

# Operational Procedures of Varian NMR

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# For all experiments

- Sample preparation:
  - For  $^1\text{H}$  spectrum: 3 ~ 10 mg of sample;
  - For  $^{13}\text{C}$  spectrum: 20 ~ 50 mg of sample;
  - For 2D spectrum: the more the better, typically 50 mg.
- Solvent: ~ 0.7 ml

# For all experiments

- Insert sample (input “i” in command line or press “Insert” button);
- Write down sample information;

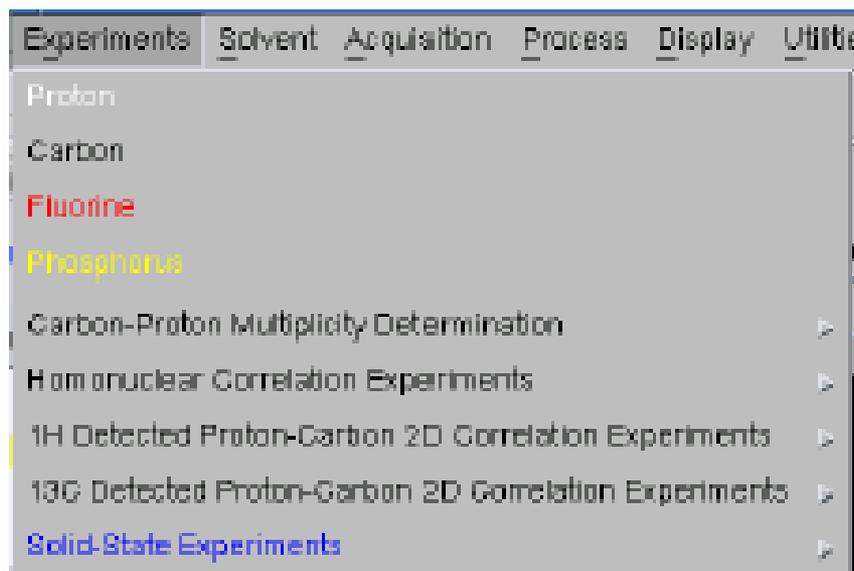


# For all experiments

- Find Z0: Lock power and lock gain can be increased if the lock status is “Not regulated” (It may occur when using  $\text{CDCl}_3$  as solvent, or high concentration).
- Gradient shim.
- Spin control: Only  $^1\text{H}$  spectrum needs spin.

# For all experiments

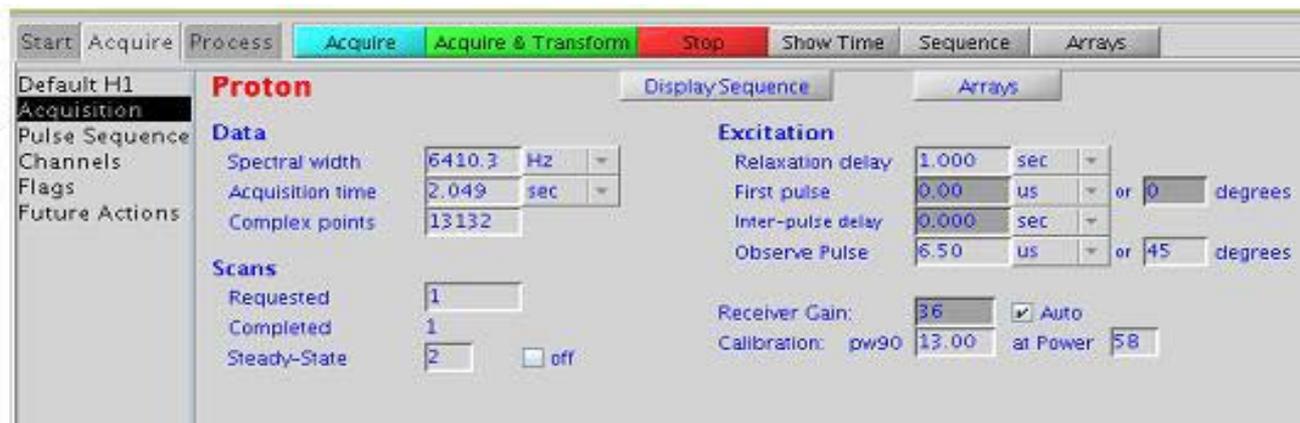
- Select experiment from “Experiments” menu.



- Save data: Select File → Save or press Ctrl + S.

