

2003 NMR User Training Course

National Program for Genomic Medicine

High-Field NMR Core Facility,

The Genomic Research Center, Academia Sinica

09/29-09/30, 2003

09/30, 2003 Course Handout

Software Training: NMRPipe & Sparky

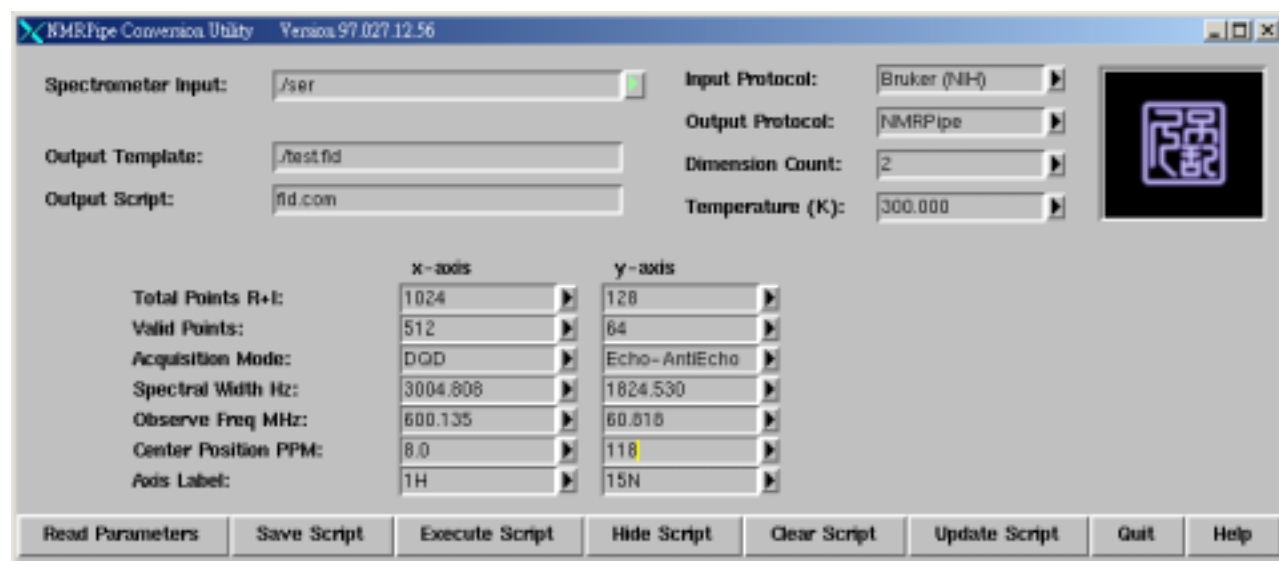
NMRPipe/NMRDraw Practical

LBD 1/1 2D 1H, 15N-HSQC experiment:

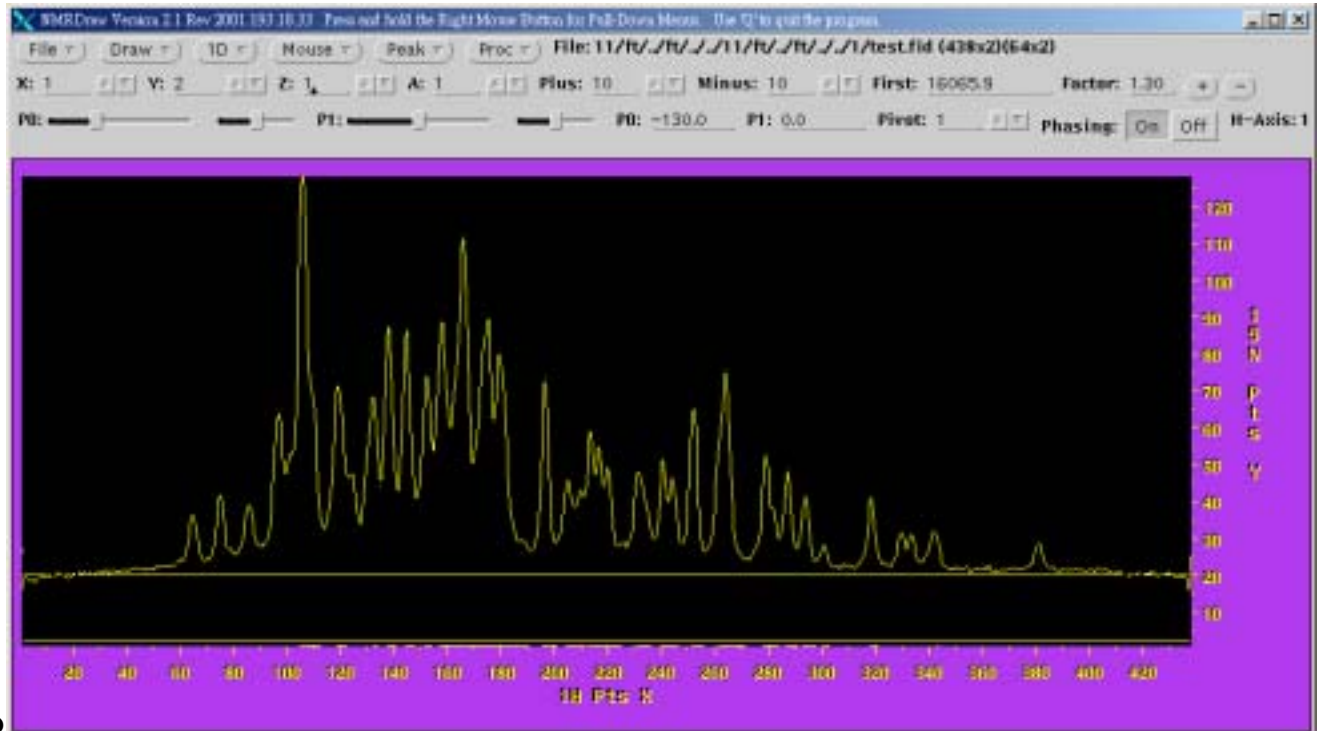
1H, 15N-hsqc, echo-antiecho, SW=3004.808 (1h), 1824.53 (15N)

