PINMRF

Varian 300 MHz NMR Spectrometers
User Guide for Advanced 1D and Basic 2D NMR Experiments

INCLUDING:
Inova-300-1 w/ 5mm 4-nucleus probe – 365 WTHR
Inova-300-2 w/ 5mm 4-nucleus probe – 4100 BRWN

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7-15-2009: Update - JSH.
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User Guide for Advanced 1D and Basic 2D NMR Experiments
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Please note the following generalities

• Under no circumstances lean against or otherwise move the magnet.

• The Varian Vnmr software enables the user to easily access and change functions, options, and their properties. If you are not sure about an option, button or command contact PINMRF staff for assistance.

• If you encounter problems at any time during locking, shimming, acquiring you should halt the experiment and reload the standard parameters for your experiment.

• In order for shimming to work well, samples should be filtered and prepared in good-quality tubes. Sample volume should be at least 0.7 mL. Do not use more than 0.1% TMS.

• The VNMR software works best with the Common Desktop Environment (CDE) graphics on the Sun computer. Please check when you log in that you are using CDE.

Conventions in this guide

• Buttons are depicted with small caps in bold, e.g. **ABORT ACQ**.

• Commands entered in the command window are described in geneva font, e.g. `h1cdcl`, and are executed by hitting the return key.

• This guide assumes that the reader is checked out for using the PINMRF Varian 300-MHz NMR spectrometers using the methods presented in the Training Guide for 1D Spectroscopy. If you checked out prior to the implementation of these methods you must familiarize yourself with these methods before attempting any of the experiments presented in this guide.
Advanced 1D and Basic 2D Experiments - Overview

This booklet provides the information necessary to run some advanced 1D and basic 2D NMR experiments on routine samples using PINMRF’s Varian 300-MHz NMR Spectrometers. These experiments require a minimum of operator intervention or optimization, and as such they are appropriate for the non-expert NMR user to carry out. These experiments are set up using customized setup macros designed to work with PINMRF’s dedicated parameter sets. These should provide good results, with minimal operator effort, for normal organic and organometallic samples in typical organic solvents or D_2O. However, if the ultimate in sensitivity or resolution is required in your work, or your sample is unusual or demanding in some way, please contact PINMRF staff for assistance with more highly specialised experiments.

The advanced 1D experiments presented here include the following:
1. The $^{13}$C DEPT experiment;
2. $^1$H homonuclear decoupling;
3. $^1$H nOe-difference spectroscopy;
4. peak suppression in $^1$H NMR using presaturation.

The basic 2D experiments include:
5. $^1$H – $^1$H COSY;
6. $^{13}$C-detected HETCOR.

Each experiment section includes all relevant instructions so that each one can be followed without referring to another experiment in this booklet. These notes assume that the reader is already checked out for basic 1D operation on PINMRF’s Varian 300-MHz NMR Spectrometers using the current instruction methods.
Varian 300 MHz NMR Spectrometers - $^{13}$C DEPT Instructions

For an overview of the DEPT experiment, see NOTE a) at the end of these instructions. You must run the conventional carbon-13 spectrum using the standard procedures prior to running a DEPT experiment.

1. Run the proton spectrum in experiment 1 (jexp1) using the standard parameters as described elsewhere. Process and phase the proton spectrum as normal.
2. Run the carbon-13 spectrum in experiment 2 (jexp2) using the standard parameters. Process and phase as normal.
3. Copy the carbon-13 spectrum from experiment 2 to experiment 3 using the following string of commands: \texttt{mf(2,3) jexp3 wft}.
4. \texttt{deptjsh} (sets up the DEPT-135 experiment from the current carbon-13 experiment)
5. \texttt{nt=xx} (sets number of scans; default is 256).
6. \texttt{ga} (starts acquisition) - wait for acquisition to finish (beep will sound).

Phasing and other processing procedures are the same as for normal carbon-13 spectra. Keep in mind that the DEPT-135 spectrum will have both positive and negative peaks (see NOTE a)). Print commands for DEPT-135 are special as shown below (other DEPT experiments use the normal print commands):

7. \texttt{print135, print135p} (p = with peak picking).

