2023 NMR Users Training (II)

Basic NMR SOP for
Small Molecules & Metabolomics Analysis

Data Collection & Processing using Topspin

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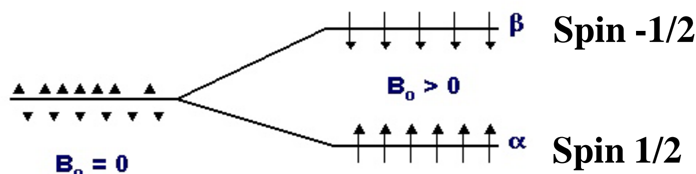
QA1: Do I need high field for better resolution ?

NMR field	<= 500MHz	>= 600 MHz
chemical shift(ppm)	the same	
coupling constant(J, Hz)	the same	
Sensitivity*	lower	higher
Resolution**	lower	better



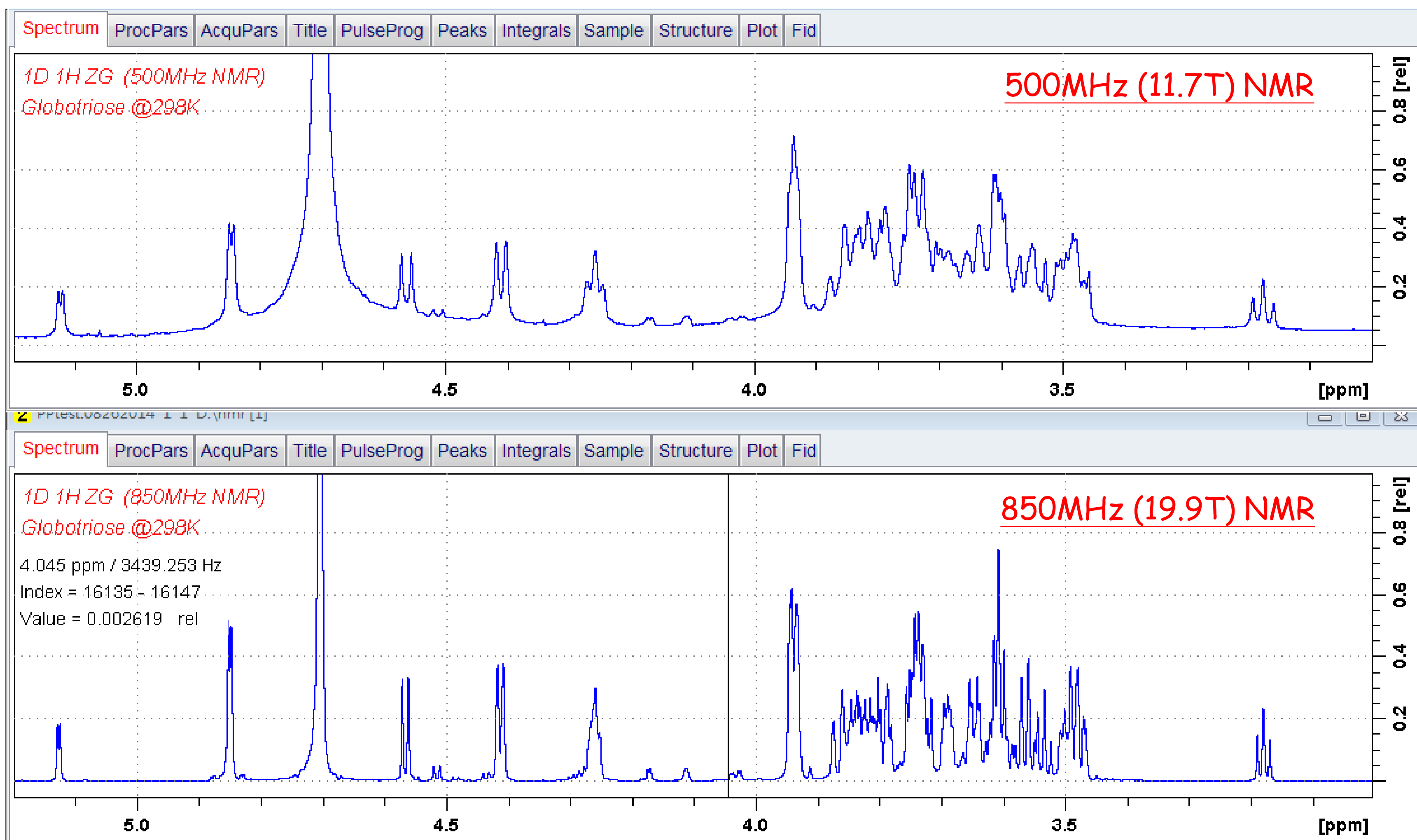
$$N_\beta/N_\alpha = \exp(-\Delta E/kT) = \exp[(\gamma h B_o)/(2\pi kT)]$$

$$S/N = \frac{N\gamma_{exc}T_2(\gamma_{det}B_0)^{3/2}\sqrt{ns}}{T}$$

S/N = signal to noise ratio
N = number of spins in the system (sample concentration)
γ_{exc} = gyromagnetic ratio of the excited nucleus
γ_{det} = gyromagnetic ratio of the detected nucleus
ns = number of scans
B_0 = external magnetic field
T_2 = transverse relaxation time (determines the line width)
T = sample temperature

* Higher Sensitivity is needed for low concentration sample
** Better resolution is needed for overlap peaks

1D 1H Spectrum in ppm

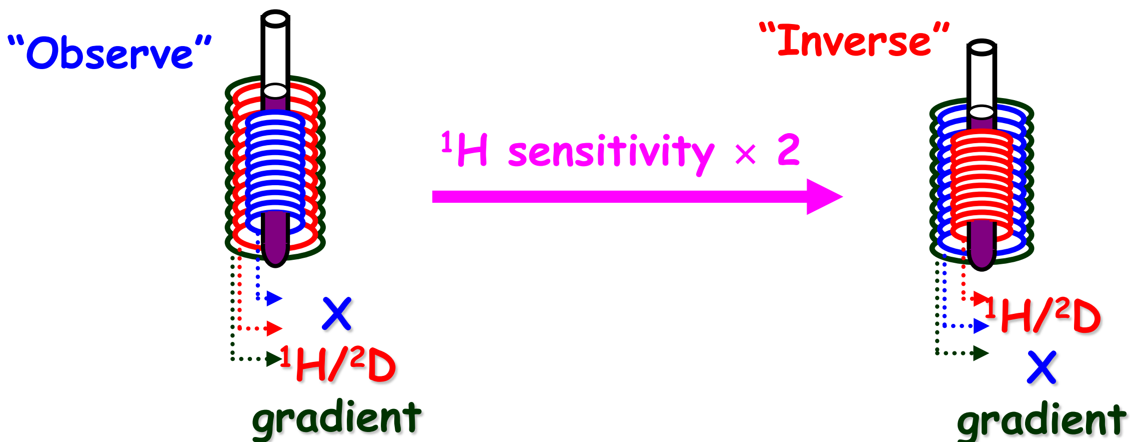


Higher the field, better the sensitivity and resolution

QA2: Which Probe could provide information I need?

