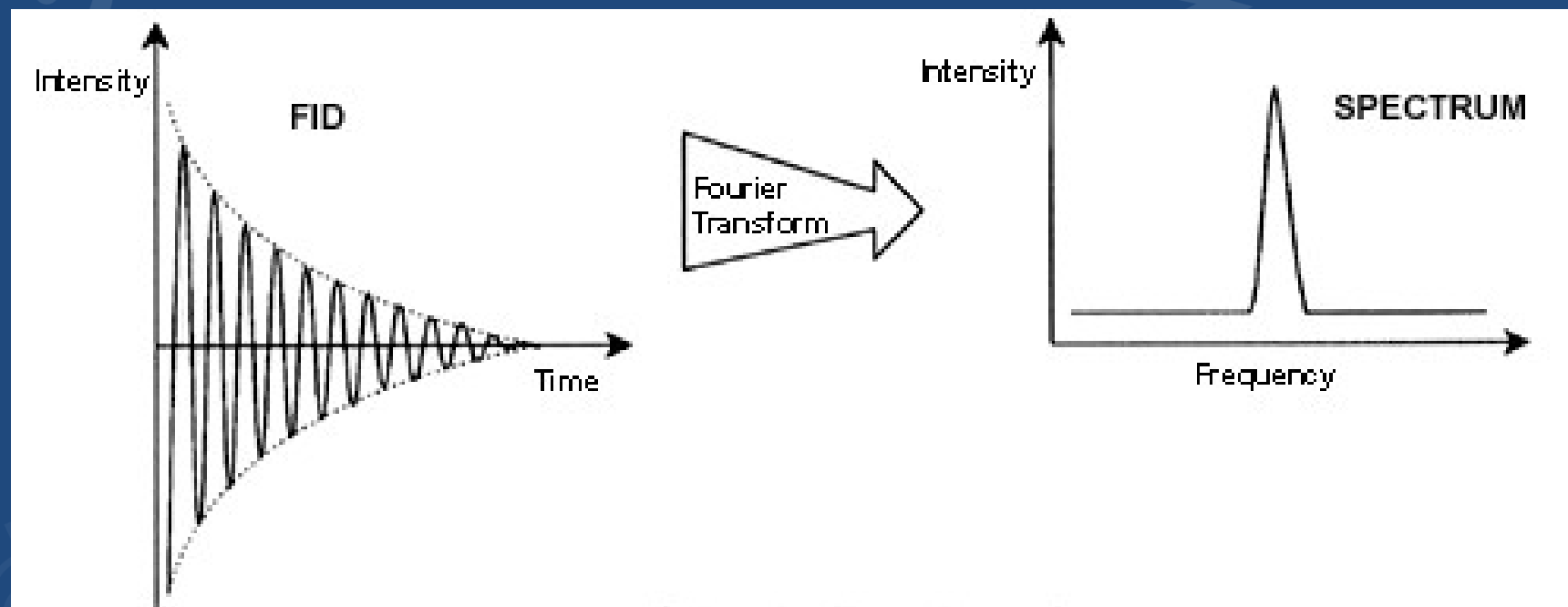


A large, stylized white cloud shape is centered on the page. Inside the cloud, the text "NMR Data Processing" is written in a bold, dark blue font. Surrounding the cloud are various white line-art icons: a globe, a lightning bolt, a lightbulb, a pencil, a question mark, a computer mouse, and an @ symbol. The background is split horizontally into an orange top half and a dark blue bottom half.

# NMR Data Processing

KAIST 화학과 NMR center // 신재선

# NMR spectrum



# NMR processing?



<http://www.stockvault.net/photo/104139/toothless-lew>

**Do you...**

- Shoot Through Light Polluted Skies?
- Get Noisy Images?
- Like Diffraction Spikes Around Stars?

**These Actions Are For You!**

[http://www.prodigitalsoftware.com/Astronomy\\_Tools\\_For\\_Full\\_Version.html](http://www.prodigitalsoftware.com/Astronomy_Tools_For_Full_Version.html)

# Software



## ○ NMR manufacture

- Topspin / VNMRJ / Delta

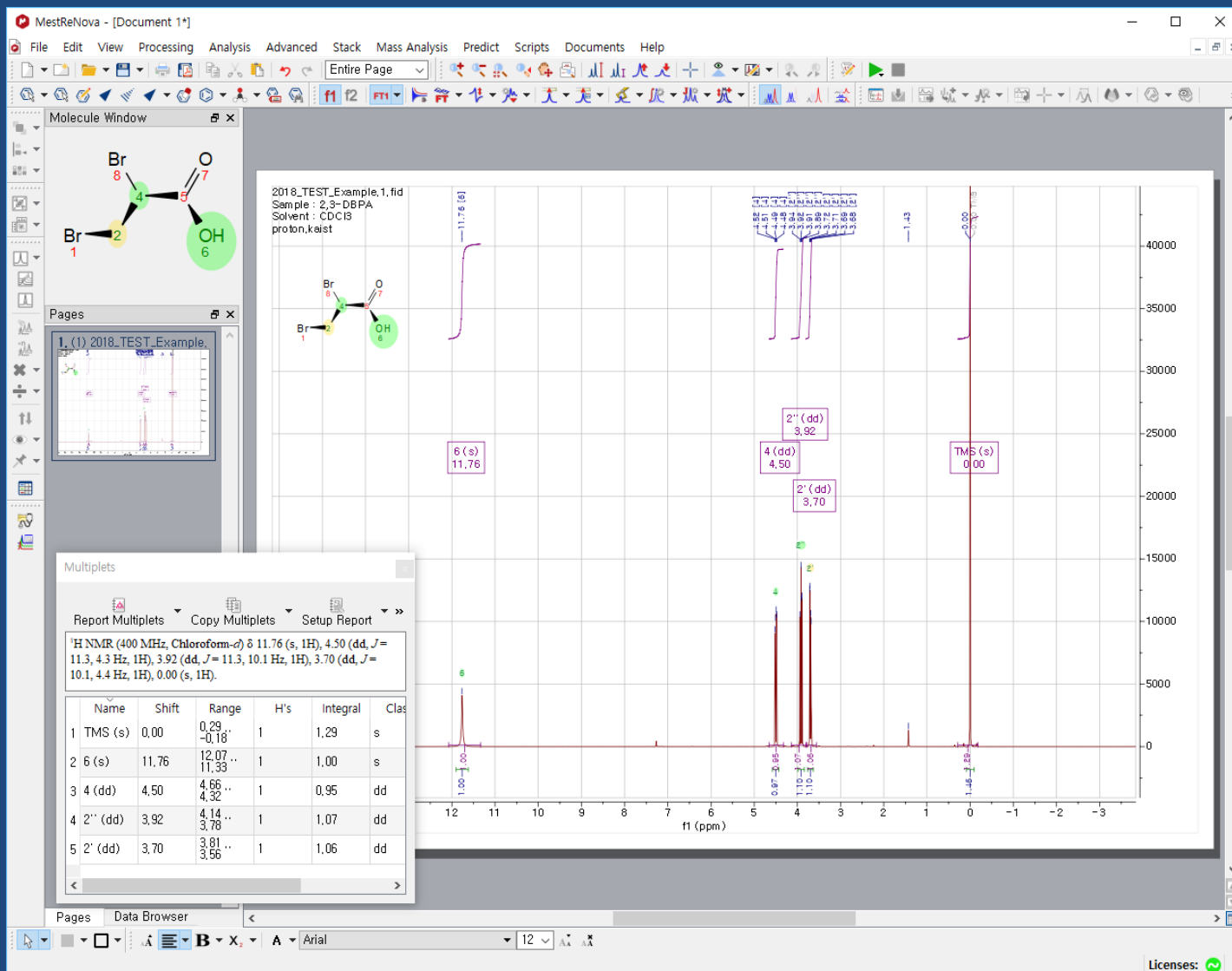
## ○ Free Software

- ACD NMR processor, SpinWorks 4, iNMR
- Sparky, CCPN-NMR, NMRViewJ

## ○ Commercial Software

- Nuts, MestReNova

# MestReNova



# MestReNova



- Version 14.0.1
- Campus license
  - Actually unlimited users can use in KAIST campus
  - 90 days : out of KAIST campus



# Installation



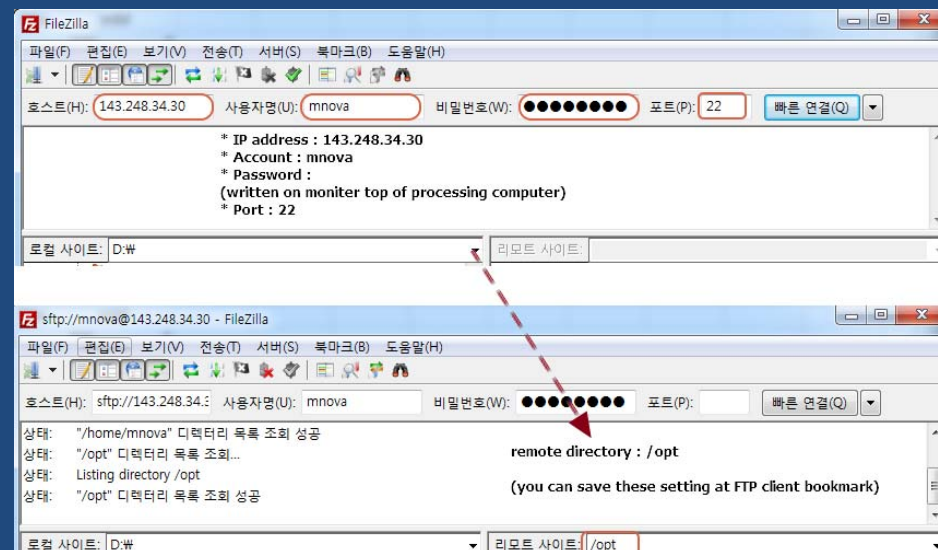
## ○ Download Mnova

○ <http://mestrelab.com/download/mnova/>

## ○ Or ...

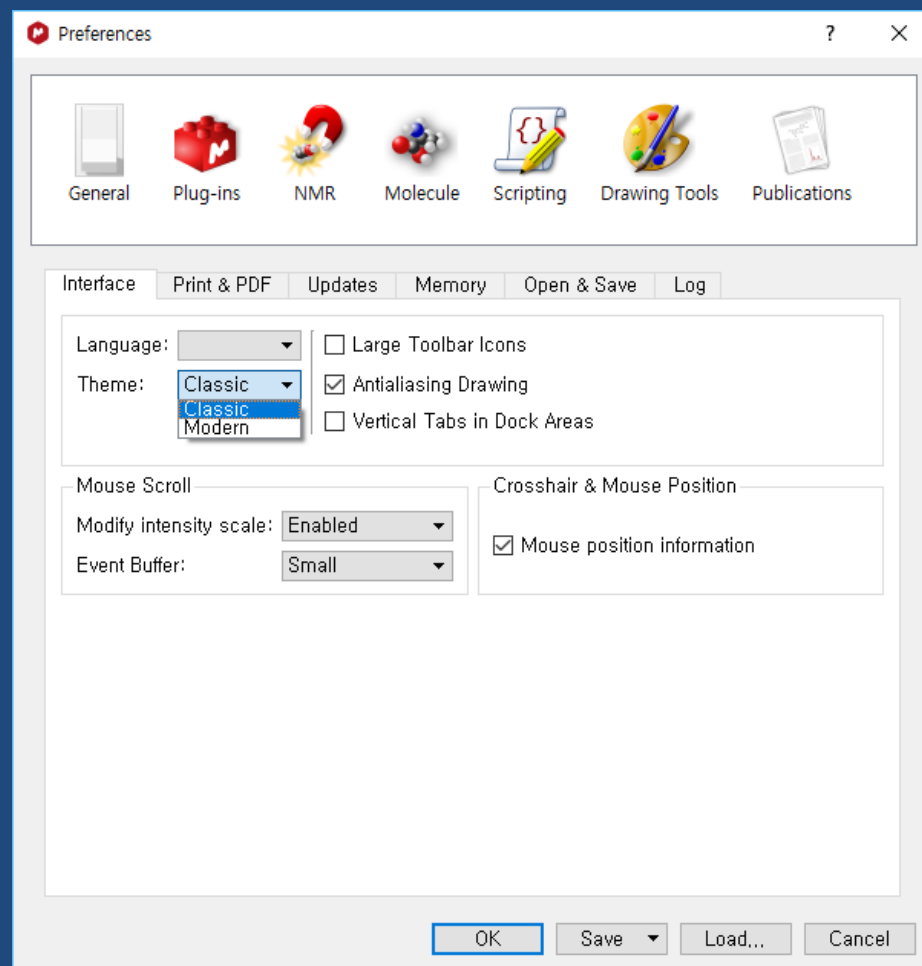
○ Install Filezilla (FTP client)

