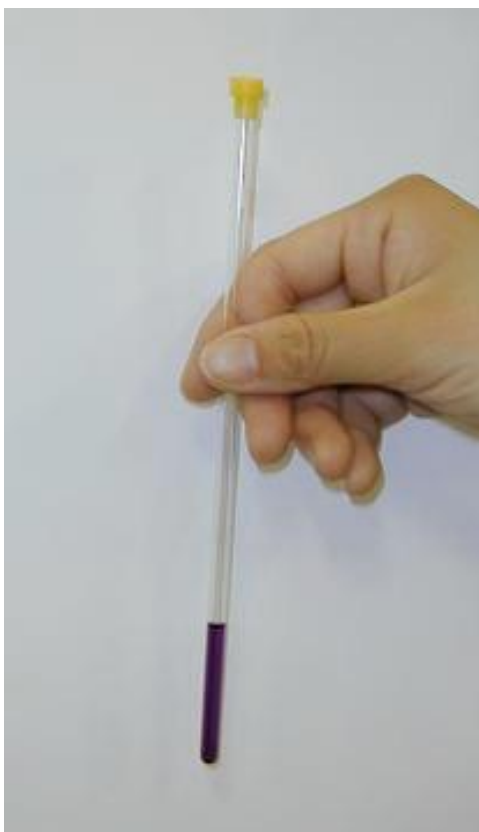


A Procedure of NMR Measurement



Solution NMR Sample Tube
(\varnothing 5 mm O.D.)

Volume: Ca. 0.5 ml

Sample should be dissolved in
deuterated solvent in principle.

*Common deuterated solvents

Chloroform- d (CDCl_3)

Acetone- d_6

DMSO- d_6

Methyl Alcohol- d_4 (CD_3OD)

Deuterium Oxide (D_2O)

Sample Preparation

- **Dissolve Sample Homogeneously**
 - ✓ A totally homogeneous concentration is required.
 - ✓ An appropriate concentration is required.
- **Remove Insoluble**
 - ✓ A floating materials in solution should not be exist.
- **Remove (Beware) Impurities**
 - ✓ Avoid signal line broadening due to paramagnetic impurities (organic radicals, metallic ions).
 - ✓ Avoid influence of water or acid in solvent.
- **Select Purposeful Sample tube**

Important Points of NMR Sample Preparation

"Homogeneous"
concentration

"Inhomogeneous"
concentration



An Inhomogeneous concentration
causes local magnetic susceptibility
distortion, and make shimming
impossible.



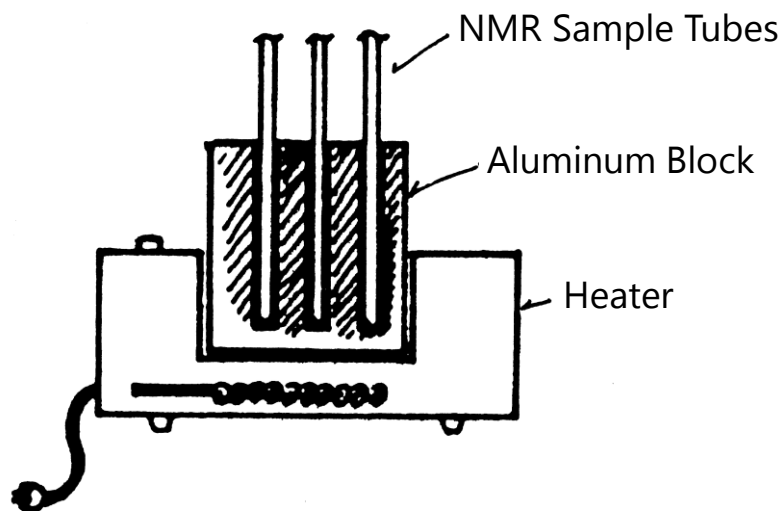
Signal line broadening
Abnormal signal splitting

Locally distorted magnetic susceptibility

Homogenous magnetic susceptibility

Dissolve Sample Homogeneously

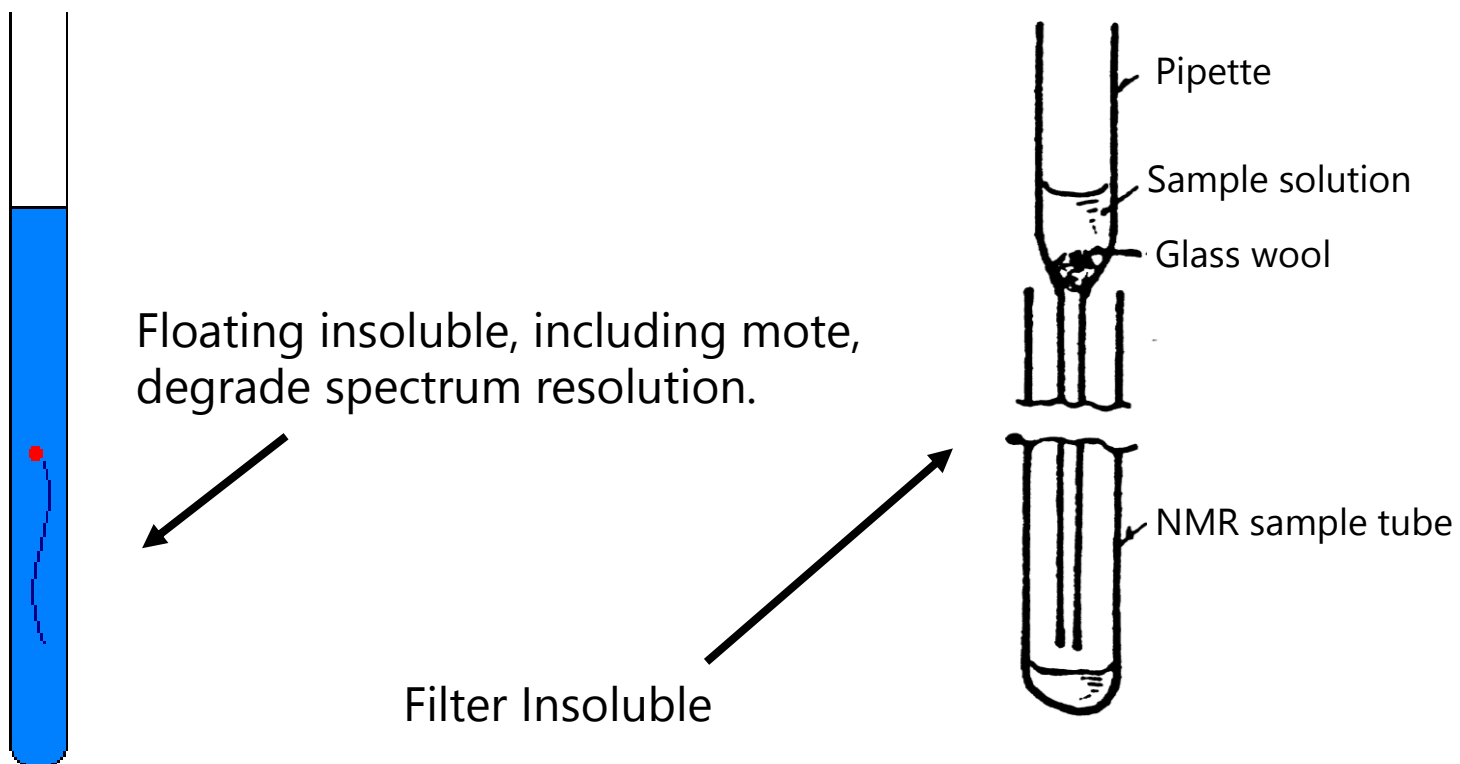
It is difficult to homogenize concentration due to high viscosity usually. In case of high resolution required, homogenize concentration carefully using long term diffusion.



Convection flow with long term heating is effective.

*Replacing Nitrogen gas to avoid oxidation is recommended.

Notification for Polymer Solution



Remove Insoluble

Paramagnetic Impurities (* dissolved oxygen is paramagnetic!)

- ✓ Paramagnetic component such as metallic ion or organic radicals broaden NMR signals, let alone high concentration of those causes signal disappear and make NMR lock impossible.

Impurities of solvent

- ✓ A chloroform produces a hydrochloric acid as a photodecomposition product during storage. Thus it should be stored in a cool, dark place. A hydrochloric acid has to be removed before dissolving especially for an acid sensitive sample.
- ✓ Carefully handle pipetting to avoid contamination of solvent.
- ✓ Trace amount of water easily contaminate from used pipette.

Remove (Beware) Impurities

Special Sample Tubes

- **Quartz Tube, PTFE Tube**

Background signals of ^{29}Si or ^{11}B from sample tube will not be obtained.

(*Materials of a probe may be obtained)

- **Shigemi Symmetrical NMR Microtube**

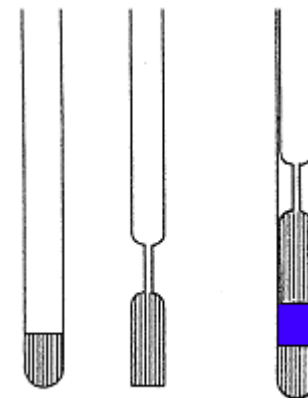
Reducing resolution degradation due to insufficient sample solution volume.

- **Special Use**

High pressure sapphire cell, etc..

- * Disposable Tubes

"Cheap" NMR tube may have distortion, and degrade spectrum resolution.



The magnetic susceptibility matched symmetrical NMR microtube assembly by Shigemi Co., Ltd

NMR sample tubes

Clean away factors to degrade resolution.

Scratch

!! Do not scrub tubes with a brush.