Probe Type	Regular	Cryoprobe
Sensitivity* (organic solvent)	1	~4
(aqueous solution)	1	~2.5

Probe Coil	Observe (ex:TBO,BBO,Dual)	Inverse (ex: TXI, TCI)
	X-nuclei observed Experiment (1D 13C, 1D 31P)	1H-detected Experiments (1D 1H, 2D COSY/TOCSY 2D HSQC/HMBC)



NMR & Probes in HFNMRC

NMR & Probe	Topspin	1H (EB)	Others
NEO500_IBMS (Cryo QNP)	TP4.2	2,000	1,000 (13C) 988 (31P) 1,000 (19F)
AVIII600_IBMS (Cryo TCI_005)	TP2.x TP3.x	5,700	710(13C)
NEO600_IBMS (Cryo TCI_121)	TP4.1	6,530	950(13C)
AV600_CHEM (regular TBO)	TP2.x	406	377(13C) 241(31P) 342(19F)
AV800_IBMS (Cryo TXI)	TP2.x	6,200	N/A
AVIII850_IBMS (Cryo TCI)	TP2.x TP3.x	8,500	1,600(13C)

Regular Probe*	1H (EB)	Others
500MHz_TXI	450	
600MHz_TXI	1,218	
600MHz_BBO	465	465 (13C)
600MHz_QXI	1,193	85(31P)
600MHz_TBO		
800MHz_TXI	2,077	

CRYO Probe*	1H (EB)	Others
500MHz_TXI	4,196	
500MHz_QNP	2,000	1,000 (13C) 988 (31P)
600MHz_TCI_005	5,700	710(13C)
600MHz_TCI_121	6,530	950(13C)
800MHz_TXI	6,200	
850MHz_TCI	8,500	1,600(13C)

*Signal to Noise (S/N)value @ installed date

Data Collection using Topspin

Simple Operation Guide for HFNMRC Users

by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

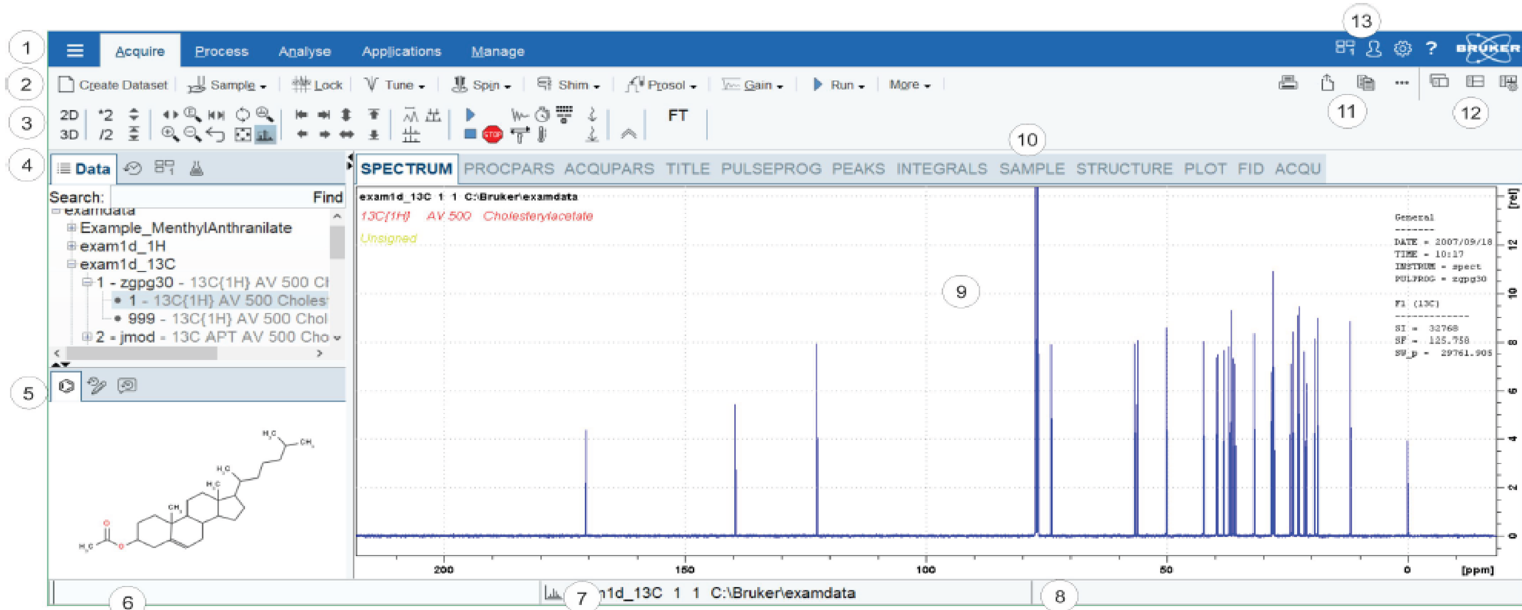
Topspin Software

	HFNMRC	Version	Upgrade in 2024
1	NEO500	Topspin4.2	Topspin4.3
2	AVIII600	Topspin2.1 Topspin3.2	
3	AV600_CHEM	Topspin2.1	
4	NEO600	Topspin4.1	Topspin4.3
5	AV800	Topspin2.1	Topspin2.1 Topspin3.2
6	AVIII850	Topspin2.1 Topspin3.2	Topspin4.3

	GRC	Version
1	AV600R	Topspin2.1
2	AV600L	Topspin2.1 Topspin3.1

What's new for Topspin4.x

➤ NEO must use Topspin4.x or higher version



◆ Routinely used commends are the same as Topspin2.x or Topspin3.x
指令與Topspin2.x or Topspin3.x 相通

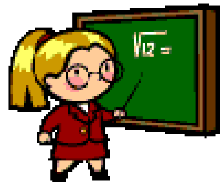
◆ Interface is different but not too difficult to follow
介面稍有不同, 熟悉即可

◆ HFNMRC Standard Experiments and SOP are the same
實驗設定方式與本核心其它NMR相同

Simple Operation Guide for HFNMRC Users

by Dr. Chi-Fon Chang for small molecules (2022.03.10 updated)

PART I: Preparation (Software & Hardware)



<Lock> The 2H signal is collected by "lock channel" that operates in parallel with the principle channels. "**Lock**" maintain the center of 2H resonance at a constant frequency.

- Deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used.
- The lock system uses a receiver to monitor this deuterium frequency and makes adjustments to the magnetic field strength accordingly.
- The deuterium frequency is measured several thousand times per second. Hence, as long as the system is locked, the user can be confident that the field is maintained at a constant strength during acquisition.

***Important: you must lock the correct solvent, otherwise, the spectrum chemical shift might be incorrect.**

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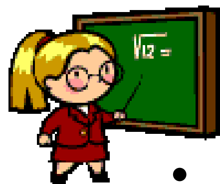
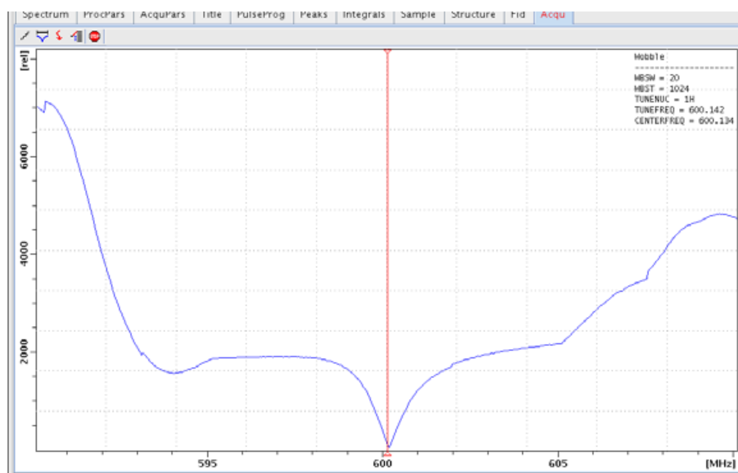
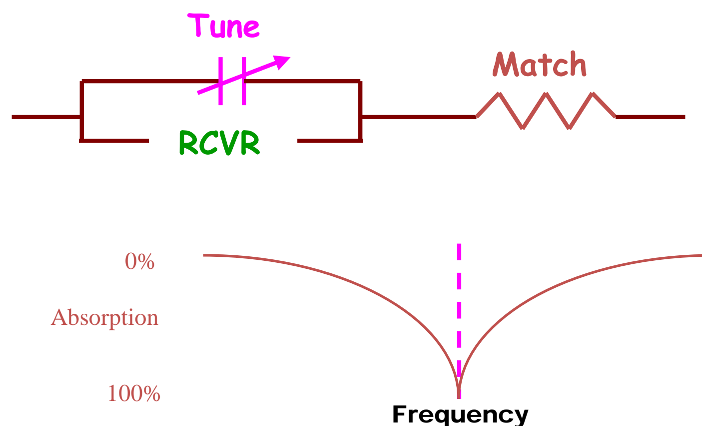


<Wobble> Wobble is to carry out "tuning" and "matching" simultaneously. Type "**wobb**" for old probe, "**atma**" or "**atmm**" for probe with ATM

- **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.)
- **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe.





*Important: for QNP or BBO probe, you should wobble for the X-nuclei you like to observe

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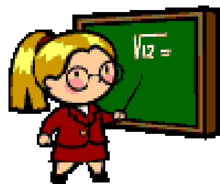
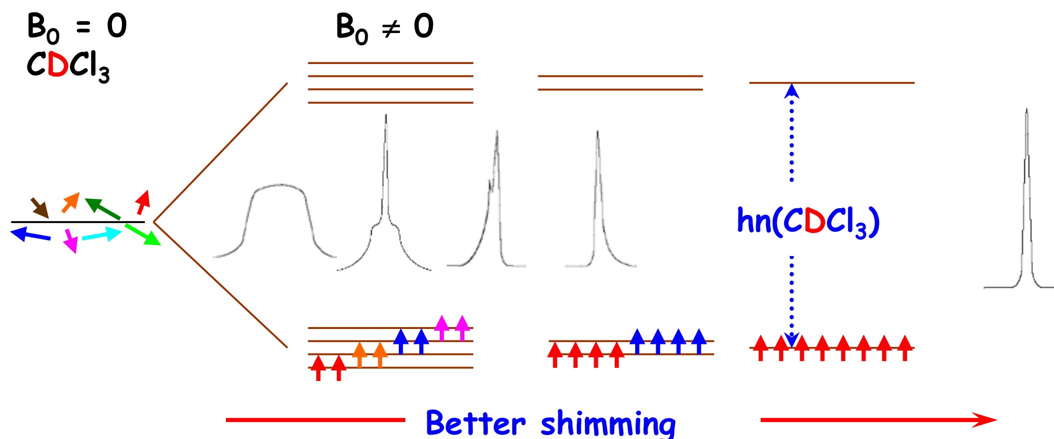


<Shimming> **Shimming** is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution.

- Shimming is to adjust the shim coil circuits (ex: Z, Z2, Z3…X,Y….) which are designed to create small magnetic fields that will cancel out inhomogeneity in the Bo magnetic field.



- Type “**topshim**” for automation, manually shim might be needed

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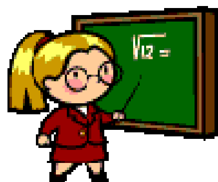
PART II: Experiment Set up & Data Collection



<getprosol> **getprosol** is to read in pulse parameters saved in the "prosol"

- **prosol** is a file (table) containing **Probe/Solvent** parameters (calibrated using standard sample, ex: Urea/Methanol) for the probehead installed on a specific NMR system.

edprosol

File Edit View Help

Saved Observe and Saved Decouple Prosol Parameter Set for:

Probe: Z44896_0121 CP TCI 600S3 H-C/N-D-05 Z Select...

Solvent: generic

Observe Nucleus Decouple

Observe: 1H Decouple: 1H

Observe Comment: Default 1H obs 600 Decouple Comment: Default 1H dec 600

90 deg. Pulses | HR Square Pulses | HR Shape Pulses | Others

Nucleus	Observe Pulse Width[μs]	Observe Att. Lvl.[dB]	Set	Decouple Pulse Width[μs]	Decouple Att. Lvl.[dB]	Set	Nucleus
1H	8.00	-7.32		8.00	-7.32		1H
2H	68.00	-14.81		68.00	-14.81		2H
13C	12.00	-19.55		12.00	-19.55		13C
15N	35.00	-19.23		35.00	-19.23		15N

*Important: the pulse parameters in "HFNMRC standard parameter set" won't be the same for current probe. Thus, you must "getprosol" to read in the correct parameters for current probe you are using.

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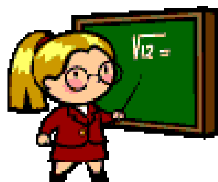
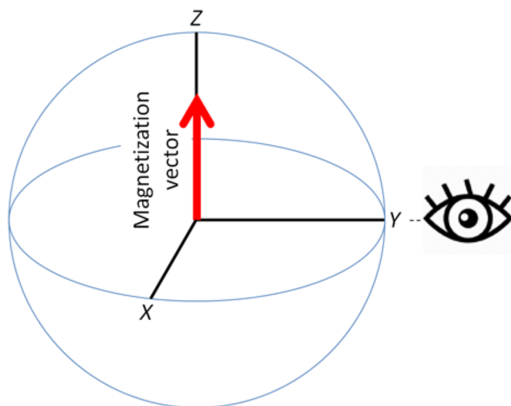
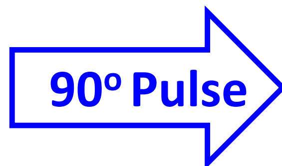
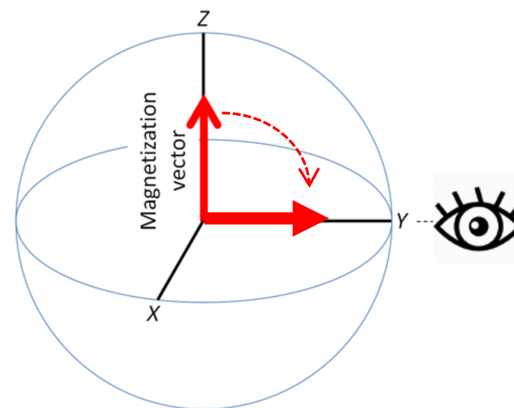
PART II: Experiment Set up & Data Collection



<pulsecal> is a command to determine 90 degree pulse for your sample





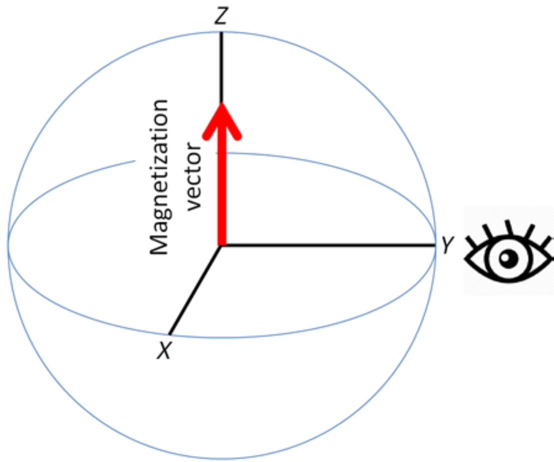


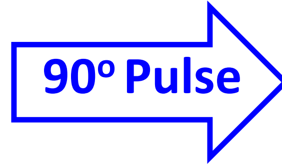
- Write down the **power level (dB)** and **corresponding pulse-length(us)**
- Instead of "getprosol" only, you should type

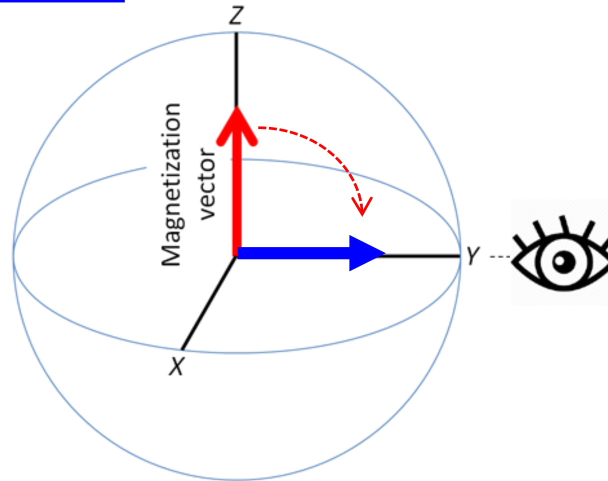
  "getprosol 1H <90º pulselength> < corresponding power level> "

***With optimized 90 degree pulse, the spectrum quality could be better. Especially for multi-dimensional experiments or experiments with specific pulse angles (ex: dept90, dept135, cosy45)!**

90° vs. 30° Pulse

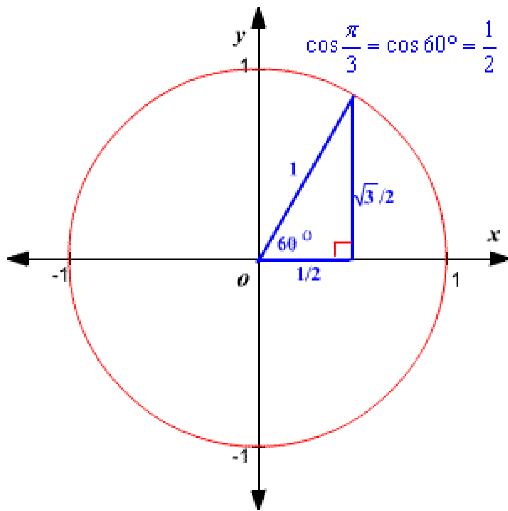


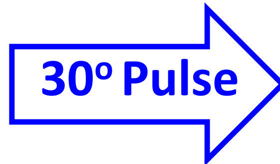


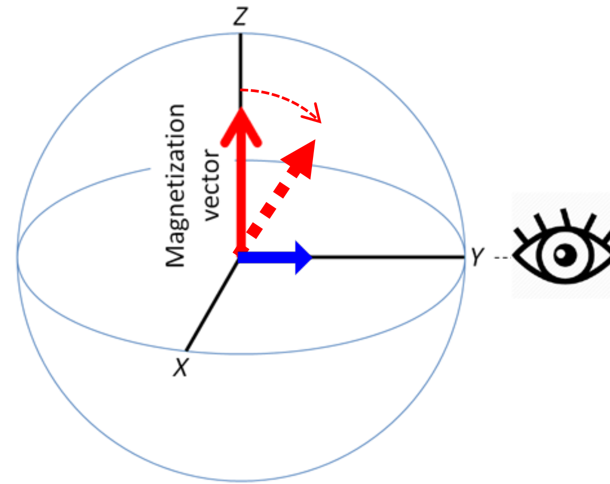


Signal Intensity=Full →

But need to wait longer time for next scan



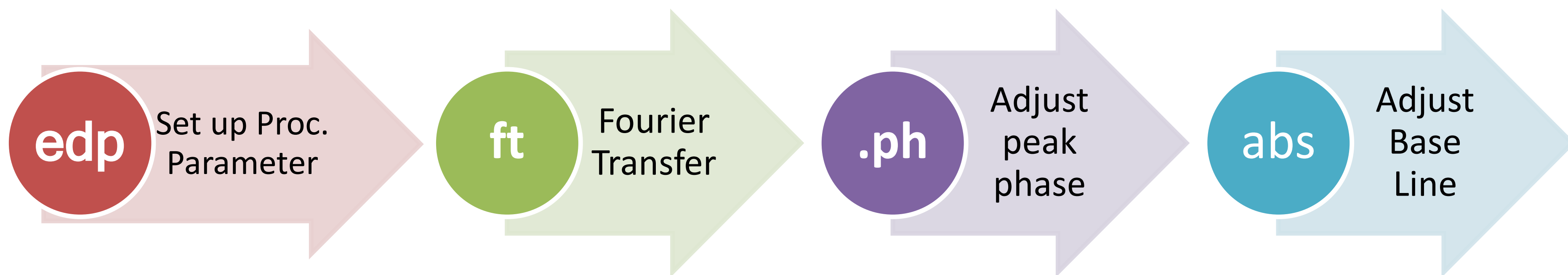




Signal Intensity=1/2 →

But back to Z faster, can collect more scan

Data Processing using Topspin

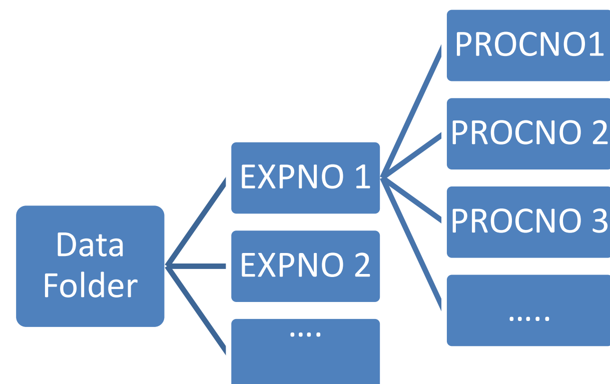


NOTE: Topspin file structure 檔案編排方式

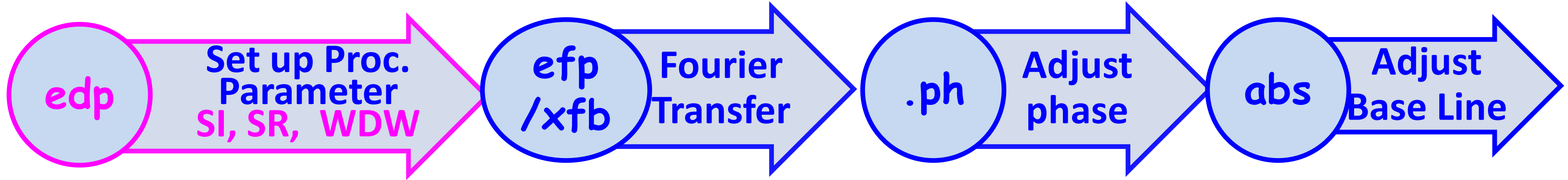
Data Folder / Experiment Number / Process Number
Data 檔名 / 實驗編號 (EXPNO) / 圖譜處理編號 (PROCNO)

Browser | Last50 | Groups

ProteinTrain.2019 (Data Folder)
- 1 - zggppr - 1D 1H ZGGPPR / UB standard (white label) @:
- 2 - hsqcetfpf3gpsi - 2D 15N HSQC hsqcetfpf3gpsi / getpro:
  - 1 - 2D 15N HSQC hsqcetfpf3gpsi / getprosol 1H 9.05 -9
- 3 - hsqcetgpsisp2.2 - 2D 13C HSQC hsqcetgpsisp2.2
  - 1 - 2D 13C HSQC hsqcetgpsisp2.2
- 11 - hncagp3d - HNCA hncagp3d
- 12 - hncocagp3d - HN(CO)CA hncocagp3d
- 13 - hncacbgp3d - HNCACB hncacbgp3d (EXPNO 13)
  - 1 - HNCACB hncacbgp3d (PROCNO 1 → reserved for final 3D processing)
  - 13
  - 23 (other PROCNO number → can be used for other purpose)
- 14 - cbcaconhgp3d - CBCA(CO)NH cbcaconhgp3d
- 15 - hncogp3d - HNCO hncogp3d
- 16 - hncacogp3d - HN(CA)CO hncacogp3d
- 21 - hbhaconhgp3d - 3D HBHA(CO)NH hbhaconhgp3d
- 22 - hcchcogp3d - 3D HCCHCOSY hcchcogp3d
- 23 - hcchdigp3d - 3D HCCHTOCSY hcchdigp3d (H-C-H)
- 24 - hccconhgp3d3 - 3D HCCCONH (H-N-C) hccconhgp3d
- 25 - hccconhgp3d2 - 3D HCCCONH (H-N-H) hccconhgp3d
- 26 - dipsihsqcf3gpsi3d - 3D 15N-TOCSYHSQC dipsihsqcf
- 27 - ccconhgp3d - CCCONHGP3D



Workflow for Data Processing (1)



1D

SI: > 2*TD
(Size of spectrum)

SR: (check)
(Spectrum Reference)

Use internal or external standard (ex: TPP, DSS) to do calibration

WDW: EM or QSINE
(Window Function)

Bruker TopSpin 3.6.0 on NB-000112 as cfchang

Start | Process | Analyse | Publish | View | Manage

Create Dataset | Find Dataset | Open Dataset | Paste Dataset | Read Pars.

Protein Train.2019 1 1 G:\data

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Plot | Fid

Reference
Window
Phase
Baseline
Fourier
Integration
Peak
Deconvolution
Automation
Miscellaneous
User

Reference

Parameter	Value	Description
SI	32768	Size of real spectrum
SF [MHz]	600.1400000	Spectrometer frequency
OFFSET [ppm]	12.71319	Low field limit of spectrum
SR [Hz]	0	Spectrum reference frequency
HZpPT [Hz]	0.293438	Spectral resolution
SPECTYP	UNDEFINED	Type of spectrum e.g. COSY, HMQC, ...