○ Download License



# Interface

## Modern vs classic





# How to use?

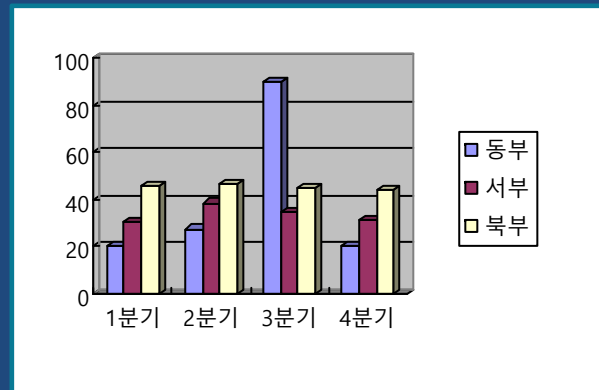
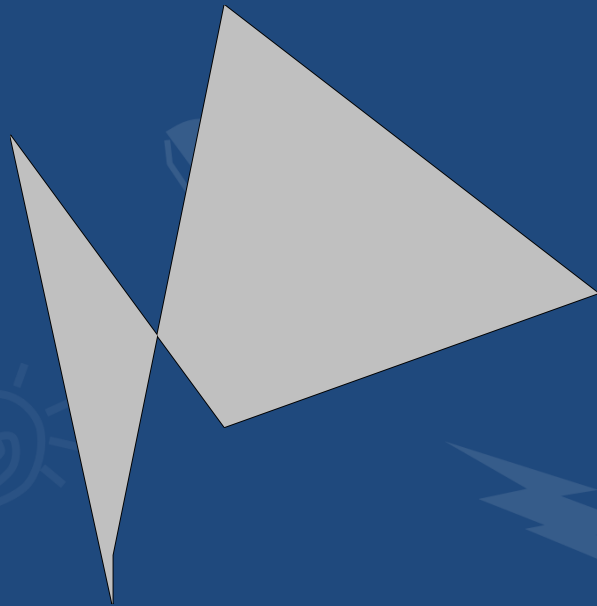


- 1D processing
  - qNMR, water suppression
- 2D processing
  - NUS, assignment
- Multiple Spectrum Analysis
  - Reaction Monitoring // Kinetics
  - DOSY
  - T1/T2 relaxation
- Etc...

# Home



- Drawing
- Insert Object



# View

○ Spectrum Toolbar

○ Shortcuts

○ Audit Trail

○ Tables

Shortcuts

	Command	Shortcut
1	Analysis > Assignments > Manual Assignment	A
2	Analysis > Assignments > Swap Assignments	S
3	Analysis > Integration > Delete Manually	Ctrl+Sh...
4	Analysis > Integration > Manual	I

Audit Trail

Show For: Document

Filter

Operation	Date	User	Version
> Import Item	2018-09-13T00:21:00	whoa	12.0.3-21384
Apodization	2018-09-13T00:46:07	whoa	12.0.3-21384
Zero Filling and LP	2018-09-13T00:46:13	whoa	12.0.3-21384

Tables

Filter

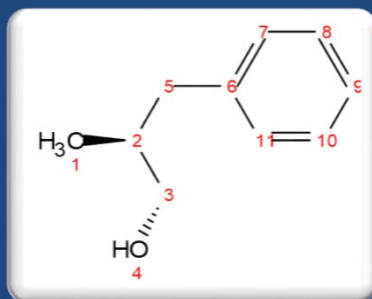
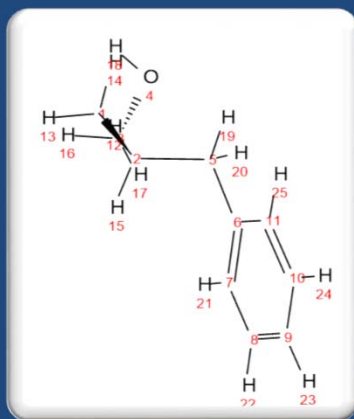
OK Cancel

- Common
  - Cursor Info
  - Audit Trail
  - Data Browser
  - Parameters
  - Stacked Items
- Mass
  - Mass Prediction
  - MS Browser
  - Elemental Composition
  - Molecule Match
  - Mass Peaks
  - Peak Purity Result
- Molecule
  - 3D Molecule
  - Compounds
  - Molecule Window
  - SDF Browser
- NMR
  - Arrayed Data
  - Assignments
  - Integrals
  - Multiplets
  - Peaks
  - Blind Regions
  - Line Fitting
  - Data Analysis
  - Integral Manager
  - Multiplet Manager
  - PCA Results
  - Phase Correction
  - Time Domain NMR Quantitati...
- NMR Prediction
  - 13C Prediction
  - 1H Prediction
  - X-Nuclei Prediction
  - Prediction DB Browser
  - Spin Simulation

# Molecule

## Draw

- 2D molecule
- 3D molecule with energy minimization
- View → Tables



3D Molecule

Report Copy Delete Select

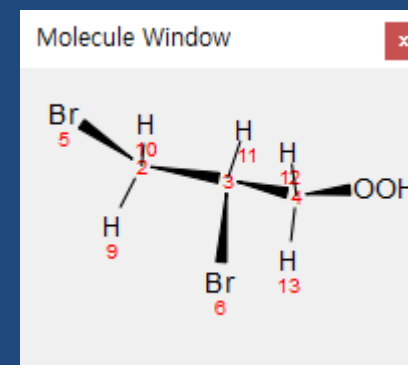
Energy (kcal/mol): Population (%):

3D ball-and-stick model of a molecule.

Compounds

Report Add Delete Setup Graphical Props

Molecule	Properties
	<b>Molecular F...</b> C <sub>3</sub> H <sub>6</sub> Br <sub>2</sub> O <sub>2</sub>
	<b>Average M...</b> 233.89
	<b>Monoisoto...</b> 231.87
	<b>Name:</b>
	<b>Label:</b> Compound(f...
	<b>Color:</b> <input checked="" type="checkbox"/> None
	<b>Assignments:</b> <input type="checkbox"/>



# 1D processing



- Apodization
- Zero filling
- Phase correction
- Baseline correction
- Referencing
- Peak picking
- Integration
- Multiplet Analysis

# Open files (Ctrl + O)



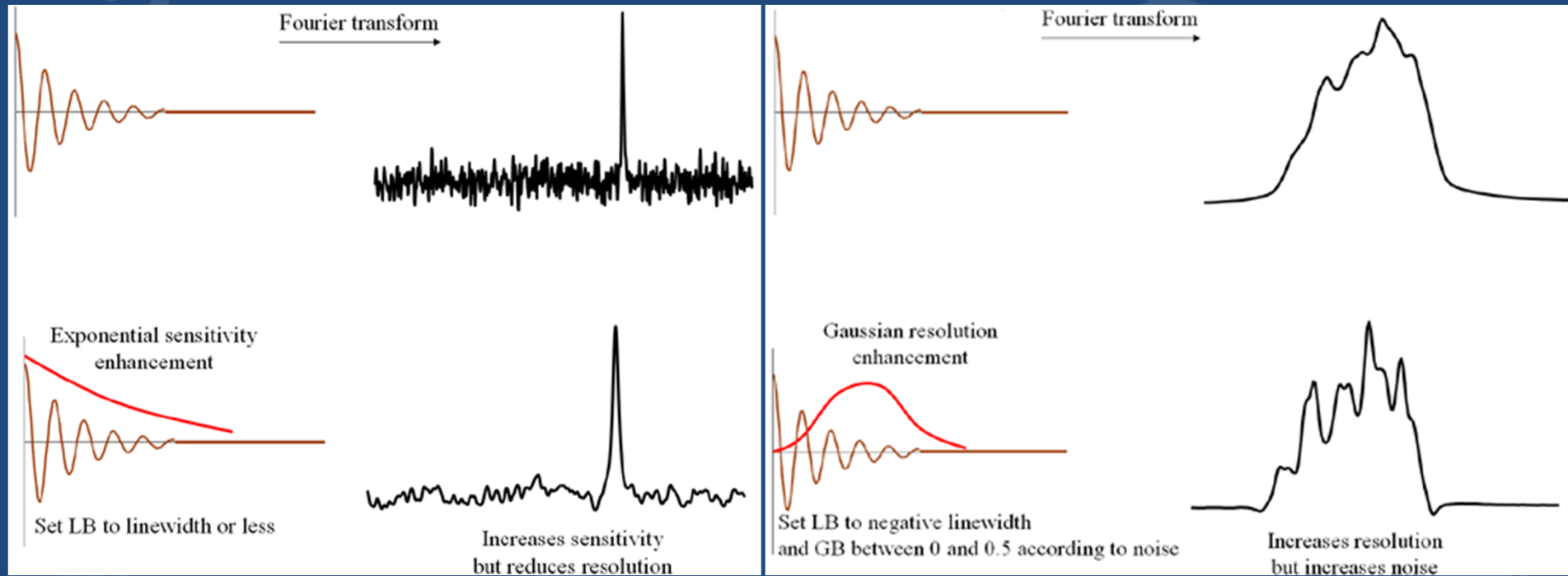
- Shortcut key : Ctrl+O
- Data type
  - Agilent / Varian : fid
  - Bruker
    - 1D : fid
    - nD : ser
  - Jeol : .jdf
- Or just drag-N-drop folder to Mnova



# Apodization(W)



$$d'_k = a_k d_k$$



# Apodization(W)

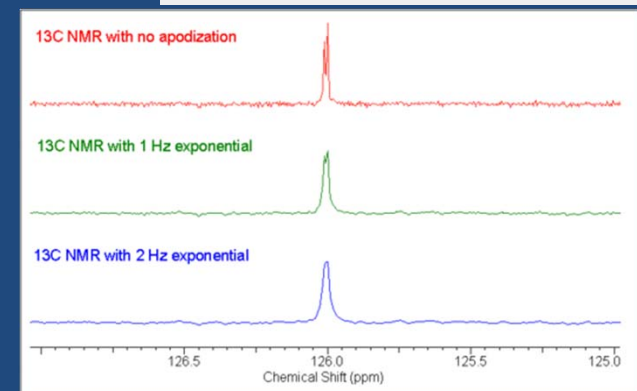
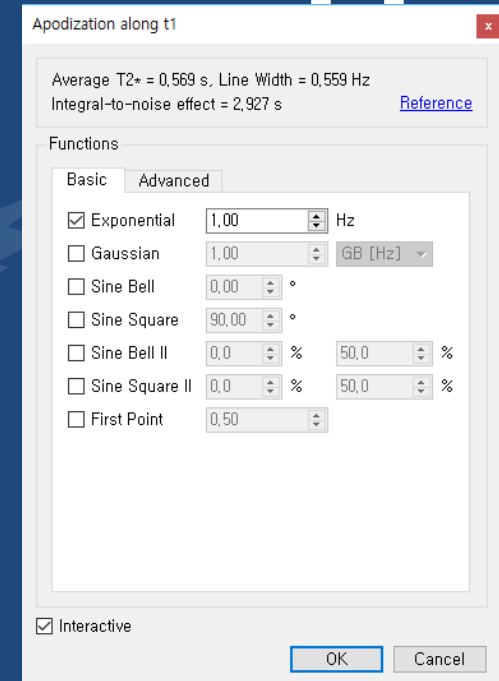
## 1D : optional