# $^1\text{H}$ and $^{13}\text{C}$ Spectrum

- ▶ Enter “nt=scan times bs=block size ga” in command line.



The screenshot shows the 'Proton' acquisition settings window. The interface includes a top menu bar with 'Start', 'Acquire', 'Process', 'Acquire', 'Acquire & Transform', 'Stop', 'Show Time', 'Sequence', and 'Arrays'. The left sidebar lists 'Default H1', 'Acquisition', 'Pulse Sequence', 'Channels', 'Flags', and 'Future Actions'. The main area is divided into 'Data' and 'Excitation' sections.

**Data**

- Spectral width: 6410.3 Hz
- Acquisition time: 2.049 sec
- Complex points: 13132

**Excitation**

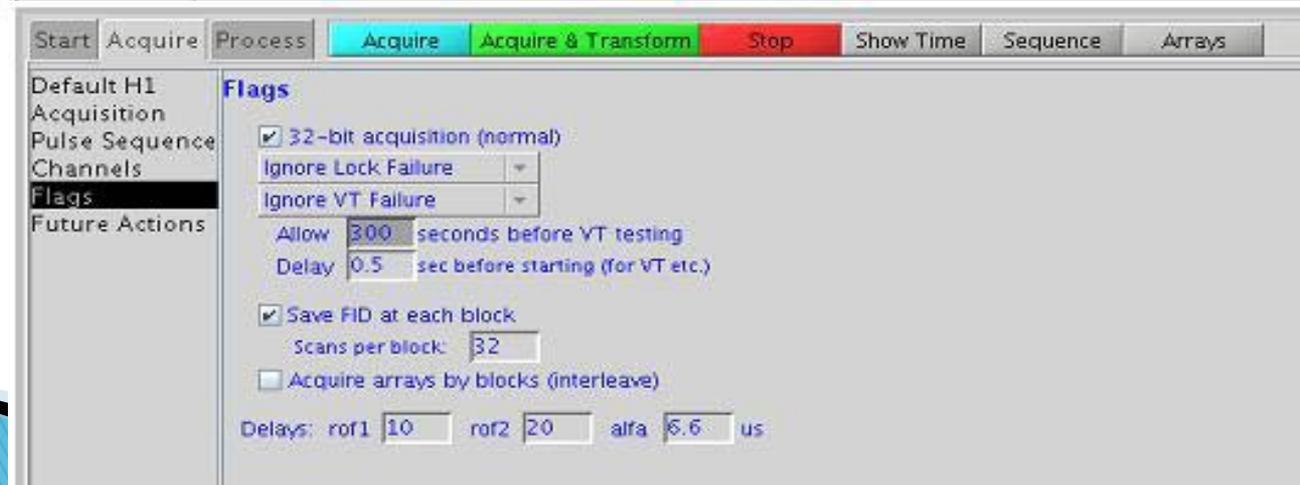
- Relaxation delay: 1.000 sec
- First pulse: 0.00 us or 0 degrees
- Inter-pulse delay: 0.000 sec
- Observe Pulse: 6.50 us or 45 degrees

**Scans**

- Requested: 1
- Completed: 1
- Steady-State: 2  off

**Receiver Gain:** 36  Auto

**Calibration:** pw90 13.00 at Power 58



The screenshot shows the 'Flags' settings window. The interface includes a top menu bar with 'Start', 'Acquire', 'Process', 'Acquire', 'Acquire & Transform', 'Stop', 'Show Time', 'Sequence', and 'Arrays'. The left sidebar lists 'Default H1', 'Acquisition', 'Pulse Sequence', 'Channels', 'Flags', and 'Future Actions'. The main area contains various acquisition flags and parameters.

**Flags**

- 32-bit acquisition (normal)
- Ignore Lock Failure:
- Ignore VT Failure:
- Allow: 300 seconds before VT testing
- Delay: 0.5 sec before starting (for VT etc.)
- Save FID at each block
  - Scans per block: 32
- Acquire arrays by blocks (interleave)

