
JUL • 64

NMR Operation at NYSBC

NYSBC Solid State NMR
Short Course

Schedule and outline, day 1

Bruker Acquisition and Processing Software

Navigating in TOPSPIN

Topspin Directory Structure

Data structure
Back-up/housekeeping protocols
Data server

Instrumentation

Magnet safety
Console components
Probes and probe safety

Initial setup

Samples and rotors: packing and spinning
Tuning and matching using Wobb
High power tuning and matching using oscilloscope
Variable temperature and coil air flow

Schedule and outline, day 2

Basic solid state NMR experiments

Setting up Magic Angle with KBr

Power handling

Direct excitation with CW decoupling

Composite pulse decoupling (CDP)

Cross Polarization (CP) with CPD

Shimming

More advanced solid state NMR experiments

Hahnecho

1D double CP experiment

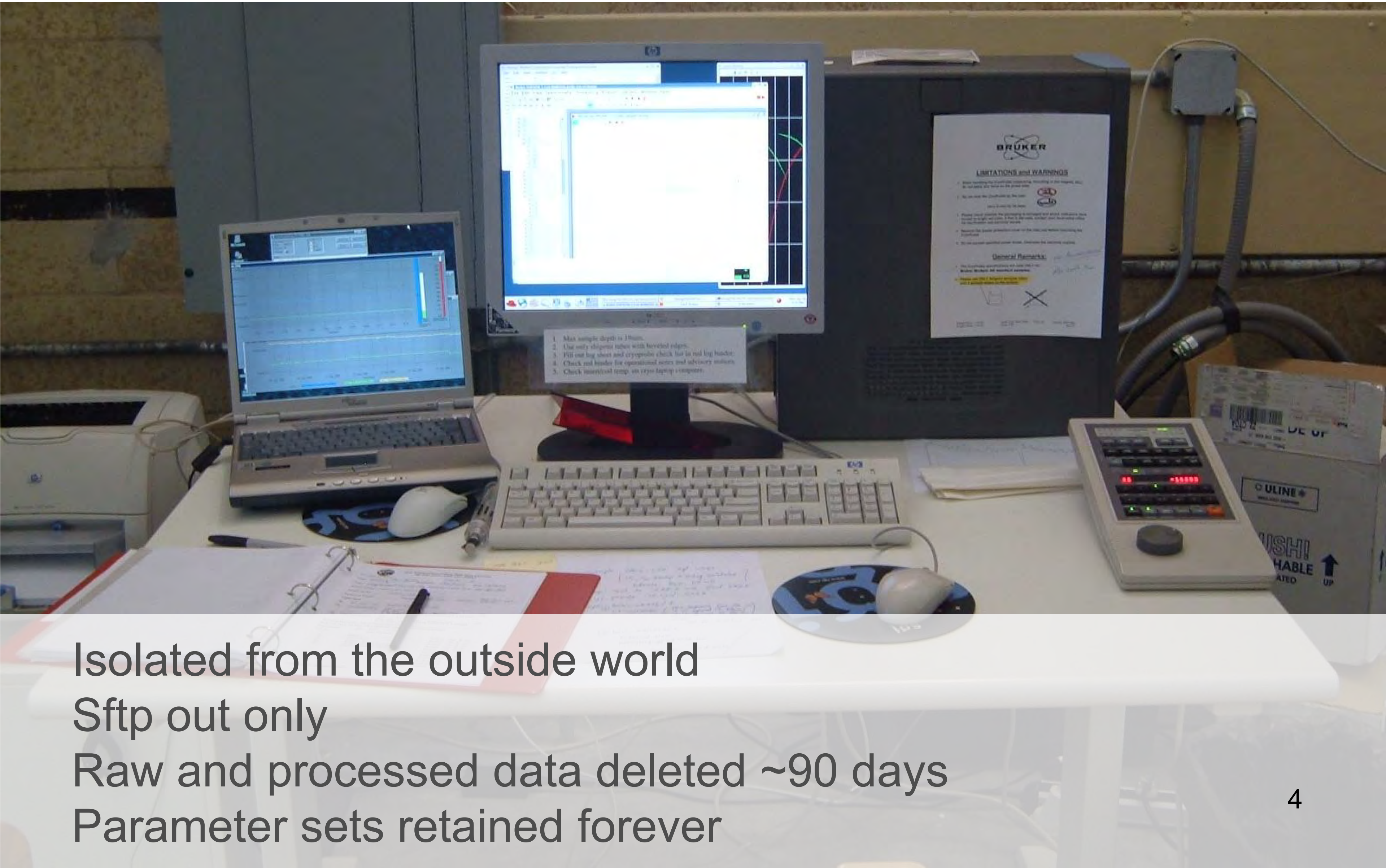
13C-15N correlation – 2D double CP experiment

13C-13C correlation – RAD type polarization transfer

13C-13C spin pair correlation – RFDR

13C-13C-15N correlation – Doubcp-Radmix experiment

IT policies: spectrometer PC



Isolated from the outside world
Sftp out only
Raw and processed data deleted ~90 days
Parameter sets retained forever