### Proton :

- Recommended for bad peak shape
- Exponential // 0 ~ 0.2 Hz

### Carbon :


- Recommended for low S/N
- Exponential // 1 ~ 2 Hz

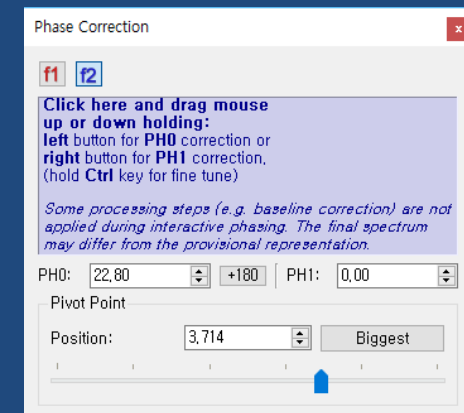




# Phase correction(Shift + P)



- Auto phase correction :  $^1\text{H}$ ,  $^{13}\text{C}$ 
  - Bruker : apk, apk0
  - Agilent : aph or [Autoprocess]
  - Mnova : 
- Manual correction
  - Spectrum of Hetero nuclei ( $^{11}\text{B}$ ,  $^{19}\text{F}$ , etc ... )
    - NMR tube : Borosilicate with teflon coating
  - Shortcut key (Shift + P)
    - Global correction ( $0^{\text{th}}$  order)
    - Gradient correction ( $1^{\text{st}}$  order)



# Baseline correction(B)



## ○ Auto baseline correction : $^1\text{H}$ , $^{13}\text{C}$

- Bruker : abs , absn, absf

- Varian : bc

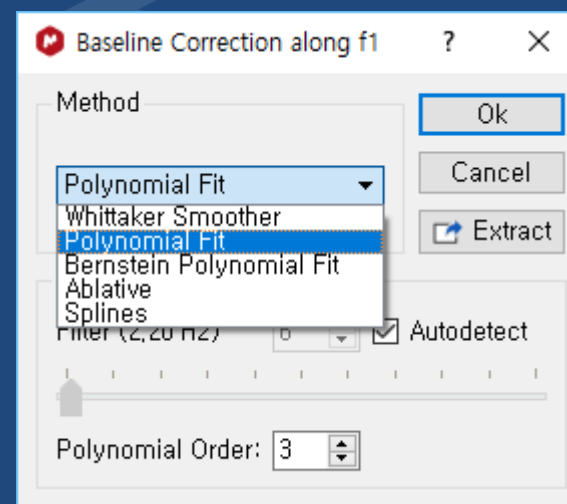
- Mnova 

## ○ Manual correction

### ○ Polynomial Fit

- Order : < 5 is recommended

- Filter : Autodetect is recommended



# Baseline correction(B)



- Manual correction
  - Splines
  - Whittaker smoother
    - Don't use if broad peak is concerned
  - Bernstein Polynomial
  - Ablative
  - Multipoint Baseline Correction
    - Pick Point

# Reference (L)



- Reference (L) or Graphic Reference (R)
  - TMS or DSS
  - Solvent peak



# Absolute Reference

## ○ IUPAC 2001 (modified 2008)

- Reference of  $^1\text{H}$  resonance of TMS for all nuclei

- <http://www.iupac.org/publications/pac/80/1/0059/>

## ○ Absolute Reference

### ○ Bruker : $\text{SR}_x = \nu_x - \text{BF1}_x$

- Ex>  $\nu_{\text{TMS}}^{\text{obs}} = \text{SF}_\text{H} = 400.1300082 \text{ Mhz}$

- $\text{BF1} + \text{SR} = 400.13 \text{ Mhz} + 8.24 \text{ Hz}$

- $\nu_\text{C} = \nu_{\text{TMS}}^{\text{obs}} \times \frac{\gamma_\text{C}}{\gamma_\text{H}} = 100.6127705 \text{ Mhz}$

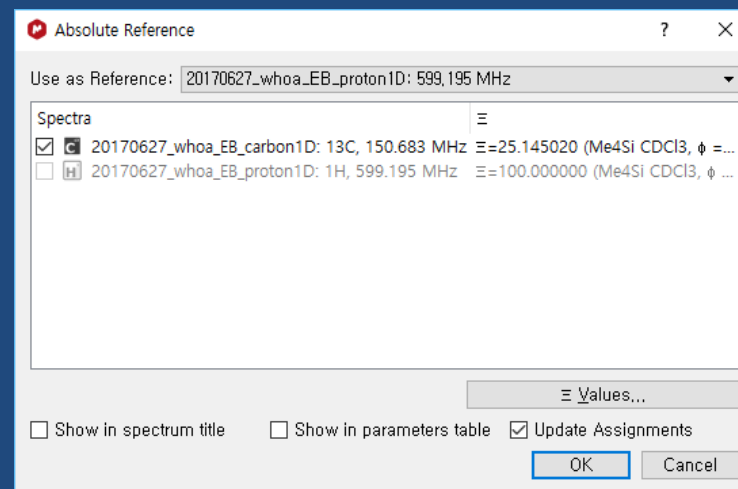
- $400.1300082 \times 25.145020 / 100 \text{ Mhz}$

- $\text{SR}_\text{C} = \nu_\text{C} - \text{BF1}_\text{C} = 1.5 \text{ Hz}$

- $100.6127705 - 100.6127690 \text{ Mhz}$

- → SR 1.5

### ○ Mnova :



# Zoom (Z)



## ○ Zoom

### ○ Zoom in

(Z)

○ Horizontal → Vertical → 2D

### ○ Zoom out

(Shift + Z)

### ○ Manual Zoom

(M)

## ○ Full Screen

(F)

## ○ Crosshair

(C)

## ○ Back to cursor

(ESC)

# Peak Picking(K, Ctrl+K)



## Automatic Peak Picking

Mnova: 

## Manual

Threshold

: K

Peak by peak

: Ctrl + K

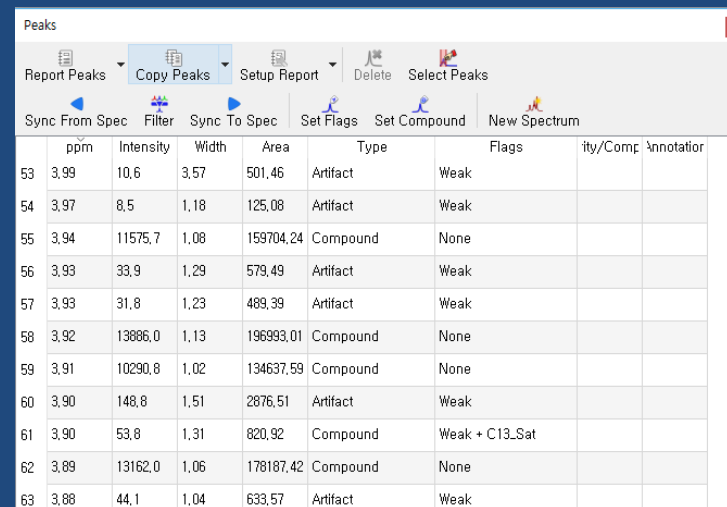
Merge

Delete

: Ctrl + Shift + K

Delete all

Tables → Peaks



	ppm	Intensity	Width	Area	Type	Flags	ity/Comp	Annotation
53	3.99	10.6	3.57	501.46	Artifact	Weak		
54	3.97	8.5	1.18	125.08	Artifact	Weak		
55	3.94	11575.7	1.08	159704.24	Compound	None		
56	3.93	33.9	1.29	579.49	Artifact	Weak		
57	3.93	31.8	1.23	489.39	Artifact	Weak		
58	3.92	13886.0	1.13	196993.01	Compound	None		
59	3.91	10290.8	1.02	134637.59	Compound	None		
60	3.90	148.8	1.51	2876.51	Artifact	Weak		
61	3.90	53.8	1.31	820.92	Compound	Weak + C13_Sat		
62	3.89	13162.0	1.06	178187.42	Compound	None		
63	3.88	44.1	1.04	633.57	Artifact	Weak		

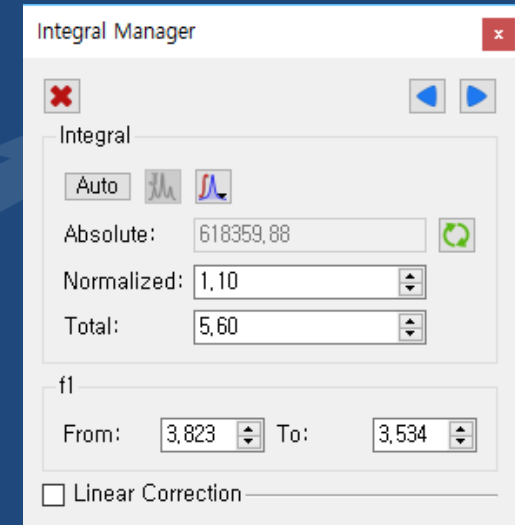
# Integration (I)

## Integration

- Bruker : .int
- Agilent : intmod
- Mnova : I

## Rule

- Apply same rule when drag region of peak
- First integration : 1.00
  - Integral Manager
  - Tables → Integrals



Integrals			
	Range	Normalizer	Absolute
1	11.91 .....	1.00	561031...
2	4.59 .. ...	0.96	540405...
3	4.06 .. ...	1.09	611425...
4	3.82 .. ...	1.10	618359...
5	0.10 .. -...	1.44	808386...



# Multiplet(J)

## Multiplet analysis (J)

Multiplets

Report Multiplets Copy Multiplets Setup Report Delete

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 11.76 (s, 1H), 4.50 (dd, *J* = 11.3, 4.3 Hz, 1H), 3.92 (dd, *J* = 11.3, 10.1 Hz, 1H), 3.70 (dd, *J* = 10.1, 4.3 Hz, 1H), 0.00 (s, 1H).