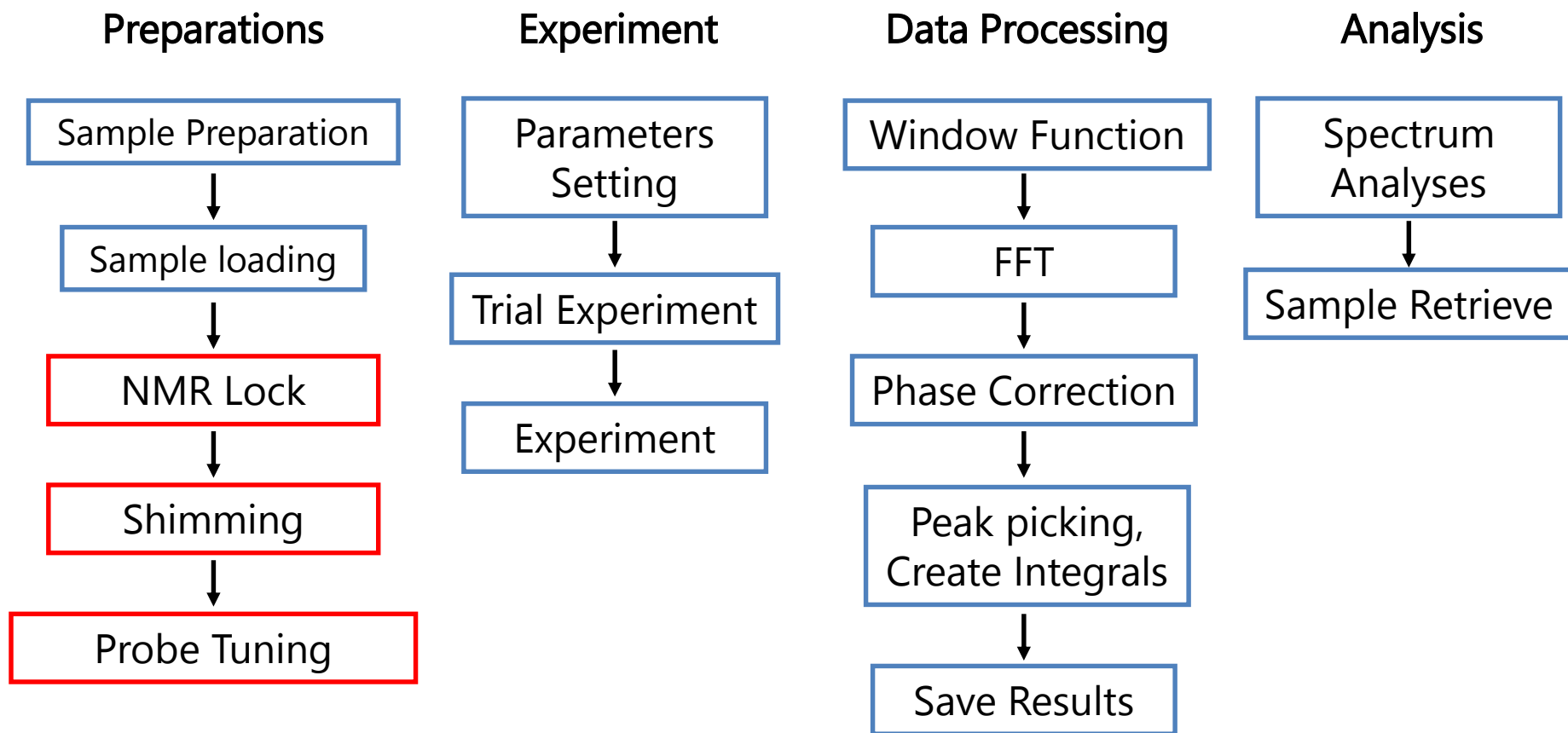
Distortion

!! Do not dry tubes in a laboratory oven.

Dust

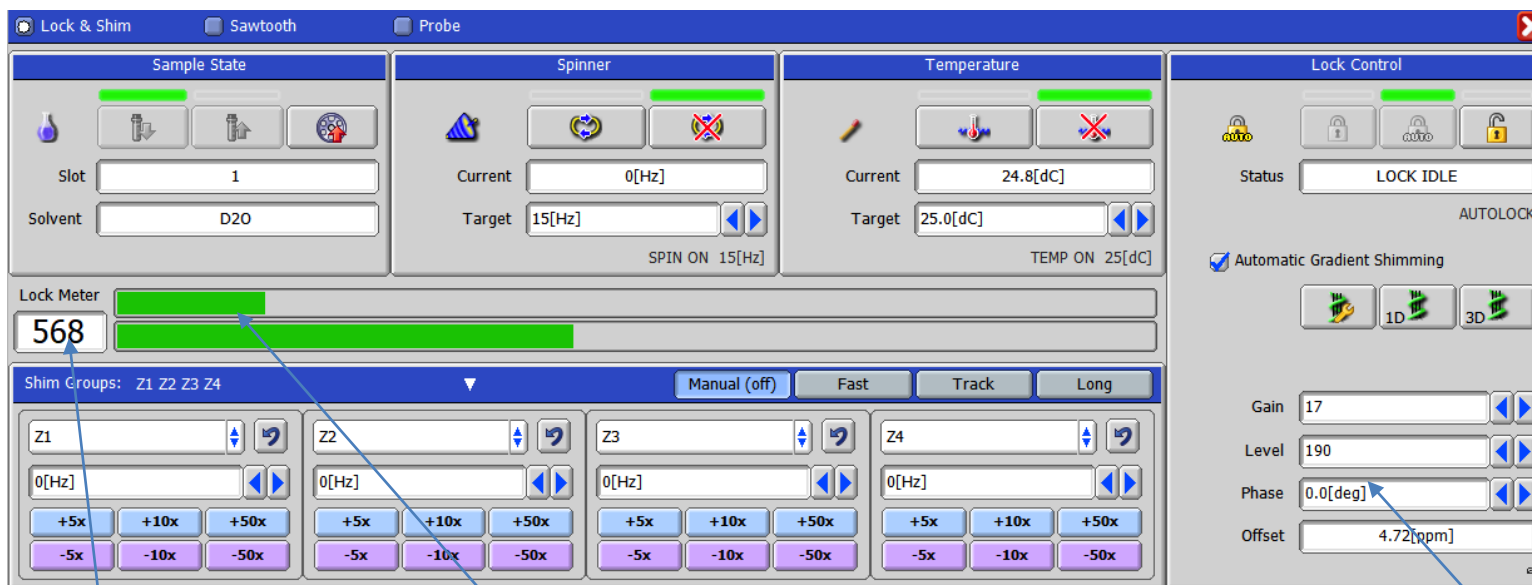
!! Cap it to keep out dusts.

Cleaning, Drying, and Storing of Tubes



A Procedure of NMR Measurement

A magnetic field strength of NMR SCM (Super Conducting Magnet) slowly decreases at constant speed, and this phenomenon is called a 'field drift'. NMR signal in a long term accumulation will spread because a resonance frequency of signal is proportional to the magnetic field. This is a serious problem for high resolution NMR experiment. To avoid this problem, NMR system monitors and corrects magnetic field by referencing particular NMR signal. This correction procedure is called a 'NMR Lock'. The deuterium signal of deuterated solvent is used for referencing in general, and thus it is also called D-Lock.