1024 (1H, DQD) by 128 ni in 15N(echo-antiecho)

- `cd /goat/data/winston/Training/LBD_NC/1`
- (source NMRpipe (“snp”, have x-window running, set DISPLAY.)
- Type in bruker and update parameters to the values below including
- change 1H center position to 8.0 ppm (this experiment have 01p at 8 ppm (center of NH region, and use only 5 ppm SW in 1H to increase digital resolution.)
- and 15N to 118 ppm



- clear scrip, updata script, save script and execute script.
- Read in test.fid, 1D Horizontal and choose the 2nd fid, (Y:2),
- Proc-auto process 1D, and phase the spectrum, p0=-130



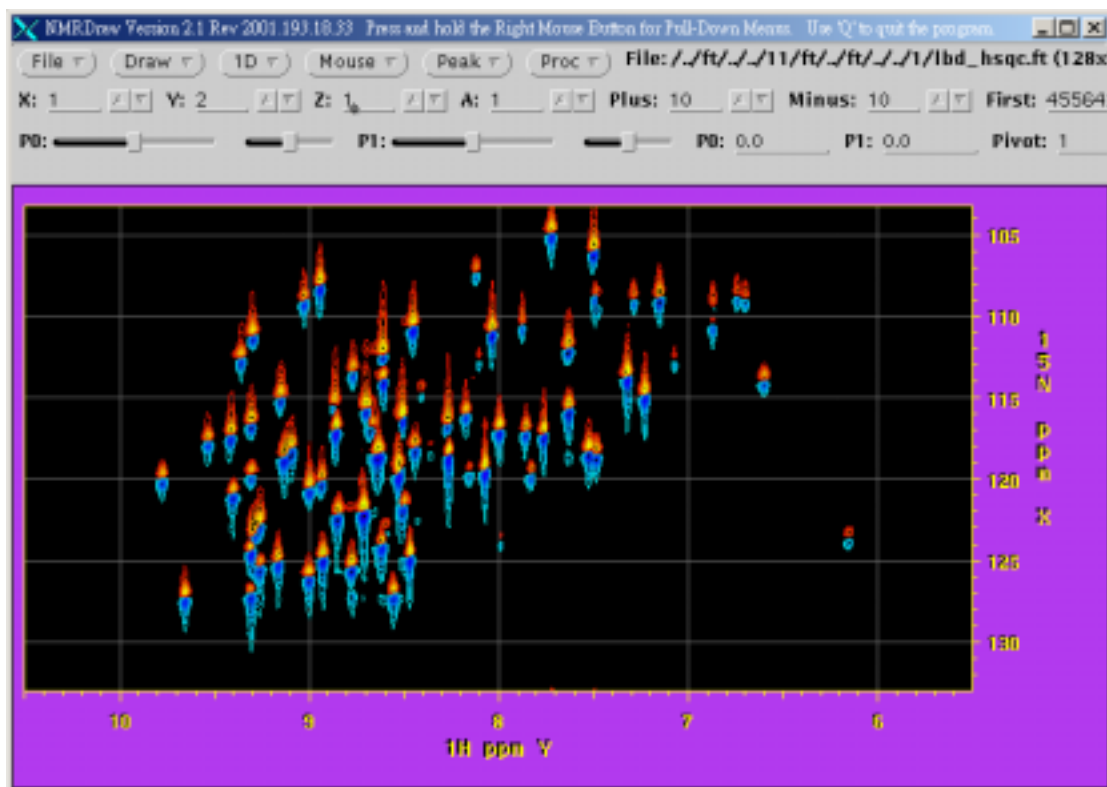
● Edit hsqc_pipe and input p0=-130 in proton