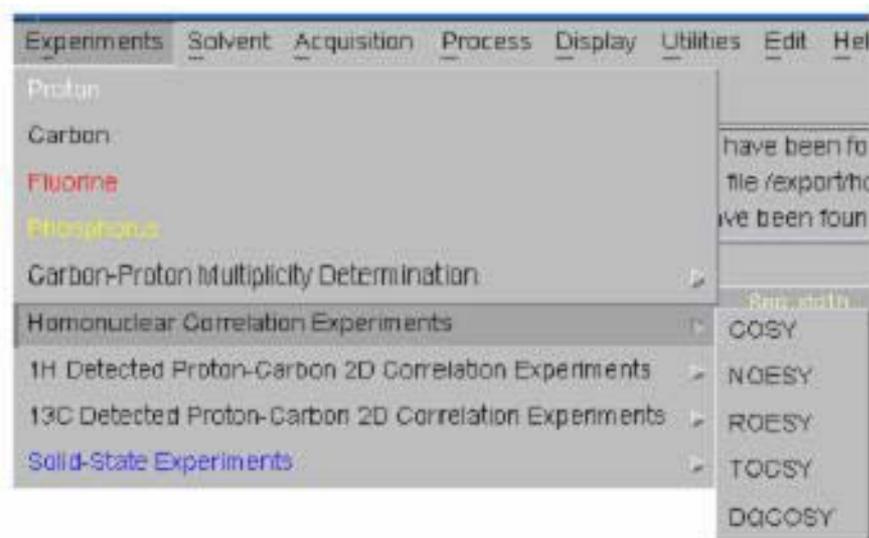
**Delays:** ror1 10 ror2 20 alfa 6.6 us

# $^1\text{H}$ and $^{13}\text{C}$ Spectrum

- ▶ Data process: Enter “wft aph” to display spectrum and carry out phase correction.
- ▶ Set reference and integrate (only for  $^1\text{H}$ ).
- ▶ Set peak display threshold: Press  button in toolbar on the right.
- ▶ Plot: Enter “phs” (for  $^1\text{H}$ ) or “pcs” (for  $^{13}\text{C}$ ).

# $^1\text{H}$ - $^1\text{H}$ correlation

- Scan  $^1\text{H}$  spectrum or load saved spectrum in one experiment zone at first;
- Jump to another zone using `jexp+number` command (for example: `jexp2` to jump to zone 2);
- Write down sample information, choose the solvent used and select experiment from “Experiments” menu.



# $^1\text{H}-^1\text{H}$ correlation

- Recommended parameters for  $^1\text{H}-^1\text{H}$  correlation spectrum:

COSY	Scans per increment(nt)	4(min),multiple of 4(max)
	Number of increment(ni)	128 or more
NOESY	Scans per increment(nt)	8(min),multiple of 32(max)
	Number of increment(ni)	128 or more
	Mixing time(mix)	
DQCOSY	Scans per increment(nt)	8(min),multiple of 32(max)
	Number of increment(ni)	128 or more
TOCSY	Scans per increment(nt)	8(min),multiple of 32(max)
	Number of increment(ni)	128 or more
	Mixing time(mix)	0.08, range:0.03~0.1
ROESY	Scans per increment(nt)	8(min),multiple of 8(max)
	Number of increment(ni)	128 or more
	Mixing time(mix)	0.3, range:0.1~0.8
gCOSY	Scans per increment(nt)	4(min),multiple of 4(max)
	Number of increment(ni)	128 or more

# gCOSY

The screenshot displays the gCOSY software interface. At the top, there is a navigation bar with buttons for Start, Acquire, Process, Acquire (highlighted in cyan), Acquire & Transform (highlighted in green), Stop (highlighted in red), Show Time, Sequence, and Arrays. Below this is a sidebar menu with options: Defaults (selected), Acquisition, Pulse Sequence, Channels, Flags, and Future Actions. The main area shows the Pulse Sequence set to 'Gcosy'. A 'Display Sequence' button is visible. Parameters are set as follows: Scans per increment: 1; Number of increments: 128; Spectral Width [ppm]: Downfield 7.9, Upfield -2.8; Fourier Number in F2 & F1: 1k x 1k; Linear Prediction in t1: ni \* 2; Window functions: (empty); Plotting turned off; Parameters: (empty); Plot contours: Positive Only; Plot 1D: F1 spectrum, F2 spectrum.