Lock Signal Strength

Lock indicator

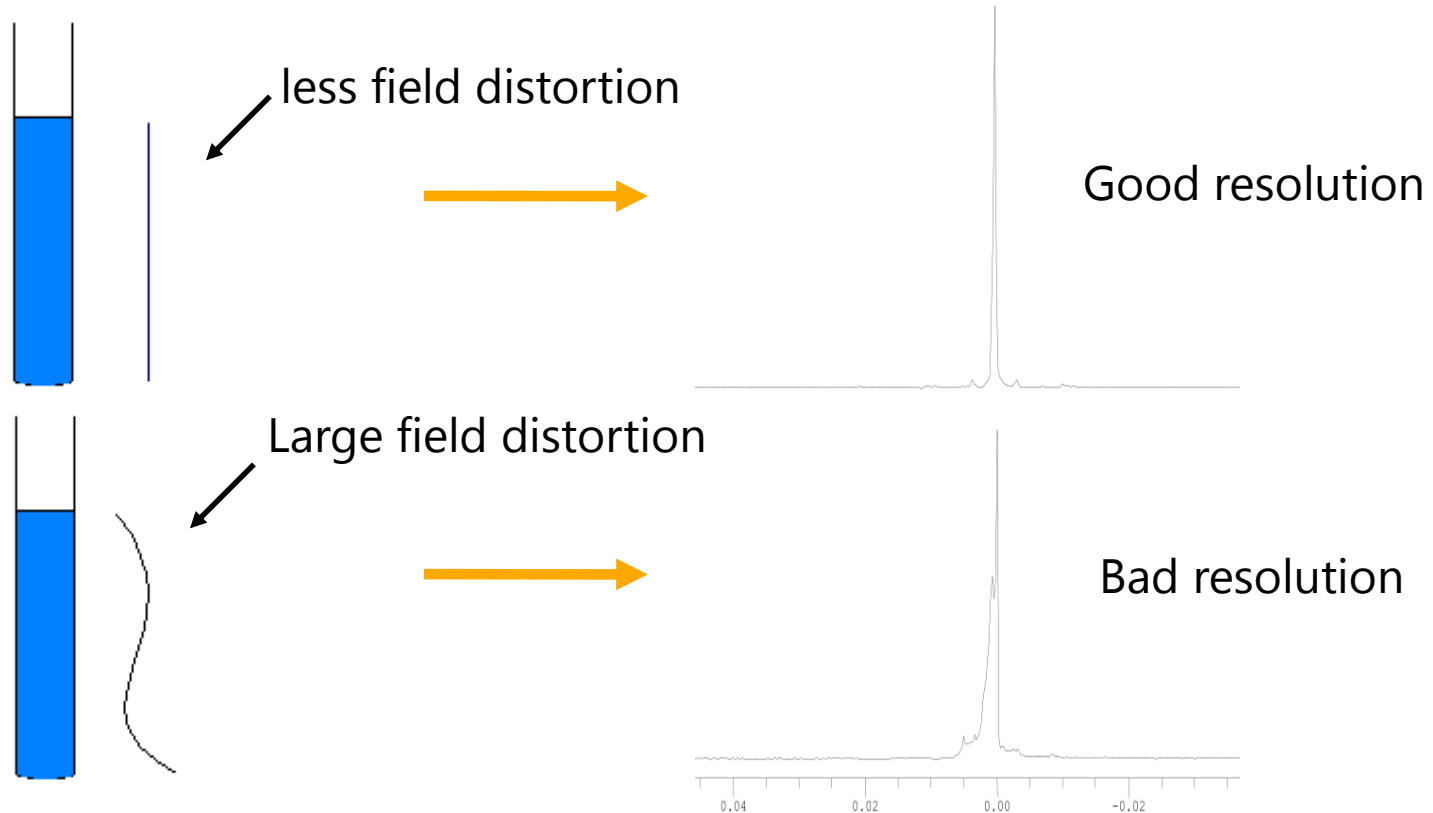
Lock Parameters

NMR Lock

A resonance frequency of NMR signal is proportional to the magnetic field.

$$(\omega = \gamma H)$$

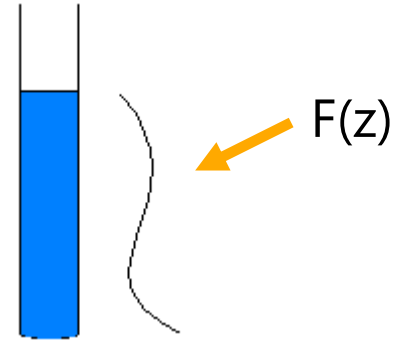
Magnetic field distribution in detectable sample space is reflected in signal shape.



Shimming

The purpose of shimming is to obtain coefficients of power series expansion of field distribution function.

$$F(z) = aZ + bZ^2 + cZ^3 + dZ^4$$



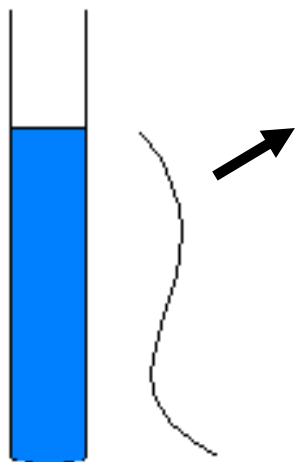
Z^1 shim value = $-a$

Z^2 shim value = $-b$

Z^3 shim value = $-c$

Z^4 shim value = $-d$

Shimming

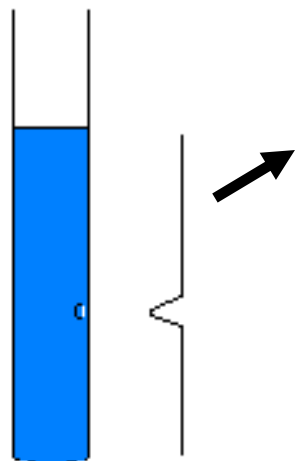


Low-order distortion

- SCM derived
- Volume difference
- Solvent difference

$F(z)$ can be approximated with $Z-Z^6$.

Shimming is possible to optimize in combination of Z^1-Z^6



High-order distortion

- Inhomogeneity
- Bubbles
- Scratches

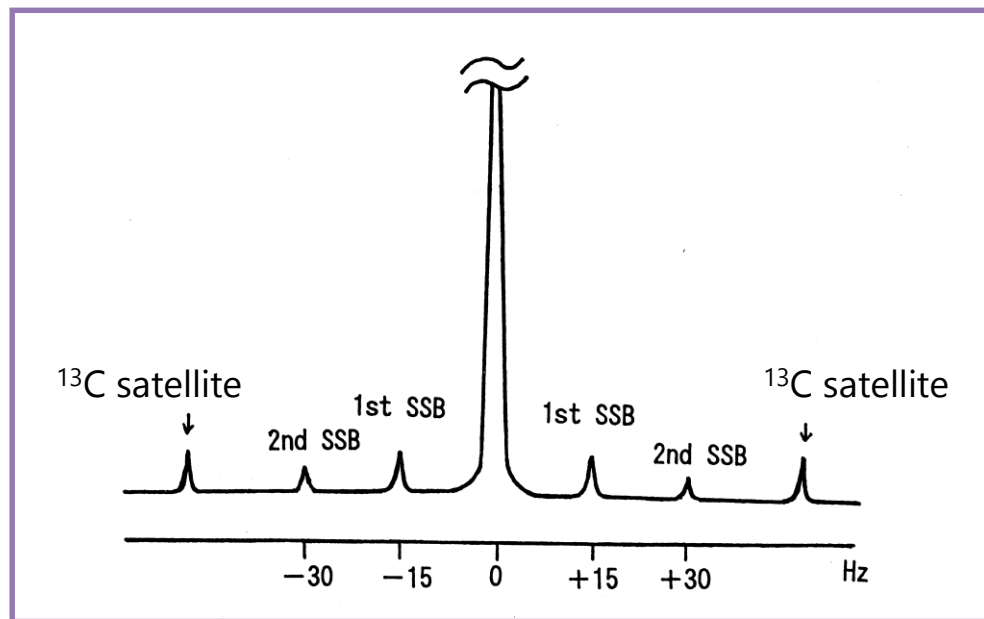
$F(z)$ cannot be approximated without super high-order terms such as Z^{100} .

There is no way to optimize in combination of Z^1-Z^6 , and sample itself should be re-prepared.

Possible Case and Impossible Case of Shimming

Field inhomogeneity in radial direction (X, Y) gives spinning sidebands (SSB) around signals.

- 1st SSB are obtained at the position of sample spinning frequency far from each signals. 1st order shims for X and Y shim term such as X, Y, XZ, YZ, and etc. should be optimized
- 2nd SSB are obtained at the position of double spinning frequency far from, each signals. 2nd or higher shims for X and Y should be optimized.

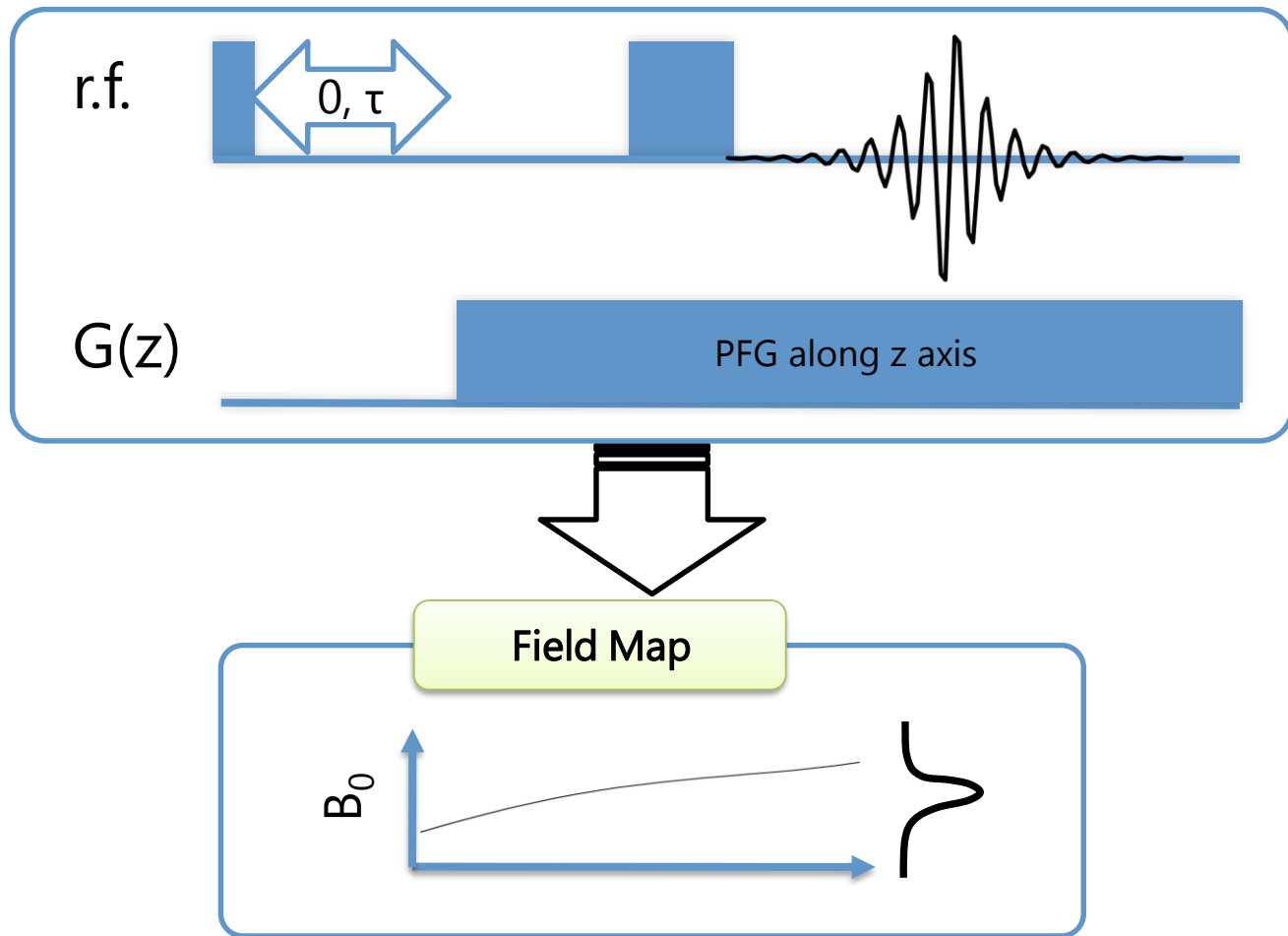


Sideband with other reasons

- Sidebands which has distorted phase from main signal might be due to the rotational motion fault.
- An Insufficient decoupling in multi-nuclear experiment causes decoupling sideband.