● hsqc_pipe processing script

```
#!/bin/csh
```

```
nmrPipe -in ./test.fid \
| nmrPipe -fn SP -off 0.33 -end 1 -pow 1 -c 1 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -130 -p1 0.0 -di -verb \
#| nmrPipe -fn EXT -left -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.33 -end 1 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -0.0 -p1 -0.0 -di -verb \
| nmrPipe -out ./lbd_hsqc.ft -ov
```

● Read in lbd_hsqc.ft



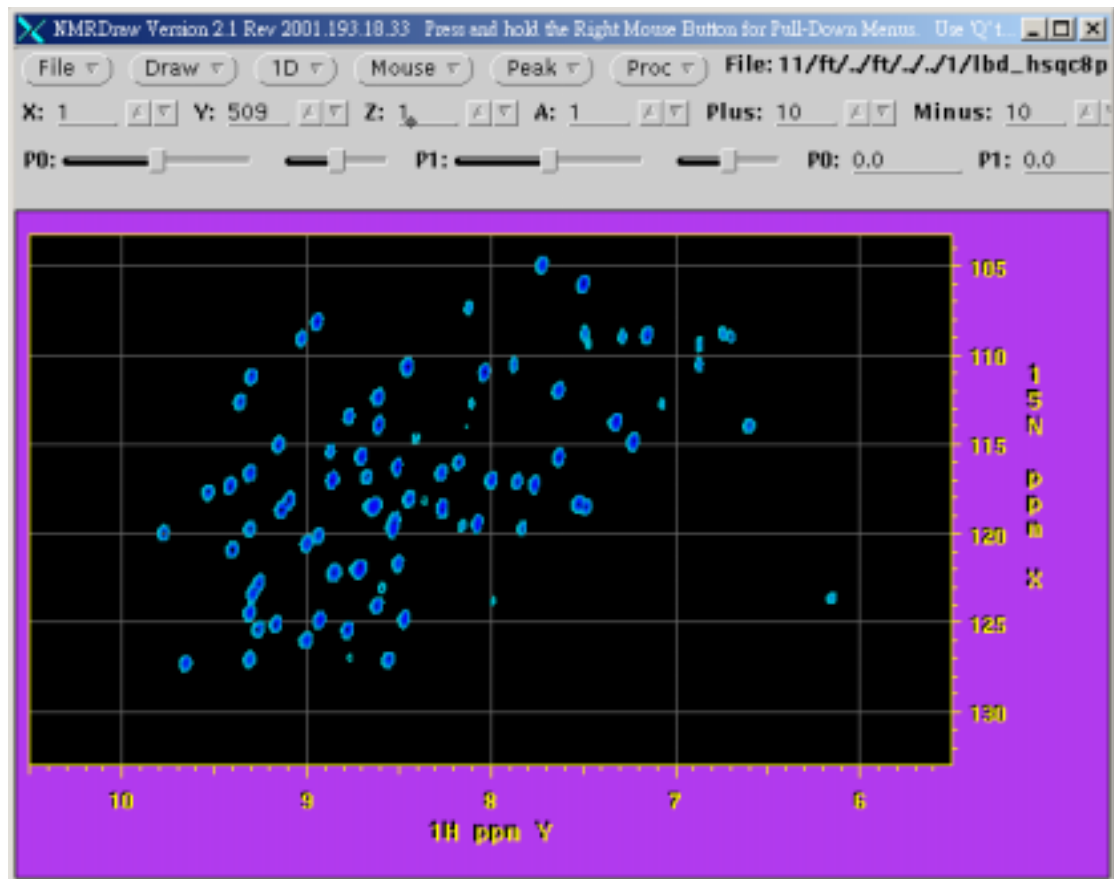
-
- choose Mouse-1D vertical, and phase a 15N vector.
- Edit the hsqc_pipe script, put p0=-85 for 15N, let's rename the script as "hsqc_pipe_ph_final".

- **The "hsqc_pipe_ph_final" script:**

```
#!/bin/csh
```

```
nmrPipe -in ./test.fid \
| nmrPipe -fn SP -off 0.33 -end 1 -pow 1 -c 1 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -130 -p1 0.0 -di -verb \
#| nmrPipe -fn EXT -left -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.33 -end 1 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -85.0 -p1 -0.0 -di -verb \
| nmrPipe -out ./lbd_hsqc8ppm.ft -ov
```

- Execute the hsqc_pipe_ph_final script, and check the spectrum in NMRDraw.