To acquire other DEPT spectra:
8. Copy the current DEPT experiment to the next available experiment using e.g. \texttt{mf(3,4) jexp4 wft}.
9. \texttt{mult=x.x} (x.x= 1.0 for DEPT-90, 0.5 for DEPT-45; default is 1.5 for DEPT-135).
10. Repeat steps 5 - 7, using the standard print commands in step 7.
11. Repeat steps 8 - 10 again to complete all three DEPT experiments.
Notes - DEPT:

a) The DEPT (Distortionless Enhancement by Polarization Transfer) experiment is a multipulse experiment that allows the determination of the different types of carbon atoms present in a molecule. The number at the end of the DEPT string (e.g. DEPT-45, DEPT-135) refers to the duration in degrees of the last $^1$H pulse in the pulse sequence, which is the part of the pulse sequence which actually differentiates between the types of carbon atoms. The DEPT-45 experiment shows all of the protonated carbons with positive phase. The DEPT-90 experiment shows only the -CH- carbons, with positive phase. The DEPT-135 experiment shows -CH$_3$ and -CH- carbons with positive phase and -CH$_2$- carbons with negative phase. Quaternary carbons do not appear in any DEPT spectrum. Appropriate combinations of the different DEPT spectra on a given molecule can provide for subspectra of each different type of carbon atom to be generated.

b) The sensitivity of the DEPT experiment is higher than that of a normal $^1$H-decoupled $^{13}$C spectrum, up to a factor of two. DEPT spectra by default are also $^1$H-decoupled.

c) Other than the $^1$H pulse duration, the only other variable in the DEPT pulse sequence is the one-bond $^1$H-$^{13}$C J-coupling. Usually an approximate average value is used, which for most small organic molecules is on the order of 130 - 160 Hz. On the Varian the J value can be changed by entering $j=xxx$.

Varian 300 MHz NMR Spectrometers - Proton Homonuclear Decoupling Instructions

1. Run the proton spectrum in experiment 1 (jexp1) using the standard parameters as described elsewhere. Process and phase the proton spectrum as normal.
2. Copy the proton spectrum from experiment 1 to experiment 2 using the following string of commands: mf(1,2) jexp2 wft.
3. Place the cursor on the peak you want to decouple (expand the spectrum if necessary) and type sd.
4. homodecjsh (sets up the homodecoupling experiment using the dof value set in step 3).
5. nt=xx (set number of scans; default is the same as used for previous proton spectrum).
6. ga (starts acquisition) - wait for acquisition to finish (beep will sound).
7. After the acquisition completes process the data in the normal fashion.
8. Expand the decoupled peak to check for complete decoupling. If decoupling is not complete, adjust the decoupler power. To do this, type `dpwr?` and note the value returned (or look at the text field below the spectrum - update this with `dg`). Now enter `dpwr=xx`, where `xx` is the new `dpwr` value that is 3 units higher numerically than the current value (i.e., if the current value is 12, enter 15). Do not enter a `dpwr` value higher than 21! Repeat the experiment by re-running it using the `ga` command, then re-check for complete decoupling.
9. Repeat step 3 to set a new decoupler frequency, if desired, then re-run the experiment with `ga`.
10. To finish up go back to experiment 1 (`jexp1`) before giving the standard command.

**Varian 300 MHz NMR Spectrometers - Proton 1D nOe-Difference Experiment Instructions**

Please read the notes at the end of these instructions for some discussion and hints regarding this experiment.

1. Run the proton spectrum in experiment 1 (`jexp1`) using the standard parameters as described elsewhere. Process and phase the proton spectrum as normal.
2. Copy the proton spectrum from experiment 1 to experiment 2 using the following string of commands: `mf(1,2) jexp2 wft`.
3. Place the cursor on the peak you want to irradiate (expand the spectrum if necessary) and type `sd`.
4. `noediffjsh` (sets up the nOe-difference experiment using the dof value set in step 3).
5. `nt=xx` (set number of scans; default is set to two-times the current value).
6. Open the interactive acquisition window (`acqi`) and turn off the sample spinning. Check the lock level is acceptable - increase lock power and/or gain to give a lock level of at least 40%. Check the Z1 and Z2 shims.
7. Turn on the VT control if the data acquisition will last longer than approximately 15 minutes.
8. **ga** (starts acquisition) - wait for acquisition to finish (beep will sound).