Name	Shift	Range	H's	Integral	Class	J's
1 E (s)	0.00	0.08 .. -0.17	1	1.34	s	
2 D (dd)	3.70	3.80 .. 3.53	1	1.11	dd	4.35, 10.10
3 C (dd)	3.92	4.01 .. 3.80	1	1.09	dd	10.08, 11.33
4 B (dd)	4.50	4.61 .. 4.35	1	0.98	dd	4.35, 11.32
5 A (s)	11.76	11.92 .. 11.56	1	1.00	s	

Multiplet Manager

3,92 (dd, *J* = 11.3, 10.1 Hz, 1H) Report Multiplets

Name: C Class: dd

δ: 3,916 ppm  Middle

J-List: 11,33, 10,08  Discard Peaks

Color: Purple

Total Nuclides = 5

Nuclides: |1| Auto

Integral: 1,09

Absolute: 669522

From: 4,013 To: 3,805

Multiplet Report

J. Am. Chem. Soc.

All as Ranges

m's as Ranges

Ascending Order of Shifts

Ascending Order of Js

Report Js

Reduce J List

Use Extended Solvent Names

Report Assignments

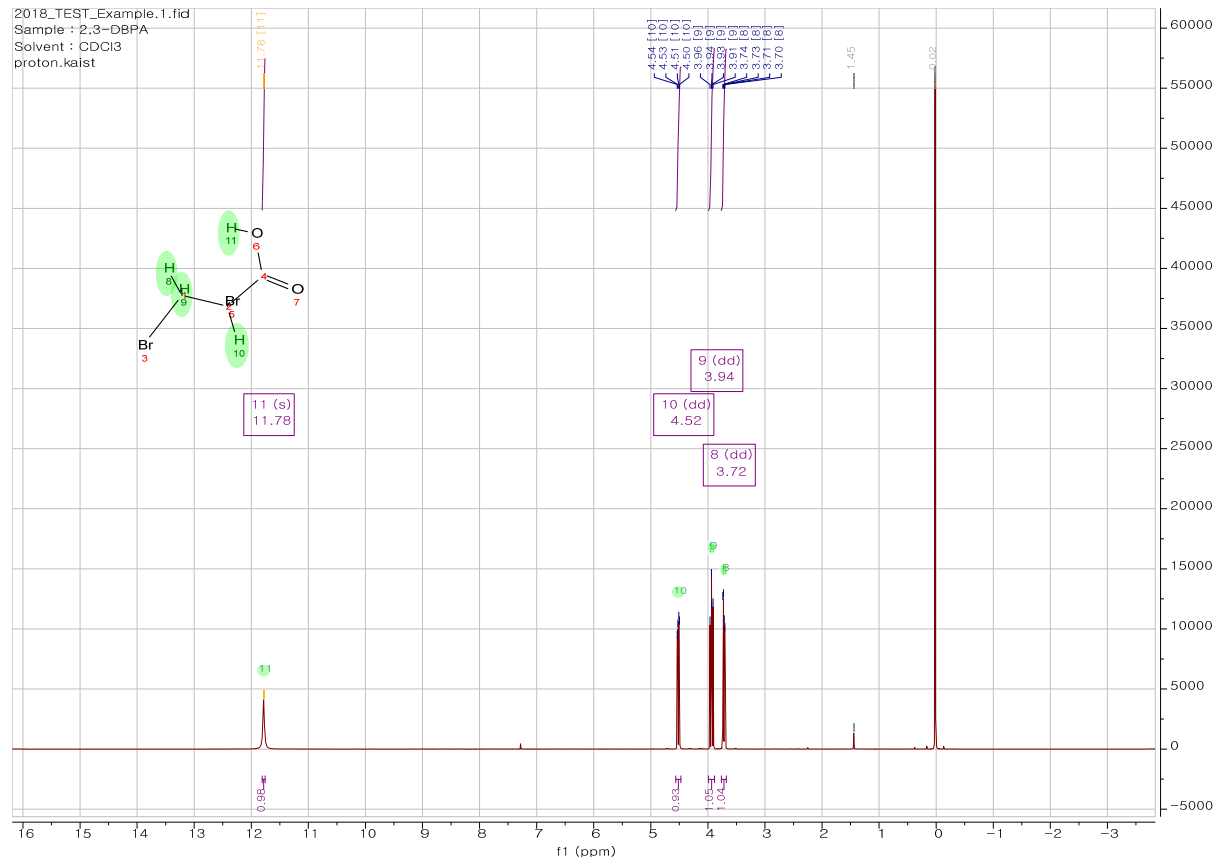
Shift Number of Decimals: 2

Js Number of Decimals: 1

Fill Style: Transparent

OK Cancel

# Assignments(A)



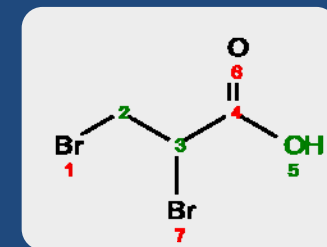


# qNMR

## ○ Reproducible integration

- Relaxation delay : 3 ~ 7 X T1
- Phase correction
- Baseline correction : Polynomial (oth order)

Intensity	5-OH	3-H	2a-H	2b-H	TMS	RMSD(%)
T1(s)	5.37 sec	8.22 sec	1.49 sec	1.36 sec	11.18 sec	
D1=2s	0.68	0.66	0.78	0.79	1.00	7.8971
D1=24s	0.69	0.67	0.71	0.71	1.00	2.7994
D1=60s	0.62	0.64	0.64	0.64	1.00	0.1570



400Mhz, 298K, 90 pulse, 4 s acquisition time, 8 scan

# qNMR

○ Advanced → qNMR → Concentration  
○ Settings

Concentration

Paste Report Settings Autoselection

Concentration Average: 0,2857 %  
RMSD(%): 5,57

	Multiplet	Autoselected	Shift	Range	Hs	Abs. Integral	Concentration	SNF
1	<input checked="" type="checkbox"/> B(dd)	1	4.50	4.61..4.35	1	600068.6946	0.2634	18..
2	<input checked="" type="checkbox"/> C(dd)	1	3.92	4.01..3.80	1	669522.2657	0.2939	22..
3	<input checked="" type="checkbox"/> D(dd)	1	3.70	3.80..3.53	1	682761.2824	0.2997	22..
4	<input type="checkbox"/> A(s)	0	11.76	11.92..11.56	1	613780.2374	0.2694	68..

Alerts

```
=====[2018-09-09T21:10:37]=====  
Multiplet A(s) not used in calculations because it is labile  
=====[2018-09-09T21:10:13]=====  
Multiplet A(s) not used in calculations because it is labile
```

Clear

Settings

Load Settings Save Settings

Calculations Options Report

Concentration Method

Internal Reference  Concentration Conversion Factor

From: -0,50 To: 0,50 ppm

Reference Peak is a Solvent  
 Apply Line Fitting to the Reference Peak

Reference Concentration: 0,03 %

Nuclides of Reference: 12

Autoselection Algorithm

OK Cancel

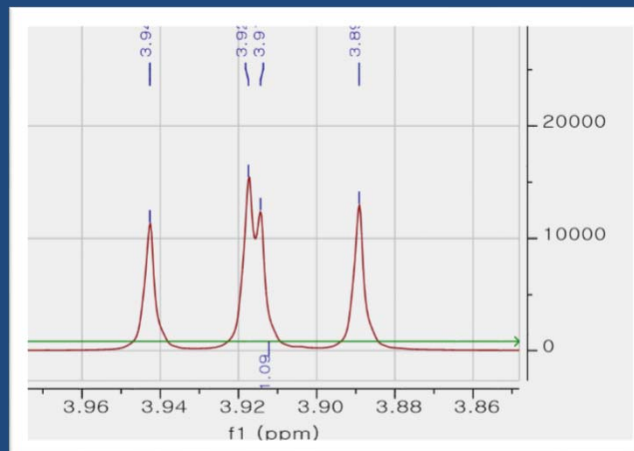
# Additionally...



## View

- Expansion (E)
- Cut (X)
- Restore (V)

## Properties



# Stack



○ Select pages → Menu : Stack -->

- Stack items
- Superimpose items
- Bruker : .md

○ Arithmetic

- 1D NOE experiment : Steady-state NOE

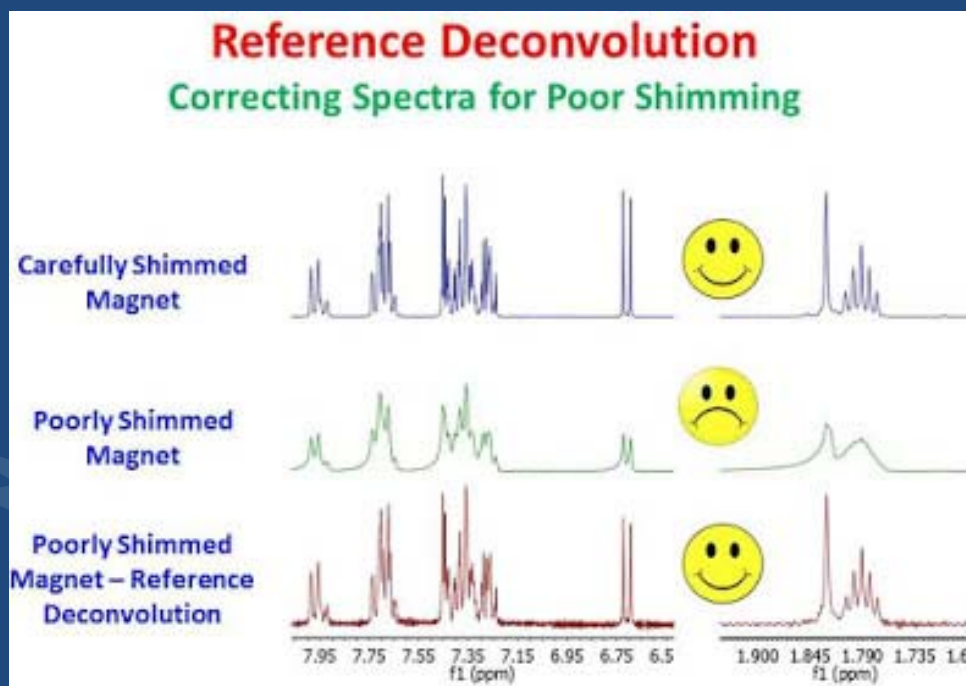
	Title	T/G	Ratio	orm. Fact	Δ (Hz)
2	<input checked="" type="checkbox"/> 2018_TEST_Example...	0.00e+00	1.00e+00	1.00e+00	7.80
1	<input checked="" type="checkbox"/> 2018_TEST_Example...	0.00e+...	1.00e+...	1.00e+...	0.00

# Reference Deconvolution



## ○ Improve “spectra of Bad shim”

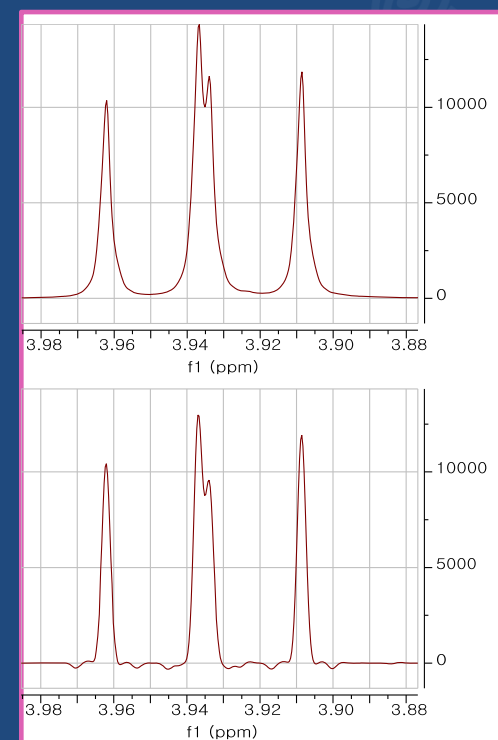
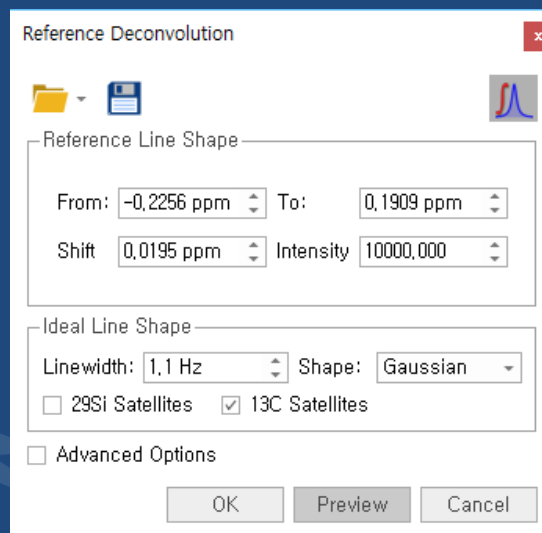
- Good : Bad lineshape → ideal lineshape
- Bad : S/N will be sacrificed