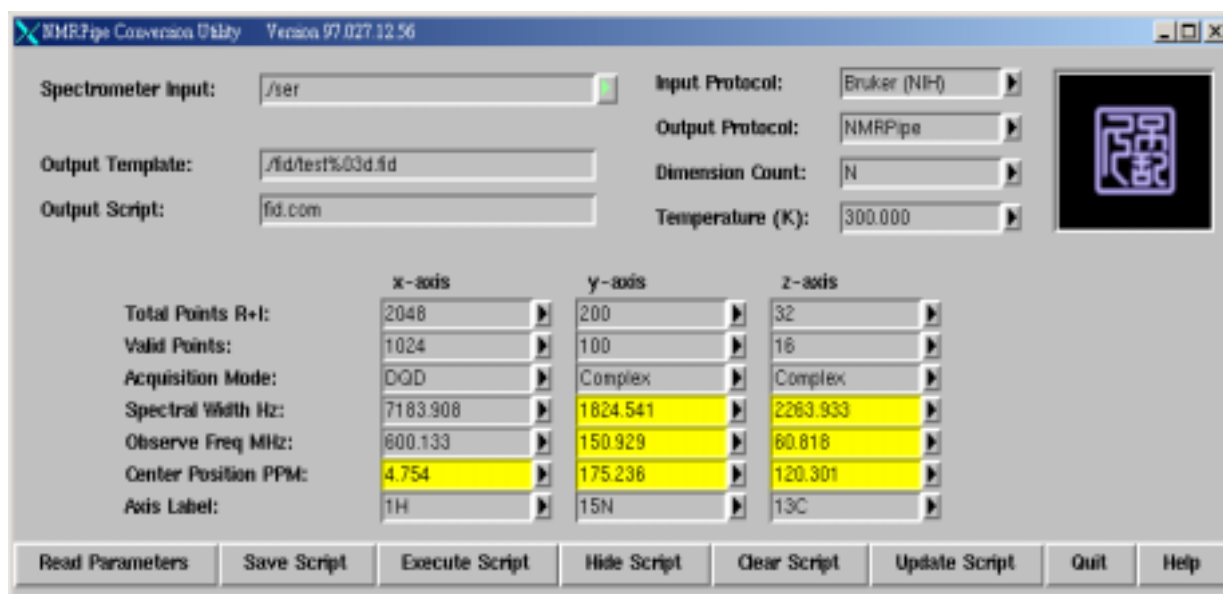


- pipe2ucsf lbd_hsqc8ppm.ft lbd_hsqc8ppm.ucsf (convert to Sparky format).
- Try using Sparky to view the file. (type in “sparky” directly).

LBD_NC 11/1, HN_CO experiment

- Source NMRPipe (snp), have x-window program running, and set environment display.
- `cd /goat/data/winston/Training/LBD_NC/11`
- type in “bruker” (figure 1 will show up).
- change the parameter below to be exactly the same as figure 1.

Figure 1 (incorrect parameters, need to be changed to the values in figure 2):