Parameter	Value
Pulse Sequence	Gcosy
Scans per increment	1
Number of increments	128
Spectral Width [ppm]	Downfield 7.9, Upfield -2.8
Fourier Number in F2 & F1	1k x 1k
Linear Prediction in t1	ni * 2
Window functions	
Plotting turned off	
Parameters	
Plot contours	Positive Only
Plot 1D: F1	spectrum
Plot 1D: F2	spectrum

# NOESY



Start Acquire Process Acquire Acquire & Transform Stop Show Time Sequence Arrays

Defaults  
Acquisition  
Pulse Sequence  
Channels  
Flags  
Future Actions

Pulse Sequence: **Noesy**

Scans per Increment 8

Number of Increments 128

Mixing time [ms] 400

Relaxation time [s] 1

Spectral Width [ppm] 14 → -2

Downfield 14.0 Upfield -2.0

Zero Quantum Suppression

Fourier Number in F2 & F1: 1k x 1k

Linear Prediction in t1: ni\* 2

Window functions:

Plotting turned off

Parameters:

Plot contours:

Plot ID: F1 F2

# TOCSY

Start Acquire Process **Acquire** **Acquire & Transform** Stop Show Time Sequence Arrays

**Defaults**  
Acquisition  
Pulse Sequence  
Channels  
Flags  
Future Actions

Pulse Sequence: **Tocsy**

Scans per increment: 4  
Number of increments: 128  
Spectral Width [ppm]: 14 → -2  
Downfield: 14.0 Upfield: -2.0  
Mixing time [ms]: 80

Z-TOCSY with ZQ suppression  XY-TOCSY

Fourier Number in F2 & F1: 1k x 1k  
Linear Prediction in t1: ni \* 2  
Window functions:

Plotting turned off

Parameters:  
Plot contours:  
Plot 1D: F1  
F2

# Data process for $1\text{H}-1\text{H}$ correlation

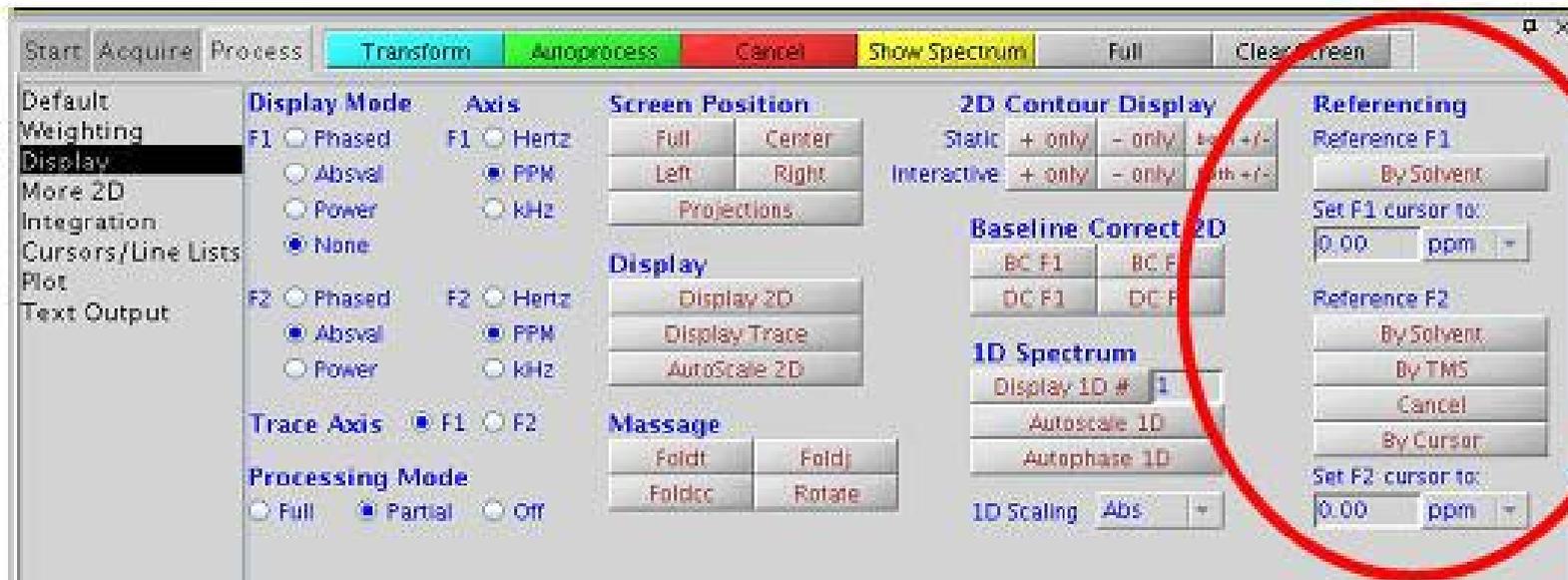
- ▶ During the scan: Jump to a third zone, and use “mf (the scan zone, the third zone)” (for example, “mf(2,3)”) to transfer data to the target zone.
- ▶ Press “Autoprocess” button to display 2D spectrum.