9. After the acquisition completes process the data in the normal fashion. Note that this experiment gives the difference spectrum directly, so it should have a large negative peak present at the irradiation site.

10. Look at the spectrum closely to check that the desired peak is fully saturated. If it is, you should see no intensity from the peaks in your sample which CANNOT have an nOe to the irradiated peak. If all the other sample peaks are present, but weak, most likely the irradiation is not complete. If you suspect that the irradiation is not complete, adjust the saturation power. To do this, type `satpwr?` and note the value returned (or look for the parameter listed in the text field below the spectrum - update this field with `dg`). Now enter `satpwr=xx`, where `xx` is the new satpwr value that is 3 units higher numerically than the current value (i.e., if the current value is 12, enter 15). **Do not enter a satpwr value higher than 24!** Repeat the experiment by re-running it using the **ga** command, then re-check for complete saturation.

11. If you want to repeat the experiment for another irradiation frequency, copy the current data to a new experiment number, e.g.: `mf(2,3) jexp3 wft`. Repeat step 3 to set the new irradiation frequency, if desired, then run the new experiment with **ga**.

**Notes:**

a) In general, an nOe difference measurement will require at least two irradiation frequencies: the reference (off-resonance) frequency, and the peak of interest, and (ideally) its nOe partner(s). It is usually beneficial to obtain difference spectra with irradiation of all nOe-interacting partners. In the PINMRF Varian implementation, the transmitter location is used by default as the reference frequency. However, it is important to note that if your sample has a resonance directly on the transmitter location - approximately 5.6 ppm - you will not get accurate results from this experiment. The exact transmitter location can be determined using the command sequence `ds f full centersw`. This will display the cursor on the transmitter frequency. If your nOe-difference spectrum has a peak under or close to the cursor, you will need to alter the parameter **control** to move this frequency away from any peak. Move the cursor away from
the peak near the center of the spectrum, then use the `sd` command as described above. Enter the new value of dof for the control value (i.e., `control=dof`). Then re-acquire the data.

b) When setting the irradiation power, keep in mind the following. Broader peaks require more power than do narrow ones, and peaks that are very close to another resonance should be irradiated with a minimum amount of power in order to reduce the possibility of partly irradiating another peak. In this case a longer irradiation time may be beneficial (parameter `sattime`).

c) The sample quality is particularly important for nOe-based experiments. For more information on this, please see “Sample-Related Considerations” on pp. 11-12 of the PINMRF manual Bruker Avance / ARX NMR Spectrometers - Routine/Survey 2D Spectra using Standard Parameter Sets.

**Varian 300 MHz NMR Spectrometers - Proton Presaturation Experiment Instructions**

1. Run the proton spectrum in experiment 1 (`jexp1`) using the standard parameters as described elsewhere. Process and phase the proton spectrum as normal.
2. Copy the proton spectrum from experiment 1 to experiment 2 using the following string of commands: `mf(1,2) jexp2 wft`.
3. Place the cursor on the peak you want to irradiate (expand the spectrum if necessary) and type `sd`.
4. `presatjsh` (sets up the presaturation experiment using the dof value set in step 3).
5. `nt=xx` (set number of scans; default is the same as used for previous proton spectrum).
6. Open the interactive acquisition window (`acqi`) and turn off the sample spinning. Check the lock level is acceptable - increase lock power and/or gain to give a lock level of at least 40%. Check the Z1 and Z2 shims.
7. `ga` (starts acquisition) - wait for acquisition to finish (beep will sound).
8. After the acquisition completes process the data in the normal fashion.
9. Expand the irradiated peak to check for complete (enough) saturation, i.e., the peak should be removed or at least significantly reduced in intensity. If the saturation is not complete, adjust the decoupler power. To do this, type `dpwr?` and note the value returned (or look at the text field below the spectrum - update this with `dg`). Now enter `dpwr=xx`, where xx is the new dpwr value that is 3 units higher numerically than the current value (i.e., if the current value is 12, enter 15). Do not enter a `dpwr` value higher than 24! Repeat the experiment by re-running it using the `ga` command, then re-check the saturation.