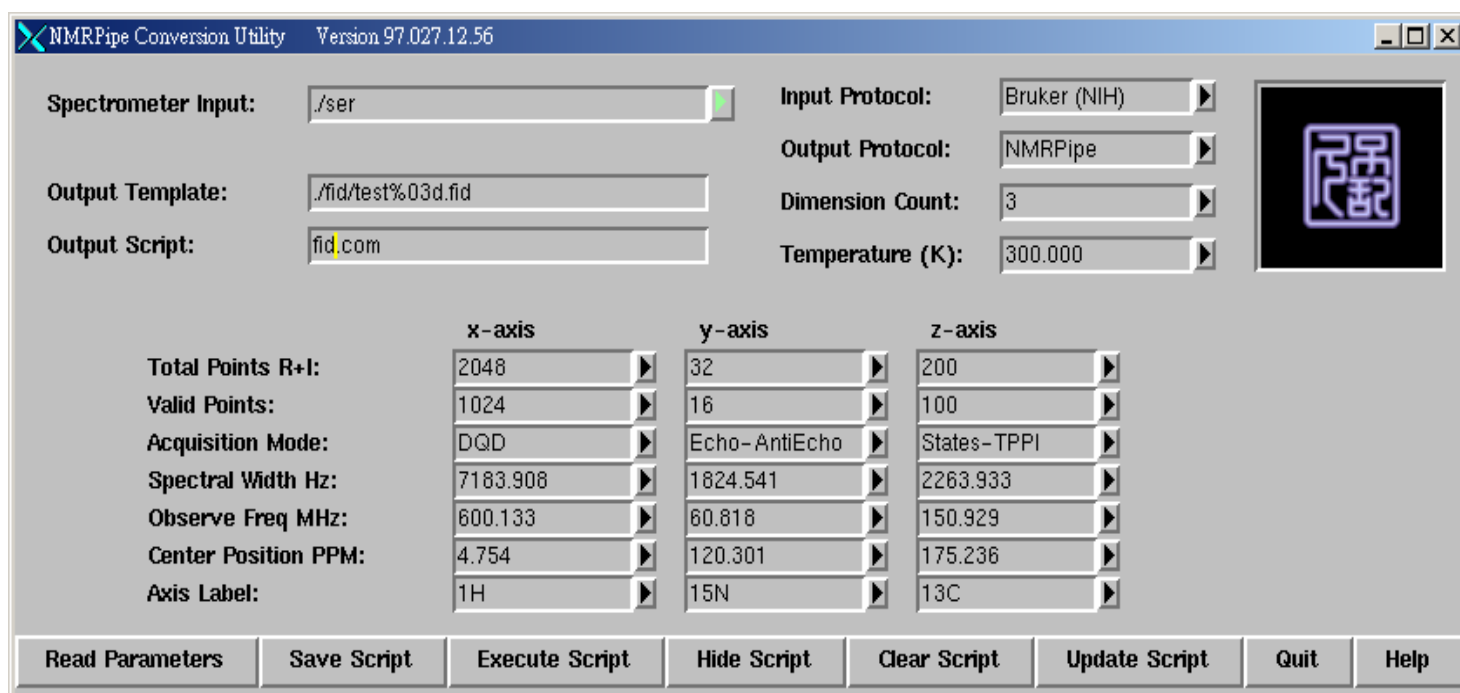


The screenshot shows the NMRPipe Conversion Utility interface with the following parameters:

	x-axis	y-axis	z-axis
Total Points R+I:	2048	200	32
Valid Points:	1024	100	16
Acquisition Mode:	DQD	Complex	Complex
Spectral Width Hz:	7183.908	1824.541	2263.933
Observe Freq MHz:	600.133	150.929	60.818
Center Position PPM:	4.754	175.236	120.301
Axis Label:	1H	15N	13C

- Change parameter to the values in figure 2.

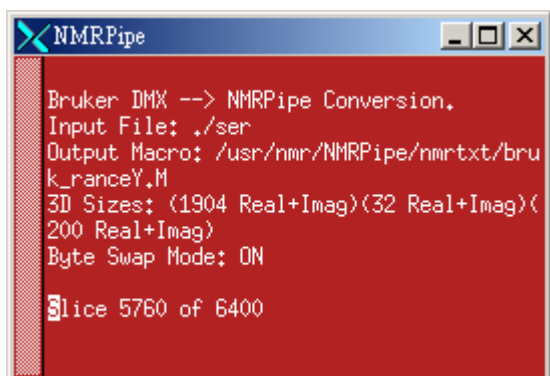
Figure 2:



The screenshot shows the NMRPipe Conversion Utility interface with the following parameters:

	x-axis	y-axis	z-axis
Total Points R+I:	2048	32	200
Valid Points:	1024	16	100
Acquisition Mode:	DQD	Echo-AntiEcho	States-TPPI
Spectral Width Hz:	7183.908	1824.541	2263.933
Observe Freq MHz:	600.133	60.818	150.929
Center Position PPM:	4.754	120.301	175.236
Axis Label:	1H	15N	13C