# Data process for $1\text{H}-1\text{H}$ correlation

- ▶ Phase correction: Enter “aph” for auto phase correction.
- ▶ Adjusting vertical height using  or  button in toolbar on the right.
- ▶ Display 2D spectrum: Enter “dconi” command.

# Data process for $1\text{H}-1\text{H}$ correlation

## ► Referencing:

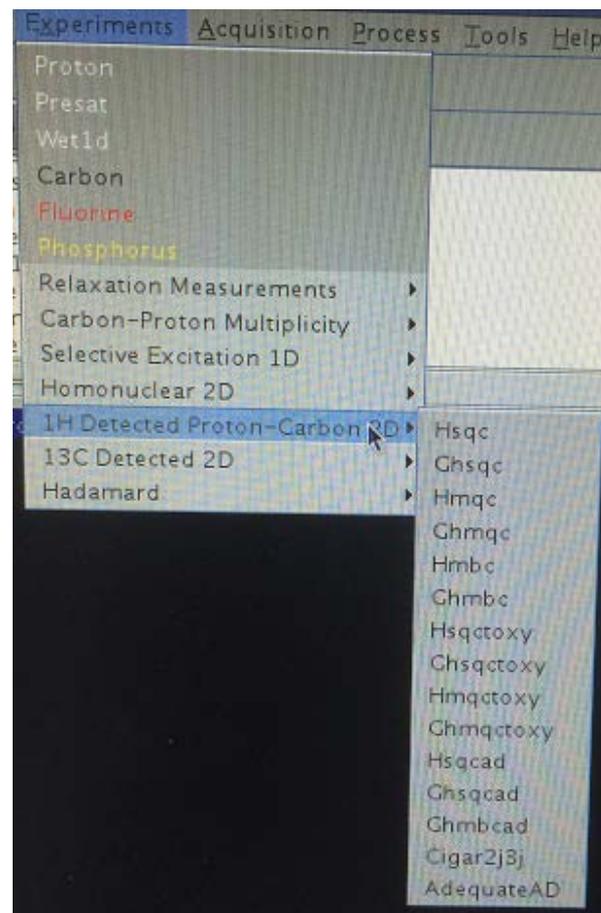


# Plot $^1\text{H}$ - $^1\text{H}$ spectrum

- ▶ Enter “`plcosy(15,1.2,experiment zone of loaded  $^1\text{H}$  spectrum)`” (for example, `plcosy(15,1.2,1)` if the  $^1\text{H}$  spectrum is in zone 1) to plot 2D spectrum.

# HSQC and HMBC

- ▶ Scan  $^1\text{H}$  and  $^{13}\text{C}$  spectrum or load saved spectrum in two experiment zones at first;
- ▶ Jump to another zone;
- ▶ Write down sample information, choose the solvent used and select experiment from “Experiments” menu.
- ▶ Data process of HSQC and HMBC is similar to  $^1\text{H}$ - $^1\text{H}$  correlation.



# gHSQC and gHMBC

- ▶ Recommended parameters for HSQC and HMBC:

gHSQC	Scans per increment(nt) Number of increment(ni) C13 Spectral Width [ppm] (sw1) C-H Multiplicity Edit(mult)	4(min),multiple of 4(max) 128 or more 160ppm~-10ppm, selectable mult=2(CH,CH3 up, CH2 down)
gHMBC	Scans per increment(nt) Number of increment(ni) C13 Spectral Width [ppm] (sw1) Coupling constang(jnxh)	8(min),multiple of 4(max) 200 or more 225ppm~-15ppm, selectable 8,range 5-10Hz

# gHSQC

Start	Acquire	Process	Acquire	Acquire & Transform	Stop	Show Time	Sequence	Arrays
Defaults	Pulse Sequence: <b>Ghsqc</b>		Fourier Number in F2 & F1: 2k x 1k					
Acquisition	Scans per increment: 2		Linear Prediction in t1: ni * 2					
Pulse Sequence	Number of increments: 128		Window functions:					
Channels	C13 Spectral Width [ppm]: 160 → -10		Plot when done:					
Flags	Downfield: 160	Upfield: -10	Parameters: Basic, Top Left					
Future Actions	C-H Multiplicity Edit: Yes		Plot contours: Positive & Negative					
			Plot 1D: F1 (X) spectrum					
			F2 (H) spectrum					