IT policies: data backup

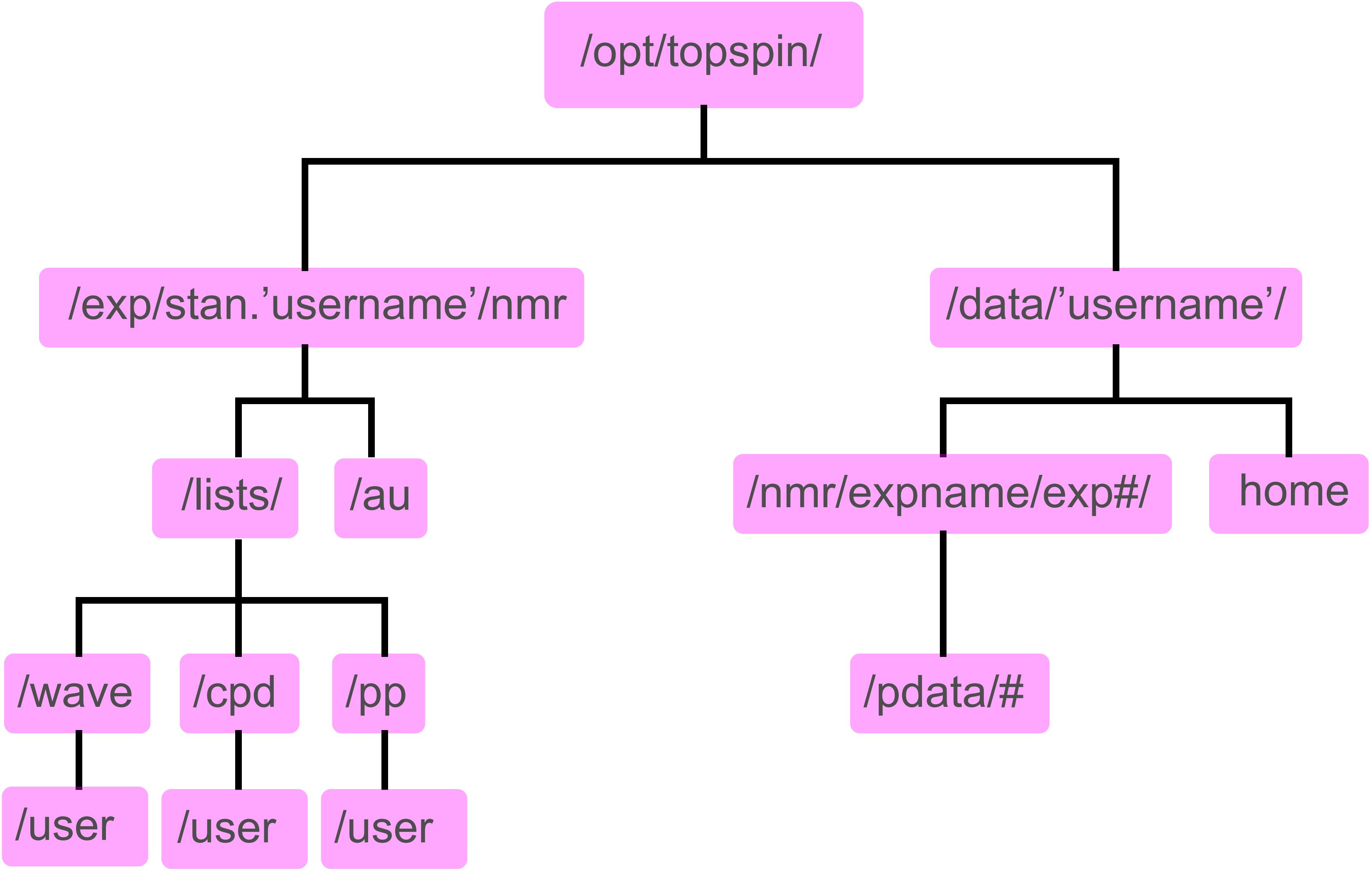
File not Found

Current user backed up to server every 10 min
Entire spectrometer backed up to a server once a week
Backed up to tape once a month
Intended for disaster recovery
Not intended for long term archiving

IT policies: data server

Net access to data
Sftp ‘username’@sftp.nysbc.net
Replication of each spectrometer PC
Check on progress of data acquisition

Topspin directory structure



/opt/topspin/

/exp/stan.’username’/nmr

/data/’username’/

/lists/

/au

/nmr/expname/exp#/

home

/wave

/cpd

/pp

/pdata/#

/user

/user

/user

Twiki pages

http://www.nysbc.net/twiki/view/Main

http://www.nysbc.net/twiki/register.html

http://www.nysbc.net/twiki/bin/view/Main/NmrfacGroup

http://www.nysbc.net/twiki/bin/view/Main/NmrSchedule

http://www.nysbc.net/twiki/bin/view/Main/CaL600

Topspin

Starting topspin

Tab/window layout

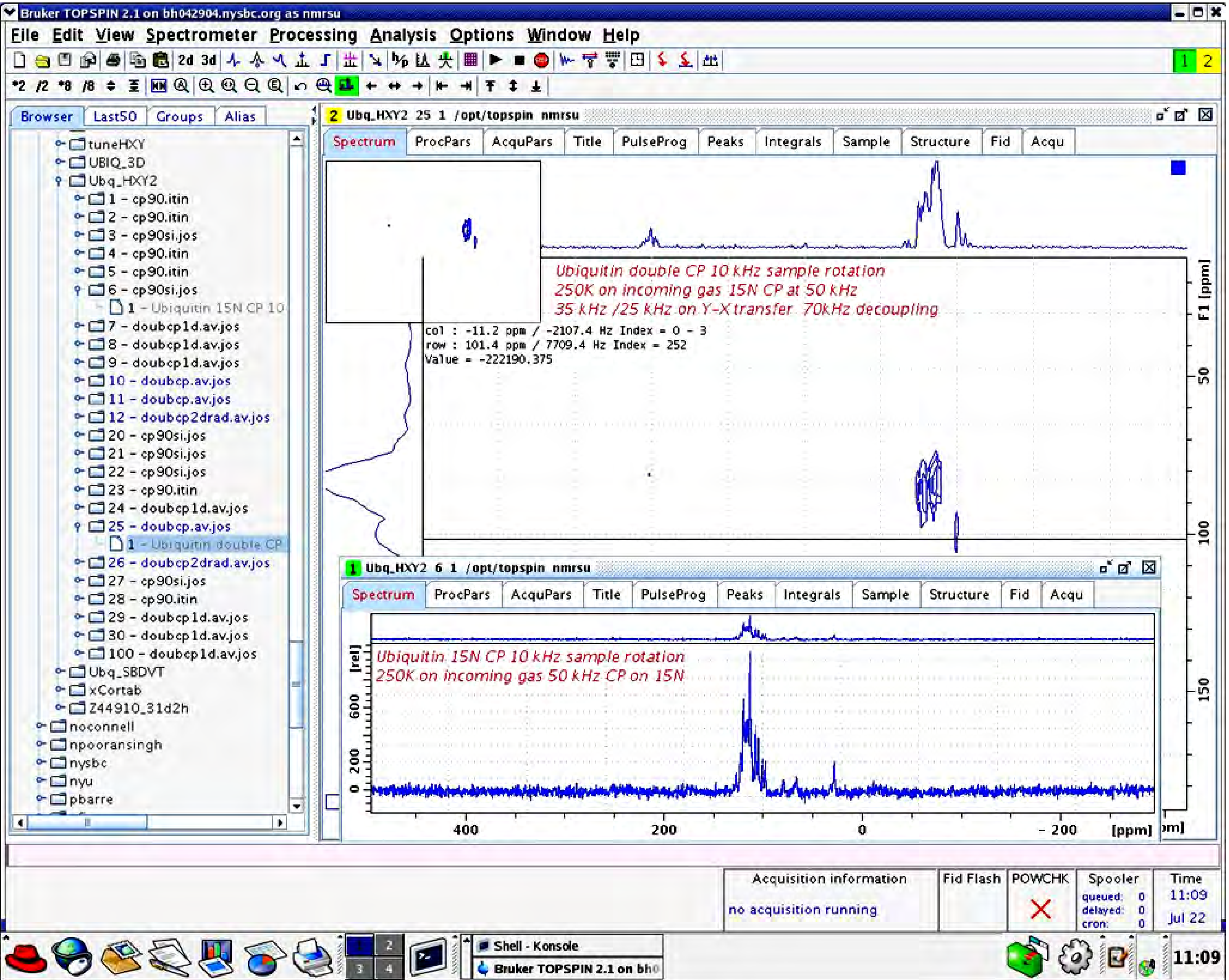
Browser

Frequently used buttons

Manipulation of processed data

Miscellaneous features

Topspin interface



Bruker TOPSPIN 2.1 on bh042904.nysbc.org as nmrsu

- tuneHXY
- UBIQ_3D
- Ubq_HXY2
  - 1 - cp90.itin
  - 2 - cp90.itin
  - 3 - cp90si.jos
  - 4 - cp90.itin
  - 5 - cp90.itin
  - 6 - cp90si.jos
    - 1 - Ubiquitin 15N CP 10
  - 7 - doubcp1d.av.jos
  - 8 - doubcp1d.av.jos
  - 9 - doubcp1d.av.jos
  - 10 - doubcp.av.jos
  - 11 - doubcp.av.jos
  - 12 - doubcp2drad.av.jos
  - 20 - cp90si.jos
  - 21 - cp90si.jos
  - 22 - cp90si.jos
  - 23 - cp90.itin
  - 24 - doubcp1d.av.jos
  - 25 - doubcp.av.jos
    - 1 - Ubiquitin double CP
  - 26 - doubcp2drad.av.jos
  - 27 - cp90si.jos
  - 28 - cp90.itin
  - 29 - doubcp1d.av.jos
  - 30 - doubcp1d.av.jos
  - 100 - doubcp1d.av.jos
- Ubq_SBDVT
- xCortab
- Z44910_31d2h
- noconnell
- npooransingh
- nysbc
- nyu
- pbarre

2 Ubq_HXY2 25 1 /opt/topspin nmrsu

Ubiquitin double CP 10 kHz sample rotation
250K on incoming gas 15N CP at 50 kHz
35 kHz /25 kHz on Y-X transfer 70kHz decoupling

```
col : -11.2 ppm / -2107.4 Hz Index = 0 - 3
row : 101.4 ppm / 7709.4 Hz Index = 252
Value = -222190.375
```

1 Ubq_HXY2 6 1 /opt/topspin nmrsu

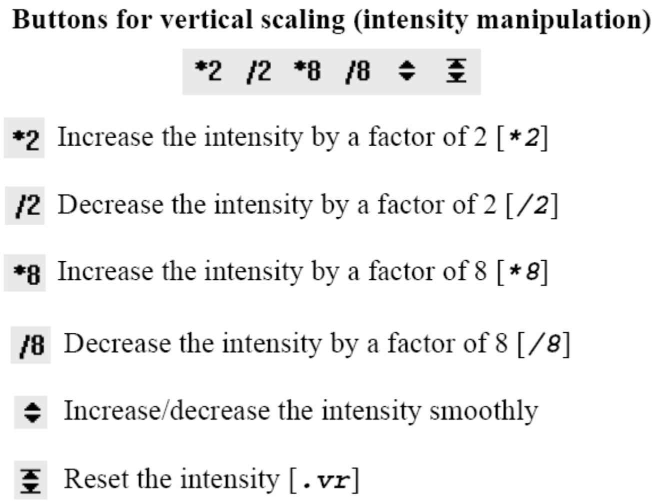
Ubiquitin 15N CP 10 kHz sample rotation
250K on incoming gas 50 kHz CP on 15N

Acquisition information: no acquisition running

Spooler: queued: 0, delayed: 0, cron: 0

Time: 11:09, Jul 22

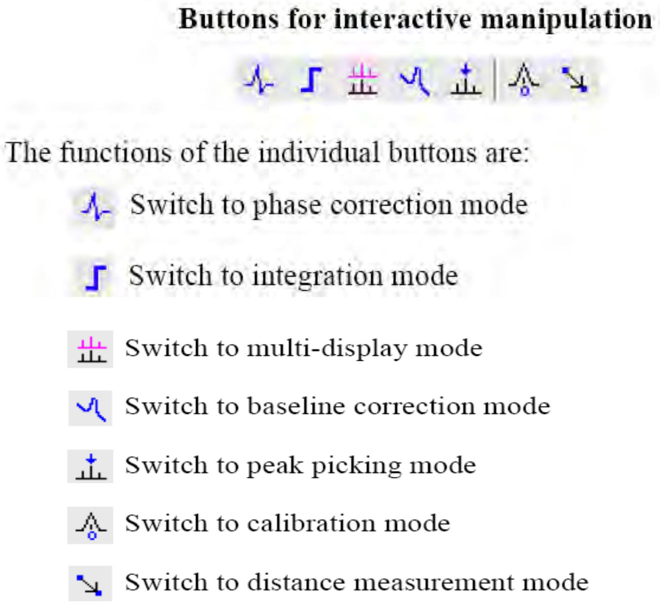
Topspin buttons



Buttons for vertical scaling (intensity manipulation)

*2 /2 *8 /8 ⬍

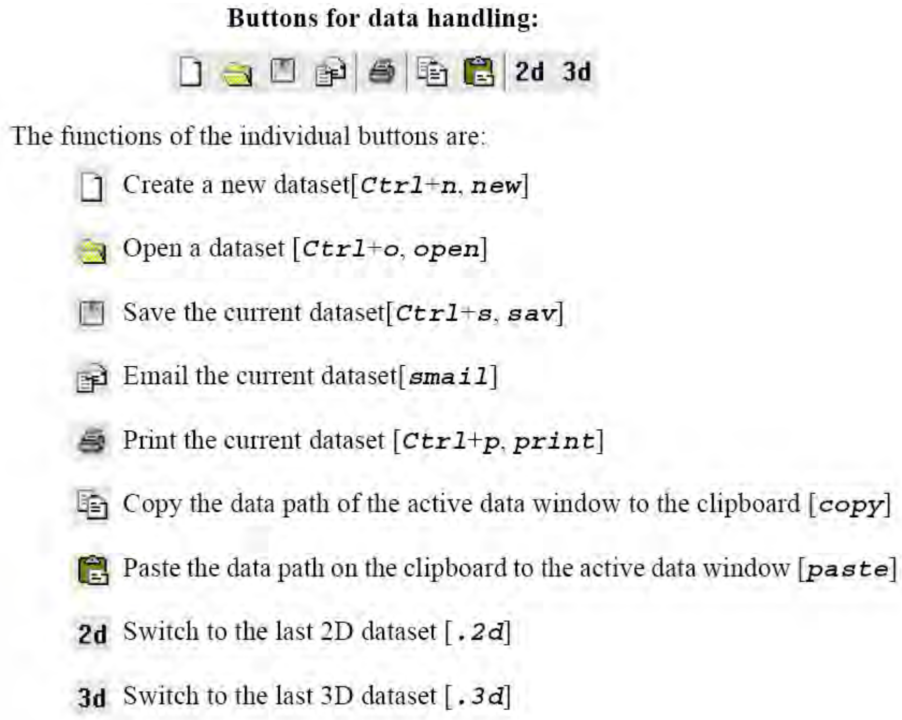
- ***2** Increase the intensity by a factor of 2 [`*2`]
- **/2** Decrease the intensity by a factor of 2 [`/2`]
- ***8** Increase the intensity by a factor of 8 [`*8`]
- **/8** Decrease the intensity by a factor of 8 [`/8`]
- ⬍ Increase/decrease the intensity smoothly
- Reset the intensity [`.vr`]



Buttons for interactive manipulation

The functions of the individual buttons are:

- Switch to phase correction mode
- Switch to integration mode
- Switch to multi-display mode
- Switch to baseline correction mode
- Switch to peak picking mode
- Switch to calibration mode
- Switch to distance measurement mode

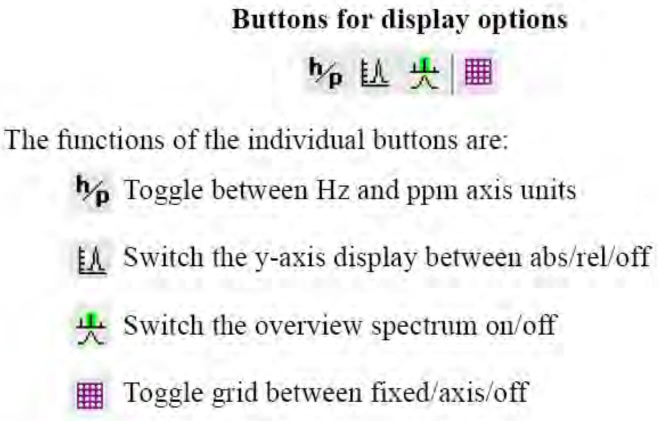


Buttons for data handling:

2d 3d

The functions of the individual buttons are:

- Create a new dataset[`Ctrl+n`, `new`]
- Open a dataset [`Ctrl+o`, `open`]
- Save the current dataset[`Ctrl+s`, `sav`]
- Email the current dataset[`smail`]
- Print the current dataset [`Ctrl+p`, `print`]
- Copy the data path of the active data window to the clipboard [`copy`]
- Paste the data path on the clipboard to the active data window [`paste`]
- **2d** Switch to the last 2D dataset [`.2d`]
- **3d** Switch to the last 3D dataset [`.3d`]



Buttons for display options

The functions of the individual buttons are:

- **h/p** Toggle between Hz and ppm axis units
- Switch the y-axis display between abs/rel/off
- Switch the overview spectrum on/off
- Toggle grid between fixed/axis/off

NYSBC ultra high field magnets

800US#2_NE

800US#1_NW

900#2

800CONV

750WB

900#1

NYSBC high field magnets

500

600

700

Basic magnet safety

Magnetic objects

Keep ferrous/steel items outside 5G stray field (red line)

Gas tanks

Chairs
Electronics
Tools
Pens
Razor blades
Hair clips
Earrings

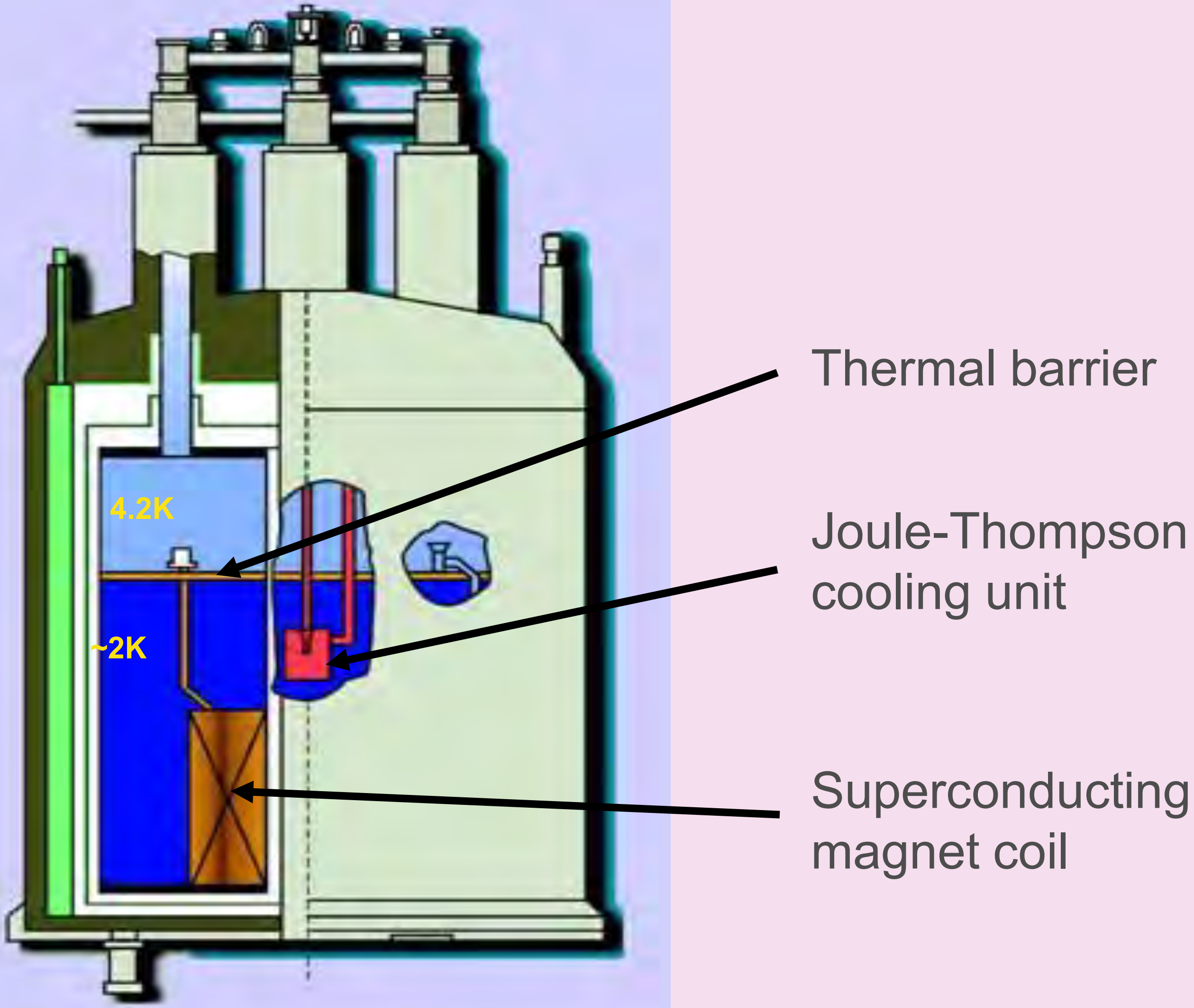
Medical implants

Keep medical implants outside 5G stray field (red line)

Magnet Quench

Evacuate the room immediately:
large volumes of N_2 and He may be released very quickly and O_2 concentration in the air will drop dramatically.
You will keel over and die.