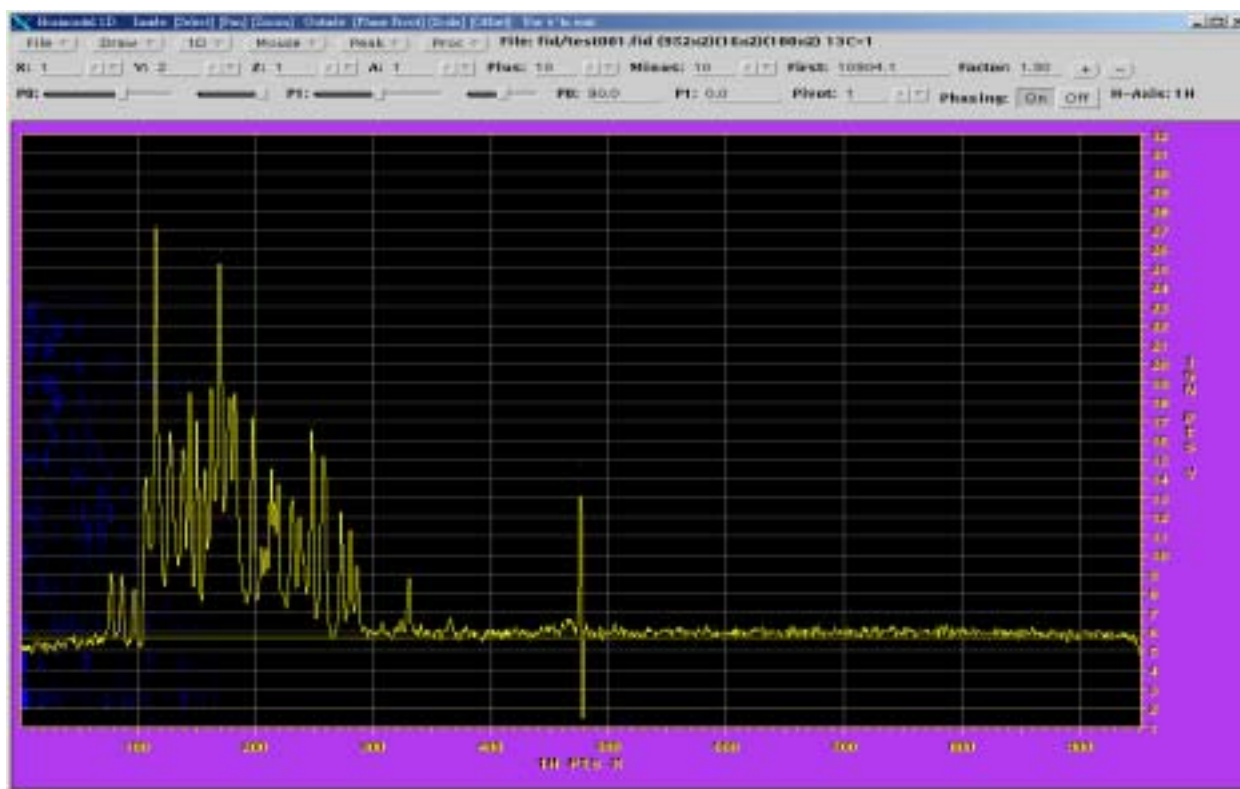
- Clear script and update script, save script and execute script.



- Type in "nmrDraw" to bring up NMRDraw
- Read in test001.fid and read in 1D horizontal and chose Y:2 (the 2nd fid)

(In this experiment the 2nd fid contains bigger signals than the first)
- Proc-auto process 1D.
- Turn the phasing on and phase the spectrum, p1~90 (figure 3)

(Figure 3)



● **ft_xyz.com**

#!/bin/csh

#

3D States-Mode HN-Detected Processing.

```

xyz2pipe -in fid/test%03d.fid -x -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 90 -p1 0.0 -di \
| nmrPipe -fn EXT -x1 10.8ppm -xn 6.0ppm -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -rs 1.7ppm -sw \
#| nmrPipe -fn POLY -auto -ord 0 \
#| nmrPipe -fn TP \
#| nmrPipe -fn POLY -auto \
$| nmrPipe -fn TP \
| pipe2xyz -out ft/B%03d.ft2 -y -ov

```

```

xyz2pipe -in ft/B%03d.ft2 -z -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -ls 0.2ppm -sw \
#| nmrPipe -fn POLY -ord 0 -auto \
| pipe2xyz -out ft/C%03d.ft2 -z -ov

```

● [winston@GOAT 11]\$ ft_xyz.com (execute the script)

XYZ2Pipe Partition: Plane 1 to 200 of 200

FT 6384 of 6400

FT 6400 of 6400

XYZ2Pipe Partition: Plane 1 to 32 of 32

ZX Plane 1 of 32

NMRPipe Fourier Transform Warning, Function 3:

The detection mode seems to require sign alternation.

This requires a Sign-Alternated Fourier Transform.

Consider using the -alt flag.

FT 25968 of 25984

FT 25984 of 25984

● We need to have **-alt** on for the z-axis according to the message above.

