoupling (probe dependent; see Table on wall or /vnmr/probes/*probe*/*probe*) and find **pwx** where antiphase doublet nulls; set **dpwr = pwxlvl** and **dmf = 1/(pwx*1e-6)**

E. Data Workup and Plotting

- work-up essentially identically to dqcosy (see end of DQCOSY section for phase-sensitive work-up)

XV. HMBC – 2d Long-Range Heterocorrelation Spec. (inverse detection)

(4-Aug-98)

A. Discussion

- HMBC and gHMBC are powerful assignment techniques

B. Critical Parameters

- **pw** – ^{1}H 90° pulse width at power **tpwr**
- **pwx** – *X* 90° pulse width at power **pwxlvl**
- **j** – J-coupling for one-bond couplings (these will be suppressed; ~140 Hz)
- **taumb** – 0.5-0.6 for ^{13}C experiments (=1/2$J_{\text{long-range}}$); use ~ 0.1 for ^{15}N

C. HMBC Acquisition

- the macro should setup the following:
  - **dm='n'** ; HMBC cannot be acquired with ^{13}C decoupling
  - **null**=0 ; BIRD suppression cannot be performed (so gHMBC should be superior)
  - increase **at** = 0.3 to 0.4 s
  - increase **nt** ≈ 8×nt from HMQC experiment
  - **ss** = -4 ; steady-state pulses before every increment
  - **mbond**='y' ; defines sequence as HMQC
  - always perform linear prediction (use button under UWMACROS MORE) in F1 on HMQC and HMBC

D. Calibration

- excellent calibrations of both ^{1}H pw90/tpwr (should always be done before acquiring an HMBC) and ^{13}C pwx/pwxlvl (calibration should be recent) are required

E. Data Workup and Plotting

- HMBC is often worked in **av ph1** mode even though both dimensions are acquired phase-sensitive; gaussian filters are typically applied in both dimensions
- linear prediction in F1 to 4×ni is recommended; resolution and sensitivity should increase by roughly a factor of 2

XVI.Other Heteronuclear Correlation Experiments

A. Discussion

- section not ready yet

B. Critical Parameters

- section not ready yet

C. DEPT Acquisition

- section not ready yet

D. Calibration

- section not ready yet

E. Data Workup and Plotting

- section not ready yet

XVII.Fitting Partially Overlapping Peaks – Deconvolution in VNMR

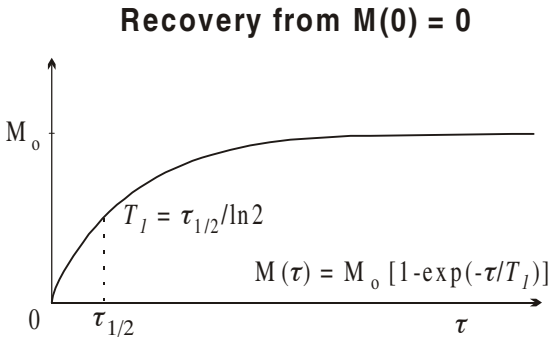
- prepare baseline region by clearing all reset points: type **cz**
- click on PARTIAL INTEGRALS
- click on DISPLAY NEXT RESET and pick integrals over all sections that are NOT good baseline regions (i.e., this is not to get good integrals, but to define regions of good baseline)
- resave the data to allow simple restarting at this point; use **svf('...')** command
- use **bc(5)** or similar; I recommend you *not* use **bc** which applies a spline fit (this is a very dangerous command that can seriously modify the shapes and integrals of peaks)
- click on DISPLAY INTERACTIVE NEXT TH to change the threshold level; you want to go just below the smallest obvious peak, but not down to where a lot of noise from a large peak will be picked up
- enter **mark('reset')** to ensure that all previous marks are deleted
- move the cursor to each "normal" peak and press MARK (this enter the center frequency and uses a default linewidth)
- move right and left cursor to the approximate half-height points of broad peaks and click MARK (this enters the average of right and left cursors for the center position, and the difference between right and left cursors as the width)
- by opening a shell tool and entering **cd ~/vnmrsys/exp1** **more mark1d.out** you can see the file that the MARK command built and will use
- click on MAIN ANALYZE DECONVOLUTE USE MARK FIT to perform the fit
- if you get a "too many points" error message, enter FN=FN/2 WFT BC(5) and click through to the FIT command again; keep reducing FN until it will fit
- use the PLOT button to get a quick plot
- use the commands CLRADD SPADD ADDI to compare two spectra

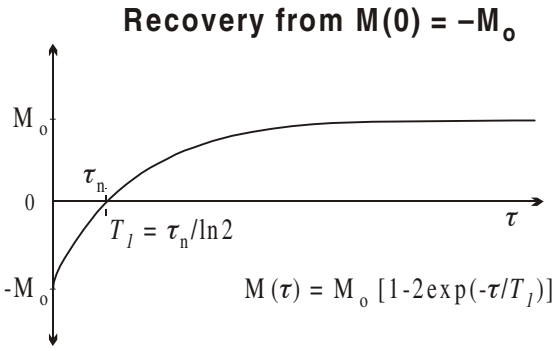
XIX. ^1H Spin-Lattice Relaxation, T_1

(updated 00.07.19)

I. Discussion

- Spin-lattice relaxation, T_1, is nominally an exponential process, and is important as the determining factor in all experiments' repetition delay, **d1**. Suggestions for how "abusable" (borrowed from Varian) **d1** is for various experiments is provided below.
- T_1 becomes critically important for any quantitative, exchange, or cross-relaxation type of experiment such as NOE. The relaxation from zero magnetization and from an inversion pulse are both shown in the figures below.