Bruker Ultra Stabilized magnet



Thermal barrier

Joule-Thompson cooling unit

Superconducting magnet coil

Bruker magnet PC

BMPC for ultra high field magnets (900s, 800s, 750wb)

Monitor magnet parameters:

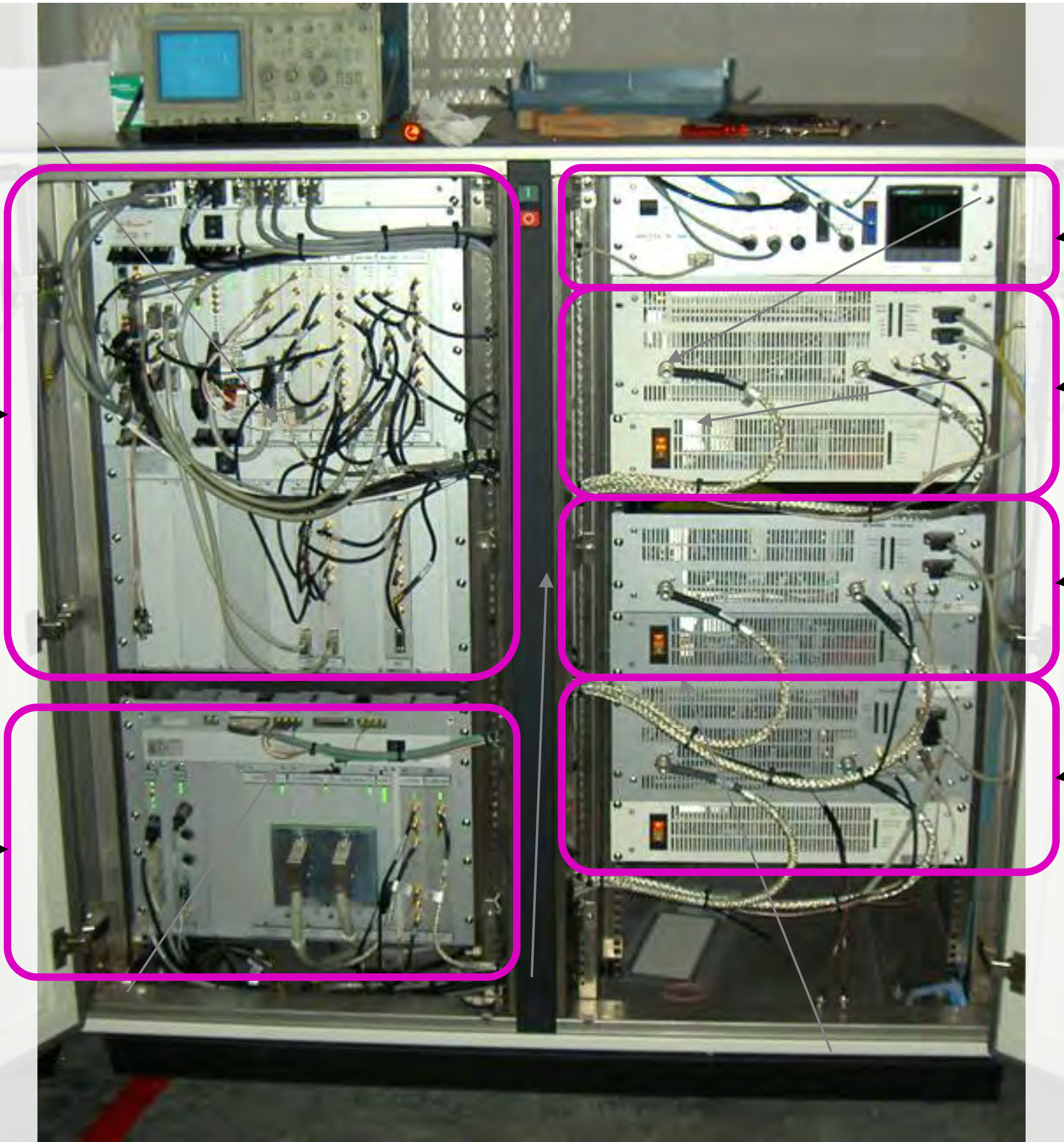
P1, P2, P3, T1, T2, He flow

Control pumps for helium flow

Call out in event of problems

Spectrometer console

750wb spectrometer console



Bruker
VT unit
BVT 3000

Low power
RF units
(AQS rack)