Make Z- DIRECTION, FT -alt

Ft_xyz_alt.com

#!/bin/csh

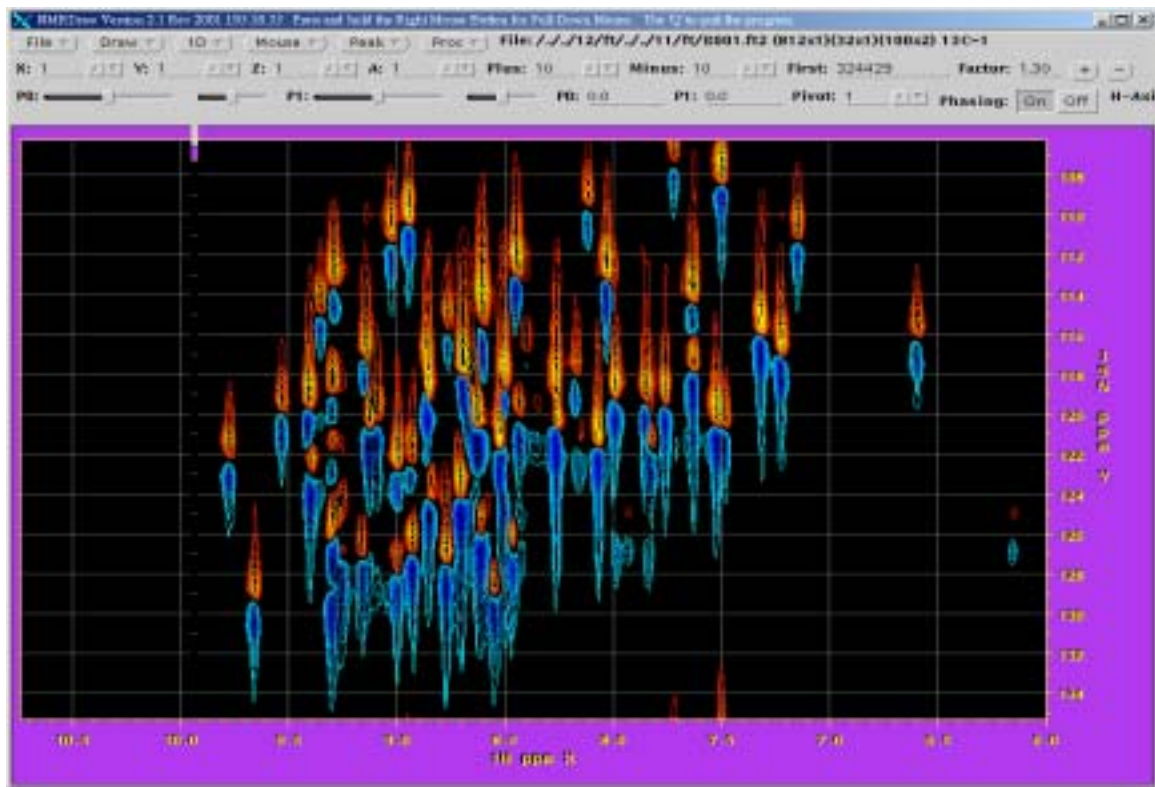
#

3D States-Mode HN-Detected Processing.

```
xyz2pipe -in fid/test%03d.fid -x -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 90 -p1 0.0 -di \
| nmrPipe -fn EXT -x1 10.8ppm -xn 6.0ppm -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -rs 1.7ppm -sw \
#| nmrPipe -fn POLY -auto -ord 0 \
#| nmrPipe -fn TP \
#| nmrPipe -fn POLY -auto \
#| nmrPipe -fn TP \
| pipe2xyz -out ft/B%03d.ft2 -y -ov
```

```
xyz2pipe -in ft/B%03d.ft2 -z -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -alt -verb \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -ls 0.2ppm -sw \
#| nmrPipe -fn POLY -ord 0 -auto \
| pipe2xyz -out ft/C%03d.ft2 -z -ov
```

- Check ft/B001.ft2, and 1D vertical to phase 15N.



- phase N15 vertical: p1=-87

● ft_xyz_alt.com

```
#!/bin/csh
#
# 3D States-Mode HN-Detected Processing.
xyz2pipe -in fid/test%03d.fid -x -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 90 -p1 0.0 -di \
| nmrPipe -fn EXT -x1 10.8ppm -xn 6.0ppm -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 -87 -p1 0.0 -di \
#| nmrPipe -fn CS -rs 1.7ppm -sw \
#| nmrPipe -fn POLY -auto -ord 0 \
```

```

#| nmrPipe -fn TP \
#| nmrPipe -fn POLY -auto \
#| nmrPipe -fn TP \
| pipe2xyz -out ft/B%03d.ft2 -y -ov

xyz2pipe -in ft/B%03d.ft2 -z -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -alt -verb \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -ls 0.2ppm -sw \
#| nmrPipe -fn POLY -ord 0 -auto \
| pipe2xyz -out ft/C%03d.ft2 -z -ov