- Usually a quick determination of the T_1 is sufficient; this allows proper setting the repetition delay, **d1**. For most liquids, use the rapid, inversion-recovery method in Section II below. This method involves finding the time at which the magnetization goes through a null, τ_n, as shown in the figure on the right below. More accurate determinations of T_1 must utilize multi-point fitting, as described in section III.

II. Rapid Determination of T_1 by Inversion-Recovery Null Method

(good for any sample—e.g. any liquid—but is semi-quantitative at best)

- Obtain a reasonable quality ^1H spectrum. Locate protons of interest for visual observation.
- 180° and 90° pulse lengths must be reasonably accurate. If your sample is highly polar (e.g., ionic water solution), you must calibrate beforehand. Otherwise, if tuned correctly, prior calibrations can be used.
- set **p1=2*pw90 pw=pw90**
- **d2**=0.01 should give large negative peaks. Lengthen **d2** until proton peaks of interest are nulled (slightly shorter **d2** should give negative peaks; slightly longer should give positive peaks). For the nulled $\mathbf{d2_n}$, $T_1 \cong d2_n/\ln 2 = d2_n * 1.44$.
- Find $\mathbf{d2_n}$ for other protons of interest.

III. Quantitative measurement of T_1 by Inversion Recovery Method

A. Comments

- A reasonable estimate of T_1 must be known to correctly setup: use the Inversion-Recovery Null Method (Section II).
- 180° and 90° pulse lengths must be accurate.
- For precise measurements, use **d1** = 10 T_1; **d1** = 5 T_1 will provide reasonable values if experimental times get long. The number of **d2** values, nd2, can also be decreased to lessen experimental times ≅ nt*nd2*(2*d1)

B. Acquisition Set-up

1. use the macro **dot1**; some randomization of the selected d2 values might be preferable
2. ensure d1> 5 * T_1

Table I. A reasonable grouping of VD delays for Inversion-Recovery T_1 sequence, where T_1^r is the T_1 estimate (from inv-rec estimate or a repetition-rate experiment).

$0.01T_1^r$	$0.5T_1^r$
$10T_1^r$	$10T_1^r$
$2T_1^r$	$5T_1^r$
$0.3T_1^r$	$0.1T_1^r$
$10T_1^r$	$0.01T_1^r$
$0.7T_1^r$	$1.4T_1^r$
$1.0T_1^r$	$10T_1^r$

C. T_1 Analysis (see Varian Subject manual, Adv. 1d section)

1. Expand about the peak(s) of interest. Adjust the threshold with the **Th**.
2. Enter **dpf** or **dll** then **fp** to locate the peak(s) and then find them through the relaxation set. **fp** writes a text file fp.out in the current experiment, e.g., ~/vnmrsys/exp1/ft.out.
3. If the dataset is a T_1 set, enter **t1**; for T_2 enter **t2**, or for a solid-state $T_{1\rho}$ enter **analyze('expfit','p3','t2','list')**
4. **t1** and **t2** will display the information on the bottom of the screen. To print, repeat using, e.g.,

   printon t1 printoff or

   print the file ~/vnmrsys/exp#/analyze.list text file (same information) from the File Manager, or in vnmr with **ptext('analyze.list')**
5. Show a plot of the data using **expl**, and plot with **pexpl page**. Note that you can look at specific peaks using **expl(3)** and **pexpl(3) page** as an example for the 3rd peak. To make a smaller plot use the sequence **expl center pexpl page**. To delete a point from the analysis, use **dels(<data#>) fp t1** or similar command.

D. T_1 "Abusability" vs Experiment Repetition (d1)

[from Varian course notes]

Likelihood of artifacts increases as go down column.

normal 1d	T_1 at 30° pulse
COSY (magnitude mode)	$< 1 \times T_1$
INADEQUATE	$> 1\text{-}1.5 \times T_1$ (^{13}C)
HMQC & HMBC	$> 1.5? \times T_1$
HOM2DJ	
HET2DJ	
HETCOR	
COSYPS	
TOCSY	
NOESY & ROESY (qualitative)	$\sim 2\text{-}3 \times T_1$
NOESY & ROESY (quantitative)	$> 3\text{-}5 \times T_1$
DQ-COSY & MQ-COSY	$> 3 \times T_1$ (+ "homospoil")
Quantitative T_1	$\geq 5 \times T_1$

XIX. Cross-Polarization/Magic-Angle-Spinning NMR

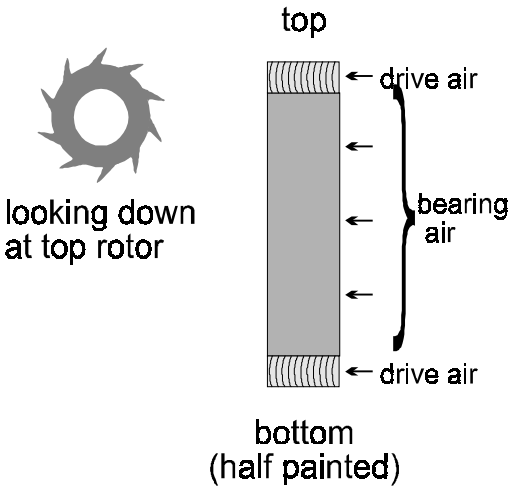
(5-Mar-96)