Non-Spinning Term Shimming

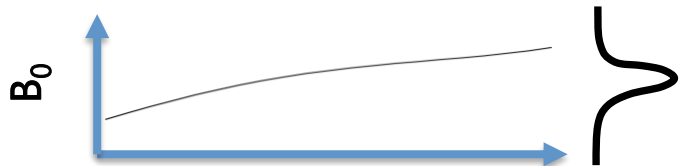
Field distortion can be described as a map (Same principle as MRI)



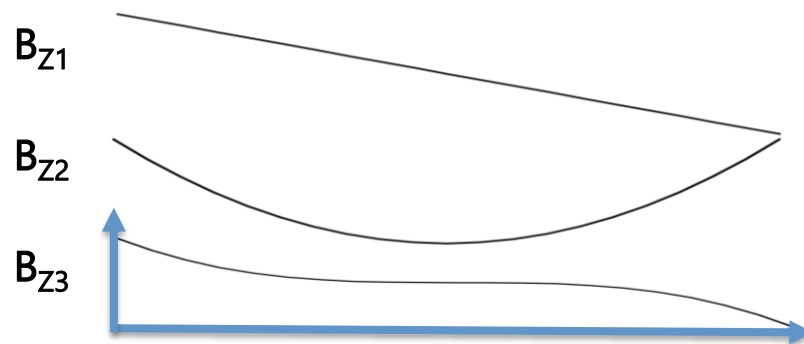
H. Barjat, P.B. Chilvers, B.K. Fetler, T.J. Horne, G.A. Morris, *J. Magn. Reson.*, **125**, 197-201 (1997).

Gradient Shimming

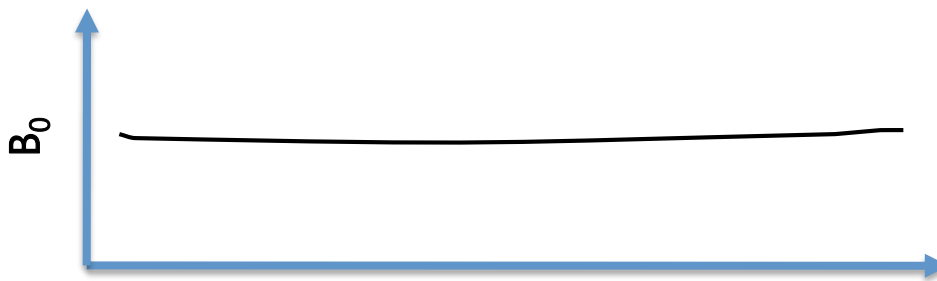
Field Map



Field contribution of each shim term



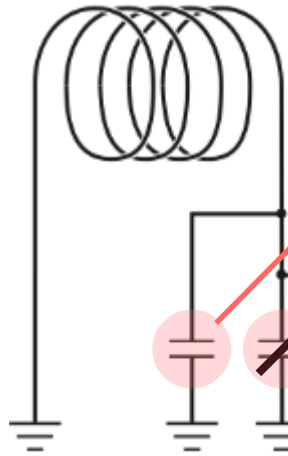
Optimized Shim Values
 $(B_{Z1}, B_{Z2}, B_{Z3}) = (-1.1, 0.7, -0.2)$



Gradient Shimming

A NMR system is a tuned circuit of oscillator, probe, cable, and sample. Optimization of resonance frequency and impedance of probe, which varies depending on sample and temperature, is probe tuning and matching.

Detection Coil



Stick

Change capacitance drastically

→ Change resonance frequency drastically

Tuning

Change capacitance of variable capacitor

→ Fine tuning of resonance frequency

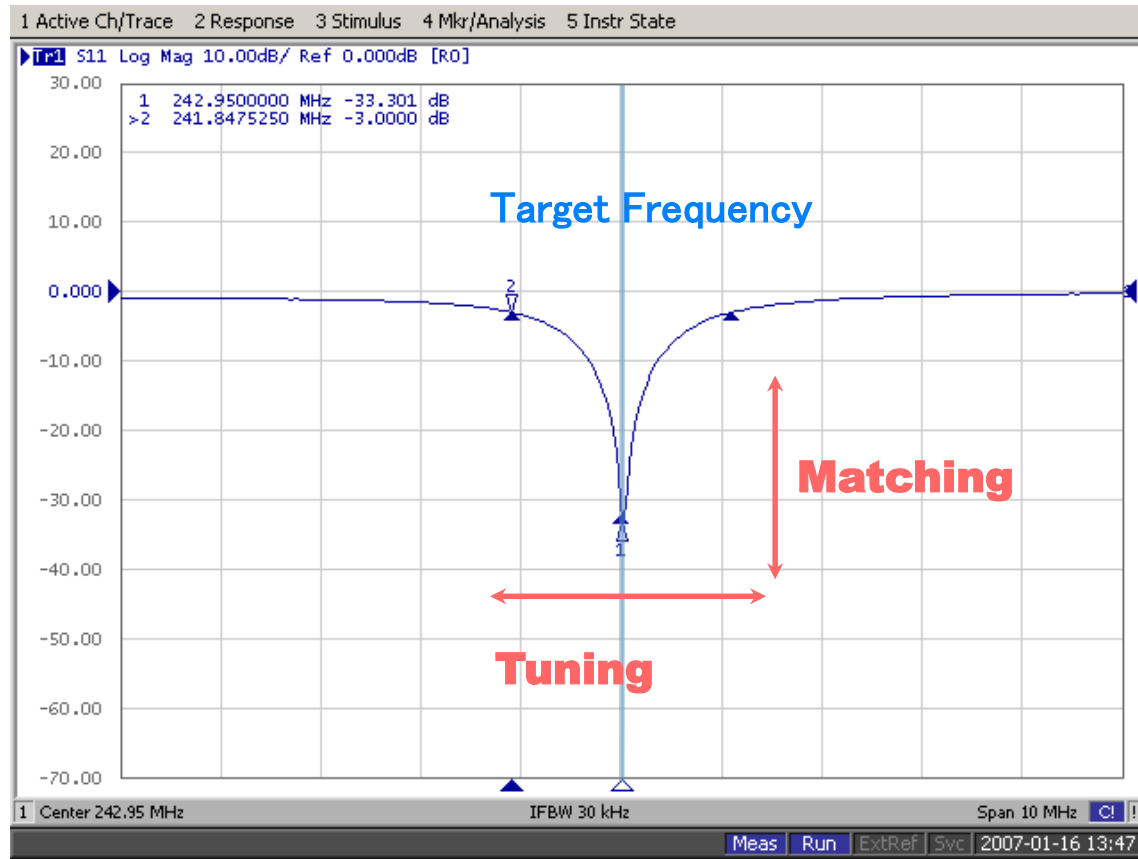
Matching

Change capacitance of variable capacitor

→ Tuning of impedance (resistance of AC circuit)

Probe Tuning

Radio frequency reflection characteristics of probe



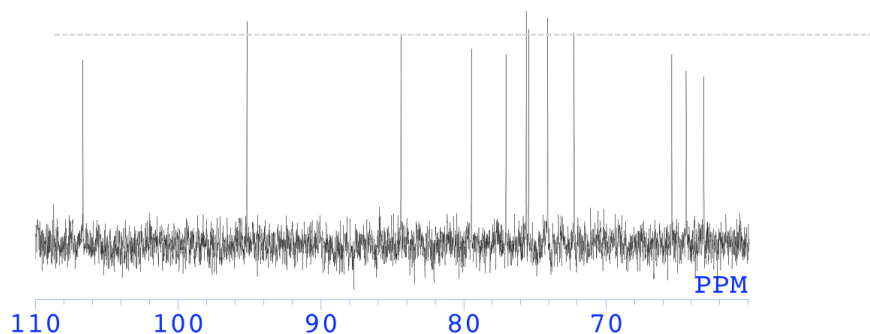
Probe Tuning

Incorrect tuning...

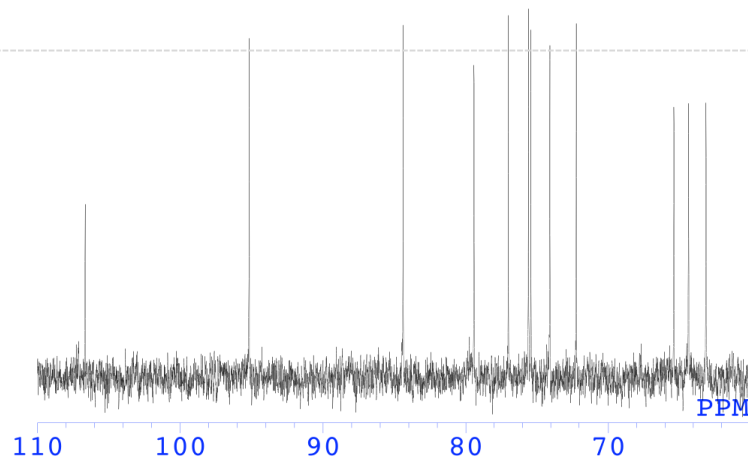
Causes sensitivity loss

Causes significant error of pulse length (90 degree pulse increases)

Causes insufficient decoupling



A spectrum of D2O solution which is probe
tuned to the other Acetone solution.