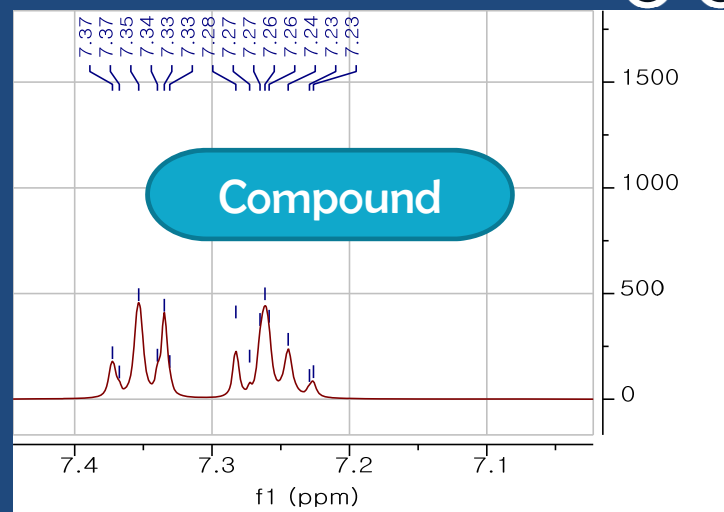
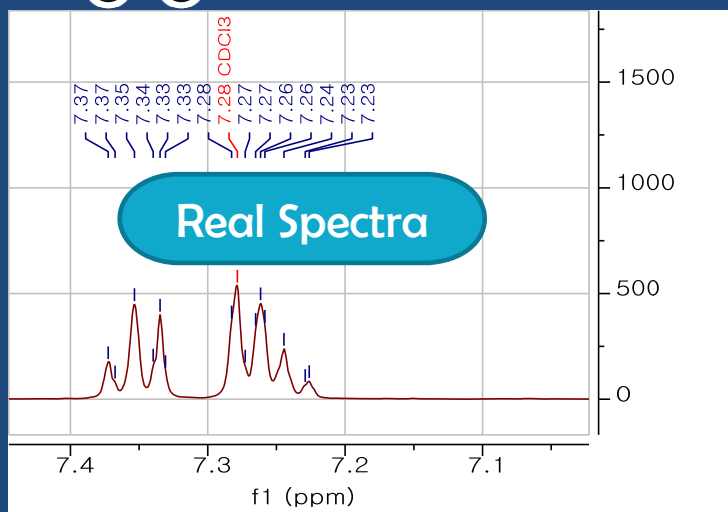


# Reference Deconvolution

- Bruker : .refdcon (Topspin ver.  $\geq 3.5$ )
- Mnova
  - Integrate “Separated strong singlet peak”
    - Ex> TMS



# Compound // Impurity

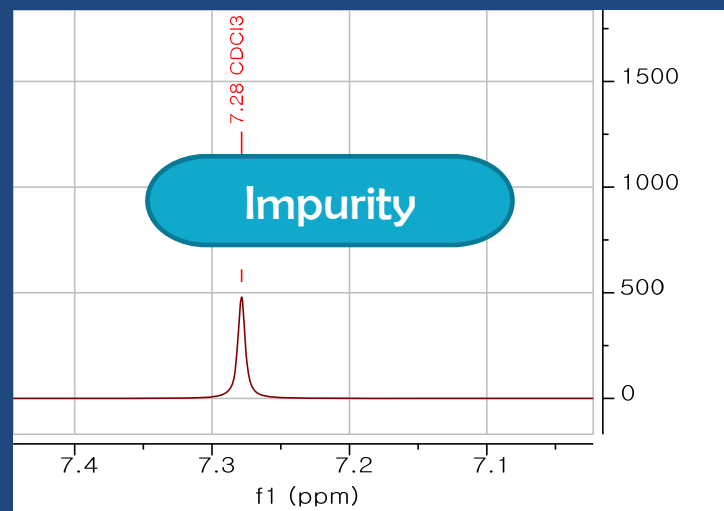


Peaks

Report Peaks Copy Peaks Setup Report Delete Select Peaks

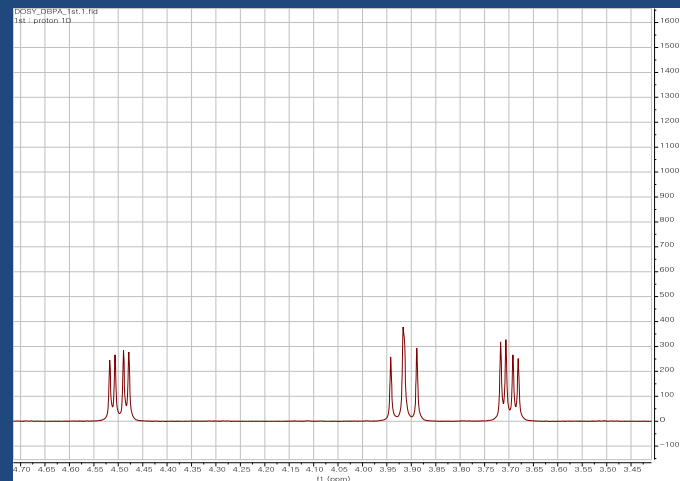
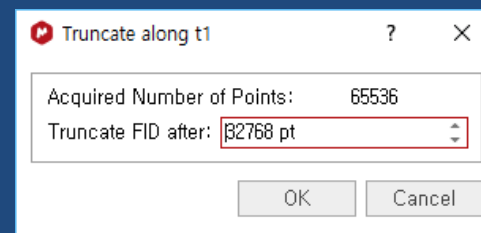
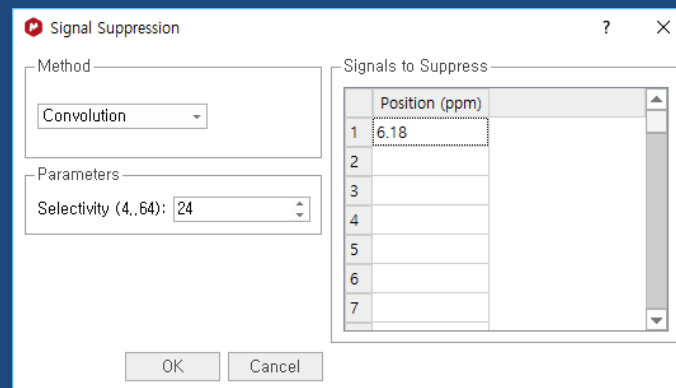
Sync From Spec Filter Sync To Spec Set Flags Set Compound

	ppm	Intensity	Width	Area	Type	Flags	ity/Comp	annotator
28	7.42	0.2	0.51	1.55	Artifact	Weak		
29	7.42	1.0	1.54	16.26	Artifact	Weak		
30	7.40	2.0	2.33	53.91	Artifact	Weak		
31	7.37	166.2	2.51	5215.40	Comp...	None		
32	7.37	30.8	1.00	390.54	Comp...	None		
33	7.35	448.1	2.93	16617.01	Comp...	None		
34	7.34	82.7	1.33	1442.72	Comp...	None		
35	7.33	390.1	1.97	10453.29	Comp...	None		



# Other functions...

- Signal suppression
- Truncate
- Binning
  - S/N  $\wedge$ , resolution  $\vee$
- Smoothing
- Resolution Booster



# 2D processing



- Apodization
- Zero filling
- Linear Prediction
- Phase correction
- Baseline correction
- Referencing
- Peak picking
- Assignment

# In advance...



## ○ Acquisition Parameter

- NS : Number of Scan
- SW : Spectral Width
- O1P, O2P : Offset
- TD : Number of point
- NUS

## ○ Experiment

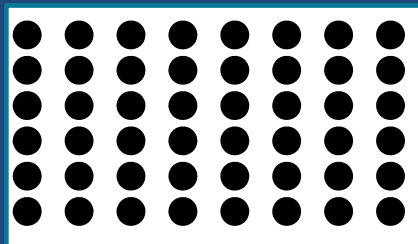
- Homonuclear : COSY, NOESY, ROESY, TOCSY
- Heteronuclear : HSQC, HMBC, H2BC,

# Non-uniform Sampling

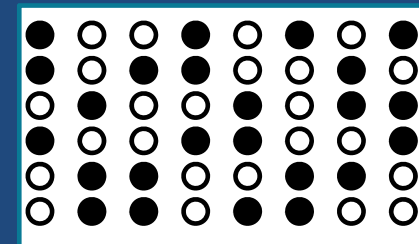


○ Save Time for 2D acquisition

○ 25 ~ 50%

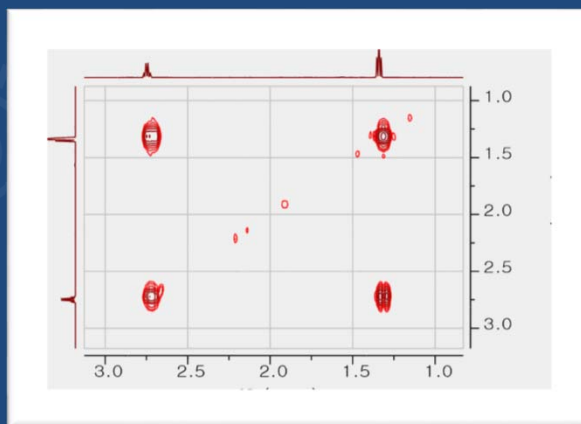
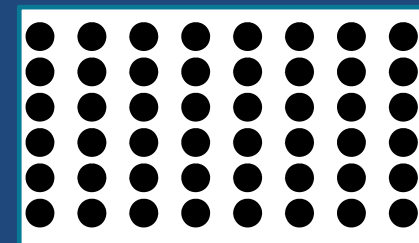