```

● ft_xyz_althc.com

```

#!/bin/csh
#
# 3D States-Mode HN-Detected Processing.
xyz2pipe -in fid/test%03d.fid -x -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 90 -p1 0.0 -di \
| nmrPipe -fn EXT -x1 10.8ppm -xn 6.0ppm -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 -87 -p1 0.0 -di \
#| nmrPipe -fn CS -rs 1.7ppm -sw \
#| nmrPipe -fn POLY -auto -ord 0 \
#| nmrPipe -fn TP \
#| nmrPipe -fn POLY -auto \
#| nmrPipe -fn TP \
| pipe2xyz -out ft/B%03d.ft2 -y -ov
xyz2pipe -in ft/B%03d.ft2 -z -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \

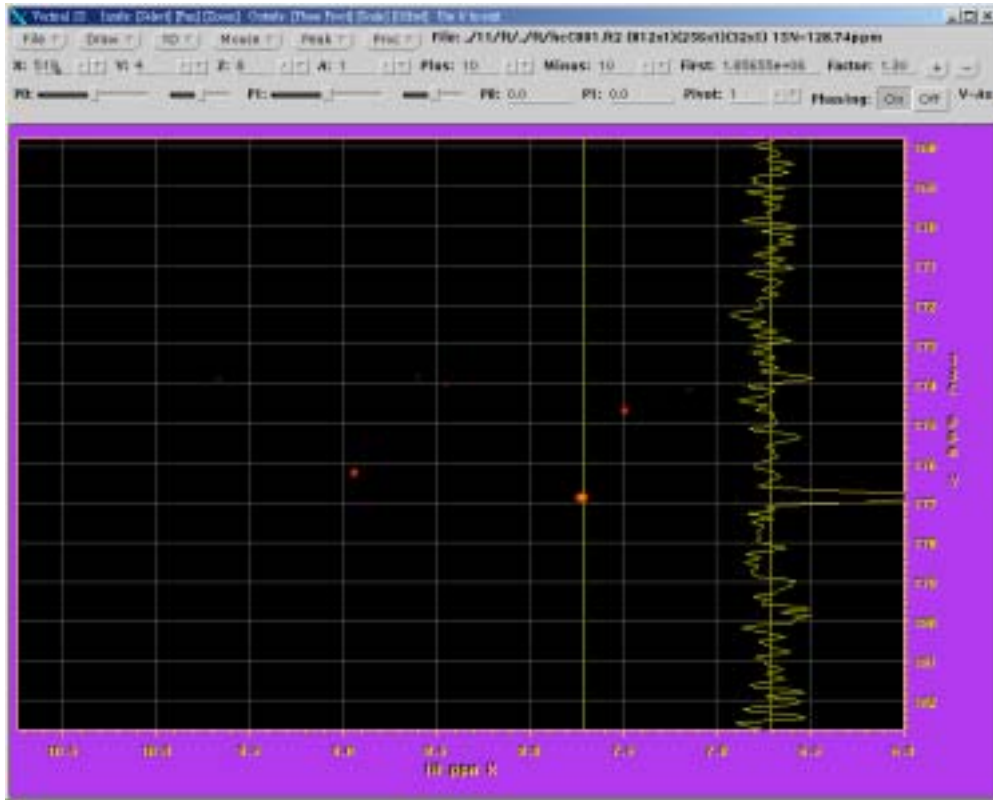
```

```

| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -alt -verb \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
#| nmrPipe -fn CS -ls 0.2ppm -sw \
#| nmrPipe -fn POLY -ord 0 -auto \
| pipe2xyz -out ft/hcC%03d.ft2 -y -ov (we have xyz=1H, 13C, 15N now).

```

- Under NMRDraw, read in hcC001.ft2 and phase the 13C.



- need p1=180 for 13C.
- now change edit ft_xyz_alt.com to ft_xyz_alt_ph.com and added 13C p1=180
- **ft_xyz_alt_ph.com** (our final script!, type it in to execute it.)

```
#!/bin/csh
```

```
#
```

```
# 3D States-Mode HN-Detected Processing.
```

```

xyz2pipe -in fid/test%03d.fid -x -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \

```

```

| nmrPipe -fn FT -verb \
| nmrPipe -fn PS -p0 90 -p1 0.0 -di \
| nmrPipe -fn EXT -x1 10.8ppm -xn 6.0ppm -sw \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 -87 -p1 0.0 -di \
#| nmrPipe -fn CS -rs 1.7ppm -sw \
#| nmrPipe -fn POLY -auto -ord 0 \
#| nmrPipe -fn TP \
#| nmrPipe -fn POLY -auto \
#| nmrPipe -fn TP \
| pipe2xyz -out ft/B%03d.ft2 -y -ov

```

```

xyz2pipe -in ft/B%03d.ft2 -z -verb \
| nmrPipe -fn SP -off 0.5 -end 0.95 -pow 1 -c 0.5 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -alt -verb \
| nmrPipe -fn PS -p0 180.0 -p1 0.0 -di \
#| nmrPipe -fn CS -ls 0.2ppm -sw \
#| nmrPipe -fn POLY -ord 0 -auto \
| pipe2xyz -out ft/C%03d.ft2 -z -ov

```

- xyz2pipe -in C%03d.ft2 > hnc0_pipe (pack all 2D planes into a single 3D file).
- pipe2ucsf hnc0_pipe lbd_hnc0.ucsf (change to sparky format)

Sparky window:

