Basic NMR Concepts

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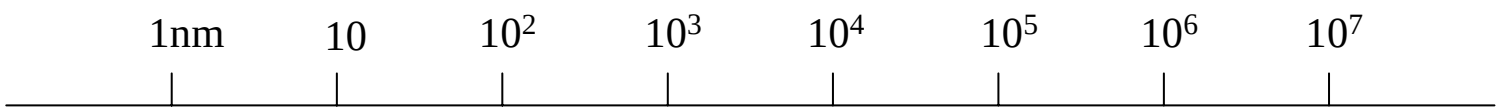
NMR Spectroscopy

Where is it?



(the wave)	X-ray	UV/VIS	Infrared	Microwave	Radio Frequency
(the transition)	Electronic Transition		Vibration	Rotation	Nuclear
(spectrometer)	**X-ray**	**UV/VIS**	**Infrared/Raman**		**NMR**
			Fluorescence		

Before using NMR

What's N, M, and R?

Properties of the Nucleus

Nuclear spin

Nuclear magnetic moments

The Nucleus in a Magnetic Field

Precession and the Larmor frequency

Nuclear Zeeman effect & Boltzmann distribution

When the Nucleus Meet the right Magnet

Nuclear Magnetic Resonance

◆ Properties of the Nucleus

Nuclear spin

• Nuclear spin is the total nuclear angular momentum quantum number. This is characterized by a quantum number I, which may be integral, half-integral or 0.

• Only nuclei with spin number $I \neq 0$ can absorb/emit electromagnetic radiation. The magnetic quantum number m_I has values of –I, -I+1, …..+I .
(e.g. for I=3/2, m_I=-3/2, -1/2, 1/2, 3/2)

1. A nucleus with an even mass A and even charge Z → nuclear spin I is zero

Example: ^{12}C, ^{16}O, ^{32}S → No NMR signal

2. A nucleus with an even mass A and odd charge Z → integer value I

Example: ^{2}H, ^{10}B, ^{14}N → NMR detectable

3. A nucleus with odd mass A → I=n/2, where n is an odd integer

Example: ^{1}H, ^{13}C, ^{15}N, ^{31}P → NMR detectable

Nuclear magnetic moments

Magnetic moment μ is another important parameter for a nuclei

$$\mu = \gamma I (h/2\pi)$$

I: spin number

h: Plank constant 6.626*10^{-34} joul-sec

γ: gyromagnetic ratio (property of a nuclei)

1H: I=1/2 , γ = 267.512 *10^{6} rad T^{-1}S^{-1}

13C: I=1/2 , γ = 67.264*10^{6}

15N: I=1/2 , γ = 27.107*10^{6}

◆ The Nucleus in a Magnetic Field

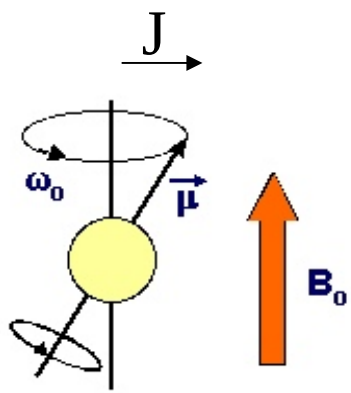
Precession and the Larmor frequency

• The magnetic moment of a spinning nucleus processes with a characteristic angular frequency called the Larmor frequency ω, which is a function of *r* and B_0

Remember $\mu = \gamma I (h/2\pi)$?

Angular momentum $dJ/dt = \mu \times B_0$

Larmor frequency $\omega = rB_0$



Linear precession frequency $\nu = \omega/2\pi = rB_0/2\pi$

Example: At what field strength do ^{1}H process at a frequency of 600.13MHz? What would be the process frequency for ^{13}C at the same field?

Nuclear Zeeman effect

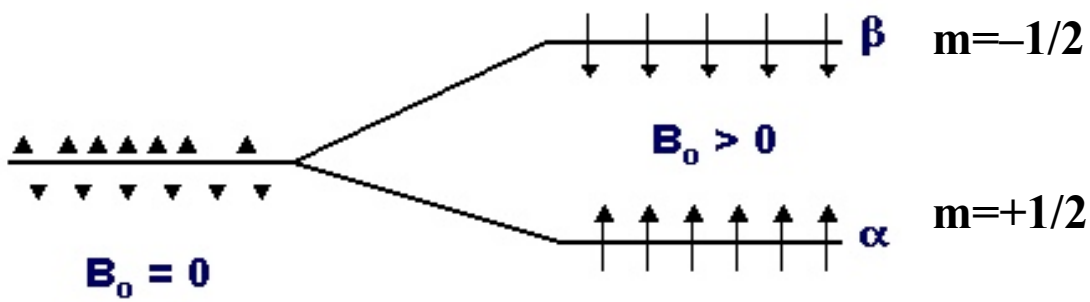
• **Zeeman effect: when an atom is placed in an external magnetic field, the energy levels of the atom are split into several states.**

• **The energy of a give spin sate (E_i) is directly proportional to the value of m_I and the magnetic field strength B_0**

Spin State Energy $E_I = -\mu . B_0 = -m_I B_0 r(h/2\pi)$

• **Notice that, the difference in energy will always be an integer multiple of $B_0 r(h/2\pi)$. For a nucleus with I=1/2, the energy difference between two states is**

$$\Delta E = E_{-1/2} - E_{+1/2} = B_0 r(h/2\pi)$$



The Zeeman splitting is proportional to the strength of the magnetic field

Boltzmann distribution

• **Quantum mechanics tells us that, for net absorption of radiation to occur, there must be more particles in the lower-energy state than in the higher one. If no net absorption is possible, a condition called saturation.**

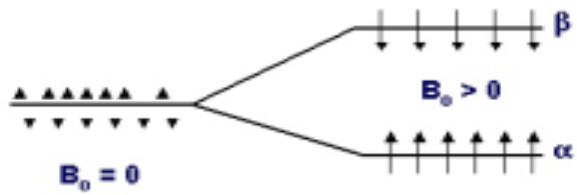
• **When it's saturated, Boltzmann distribution comes to rescue:**

$$P_{m=-1/2} / P_{m=+1/2} = e^{-\Delta E/kT}$$

where P is the fraction of the particle population in each state,

T is the absolute temperature,

k is Boltzmann constant $1.381*10^{-28}$ JK^{-1}



Example: At 298K, what fraction of ^1H nuclei in 2.35 T field are in the upper and lower states?

(m=-1/2 : 0.4999959 ; m=1/2 : 0.5000041)

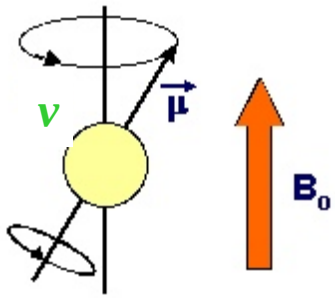
• **The difference in populations of the two states is only on the order of few parts per million. However, this difference is sufficient to generate NMR signal.**

• **Anything that increases the population difference will give rise to a more intense NMR signal.**

◆ When the Nucleus Meet the Magnet

Nuclear Magnetic Resonance

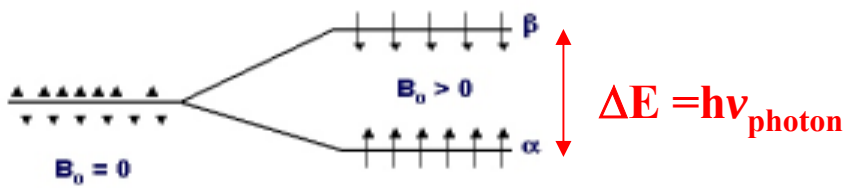
•For a particle to absorb a photon of electromagnetic radiation, the particle must first be in some sort of uniform periodic motion



• If the particle "uniformly periodic moves" (i.e. precession) at $\nu_{precession,}$ and absorb erengy. The energy is $E=h\nu_{precession}$

•For I=1/2 nuclei in B_0 field, the energy gap between two spin states:

$$\Delta E = rhB_0/2\pi$$

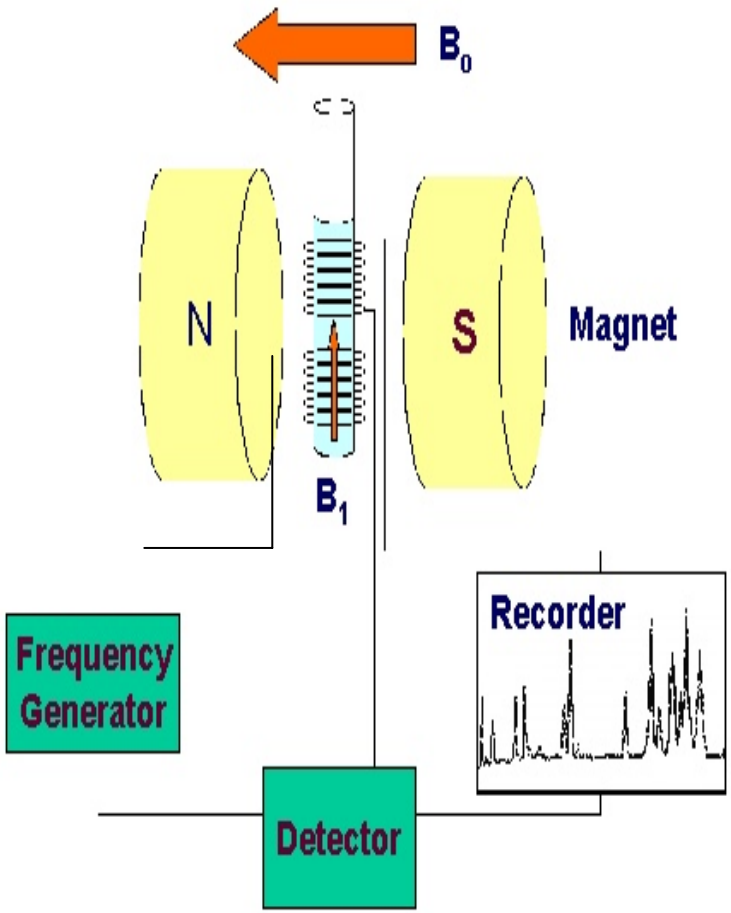


• The radiation frequency must exactly match the precession frequency

$$E_{photon}=h\nu_{precession}=h\nu_{photon}=\Delta E=rhB_0/2\pi$$

→This is the so called " Nuclear Magnetic RESONANCE"!!!!!!!!

Nuclear Magnetic Resonance Spectrometer
How to generate signals?

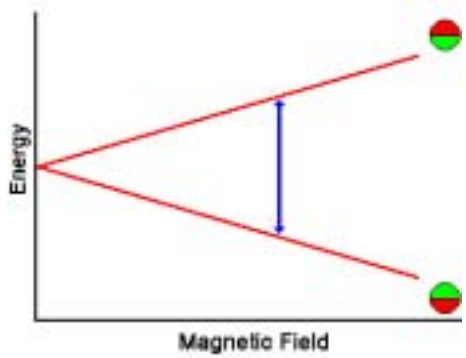


B_0：光譜儀之磁場強度

B_1：外加小磁場
(來自樣品周圍之線圈)

◆ Magnet B_0 and irradiation energy B_1

B_0 (the magnet of machine)



(1) Provide energy for the nuclei to spin

$E_i=-m_iB_0\,(rh/2\pi)$

Larmor frequency $\omega=rB_0$

(2) Induce energy level separation (Boltzmann distribution)

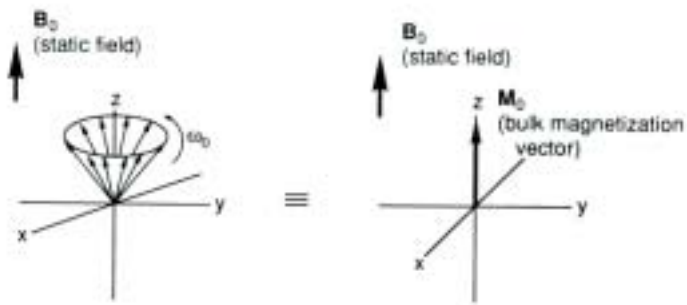
The stronger the magnetic field B_0, the greater separation between different nuclei in the spectra

$$\Delta\nu=\nu_1-\nu_2=(r_1-r_2)B_0/2\pi$$

(3) The nuclei in both spin states are randomly oriented around the z axis.

$$M_z=M,\ M_{xy}=0$$

(where M is the net nuclear magnetization)

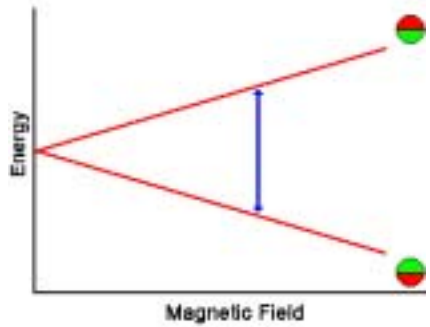


B_1 (the irradiation magnet, current induced)

(1) Induce energy for nuclei to absorb, but still spin at ω or $\nu_{precession}$

$$E_{photon}=h\nu_{photon}=\Delta E=rhB_0/2\pi=h\nu_{precession}$$

And now, the spin jump to the higher energy (from m=1/2→m= – 1/2)



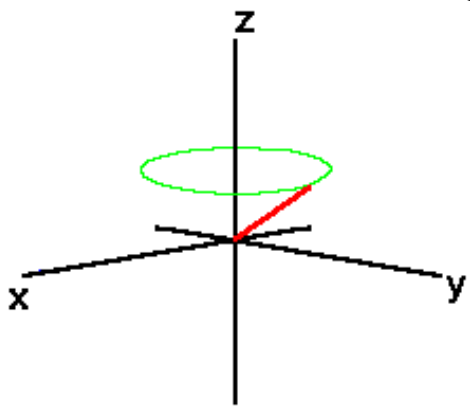
m= –1/2

m= 1/2

(2) All of the individual nuclear magnetic moments become phase coherent, and the net M process around the z axis at α angel

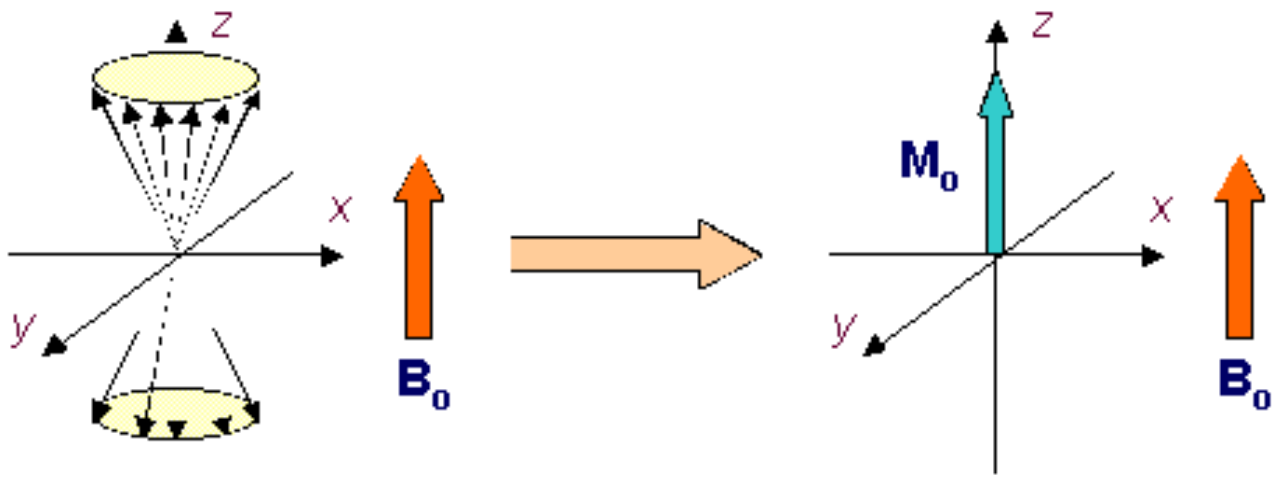
$$M_z=M\cos\alpha$$

$$M_{xy}=M\sin\alpha.$$



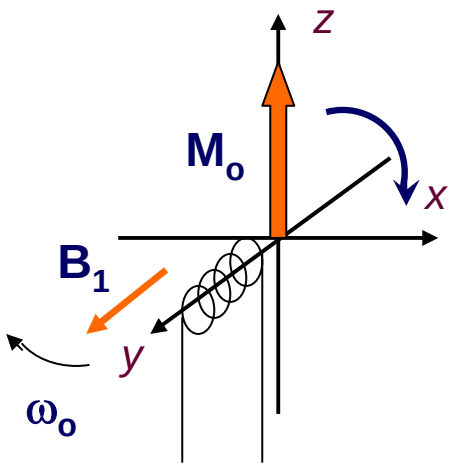
What happen before irradiation

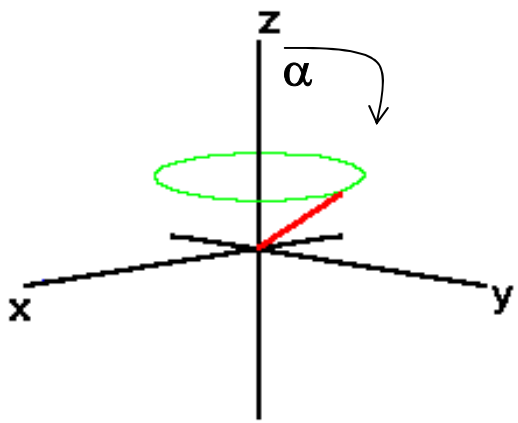
• **Before irradiation, the nuclei in both spin states are processing with characteristic frequency, but they are completely out of phase, i.e., randomly oriented around the z axis. The net nuclear magnetization M is aligned statically along the z axis ($M=M_z$, $M_{xy}=0$)**



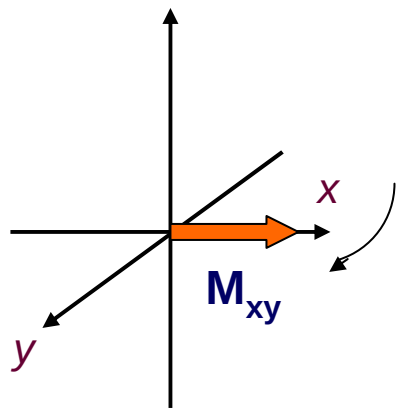
What happen during irradiation

When irradiation begins, all of the individual nuclear magnetic moments become phase coherent, and this phase coherence forces the net magnetization vector M to process around the z axis. As such, M has a component in the x, y plan, $M_{xy}=M\sin\alpha$. α is the tip angle which is determined by the power and duration of the electromagnetic irradiation.





α deg pulse



90 deg pulse

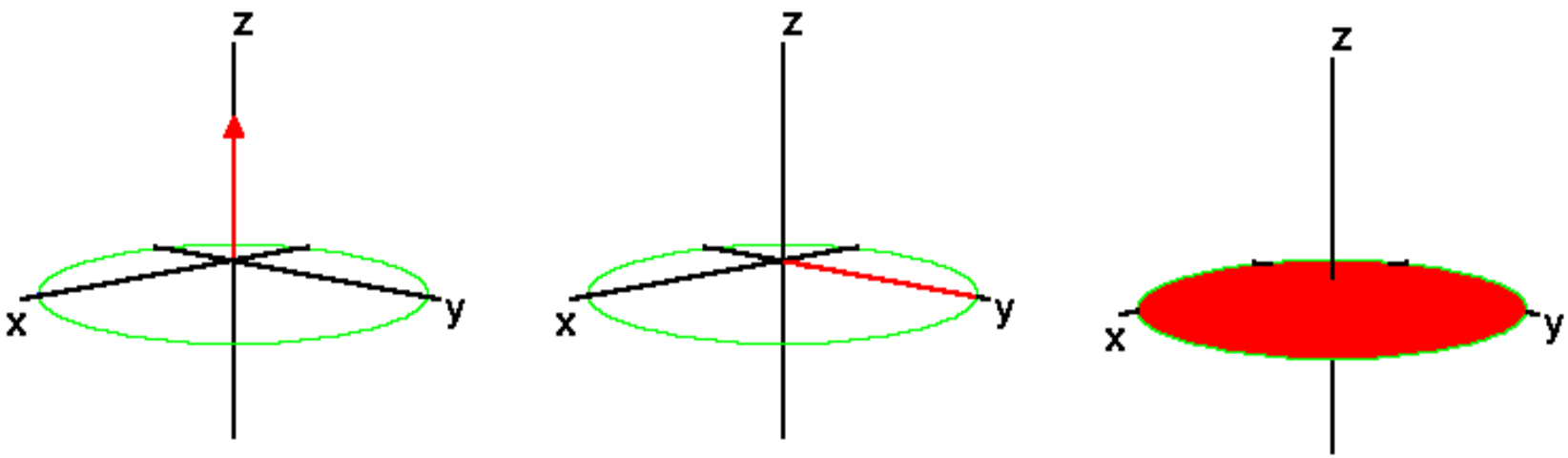
What happen after irradiation ceases

•After irradiation ceases, not only do the population of the states revert to a Boltzmann distribution, but also the individual nuclear magnetic moments begin to lose their phase coherence and return to a random arrangement around the z axis.

(NMR 的光譜其實就是在紀錄這個過程!!)

•This process is called “relaxation process” (弛緩現象)

•There are two types of relaxation process : T1(spin-lattice relaxation) & T2(spin-spin relaxation)



T1 (the spin lattice relaxation)

• How long after immersion in a external field does it take for a collection of nuclei to reach Boltzmann distribution is controlled by T1, the spin lattice relaxation time.

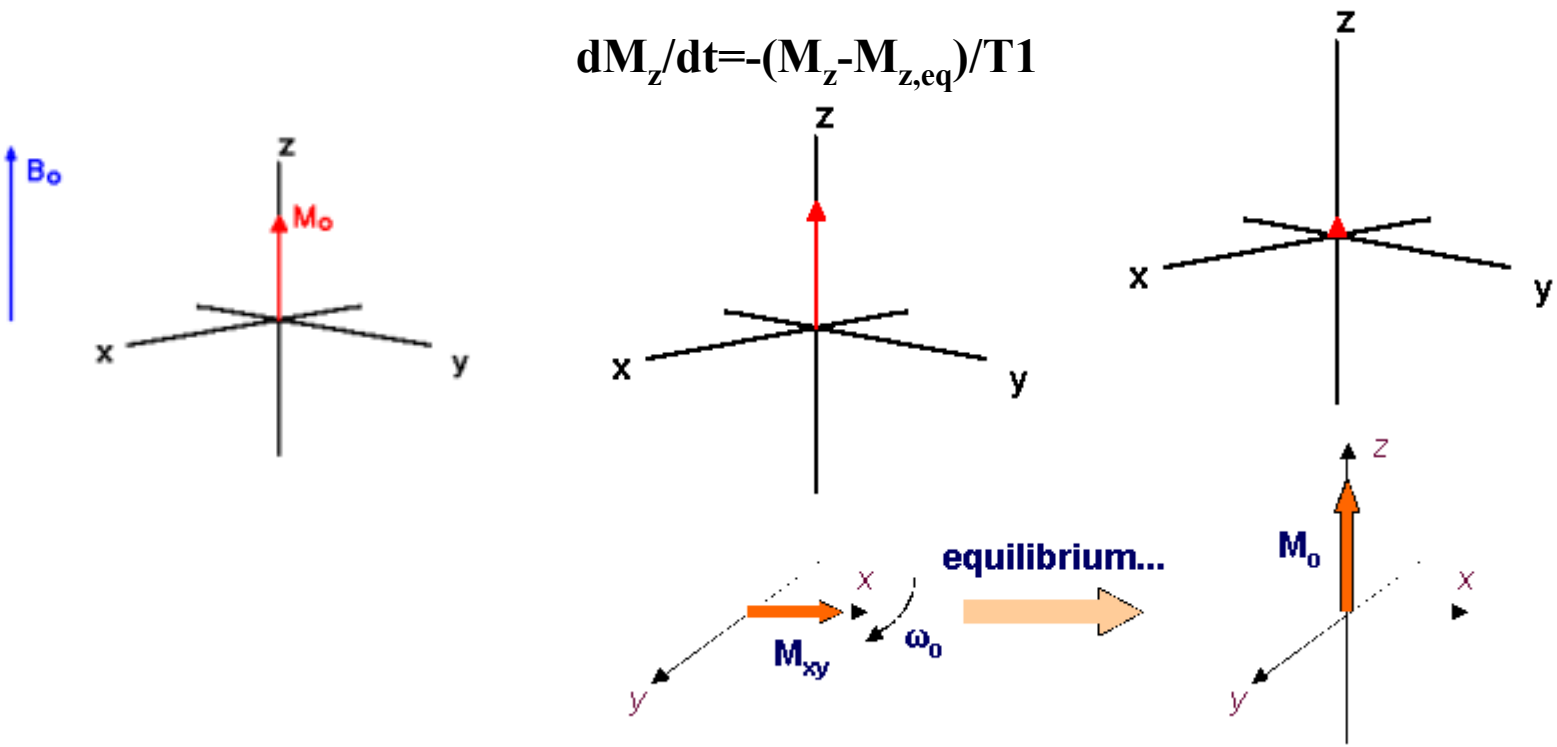
(考慮波茲曼分布的效應為主)

•Lost of energy in system to surrounding (lattice) as heat

(能量釋放的過程)

•It's a time dependence exponential decay process of Mz components

$$dM_z/dt=-(M_z-M_{z,eq})/T1$$



T2 (the spin –spin relaxation)

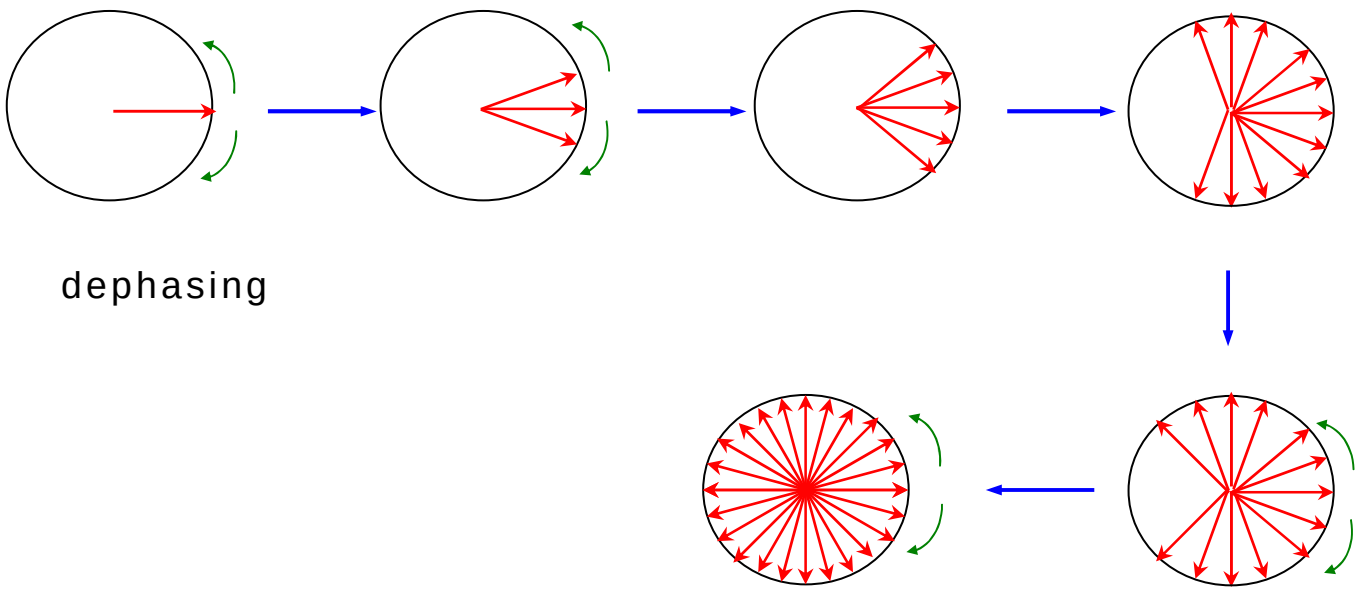
•This process for nuclei begin to lose their phase coherence and return to a random arrangement around the z axis is called spin-spin relaxation.

(考慮自旋方位由同一方向又回到 random 的過程)

•The decay of M_{xy} is at a rate controlled by the spin-spin relaxation time T2.

$dM_x/dt=-M_x/T2$

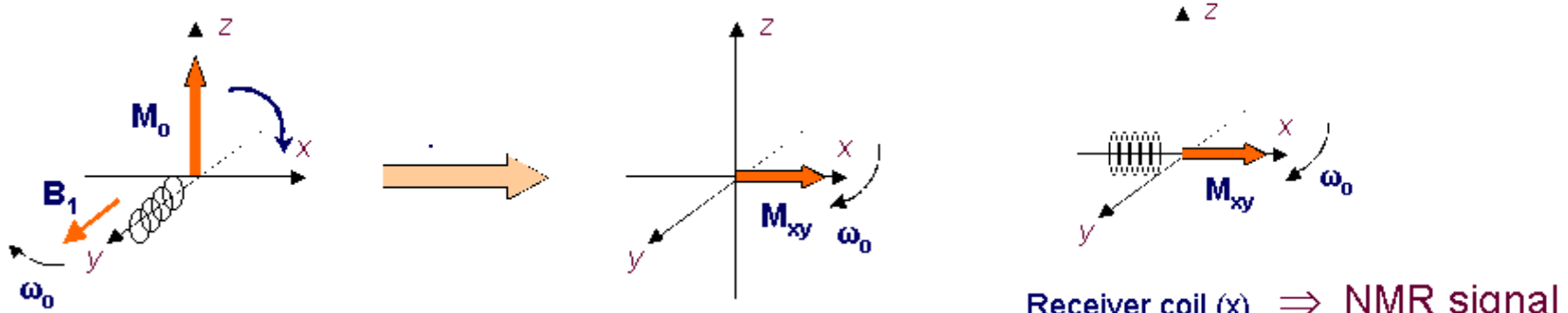
$dM_y/dt=-M_y/T2$

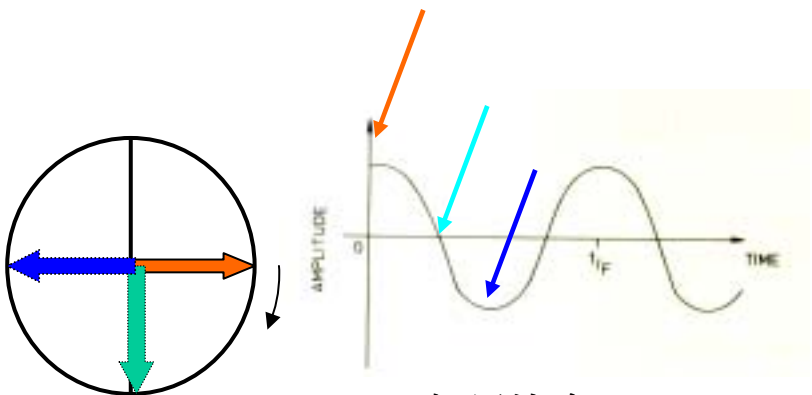


◆ Collecting NMR signals

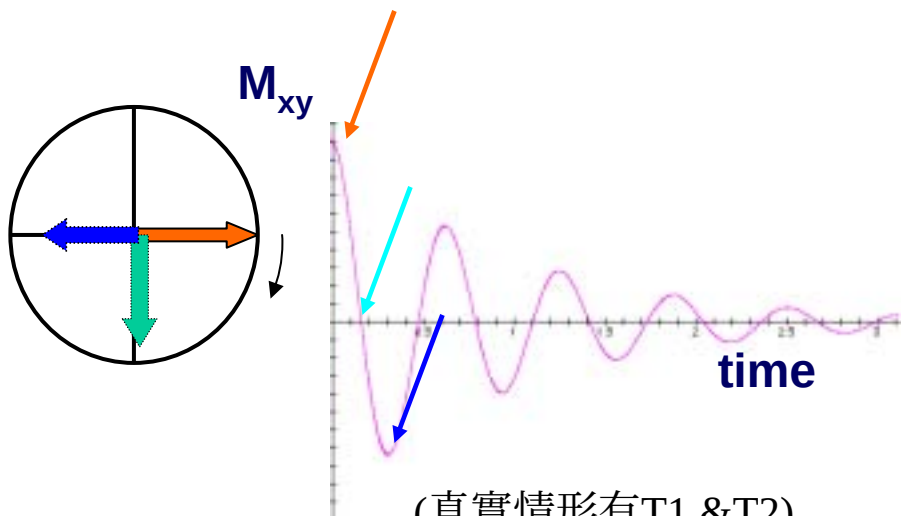
•The detection of NMR signal is on the xy plane. The oscillation of Mxy generate a current in a coil , which is the NMR signal.

•Due to the "relaxation process", the time dependent spectrum of nuclei can be obtained. This time dependent spectrum is called "free induction decay" (FID)



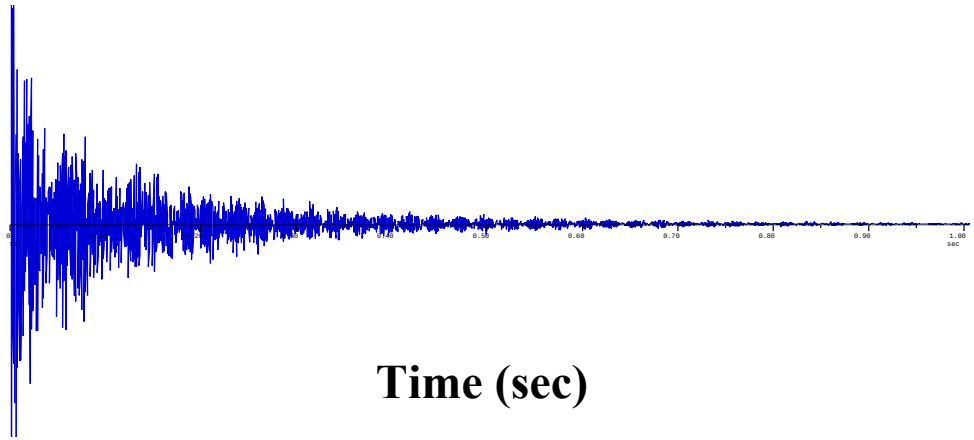


(如果沒有 relaxation)



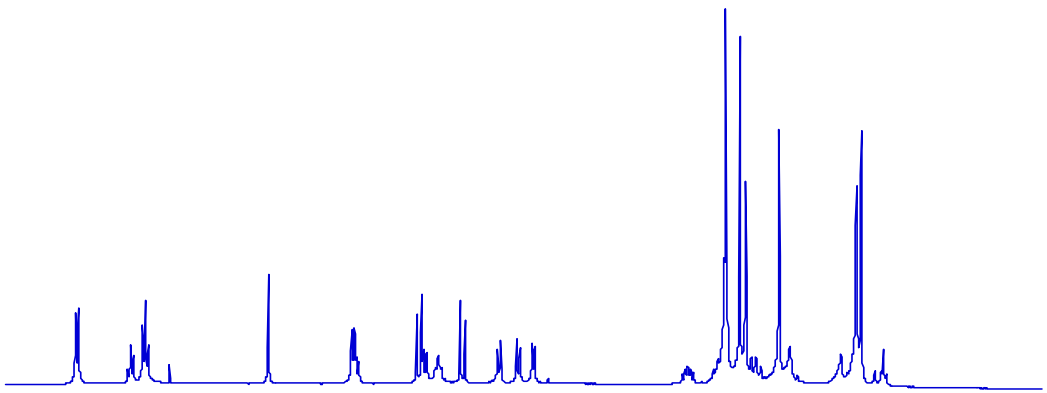
(真實情形有T1 &T2)

•**In addition, most molecules examined by NMR have several sets of nuclei, each with a different precession frequency.**



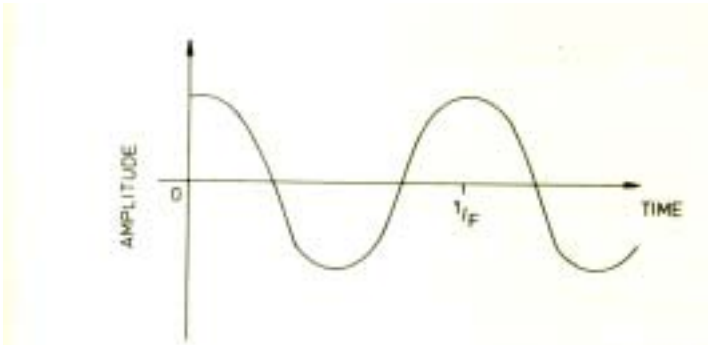
Time (sec)

•**The FID (free induction decay) is then Fourier transform to frequency domain to obtain each $\nu_{pression}$ (chemical shift) for different nuclei.**

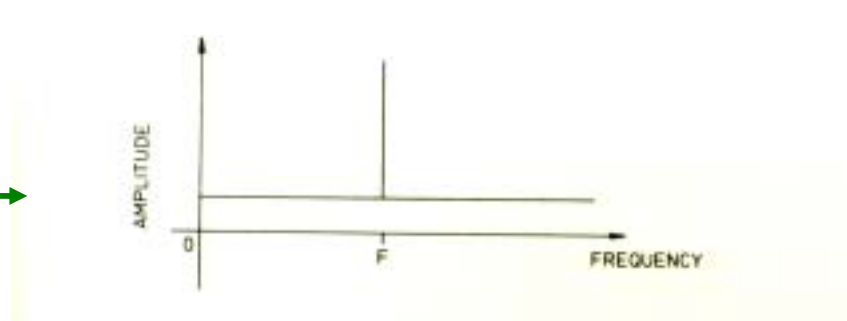


frequency (Hz)

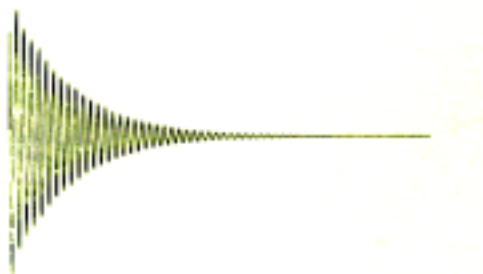
Fourier transformation (FT)



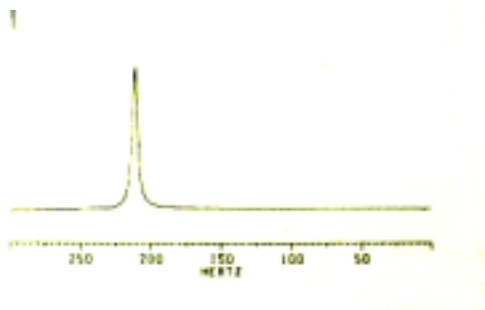
FT



$$f(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt = \int_{-\infty}^{+\infty} f(t)[\cos(\omega t) - i \sin(\omega t)] dt$$



FT



NMR signals

• We have immersed our collection of nuclei in a magnetic field, each is processing with a characteristic frequency, To observe resonance, all we have to do is irradiate them with electromagnetic radiation of the appropriate frequency.

(想要觀察核磁共振的現象只須提供適當的能量)

•It's easy to understand that different nucleus "type" will give different NMR signal.

(記得 $\nu = \omega/2\pi = \gamma B_0/2\pi$? 所以不同的 γ 就有不同的 ν !!)

•However, it is very important to know that for same "nucleus type", but "different nucleus" could generate different signal. This is also what make NMR useful and interesting.

•Depending on the *chemical environment*, there are variations on the magnetic field that the nuclei feels, even for the same type of nuclei.

•The main reason for this is, each nuclei could be surrounded by different electron environment, which make the nuclei "feel" different net magnetic field , B_{effect}

•**Electron surrounding each nucleus in a molecule serves to shield that nucleus from the applied magnetic field. This shielding effect cause the ΔE difference, thus, different *v* will be obtained in the spectrum**

$B_{eff}=B_0-B_i$ **where B_i induced by cloud electron**

$B_i = \sigma B_0$ **where σ is the shielding constant**

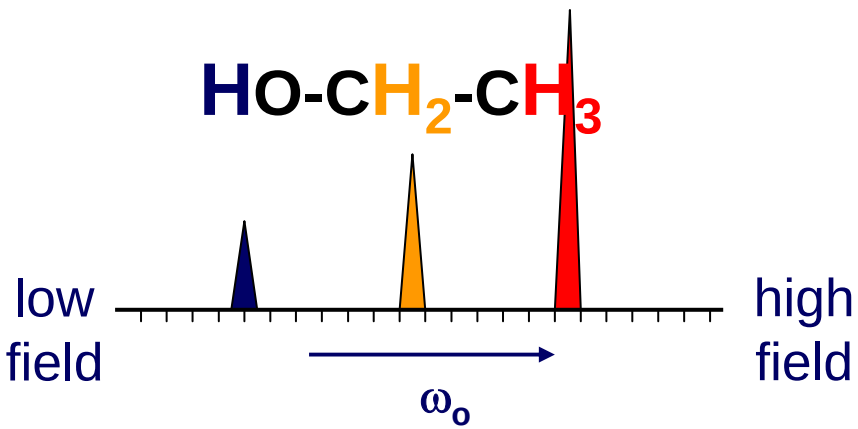
$B_{eff}=(1-\sigma)\ B_0$

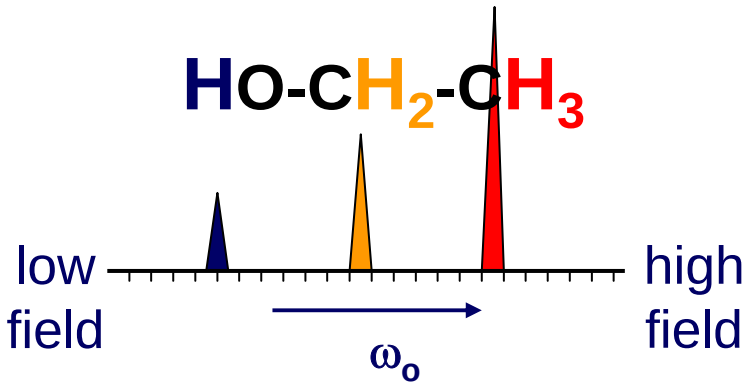
$v_{precession}= (rB_0/2\pi)\ (1-\sigma)$

σ=0 → naked nuclei

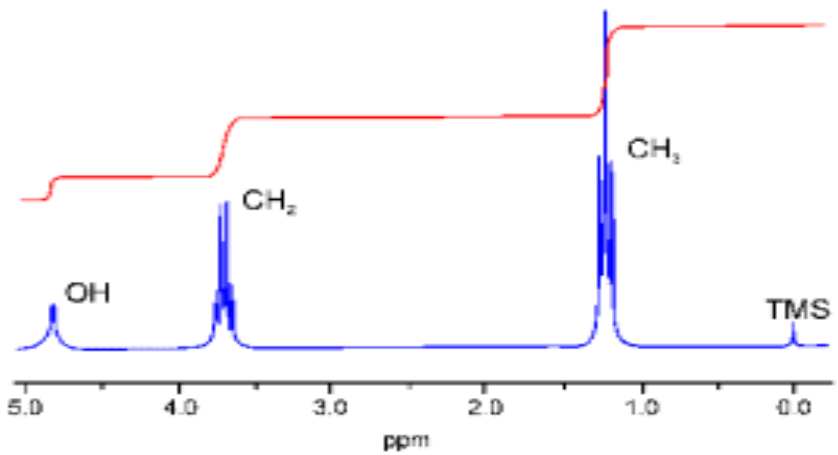
σ >0 → nuclei is shielded by electron cloud

σ <0 → electron around this nuclei is withdraw , i.e. deshielded





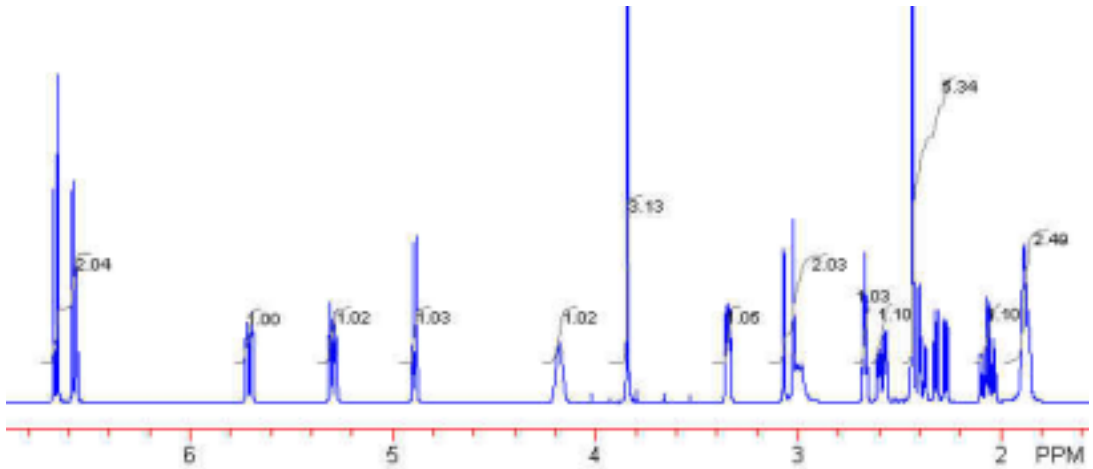
$\omega_0 = rB_{effect}$

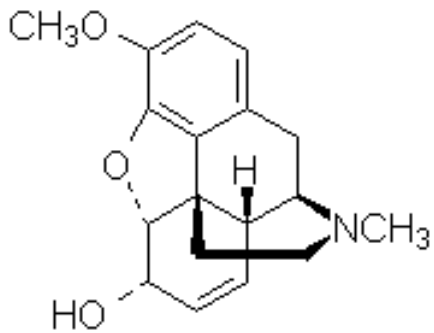
- 

Ref: Some figures copy from the web page by Guillermo Moyna, University of the Sciences in Philadelphia

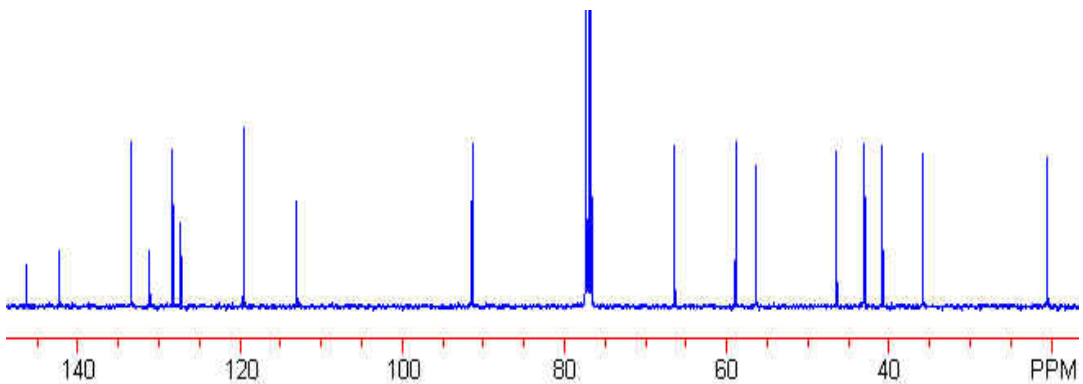
•**Example of 1D : 1H spectra, 13C spectra of Codeine $C_{18}H_{21}NO_3$, MW= 299.4**

1H

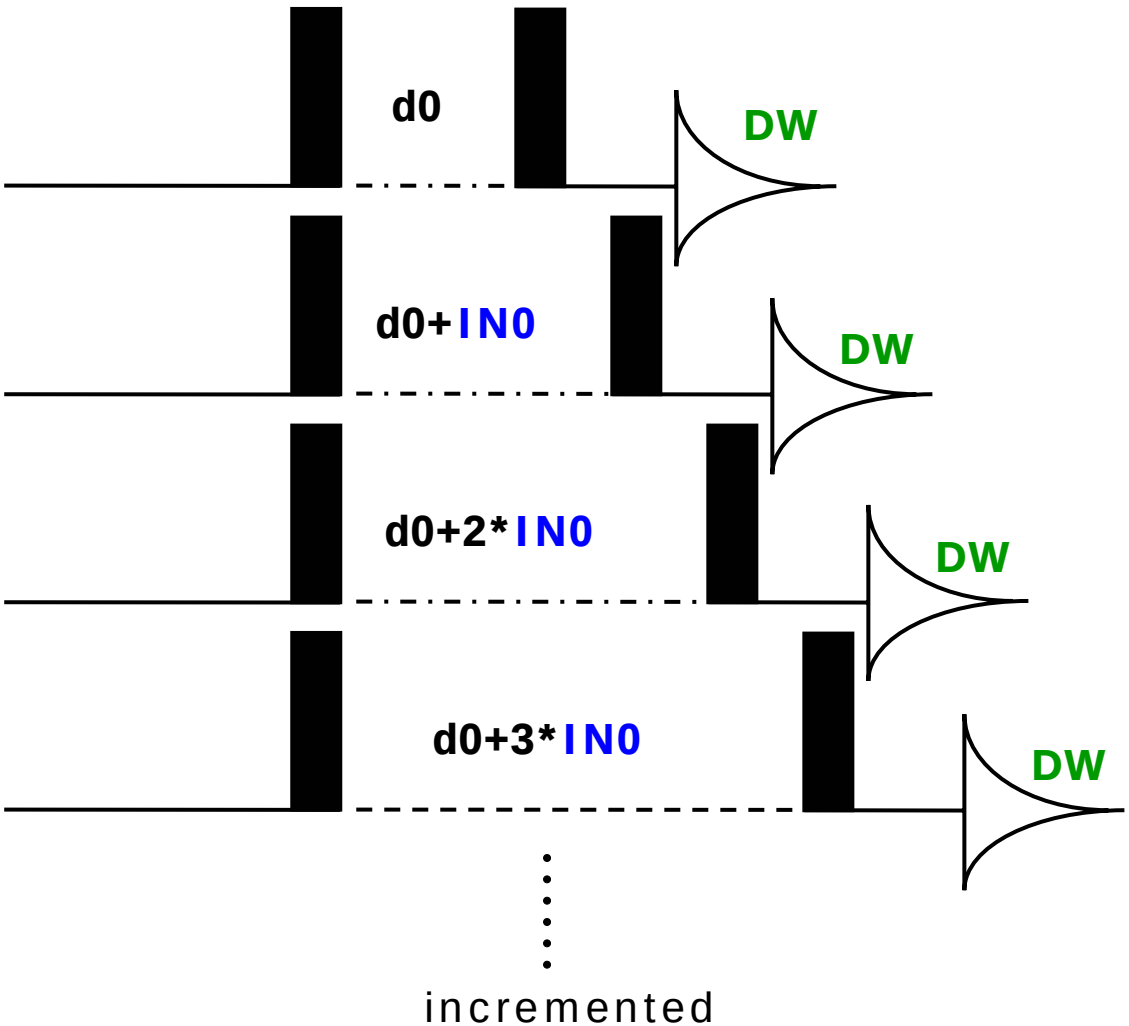




13C



•**Homo nuclear 2D NMR : a series of 1D**



F(t2) FT F(w2)

SW (f2) = 1/(2*DW)

F(t1) FT F(w1)

SW (f1) = 1/(2*IN0)

Set up 1D/homo Nuclear 2D

(Bruker AV system)

Before go into NMR LAB

1. **What's the nucleus? → Which Prohead to choose?**

   AV500 in IBMS : 5mm TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient) only

   AV600 in IBMS: 5mm QXI-Z ($^{1}H/^{15}N/^{13}C/^{31}P$) (not ready to use yet)

   AV600 in CHEM: 5mm BBO and TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient)

   DRX600 in IBMS: 5mm TXI-XYZ ($^{1}H/^{15}N/^{13}C$, with XYZ gradient) and others

   5mm : ^{1}H , $^{1}H/^{19}F$, BBO, TXI($^{1}H/^{15}N/^{13}C$) , TXI-Z ($^{1}H/^{3}C/^{31}P$)

   8mm : TXI ($^{1}H/^{13}C/^{15}N$) 8mm with Z gradient

   10mm: ^{1}H , $^{1}H/^{19}F$, BBO

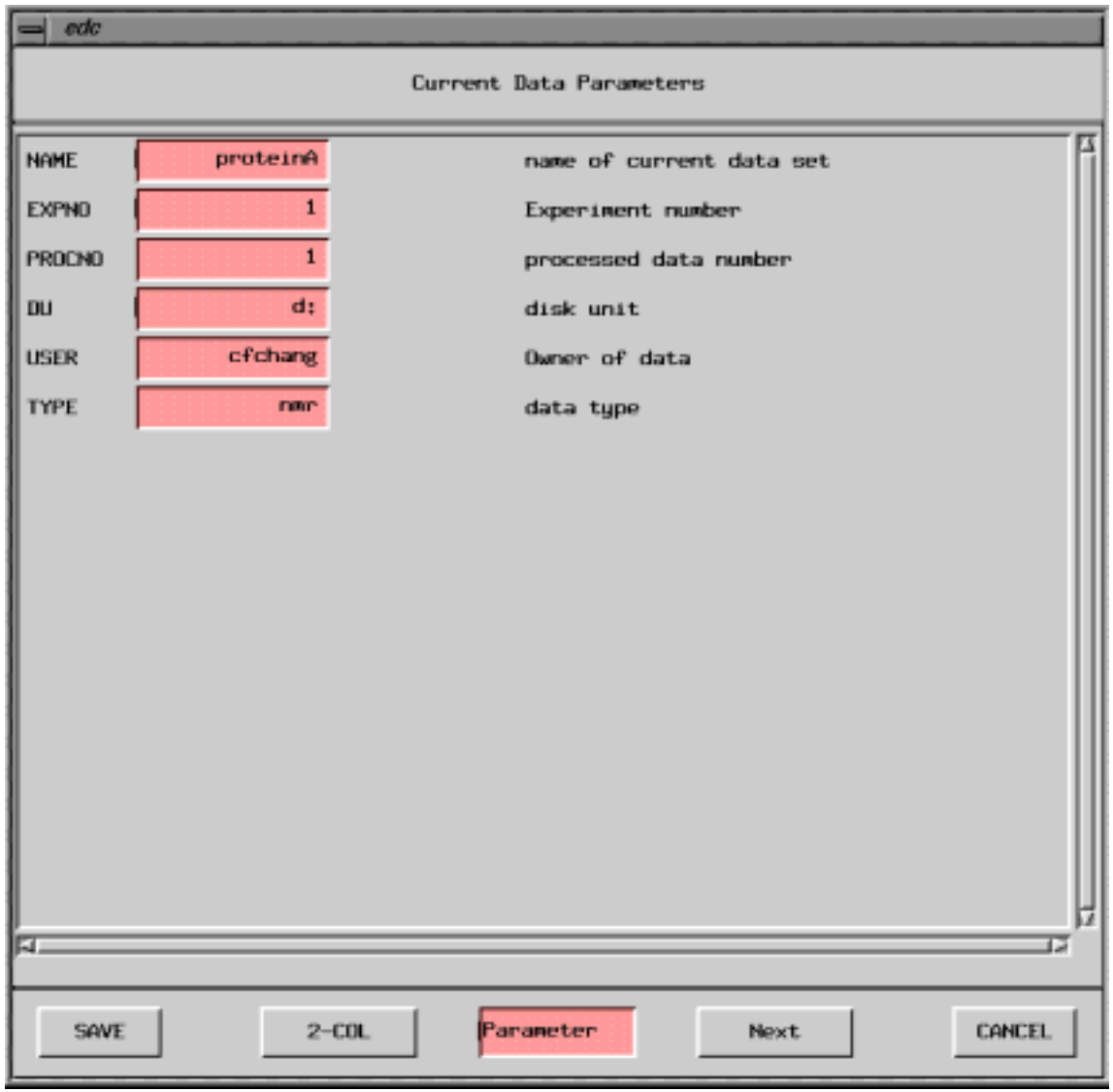
2. **What's the goal? → Which type of experiment you need?**
3. **Which parameter set to choose? → Experiment List**
4. **How to optimize condition? → Experiment Guide**

Definition of some AQ Commands & parameters

edc,new	edit current data set or generate a new data set
eda.ased	edit AQ parameters (eda: shows all, ased: shows required only)
rga	auto optimize rg value
zg	zero memory, and start to collect FID (go)
go	start to collect FID and add signals to the previous memory
stop	stop the active job (just one job)
kill	kill active job (can choose several jobs)
o1.o2,o3	center frequency of the spectrum for nuclear at f1 channel (ex: 1H), f2 channel (ex:13C), and f3 channel (ex:15N)
sw	spectrum width (1 sw : F1 dimension, 2 sw: F2 dimension…….)
td	number of points for FID collection(1 td: F1 dimension, 2 td: F2 dimension….)
d1	relaxation time (usually > 5* T1)
ns	number of scan
ds	dummy scan
rg	receiver gain (usually use the value calculated by rga)

new :New data set

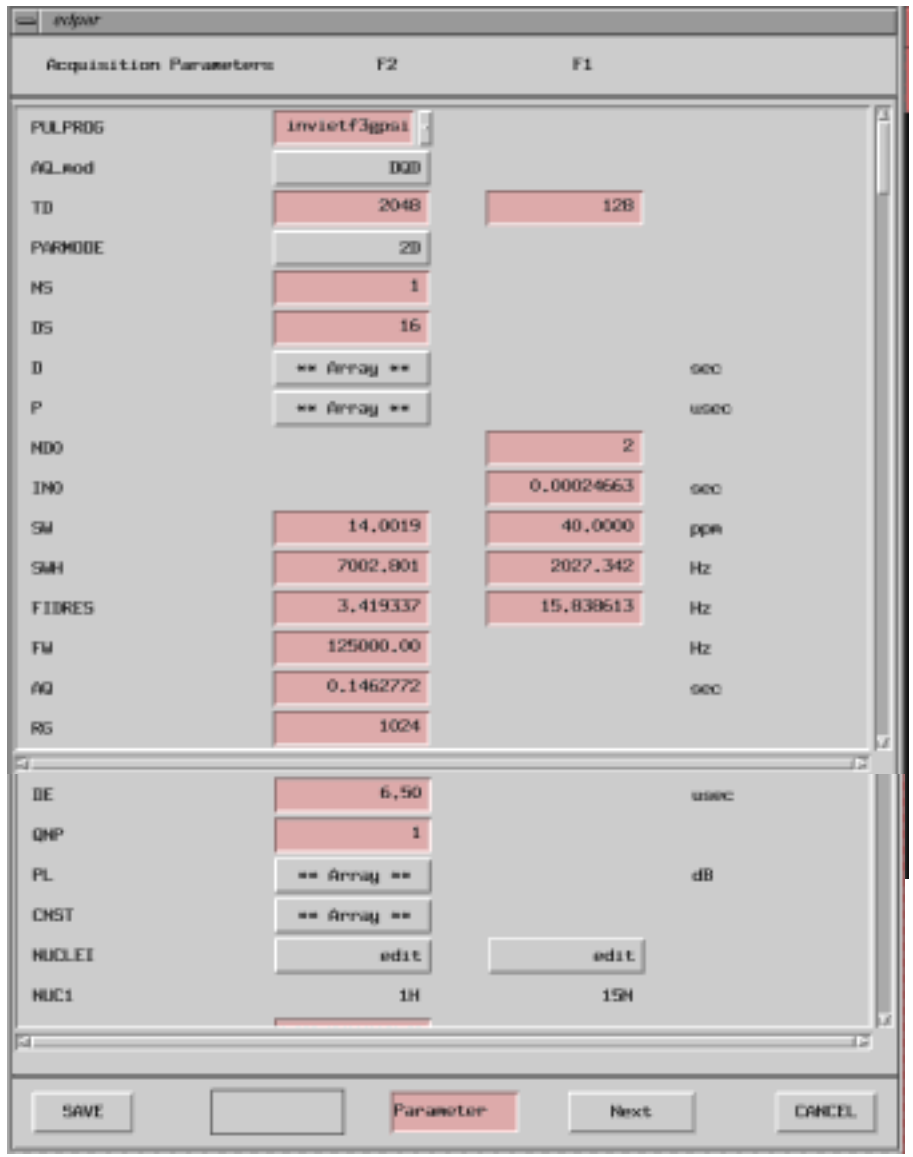
edc: Edit Current data set



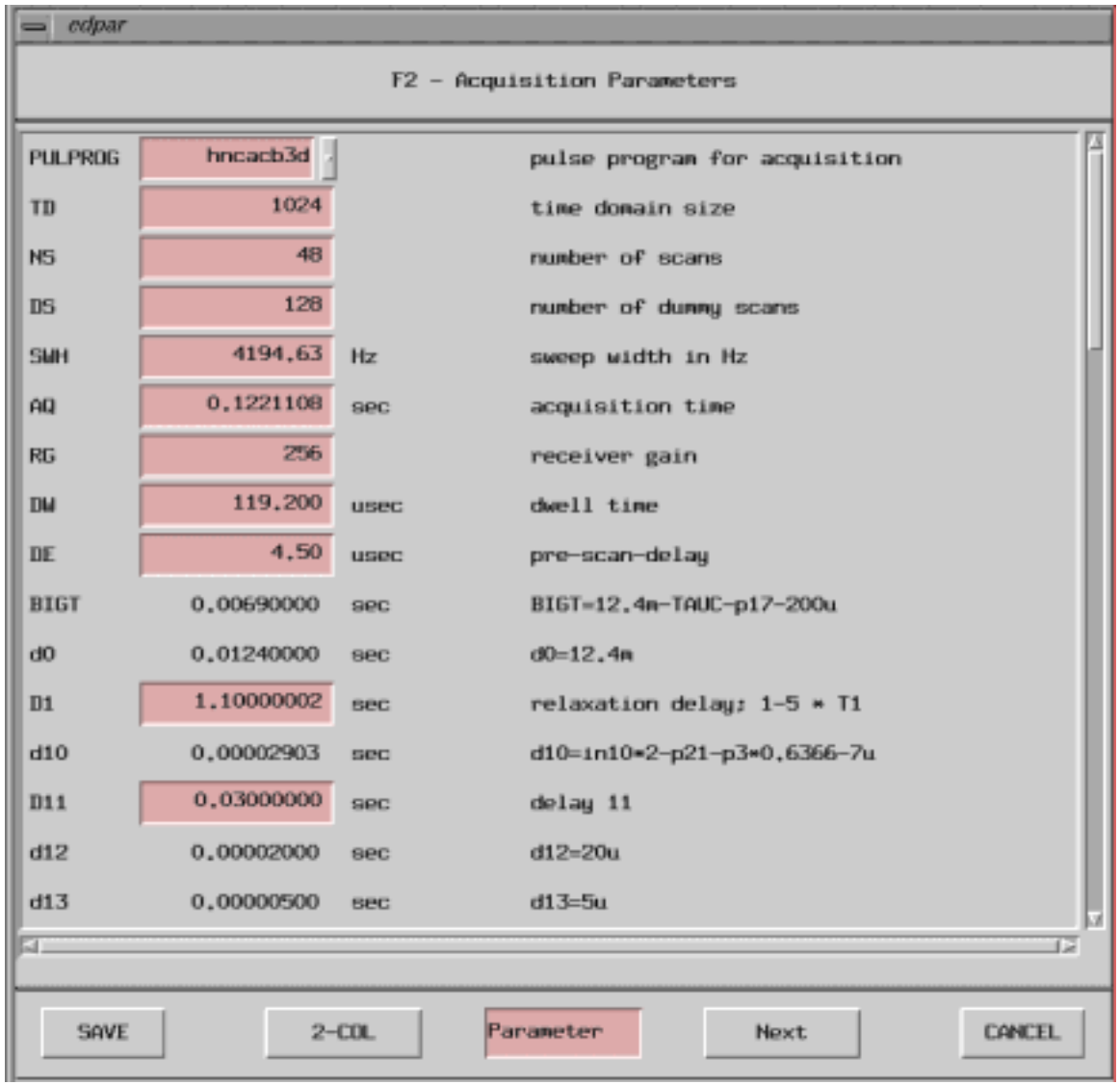
eda: Edit AQ parameter

1D

2D



ased : shows required parameters only

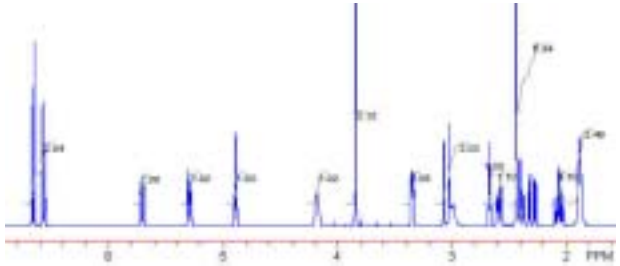


Definition of some AQ Commands & parameters

edc,new	edit current data set or generate a new data set
eda.ased	edit AQ parameters (eda: shows all, ased: shows required only)
rga	auto optimize rg value
zg	zero memory, and start to collect FID (go)
go	start to collect FID and add signals to the previous memory
stop	stop the active job (just one job)
kill	kill active job (can choose several jobs)

o1.o2,o3	center frequency of the spectrum for nuclear at f1 channel (ex: 1H), f2 channel (ex:13C), and f3 channel (ex:15N)
sw	spectrum width (1 sw : F1 dimension, 2 sw: F2 dimension…….)
td	number of points for FID collection(1 td: F1 dimension, 2 td: F2 dimension….)
d1	relaxation time (usually > 5* T1)
ns	number of scan
ds	dummy scan
rg	receiver gain (usually use the value calculated by rga)

1D

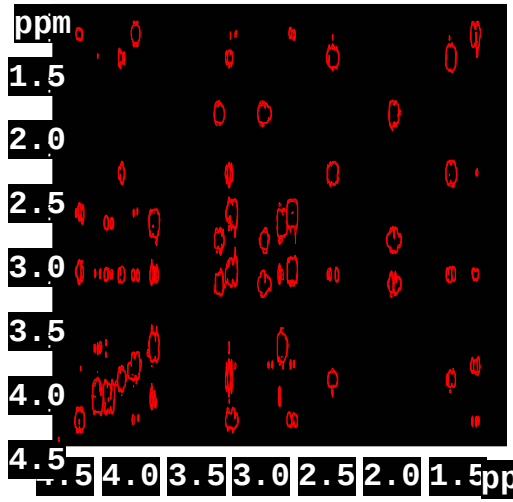


center (o1) at 4.5ppm

sw=6ppm

1.5ppm (=o1-sw/2) to 7.5ppm(=o1+sw/2)

2D/homo



F1 dimension !!!

F2 dimension !!!

F2: H (f1 channel)

O1(f1 channel):3ppm

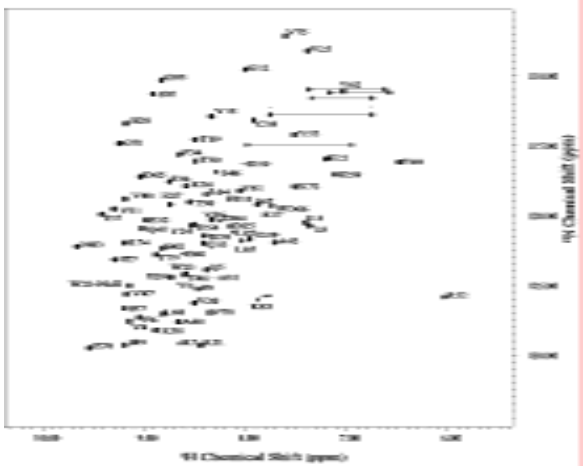
2 sw(F2 dimension): 5ppm

F1: H (f1 channel)

O1(f1 channel): 3ppm

1 sw(F1 diemnsion): 5ppm

2D/hetero



F2: H (f1 channel)

O1(f1 channel):8ppm

2 sw(F2 dimension): 5ppm

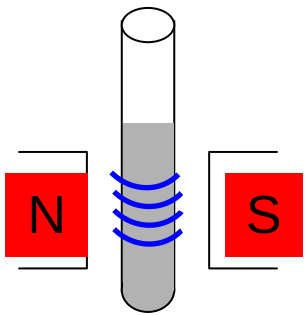
F1: N (f3 channel)

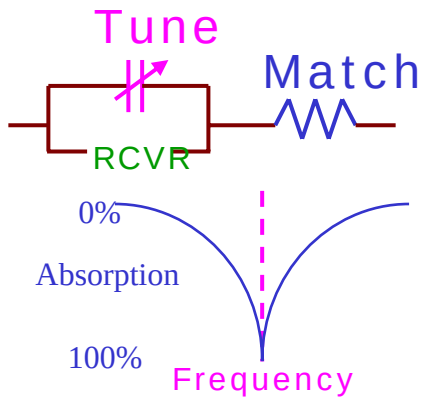
O3(f3 channel): 120ppm

1 sw(F1 diemnsion): 40ppm

In the NMR LAB

1. **The best condition for sample? → Temperature, sample position**
2. **The best condition for NMR? → Wobble : Tune & Match**



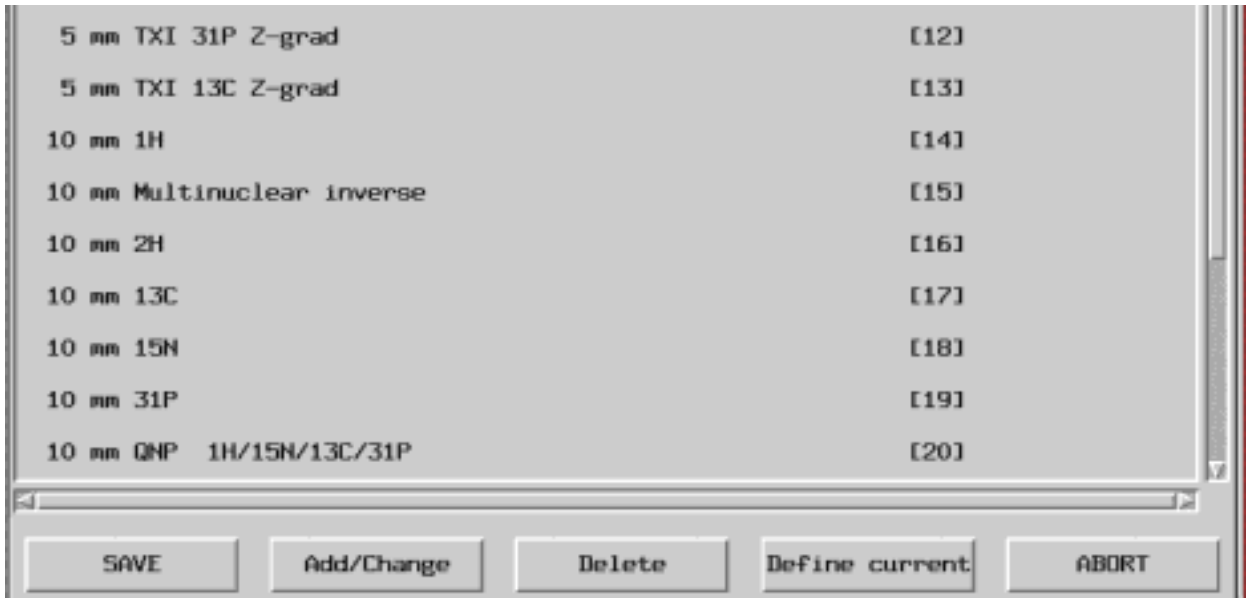


3. **The best condition for field? → Lock and shim**

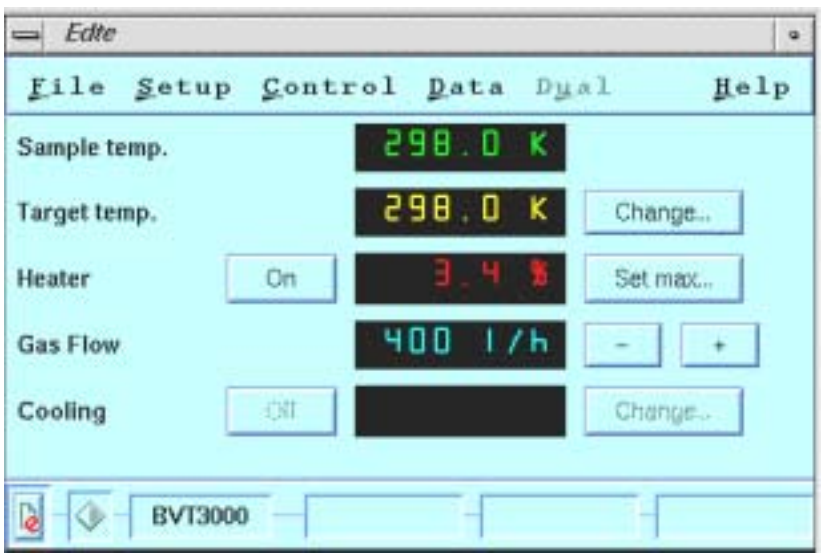
   (Simple Operation Guide for Bruker AV System)

4. **Ready to go! (LAB work sheet)**

edhead :Edit probe head

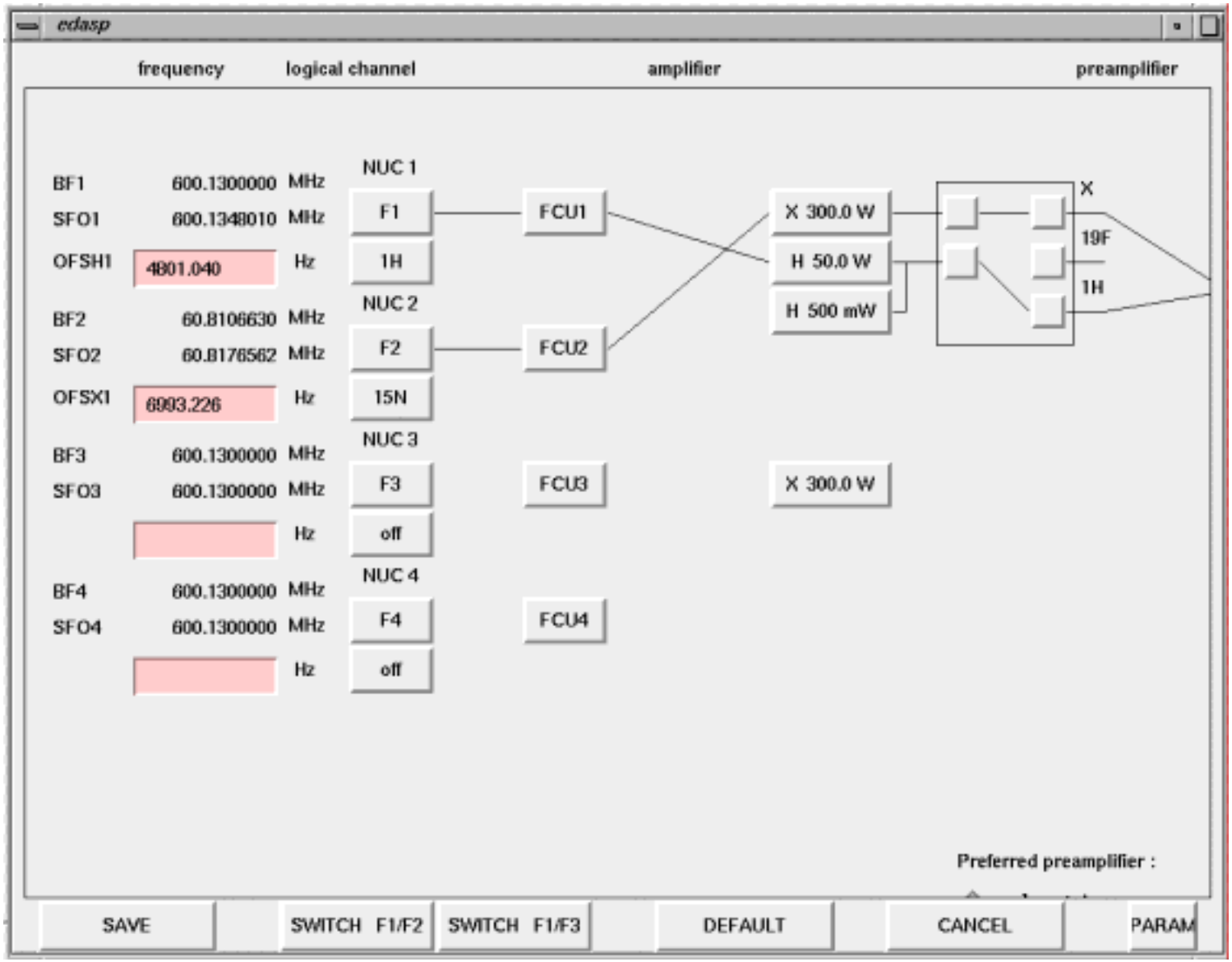


edte :Edit temperature



lock

edasp :Edit AQ Spectrometer



2002 NMR Training Course

Basic Operation for Bruker AV System

Basic Data processing for 1D/2D using xwinnmr

Yong-Li Pan

12/23 (Mon) 11:00-12:00

Topic

- **1D NMR data process**
- **2D NMR data process**

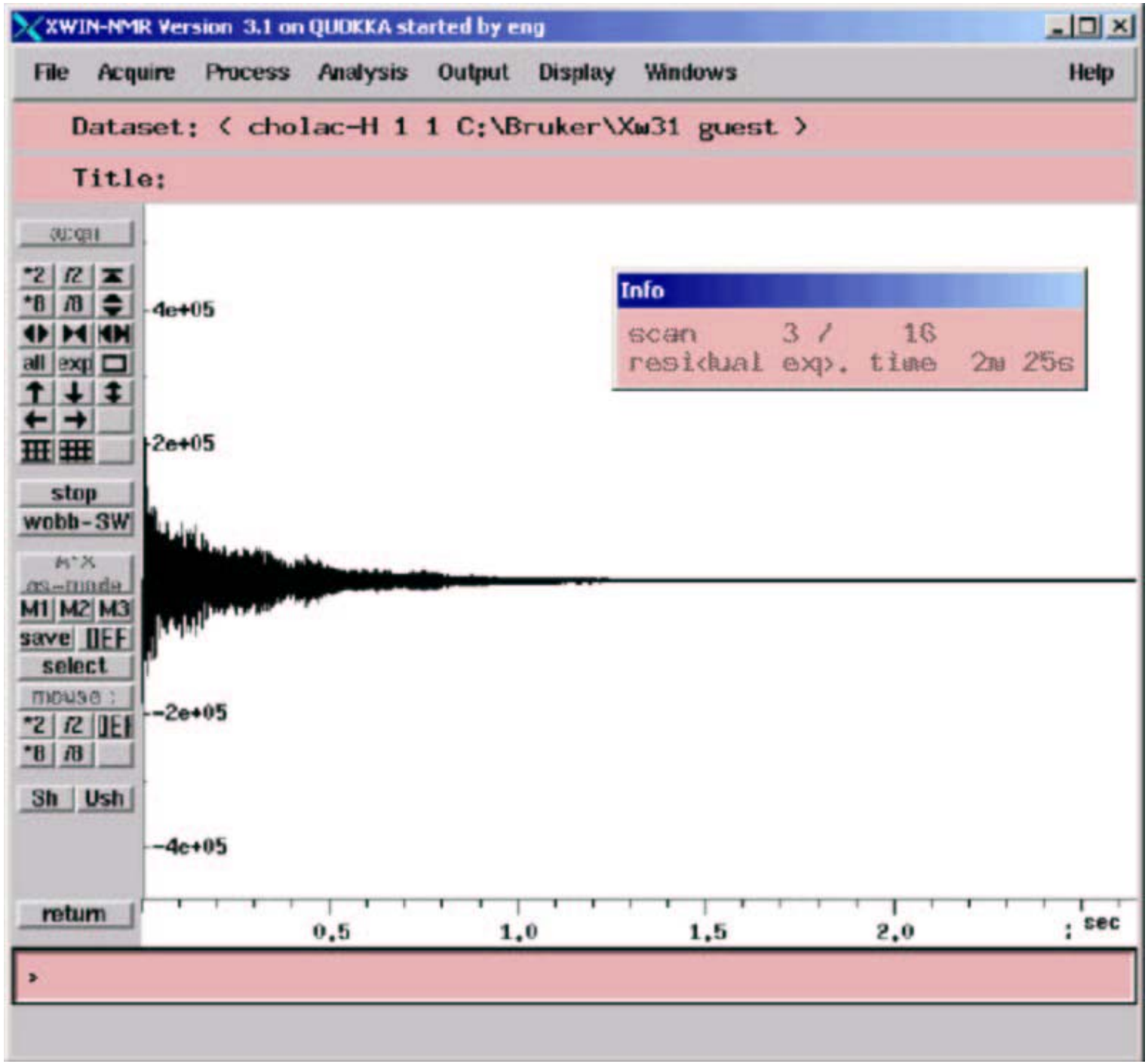
1D NMR spectrum data process

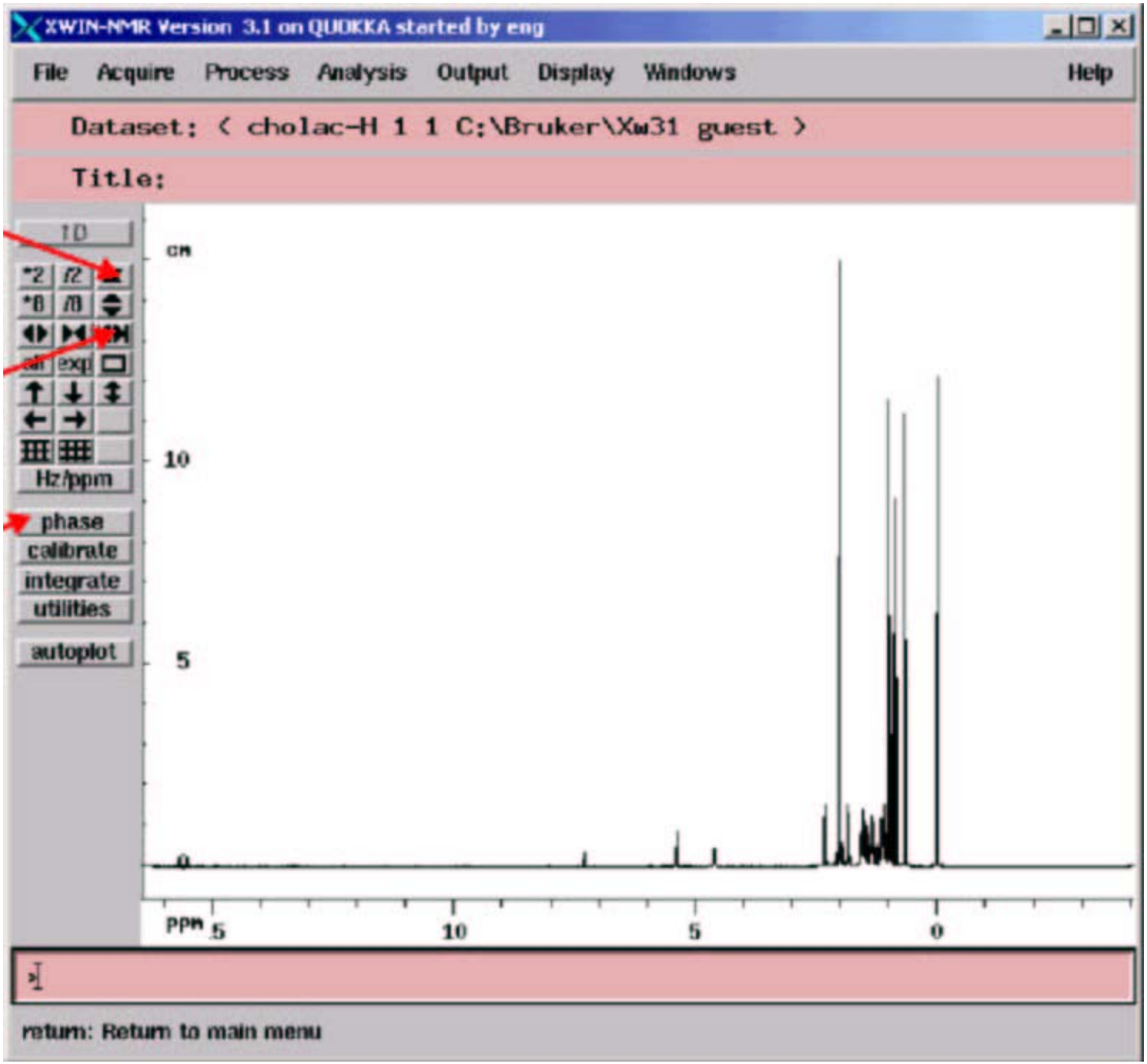
Data Acquisition on Console

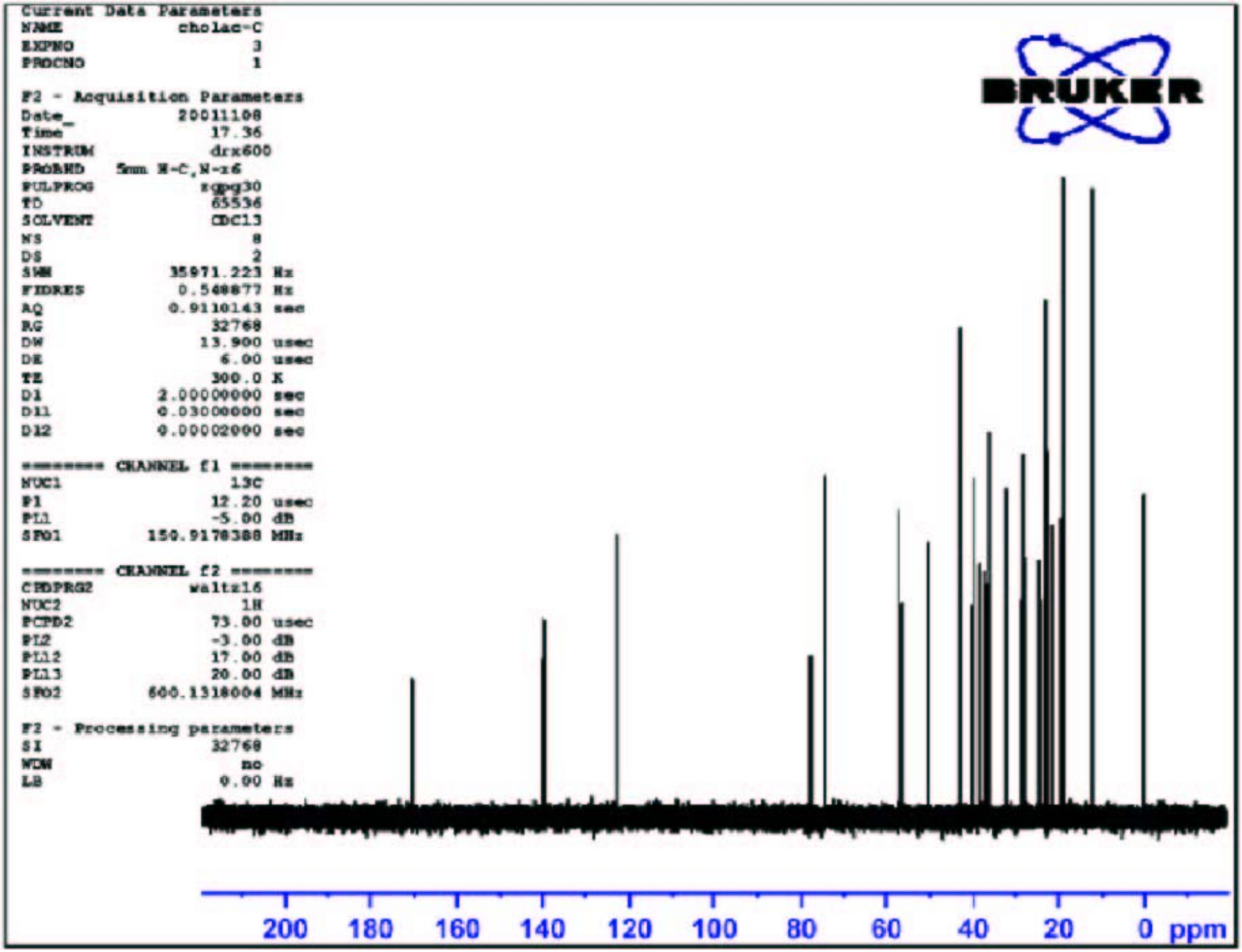
- **1D:Time domain data: FID**
- **2D:Time domain data: SER**
- **FTP: XFTP, WinSCP或網路上的芳鄰將data傳到其它工作站, 利用XwinNMR, aurelia等軟體, 進行data process**

Procedure of 1D data process

- Getting the data set: Search
- Setup parameter of Fourier transform and execute
- Phase
  - Biggest
  - Cursor
  - MC
- Use edp menu or key in window function command to improve the resolution of spectrum
- Integration
- Plot


XWIN-NMR Version 3.1 on QUOKKA started by eng
File
Acquire
Process
Analysis
Output
Display
Windows
Help
Dataset: (cholac-H 1 1 C:\Bruker\Xw31 guest)
Title:
stop
wobb-SW
save
select
Sh
Ush
return
Info
scan 3 / 16
residual exp. time 2m 25s
4e+05
2e+05
-2e+05
-4e+05
0.5
1.0
1.5
2.0
sec


XWIN-NMR Version 3.1 on QUOKKA started by eng
File
Acquire
Process
Analysis
Output
Display
Windows
Help
Dataset: (cholac-H 1 1 C:\Bruker\Xw31 guest)
Title:
1D
Hz/ppm
phase
calibrate
integrate
utilities
autoplot
cm
10
5
0
ppm
5
10
5
0
return: Return to main menu


Current Data Parameters
NAME cholac-C
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20011108
Time 17.36
INSTRUM drx600
PROBHD 5mm H-C,N-z6
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 8
DS 2
SWH 35971.223 Hz
FIDRES 0.548877 Hz
AQ 0.9110143 sec
RG 32768
DW 13.900 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
D11 0.03000000 sec
D12 0.00002000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 12.20 usec
PL1 -5.00 dB
SFO1 150.91708388 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 73.00 usec
PL2 -3.00 dB
PL12 17.00 dB
PL13 20.00 dB
SFO2 600.1318004 MHz
F2 - Processing parameters
SI 32768
WDW no
LB 0.00 Hz
BRUKER
200
180
160
140
120
100
80
60
40
20
0 ppm

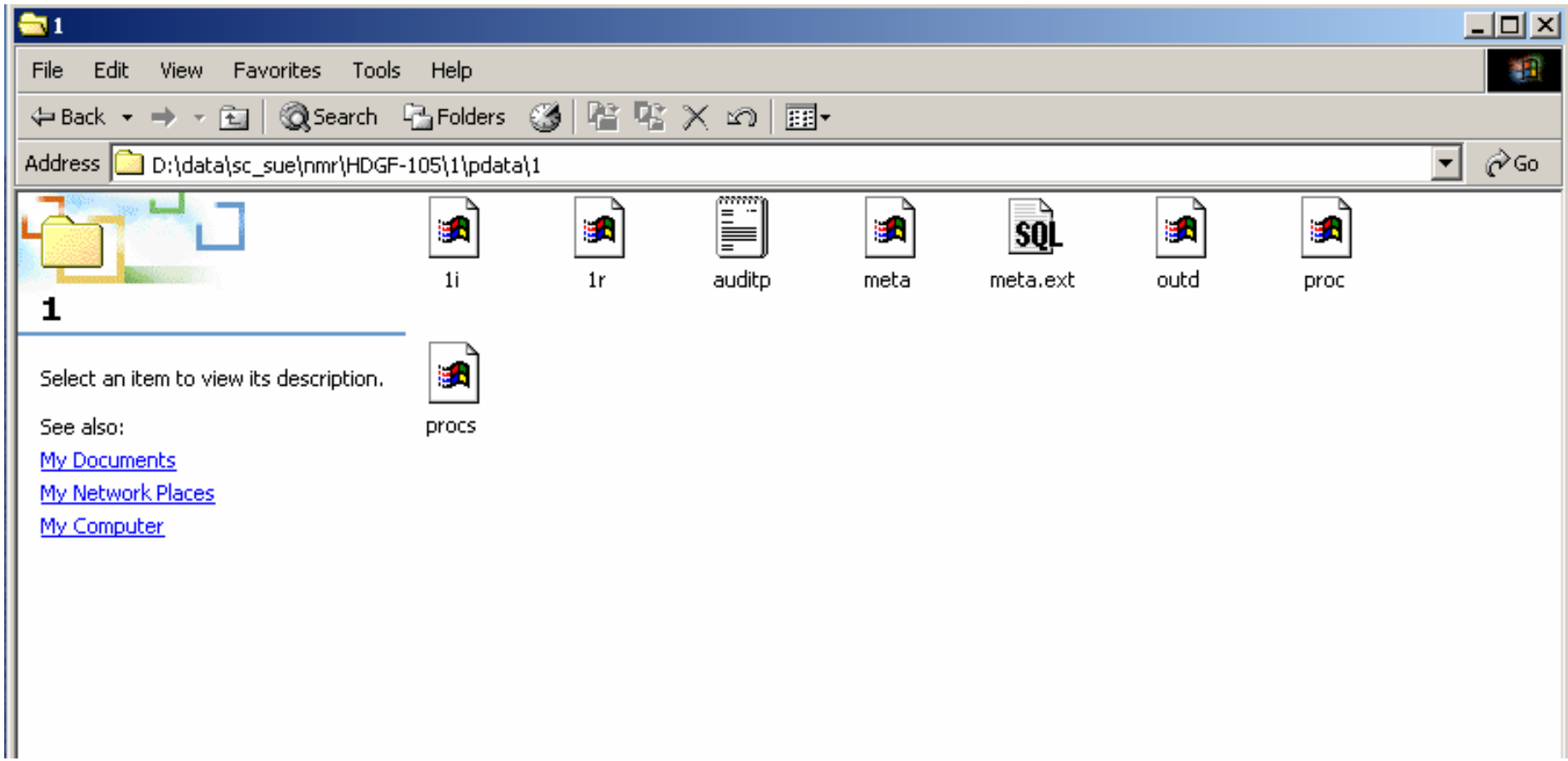
Getting the data set

- *Time domain data:*
  - */Disk/data/user/nmr/Filename/Expno/fid*
- *Frequency domain data:*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/1r*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/1i*

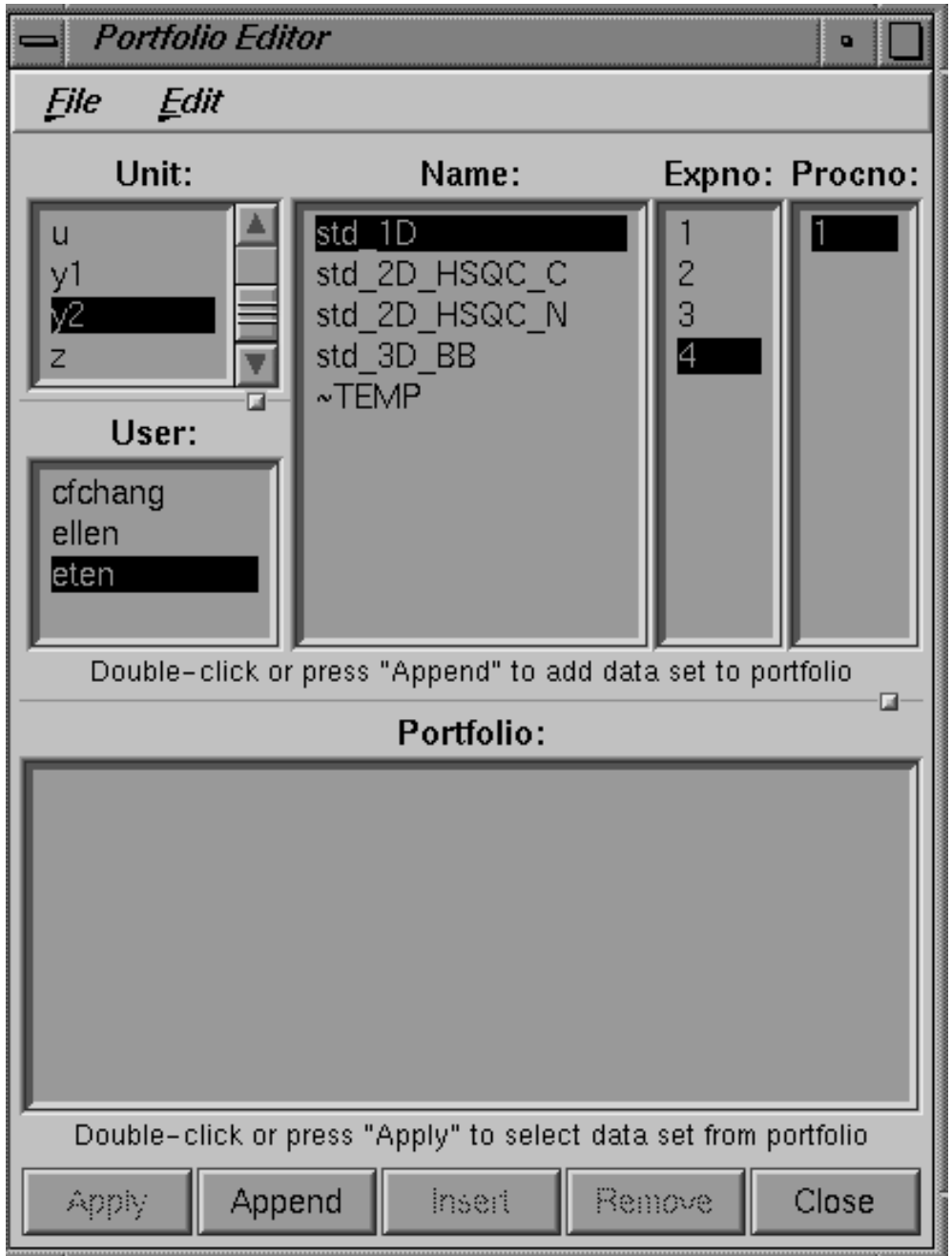
1D data file path

```
NMR5 21% pwd
/y2/data/eten/nmr/std_1D/1/pdata/1
NMR5 22% ls
1i           int          meta         parm.txt     procs
1r           intgap       meta.ext     peaks        title
auditp.txt   intrng       outd         proc
NMR5 23%
```

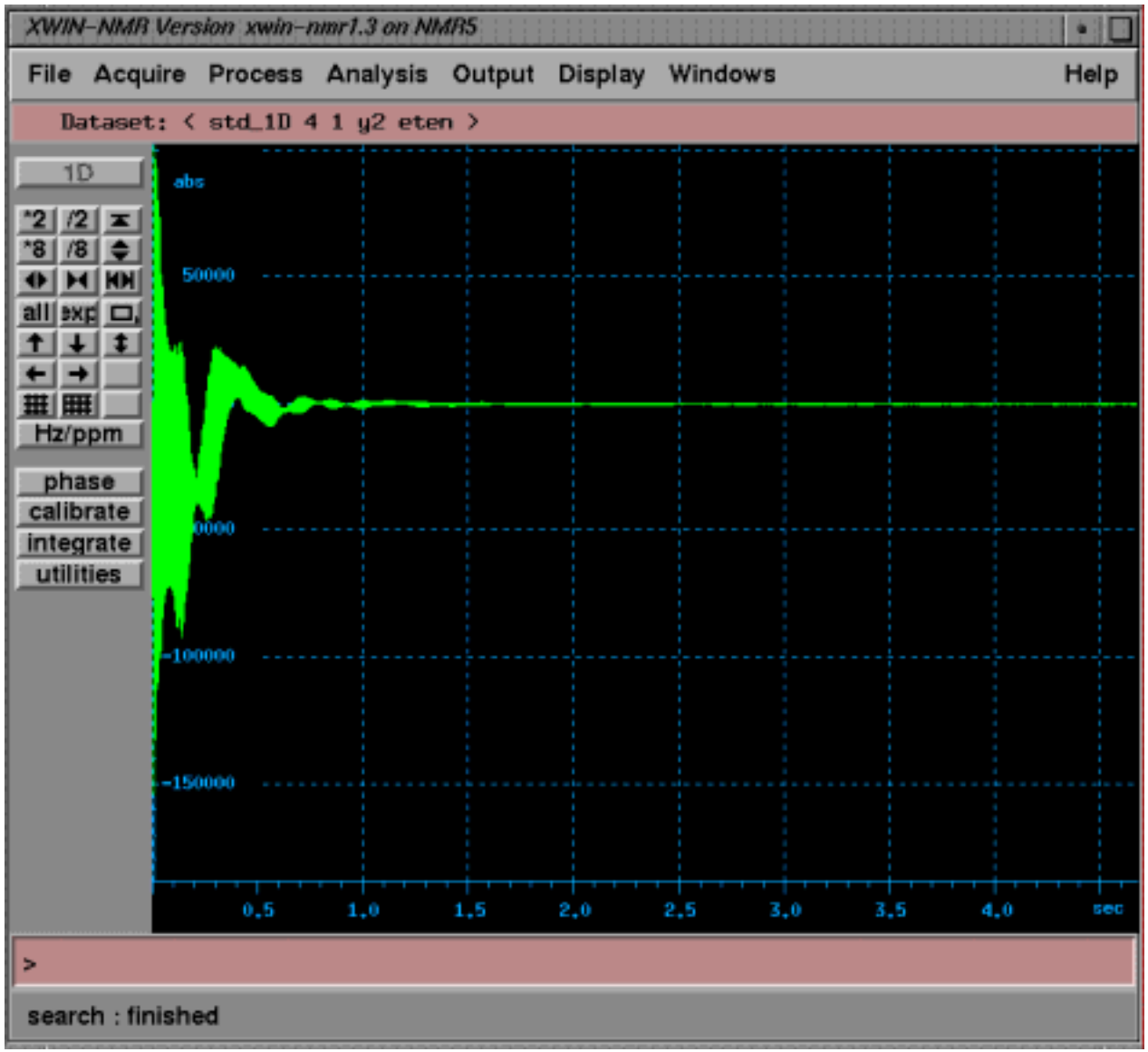
Getting the data set



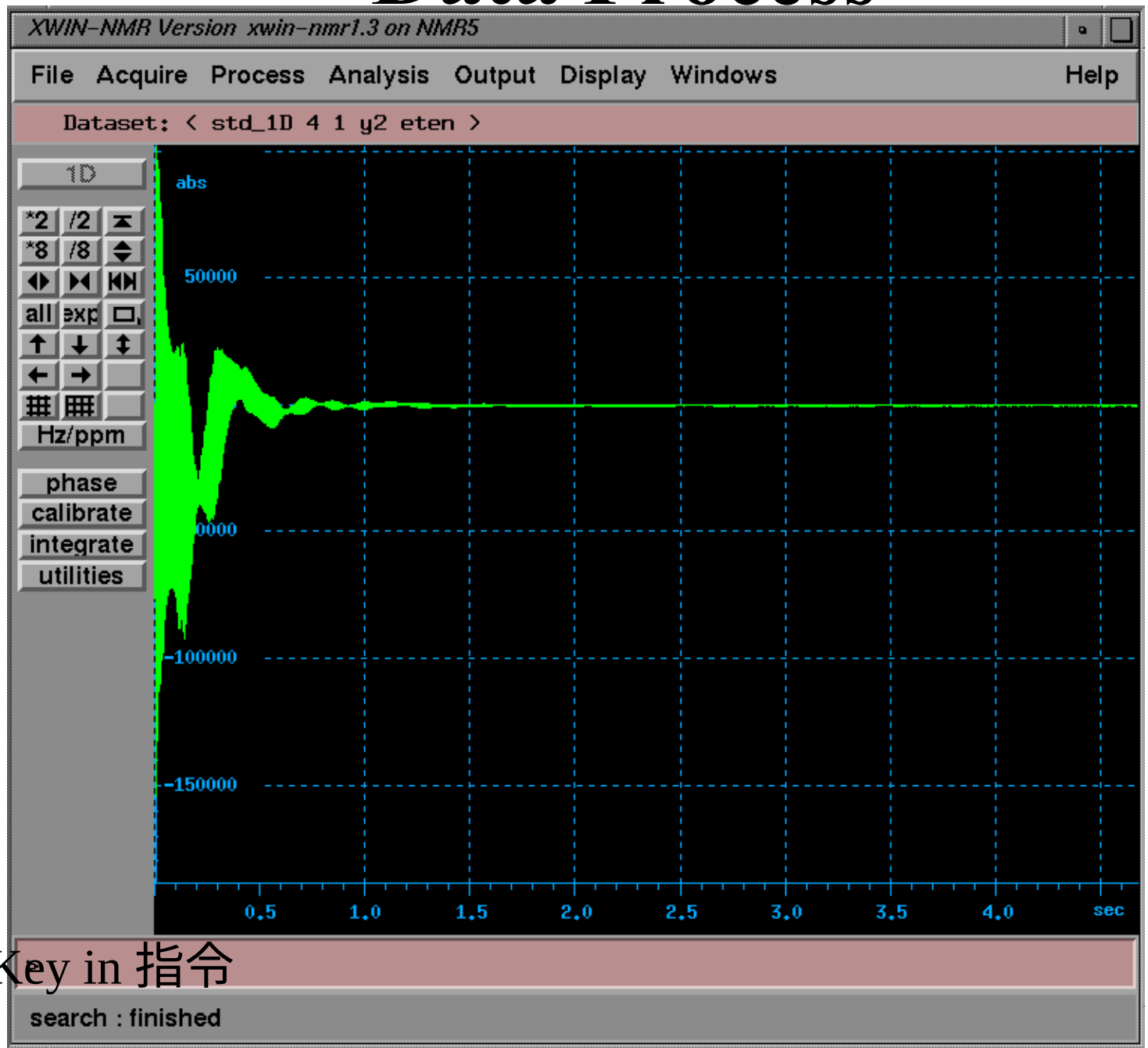
File Search


Portfolio Editor
File
Edit
Unit:
Name:
Expno:
Procno:
u
y1
y2
z
std_1D
std_2D_HSQC_C
std_2D_HSQC_N
std_3D_BB
~TEMP
1
2
3
4
1
User:
cfchang
ellen
eten
Double-click or press "Append" to add data set to portfolio
Portfolio:
Double-click or press "Apply" to select data set from portfolio
Apply
Append
Insert
Remove
Close

1D layout with fid (raw data)

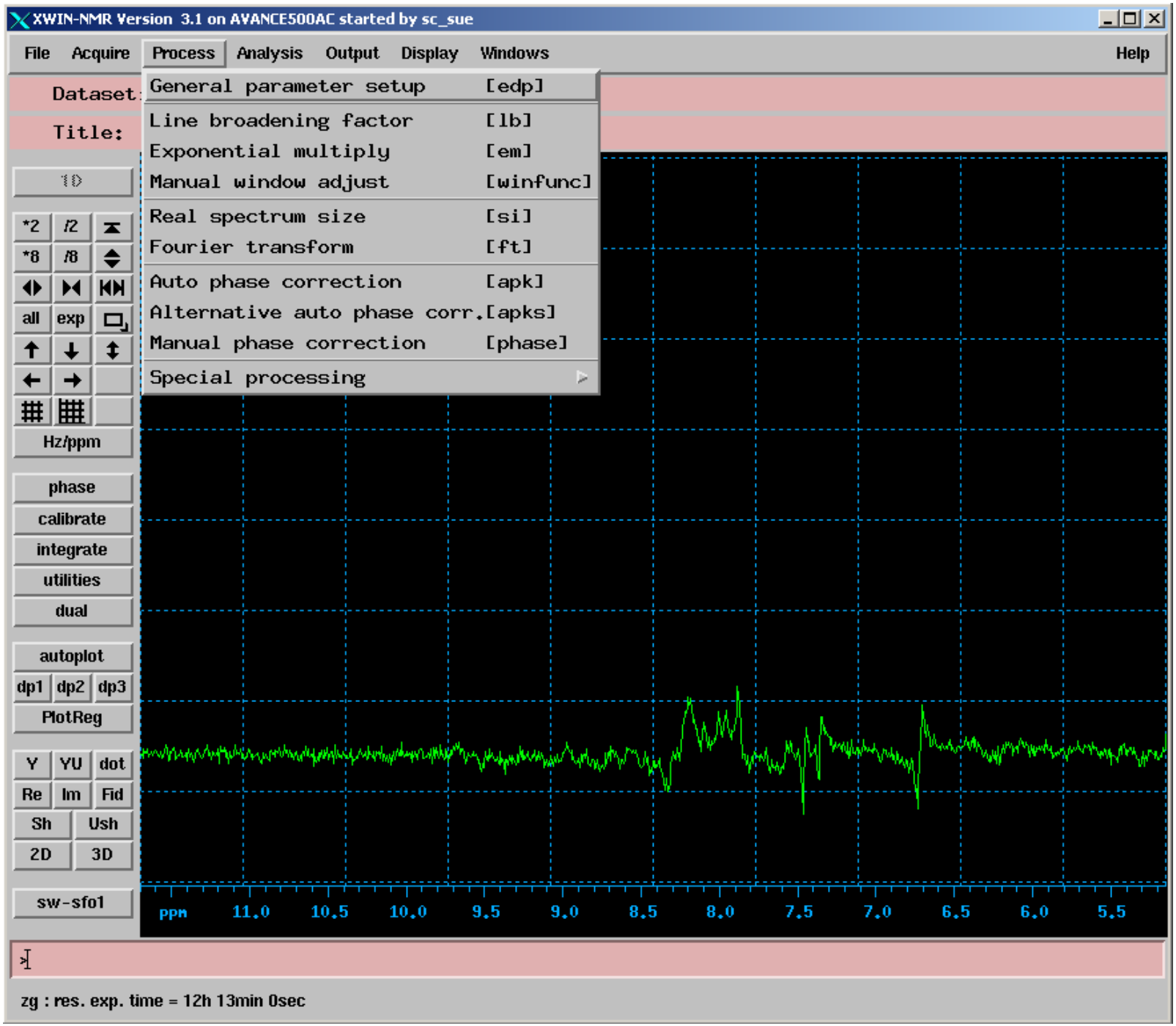


Data Process



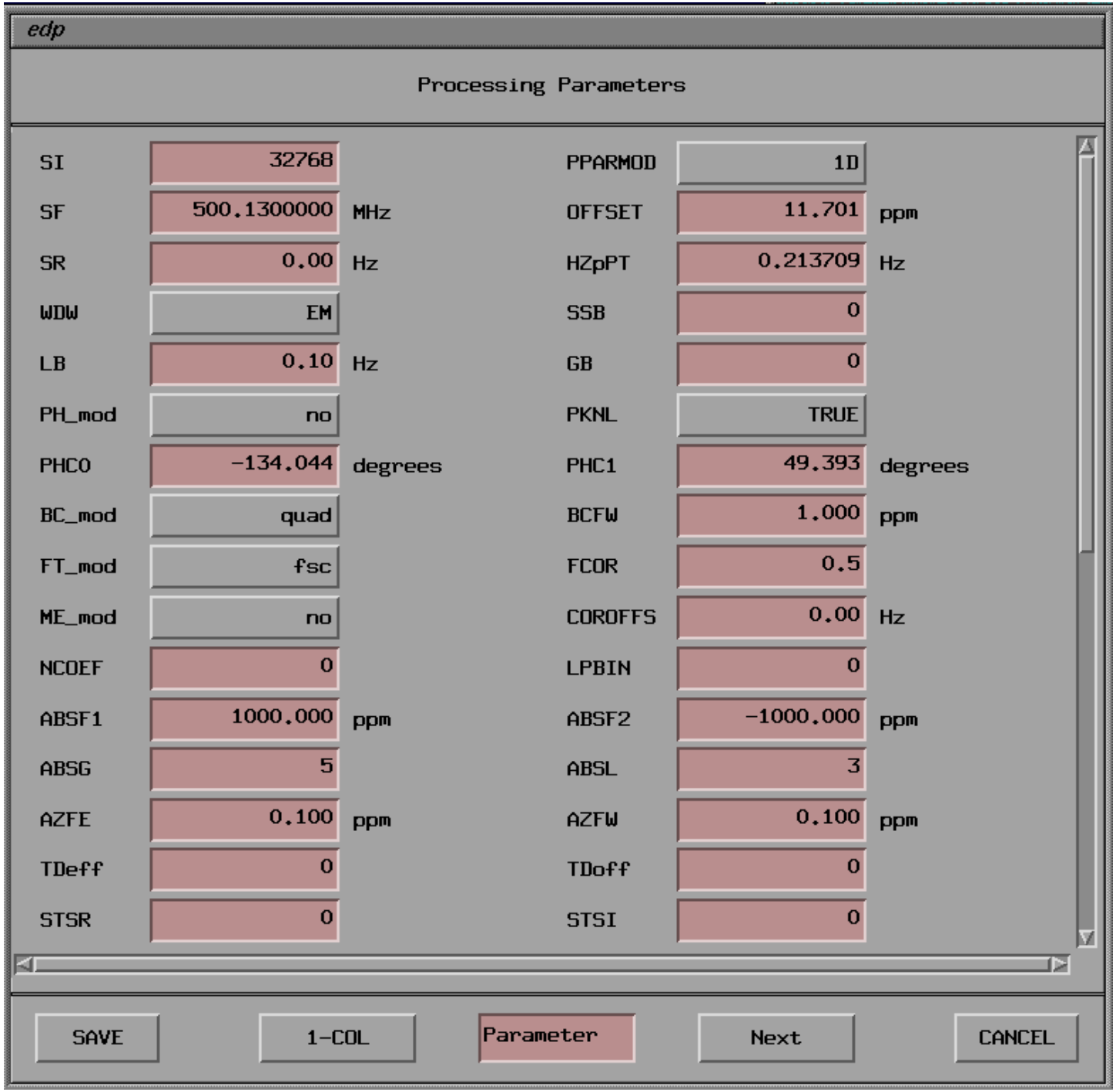
下拉式選單之process

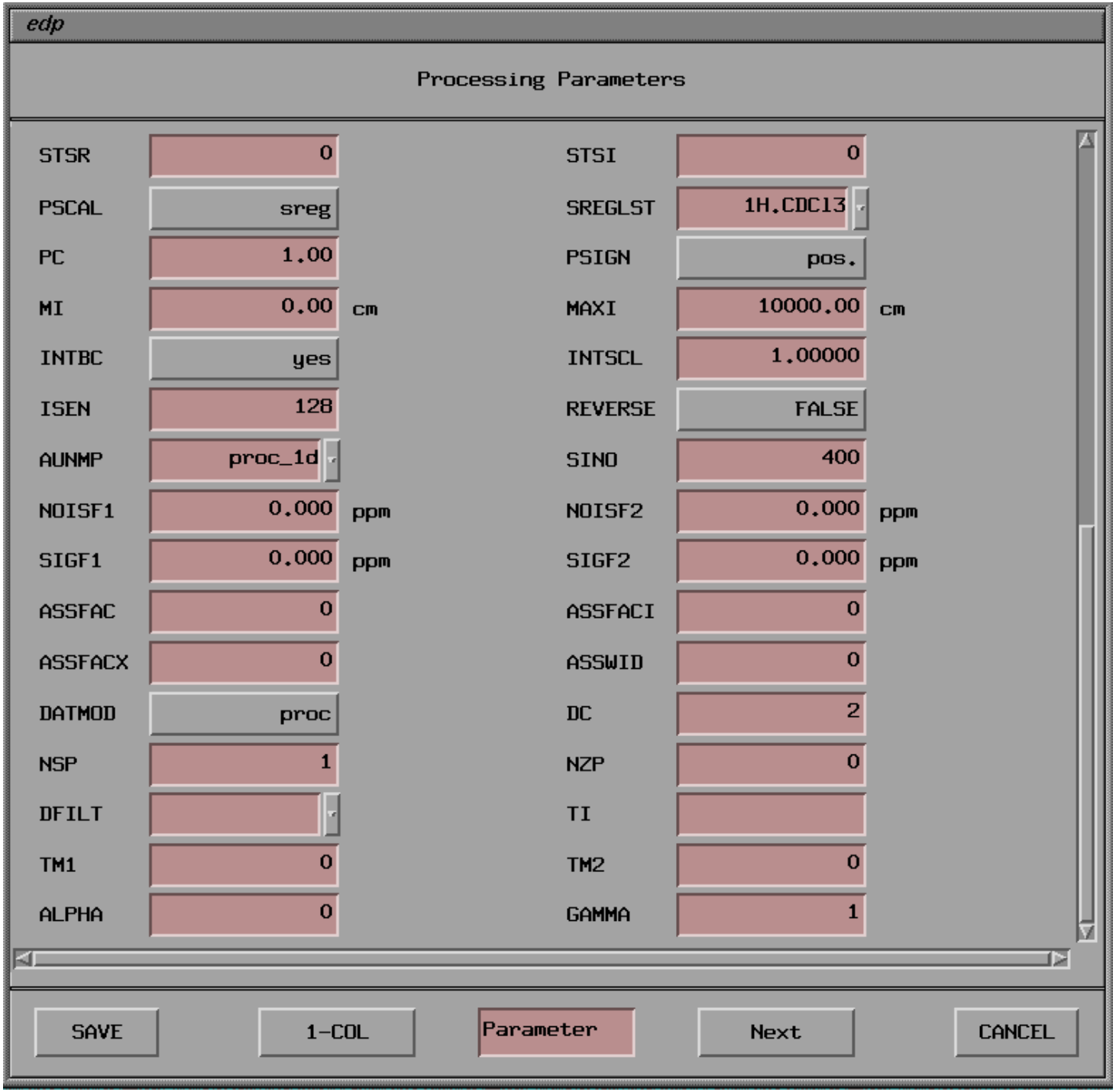
Key in 指令


XWIN-NMR Version 3.1 on AVANCE500AC started by sc_sue
File
Acquire
Process
Analysis
Output
Display
Windows
Help
Dataset:
Title:
General parameter setup [edp]
Line broadening factor [lb]
Exponential multiply [em]
Manual window adjust [winfunc]
Real spectrum size [si]
Fourier transform [ft]
Auto phase correction [apk]
Alternative auto phase corr. [apks]
Manual phase correction [phase]
Special processing
1D
*2
/2
*8
/8
all
exp
Hz/ppm
phase
calibrate
integrate
utilities
dual
autoplot
dp1
dp2
dp3
PlotReg
Y
YU
dot
Re
Im
Fid
Sh
Ush
2D
3D
sw-sfo1
ppm
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
zg : res. exp. time = 12h 13min 0sec

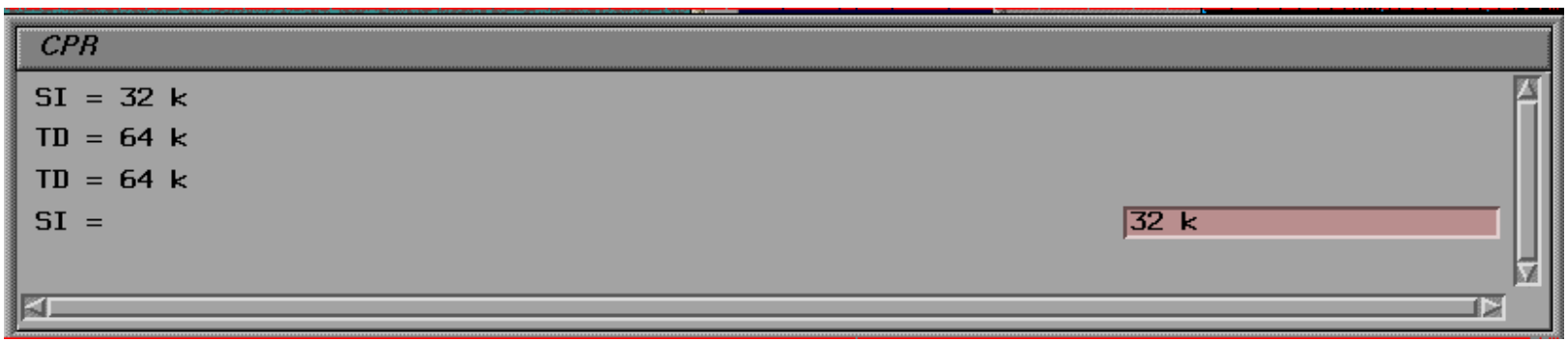
General parameter for processing (edp)

- The edp command opens a dialog box which allows you to set the parameters required by the various commands in the Process menu. The parameters are described along with the commands which make use of them.


edp
Processing Parameters
SI 32768
SF 500.1300000 MHz
SR 0.00 Hz
WDW EM
LB 0.10 Hz
PH_mod no
PHC0 -134.044 degrees
BC_mod quad
FT_mod fsc
ME_mod no
NCOEF 0
ABSF1 1000.000 ppm
ABSG 5
AZFE 0.100 ppm
TDeff 0
STSR 0
PPARMOD 1D
OFFSET 11.701 ppm
HZpPT 0.213709 Hz
SSB 0
GB 0
PKNL TRUE
PHC1 49.393 degrees
BCFW 1.000 ppm
FCOR 0.5
COROFFS 0.00 Hz
LPBIN 0
ABSF2 -1000.000 ppm
ABSL 3
AZFW 0.100 ppm
TDoff 0
STSI 0
SAVE
1-COL
Parameter
Next
CANCEL


edp
Processing Parameters
STSR
0
STSI
0
PSCAL
sreg
SREGLST
1H.CDC13
PC
1.00
PSIGN
pos.
MI
0.00
cm
MAXI
10000.00
cm
INTBC
yes
INTSCL
1.00000
ISEN
128
REVERSE
FALSE
AUNMP
proc_1d
SINO
400
NOISF1
0.000
ppm
NOISF2
0.000
ppm
SIGF1
0.000
ppm
SIGF2
0.000
ppm
ASSFAC
0
ASSFACI
0
ASSFACX
0
ASSWID
0
DATMOD
proc
DC
2
NSP
1
NZP
0
DFILT
TI
TM1
0
TM2
0
ALPHA
0
GAMMA
1
SAVE
1-COL
Parameter
Next
CANCEL

Setting the size of the real spectrum



SI (>=1/2 TD)

Process Command

- General parameter setup (edp)
- Line broadening factor (lb)
- Exponential multiply (em)
- Manual window adjust (winfunc)
- Real spectrum size (si)
- Fourier transform (ft)

Process Command

- Autophase correction (apk)
- Alternative autophase corr. (apks)
- Manual phase correction (phase)
- Special processing
  - Phase w. constants PHC0,1 (pk)
  - Magnitude spectrum (mc)
  - Power spectrum (ps)
  - Special window function:
    - Gaussian (gm), Trapezoidal ™, Sine (sine), Squared sine (qsin), Sinc (sinc)

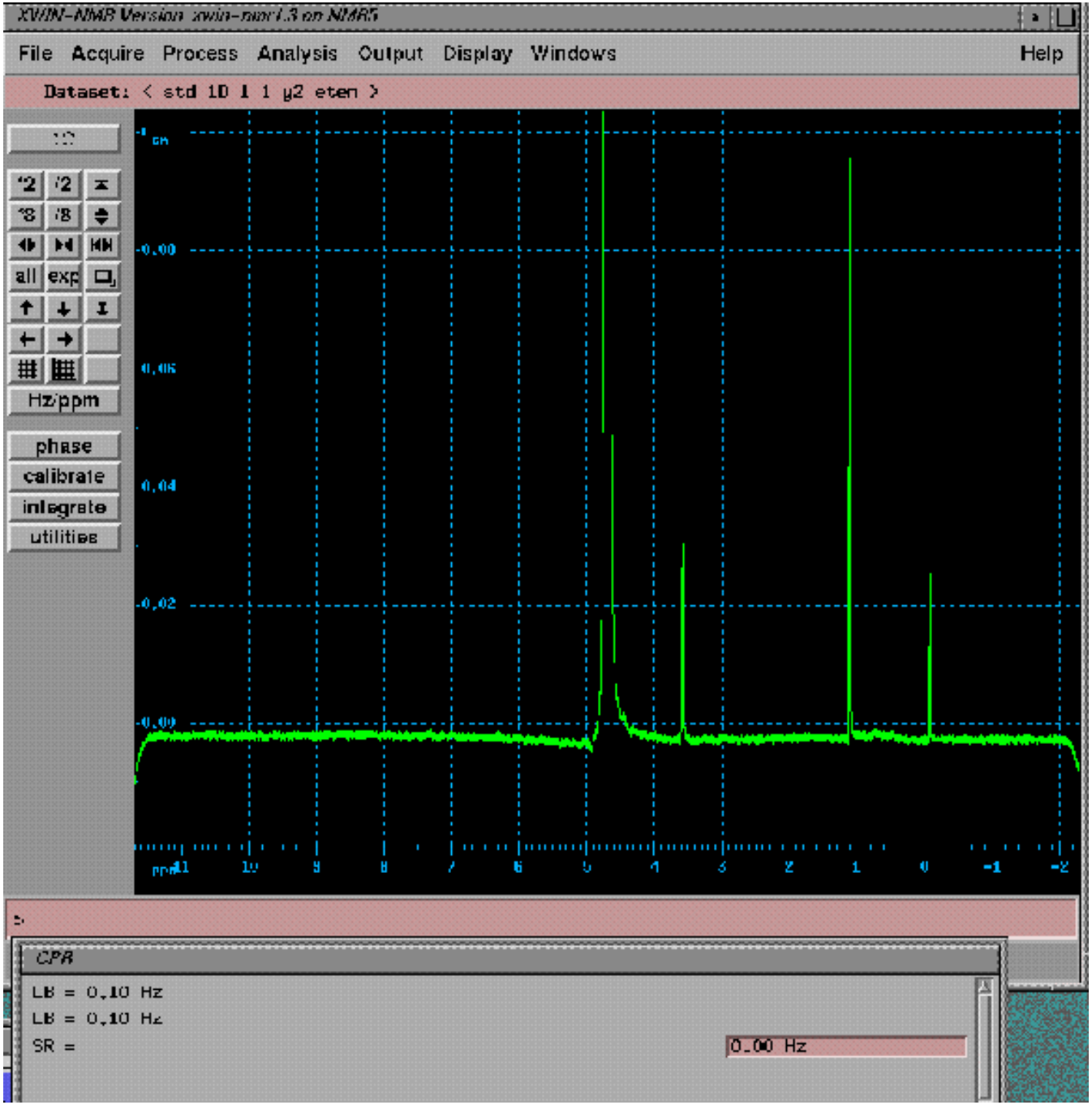
Process Command

- Baeline correction
  - Automatic full spectrum (abs)

Spectrum calibration

- H2O: MRS, 4.7ppm
- TMS: 有機小分子, 0 ppm
- DSS: 生物分子, 0 ppm

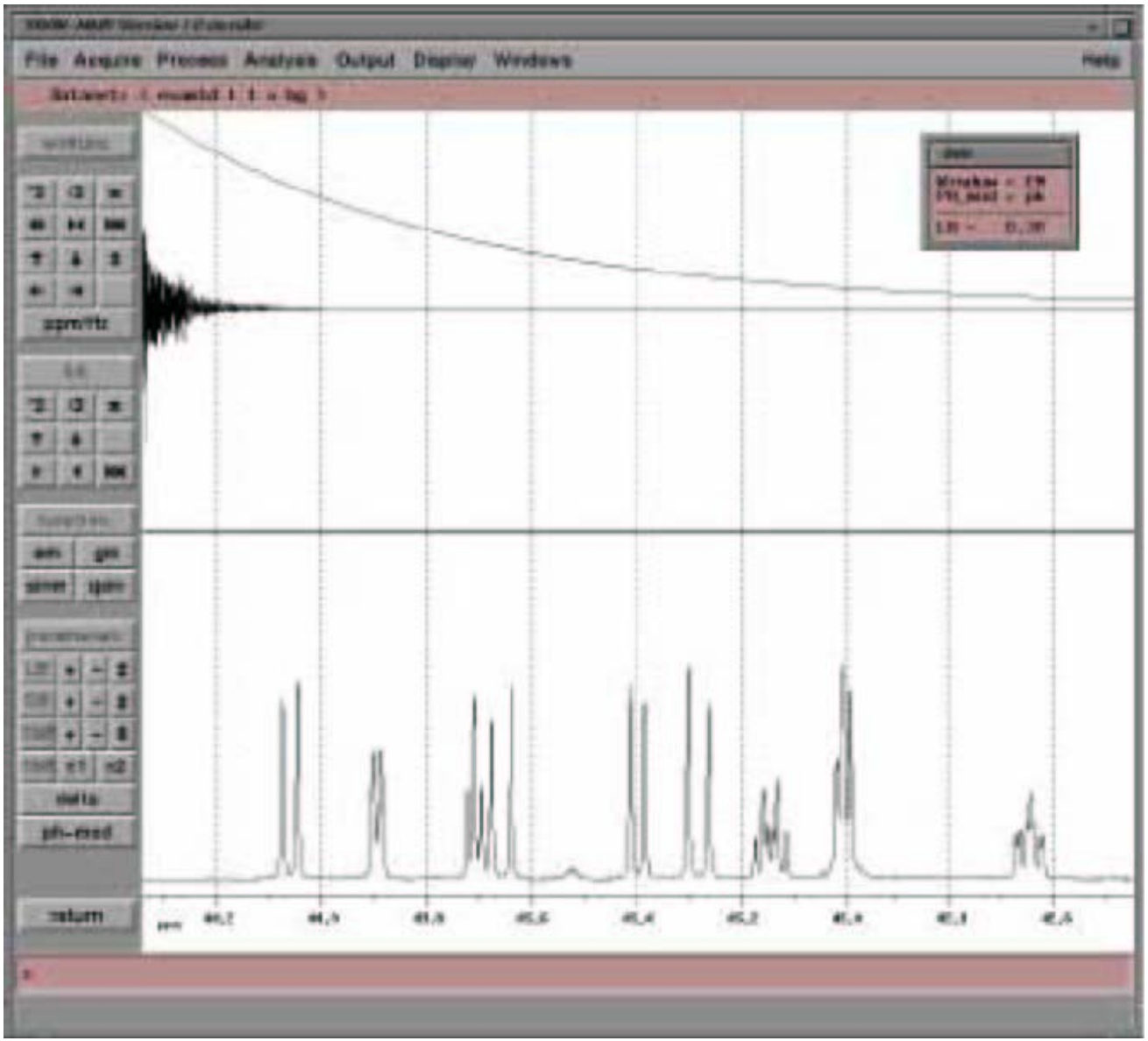
Use “SR” (edp 內之參數)根據不同溫度校正


File Acquire Process Analysis Output Display Windows Help
Dataset: < std 1D 1 1 y2 eten >
*2 /2
*8 /8
all exp
Hz/ppm
phase
calibrate
integrate
utilities
0,00
0,02
0,04
ppm
CPR
LB = 0,10 Hz
LB = 0,10 Hz
SR =
0,00 Hz

Window function

- Line broadening factor (lb)
- em, gm:window function
- key in lb =0.3, type em to execute
- MC: 取實部和虛部平方之開根號, spectrum 皆為正
- Manual adjust window function: 可先預覽各種window function之執行結果, 是否可提高spectrum之resolution

Manual adjustment of window function

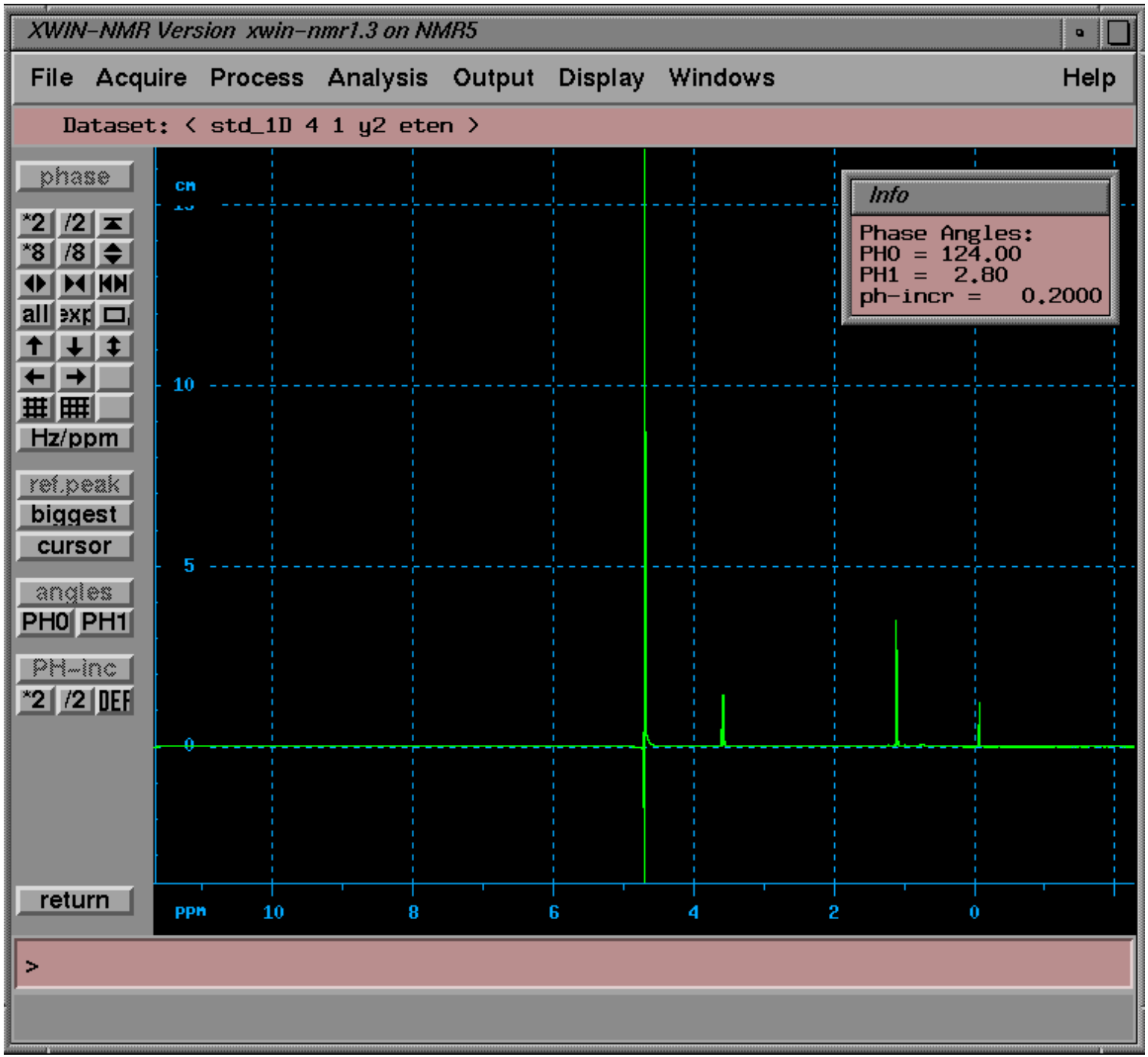


Fourier Transform Command

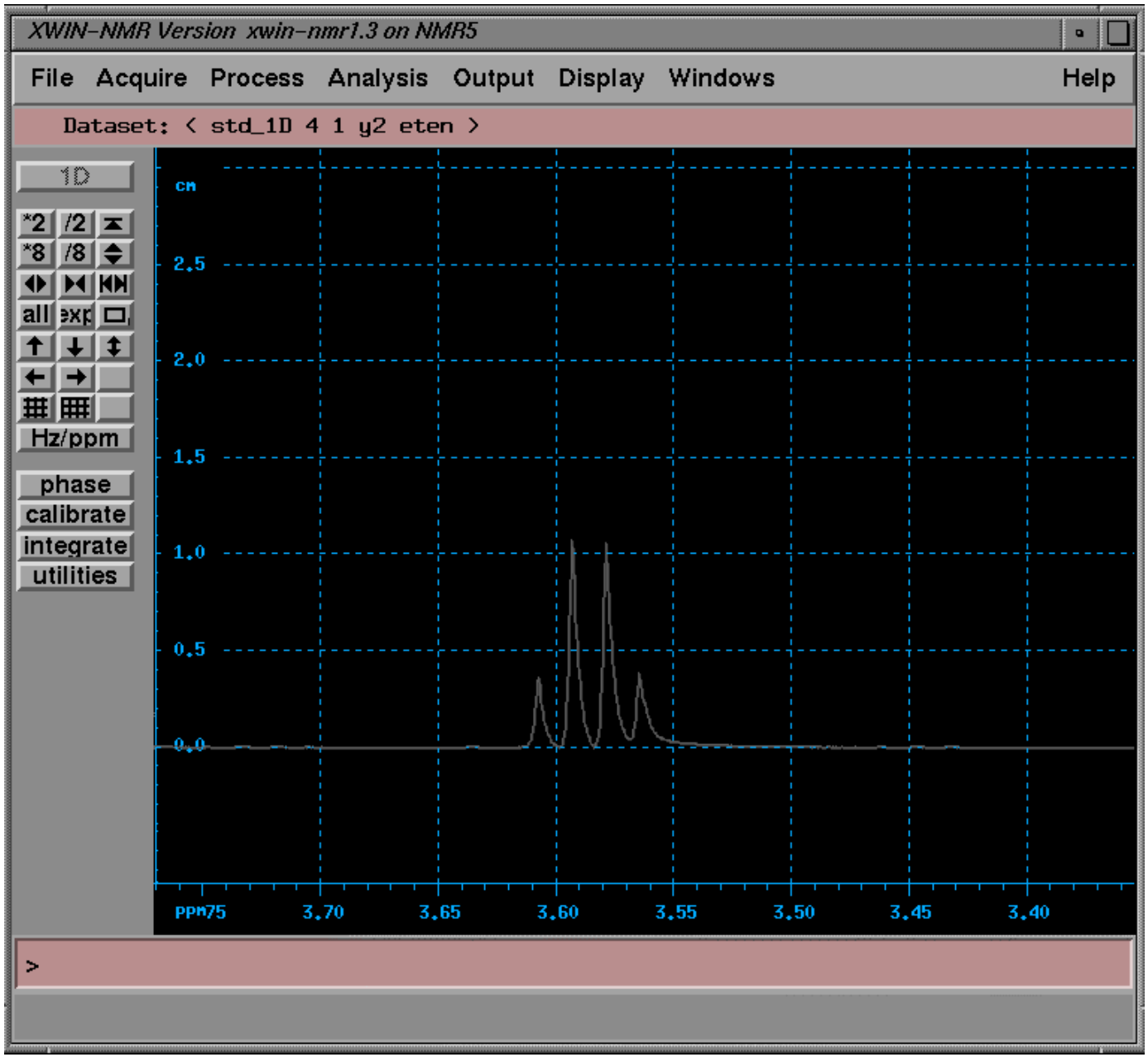
- **ft**
- **Sequential operations:**
  - **em + ft (ef)**
  - **em + ft +pk (efp)**
  - **gm +ft (gf)**
  - **gm +ft +pk (gfp)**