Uniform sampling



Non -Uniform Sampling

↓ NUS processing



# 2D spectra processing



## ○ FT

○ Bruker

: xfb

○ Varian

: wft2da

## ○ Convolution

○ Bruker

: dcon2d

○ Varian

: dconi

## ○ Extract 1D

○ Bruker

: rser

○ Varian

: ds

# Apodization (W)



○ 2D : strongly recommended

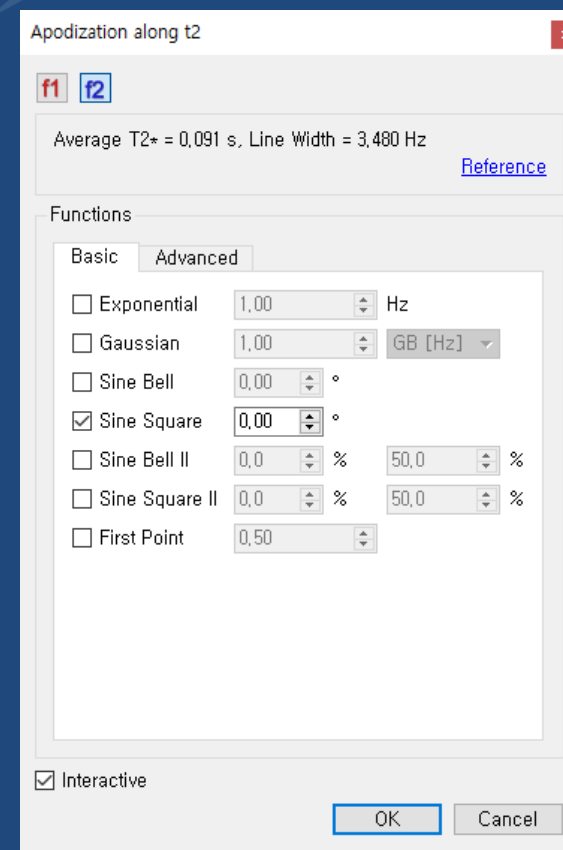
○ Magnitude : SSB 0 deg

○ COSY, HMBC

○ Phase sensitive : SSB 90 deg

○ NOESY, edited HSQC

○ Apply both F2 and F1

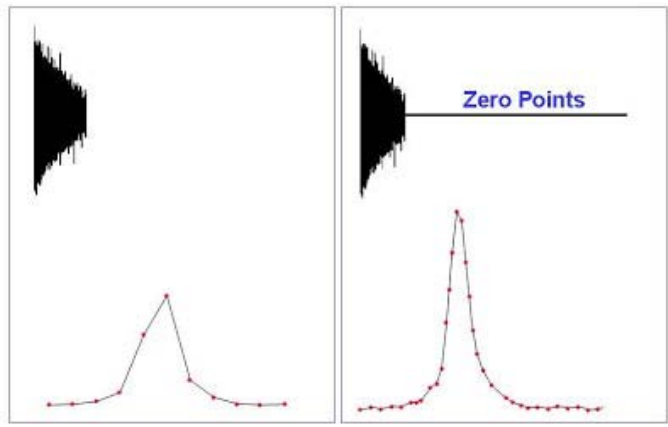




# Zero Filling

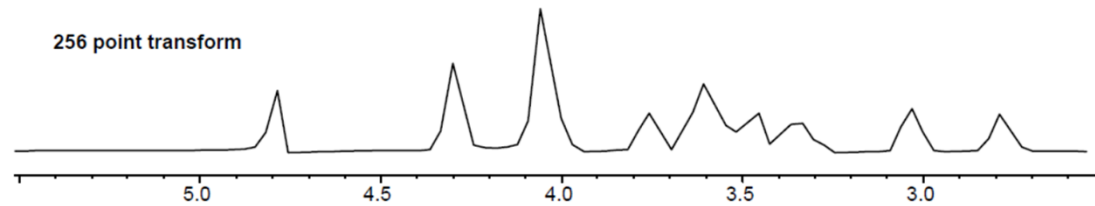


## Zero Filling

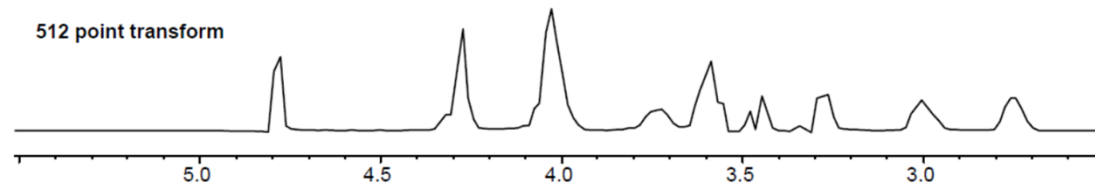


<http://u-of-o-nmr-facility.blogspot.kr/2007/11/zero-filling.html>

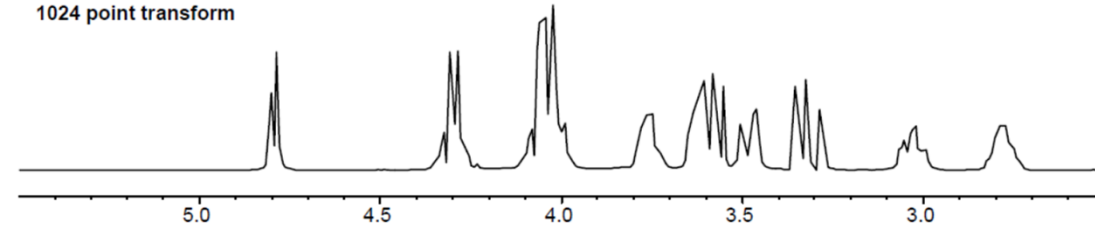
256 point transform



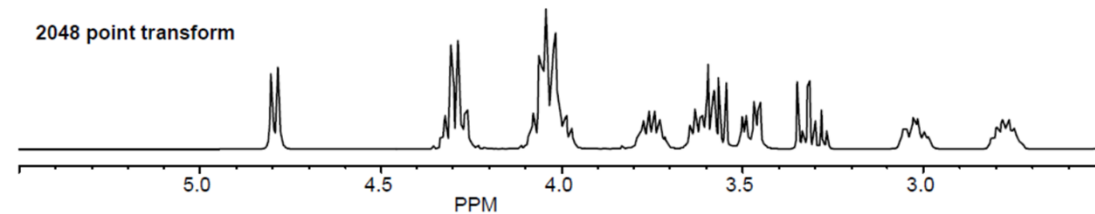
512 point transform



1024 point transform



2048 point transform



**B. Digital Resolution in 2D NMR Spectra**

<http://nmr.asu.edu/page8/files/zerofill.pdf>

# Zero Filling

○ Bruker : SI

○ Mnova

○ Select dimension : F2

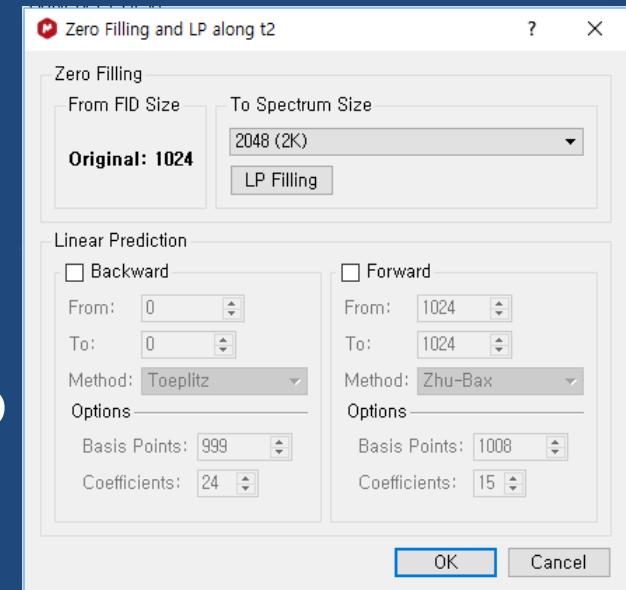
○ Processing → Zero Filling and LP

○ “From FID Size”

○ To Spectrum Size : upper size

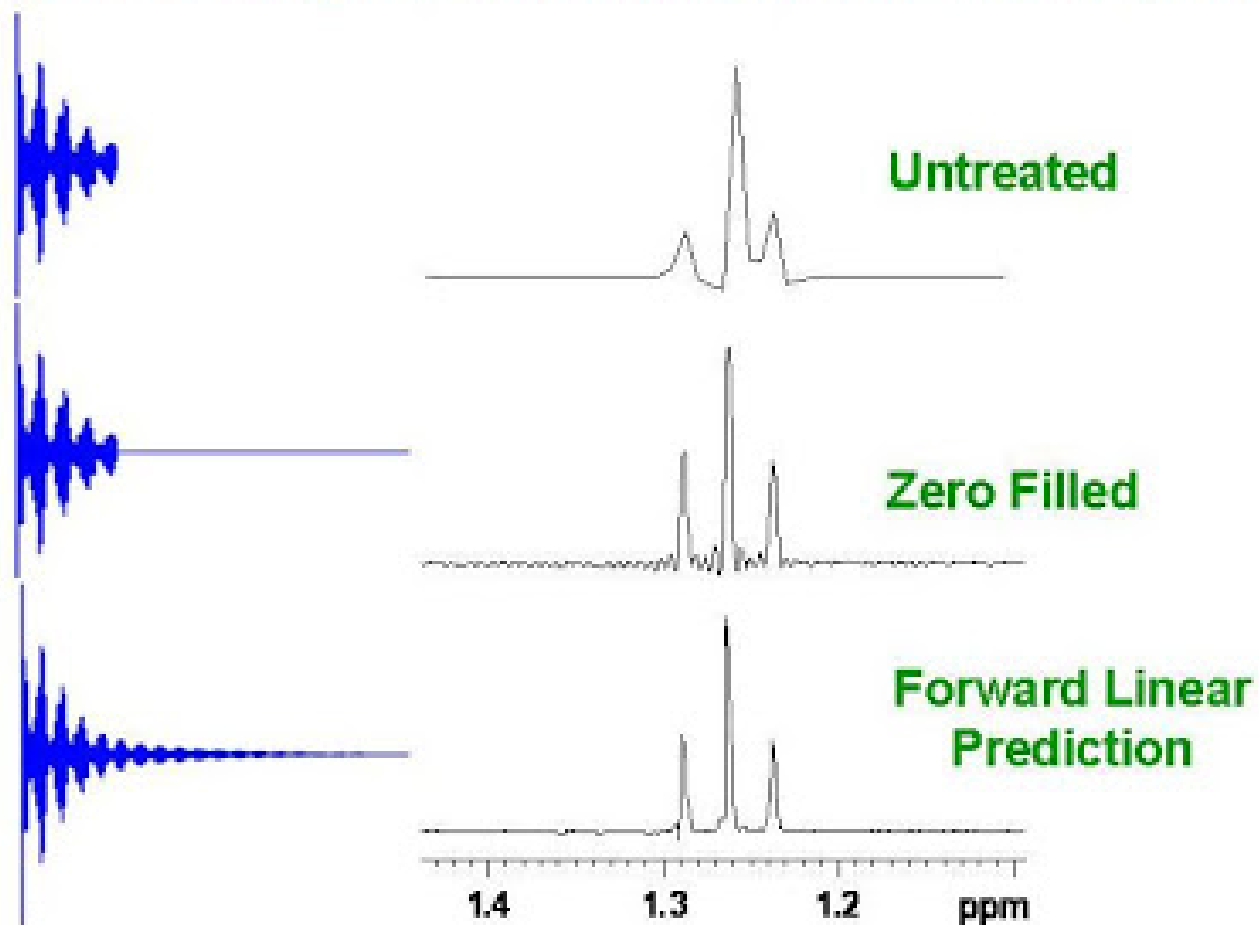
○ Ex> original : 512 ~ 972 // To : 1024

○ Check available hard disk usage



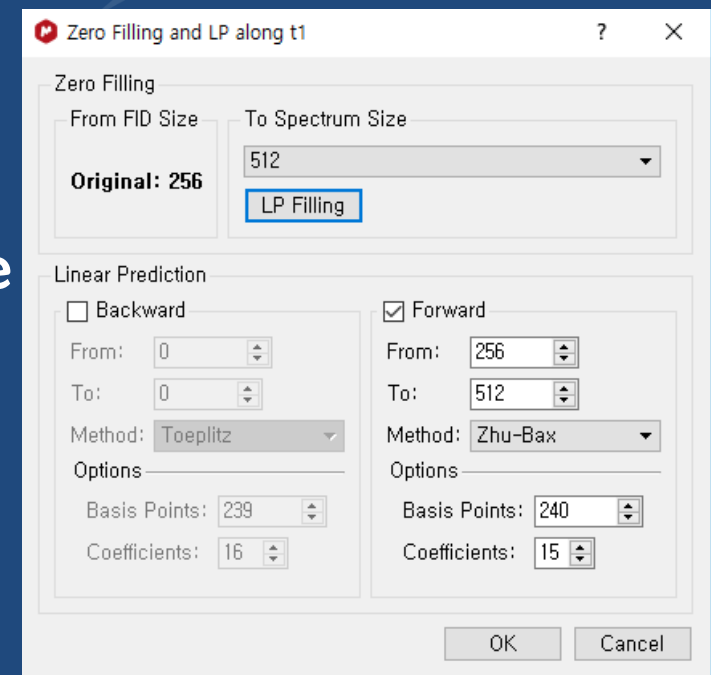
# Linear Prediction

## Zero Filling vs Forward Linear Prediction




# Linear Prediction

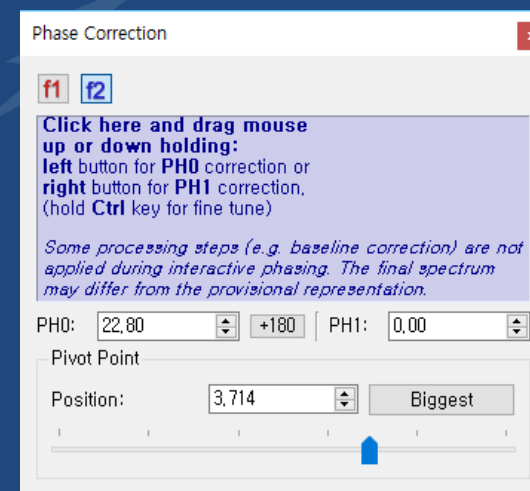
- Select dimension : F1
- Processing → Zero Filling and LP
  - “From FID Size”
    - To Spectrum Size : upper size
    - Check available hard disk usage
  - Click : LP Filling
    - “Forward” should be checked