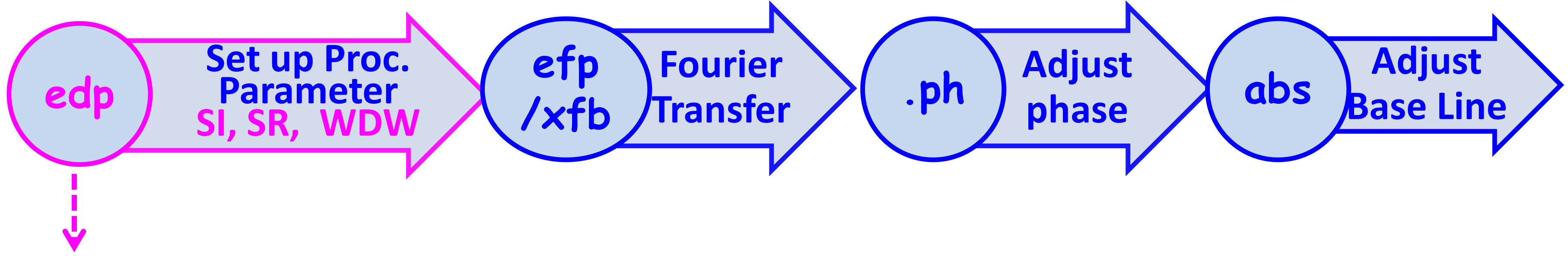
Window function

Parameter	Value	Description
WDW	EM	Window functions for trf, xfb,...
LB [Hz]	1.00	Line broadening for em
GB	0	Gaussian max. position for gm, 0<GB<1
SSB	2	Sine bell shift SSB (0,1,2,..)
TM1	0	Left limit for tm 0<TM1<1
TM2	0	Right limit for tm 0<TM2<1

Phase correction

Parameter	Value	Description
PHC0 [degrees]	98.824	0th order correction for pk

Workflow for Data Processing (1)



Bruker TopSpin 3.6.0 on NB-000112 as cfchang

Start | Process | Analyse | Publish | View | Manage

Proc. Spectrum | Adjust Phase | Calib. Axis | Pick Peaks | Integrate | Advanced

1 Protein Train.2019 2 1 G:\data

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Plot | Fid

Reference, Window, Phase, Baseline, Fourier, NUS, Peak, Automation, Miscellaneous, User

2D

	F2	F1	Frequency axis
Reference			
SI	2048	256	Size of real spec
SF [MHz]	600.1400000	60.8116580	Spectrometer fre
OFFSET [ppm]	10.70628	135.00130	Low field limit of
SR [Hz]	0	0	Spectrum refere
HZpPT [Hz]	3.521259	8.314709	Spectral resoluti
SPECTYP	HSQC		Type of spectrur
Window function			
WDW	QSINE	QSINE	Window function
LB [Hz]	0.30	0.30	Line broadening
GB	0	0.1	Gaussian max. p
SSB	2	2	Sine bell shift SS
TM1	0	0.1	Left limit for tm 0
TM2	0	0.9	Right limit for tm

4 Protein Train.2019 11 2 G:\data [3]

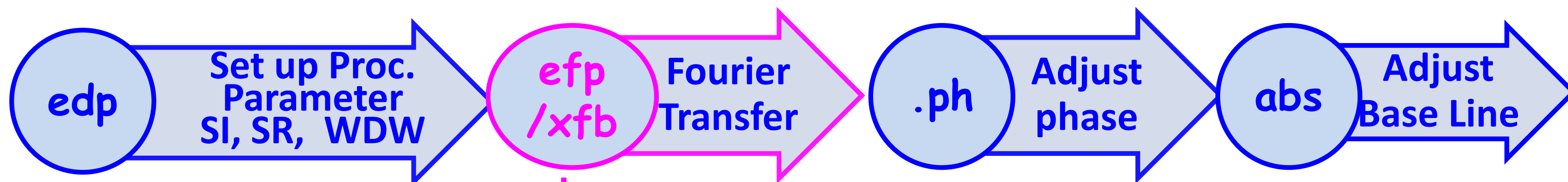
Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Plot | Fid

Reference, Window, Phase, Baseline, Fourier, NUS, Peak, Automation, Miscellaneous, User

3D

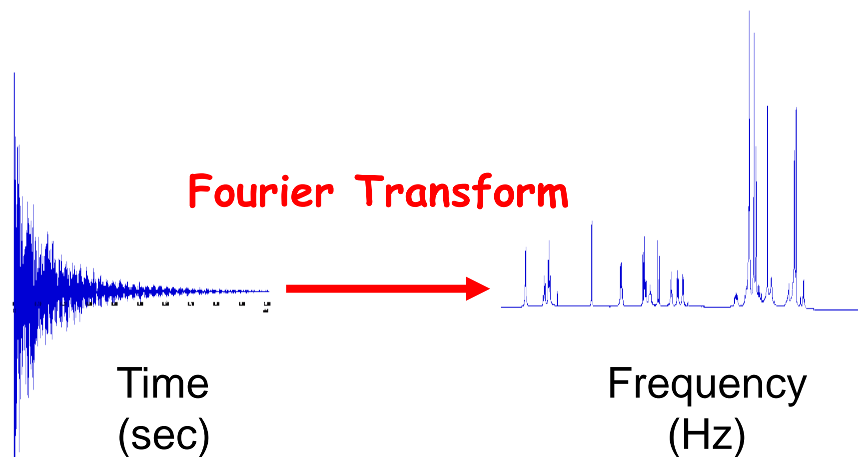
	F3	F2	F1
Reference			
SI	2048	32	128
SF [MHz]	600.1400000	60.8116580	150.9053230
OFFSET [ppm]	10.70628	135.00130	70.00701
SR [Hz]	0	0	0
HZpPT [Hz]	1.595570	66.517670	37.741547
AQORDER	3-2-1		
SPECTYP	UNDEFINED		
Window function			
WDW	QSINE	QSINE	QSINE
LB [Hz]	1.00	0.30	0
GB	0.1	0.1	0
SSB	2	2	2
TM1	0.1	0.1	0.1