A spectrum of D2O solution which is
probe tuned to itself.

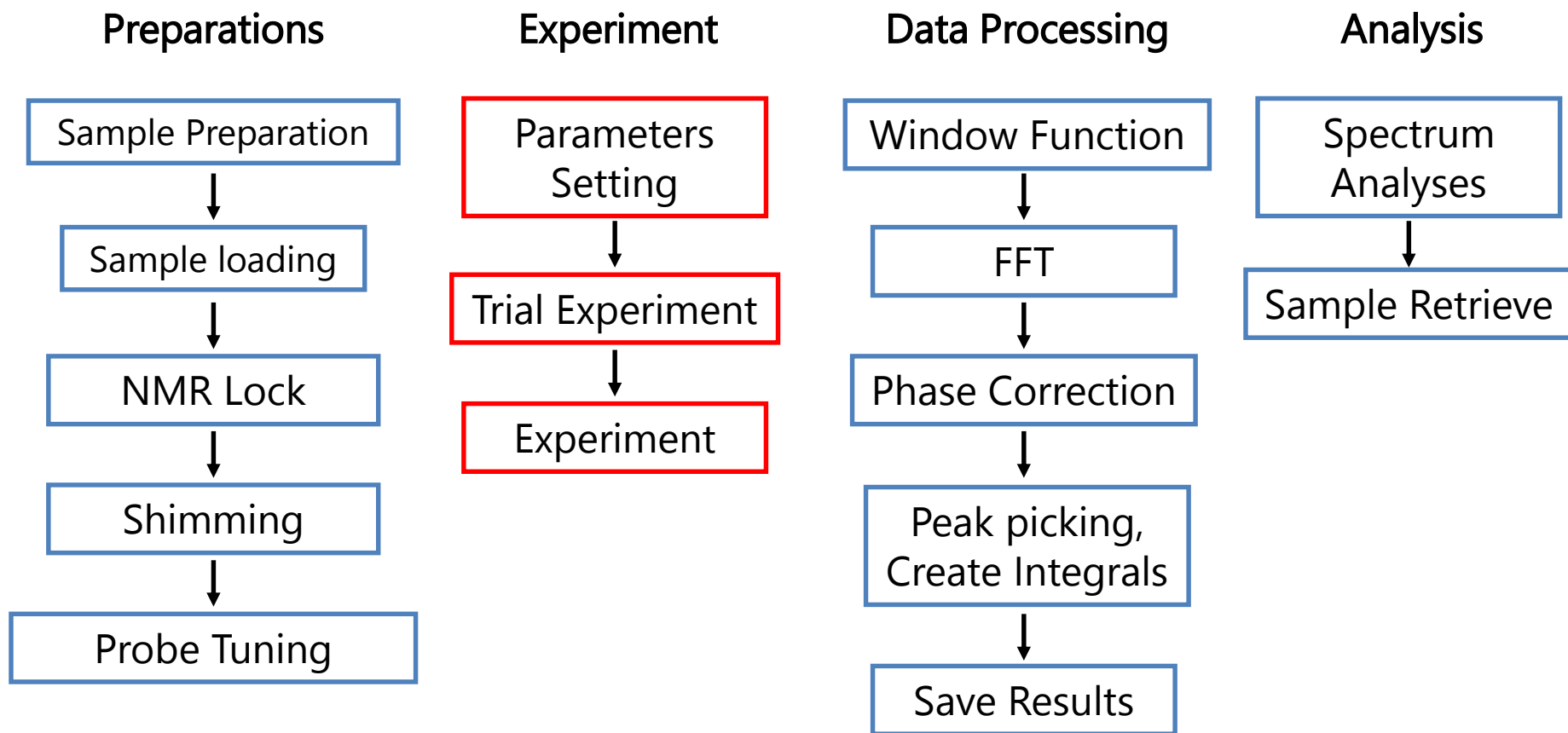
Probe tune strongly recommended cases

- Long term accumulation : sensitivity should be optimized.
- Variable temperature : different temperature varies tune.
- Multi-pulses experiments : pulse length should be correct.
- High-dielectric loss sample : high-dielectric loss varies tune.

Solvent	Water	Acetone	Benzene	Toluene	Ethanol	Methanol
Dielectric loss	3.19	0.31	0.00	0.00	9.59	6.63

- ✓ The dielectric loss of ethanol and methanol are significantly higher than others.
- ✓ Too high-dielectric loss sample gives pulse length error even though probe tuned.
- ✓ The dielectric loss of water containing ionic solute is very high although pure water is not so high.

Dielectric loss difference of solvents



A Procedure of NMR Measurement

Experiment Parameters

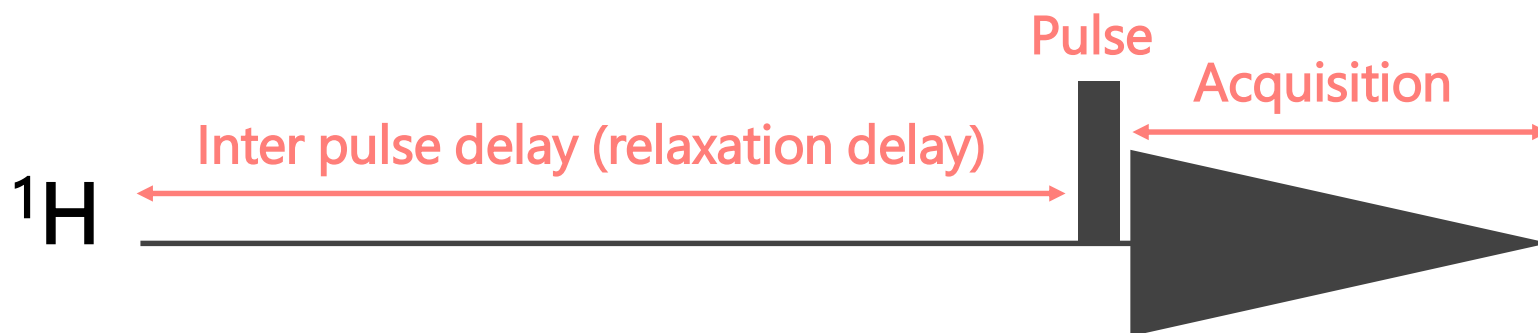
Experiment Parameters

Header Instrument Acquisition Pulse Diagram ★ Favorites

+ Add Parameters ?

storage_filename	test_proton	\$(SAMPLE)_\$(EXP.filename)
filename	proton	
storage_comment	single_pulse	\$(SAMPLE.comment) \$(EXP.comment)
comment	single_pulse	
auto_gain	<input type="checkbox"/>	
filter_limit	16	
force_dual_mode	<input type="checkbox"/>	
force_tune	<input type="checkbox"/>	

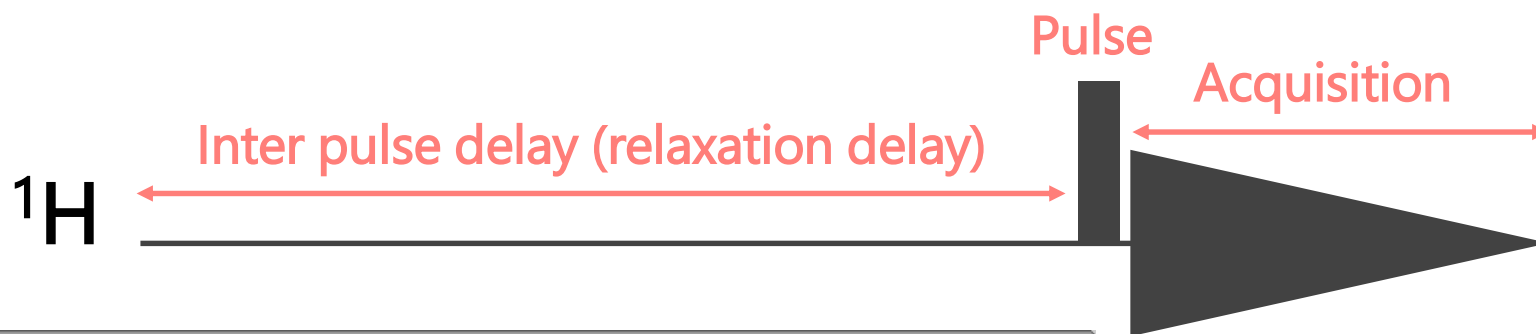
^1H NMR



^1H NMR Information

- Integrals: Number of Protons
- Spin-spin coupling: Structure connectivity
- Chemical shift: Functional groups
- (deuterium exchange): exchangeable protons

^1H -NMR Parameters



The screenshot shows the "Experiment Parameters" window with the following settings:

Parameter	Value
x_domain	Proton
x_offset	5[ppm]
x_sweep	15[ppm]
x_points	16384
x_prescans	1
scans	8
relaxation_delay	5[s]