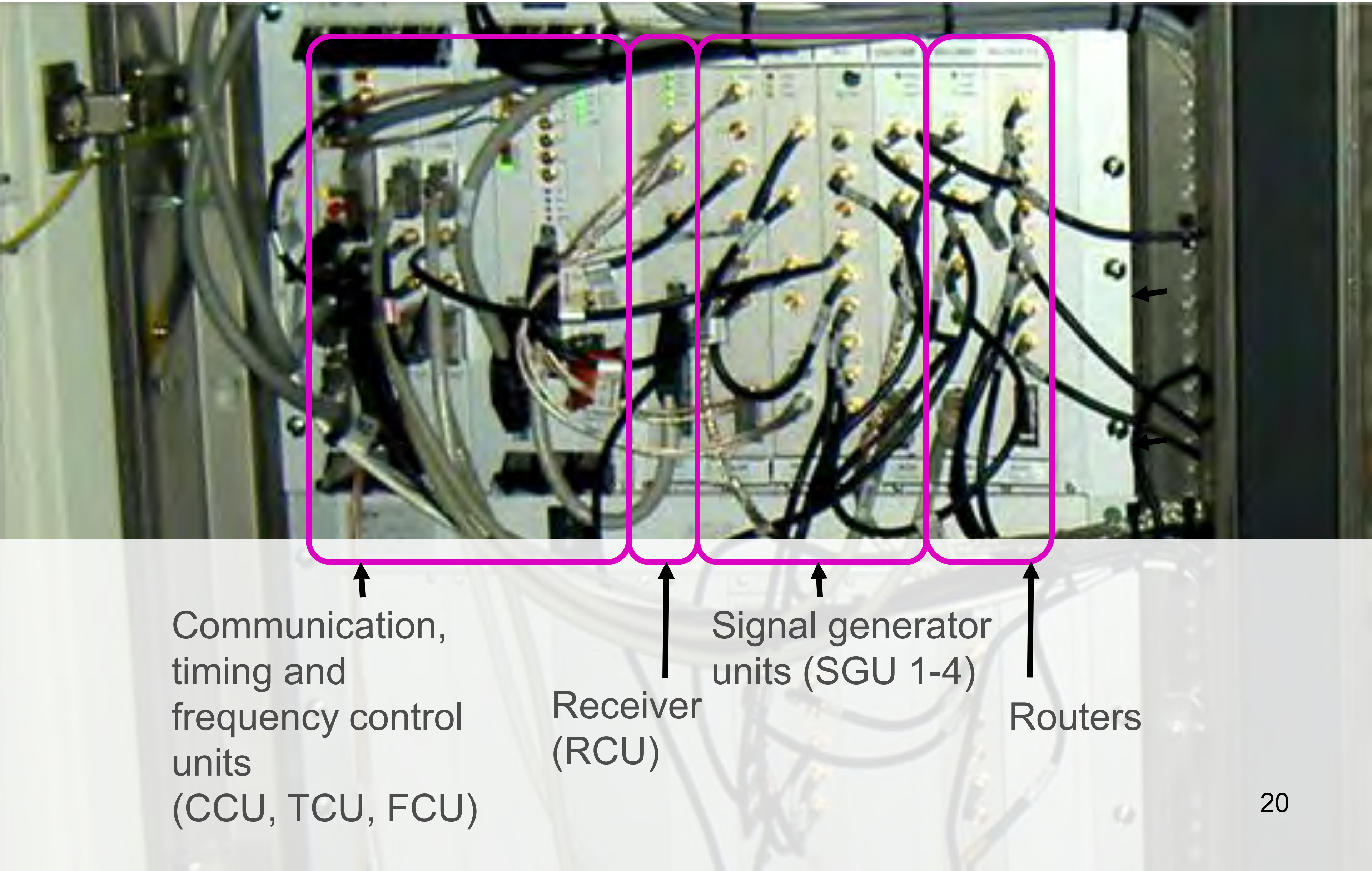
BLAX1
amplifier

BLAH
amplifier

BLAX2
amplifier

Bruker
shimming unit
BMPC

750wb spectrometer console



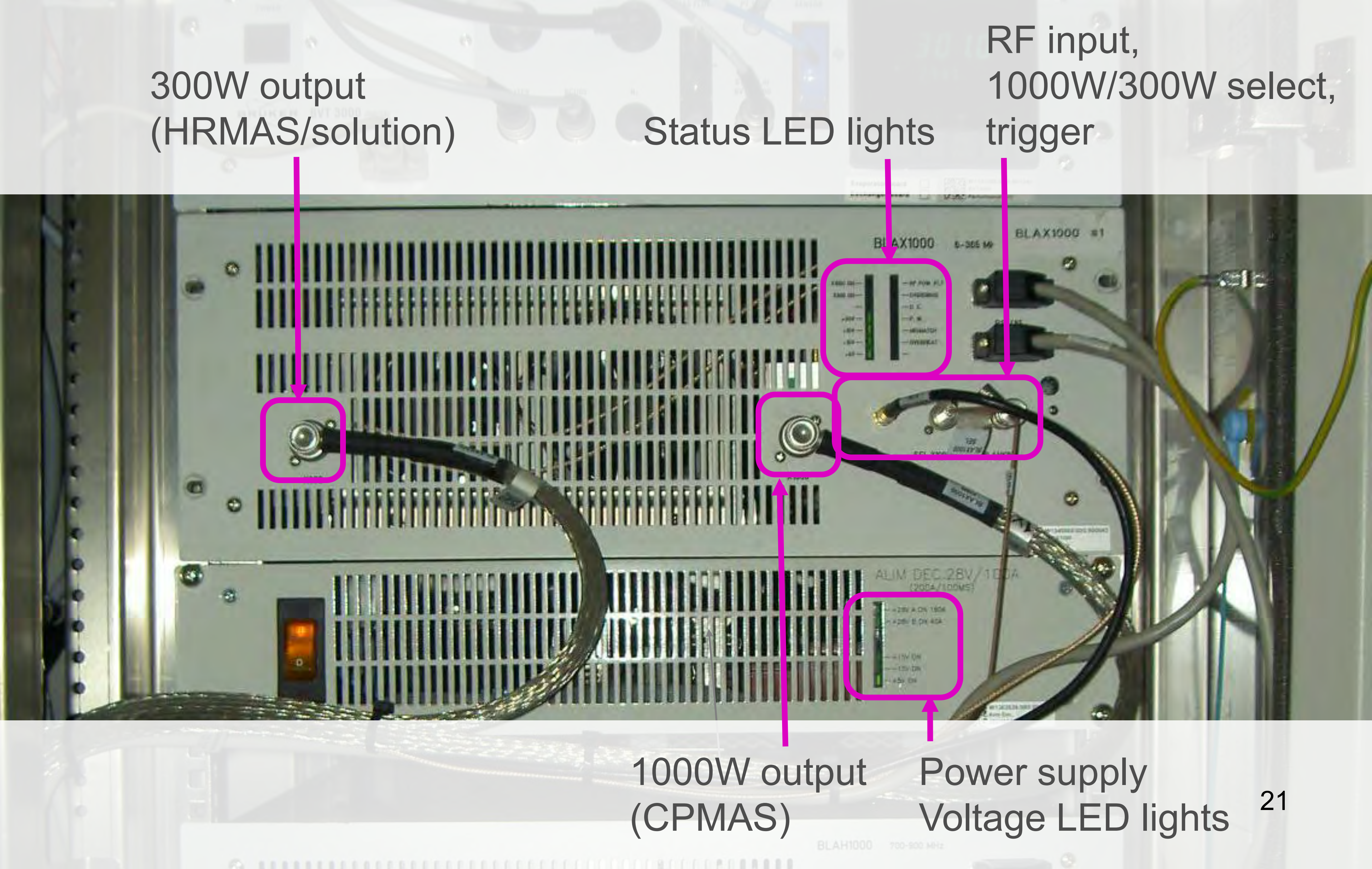
Communication, timing and frequency control units (CCU, TCU, FCU)