**Varian 300 MHz NMR Spectrometers - 2D COSY Instructions**

**COSY data acquisition**

1. Acquire a $^1$H spectrum in experiment 1 (jexp1) using the standard parameters.
2. Phase the $^1$H spectrum, then expand the spectrum such that the peaks of interest are displayed on the screen. DO NOT MOVE THE CURSORS AFTER EXPANDING THE DISPLAY.
3. `movesw` sets up an optimized acquisition window for your sample.
4. Re-acquire the spectrum (ga), phase and display it. Click the FULL button above the spectrum window. Check that it looks correct.
4a. If you are planning on saving this COSY data, you need to now save the 1D $^1$H spectrum: `svf('sample-1-h1')`
5. Copy the proton spectrum from experiment 1 to experiment 3 using the following string of commands: `mf(1,3) jexp3 wft` .
6. `cosyjsh` will set up the magnitude COSY spectrum. The COSY takes about 10 - 30 minutes, depending on the spectrometer (the INOVA-300’s use a gradient-COSY, which is quicker).
7. Open the interactive acquisition window (`acqi`) and turn off the sample spinning. Check the lock level is acceptable - increase lock power and/or gain to give a lock level of at least 40%. Check the Z1 and Z2 shims.
8. Type `go` to start the acquisition - DO NOT USE `ga`!
COSY data processing and plotting

9. wft2d carry out a 2D Fourier transform and display the 2D spectrum.

9a. If your experiment looks OK and you want to save the COSY data, do it now:

```
svf('sample-1-cosy')
```

10. Click the MAIN MENU button above the spectrum window, then click the DISPLAY, SIZE, FULL SCREEN buttons.

11. Use the VS-20% and the VS+20% buttons above the spectrum to adjust the vertical scaling of the intensity image prior to plotting.

12. Type plcosy(8,1.5,1) to plot the 2D spectrum along with the 1D spectrum along the axes.

13. Expansions are done in the same fashion as for 1D spectra, with the left and right mouse buttons and the EXPAND menu button.

Finishing up

14. Go back to experiment 1 (jexp1) and restart sample spinning.

Varian 300 MHz NMR Spectrometers - 2D HETCOR Instructions

HETCOR data acquisition

1. Acquire a 1H spectrum in experiment 1 (jexp1) using the standard parameters.

2. Phase the 1H spectrum, then expand the spectrum such that the peaks of interest are displayed on the screen. DO NOT MOVE THE CURSORS AFTER EXPANDING THE DISPLAY.

3. movesw sets up an optimized acquisition window for your sample.

4. Re-acquire the spectrum (ga), phase and display it. Click the FULL button above the spectrum window. Check that it looks correct.

4a. If you are planning on saving this HETCOR data, you need to now save the 1D 1H spectrum:

```
svf('sample-2-h1')
```

5. Acquire a 13C spectrum in experiment 2 (jexp2). If time permits, expand, optimize, and re-run the 13C spectrum (note that if your spectrum has downfield quaternary carbons these do not need to be included in the expanded region).
5a. If you are planning on saving this HETCOR data, you need to now save the 1D $^{13}$C spectrum: svf('sample-2-c13')

6. Copy the carbon-$^{13}$ spectrum from experiment 2 to experiment 3 using the following string of commands: mf(2,3) jexp3 wft .

7. hetcorjsh(1) set up the magnitude-mode heteronuclear correlation spectrum - check that the nt and d1 values are appropriate for your sample (usually the defaults are fine).

8. go start the acquisition - DO NOT USE ga!

HETCOR data processing and plotting

9. wft2d carry out a 2D Fourier transform and display the 2D spectrum.

9a. If your experiment looks OK and you want to save the HETCOR data, do it now: svf('sample-2-hetcor')

10. Click the MAIN MENU button above the spectrum window, then click the DISPLAY, SIZE, FULL SCREEN buttons.

11. Use the VS-20% and the VS+20% buttons above the spectrum to adjust the vertical scaling of the intensity image prior to plotting.