Phase correction

- 1DZG: 量line width, 觀察shimming情形
  - Phase時, 可選H2O當biggest,再phase
- 1DZGPR: one pulse with f1 presaturation, 因為有打水,所以要用cursor選其它peak, 再進行phase
- PH0: first order
- PH1: second order


XWIN-NMR Version xwin-nmr1.3 on NMR5
File Acquire Process Analysis Output Display Windows Help
Dataset: < std_1D 4 1 y2 eten >
phase
*2 /2
*8 /8
all exp
Hz/ppm
ref.peak
biggest
cursor
angles
PH0 PH1
PH-inc
*2 /2 DEF
return
cm
10
5
0
Info
Phase Angles:
PH0 = 124.00
PH1 = 2.80
ph-incr = 0.2000
ppm 10 8 6 4 2 0
>

1D NMR spectrum scale adjust



Baseline Correction

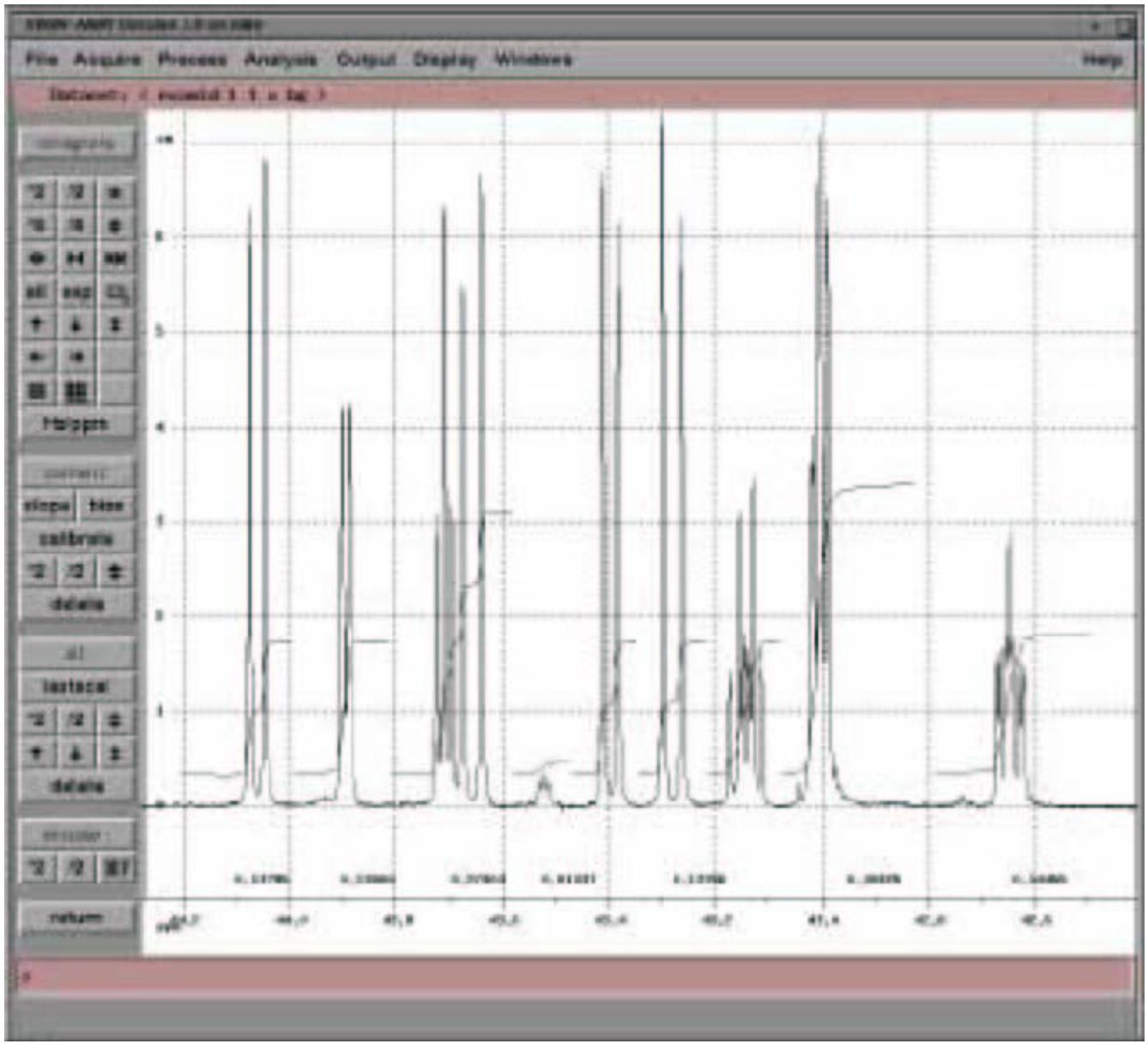
- Automatic baseline correction

  -abs

Spectrum integration

- 點選integration之button, 再用滑鼠來點選欲積分之範圍, 於光譜下方會出現積分值

Manual integration



2D NMR Spectrum data process

Procedure of 2D data process

- Getting the data set: Search
- Setup parameter of Fourier transform and execute (xfb)
- Phase
- Baseline Correction
- Setup contour level (edlev)
- Plot

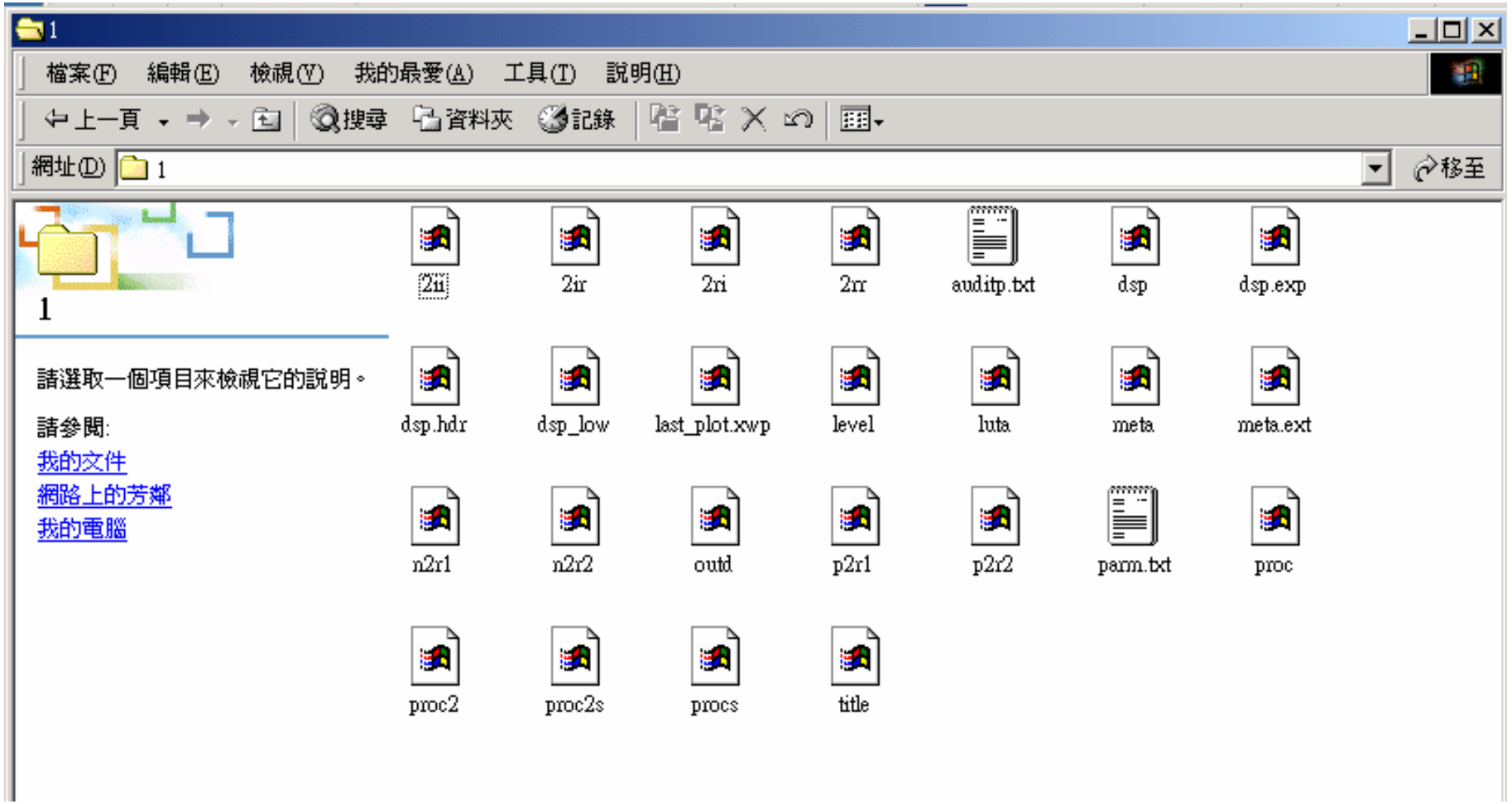
Getting the data set

- *Time domain data:*
  - */Disk/data/user/nmr/Filename/Expno/ser*
- *Frequency domain data:*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/2rr*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/2ri*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/2ir*
  - */Disk/data/user/nmr/Filename/Expno/pdata/procno/2ii*

2d NMR data file

```
NMR5 41% pwd
/y2/data/eten/nmr/std_2D_HSQC_N/2/pdata/1
NMR5 42% ls
2ii          Arr.fb       dsp_low      meta.ext     p2r1         proc2
2ir          auditp.txt   level        n2r1         p2r2         proc2s
2ri          dsp          luta         n2r2         parm.txt     procs
2rr          dsp.hdr      meta         outd         proc         title
```

2d NMR data


1
檔案(F) 編輯(E) 檢視(V) 我的最愛(A) 工具(T) 說明(H)
上一頁 搜尋 資料夾 記錄
網址(D) 1
移至
1
請選取一個項目來檢視它的說明。
請參閱:
我的文件
網路上的芳鄰
我的電腦
2ii
2ir
2ri
2rr
auditp.txt
dsp
dsp.exp
dsp.hdr
dsp_low
last_plot.xwp
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luta
meta
meta.ext
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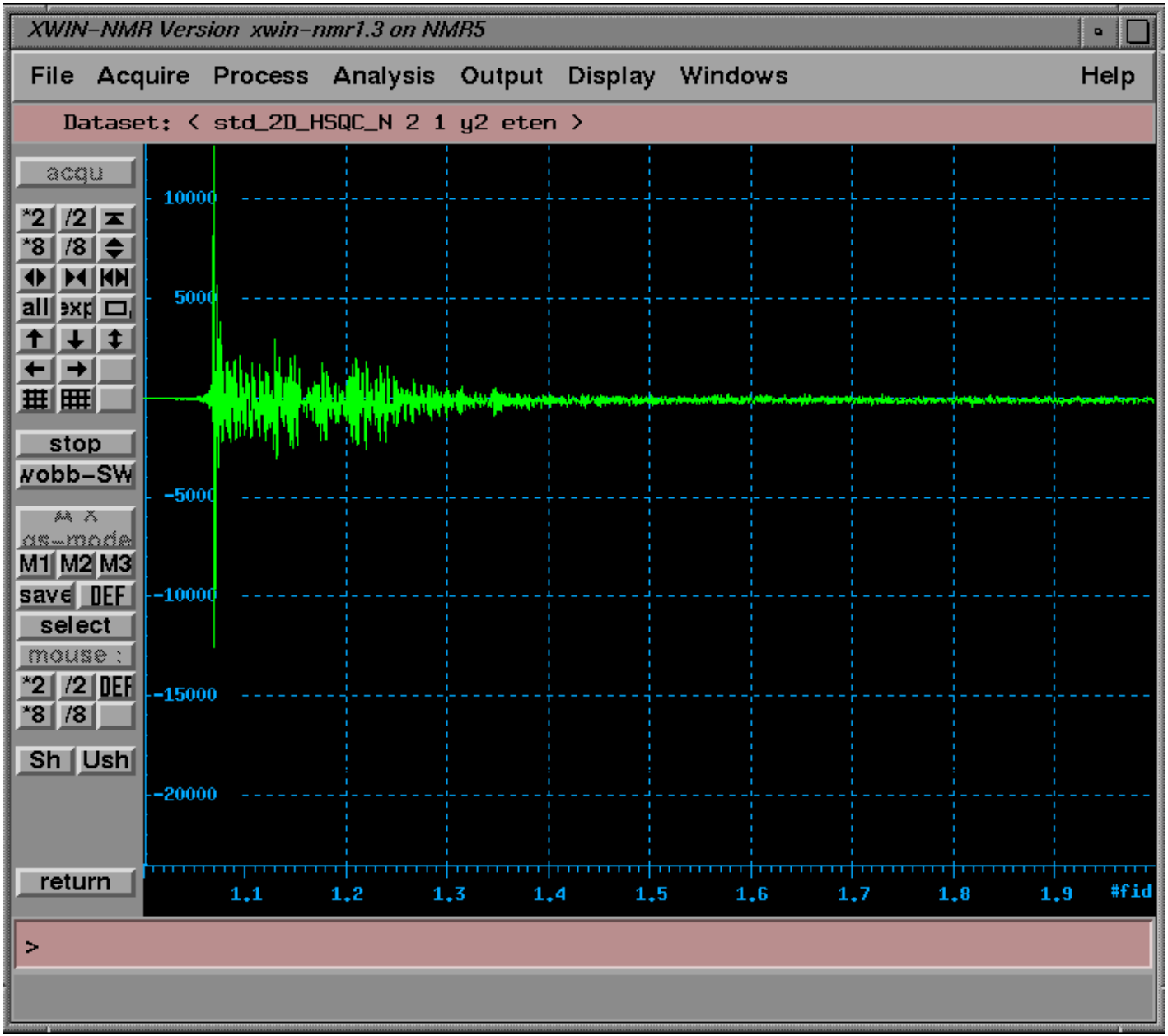
n2r2
outd
p2r1
p2r2
parm.txt
proc
proc2
proc2s
procs
title

2D FID (SER)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save DEF
select
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*8 /8
Sh Ush
return
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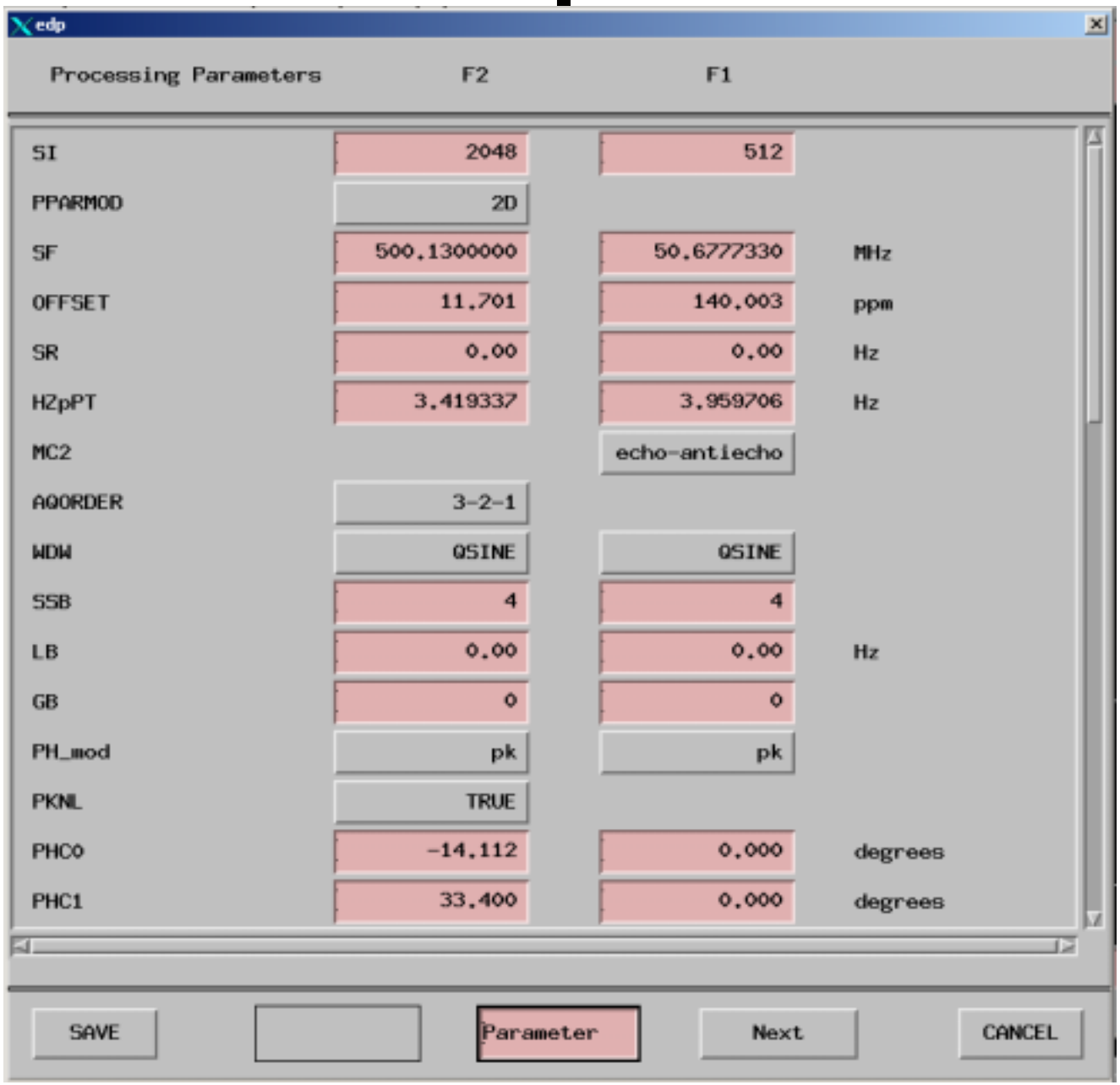
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SSB
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degrees
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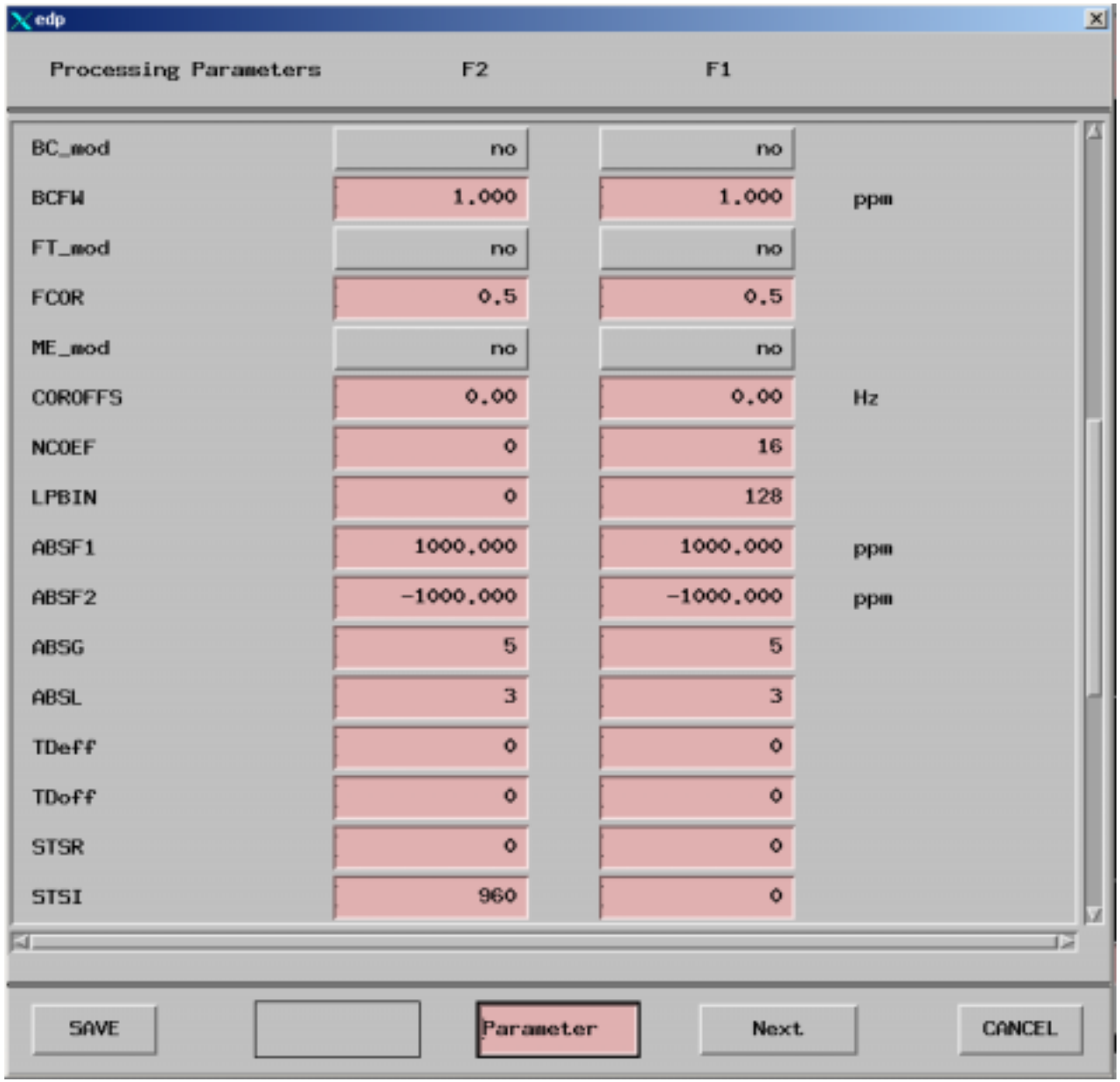
edp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edp


edp
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TM2
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ALPHA
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GAMMA
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1
NLEV
6
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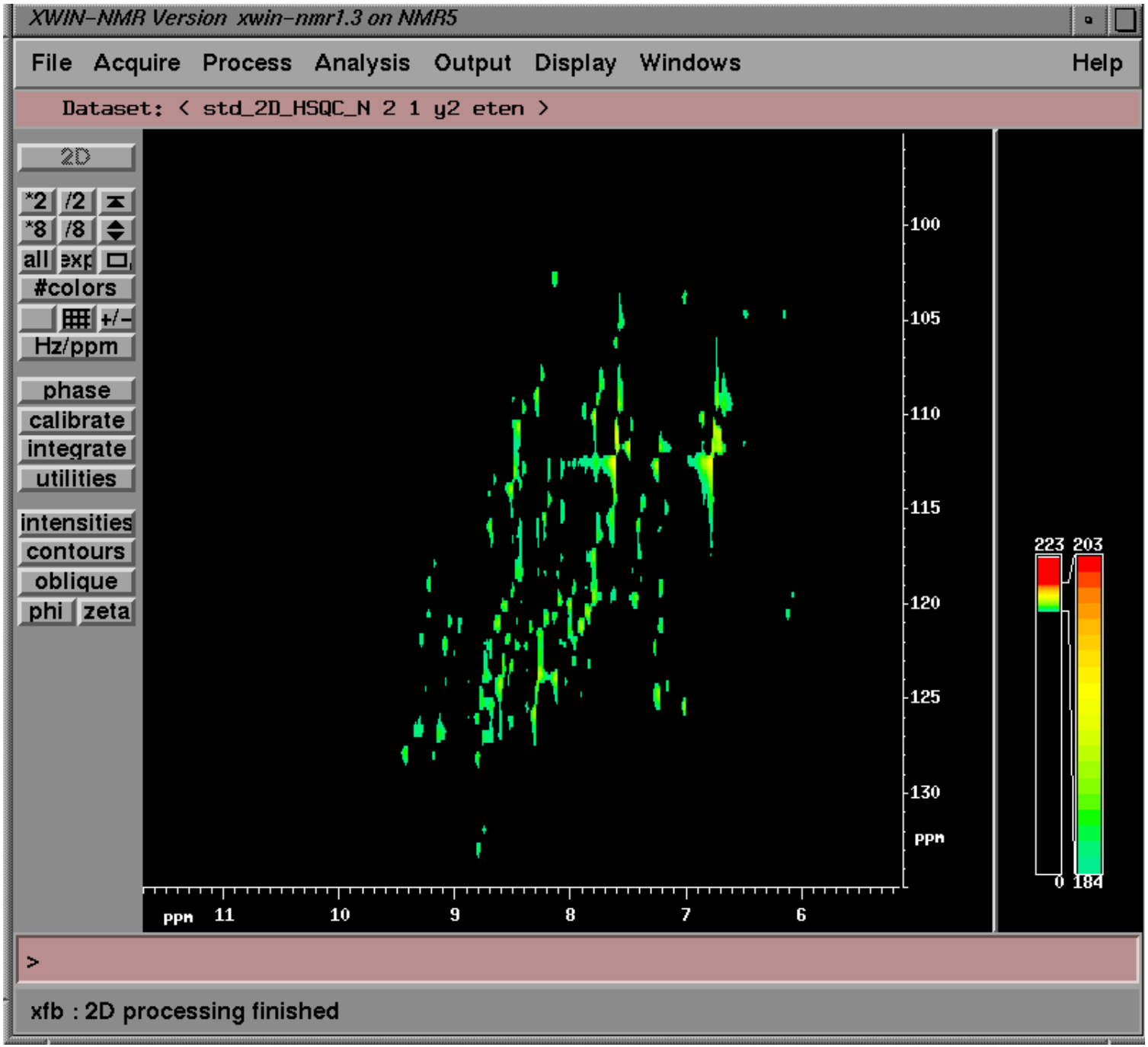
%
SAVE
Parameter
Next
CANCEL

Fourier transform

- SI (>=1/2 TD)
- xfb (execute Fourier transform both)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*8 /8
all exp
#colors
+/-
Hz/ppm
phase
calibrate
integrate
utilities
intensities
contours
oblique
phi zeta
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110
115
120
125
130
ppm
ppm 11 10 9 8 7 6
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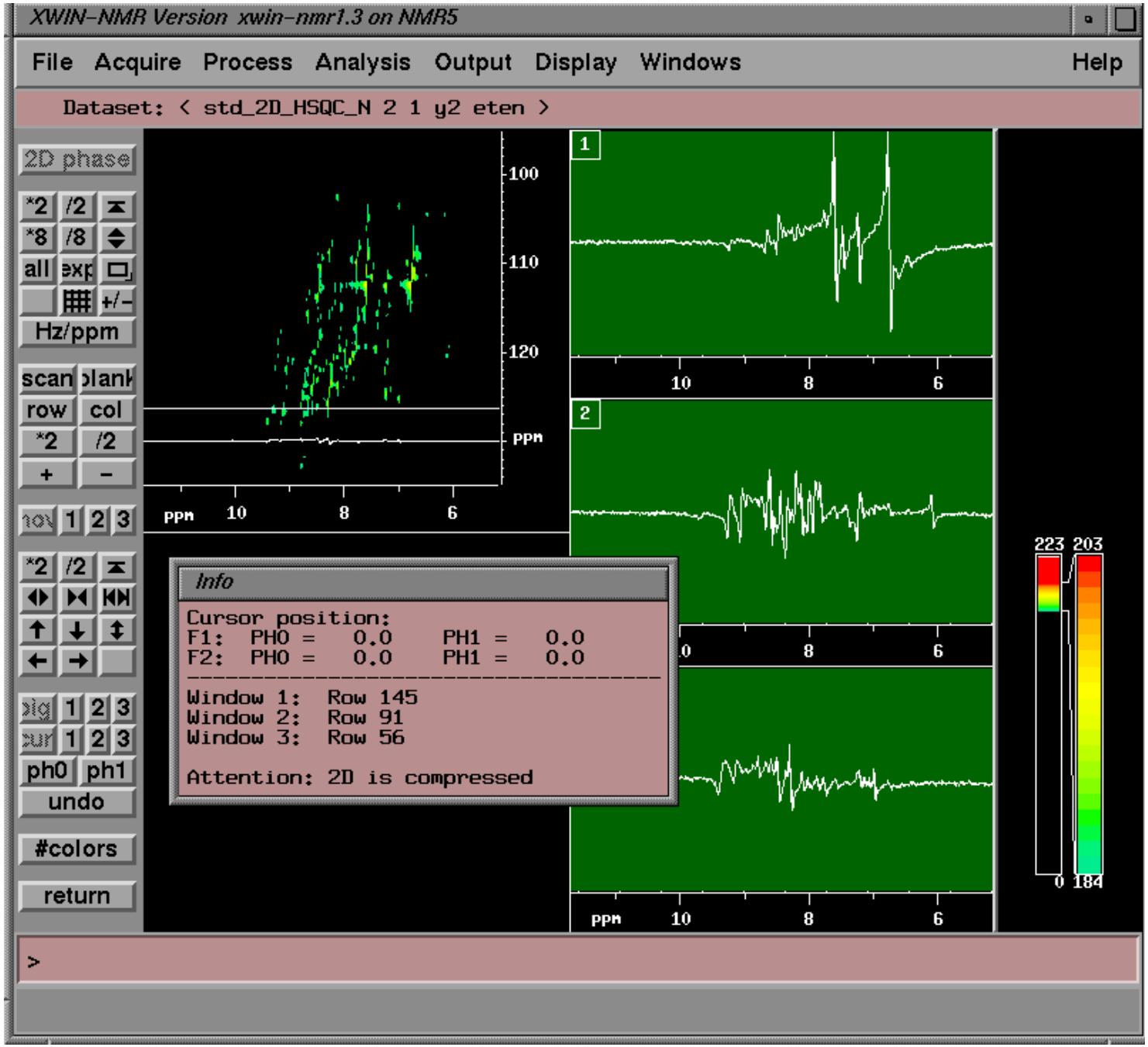
0 184
>
xfb : 2D processing finished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*8 /8
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+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
1 2 3
*2 /2
1 2 3
1 2 3
ph0 ph1
undo
#colors
return
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120
ppm
10 8 6
1
2
10 8 6
0 8 6
ppm 10 8 6
223 203
0 184
Info
Cursor position:
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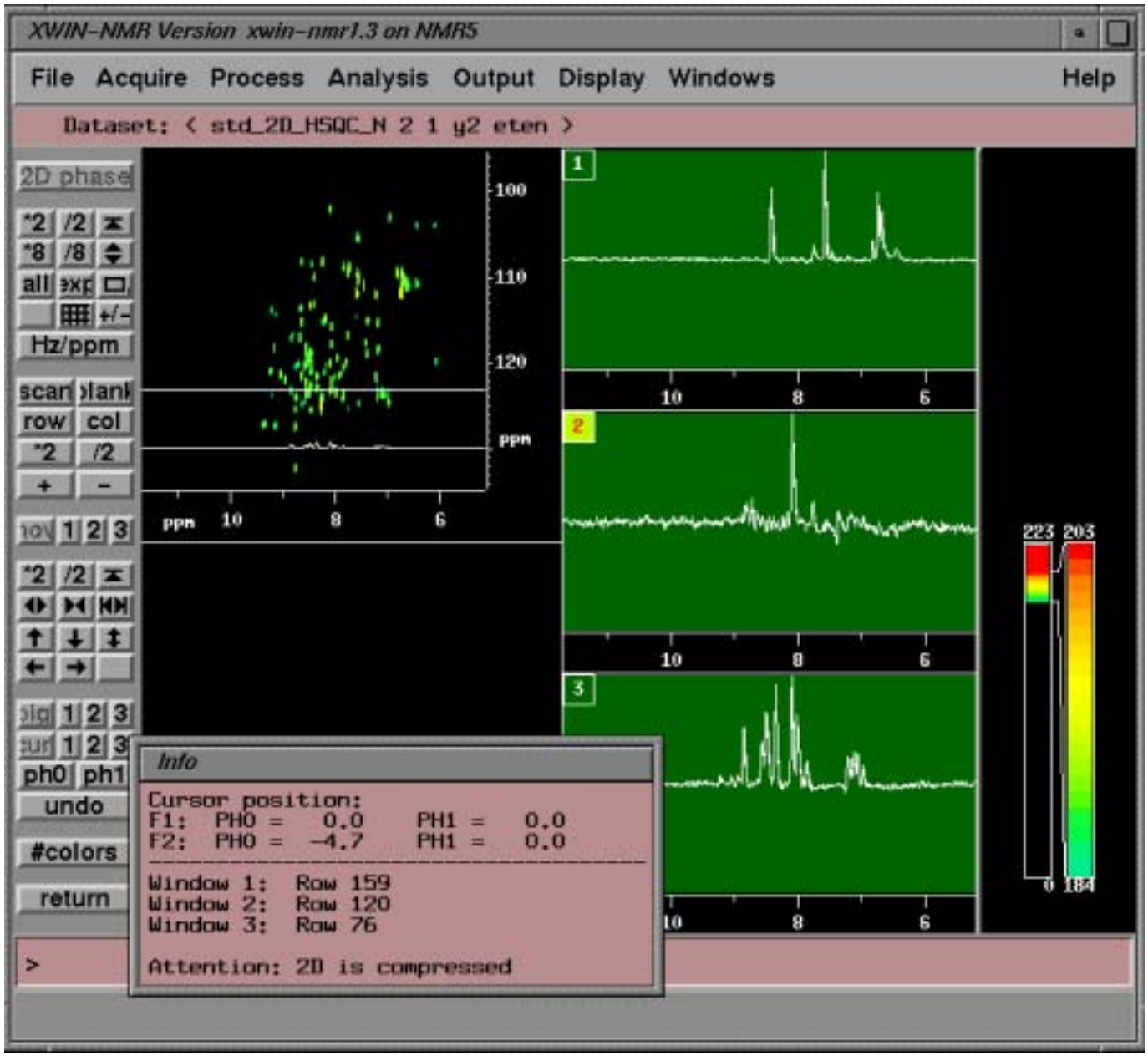
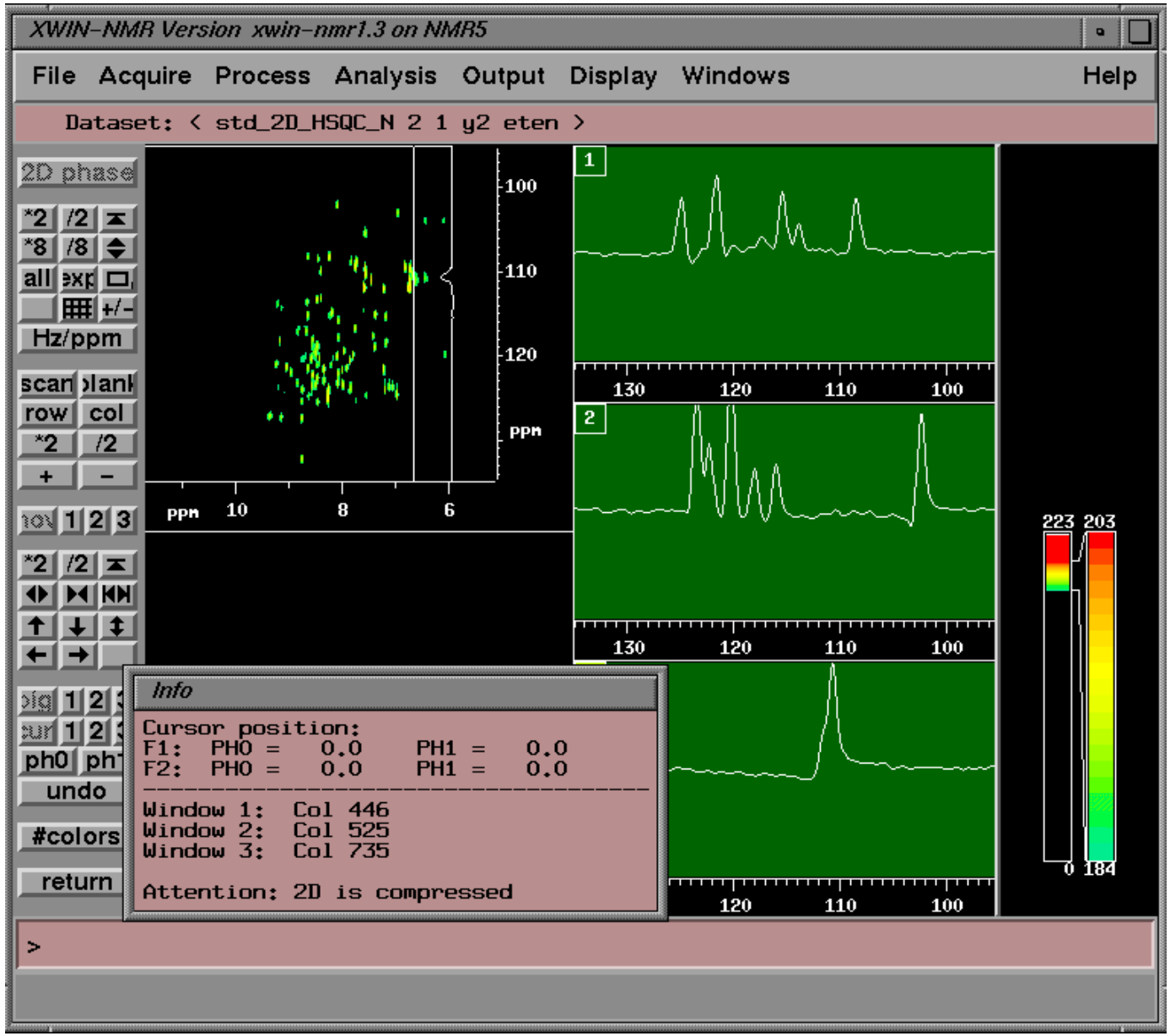
Window 2: Row 91
Window 3: Row 56
Attention: 2D is compressed
>

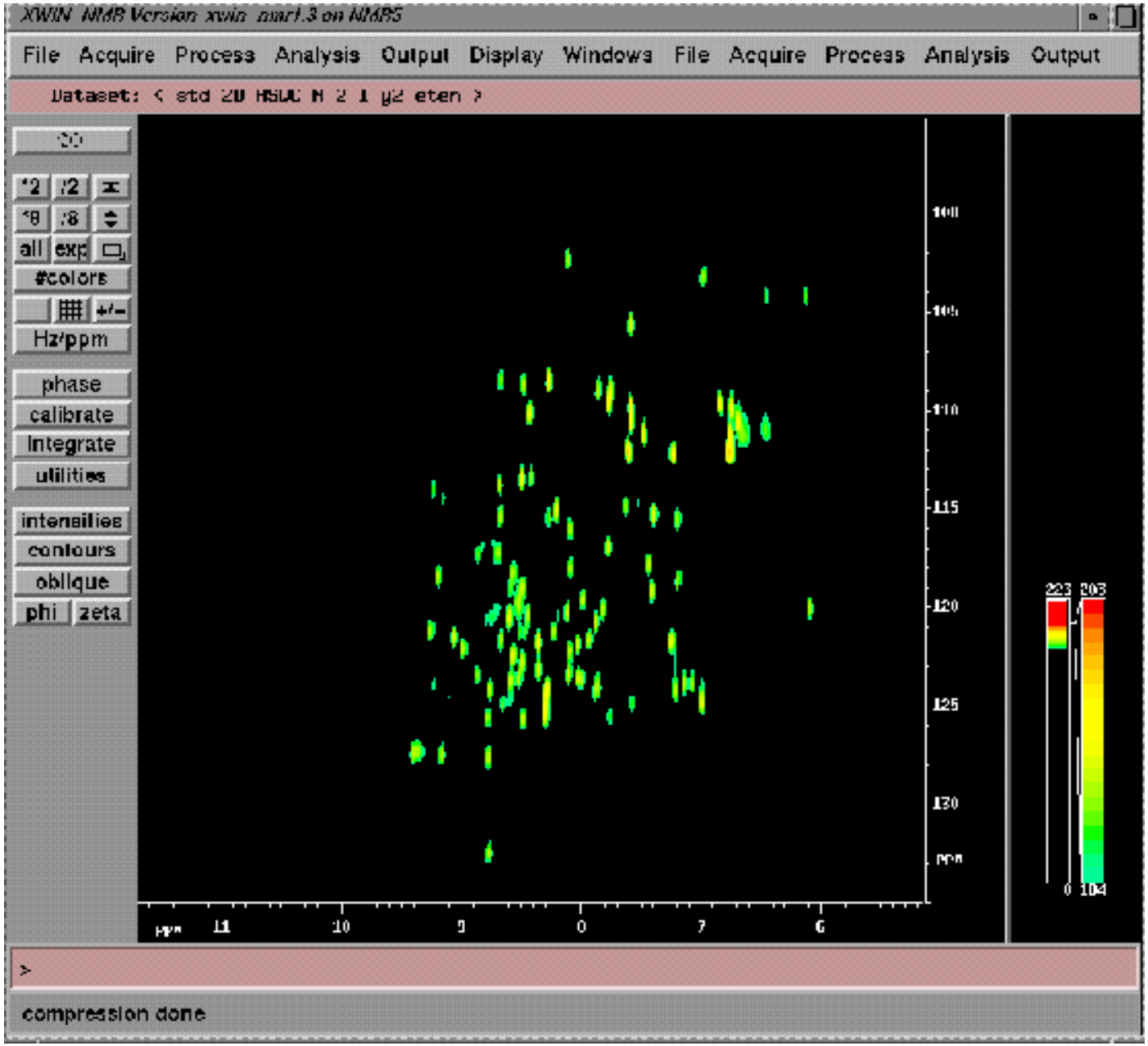
2D NMR Phase (Row)



2D NMR Phase (column)