# Phase correction(Shift + P)



- For Phase-sensitive spectrum
  - For NOESY, edited HSQC
    - Cf> mag COSY, HMBC
- Auto phase correction
  - Bruker : .ph
  - Mnova : 
- Manual correction
  - Shortcut key (Shift + P)
    - Global correction (0<sup>th</sup> order)
    - Gradient correction (1<sup>st</sup> order)



# Baseline correction(B)



## ○ Auto baseline correction : 1H, 13C

- Bruker : abs , absn, absf , abs2

- Mnova



## ○ Manual correction

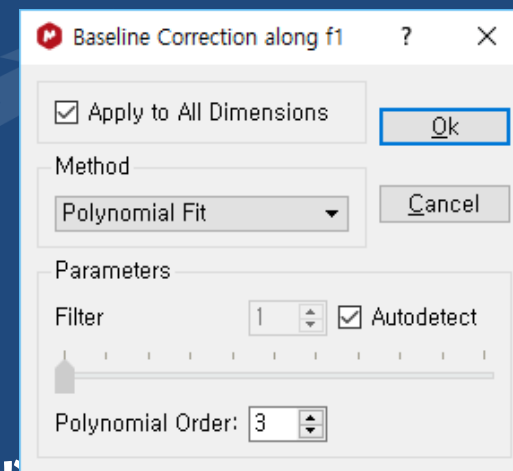
### ○ Polynomial Fit

- Order : 0 ~ 20 (<5 recommended)

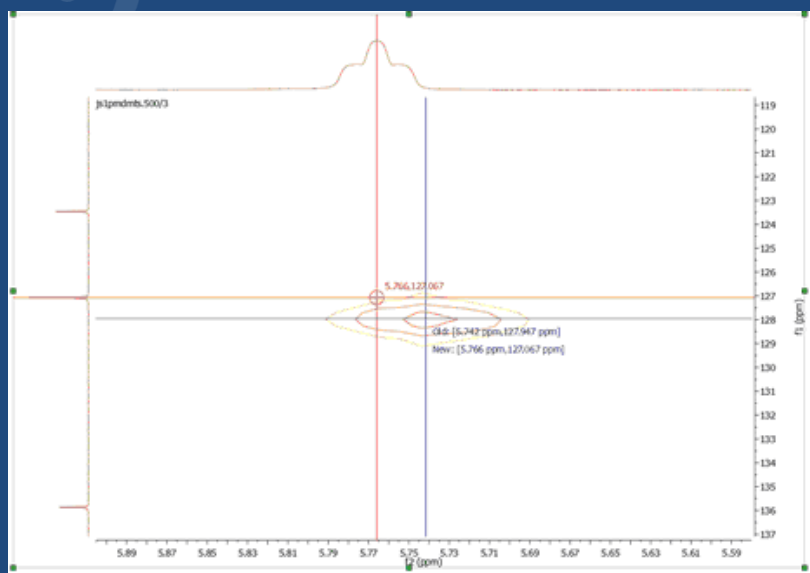
- Filter : autodetect

### ○ Check “Apply to All Dimensions”

- If user want, can apply f2, f1 separately.



# 1D external trace



**Setup Traces**

Available 1D Spectra:

- 1H js1pm24luti
- 13C js1pm24luti

Horizontal Trace

1H

Internal Trace: 0

Vertical Trace

13C

Internal Trace: 0

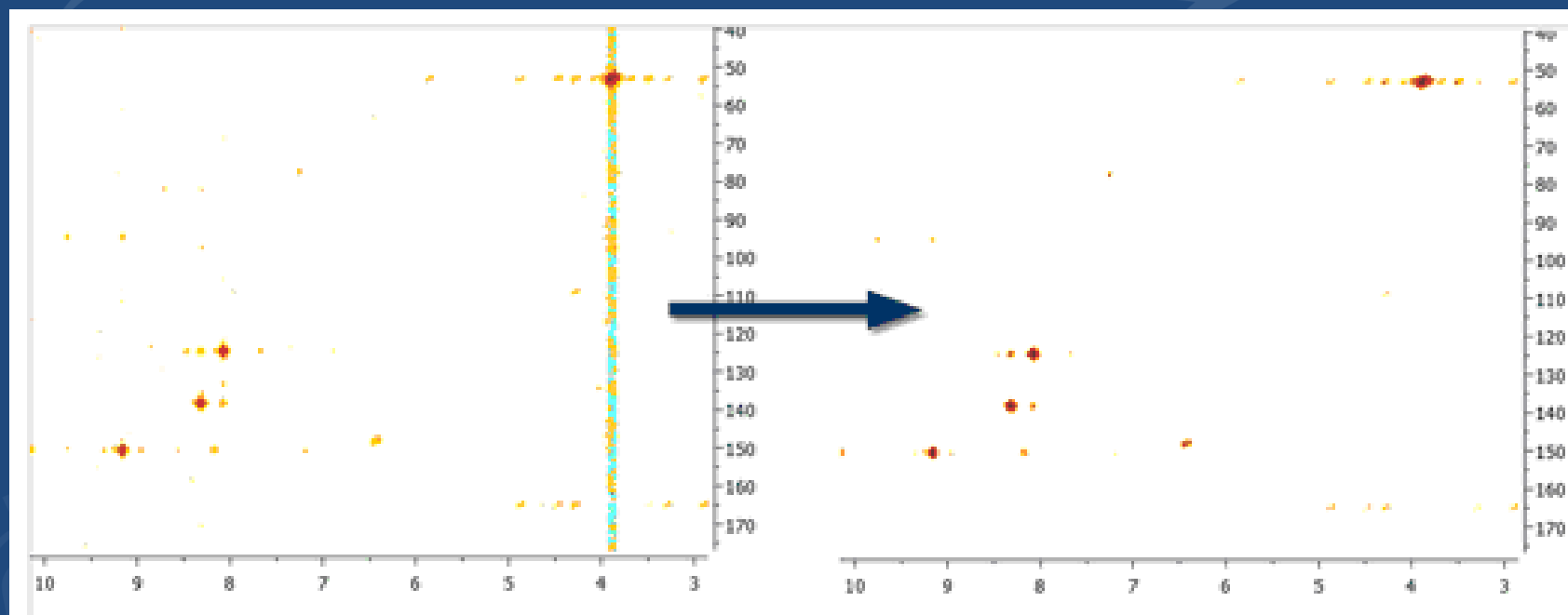
Use External 1H Trace as Reference

OK Cancel

# Reduce t1 Noise

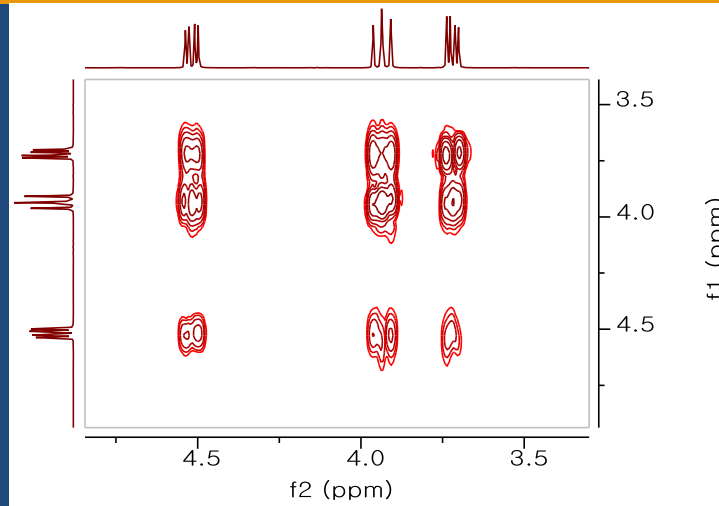


○ For heteronuclei spectra(ex>HSQC)