# gHMBC

Start Acquire Process **Acquire** **Acquire & Transform** Stop Show Time Sequence Arrays

**Defaults**  
Acquisition  
Pulse Sequence  
Channels  
Flags  
Future Actions

Pulse Sequence: **ghmhc**

Scans per increment 8  
Number of increments 200  
C13 Spectral Width [ppm] 225 → -15  
Downfield 225 Upfield -15  
Coupling constant 8 Hz

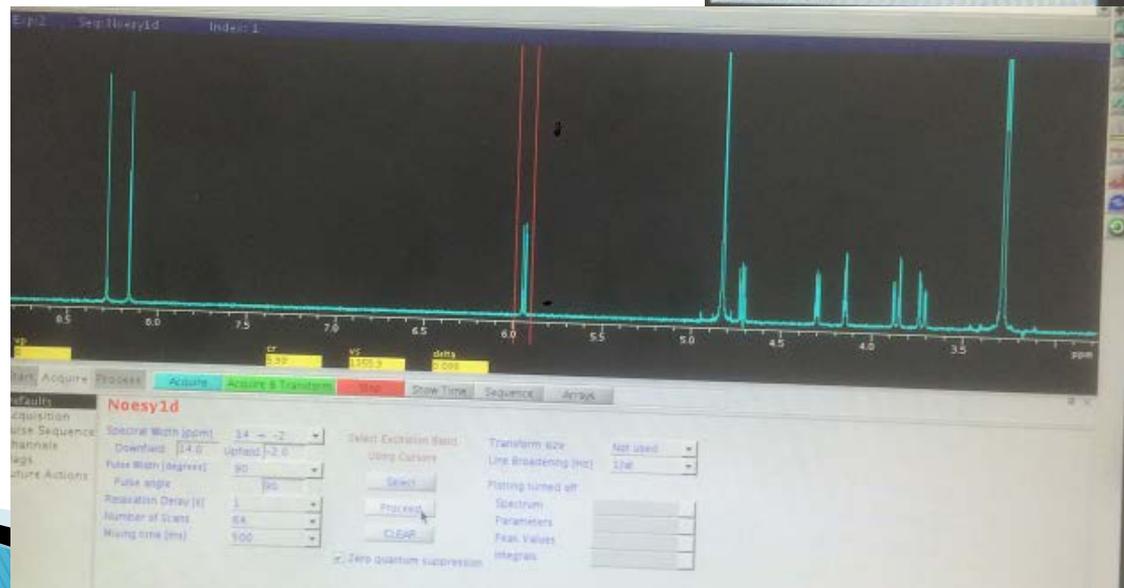
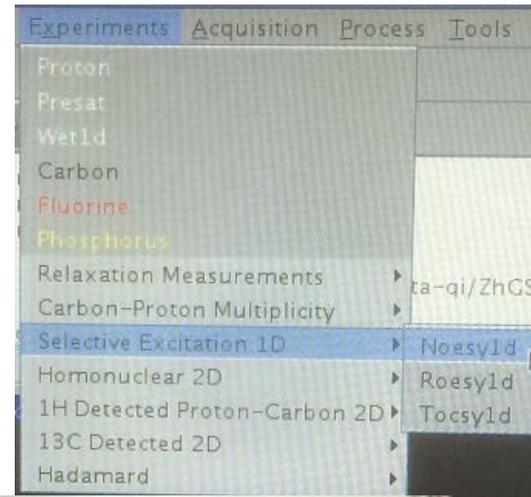
Fourier Number in F2 & F1 2k x 1k  
Linear Prediction in t1 ni + 2  
Window functions  
Plotting turned off  
Parameters  
Plot contours  
Plot 1D F1 (X)  
F2 (H)

# Plot HSQC and HMBC

- ▶ Enter “plhxcor(15,1.2,experiment zone of loaded  $^1\text{H}$  spectrum,experiment zone of loaded  $^{13}\text{C}$  spectrum)” (for example, plhxcor(15,1.2,1,2) if the  $^1\text{H}$  spectrum is in zone 1 and the  $^{13}\text{C}$  spectrum is in zone 2) to plot 2D spectrum.