2D spectrum after phase



Baseline Correction

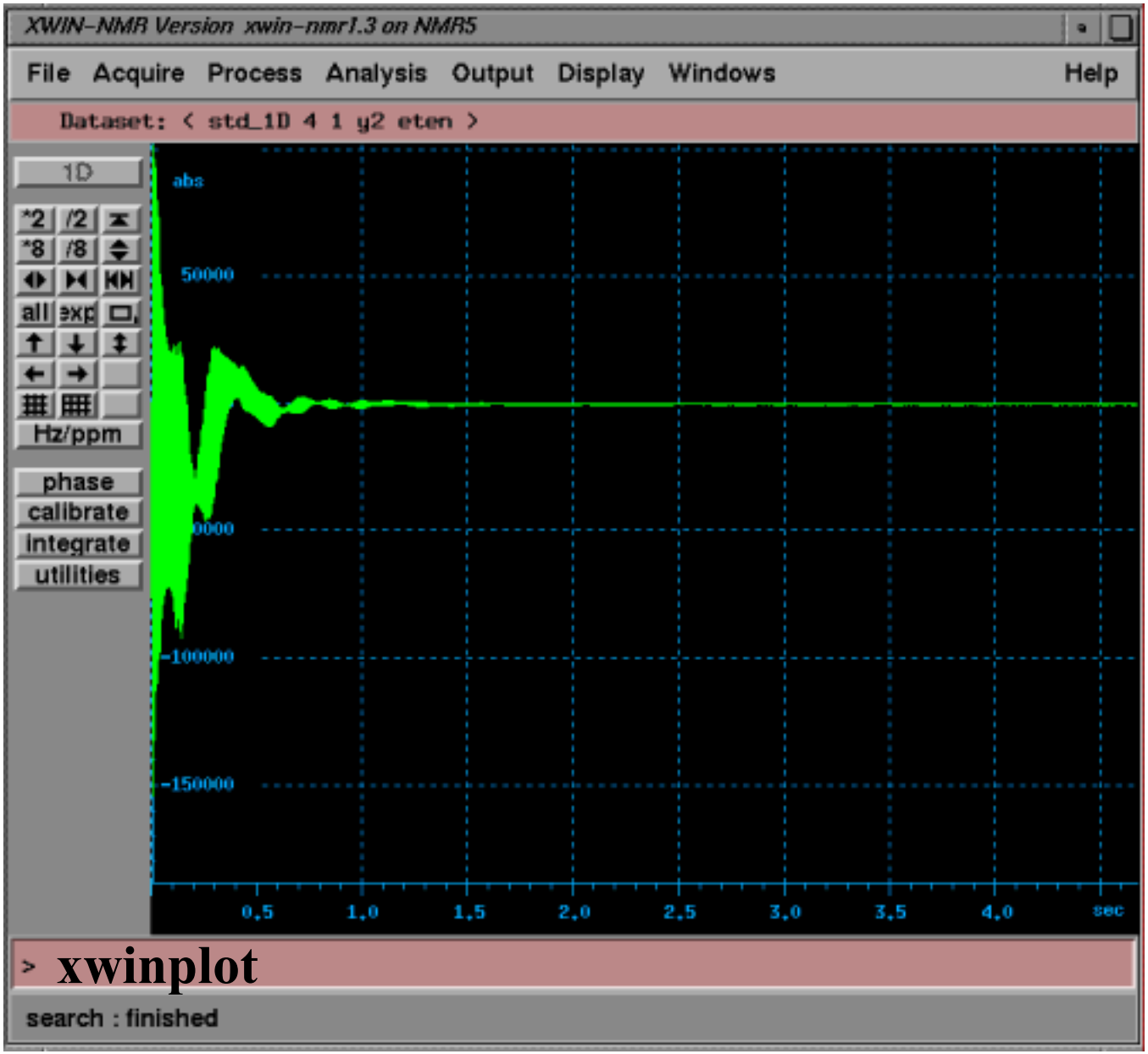
- **Automatic baseline correction**
  - abs2, abs1

Contour level (edlev)

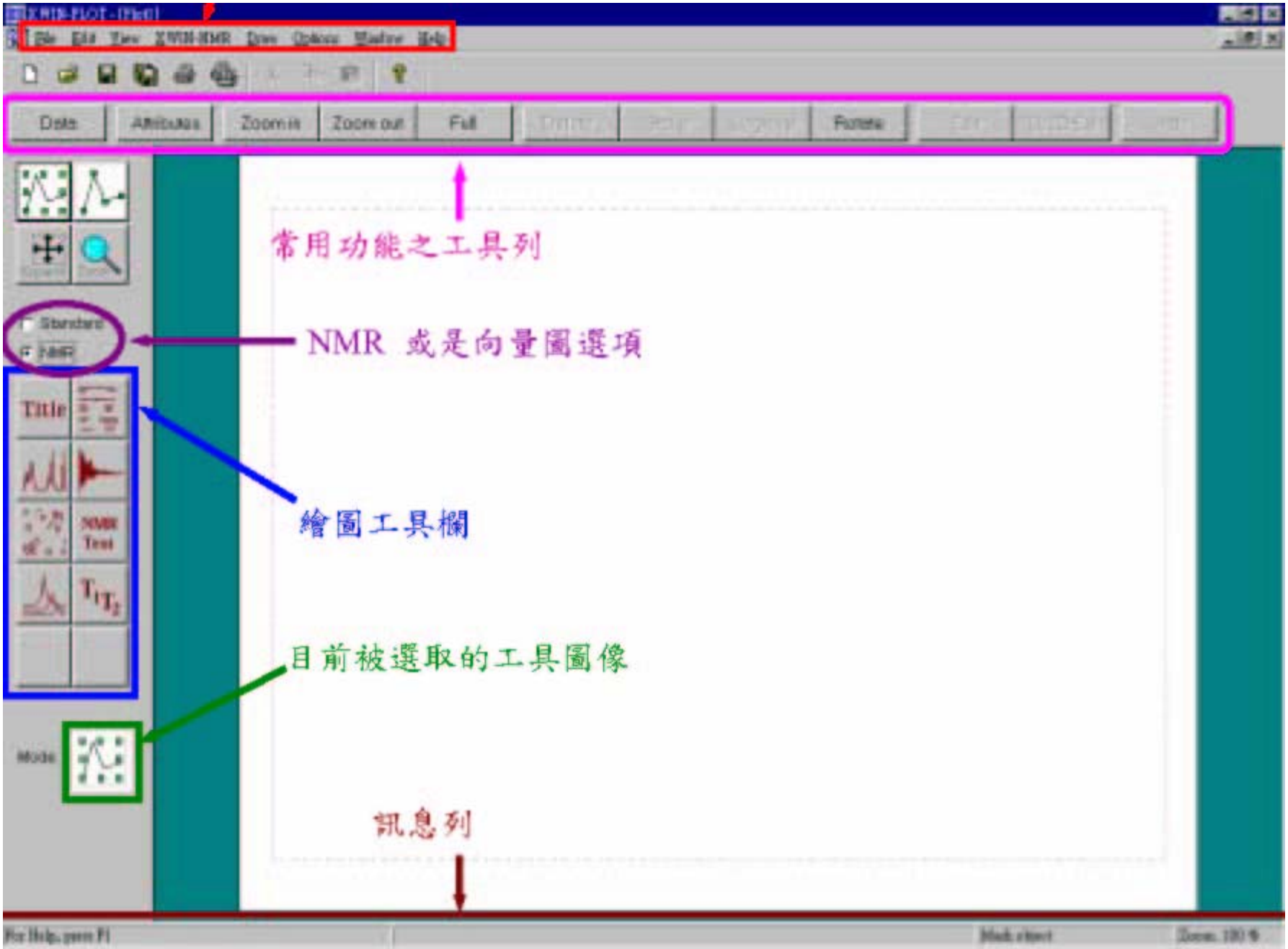
XWIN-PLOT

- 啟動XWIN-PLOT應用程式
  - Windows menu→plot-editor
- 畫出一維頻譜及參數檔內容
- 標題的設定 (title)
- 加上Peak Picking
- 加上積分值
- 列印一維頻譜

Start XwinPlot



Plot editor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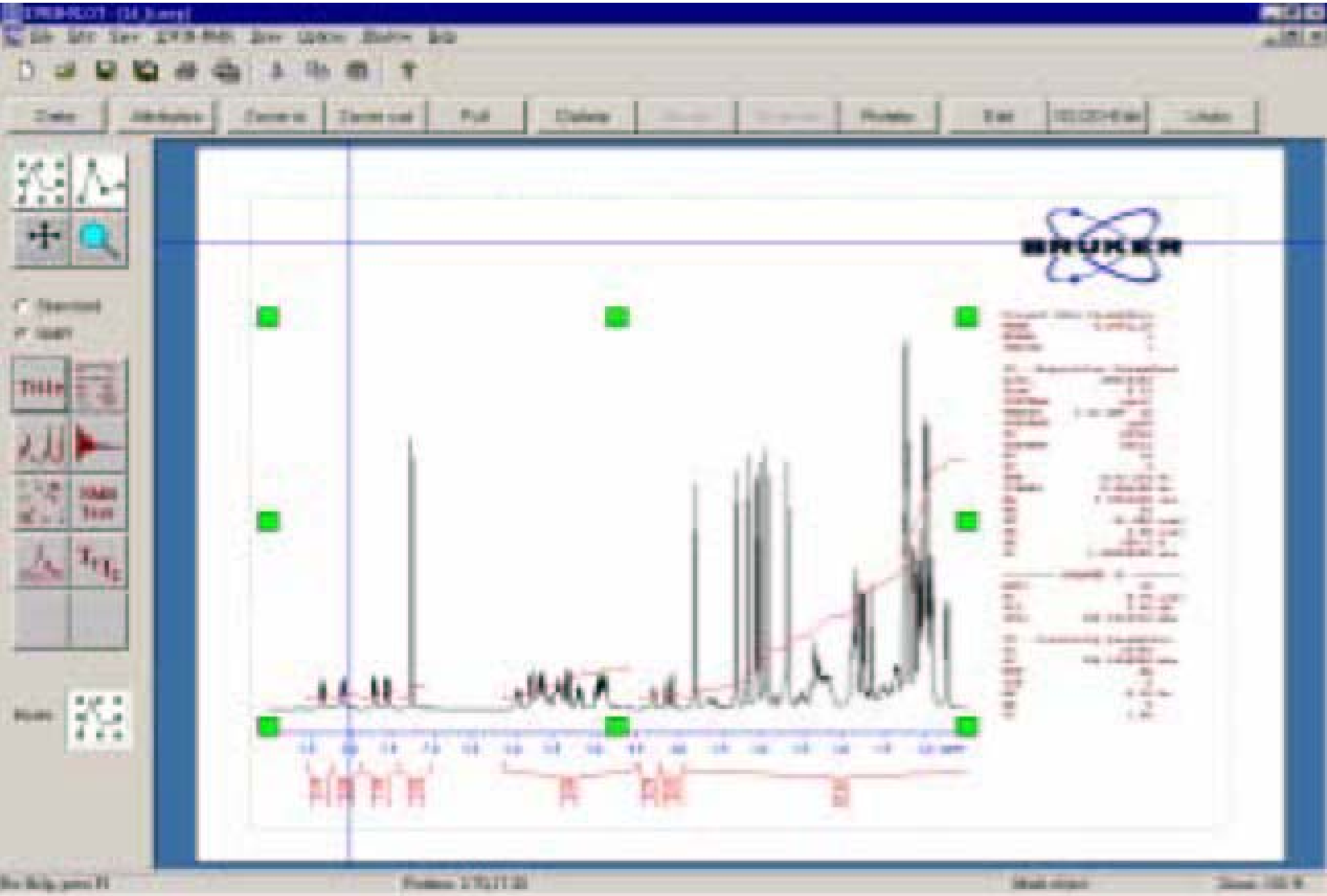
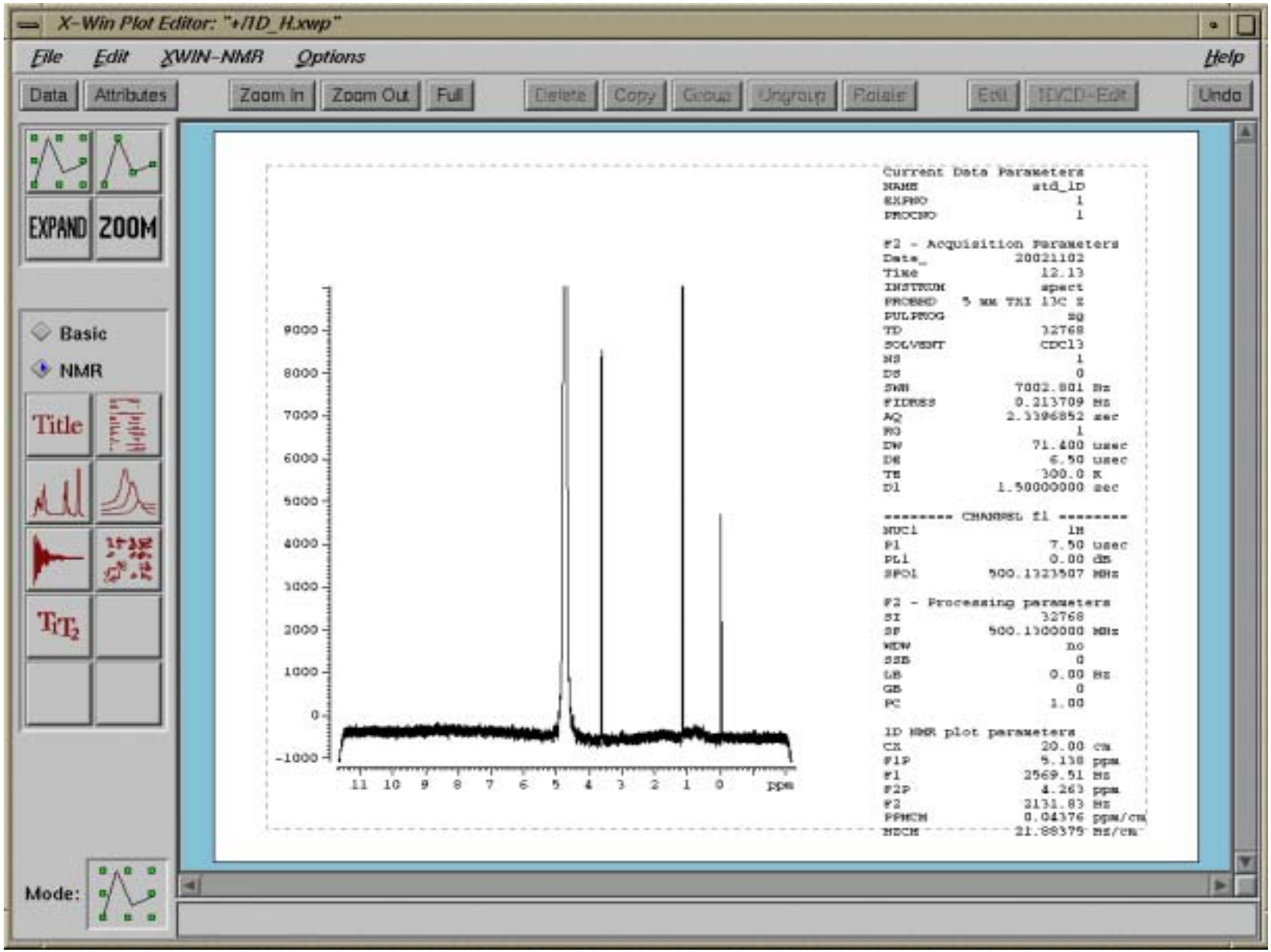
Attributes
Zoom in
Zoom out
Full
Printer
常用功能之工具列
Standard
NMR
NMR 或是向量圖選項
Title
NMR Test
繪圖工具欄
目前被選取的工具圖像
Mode
訊息列


BRUKER

XwinPlot 1D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Options
Help
Data
Attributes
Zoom In
Zoom Out
Full
Delete
Copy
Group
Ungroup
Rotate
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Undo
EXPAND
ZOOM
Basic
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Mode:
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PROCNO 1
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PULPROG zg
TD 32768
SOLVENT CDCl3
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DE 6.50 usec
TE 300.0 K
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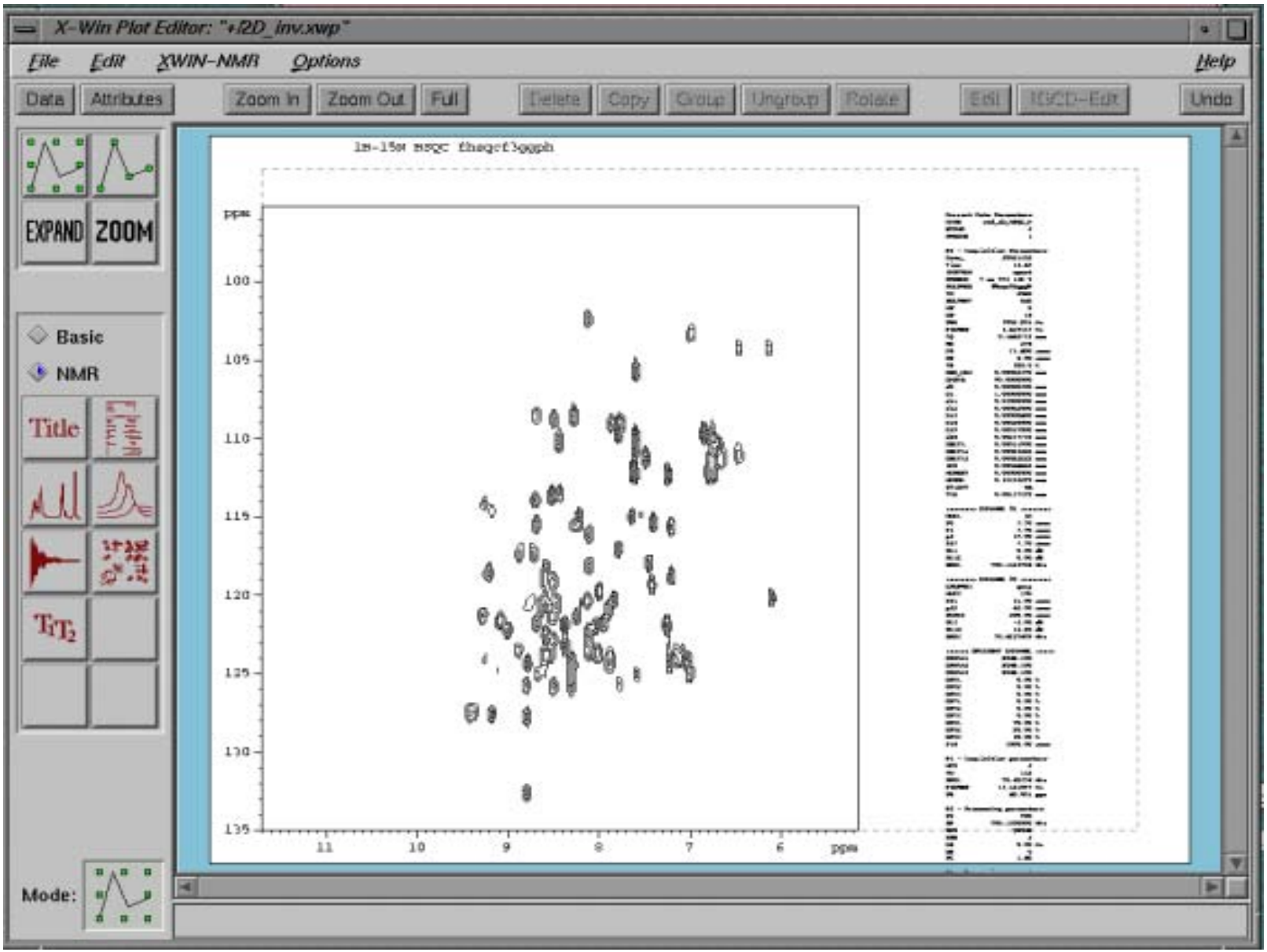
2D NMR Plot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XWIN-NMR
Options
Help
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Zoom Out
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Ungroup
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Edit
ICSD-Edit
Undo
EXPAND
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Basic
NMR
Title
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ppm
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105
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130
135
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10
9
8
7
6
ppm

Experiments Set up for hetero nuclear 2D/3D

Chi-Fon Chang

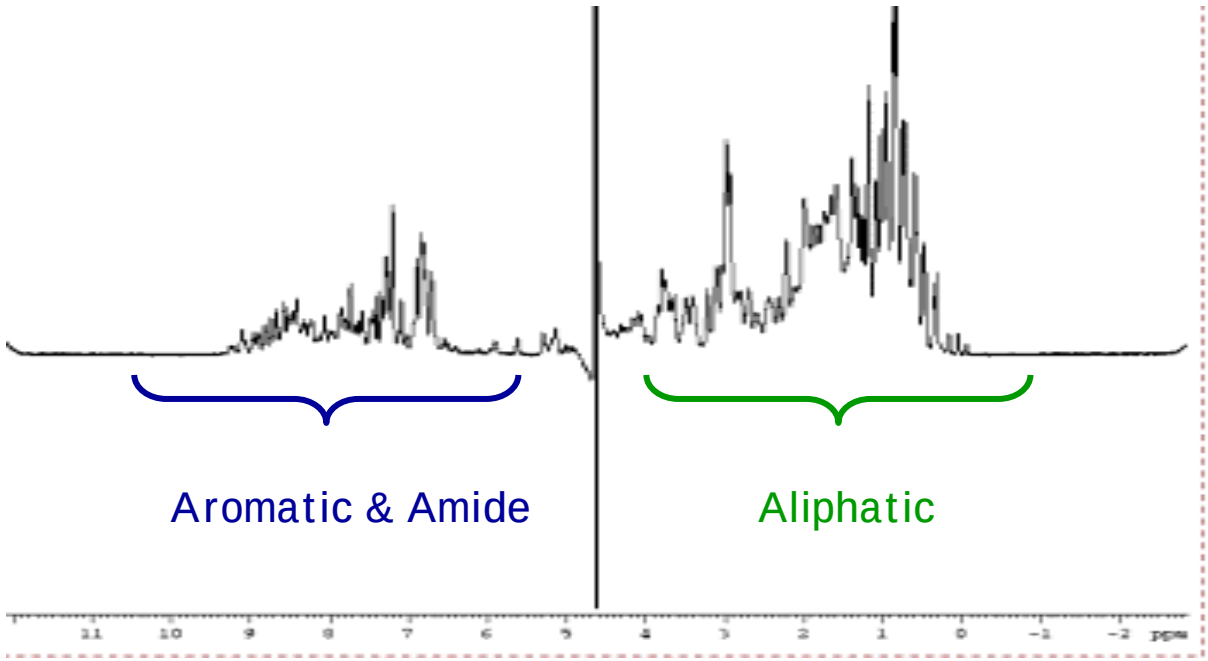
NMR Core Facility

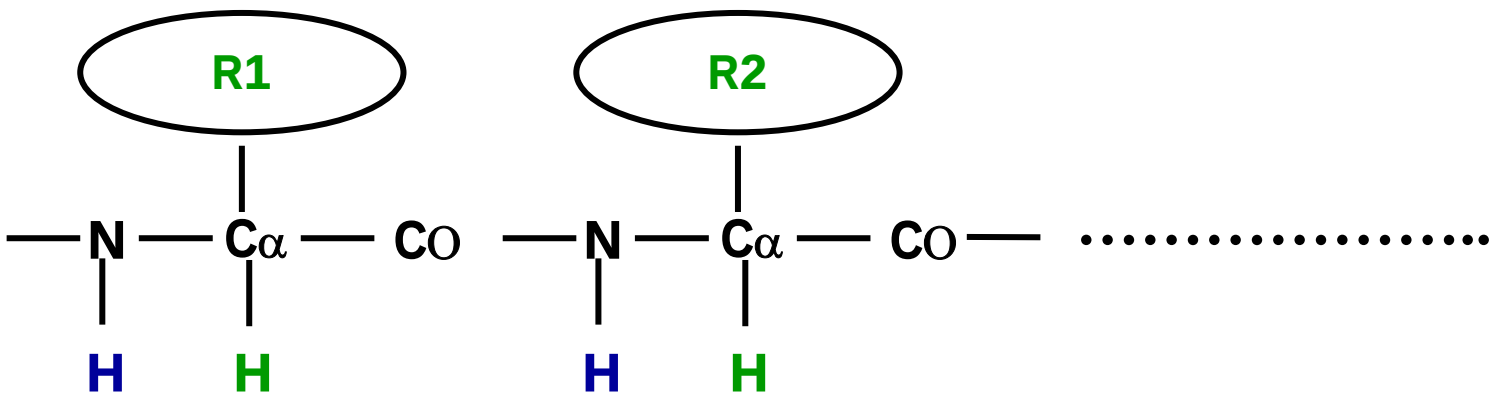
2002/12/24

Hetero Nuclear 2D/3D NMR

Why are they?

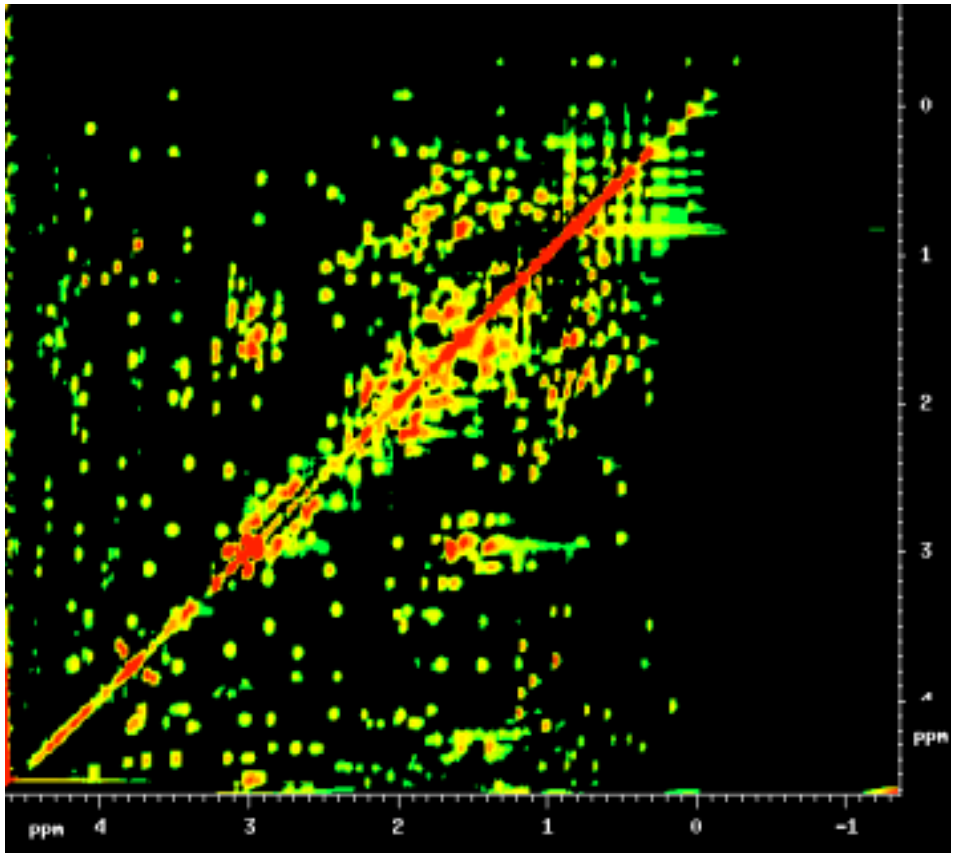
1D 1H

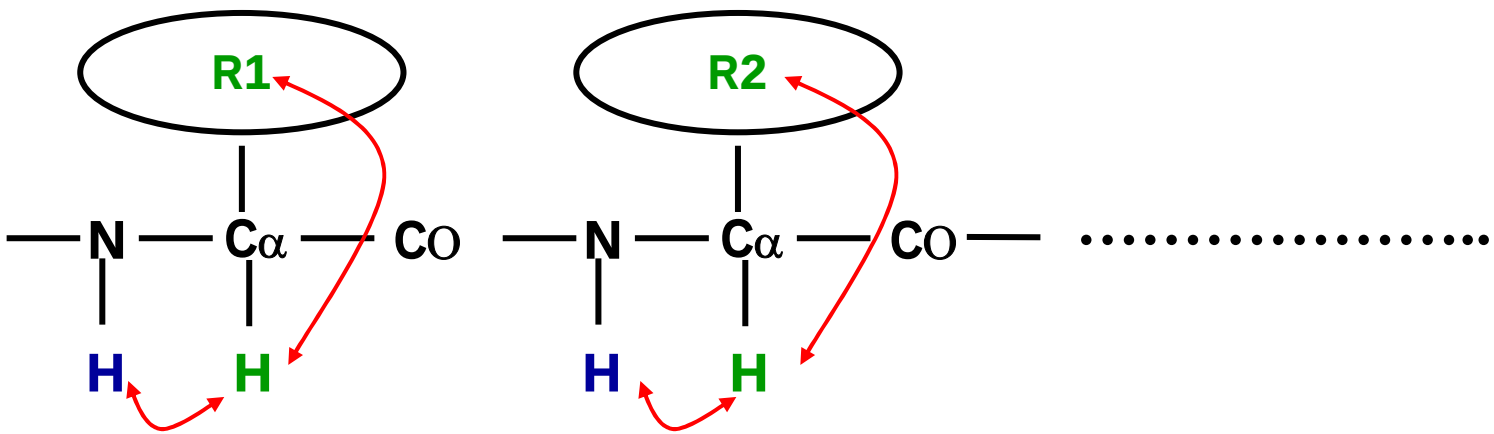




2D Homo Nuclear

1H-1H

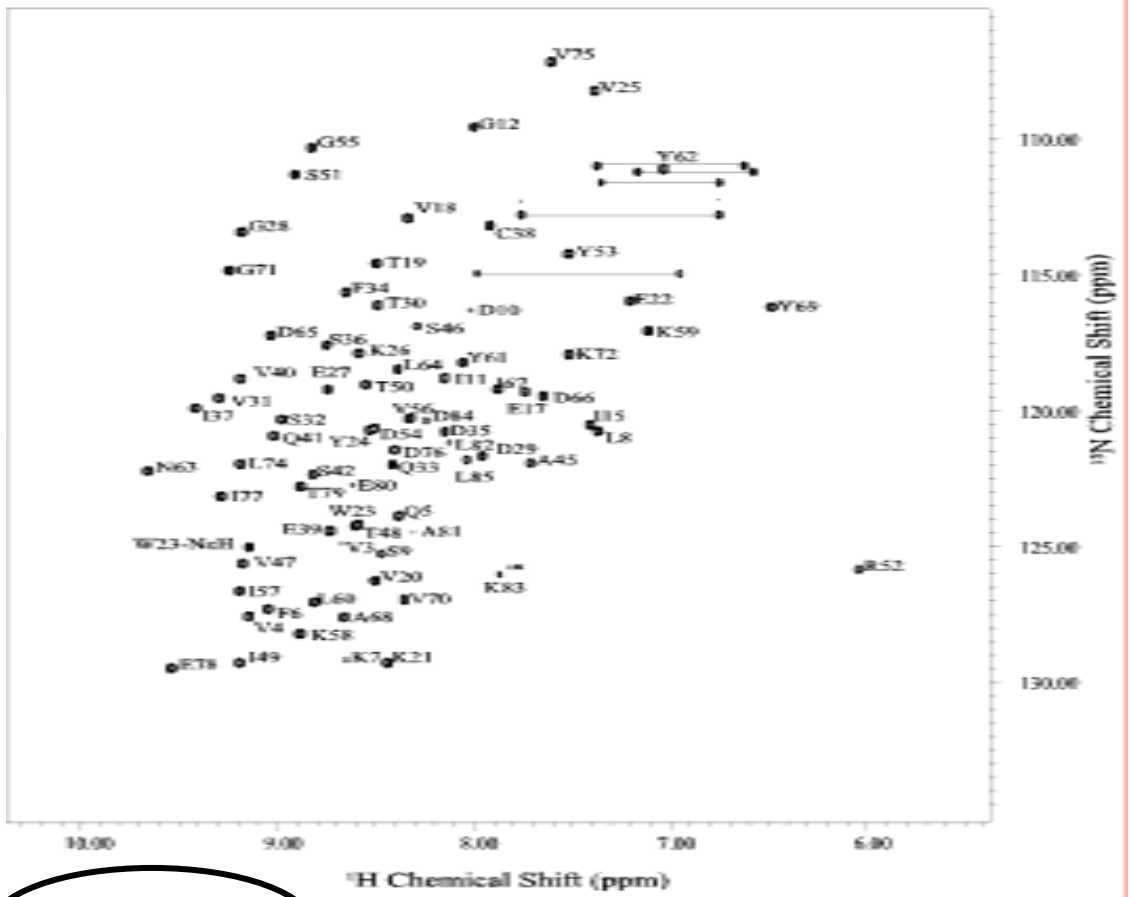


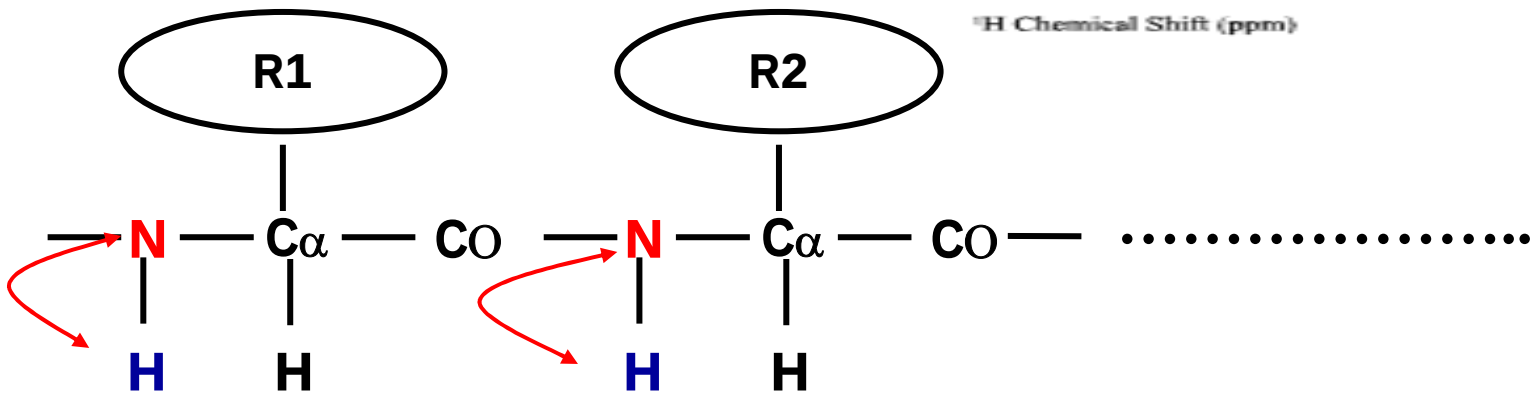


2D Hetero Nuclear

1H-15N

2D HSQC

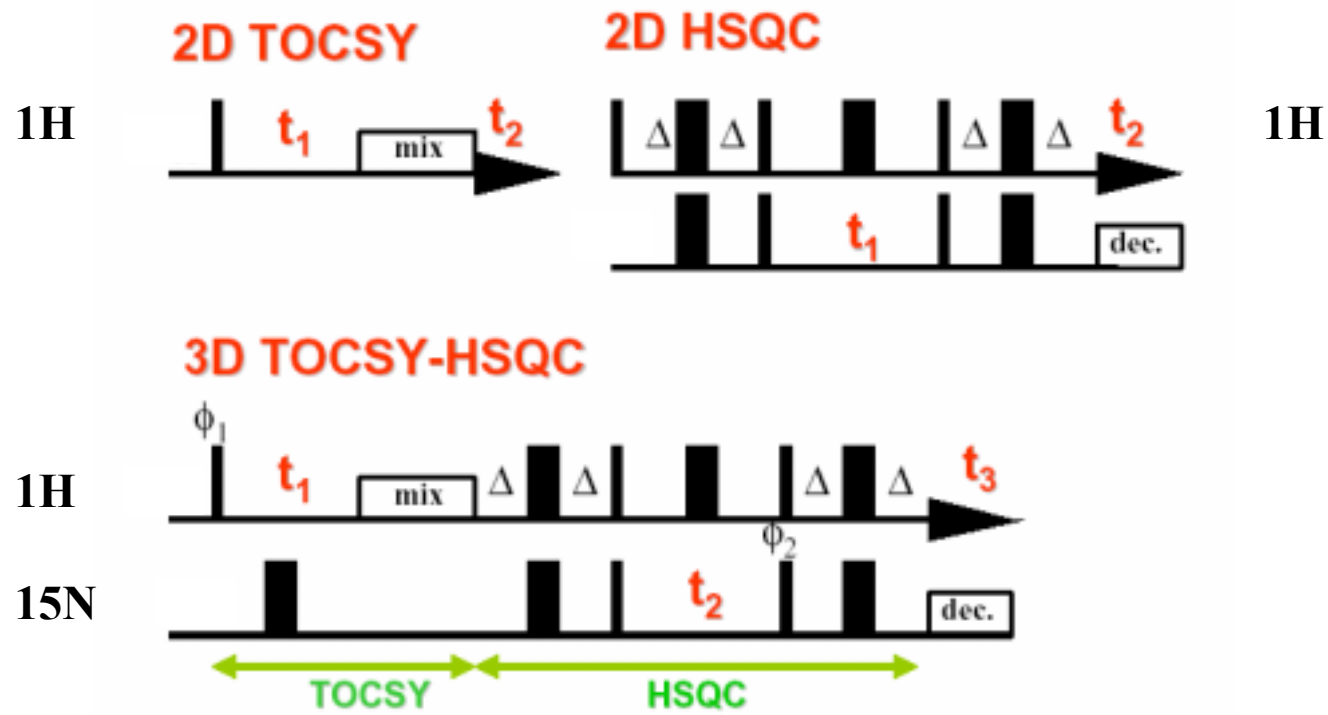


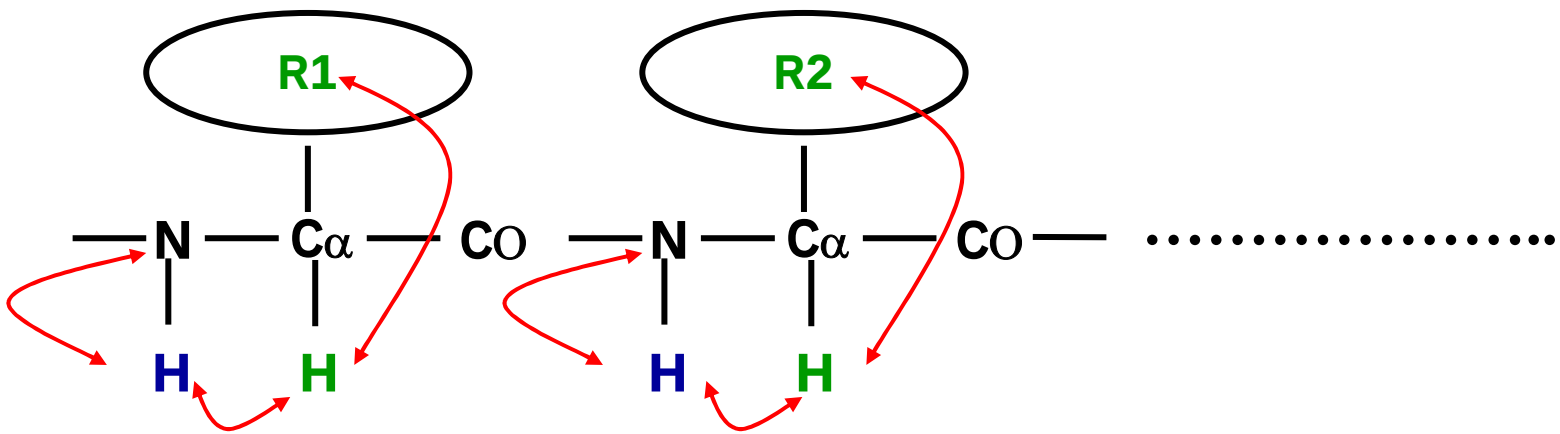


3D Hetero Nuclear

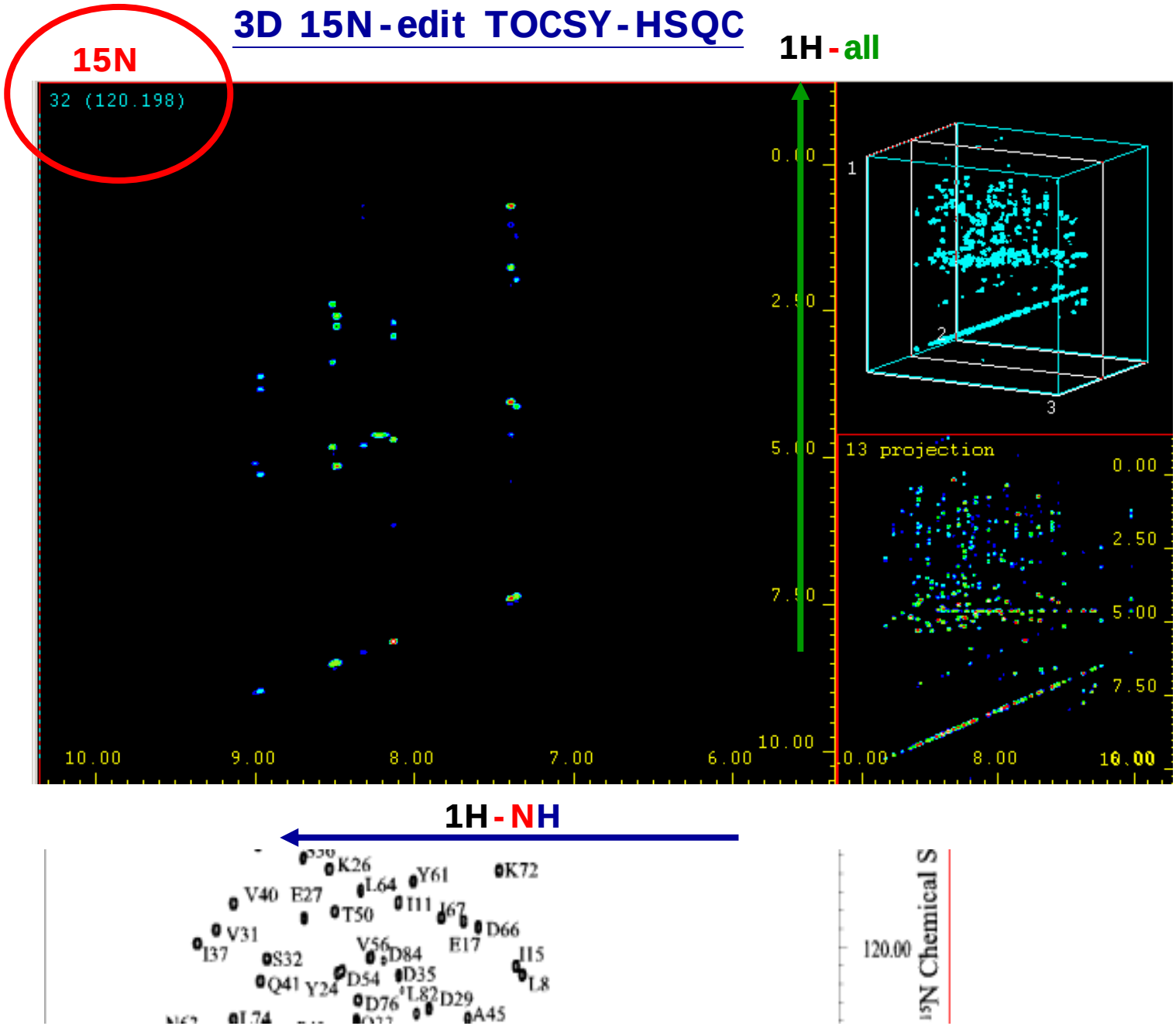
1H-15N-1H

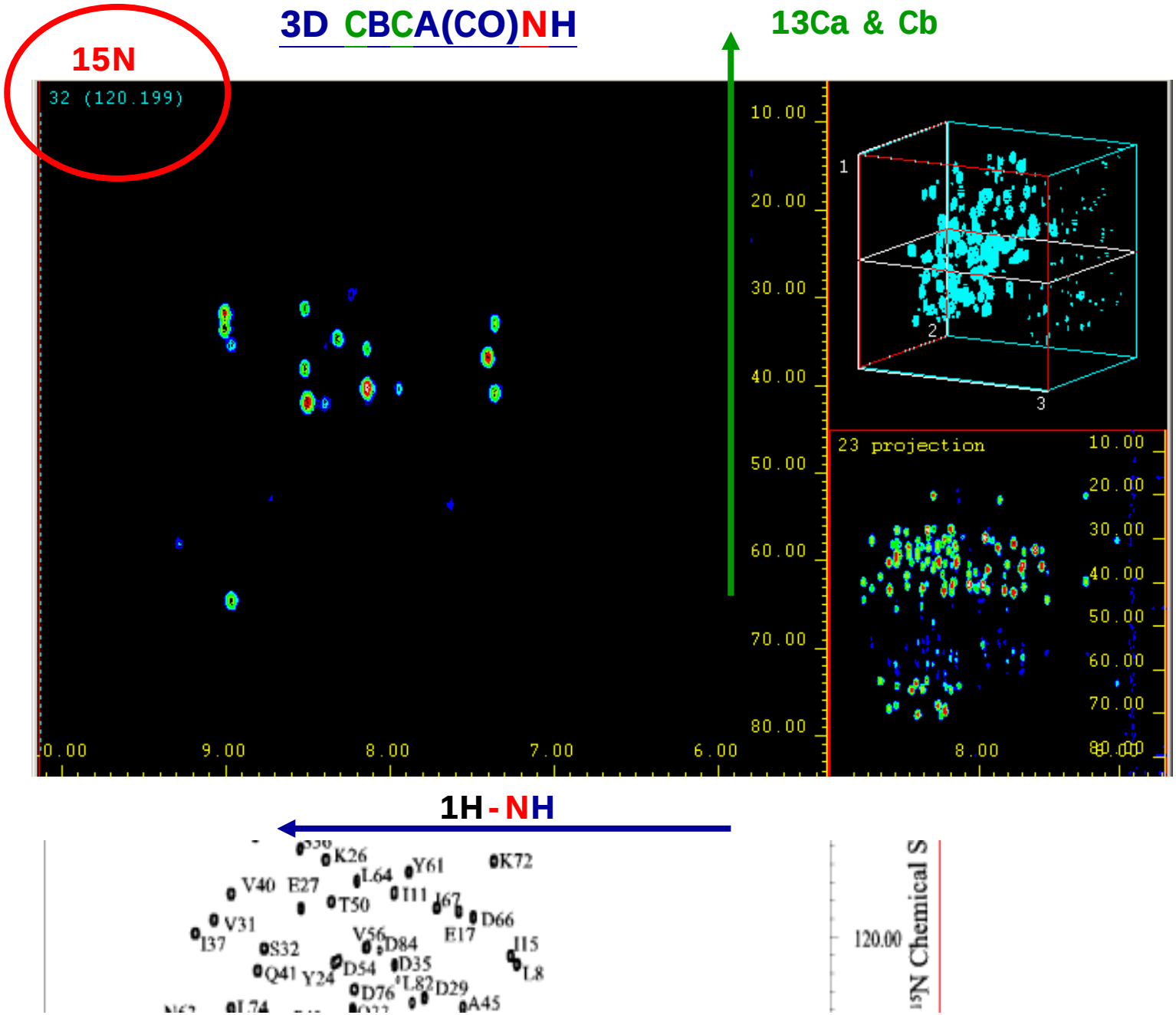
Fig. 1. The 3D pulse sequence.