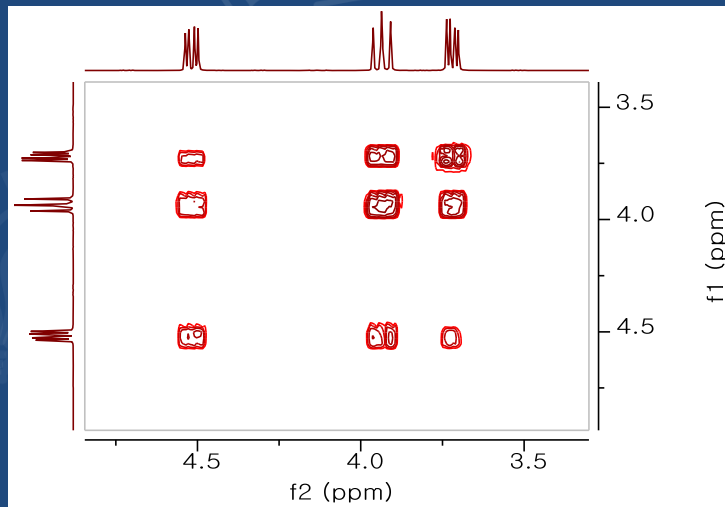




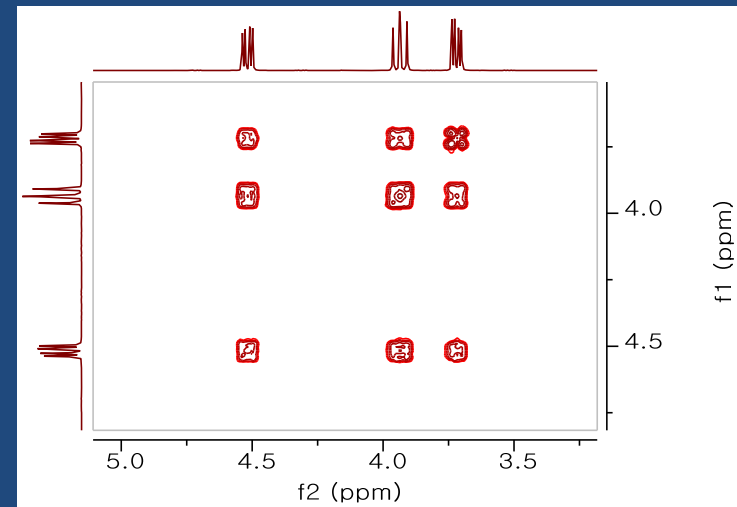
# Symmetrization vs Covariance



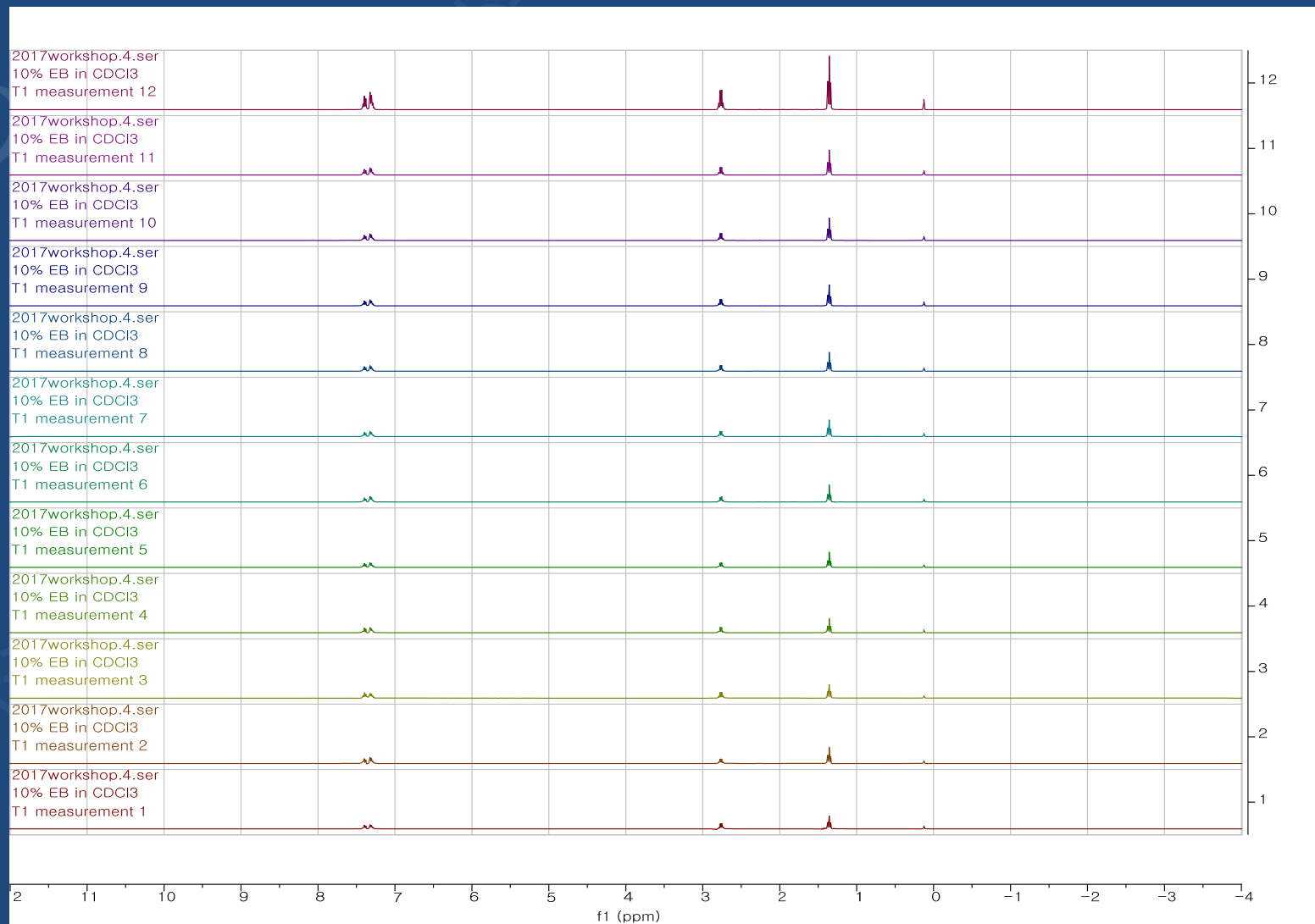
Symmetrization



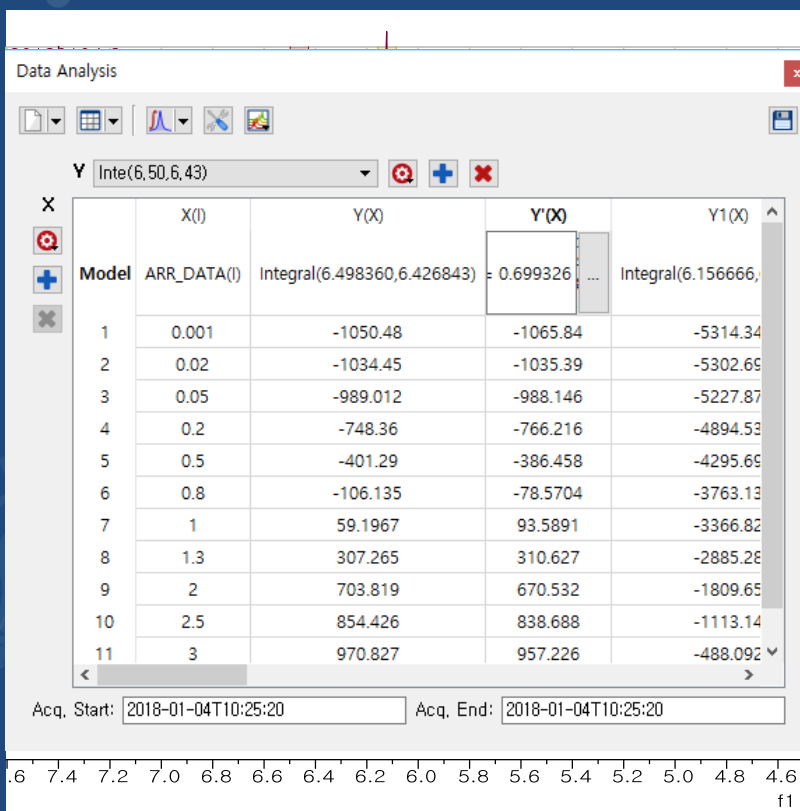
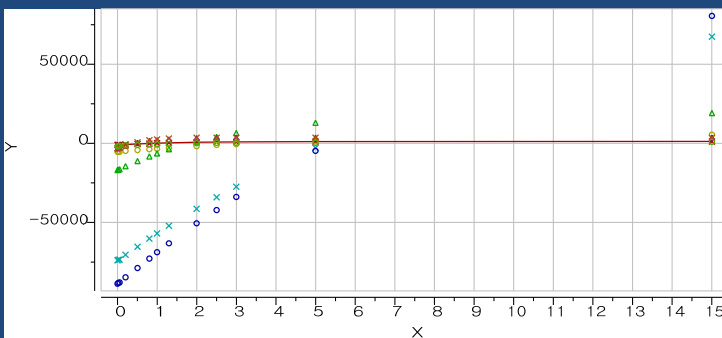
Covariance



# T1 relaxation



# T1 relaxation



Y'-Column Model Function

Functions

Name	Function	Initialization	Report
1 Linear Fit	$A+B*x$	$A= 0, B= 0$	Zero Order R
2 Mono-exponential Fit	$B*\exp(-x*F)$		Exponential t
3 Three Parameter Exponential Fit	$B+F*\exp(-x*G)$		Exponential t
4 Inverse Linear Fit	$1/(A+B*x)$	$A= 1, B= 0$	Second Order
5			

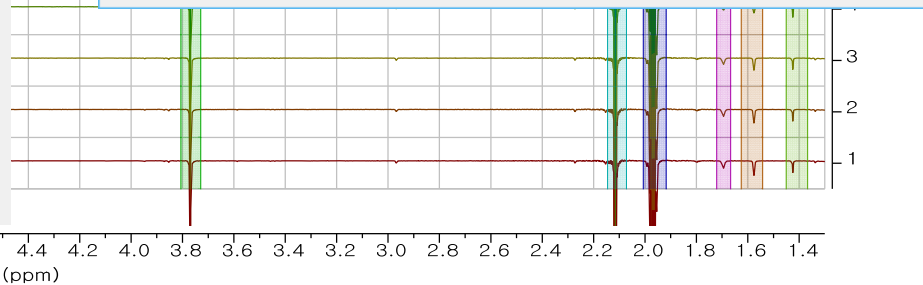
Restore Defaults

Fitted Parameters

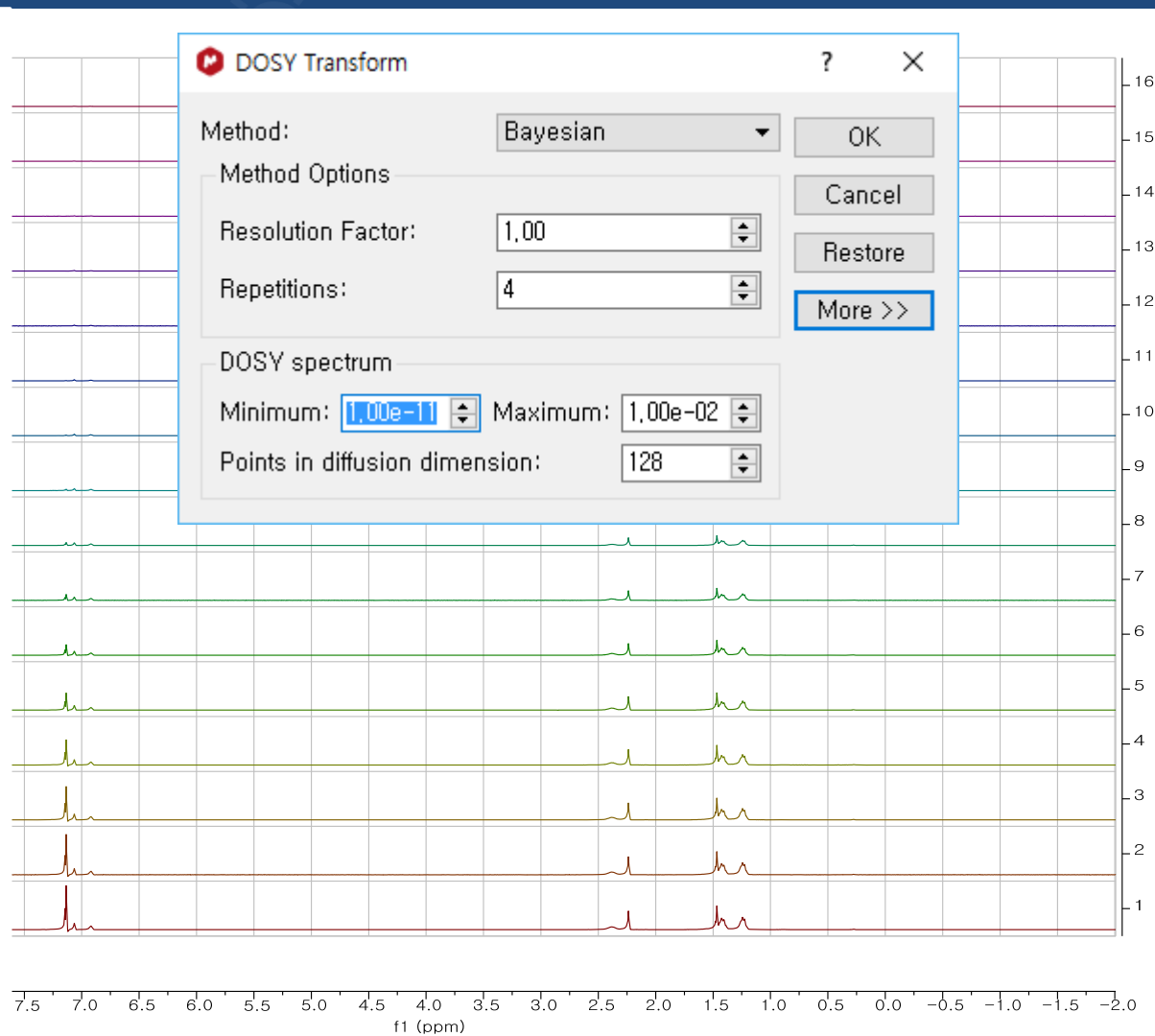
Calculate

B= 1240,41, F= -2307,86, G= 0,699326  
 rError = 0,0114327, probnotmono = 0,79731

OK    Cancel



# DOSY Transform



# DOSY Transform