I. Computer Set-Up

- Login, enter **acqi** in the dialog box; use right mouse on background to bring up acquisition status box
- Typically, all zero shims (see with **dgs**) will work fine for solids
- Read in the file ***cpmas-setup.fid*** in /usr/fry directory for first run
- set **gain=0 temp='n' vttype=0 su** (better, use *tempoff* macro)

II. Sample Set-Up

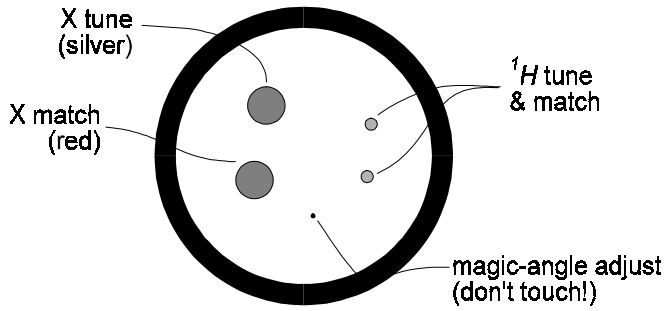
- Pack sample into rotor with special care for keeping radial symmetry during packing.
- The caps ***must*** fit all the way on; even a bit too much sample keeping the cap slightly off will prevent smooth spinning.
- Note the flutes on both caps face the same direction, with drive air coming from the right (flutes clockwise looking down onto rotor).
- The bottom of the rotor goes into the stator first; tighten the screws snug, but not with excessive force.
- With the metal cap off the probe, test the spinning by:
  i. Turning the bearing air (upper knob) to ~10 (black scale)
  ii. Slowly increase the drive air until spinning begins, at 5 on black scale sample should spin smoothly at ~2kHz. If seem to get 'stuck' at ~800, 1200 or 1500 Hz, **DO NOT** try to force spinning by turning air up. Take sample out, check cap tightness and fit, tamp the rotor a bit, try again to spin, and repack if it still will not spin.
  iii. With metal cap on probe, spin up to ~2500-3000Hz. Put probe into magnet, and spin up to desired speed. Do not spin faster than 6 kHz, or raise bearing air above 20 on black scale with NMR Director's approval.



III. Probe Tune-Up

This procedure MUST be performed for every sample change. Even slightly untuned probes may prevent reasonable CP match.

- Check tn value by entering **dg** or **tn?**. Should get *tn=C13* for ^{13}C CP/MAS.
- Set **gain=0** and enter **su** (for setup).
- Attach observe cable to middle tune BNC, switch to tune at 50 or 60. Should be able to get ~27 on meter by adjusting two large knobs on bottom of probe. Switch back to observe.
- Tune decouple channel by entering **tuneh** (macro: **tn='H1' su**). Attach decouple cable to tune, and adjust two small knobs to get ~27 on meter. Replace cable to decouple, and switch to observe. Enter **tunec** (macro: **tn='C13' gain=0 su**) check temperature control with **vttype?** (=2 for control, =0 if control unit off) and **temp?**; enter **su**.



tuning knobs on bottom of
Doty CP/MAS probe

Use of Wavetek Sweep Unit

Use this setup if you cannot find the resonance with the Varian tuning meter:

- switch oscillascope, Wavetek, and synthesizer on using the power strip switch at back of cabinet
- use appropriate *tune*x command (e.g., *tunec*) and note the sfrq reading exactly
- set the synthesizer to the sfrq setting, and the Wavetek also to ~ sfrq
- turn the level up on the synthesizer to ~0.5 dBm
- hook the BNC from the mixer to the input of the probe
- turn the Wavetek's sweep width control clkwise to increase the sweep and center the synthesizer marker on the oscillascope
- adjust the ***tune*** cap to center the resonance on the marker; the marker size can be reduced with the marker width control (small knob) on the Wavetek
- turn the ***match*** cap to make the resonance as deep as possible; it will hit the bottom line only when the sweep width on the Wavetek is very narrow, spreading out the resonance very wide (at this setting, it will be difficult to keep the resonance on screen)
- 2nd order tune the ***tune*** and ***match*** caps to only a roughly tune
- switch off the power (on the power strip, not on the instruments!), and hookup to the Varian meter to precisely finish the tune

III. CP Match

- The cross-polarization experiment requires that . The *xpolar* pulse program used by the spectrometer:

xpolar diagram

IV. Analyzing MAS Data to Obtain CSA Information

There are three distinct steps involved in analyzing MAS data:

a) prepare the 1d file to correct baseline roll, find (mark) the isotropic peak and set threshold for spinning sideband setup, and prepare three exp areas for display and plotting;
b) simulate the spinning sideband pattern to obtain good CSA and ASYM parameters

c) perform a fit to obtain best values for CSA (chemical shift anisotropy, δ_{CSA} or σ_{CSA}) and ASYM (asymmetry parameter, η)