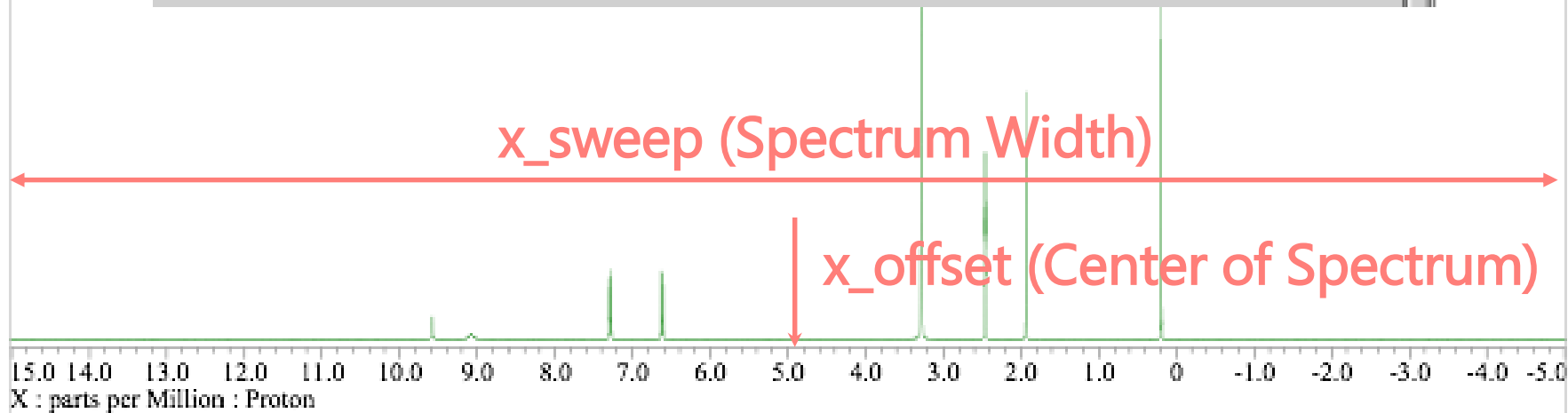
Observation Range

Number of Scans

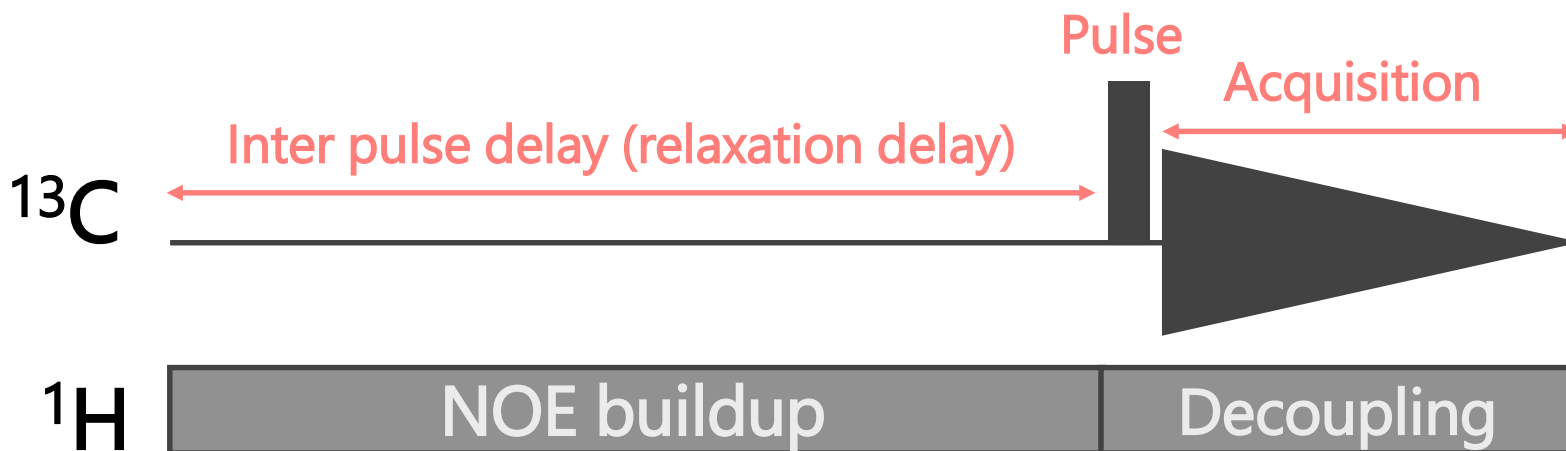
Relaxation Delay

^1H NMR

Header	Instrument	Acquisition	Pulse	Diagram	★ Favorites	+ Add Parameters	?
x_domain	Proton						▲ ▼
x_offset	5[ppm]						
x_sweep	15[ppm]						
x_points	16384						◀ ▶
scans	8						◀ ▶
x_prescans	1						◀ ▶
mod_return	1						◀ ▶
x_acq_time	2.73215[s]						
x_resolution	0.36601[Hz]						



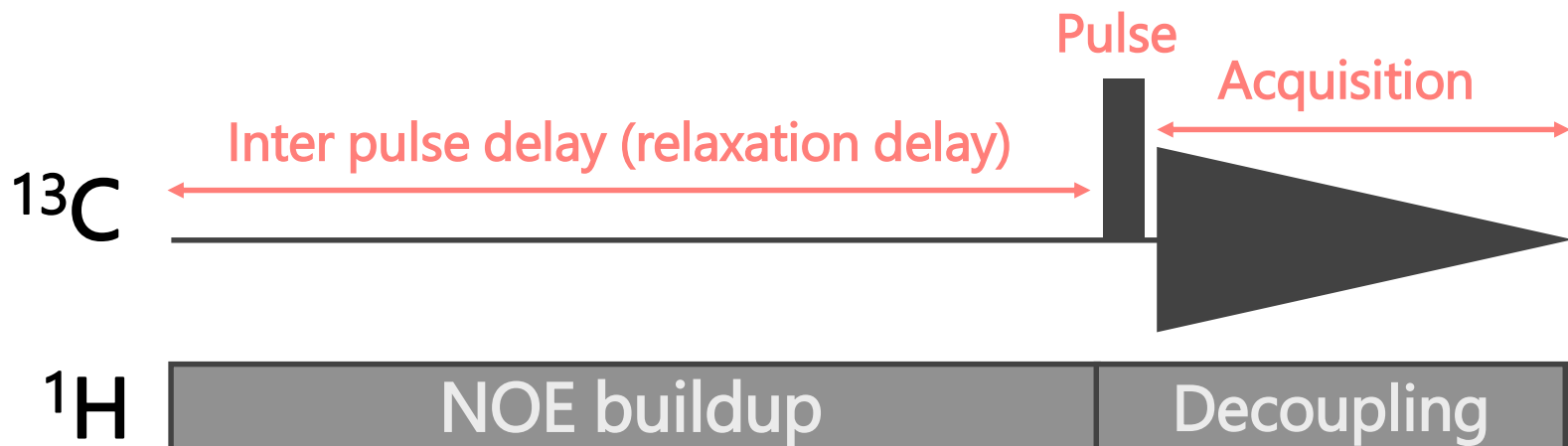
^{13}C NMR



^{13}C NMR Information

- Number of peaks: Number of Carbons
- Chemical shift: Functional groups

^{13}C -NMR Parameters



The screenshot shows the "Experiment Parameters" window with the following settings:

Parameter	Value
x_domain	Carbon13
x_offset	100[ppm]
x_sweep	250[ppm]
x_points	32768
x_prescans	4
scans	1024
relaxation_delay	2[s]