3D 15N-edit TOCSY-HSQC

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13Ca & Cb
15N
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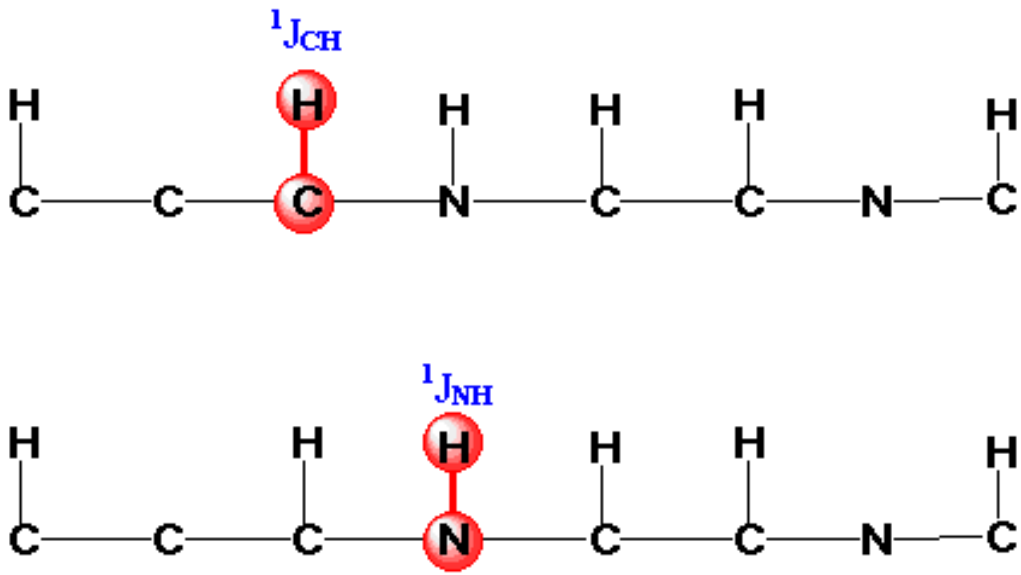
30.00
40.00
50.00
60.00
70.00
80.00
9.00
8.00
7.00
6.00
1
2
3
23 projection
1H-NH
K26
L64
Y61
K72
V40
E27
T50
I11
I67
D66
V31
I37
S32
V56
D84
E17
I15
Q41
Y24
D54
D35
L8
D76
L82
D29
A45
120.00
Chemical S

Hetero Nuclear 2D/3D NMR

What are they?

Example of 2D Hetero Nuclear NMR: HSQC or HMQC

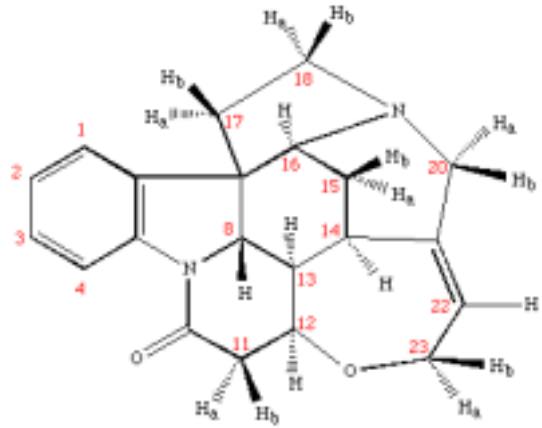
The HSQC or HMQC experiment allows to trace out directly bonded ^{1}H-X pairs via the large $^{1}J_{HX}$ coupling constant

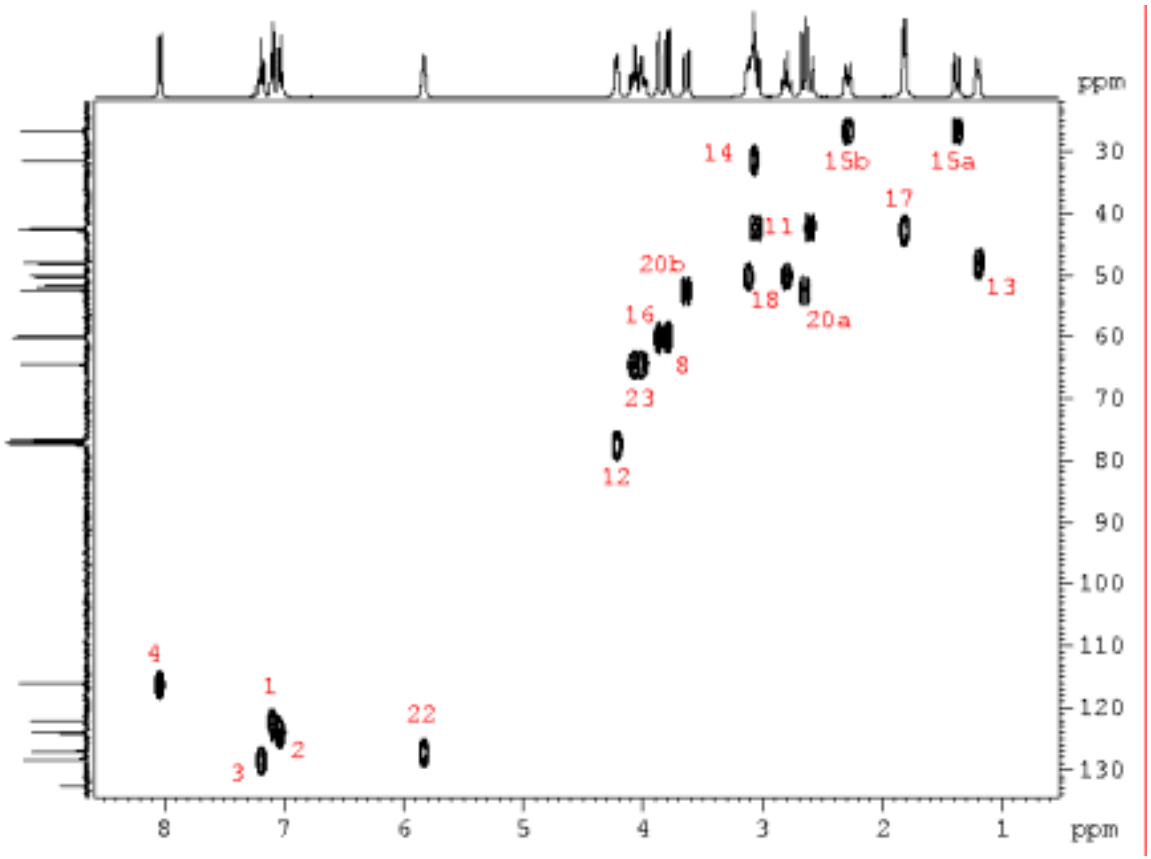


2D 1H-13C HSQC

Strychnine

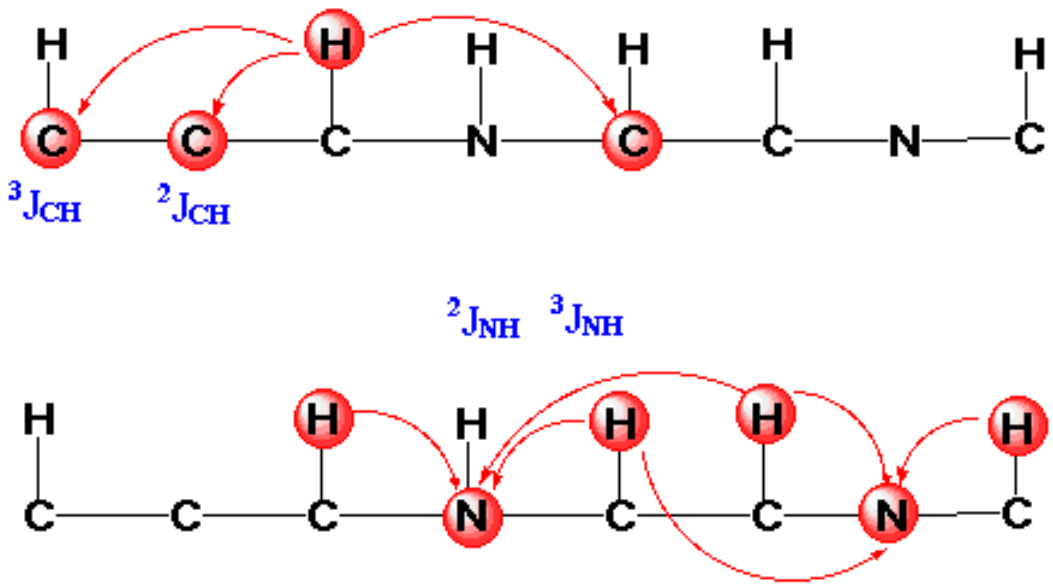
Solvent: $CDCl_3$



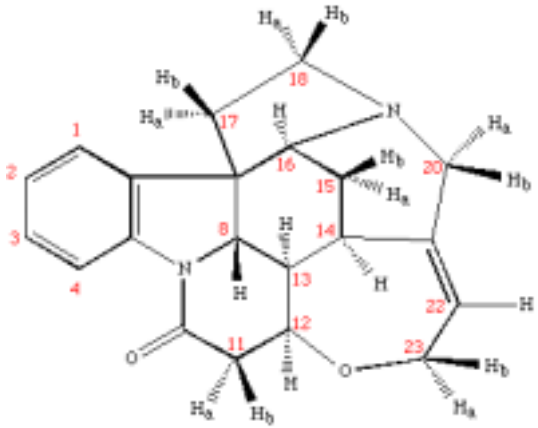


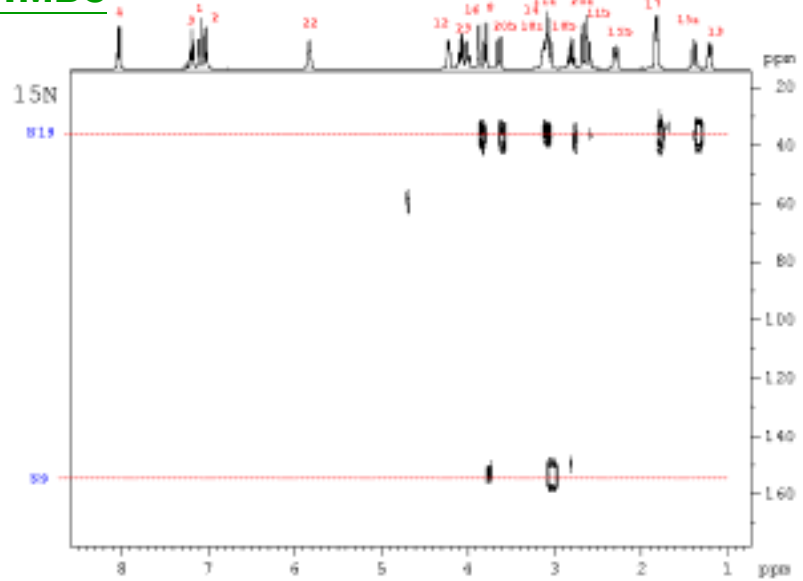
Example of 2D Hetero Nuclear NMR: HMBC

The HMBC experiment allows to trace out longa-range (typically two- and three-bonds away) ^{1}H-X pairs via the small $^{n}J_{HX}$ coupling constant.

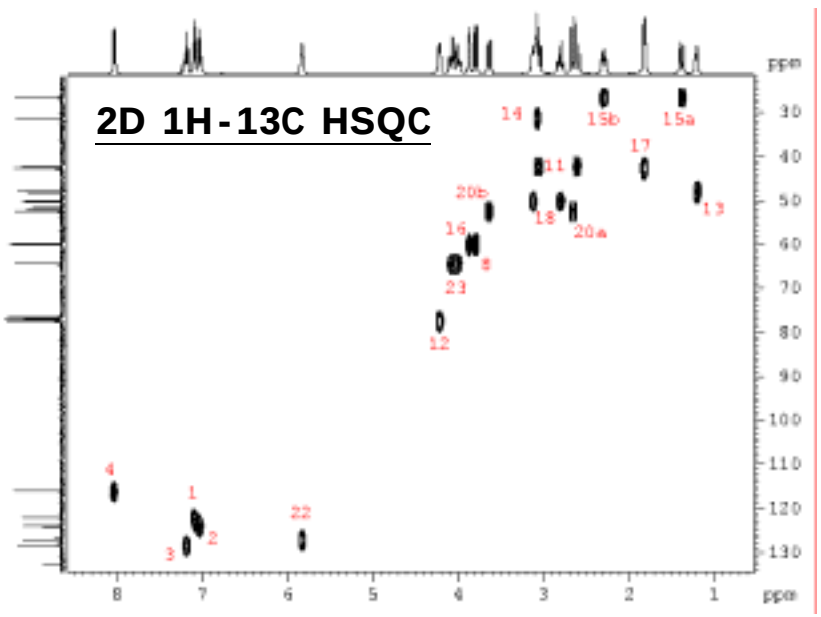


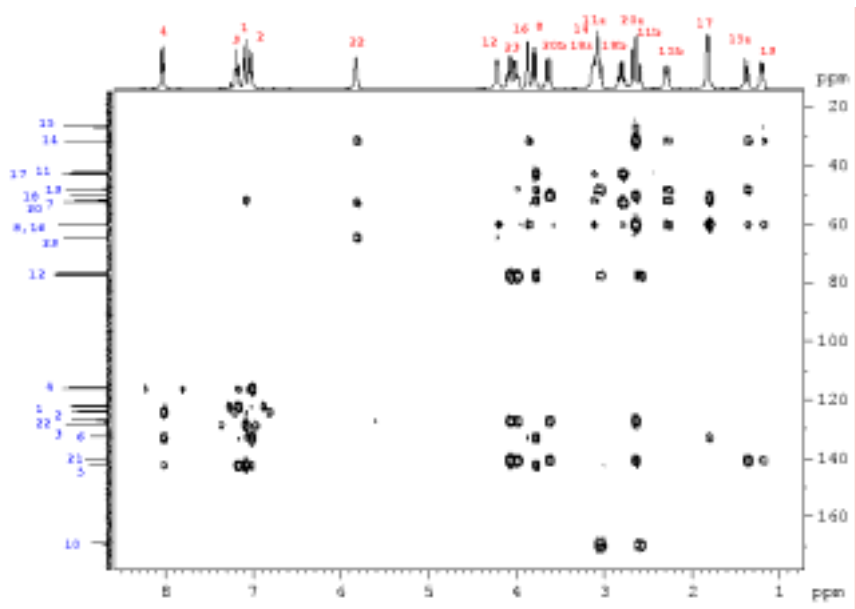
2D 1H-15N HMBC





2D 1H-13C HMBC

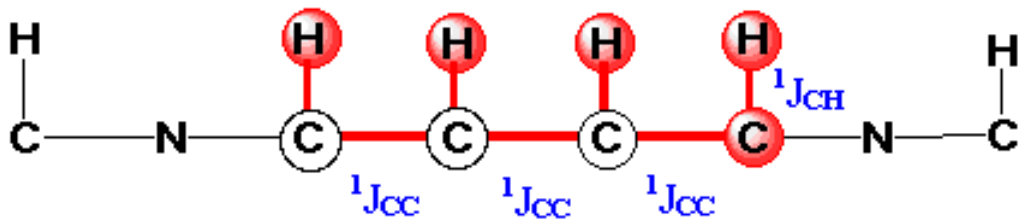


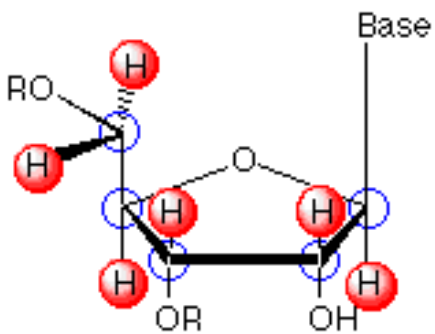


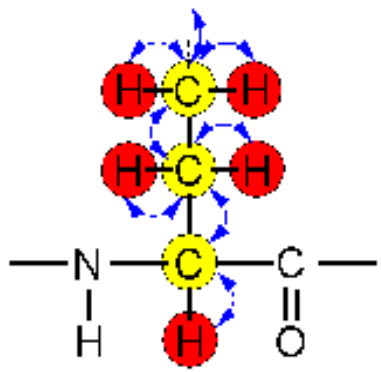
Example of 3D Double Resonance NMR: HCCH-TOCSY (side chain assignment)

The 3D HCCH-TOCSY experiment is specifically designed to correlate side-chain aliphatic proton and ^{13}C resonances via $^{1}J(CH)$ and $^{1}J(CC)$ coupling constants. The experiment provides nearly complete assignments of all aliphatic ^{1}H and ^{13}C resonances.

1H-13C



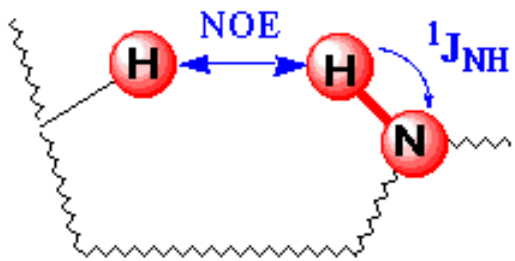




Example of 3D Double Resonance NMR: NOESY-HS(M)QC (through space)

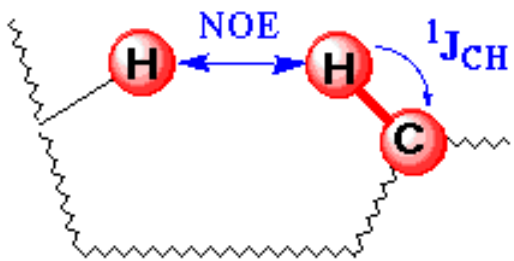
The 3D NOESY-HSQC or NOESY-HMQC experiment is specifically designed to obtain X-edited NOESY spectra of X-labeled biomolecules from which homonuclear ^{1}H-^{1}H NOEs can be clearly assigned even in overcrowded regions

3D 15N-edit NOESY-HSQC



1H-15N

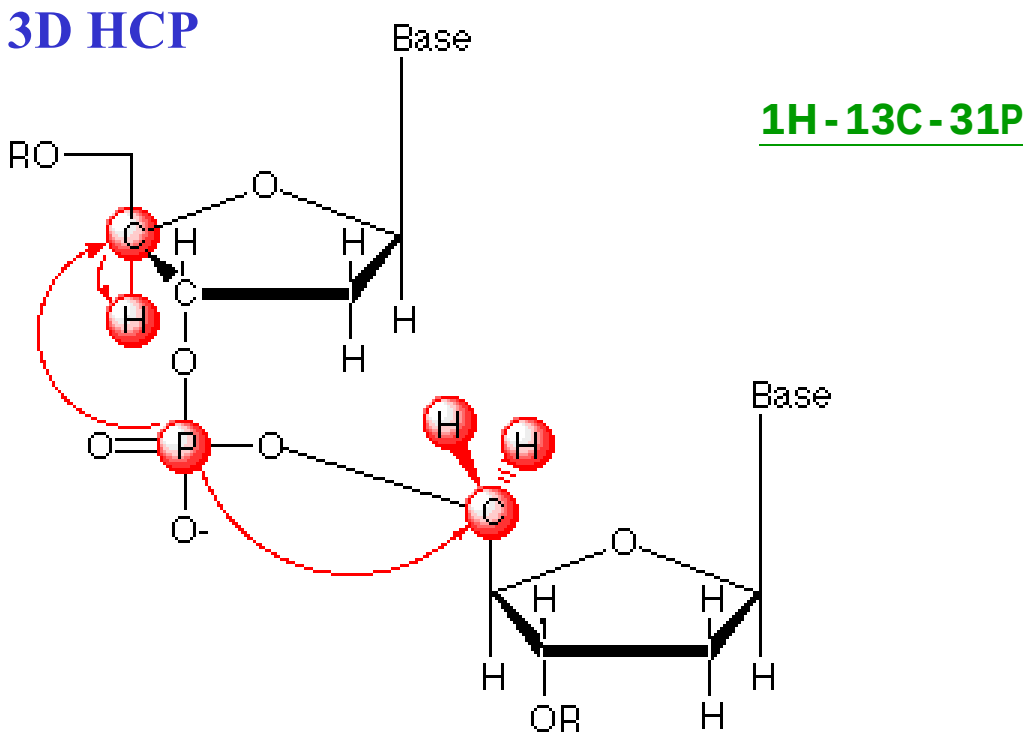
3D 13C-edit NOESY-HSQC



1H-13C

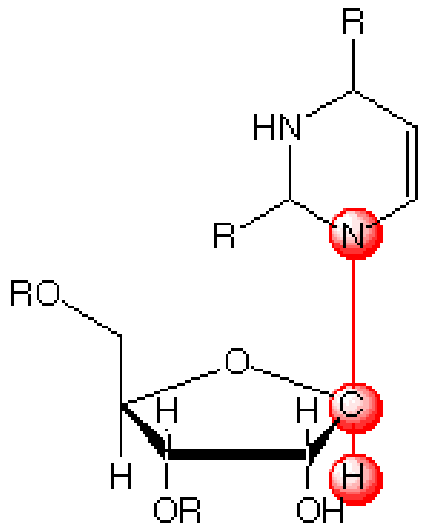
Example of 3D Triple Resonance NMR: for Nucleic Acids

The 3D HCP experiment is a ^{1}H-^{13}C-^{31}P triple-resonance experiment specifically designed to assign the ribose H3'/C3', H4'/C4' on the 5' side and, H4'/C4' and H5',H5''/C5' resonances on the 3' side of the intervening phosphorus in ^{13}C-labeled nucleic acids.



The 3D HCN experiment allows to obtain sugar-to-base correlations from the H1'(sugar) to N1/N9 (base) via C1'(sugar) ($H_sC_sN_b$ experiment)

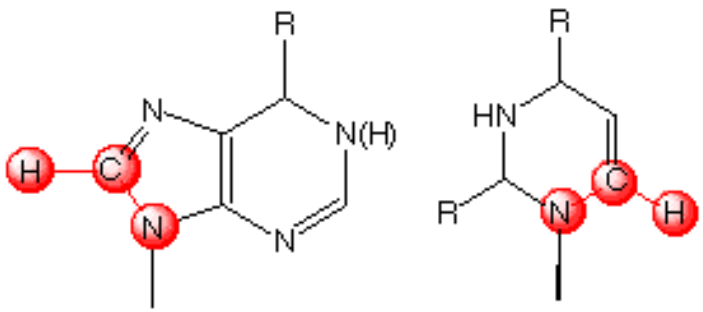
3D HCN



1H-13C-15N

HsCsNb

or non-exchangeable base proton assignments from the H6/H8 (base) to N1/N9 (base) via C6/C8 (base) ($H_bC_bN_b$ experiment) in ^{13}C,^{15}N-labeled nucleic acids.



1H-13C-15N

HbCbNb

Example of 3D Triple Resonance NMR: for protein/peptide sequential

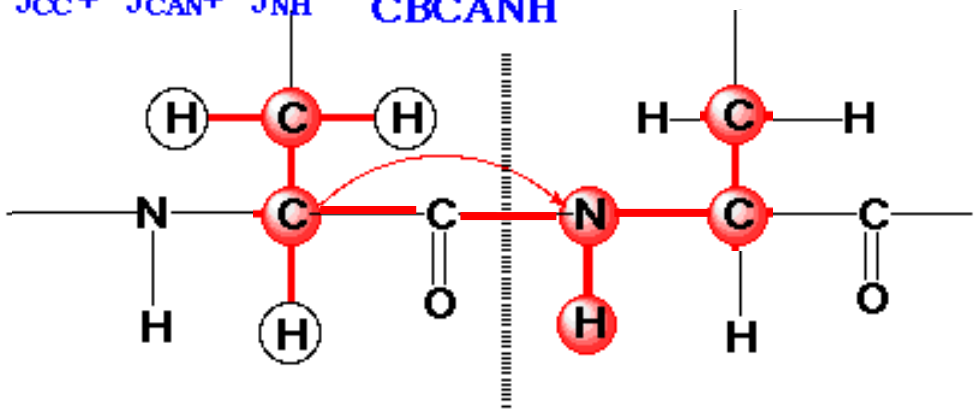
1H-13C-15N

- Sequential Assignment
- Chemical Shifts of Cα , Cβ
- H^N & N^H chemical Shifts

$^1J_{CH} + {}^1J_{CC} + {}^1J_{CAN} + {}^1J_{NH}$

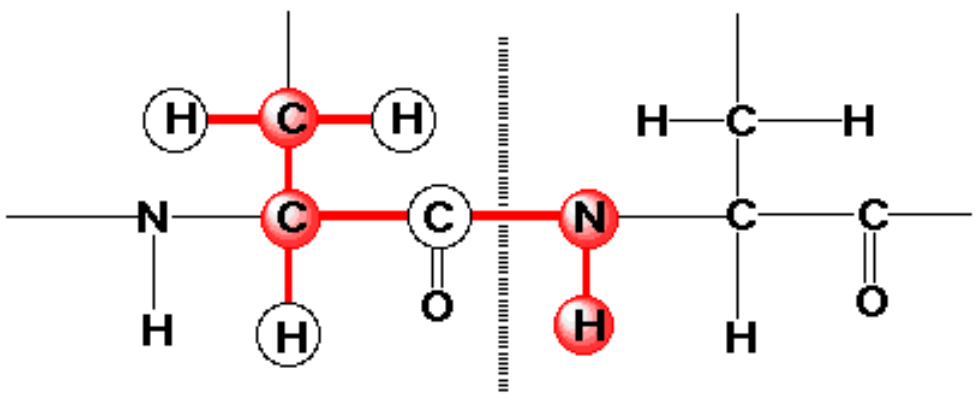
$^1J_{CH} + {}^1J_{CC} + {}^2J_{CAN} + {}^1J_{NH}$

CBCANH



$^1J_{CH} + {}^1J_{CC} + {}^1J_{CON} + {}^1J_{NH}$

CBCA(CO)NH

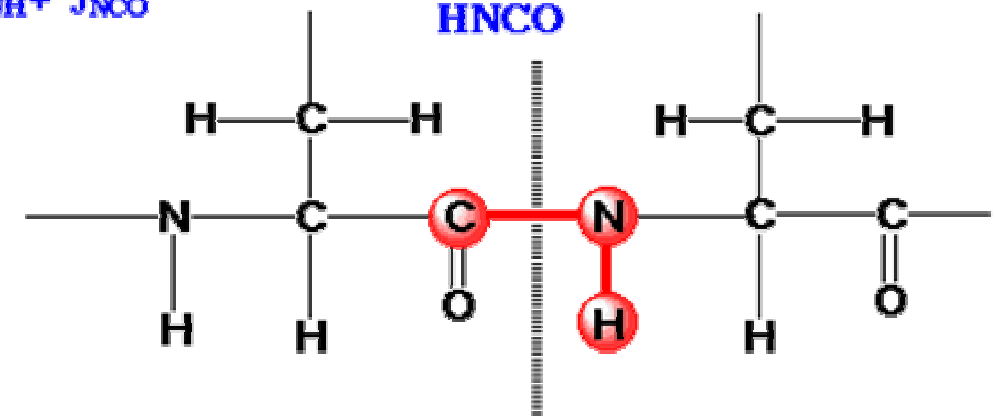


1H-13C-15N

- Sequential Assignment
- Chemical Shifts of CO
- H^N & N^H chemical Shifts

$^1J_{NH} + {}^1J_{NCO}$

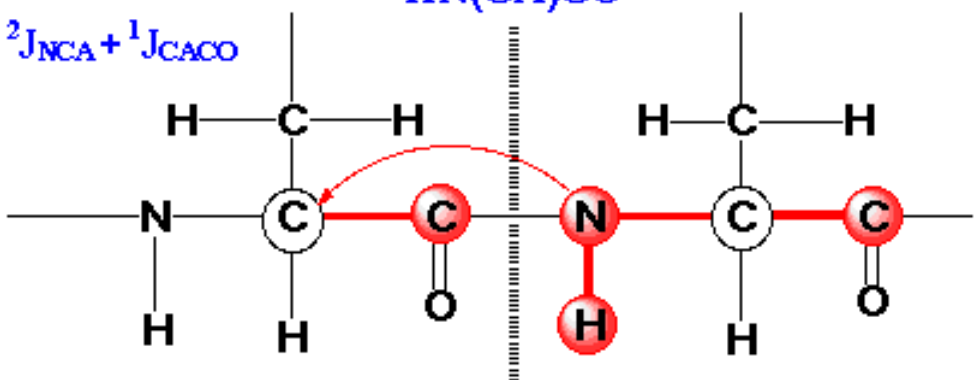
HNCO



$^1J_{NH} + {}^1J_{NCA} + {}^1J_{CACO}$

$^1J_{NH} + {}^2J_{NCA} + {}^1J_{CACO}$

HN(CA)CO



Hetero Nuclear 2D/3D NMR

How to get spectra?

- **What's the sample? → Which Prohead to choose?**

  AV500 in IBMS : 5mm TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient) only

  AV600 in IBMS: 5mm QXI-Z ($^{1}H/^{15}N/^{13}C$ $/^{31}P$) (not ready yet)

  AV600 in CHEM: 5mm BBO and TXI-Z ($^{1}H/^{15}N/^{13}C$, with Z gradient)

  DRX600 in IBMS:

  5mm : ^{1}H , $^{1}H/^{19}F$, BBO, TXI($^{1}H/^{15}N/^{13}C$) , TXI-Z ($^{1}H/^{3}C/^{31}P$), TXI-XYZ ($^{1}H/^{15}N/^{13}C$, with XYZ gradient)

  8mm : TXI ($^{1}H/^{13}C/^{15}N$) 8mm with Z gradient

  10mm: ^{1}H , ^{1}H $/^{19}F$, BBO

- **What's the goal? → Which type of experiment you need?**
- **Which parameter set to choose? → Experiment List**

- **How to optimize condition? → For users: Experiment Guide**

•Experiment Name: 3D HNCO

•Experiment Type: Using Echo/antiecho , f1: H, f2:C, f3:N, F1(CO), F2(N), F3(H)

•Standard Parameter Set: std_3D_HNCO

•Pulse Program: hncogp3d.2

•AQ parameters to check

1H pulses

pl1 (high power, ex: 0db), p1(90deg at pl1), p2(180deg at pl1)

pl19 (low power for dipsi2,pcpd1), p26(90deg at pl19), pcpd1(90deg ,ex: 40-50usec)

sp1 (shape pulse power for Sinc.1000) , p11(pulse length for sp1, ex: 2m)

Others

o1 (for 1H), o2 (for 13CO), o3 (for 15N)

1 sw, 1 td (for F1 dimension, ie: 13C)

2 sw, 2 td (for F2 dimension, ie:15N)

3 sw, 3 td (for F3 dimension, ie:1H)

d1, rg, ns(=8*n), ds (≧ 16)

Users need to adjust parameters in "red" (meaning of the parameter in "green")

- **How to optimize condition? → For operators : pulse program**

(1) hard pulse calibration for hetero nuclei

(2) Shape pulse calibration for hetero nuclei

(3) other "uniform" (sample independent) parameters set up

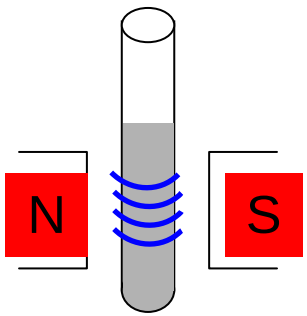
ex: delays, decouple program, gradient program, frequency jump……

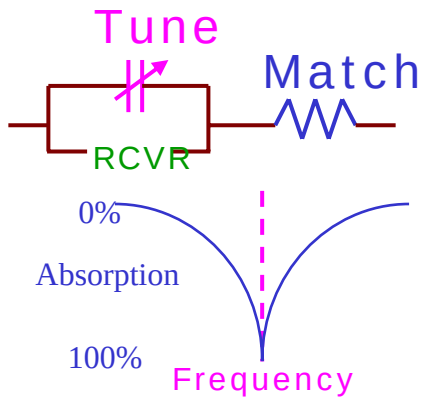
```
;hncogp3d.2
;avance-version (01/05/09)
;HNCO
…….( skips)
;     F1(H) -> F3(N) -> F2(C=O,t1) -> F3(N,t2) -> F1(H,t3)
;
;on/off resonance Ca and C=O pulses using shaped pulse
;phase sensitive (t1)
;phase sensitive using Echo/Antiecho gradient selection (t2)
…….( skips)
;sp1: f1 channel - shaped pulse  90 degree  (H2O on resonance)
;sp2: f2 channel - shaped pulse  90 degree  (C=O on resonance)
;sp3: f2 channel - shaped pulse 180 degree  (C=O on resonance)
;sp5: f2 channel - shaped pulse 180 degree  (Ca off resonance)
;sp8: f2 channel - shaped pulse  90 degree  (C=O on resonance)
;              for time reversed pulse
…….( skips)
;d21: 1/(2J(NH)                          [5.5 msec]
;d23: 1/(4J(NCO)                          [12 msec]
;d26: 1/(4J'(NH)                         [2.3 msec]
…….( skips)
;cpds1: decoupling according to sequence defined by cpdprg1
;cpd3: decoupling according to sequence defined by cpdprg3
;pcpd1: f1 channel - 90 degree pulse for decoupling sequence
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence

;use gradient ratio:    gp 1 : gp 2 : gp 3 : gp 4 : gp 5
;                              60 :  -40 :   10 :   80 :  8.1
```

In the NMR LAB

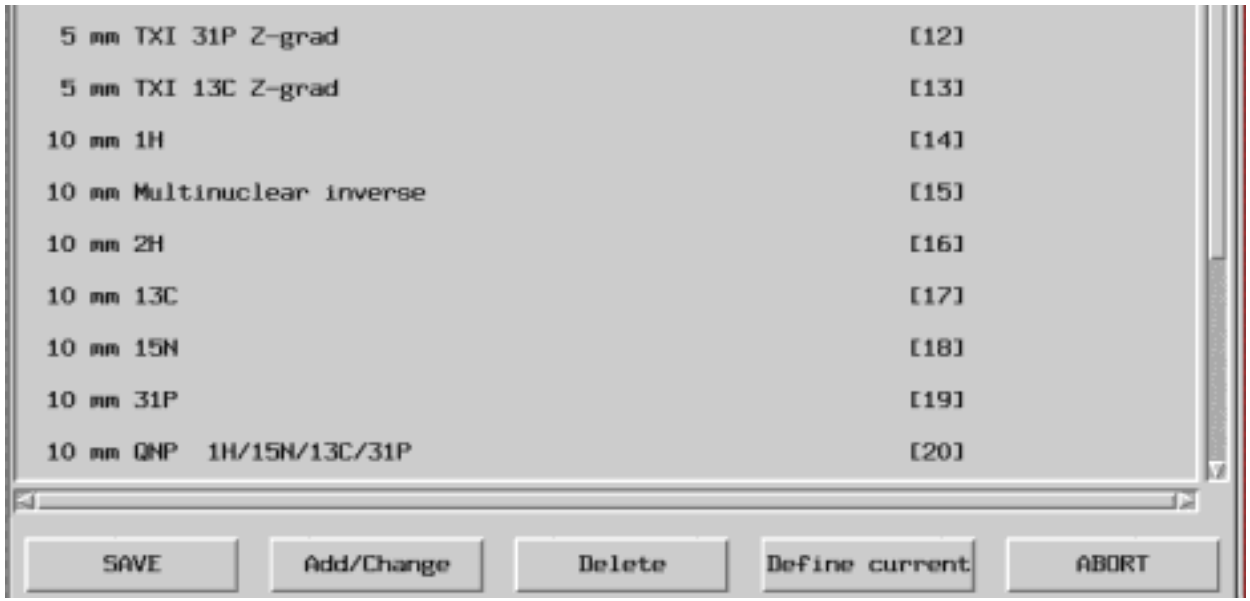
1. **The best condition for sample? → Temperature, sample position**
2. **The best condition for NMR? → Wobble : Tune & Match**



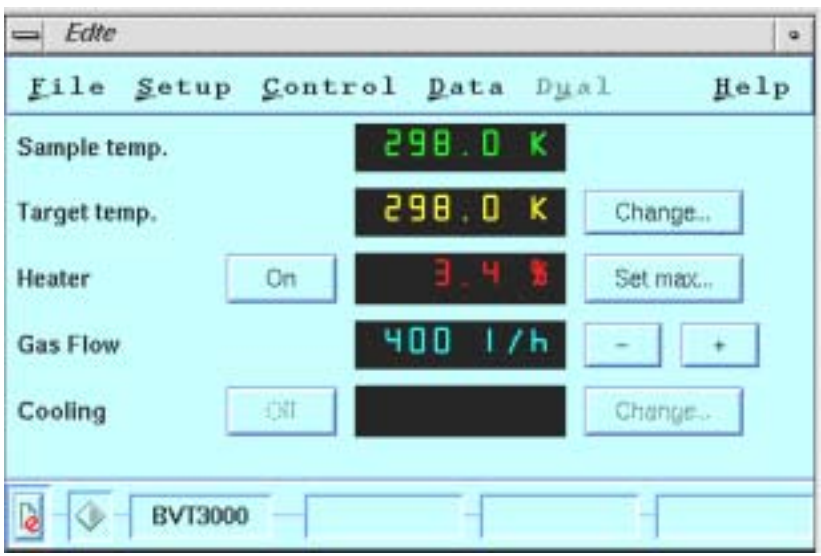


3. **The best condition for field? → Lock and shim**
4. **Ready to go! (LAB work sheet)**