A. Preparation of MAS spectra

- read in the data (use menu items MAIN MENU FILE SET DIRECTORY to change default dir)
- expand to exclude baseline (i.e., to edges of SSB's)
- integrate spectrum to exclude all peaks from baseline correction routine:
  - type **cz** to zero all integral regions
  - click on DISPLAY INTERACTIVE NEXT RESETS , then click (left button) on each side of all peaks in spectrum; the idea here is to remove everything from a baseline calculation (all integral regions are ignored for baseline correction)
  - type **bc** to correct baseline (see VNMR online for more bc options)
- write down the isotropic peak (compare two spectra at non-multiple srates; the peak that does *not* move is the isotropic peak) in ppm (use macro **axisp**),
- write down the spinning speed using BOX in hertz (use macro **axish** and check parameter DELTA)
- write down the value of *fn*
- set the threshold (click on MAIN MENU DISPLAY INTERACTIVE NEXT TH) to just under the smallest spinning sideband peak to be included in the fit
- save the new spectrum using **svf** command
- to setup for comparing simulations and fits to the data, use the commands ...**clradd** and then **spadd** (for more info, look under *addi* in VNMR online) to move the experimental data to exp5

B. Simulation of spinning sideband patterns

You normally will always run simulations of the MAS data, to gain reasonable estimates of CSA and ASYM. Only if you are very confident of these two parameters should you go directly into a fit. If the data is "messy," you might use simulation alone to give estimates of CSA and ASYM.

- join another experiment (assuming section A done in exp1), e.g. **jexp2** and load the file in that you just saved at the end of section A
- click on MAIN MENU ANALYZE SOLIDS SETUP FOR SOLIDS then MAS
- enter the values written down for *isotr, srate* and *fn*, and make best guesses for *csa* and *asym*
- set *np* equal to 2048 up to 8192 ($\leq$ *fn*)
- set *nlat* = 18 to 72 (bigger is more accurate, but takes longer; for estimates to put into the fit algorithm, I recommend *nlat* = 18)
- click on SIMULATE CALC(FG) and wait; should take less than a minute unless *nlat* is large
- once done, click on SIMULATE DISPLAY [can be replaced with command **wft('nodc')**]
- to compare to the experimental spectrum, type **addi**
- change CSA and ASYM until the simulation is fairly reasonable

C. Least-squares fitting of spinning sideband patterns

- join a new experiment: e.g., **jexp3**
- click on MAIN MENU ANALYZE SOLIDS SETUP FOR SOLIDS MAS
- enter the correct values for *csa, asym, isotr, srate*
- enter same values for *np* and *fn* as used for the simulations
- the first time, I like to use *nlat* = 18; once the fit looks OK, then I increase *nlat*
- click on FIT MARK ISOTROPIC, then click (left button) line onto isotropic peak and click MARK

- click on FIT FIND SSB; if threshold was set right, and good s/n spectrum, only the correct peaks should be labelled; if they aren't, use the EDIT SSB to delete extra labelled peaks
- click on FIT CALC ; the program will ask for a file name – use something descriptive and unique
- then open a window (click and hold *right* button on background and slide to WINDOW) and type **simulate**
- when finished, join yet another experiment, and use MAIN MENU FILE (click on fit filename) LOAD
- use **wft('nodc')** to transform and display, and **addi** to compare the fit to experimental
- the *log* file in the fit filename directory has η, and σ_{11}, σ_{22}, σ_{33} info at the end of the file (use the unix command **more log**)

V. Analyzing Relaxation Data (see Varian Subject manual, Adv. 1d section)

1. Expand about the peak(s) of interest. Adjust the threshold with the **Th**.
2. Enter **dpf** or **dll** then **fp** to locate the peak(s) and then find them through the relaxation set. **fp** writes a text file fp.out in the current experiment, e.g., ~/vnmrsys/exp1/ft.out.
3. If the dataset is a T_1 set, enter **t1**; for T_2 enter **t2**, or for a solid-state $T_{1\rho}$ enter **analyze('expfit','p3','t2','list')**
4. **t1** and **t2** will display the information on the bottom of the screen. To print, repeat using, e.g.,
   printon t1 printoff or
   print the file ~/vnmrsys/exp#/analyze.list text file (same information) from the File Manager, or in vnmr with **ptext('analyze.list')**
5. Show a plot of the data using **expl**, and plot with **pexpl page**. Note that you can look at specific peaks using **expl(3)** and **pexpl(3) page** as an example for the 3rd peak. To make a smaller plot use the sequence **expl center pexpl page**. To delete a point from the analysis, use **dels(<data#>) fp t1** or similar command.

Data Backups from UNIX Workstations

created 4/10/96 – updated 4/10/96

I. Backups to Colorado Systems Tape Drive on Windows NT Network

A. Perpare tar file on UNIX workstations

- cd to the directory that the .fid directories reside in [you **must not** use full paths for the tar statement; when bringing back data tar tries to follow the path explicitly]
- tar the fid's into a single or more tar sets using a command similar to:
  tar -cvf /destination-dir/tarfile.tar [directory names to be tarred; wildcards ok]
- I often move fid's to a separate directory to make a single tar file from all of them easier:
  mv -r directory.name ~/tardir/.
  cd ~/tardir
  tar -cvf /dest-dir/tarfile.tar *.*
- compress the file:
  cd /dest-dir
  compress tarfile.tar (adds .Z to end of filename)
- Give file a DOS-compatible filename
  mv tarfile.tar.Z tarfile.taZ