Observation Range

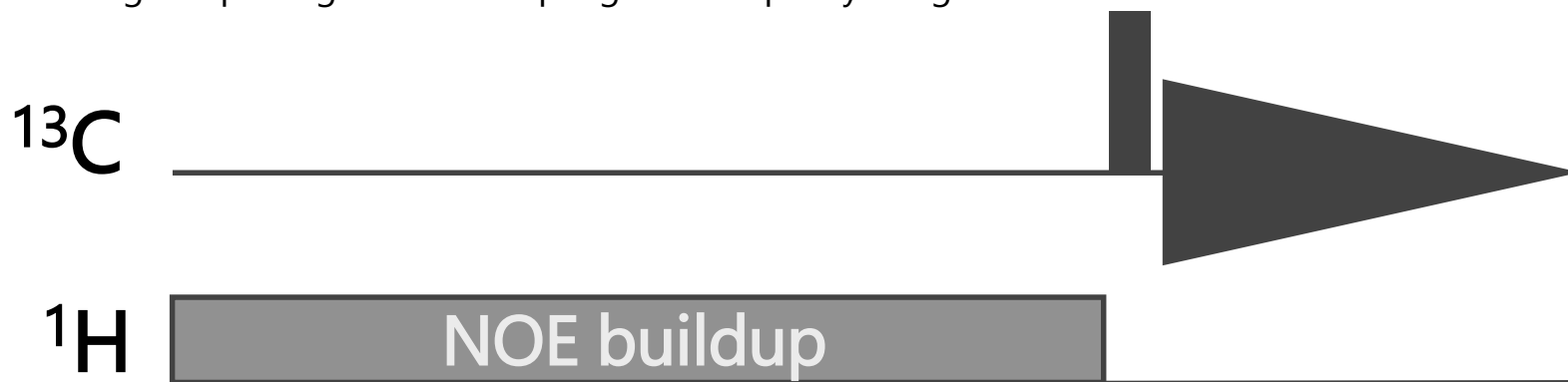
Number of Scans

Relaxation Delay

A kind of specific ^{13}C NMR

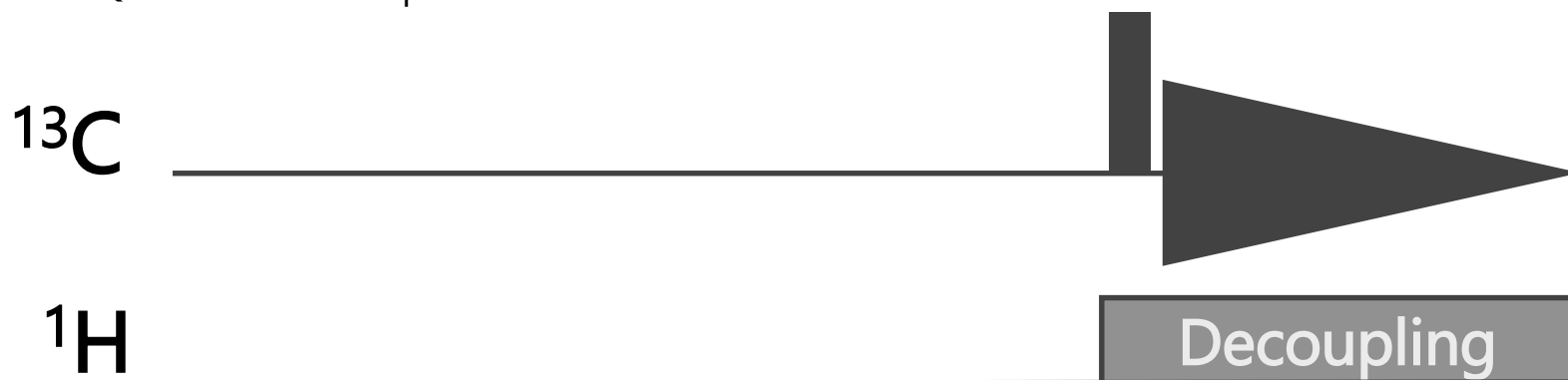
Gated Decoupling

- Signal splitting with ^1H coupling \rightarrow Multiplicity assignment

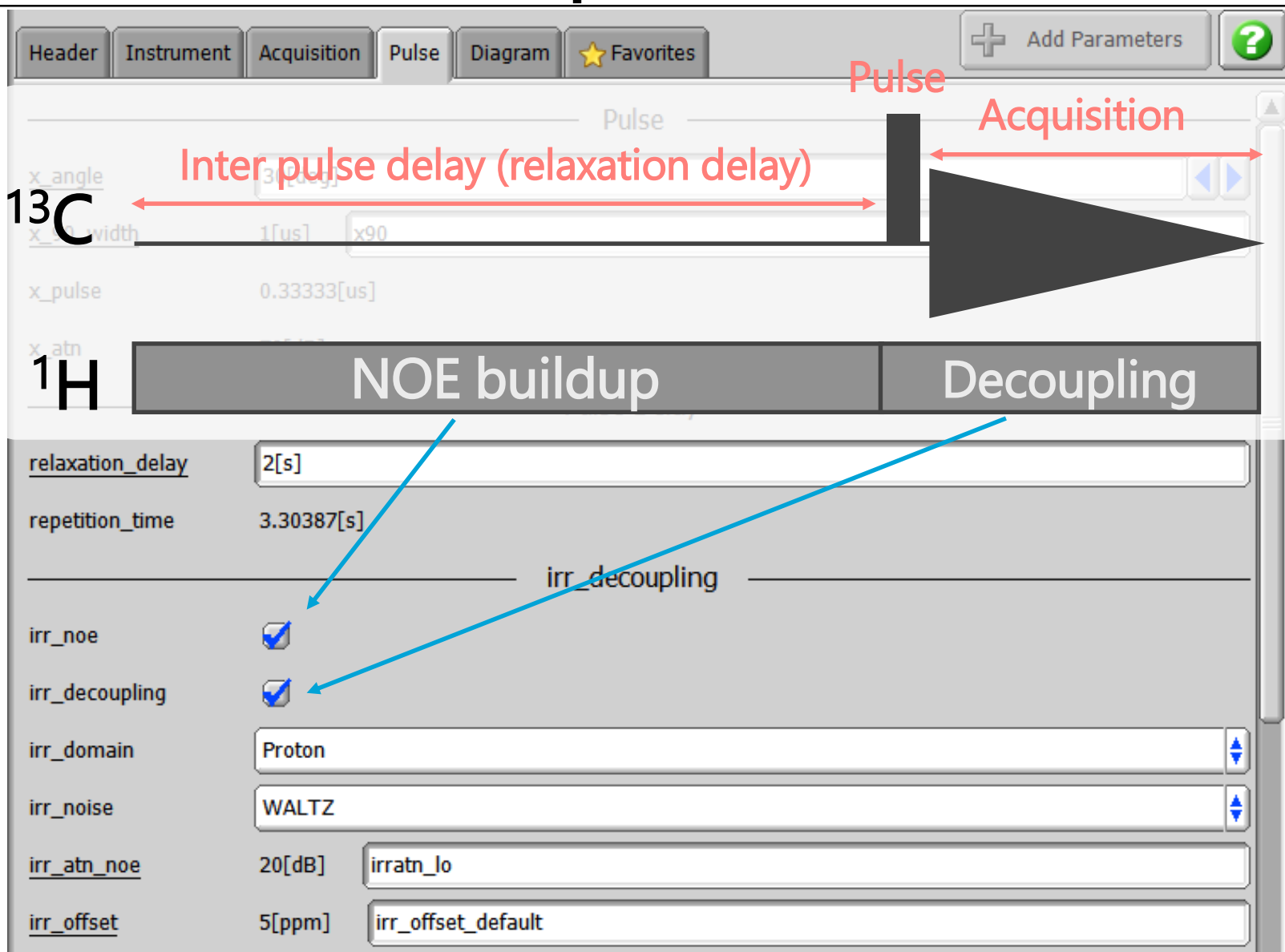


Inverse Gated Decoupling

- Quantitative ^{13}C experiment



A kind of specific ^{13}C NMR



* Sample Spinning










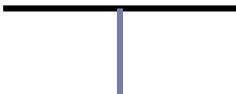


The sample spinning should be ONLY applied to 1D experiment !

- The sample spinning should not be applied to multi-pulse experiment even though it is 1D.
- It will cause sensitivity decrease.
- It may cause artifact signals.

^{13}C -DEPT NMR

^{13}C -DEPT NMR Information

– Multiplicity of carbons

	Primary	Secondary	Tertiary	Quaternary
	$-\text{CH}_3$	$-\text{CH}_2-$	$\begin{array}{c} \\ -\text{CH}- \end{array}$	$\begin{array}{c} \\ -\text{C}- \\ \end{array}$
DEPT45				
DEPT90				
DEPT135				

DEPT Parameters

Experiment Parameters

Header Instrument Acquisition Pulse Diagram ★ Favorites + Add Parameters ?

Pulse

x_pulse 1[us] x90

x_atn 79[dB]

Irr Pulse

irr_domain Proton

irr_offset 5[ppm]

irr_pulse 5[us] irr90

irr_atn 3[dB]

selection_angle 135[deg]

selection_factor 1

selection_pulse 7.5[us]

Pulse Delay

j_constant 140[Hz]

base_line_correct ☒

relaxation_delay 2[s]

irr_Decoupling

irr_decoupling ☒

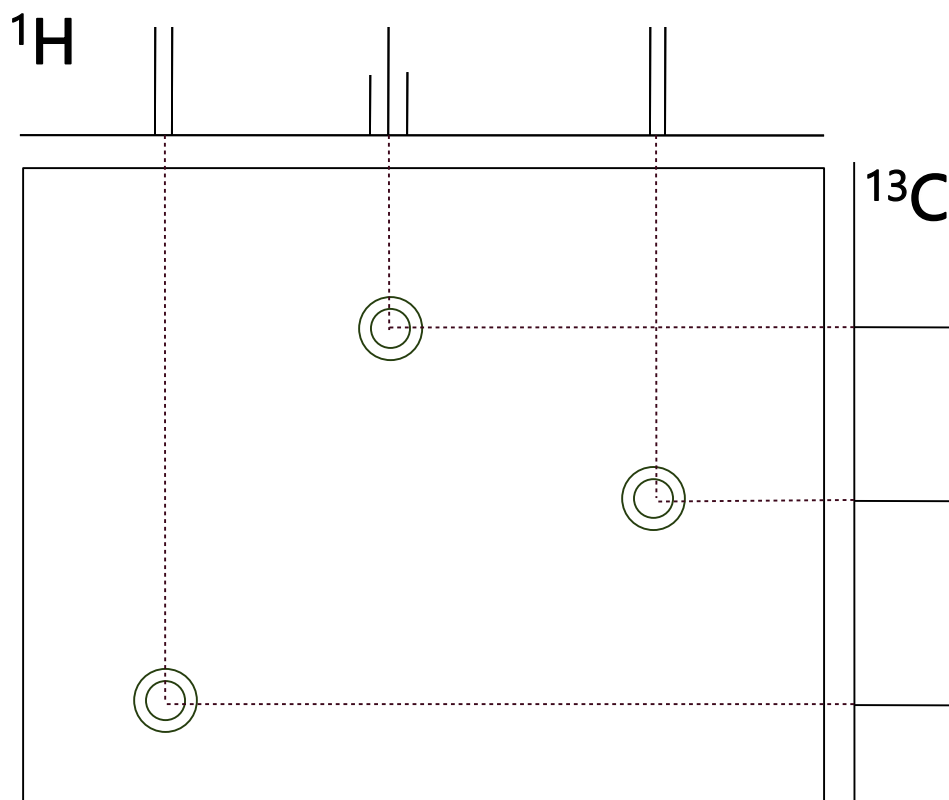
irr_noise WALTZ

Selection Angle
(45°, 90°, 135°)