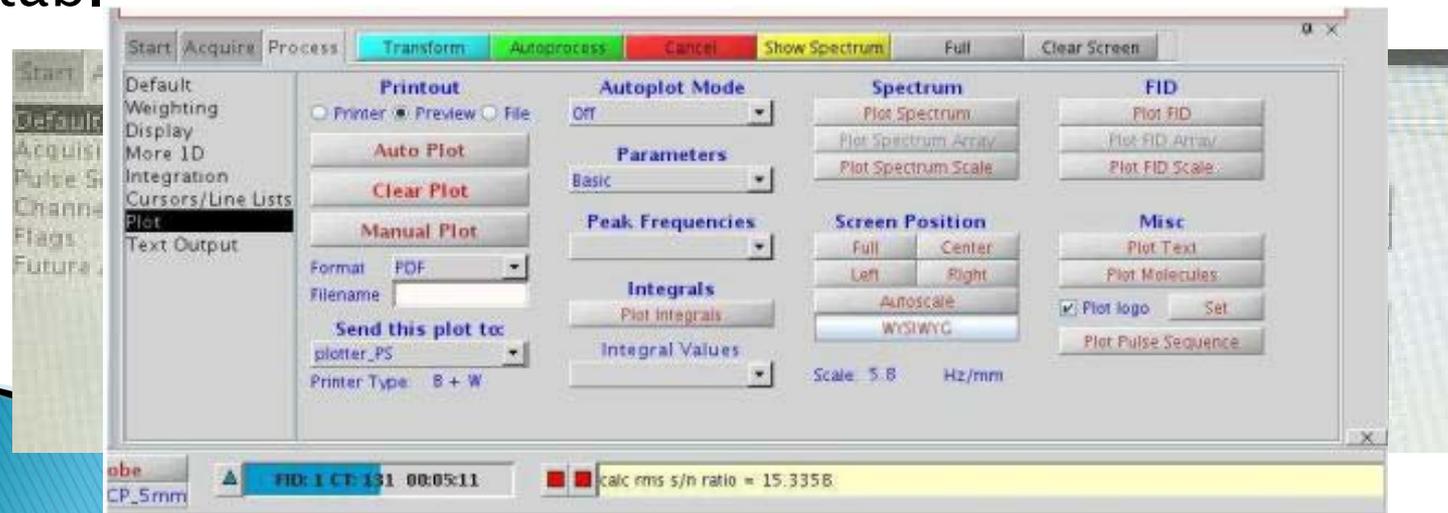
# 1D NOESY (ROESY and TOCSY)

- ▶ Select experiment;
- ▶ Load a  $^1\text{H}$  spectrum and conduct referencing at first, then select H in the spectrum to be excited;
- ▶ Press “Select” button and “Proceed” button;



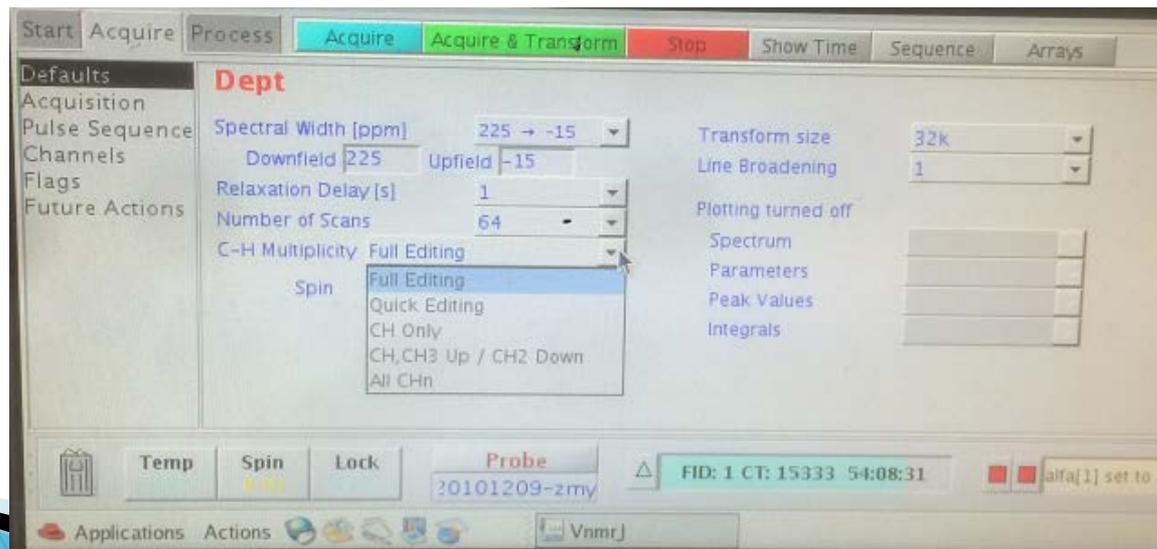
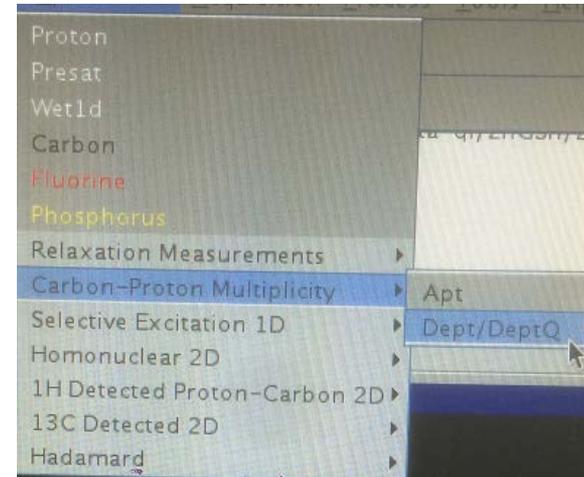
# 1D NOESY (ROESY and TOCSY)