B. Move file to NT server

- Log into NT server:
  ftp babylon
  username: **anonymous**
  password: **<email address: e.g., fry@chem.wisc.edu>**
- change to writable directory and image transfer type
  ftp> **cd /waste/ftp**
  ftp> **binary**
- put file in directory:
  ftp> **put tarfile.taZ**

C. Backup file to Colorado Systems tape drive

- Go to the PC named Vulcan in rm. 2224, exit Windows
- **cd /waste/ftp**
- type **tape** at the DOS prompt and follow the prompts (should be obvious from here)

D. Recovering backup'ed up data

- essential follow the previous steps in reverse
- use **get tarfile.taZ** instead of the put statement in ftp
- remember to rename the file back to a compressed filename when you get it on UNIX:
  mv tarfile.taZ tarfile.tar.Z
  uncompress tarfile.tar
- use the following tar statement:

cd /<directory you want .fid's put into>
tar -xvf /tarfile-dir/tarfile.tar

II. Backups to IOmega Zip Drive on Windows NT Network (currently installed on Morder, Terminus, Drazi)

A. Prepare a tar file

see section I.A above

B. Backup file to IOmega Zip drive

- Go to any (One with a Zip is preferred) of the PC's in 2224 or 2210
- Insert your Zip disk in the drive you intend to use.
- open WSFTP (Winsock FTP 32 bit)
- connect to the host computer of your VNMR account (ex: zhadum.chem.wisc.edu) as the remote system using your login name and password.
- set the Zip drive as the local directory in WSFTP
- Select the tar file you wish to backup in the remote window ;hold the <CNTL> key down to select many files, then transfer it to the local window using the (left→ right arrow).;
-

D. Recovering backup'ed up data

see section I.D above

III. Backups to Pinnacle Optical Disk (on Tango on 5th floor)

[You must obtain specific permission from the Kiessling group to use this device.]

A. Format a New Optical Disk

- enter the following commands
  login as root (see Director for root password; **exportfs -a** should re-establish connection to opdisk if lost somehow)
  # **cd /usr/local/pmo**
  # **pmo_format.sun4 -x /usr/local/pmo/pmo.dat**
  # Enter Choice 0 (sd0 @ esp0 slave 24)
  (SCSI ID = 3 = slave/8 = 3)
  Enter Choice 0 (PMO 650)
  format> **defect**
  defect> **commit**
  defect> **yes** (0 defects usually)
  defect> **quit**
  format> (answer **y** ← small case)
  format> **partition**
  partition> **select** ..... (0 is whole disk)
  partition> **label**
  partition> **quit**

format> **format** (takes ~15 min)
format> **quit**

B. Create File System on New Optical Disk

- enter the following commands
  # mv /usr/etc/mkfs /usr/etc/mkfs.orig
  # cp -p /usr/local/pmo/mkfs.sun4 /usr/etc/mkfs
  # newfs -c 336 -d 0 /dev/rsd0c

C. Backup to Optical Disk

- insert the disk into the drive
- mount the optical disk with the command **optmnt**
- use the **cp** command to copy directories
  e.g., **cp -rp /home/fry/newdata/* /opdisk/newdata/.**
- unmount drive when finished **optdmnt**

IV. Backups to Pinnacle CD Writer (on Twiddle on 9th floor)

[See Brad Spencer for specific questions about this device.]

A. Transfer Data to Twiddle

- Twiddle is the Dell with the 21" (huge) monitor in the far corner of the 9th floor computer room
- Twiddle

OPEN (or CONNECT??) and it will ask for your password

- you should see shadow in one window and the L: drive (empty) in the other
- click on the directory(s) that you want transferred in the shadow window; hold the SHIFT key down to select a range of directories, or hold the CNTL key down to select random directories; make sure BINARY transfer is selected
- press the ← or → arrow button sending the data from shadow to l:
- the transfer runs at ~0.5Mb/s, so 600Mb takes ~20 min
- close WS_FTP32 when the transfers are finished

B. Transfer Directories/Files to Easy-CD Window

- insert your CD using a CD caddy into the Pinnacle drive; transparent side of caddy to right
- unhook the ethernet (phone line) connection at the back of the PC; this insures that no one will demand CPU cycles from the PC during the CD write and ruin your CD
- on the Microsoft Office Taskbar, click on the ECD button (again toward bottom)
- in the Easy-CD window, click on CD-R and the Properties
  - change temp to c:\temp
  - make sure 1× speed is selected (Brad believe higher speeds are unsafe for writes)

  - make sure Joliet filenames is selected–not sure about TEST BEFORE WRITE button; Prakash didn't use but Brad seemed to think it should be on
- all should look ok at this point; all items under CD-R menu should be ready except WRITE
- open MS Explorer window (button on MS Office Taskbar that is a folder with a magnifying glass on it)
- click onto l: drive; select all your directories that were FTP'ed; click on top directory, then hold SHIFT key down while clicking on last directory; all directories should be selected
- click and hold the mouse button down on any of the directories; drag the mouse into the Easy-CD window and let go; all the directories should appear in the Easy-CD window
- select WRITE TO CD under the CD-R menu
- either stay next to the PC (could take an hour) or leave a sign on the PC for all to keep hands off
- make sure to come back before the write finishes
- I recommend writing a second, identical backup CD
- MAKE CERTAIN!!!! THAT YOUR CD IS READABLE BEFORE DELETING ORIGINAL DATA
- rehookup the ethernet cable at the back of the PC
- delete your files from the l: drive (select all directories in Explorer window and hit DEL key)