C-H Correlation

HMQC, HSQC, CH-COSY

- Correlation between directly bonded proton and carbon



HMQC Parameters

Parameter	Value
x_domain	Proton
x_offset	5[ppm]
x_sweep	15[ppm]
x_points	1024
y_domain	Carbon13
y_offset	85[ppm]
y_sweep	170[ppm]
y_points	256
x_prescans	4
scans	4
relaxation_delay	1.5[s]

Observation
Range

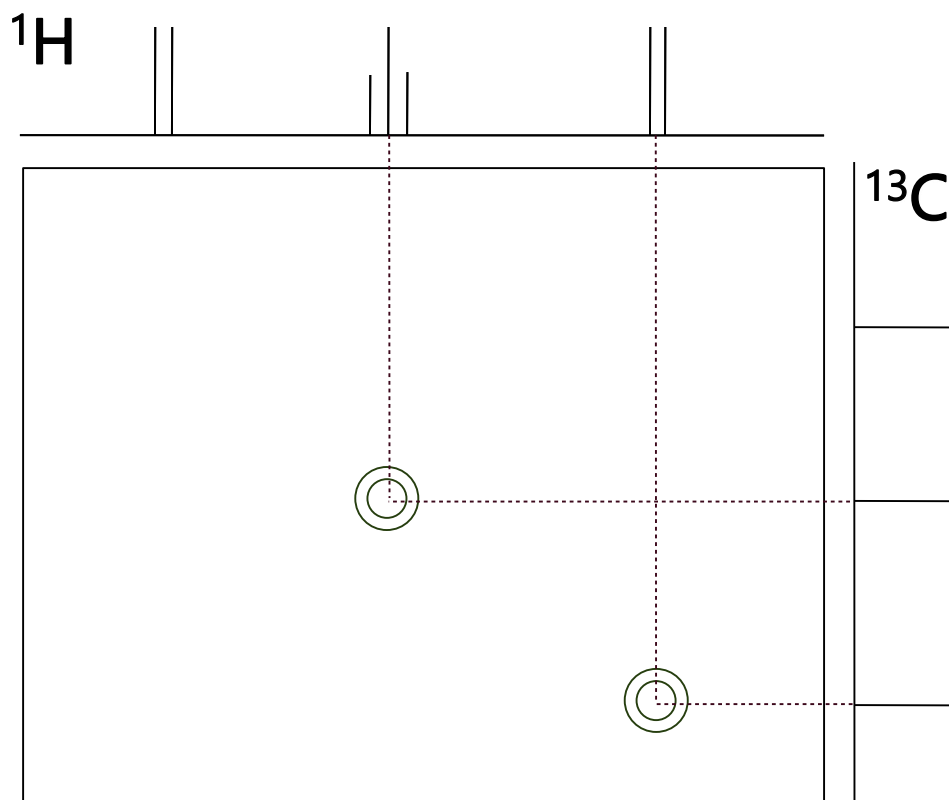
Number of Scans

Relaxation Delay

Long-Range C-H Correlation

HMBC, COLOC

- Correlation between ^1H and ^{13}C connected via 2 or 3 bonds



HMBC Parameters

Parameter	Value
x_domain	Proton
x_offset	5[ppm]
x_sweep	15[ppm]
x_points	2048
y_domain	Carbon13
y_offset	100[ppm]
y_sweep	250[ppm]
y_points	256
x_prescans	4
scans	4
long_range_j	8[Hz]
relaxation_delay	1.5[s]

Observation Range

Number of Scans

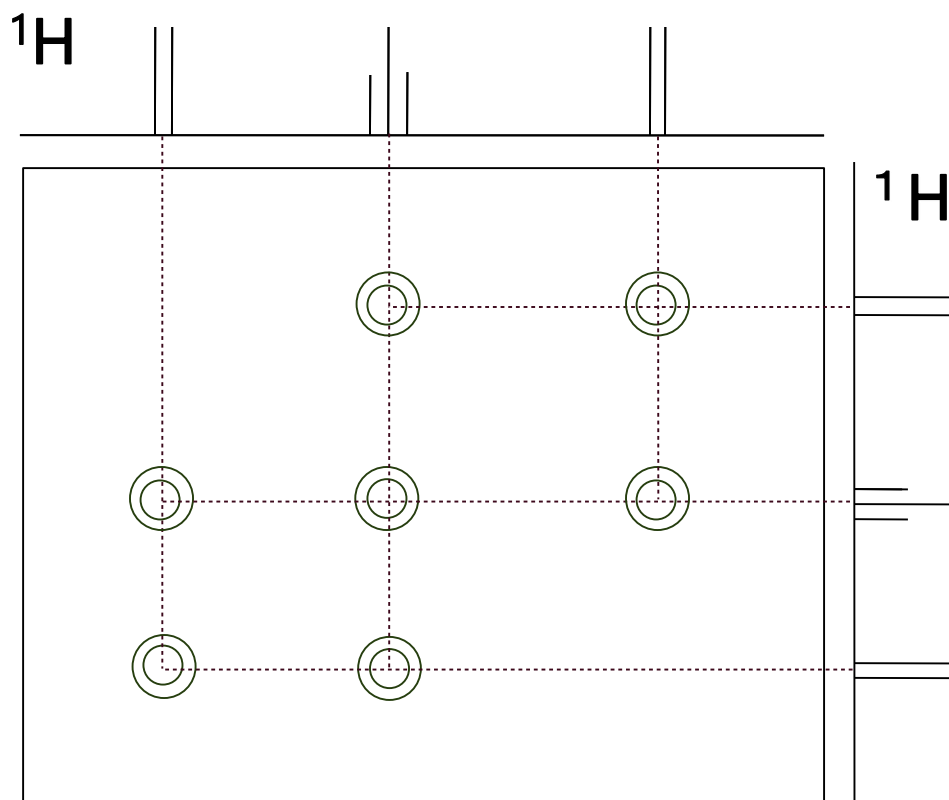
Long range J -constant

Relaxation Delay

H-H correlation

HH-COSY

- Correlation between protons which have spin-spin coupling (protonated carbons ^{13}C - ^{13}C connectivity)



COSY Parameters

Experiment Parameters

Header Instrument Acquisition Pulse Diagram ★ Favorites + Add Parameters ?

x_domain Proton

x_offset 5[ppm]

x_sweep 15[ppm]

x_points 1024

y_points 256

x_prescans 4

scans 1

relaxation_delay 1.5[s]

Observation
Range

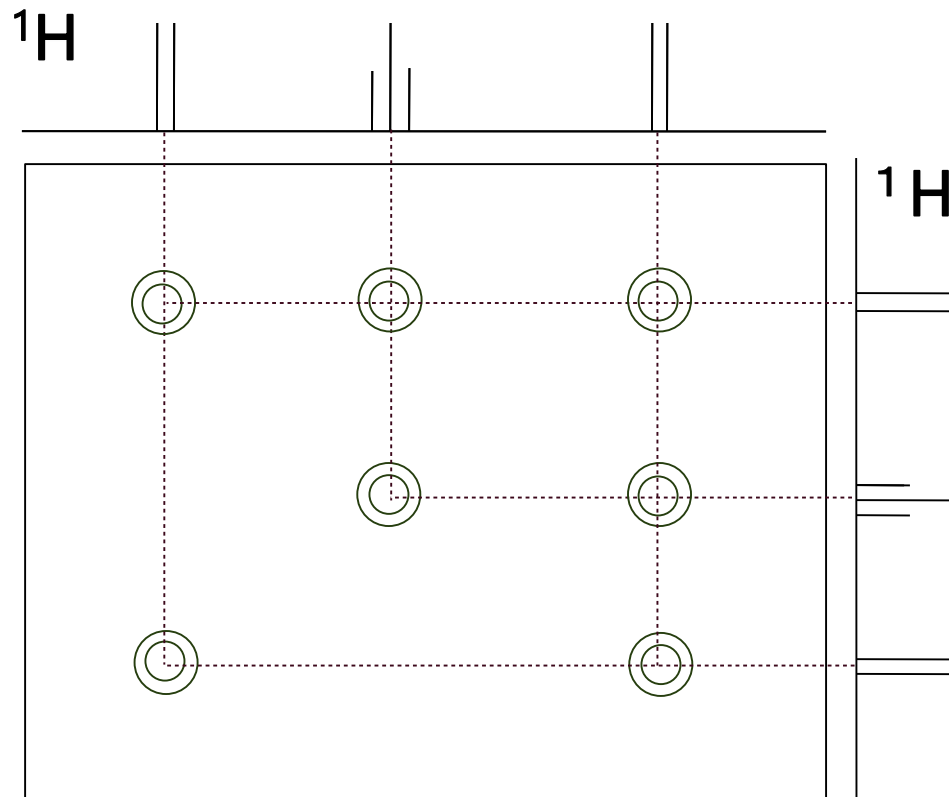
Number of Scans

Relaxation Delay

NOE correlation

HH-NOESY

- Correlation between spatially close protons ($< 6\text{\AA}$)



NOESY Parameters

Parameter	Value
x_domain	Proton
x_offset	5[ppm]
x_sweep	15[ppm]
x_points	1024
y_points	256
x_prescans	4
scans	4
mix_time	0.5[s]
relaxation_delay	1.5[s]

Observation
Range

Number of Scans

Mixing Time

Relaxation Delay

The JEOL logo is rendered in a bold, dark blue, sans-serif typeface. The letters are thick and blocky, with a distinctive design where the 'J' and 'L' have a slight inward curve at their base, and the 'O' is a simple, solid circle.

Solutions for Innovation