12. Type plhxcor(8,1.5,1,2) to plot the 2D spectrum along with the 1D spectra along the axes.

13. Expansions are done as for 1D spectra, with the left and right mouse buttons and the EXPAND menu button. NOTE: multiple printouts with different vertical scale settings may be needed to allow all of the cross-peaks to be seen.

Finishing up

14. Go back to experiment 1 (jexp1) and restart sample spinning if it was turned off.
Varian 300 MHz NMR Spectrometers

Parameter Files and Macros

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<thead>
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<th>Parameter Files</th>
<th>Macros</th>
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<td>c13meth</td>
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</table>

h2cdcl (Inova-300-1 only – requires cables to be changed)

depjtsh (sets up DEPT-135 experiment from the current $^{13}$C experiment)

homodecjsh (sets up a homonuclear decoupling experiment from the current $^1$H experiment)

noediffjsh (sets up a nOe difference experiment from the current $^1$H experiment)

presatjsh (sets up a presaturation experiment from the current $^1$H experiment)


cosyjsh (sets up a 2D-COSY experiment from the current $^1$H experiment)

hetcorjsh(1) (sets up 2D-HETCOR experiment from the current $^{13}$C experiment and the $^1$H experiment already run in experiment 1)
Commonly Used Keyboard Commands

**Experiment Setup**
- `jexpn` - join (go to) experiment #n
- `rt('abcd')` - read NMR data file “abcd”
- `rtp('abcd')` - read parameter set “abcd”
- `rts('abcd')` - read shim set “abcd”
- `svf('abcd')` - save NMR data file “abcd”
- `svp('abcd')` - save parameter set “abcd”
- `svs('abcd')` - save shim set “abcd”
- `mf(x,y)` - move NMR data (FID) from experiment “x” to experiment “y”

**Data Acquisition**
- `h1xxxx` - load acquisition and lock parameters for $^1$H experiment in solvent xxxx
  (xxxx = acet, cdcl, d2o, dmso, meth)
- `acqi` - open lock / shimming window
- `su` - set up interfaces
- `gain=xy` - set receiver gain to value “xy” (“xy” = 1 – 60)
- `nt=xy` - set number of scans to value “xy”
- `bs=xy` - set block size to “xy” scans
- `ga` - zero current data and start acquisition, Fourier transform and display result
- `go` - zero current data and start acquisition
- `aa` - abort data acquisition after next scan
- `standard` - set up spectrometer for CDCl$_3$ standard sample
- `f19xxxx` - load acquisition parameters for $^{19}$F experiment in solvent xxxx
- `p31xxxx` - load acquisition parameters for $^{31}$P experiment in solvent xxxx
- `c13xxxx` - load acquisition parameters for $^{13}$C experiment in solvent xxxx
- `deptjsh` - set up DEPT-135 experiment from current $^{13}$C experiment
- `homodecjsh` - sets up a homonuclear decoupling experiment from the current $^1$H experiment
- `noediffjsh` - sets up a nOe difference experiment from the current $^1$H experiment
- `presatjsh` - sets up a presaturation experiment from the current $^1$H experiment
- `cosyjsh` - set up 2D-COSY experiment from current $^1$H experiment
- `hetcorjsh(x)` - set up 2D-HETCOR using current $^{13}$C experiment and $^1$H experiment in experiment “x”

**Data Processing and Plotting**
- `text('abcd')` - set title for plot to be the text string “abcd”
- `ft` - Fourier transformation
- `wft` - exponential multiplication and Fourier transformation (can use after bs scans completed)
- `lb=xy` - set amount of exponential multiplication equal to “xy”
- `aph` - automatic phase correction
- `rl(x,yz*sfrq)` - set chemical shift at location of cursor to x,yz ppm
- `th` - set threshold for peak picking using mouse
- `dpf` - show peak labels
- `ds` - re-display spectrum with cursor active
- `dir` - show a listing of the current directory
- `print, printp, printi, printpi` - print spectrum with/without peak labels (p) and/or integration (i)
- `print135, print135p` - print DEPT-135 spectrum with/without peak labels (p)