Administration of Varian Spectrometers

created 5/6/96 – updated 5/6/96

I. Rebooting Sun's on Spectrometers (requires root privileges)

A. Shutting down UNIX:

- exit current login by (assuming in SunView VNMR):
  - click on EXIT in acqi, and acquisition status windows
  - type **exit** in vnmr command window
  - hold right mouse button while mouse on background, and let go on EXIT and confirm
  - at Unix > prompt, type **logout**
- login as root: > username: **root** password: (see charlie)
- shutdown system:
  - **cd /vnmr/acqbin**
  - **killacqproc** (should get a message back that Acqproc Terminated)
  - **cd /etc**
  - **mv acqpresent acqpresent.old** (or could use shorter filename, like **joe**)
  - **shutdown -h now** (be *patient*; takes a couple minutes for filesystem to stop)
- power down system; turn off hard disks, then computer, then diff box

B. Powering Up UNIX:

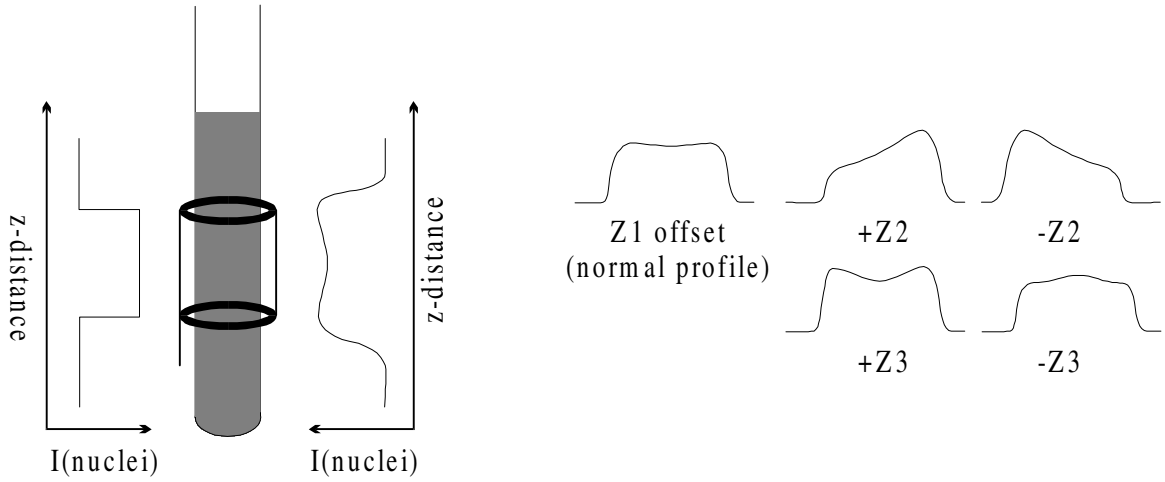
- power-up: turn on computer, then hard disks
- wait for **>** prompt to come up, then turn on diff box
- type **b** and **<enter>** and wait for system to come all the way up to login prompt
- login as root (see above)
  - **cd /etc**
  - **mv acqpresent.old** (or **joe**) **acqpresent**
  - **cd /vnmr/acqbin**
  - **startacqproc** (wait for Acquisition Ready, hit enter once, unix prompt comes back)
- login as user (could use username: fry, password:)
  - use **S**unview to get normal acquisition setup; **acqi** for the acquisition .....(lock/fid/shim) window, and right button for acquisition status

Pulsed-Field Gradient Shimming With VNMR

cg fry: created 99.01.27 – updated 99.02.01

I. General Discussion

Pulsed-field gradients allow analytically accurate, automated adjustments of shims. The technique uses "gradient profiles," more pictorially described as 1-dimensional images of the atomic density of either protons or deuterons [see Section III below for a description of how the profile is experimentally generated]. The picture below shows a sample tube with solvent (in gray), and a crude picture of a standard rf coil about the tube. On the left side of the tube, the density (intensity) of observed nuclei (either ^{1}H or ^{2}H) is shown as a function of position along the z-direction, with the assumption that the rf coil observes nuclei at equal intensities at all positions inside the coil boundaries and observes nothing outside the coil. The profile to the right is a depiction closer to the real case, where the rf coil loses intensity slowly outside its boundaries, and has a slight loss of intensity in the center of the coil compared to the intensity closer to the horizontal wires.



The normal profile is observed in the presence of a large Z1 shim offset, produced by the pulsed-field gradients (but otherwise identical in nature to the RT shim's Z1 shim setting). When higher order Z shims are misset from the "perfectly shimmed" case (denoted as the normal profile above), identifyable changes occur in the profiles. Exagerated changes are shown for Z2 and Z3 missets. The gradient shimming software analytically expresses these changes when a ***shim map*** is made; each RT z-axis shim is changed independently, providing data upon which an analytical expression can be calculated for each Z shim. From this shim map, analytically accurate changes to the Z shims can be performed to minimize shim missets—getting as close to a perfect shim as possible in the presence of noise and inaccurate non-radial (X,Y) shims.