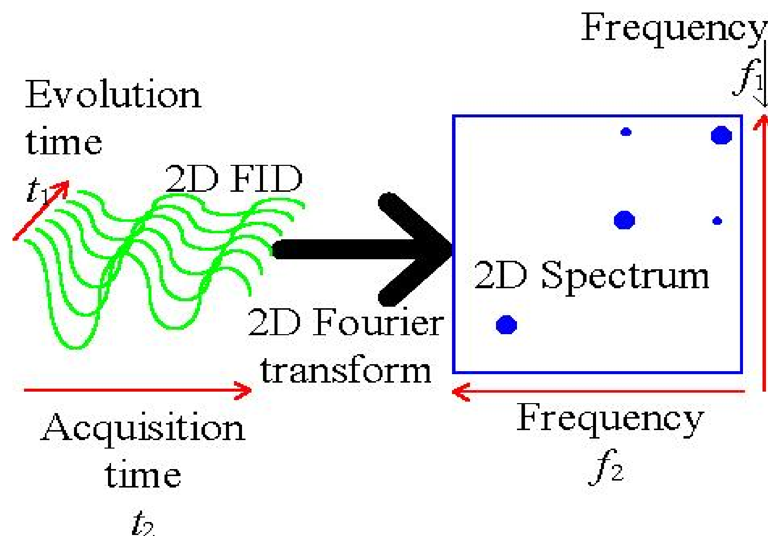
Workflow for Data Processing (2)



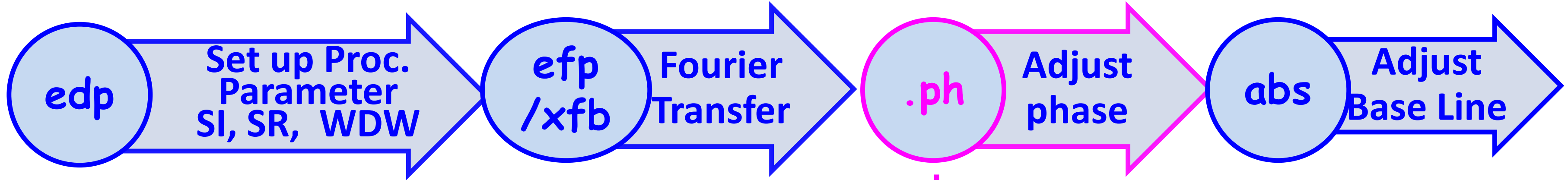
1D : efp
(Fourier Transfer on 1 dimension)

2D : xfb
3D: xfb & ft3d
(Fourier Transfer on "both", ie, 2 dimension)





Workflow for Data Processing (3)



1D → .ph
(PHC)/PHC1)

Bruker TopSpin 3.6.0 on NB-000112 as cfchang

Start | Process | Analyse | Publish | View | Manage

Proc. Spectrum | Adjust Phase | Calib. Axis | Pick Peaks | Integrate | Advanced

Adjust spectrum phase manually *(.ph)*
Enter manual phasing mode. Allows you to adjust the zero and first order correction terms ***PHC0*** and ***PHC1***.

2 PPtest_PS_HSQC.20190805 1

1 PPtest_PS_HSQC.20190805 1 1 G:\data [2]

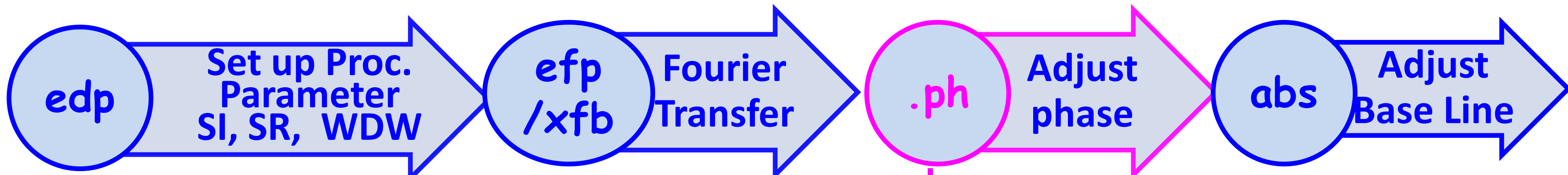
Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample

1D 1H ZG

Bad Phase !!

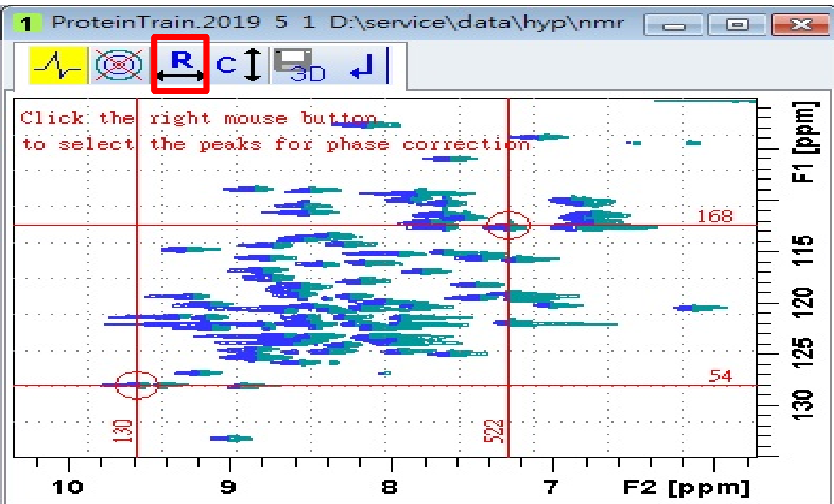
Good Phase !!

Workflow for Data Processing (3)

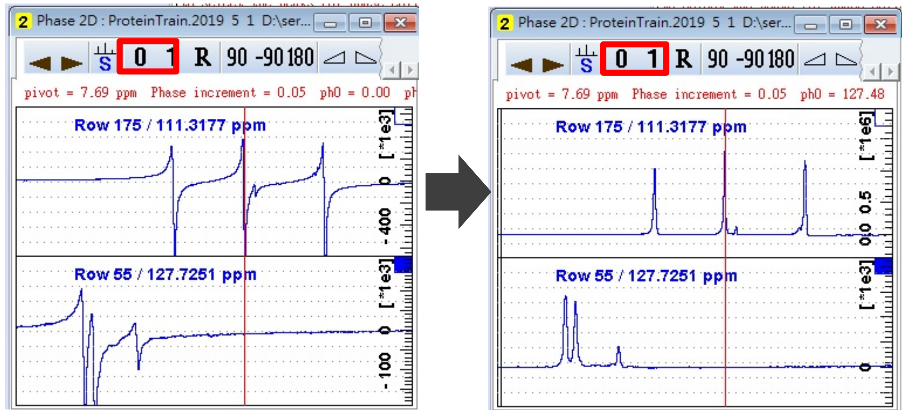


2D → select 2 or more "rows" → adjust PHC0 & PHC1 similar to 1D

Step 1: pick peaks by click right mouse and add, then click "row" to phase the row