- ▶ Type command like  $^1\text{H}$  spectrum, for example, nt=128 bs=4 ga, or select number of scans in Defaults tab and block size in Flags tab then press the green “Acquire & Transform” button to start scanning.
- ▶ Data process is similar to  $^1\text{H}$  spectrum.
- ▶ Press “Auto Plot” button in “Process” menu at “Plot” tab.



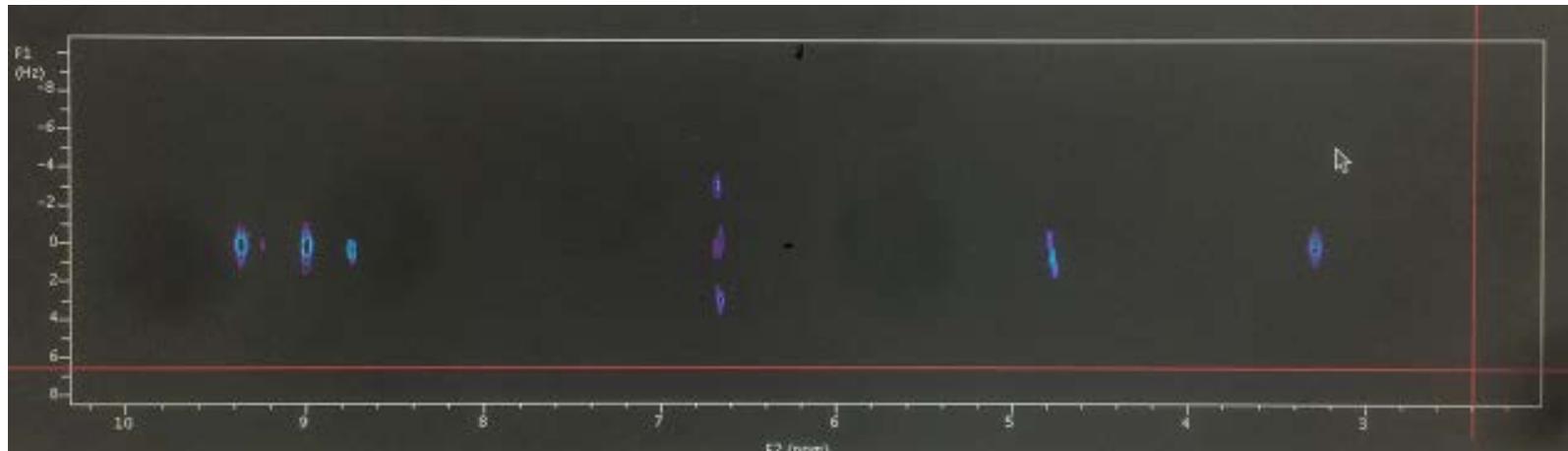
# DEPT

- ▶ Select experiment;
- ▶ Set parameters and start acquire;
- ▶ Data process is similar to  $^{13}\text{C}$  spectrum.



# Homo 2DJ

- ▶ 2D J-resolved Spectroscopy: Chemical shift in one dimension and J-value in another.
- ▶ A typical homo 2DJ spectrum as below:



# Homo 2DJ

- ▶ Set parameters as follows;
- ▶ Data process is similar to COSY.