☞ Keep in mind that linewidths are ***sample dependent***. PFG, or gradient, shimming cannot overcome aggregating or suspended samples, solvent bubbles, too little solvent height (use susceptibility inserts!!), scratched sample tubes, etc. And many samples simply will not shim to better than 1 (or even 2 or higher) Hz linewidths due to their intrinsic relaxation properties in the solvent and temperature being used; i.e. their natural linewidths are >1 or 2 Hz.

☞ ***Students needing to perform PFG shimming must make a commitment to learning the proper procedures and pitfalls involved with this method; facility staff will not entertain extended questioning or training sessions to assist students beyond a brief introduction. Moreover, it is my opinion (cg fry) that PFG shimming is not as efficient for shimming as standard methods for most samples. I and the facility staff, therefore, will not answer any questions about PFG shimming unless students can provide a good reason as to why they are not manually shimming their sample in. Some common reasons for needing PFG shimming:***

1. When working with a wide range of temperatures; PFG shimming in my experience is very helpful in adjusting to the changes in Z shims when temps change >40 degrees.

2. When working with D2O or DMSO (or similar deuterated solvent) that simply will not lock-shim well; students might (should) be FID shimming in these cases. Anyone FID shimming should look into PFG shimming, as large time savings in getting to good line shapes are likely.

3. When filter of the sample is not possible; so cloudy solutions, or some suspension, etc. are present that substantially pushes the shims away from the facility settings.

4. When forced (usually by cost) to reduced solvent quantities, such that air-solvent interfaces get close enough to the rf coil such that shims are pushed well away from the facility settings. I recommend in these cases that students try susceptibility inserts; in some cases, these cannot be used and PFG shimming will assist in obtaining optimimum lineshape [for very small solvent quantities, "good" line shapes will not be feasible].

5. When performing water suppression. I have not had great success with having PFG shimming always improve my water suppression, but Varian (and Bruker for their systems) insist PFG shimming can do better than the best spectroscopists even for these most demanding samples. So it is worth trying and experimenting with.

II. Normal PFG Shimming Procedure

1. Save your shims if they are important (use **svs<enter>** or click **FILE SAVESHIMS**).

2. Type **gmapsys** to start the gradient mapping system in the software.

   If you "lose" the **gmapsys** buttons at anytime (as happens, for example, after a **wft** or **ds**), simply re-enter the **gmapsys** command to get them back.

☞ You can run gmapsys from any experiment, but you ***must*** end the session by clicking on **QUIT** to properly exit back to your original data set/parameters.

3. Check that the parameters are setup correctly for your solvent:
   e.g., if you are working in a deuterated solvent, **tn='lk'** (INOVA) or **tn='H2'** (UNITY);
   for a protonated solvent (90%:10% H_2O:D_2O), **tn='H1'**

☞ See Section III below for **1ST-TIME USE** and a detailed explanation of parameter setup.

4. *If you are using a deuterated solvent on the UNITY only*: disconnect the lock cable at the bottom of the 2nd lock filter (attached to the back of the magnet preamp box), and connect it to the X Observe BNC. No cable switching is needed on the INOVA, or for protonated solvents on the UNITY.

5. *If you are using a different deuterated solvent than the last time you PFG shimmed*, run the macro to setup parameters for the solvent you are now using. E.g., for $CDCl_3$ enter **gmapcdcl3**, or for acetone enter **gmapacetone**.

   To see a list of available macros for various solvents and temperatures, type the following in the UNIX terminal window: **ls /vnmr/maclib/gmap*** . Parameters for deuterated solvents will be very temperature dependent. See Section III below for a detailed discussion of how to setup parameters for individual cases.

6. Click on **SETUP** on the gmapsys menu, and then on **FIND GZWIN**. gzwin is a very important parameter for proper PFG shimming; see item 8 below. gzwin is the percentage of the spectral width that contains usable data (so it would ~70 in the schematic shown above).

7. If you have a recent Shim Map in place for the probe you are using, skip to step 8 [Shim Maps done on protonated solvents will *not* work for deuterated solvents, and vice-versa. A Shim Map made with DMSO will work for $CDCl_3$, however. Since DMSO provides much better S/N with ^2H than $CDCl_3$, it is a much better choice for making a Shim Map for all deuterated solvents].

   Otherwise, click on **SHIMMAP MAKE SHIMMAP** . Depending on the solvent, the shim map will take 2 to 10 mins to create. [As noted above, it is often best to use a DMSO-d_6 sample to make the shim map.] You should check the quality of the map after it finishes by clicking on **RETURN DISPLAY DISPLAY SHIMMAP**. Each shim should show its characteristic polynomial dependence, and less noise is of course better.

8. Click on **AUTOSHIM ON Z** and wait for the procedure to finish. Do this at least once more to ensure that the autoshim procedure has converged on good shims.

☞ I have found that nearly every time the procedure fails to run (i.e., get an error message and it doesn't even start), it is because **gzwin** has been set slightly too large. For example, if **gzwin=63.2**, and the autoshim fails to run, setting **gzwin=61** will nearly always work.