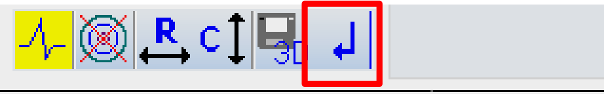


Step 2: use mouse to adjust phase 0 (zero order) and phase 1 (first order)

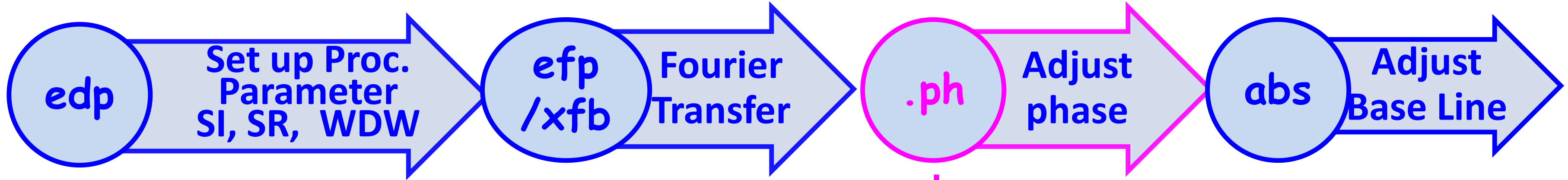


Save the good phase

Step 3 :Exit the "phase mode"



Workflow for Data Processing (3)

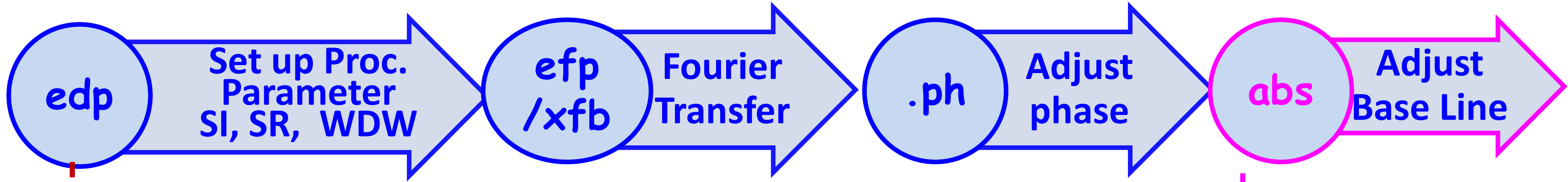


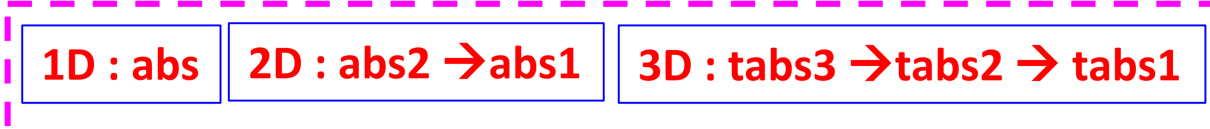
2D → select 2 or more "rows" → adjust PHC0 & PHC1 similar to 1D

Bad Phase !!

Good Phase !!

Workflow for Data Processing (4)



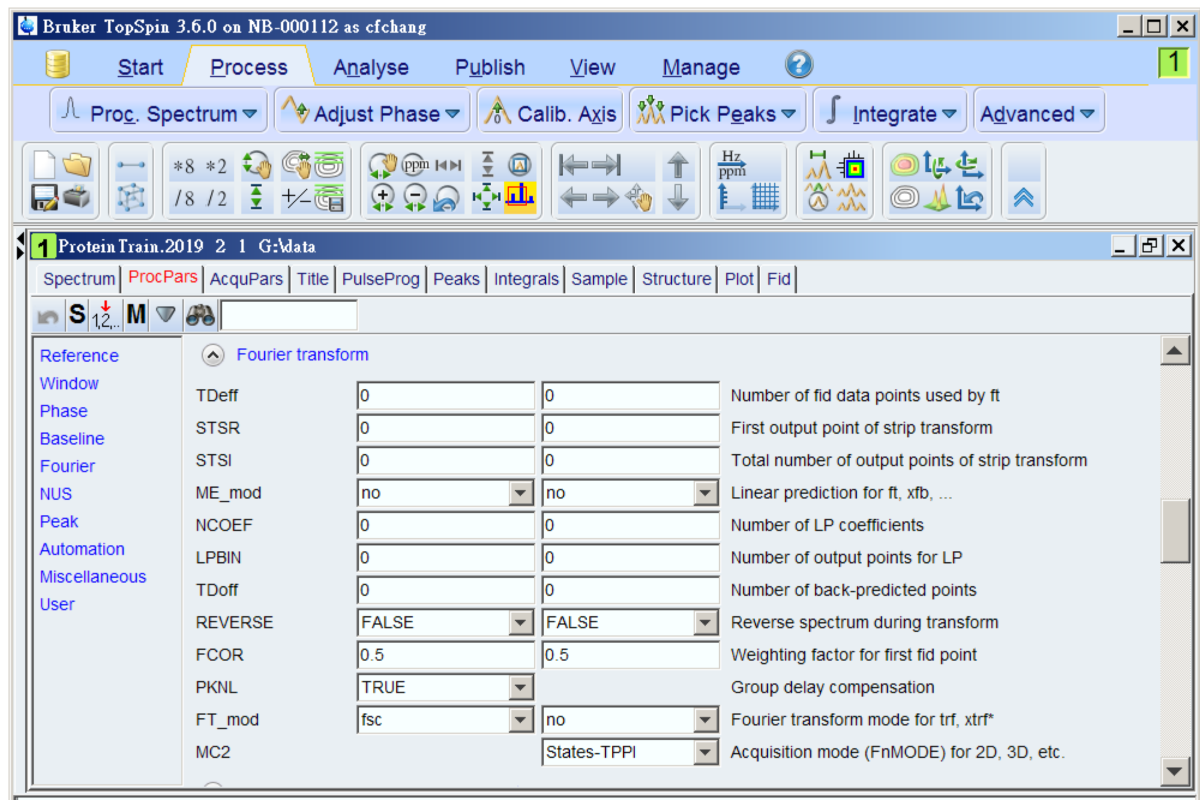


Other useful Tips

STSR / STSI : adjust window
(adjust spectrum window)

ME_mod: (ex: LPfr)
(Linear prediction)
NCOEF: 8*n (ex: 8, 16…)

REVERSE: FALSE / TURE
(check from spectrum)



Hands-on
Let's try Some Experiments

- 1D 1H one pulse (zg)
- 1D 1H solvent suppression (noesypr1d)
- 1D 13C DEPT (deptq)
- 2D 1H-1H COSY
- 2D 1H-13C HSQC