edhead :Edit probe head

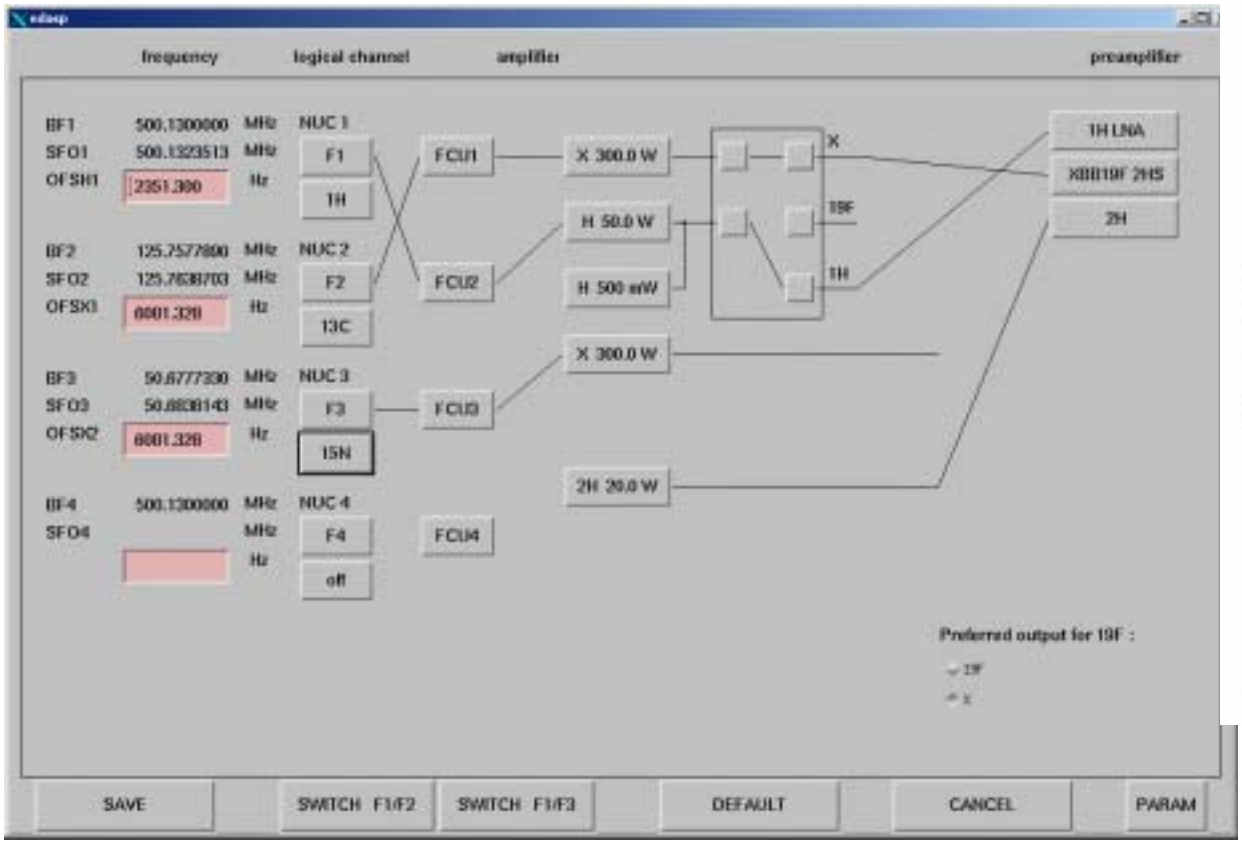


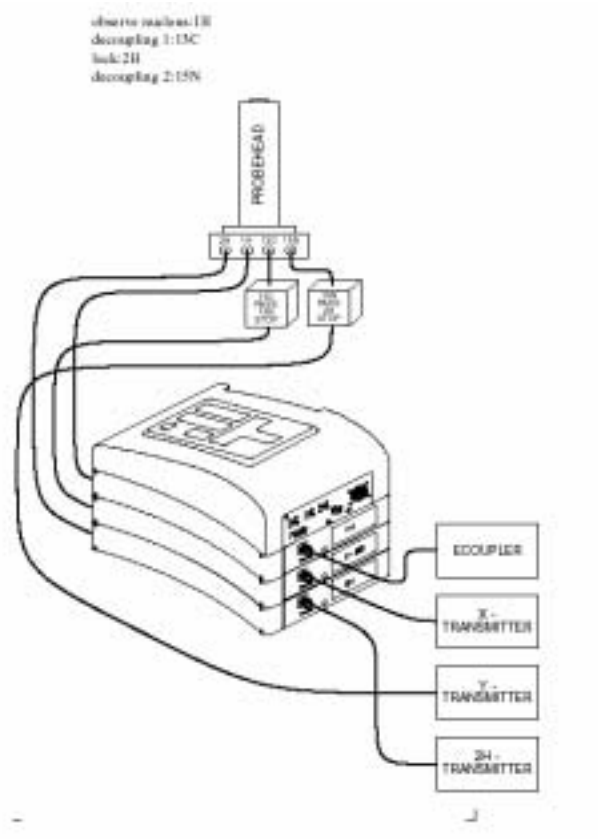
edte :Edit temperature



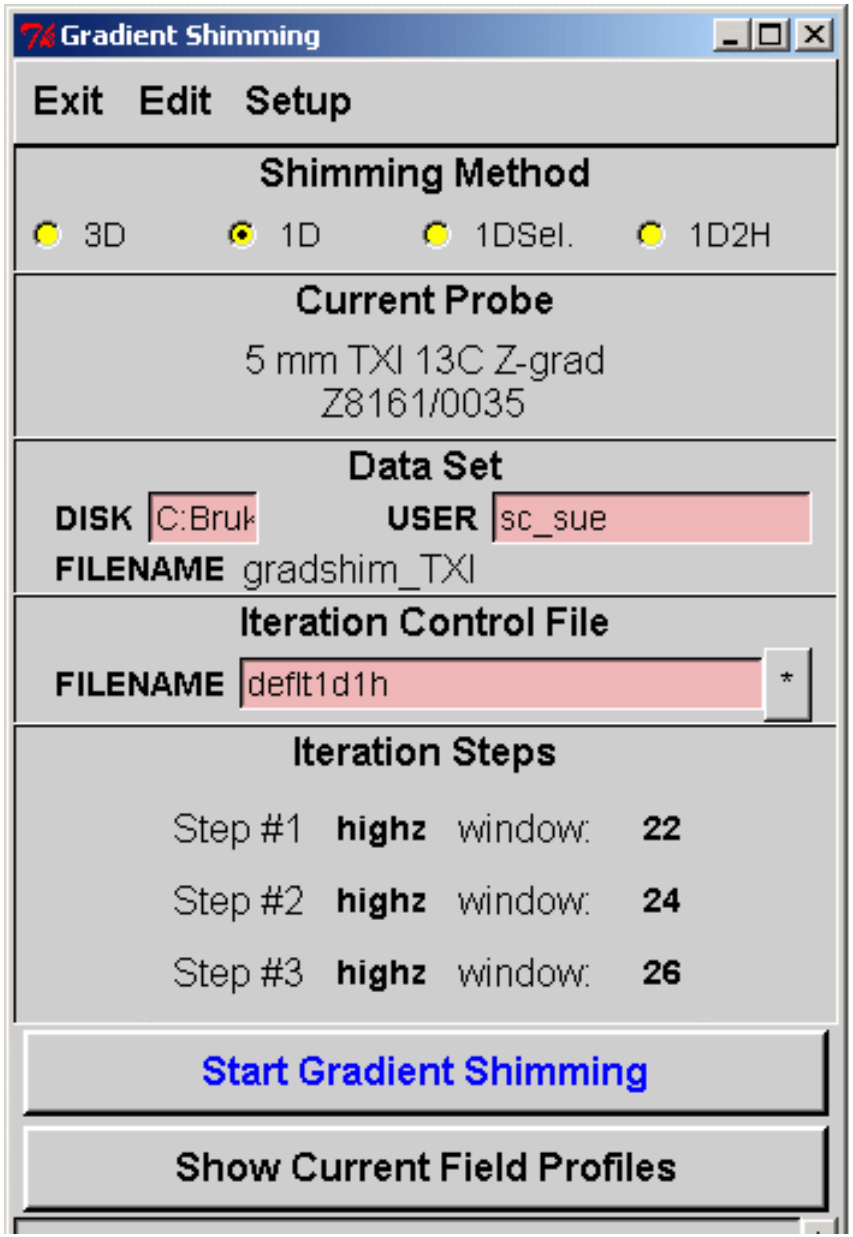
lock

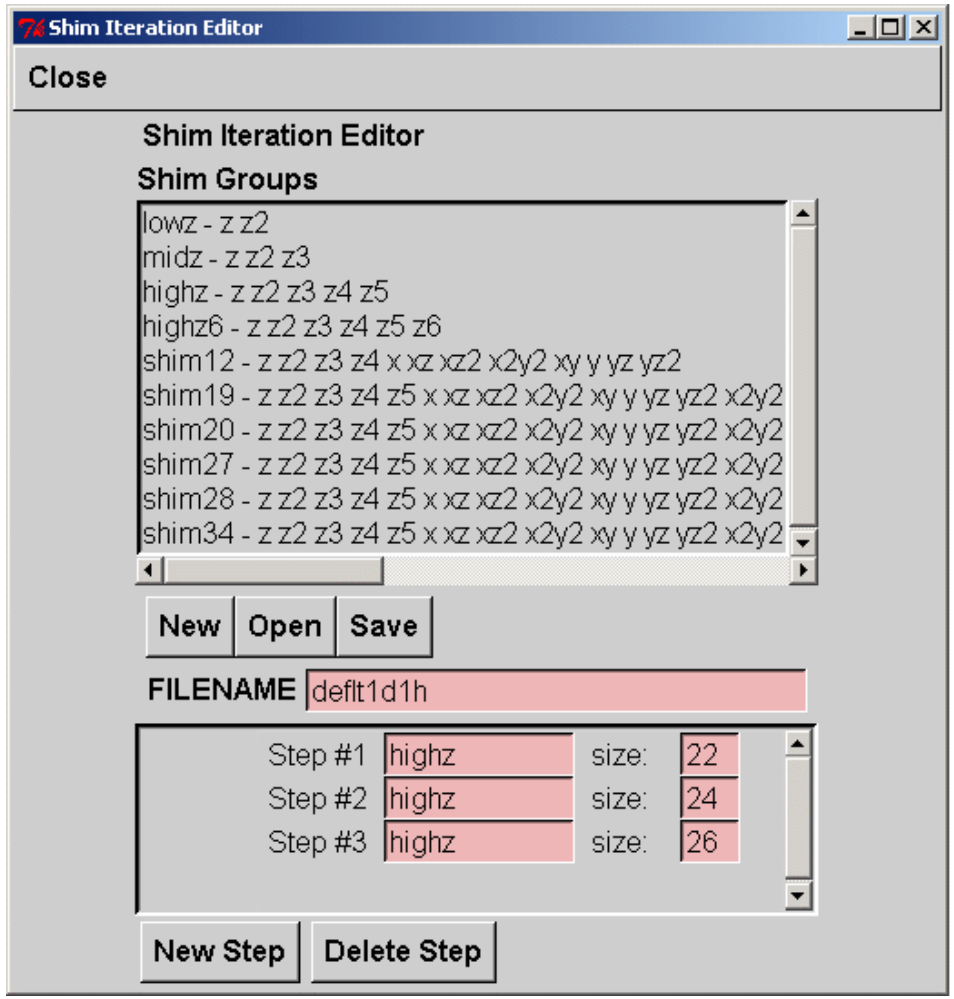
edasp :Edit AQ Spectrometer





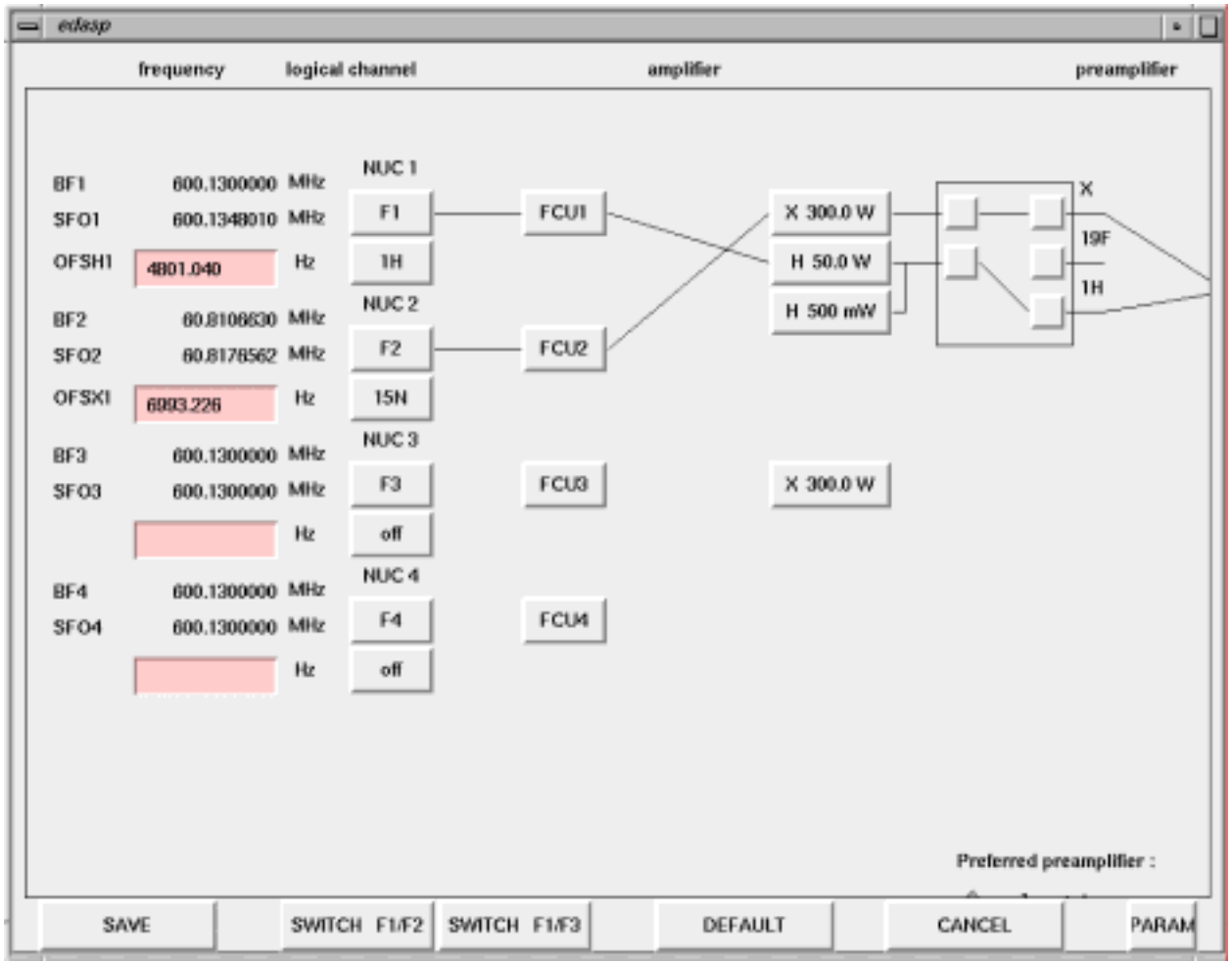
gradshim : gradient shimming





Wobble all 3 channels: 15N→ 13C → 1H

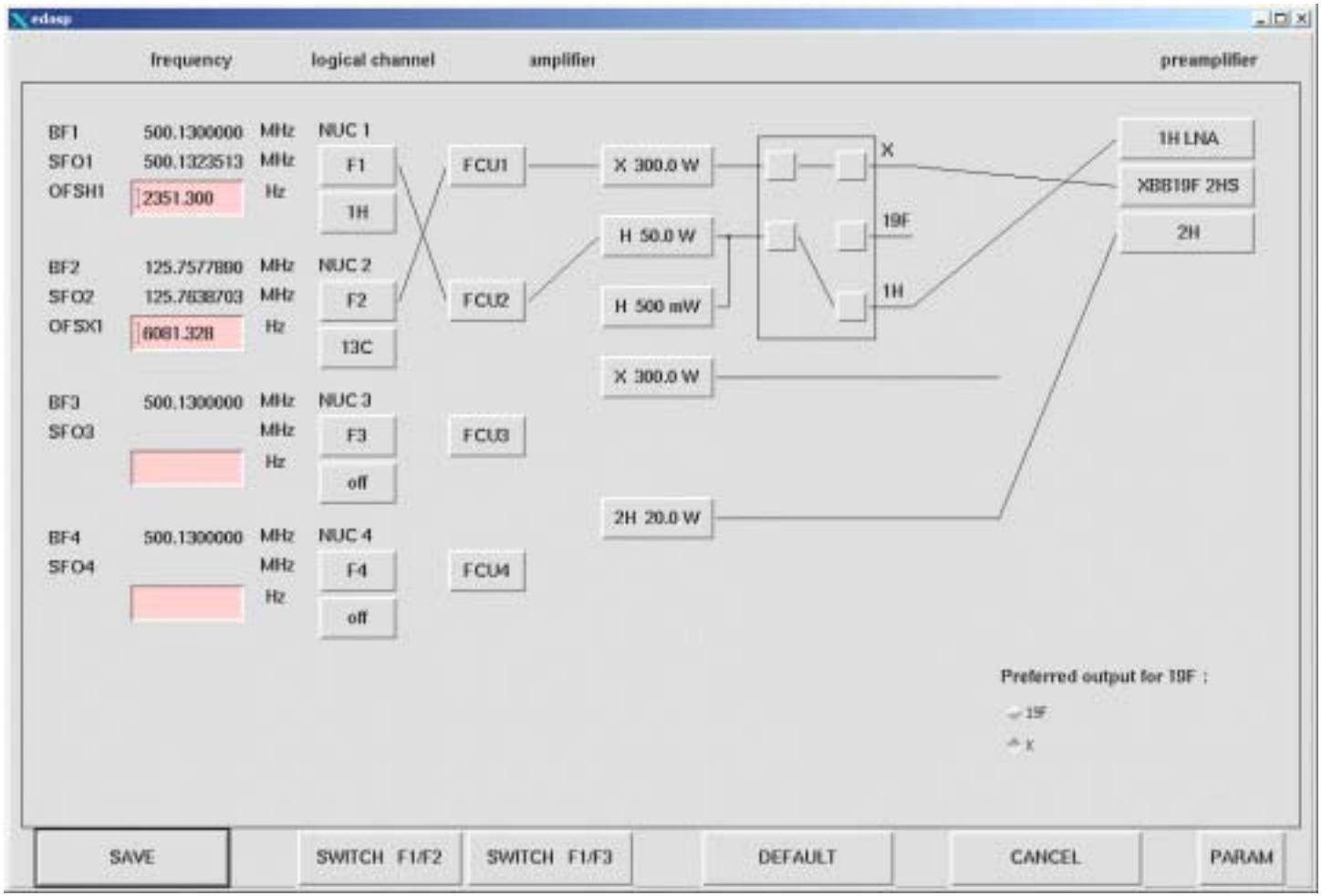
Step 1.1: (15N first) edasp to change setting and connection



Step 1.2: type wobble to wobble 15N, type stop after tune and match

Wobble all 3 channels: 15N→ 13C → 1H

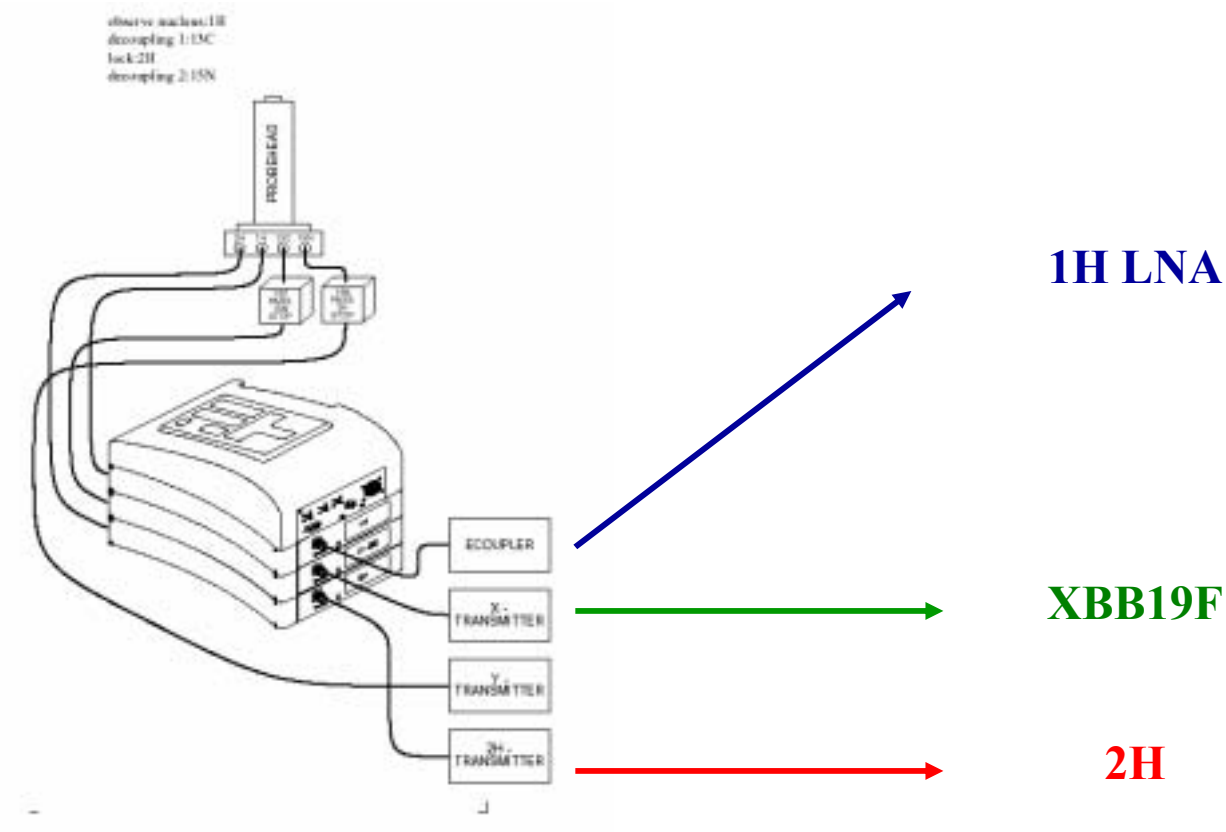
Step 2.1: (13C) edasp again to change setting and connection



Step 2.2: type wobble to wobble 13C,click any key on “HPPR” after 13C tuning and matching are done.

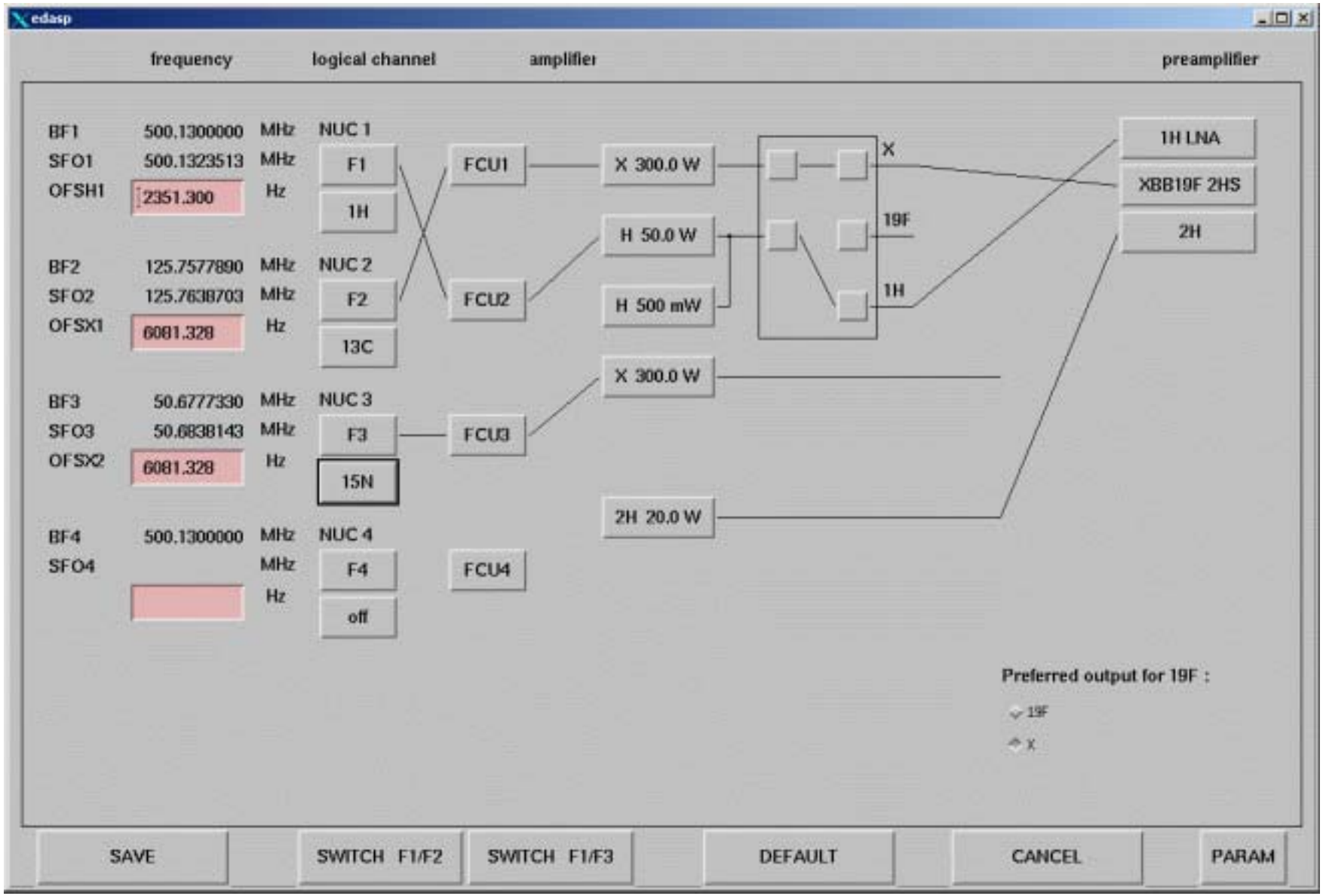
Wobble all 3 channels: 15N→ 13C → 1H

Step 3.1: click "Chn ↑" on "HPPR" , then wait until wobble on 1H pop out (this might take 10-20 sec, please be patient!)



Step 3.2: type stop after tuning and matching are done for 1H.

Step 3.3: edasp to change setting and connection for the experiment



After the LAB work

NMR data processing software

XWINNMR *(process NMR data on IRIX 6.X & Linux)*

License only available for older version

nmrPipe *(process NMR data on IRIX6.X & Linux)*

NMR data analysis software

AURELIA *(analyze NMR data on IRIX 6.X & Linux)*

License only available for older version

nmrDraw *(analyze NMR data on IRIX 6.X & Linux)*

nmrView *(analyze NMR data on IRIX 6.X & Linux)*

Sparky *(analyze NMR data on Linux)*

Thank you!!

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3D data processing and Display

Xwinnmr processing 3D data

Sparky display processed NMR spectra

NMR core tsunai yu

Strategies for protein NMR studies

Table 1: Strategies for protein studies

Protein/Size	Experiment	Information obtained	Sensitivity
Unlabeled/ less than 50.a.a.	***2D Homonuclear***		
	COSY, TOCSY	intra-residue assignments	
	NOESY	sequential connectivities NOE distance constraints $^{3}J_{HN\alpha}$ coupling constants	
	E.COSY	$^{3}J_{H\alpha\beta}$ coupling constants	
^{15}N-labeled/ ~ 50-80 .a.a.	***3D Double resonance***		
	^{15}N-TOCSY	intra-residue assignments	
*	^{15}N-NOESY	sequential connectivities NOE constraints	
*	^{15}N-HNHA	$^{3}J_{HN\alpha}$ coupling constants	
	or 2D HMQC-J	$^{3}J_{HN\alpha}$ coupling constants	
	^{15}N-HNHB	$^{3}J_{H\alpha\beta}$ coupling constants	

Protein/Size	Experiment	Information obtained	Sensitivity
^{13}C, ^{15}N-labeled/ ~ 80-150 .a.a.	*3D Double resonance*	*NB. Possibly fractionally ^{2}H-labeled*	
*	^{15}N-NOESY	NOE constraints	
*	^{15}N-HNHA	$^{3}J_{HN\alpha}$ coupling constants	
	^{15}N-HNHB	$^{3}J_{H\alpha\beta}$ coupling constants	
	^{13}C HCCH-COSY	intra-residue assignments	
*	^{13}C HCCH-TOCSY	intra-residue assignments	
	^{13}C NOESY	sidechain NOE constraints	
	3D Triple resonance		
	HNCO	sequential connectivity	100 inter
	HN(CA)CO	sequential connectivity (combine with HNCO)	13/4 inter/intra
*	HNCA	sequential connectivity $^{13}C^{\alpha}$ chemical shift constraints	50/15 intra/inter
*	HN(CO)CA	(combine with HNCA)	71 inter
	HNCAH	sequential connectivity	
	HN(CO)CAH	(combine with HNCAH)	
*	CBCA(CO)NH	sequential connectivity $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shifts	13/9 $^{13}C^{\alpha}$ / $^{13}C^{\beta}$ intra
	CBCANH	for smaller proteins (combine with CBCA(CO)NH)	4/1.7 $^{13}C^{\alpha}$ / $^{13}C^{\beta}$ intra
	HNCACB	for bigger proteins (combine with CBCA(CO)NH)	1.3/0.5 $^{13}C^{\alpha}$ / $^{13}C^{\beta}$ intra
*	HBHA(CO)NH	$^{1}H^{\alpha}$ and $^{1}H^{\beta}$ assignments	13/9 $^{1}H^{\alpha}$ / $^{1}H^{\beta}$ intra
*	H(CCCO)NH	sidechain ^{1}H assignments	
	(H)CC(CO)NH	sidechain ^{13}C assignments	

edp ↵—Display Processing parameter

Size of the raw data

(SI ≥ 1/2TD)

Spectral calibration

Window function

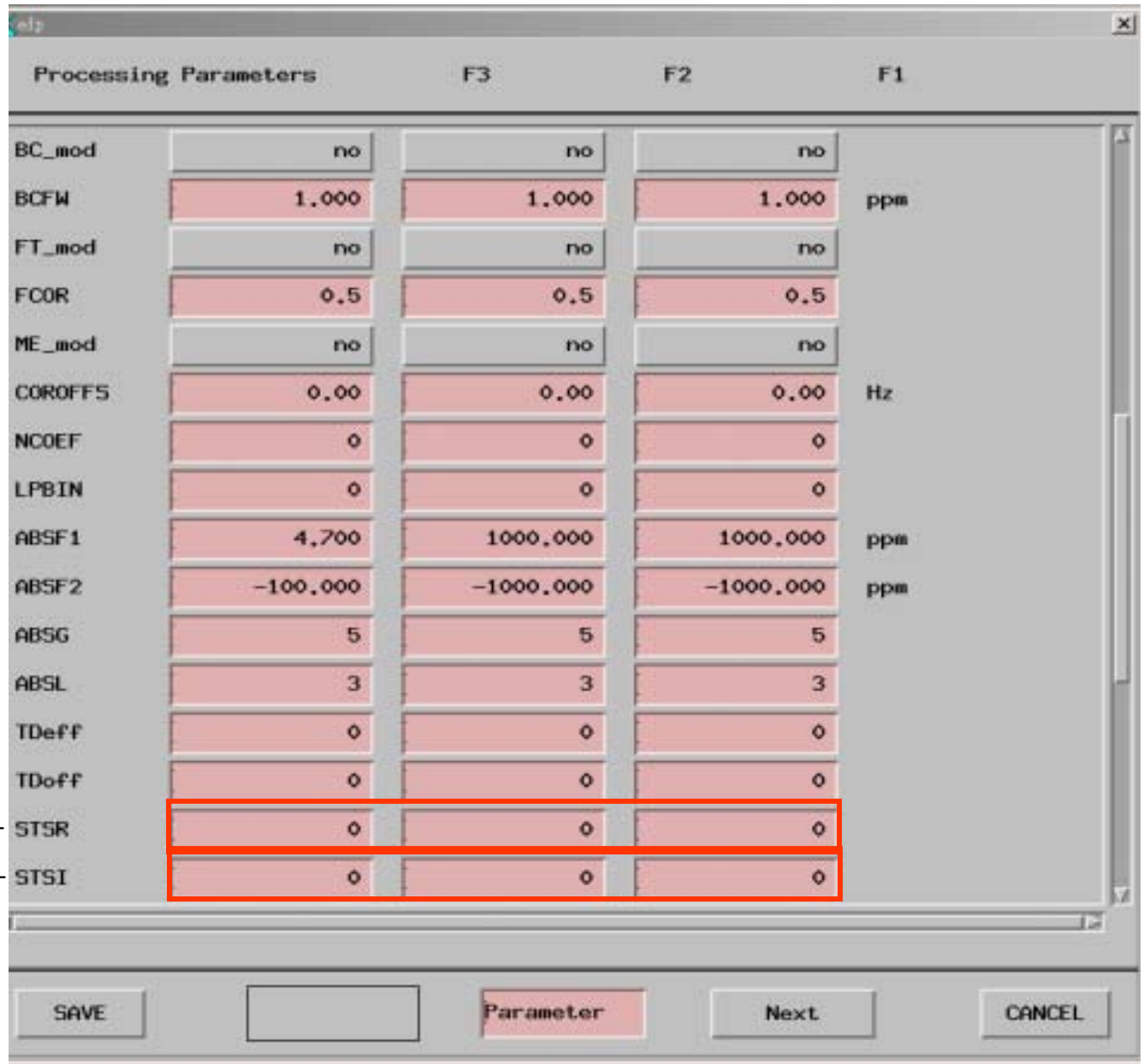
$180^0/3$

Phase correction

Processing Parameters	F3	F2	F1	
SI	1024	64	256	
PPARMOD	3D			
SF	600.1299496	60.8106385	150.9023938	MHz
OFFSET	9.841	137.905	182.856	ppm
SR	-50.40	-24.52	-355.23	Hz
HZpPT	5.868765	33.258835	8.843672	Hz
MC2		echo-antiecho	States-TPPI	
AQORDER	3-1-2			
WDW	QSINE	QSINE	QSINE	
SSB	3	3	3	
LB	1.00	0.30	0.00	Hz
GB	0.1	0.1	0	
PH_mod	no	no	no	
PKNL	TRUE			
PHC0	0.000	0.000	0.000	degrees
PHC1	0.000	0.000	0.000	degrees

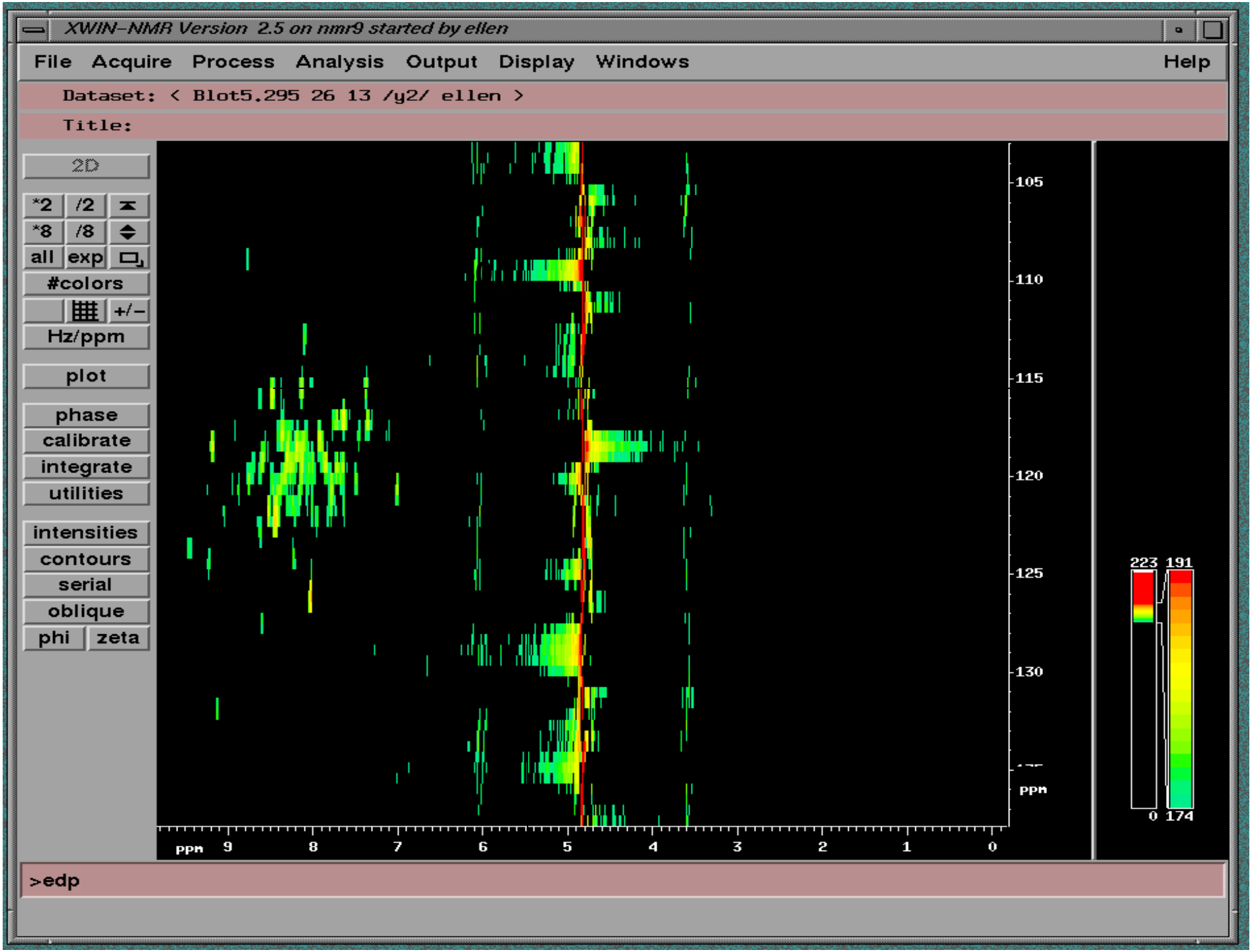
SAVE | | Parameter | Next | CANCEL

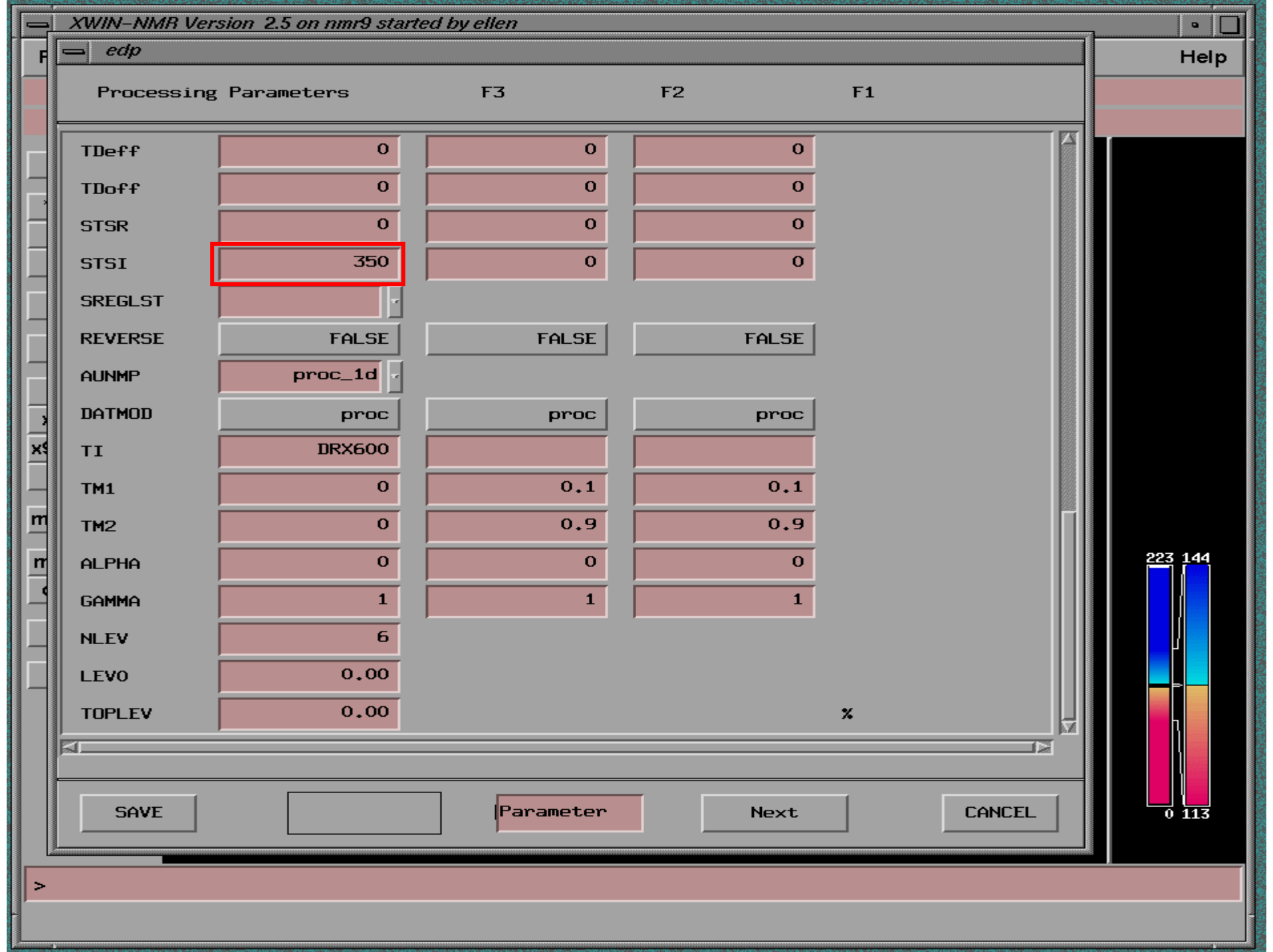
edp ↩—Display Processing parameter

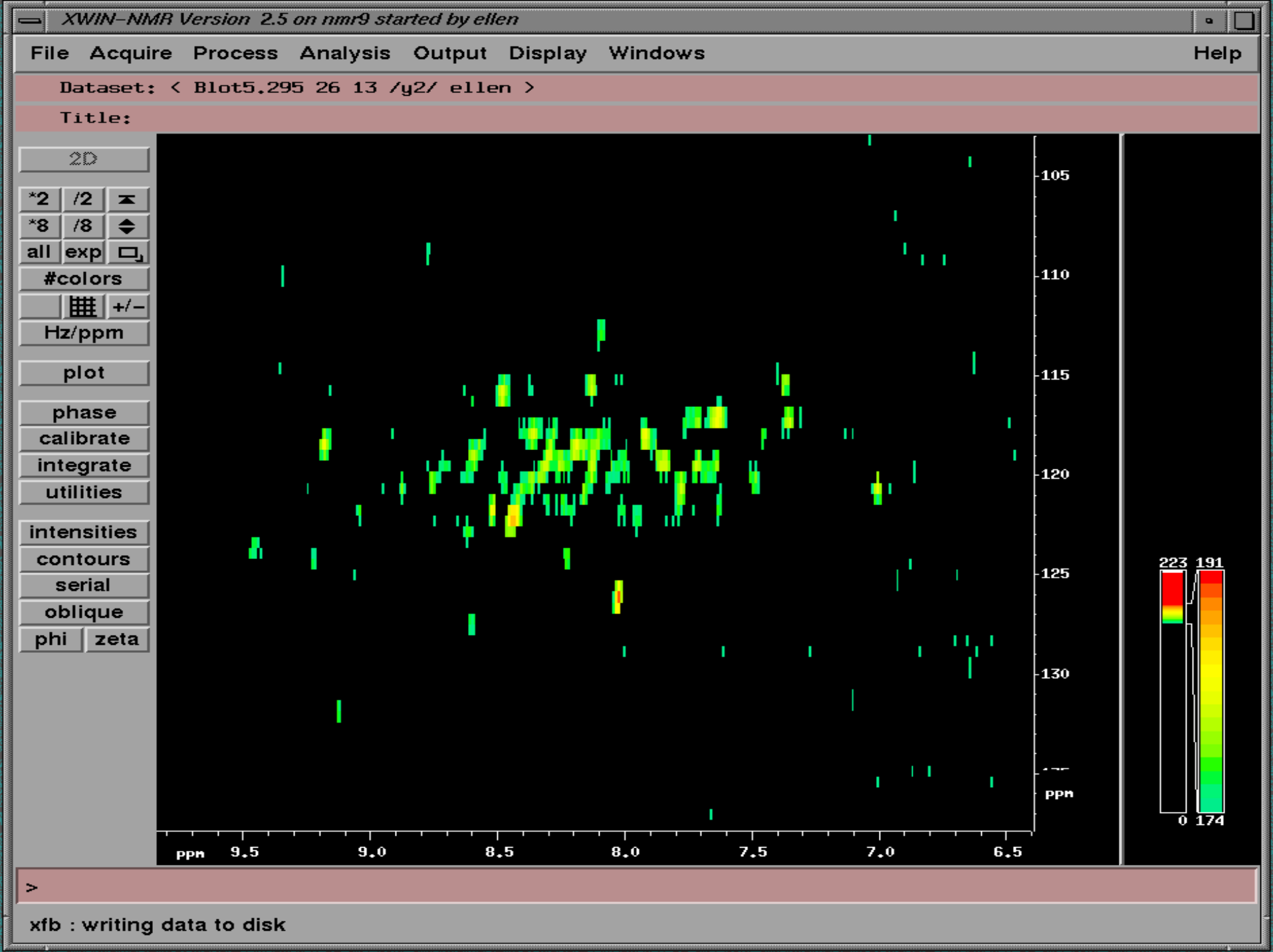


Strip start ← STSR

# of raw data points of strip transform ← STSI

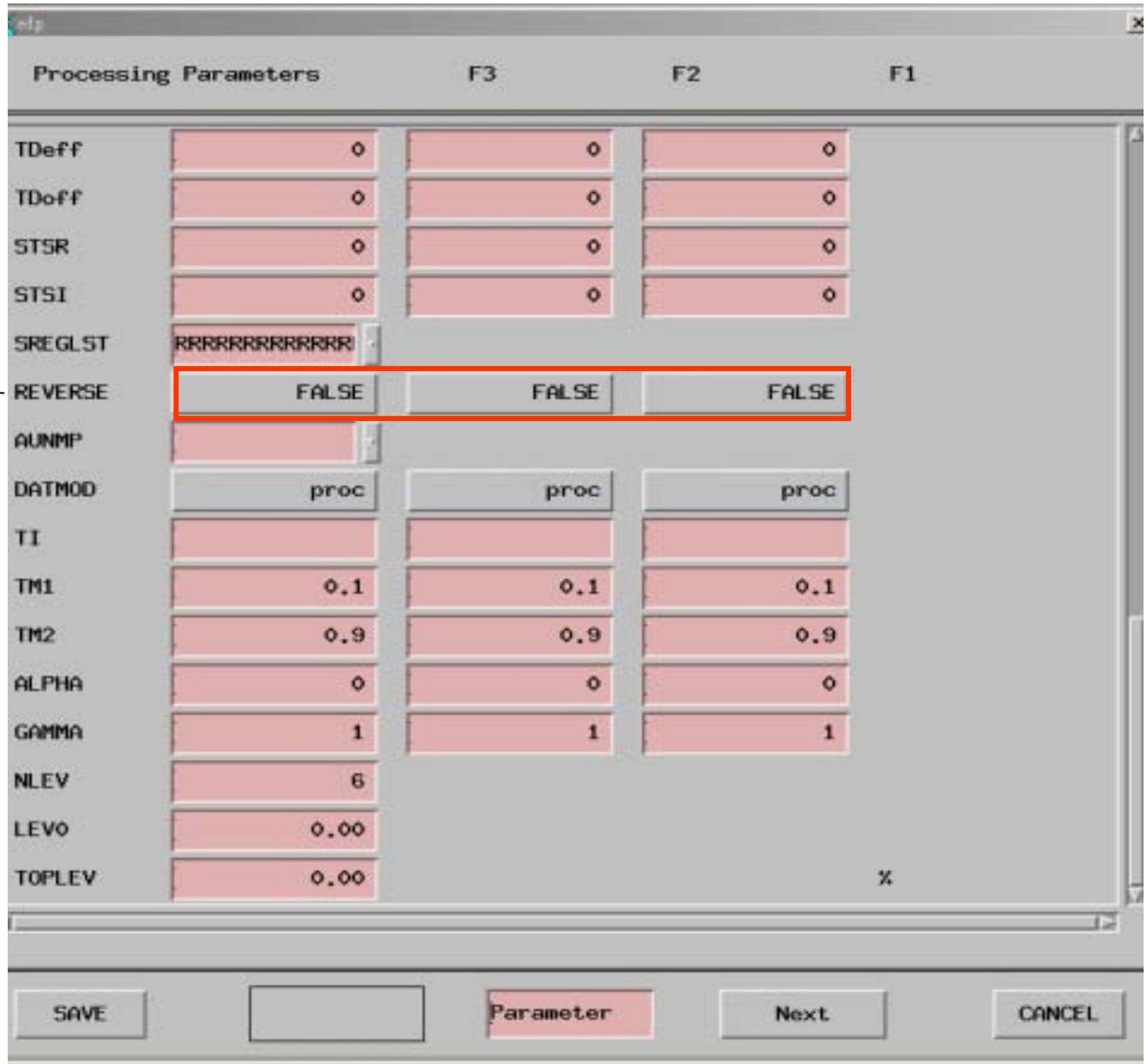

XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D
*2 /2
*8 /8
all exp
#colors
+/-
Hz/ppm
plot
phase
calibrate
integrate
utilities
intensities
contours
serial
oblique
phi zeta
105
110
115
120
125
130
ppm
223 191
0 174
ppm 9 8 7 6 5 4 3 2 1 0
>edp


XWIN-NMR Version 2.5 on nmr9 started by ellen
edp
Help
Processing Parameters F3 F2 F1
TDeff 0 0 0
TDoff 0 0 0
STSR 0 0 0
STSI 350 0 0
SREGLST
REVERSE FALSE FALSE FALSE
AUNMP proc_1d
DATMOD proc proc proc
TI DRX600
TM1 0 0.1 0.1
TM2 0 0.9 0.9
ALPHA 0 0 0
GAMMA 1 1 1
NLEV 6
LEVO 0.00
TOPLEV 0.00
%
SAVE
Parameter
Next
CANCEL
223 144
0 113
>


XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D
*2 /2
*8 /8
all exp
#colors
+/-
Hz/ppm
plot
phase
calibrate
integrate
utilities
intensities
contours
serial
oblique
phi zeta
105
110
115
120
125
130
ppm
223 191
0 174
ppm 9.5 9.0 8.5 8.0 7.5 7.0 6.5
>
xfb : writing data to disk

edp ↩—Display Processing parameter

Some expt. need to set as "TRUE" ←



3D processing

- **Phase 2D plane** (F1-F3 plane and F2 F3 plane)

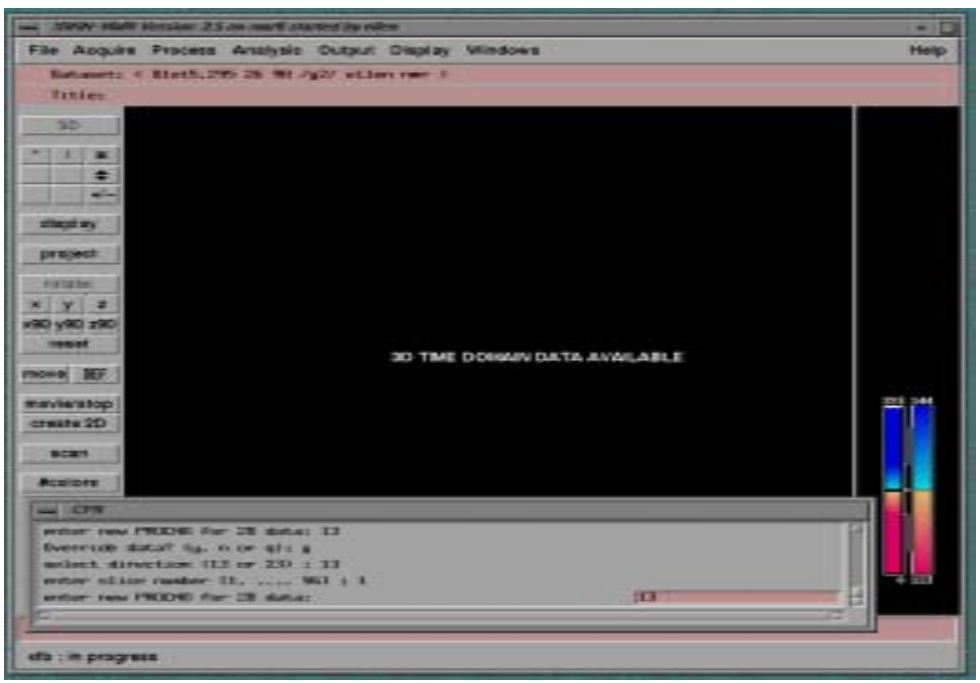
  eg. HNCACB F3(^{1}H), F2(^{15}N), F1(^{13}C)

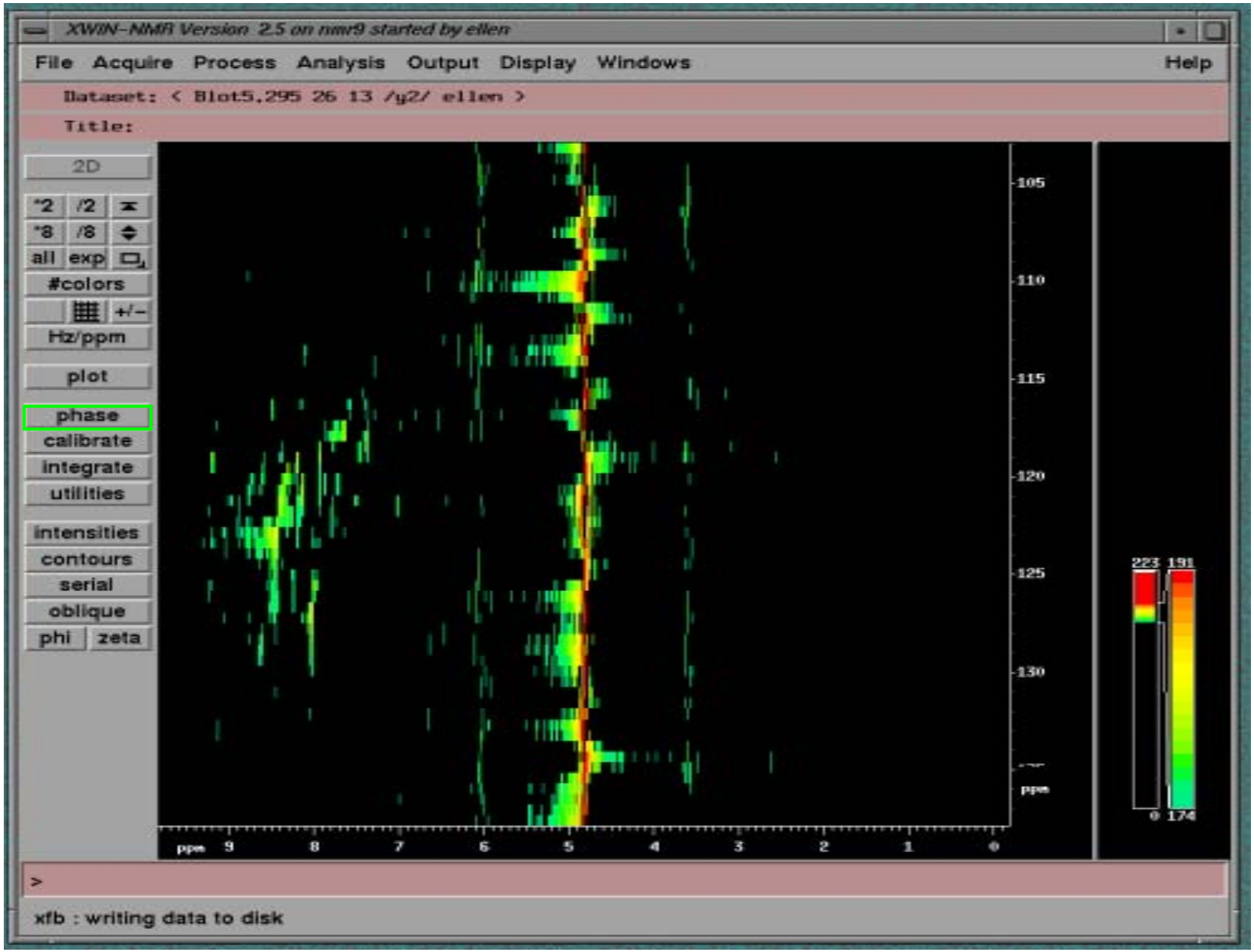
  rule: phase ^{1}H-^{15}N( ) and ^{15}N-^{13}C plane

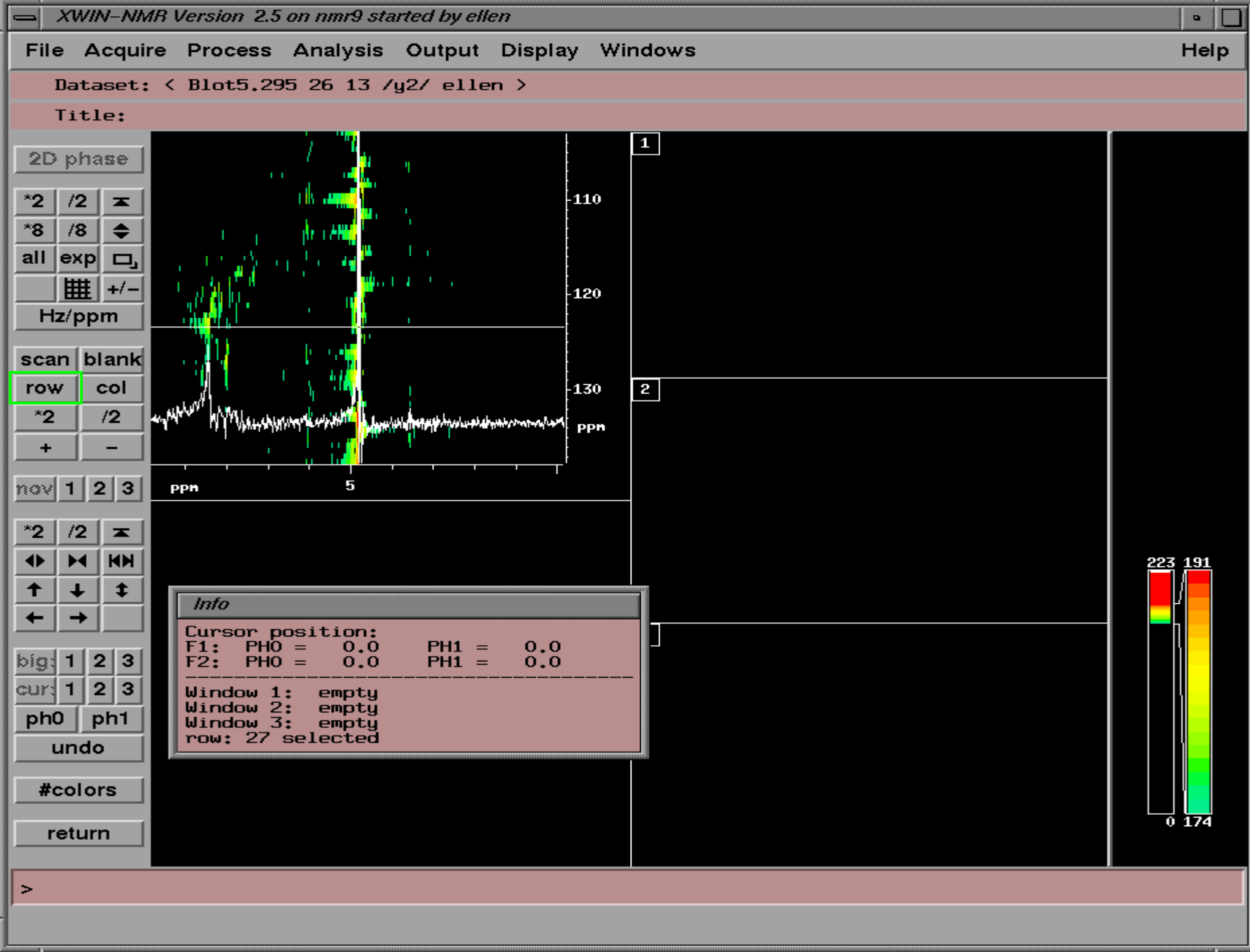
xfb↵→ select direction (13or 23) :

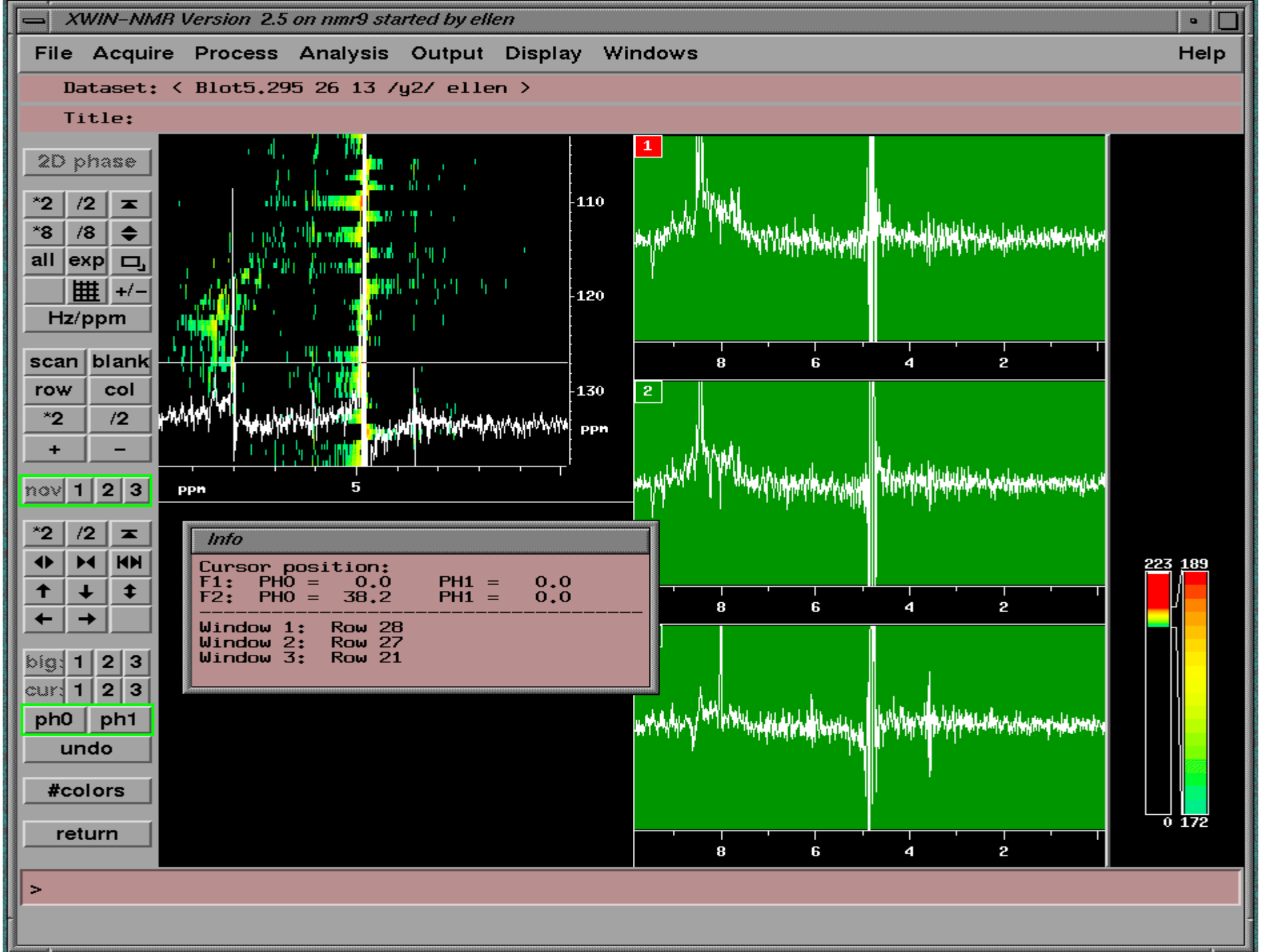
enter slice number [1, …..,96]:

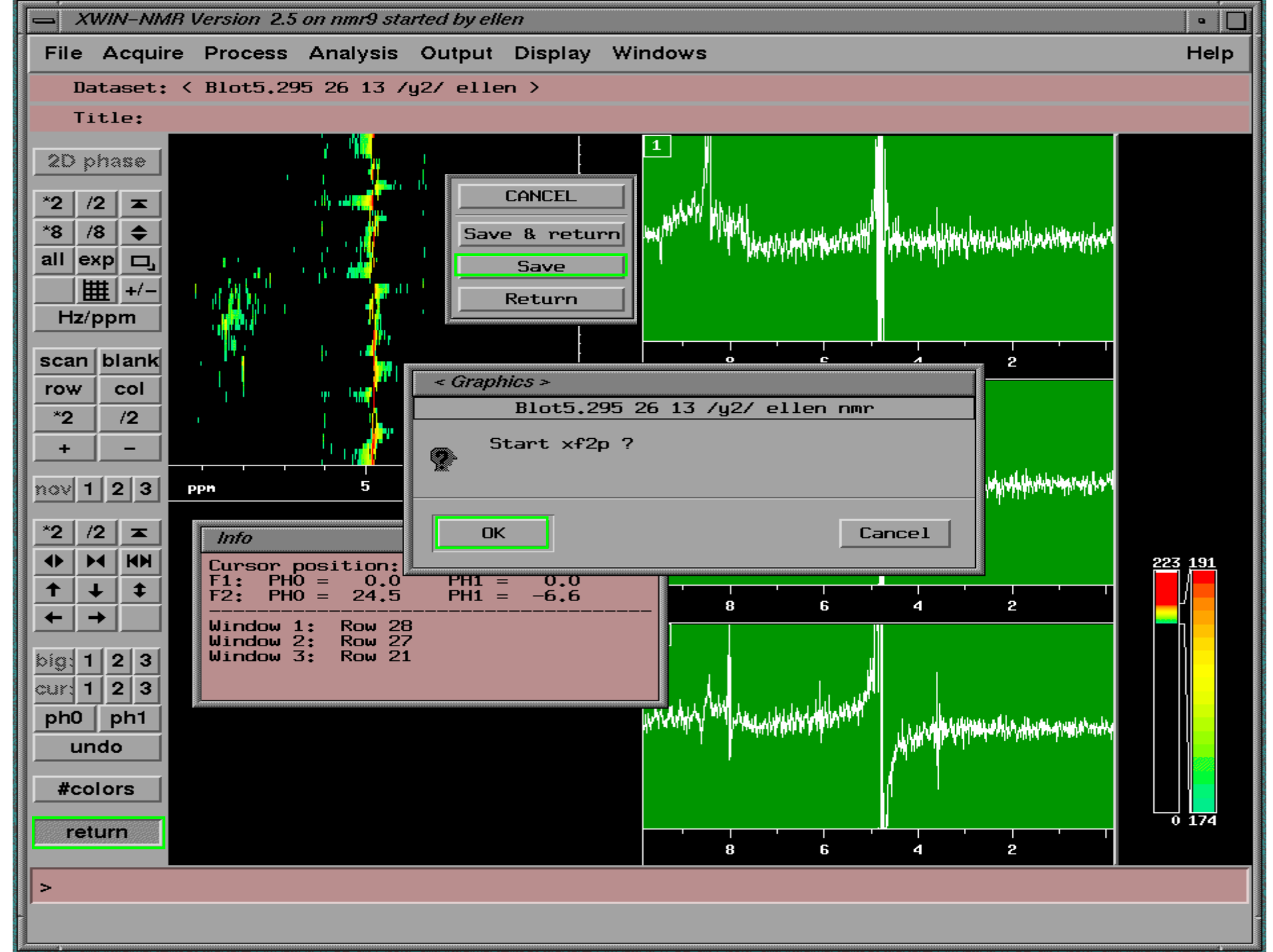
enter new PROCNO for 2D data:

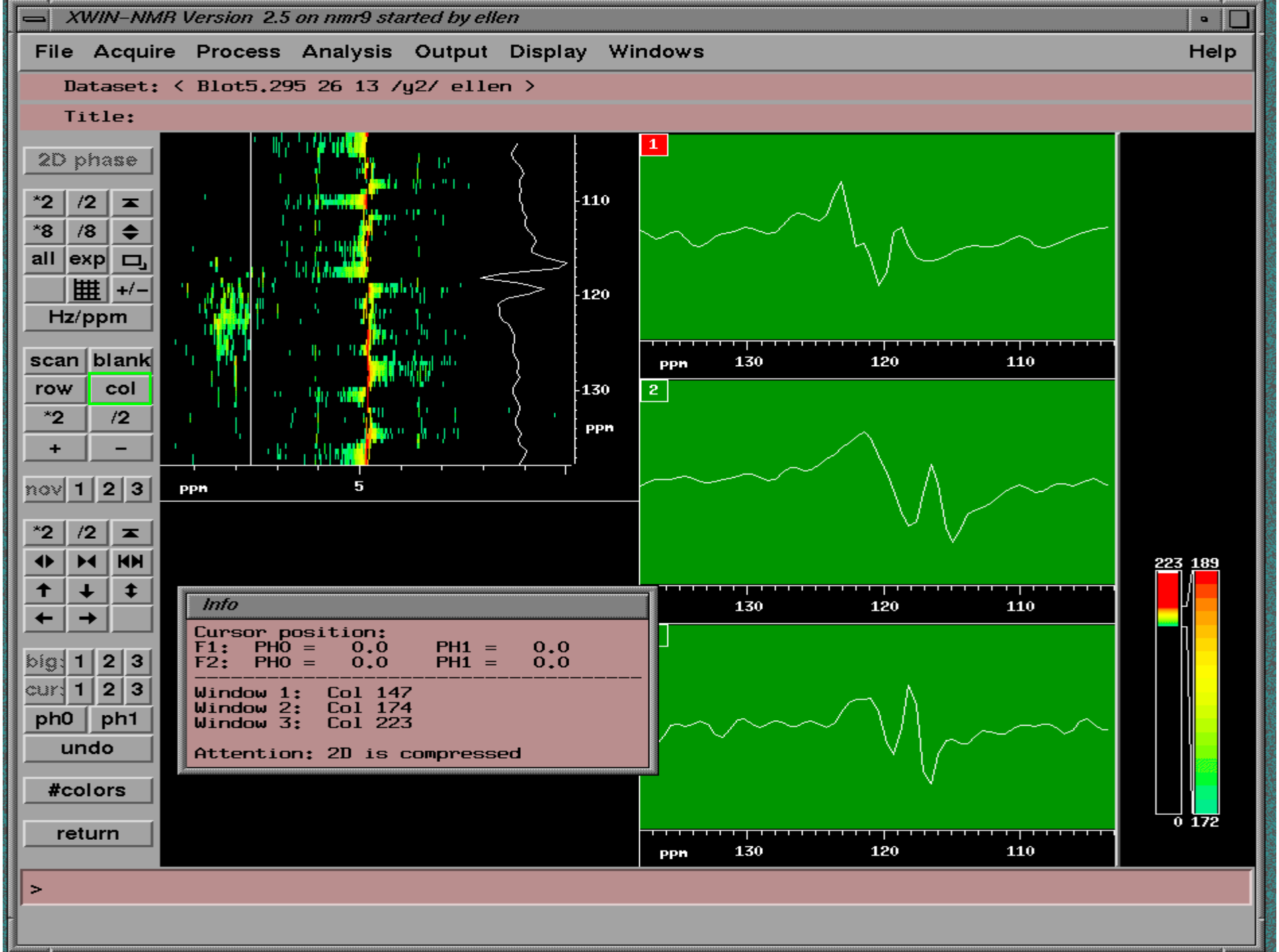


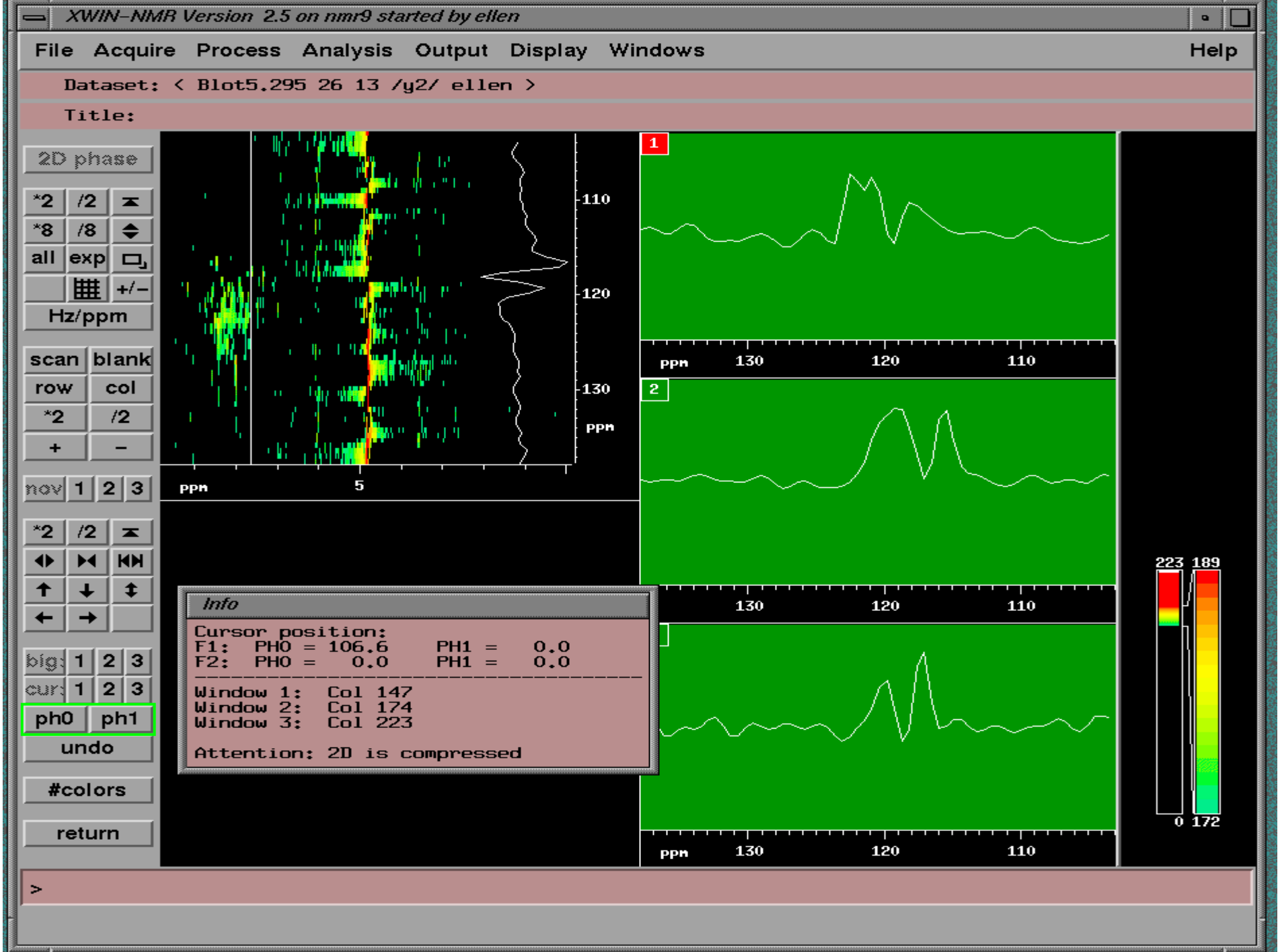

XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D
*2 /2
*8 /8
all exp
#colors
+/-
Hz/ppm
plot
phase
calibrate
integrate
utilities
intensities
contours
serial
oblique
phi zeta
105
110
115
120
125
130
ppm
223 191
0 174
ppm 9 8 7 6 5 4 3 2 1 0
>
xfb : writing data to disk

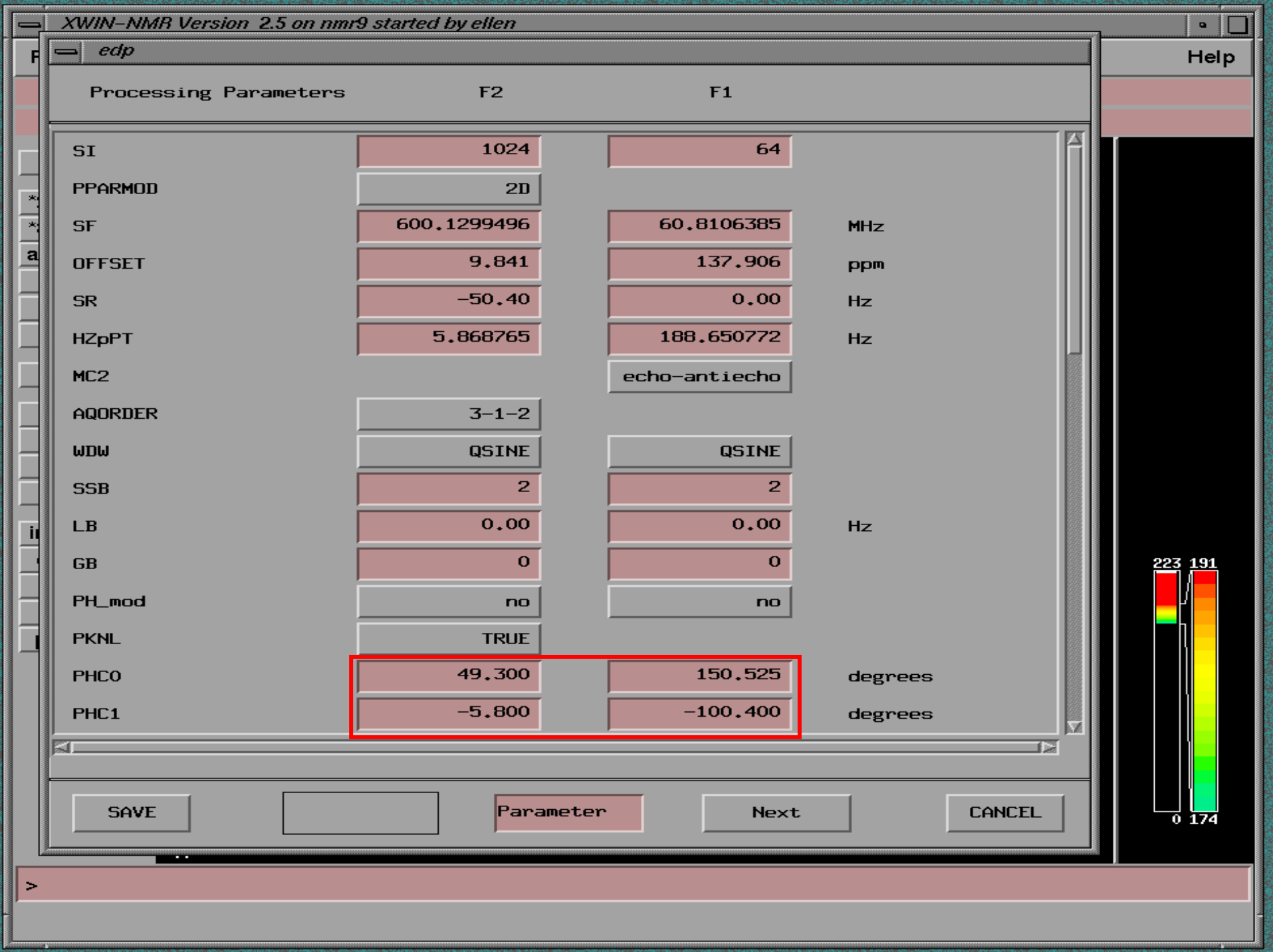

XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D phase
*2 /2
*8 /8
all exp
+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
nov 1 2 3
big 1 2 3
cur 1 2 3
ph0 ph1
undo
#colors
return
110
120
130
ppm
ppm
5
1
2
Info
Cursor position:
F1: PH0 = 0.0 PH1 = 0.0
F2: PH0 = 0.0 PH1 = 0.0
Window 1: empty
Window 2: empty
Window 3: empty
row: 27 selected
223 191
0 174
>

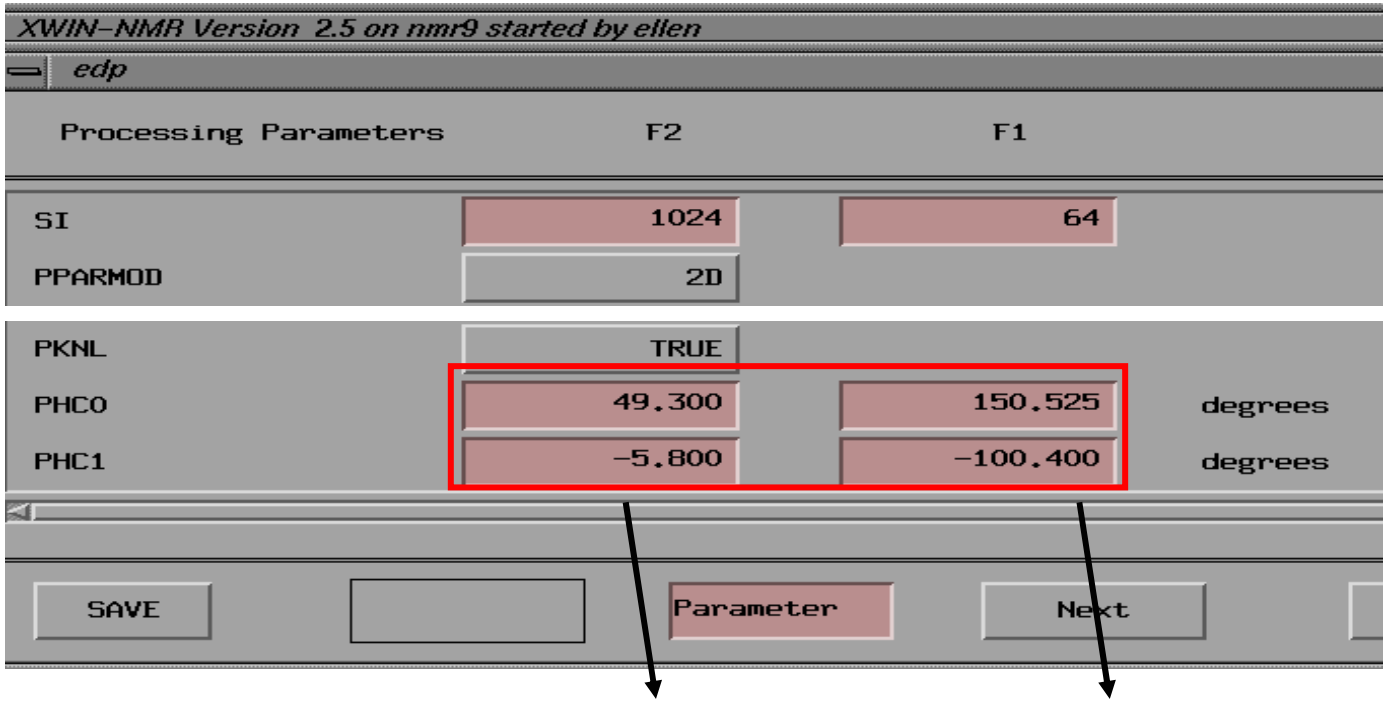

XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D phase
*2 /2
*8 /8
all exp
+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
nov 1 2 3
*2 /2
big 1 2 3
cur 1 2 3
ph0 ph1
undo
#colors
return
110
120
130
ppm
ppm
5
1
2
8 6 4 2
Info
Cursor position:
F1: PH0 = 0.0 PH1 = 0.0
F2: PH0 = 38.2 PH1 = 0.0
Window 1: Row 28
Window 2: Row 27
Window 3: Row 21
223 189
0 172
>


XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D phase
*2 /2
*8 /8
all exp
+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
nov 1 2 3
big 1 2 3
cur 1 2 3
ph0 ph1
undo
#colors
return
CANCEL
Save & return
Save
Return
< Graphics >
Blot5.295 26 13 /y2/ ellen nmr
Start xf2p ?
OK
Cancel
Info
Cursor position:
F1: PH0 = 0.0 PH1 = 0.0
F2: PH0 = 24.5 PH1 = -6.6
Window 1: Row 28
Window 2: Row 27
Window 3: Row 21
ppm
5
1
8
6
4
2
223 191
0 174
>


XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D phase
*2 /2
*8 /8
all exp
+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
nov 1 2 3
big 1 2 3
cur 1 2 3
ph0 ph1
undo
#colors
return
110
120
130
ppm
5
1
2
ppm 130 120 110
Info
Cursor position:
F1: PH0 = 0.0 PH1 = 0.0
F2: PH0 = 0.0 PH1 = 0.0
Window 1: Col 147
Window 2: Col 174
Window 3: Col 223
Attention: 2D is compressed
223 189
0 172
>


XWIN-NMR Version 2.5 on nmr9 started by ellen
File Acquire Process Analysis Output Display Windows Help
Dataset: < Blot5.295 26 13 /y2/ ellen >
Title:
2D phase
*2 /2
*8 /8
all exp
+/-
Hz/ppm
scan blank
row col
*2 /2
+ -
nov 1 2 3
big 1 2 3
cur 1 2 3
ph0 ph1
undo
#colors
return
110
120
130
ppm
5
1
2
ppm 130 120 110
Info
Cursor position:
F1: PH0 = 106.6 PH1 = 0.0
F2: PH0 = 0.0 PH1 = 0.0
Window 1: Col 147
Window 2: Col 174
Window 3: Col 223
Attention: 2D is compressed
223 189
0 172
>

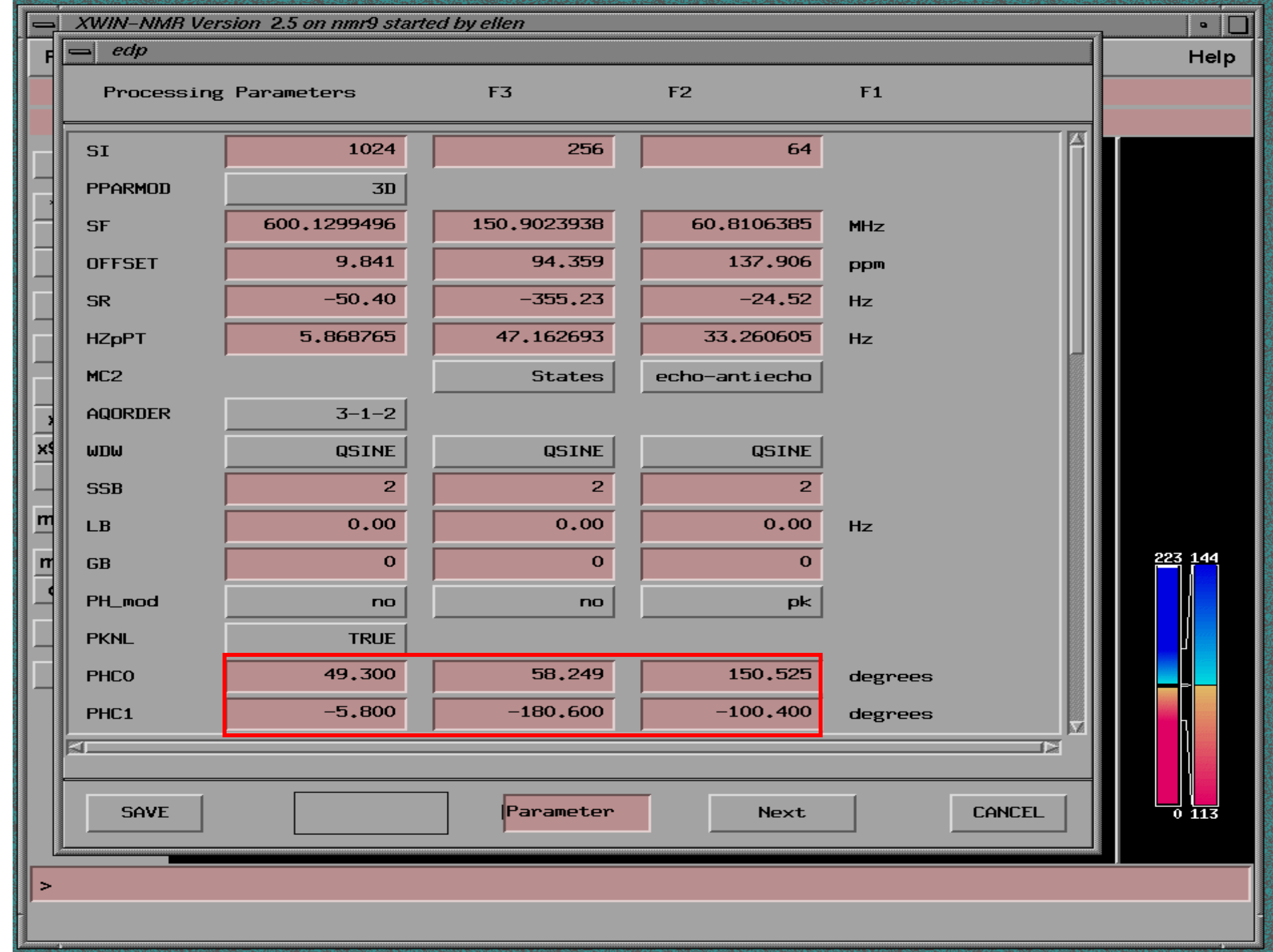

XWIN-NMR Version 2.5 on nmr9 started by ellen
edp
Help
Processing Parameters F2 F1
SI 1024 64
PPARMOD 2D
SF 600.1299496 60.8106385 MHz
OFFSET 9.841 137.906 ppm
SR -50.40 0.00 Hz
HZpPT 5.868765 188.650772 Hz
MC2 echo-antiecho
AQORDER 3-1-2
WDW QSINE QSINE
SSB 2 2
LB 0.00 0.00 Hz
GB 0 0
PH_mod no no
PKNL TRUE
PHC0 49.300 150.525 degrees
PHC1 -5.800 -100.400 degrees
SAVE
Parameter
Next
CANCEL
223 191
0 174
>



		F3(H)	F2(C)	F1(N)
13 plane	PHC0	49.3		150.252
	PFC1	-5.8		-100.400
23 plane	PHC0	X1	58.249	
	PHC1	X2	-180.6	

If X1≠13 plane PHC0 value, X2≠13 plane PHC1 value

Use the HSQC plane phase value (eg.13 plane in this case)


XWIN-NMR Version 2.5 on nmr9 started by ellen
edp
Help
Processing Parameters F3 F2 F1
SI 1024 256 64
PPARMOD 3D
SF 600.1299496 150.9023938 60.8106385 MHz
OFFSET 9.841 94.359 137.906 ppm
SR -50.40 -355.23 -24.52 Hz
HZpPT 5.868765 47.162693 33.260605 Hz
MC2 States echo-antiecho
AQORDER 3-1-2
WDW QSINE QSINE QSINE
SSB 2 2 2
LB 0.00 0.00 0.00 Hz
GB 0 0 0
PH_mod no no pk
PKNL TRUE
PHC0 49.300 58.249 150.525 degrees
PHC1 -5.800 -180.600 -100.400 degrees
SAVE
Parameter
Next
CANCEL
223 144
0 113
>

3D processing

•Applying the 3D Fourier Transform

tf3 no (processed 3D data in F3 dimension without imaginary data)

tf2 no

tf1 no

•Baseline correction

tabs3 (automatic baseline correction in the F3 dimension)

tabs2

tabs1

Sparky display processed NMR spectra

Adventage

- Using a graphical interface (in PC, linux, and unix)
- Work with 2-4 dimensional spectra
- Suitabe display with other process program
- Output suitable for other structure determination program
- Free license

Start sparky

•Under unix the command to run Sparky is “sparky”. The installation locaton is /usr/local/sparky/bin.

•Under windows(95, 98,or NT) double clicking sparky.bat in c:\progranfiles\sparky\bin c:\progranfiles\sparky\bin

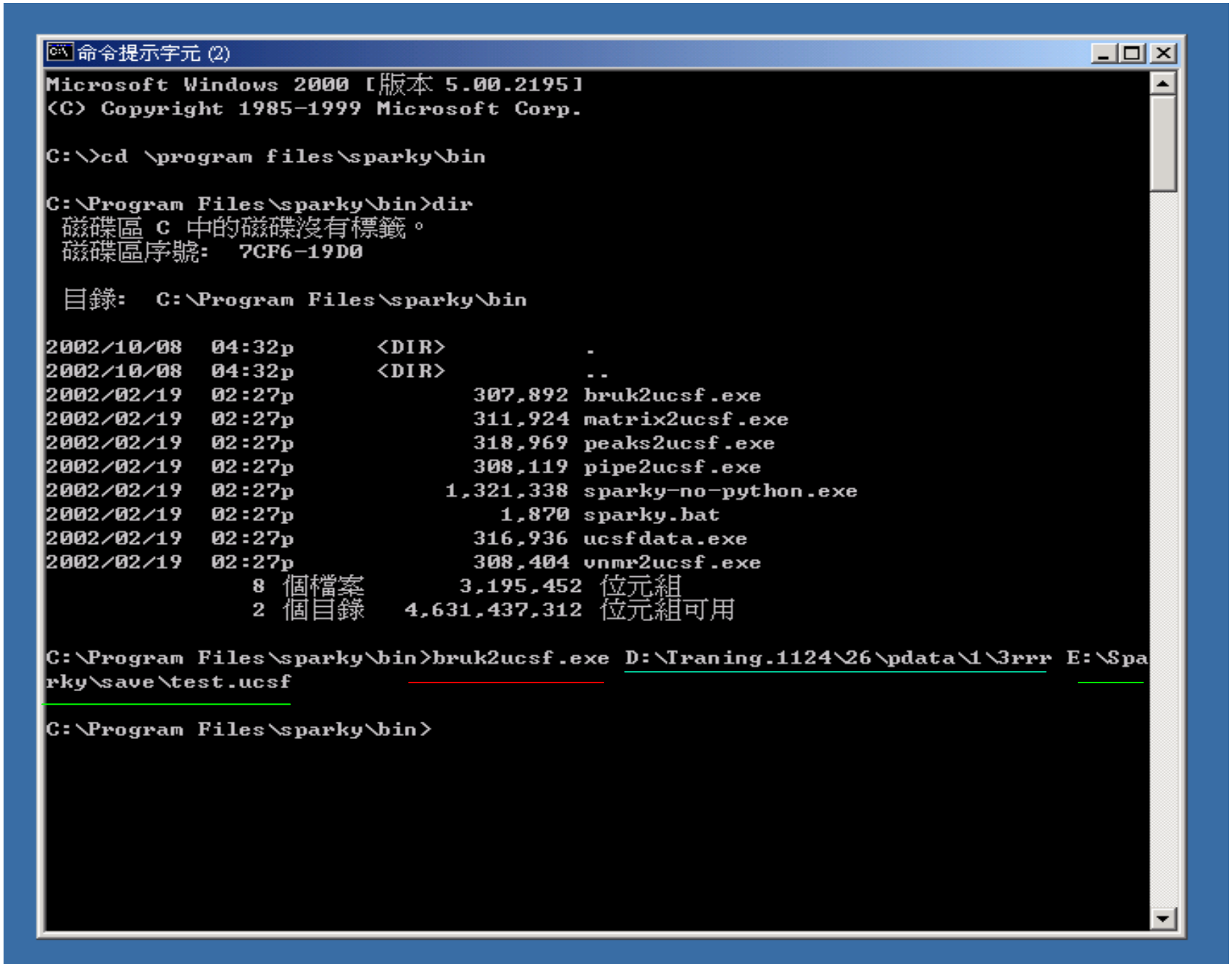
Open spectra

Converting processed data to UCSF format by bruk2ucsf, pipe2ucsf,vnmr2ucsf……

eg. in PC

c:\progranfiles\sparky\bin> bruk2ucsf d:\data\nie.310\12\pdata\1\3rrr

d:\sparky\data\noe.ucsf


命令提示字元 (2)
Microsoft Windows 2000 [版本 5.00.2195]
(C) Copyright 1985-1999 Microsoft Corp.
C:\>cd \program files\sparky\bin
C:\Program Files\sparky\bin>dir
磁碟區 C 中的磁碟沒有標籤。
磁碟區序號: 7CF6-19D0
目錄: C:\Program Files\sparky\bin
2002/10/08 04:32p <DIR> .
2002/10/08 04:32p <DIR> ..
2002/02/19 02:27p 307,892 bruk2ucsf.exe
2002/02/19 02:27p 311,924 matrix2ucsf.exe
2002/02/19 02:27p 318,969 peaks2ucsf.exe
2002/02/19 02:27p 308,119 pipe2ucsf.exe
2002/02/19 02:27p 1,321,338 sparky-no-python.exe
2002/02/19 02:27p 1,870 sparky.bat
2002/02/19 02:27p 316,936 ucsfdata.exe
2002/02/19 02:27p 308,404 vnmr2ucsf.exe
8 個檔案 3,195,452 位元組
2 個目錄 4,631,437,312 位元組可用
C:\Program Files\sparky\bin>bruk2ucsf.exe D:\Traning.1124\26\pdata\1\3rrr E:\Sparky\save\test.ucsf
C:\Program Files\sparky\bin>