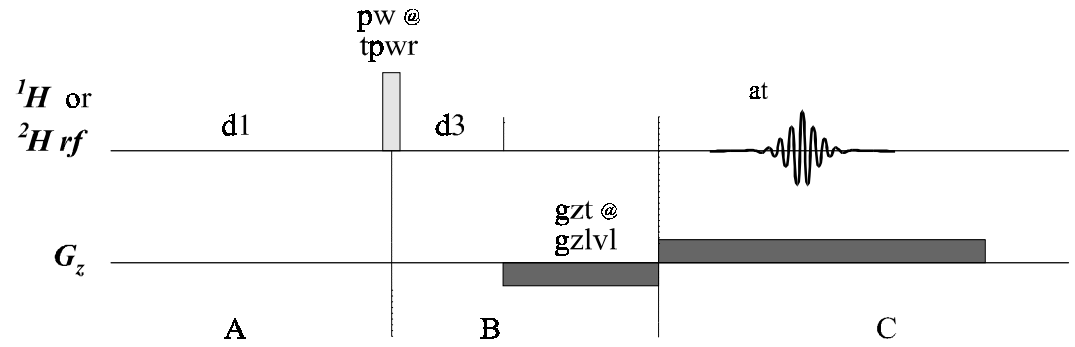
☞ If the autoshim fails to converge (i.e., the convergence factor simply will not get < 1.00), it is usually because the XY shims are very poor (re-read in the facility shim file), or because your sample/tube quality is poor: check the tube quality, especially for scratches; check solvent clarity and filter if needed; check solvent height—PFG shimming can help a lot with solvent amounts less than recommended, but cannot necessarily achieve a good shim for solvent heights < 3.5 cm (< 0.45 ml).

   In cases where autoshim fails to converge, enter **aa** or click on **ABORTACQ** to stop the acquisition. **QUIT** out of the gmapsys system, and shim the XY shims (make sure the spinner is turned off!). Then go back into **gmapsys**, remake your shimmap, and retry the autoshim. Repeat this—quit gmapsys and manually XY shim, go back in and remake shimmap and autoshim—until the shims are optimum.

III. First-Time Use and Detailed Explanation of Parameters

The pulse sequence used in the gmapsys system is gmapz, and is shown schematically below. This sequence performs a "gradient echo." Magnetization isochromats are dephased in frequency space according to the z-location of the nucleus. Assuming translational diffusion is small during the time gzt, the dephasing of the isochromats will be refocused by the opposite signed gradient a time gzt later, producing an echo. The Fourier transform of the echo, with absolute value processing, will produce intensity versus frequency: since the frequency is directly related to the z-location, the frequency axis is directly mapped to the z-location. This experiment is one example of how human and small animal images are obtained with MRI.

Gradient Echo Experiment - gmapz



A. Critical Parameters

d1 – experiment repitition rate; should be set 2-3×T_1 of the solvent ^1H or ^2H nuclei

d3 – arrayed to two values; the 2nd value should produce ~ 0.8 to 0.4 the intensity of the 1st arrayed value; use the **SETUP GO DSSH** buttons to see if **d3** is arrayed correctly; usually the 1st value = 0, but see below for more explanation

ss – usually **ss**=0, but in cases where T_1 is quite long, it may be preferable to set **ss**=-2 and **pw=30°**

gain – reduce the gain until the baseline portion is flat

nt – increase if the signal-to-noise is poor

The following should be setup correctly by a gmap*solvent* macro:

pw – a 30-90° pulse; use smaller angles for solvents having large T_1, 90° if T_1 is short

tpwr – it is absolutely critical tpwr not be > 50 when using the lock channel to shim on ^2H solvents; normal values of pw, tpwr can be used for ^1H shimming

gzlvl – ~4000 works quite well for most situations

sw – 100 kHz for ^1H shimming, ~15 kHz for ^2H shimming

gzt – actually set as **at/2 + d2** where d2 appears to be a gradient recovery time of ~1ms (not sure why it is needed)

B. Details of Correct Parameter Setup

pw, tpwr – in cases where the solvent T_1 is not known, you must first obtain a calibrated 90° pulsewidth; this is exactly the same as for other nuclei (see, for example, Experiments on the UNITY-500, Section D: ^1H pw90 Calibration, pg 26 of this manual);

When using the lock channel

- call in a standard ^2H parameter set using **MAIN MENU SETUP**
- make sure you are on-resonance to the ^2H signal
- set **tpwr** ≤ 50 if using the lock channel, and array **pw** through a 360° rotation; change tpwr such that **pw90** ~ 400-500μs on the lock channel

d1 – after determining a correct 90° pulsewidth, set **p1=2*pw90**, **pw=pw90**, **d2=0.1** , take 1 scan and make sure the magnetization is inverted; increase **d2** until the magnetization nulls (further increase in **d2** make the magnetization go positive); $\boldsymbol{T_1 \sim 1.4 \times d2_{null}}$; set $\boldsymbol{d1 = 2\text{-}3 \times T_1}$

d3 – start with $\boldsymbol{d3 = 0 , 0.6 \times T_1}$; then click in gmapsys **SETUP GO DSSH** ; if the ratio of intensities is 1:(0.8 to 0.4), the d3 array is ok. Increase **d3** if the 2nd profile is > 0.8 × the 1st; decrease **d3** if the 2nd profile is < 0.4 × the 1st, or if the 2nd profile is severely distorted compared to the first.

☞ sometimes for solvents with different ^2H nuclei (e.g., pentane-d_{12}), chemical shift and/or J-coupling of the ^2H cause intensities for d3 > 0 to be bigger than d3=0; if these cases, you just have to experiment with different d3 values to select the "best" (most sensitive) array