Receiver (RCU)

Signal generator units (SGU 1-4)

Routers

BLAX/BLAH amplifier



300W output
(HRMAS/solution)

Status LED lights

RF input,
1000W/300W select,
trigger

1000W output
(CPMAS)

Power supply
Voltage LED lights

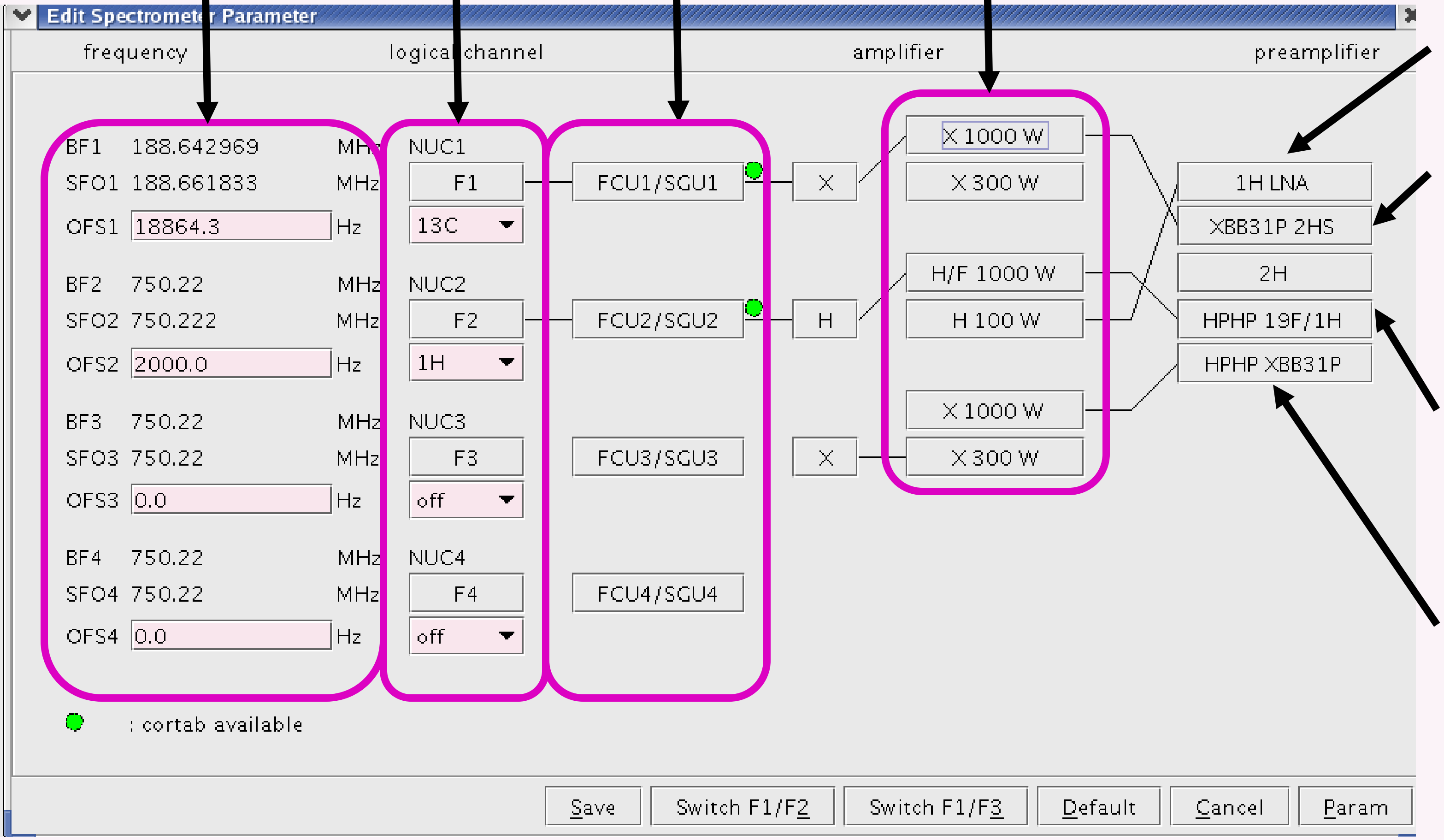
EDASP

Main Frequency and Carrier offset

Channel

Signal Generator

Amplifier



Edit Spectrometer Parameter

frequency				logical channel			amplifier		preamplifier
BF1	188.642969	MHz	NUC1	F1	13C	FCU1/SGU1 ●	X	X 1000 W X 300 W	1H LNA XBB31P 2HS
SFO1	188.661833	MHz							
OFS1	18864.3	Hz							
BF2	750.22	MHz	NUC2	F2	1H	FCU2/SGU2 ●	H	H/F 1000 W H 100 W	2H HPHP 19F/1H
SFO2	750.222	MHz							
OFS2	2000.0	Hz							
BF3	750.22	MHz	NUC3	F3	off	FCU3/SGU3	X	X 1000 W X 300 W	HPHP XBB31P
SFO3	750.22	MHz							
OFS3	0.0	Hz							
BF4	750.22	MHz	NUC4	F4	off	FCU4/SGU4			
SFO4	750.22	MHz							
OFS4	0.0	Hz							

● : cortab available

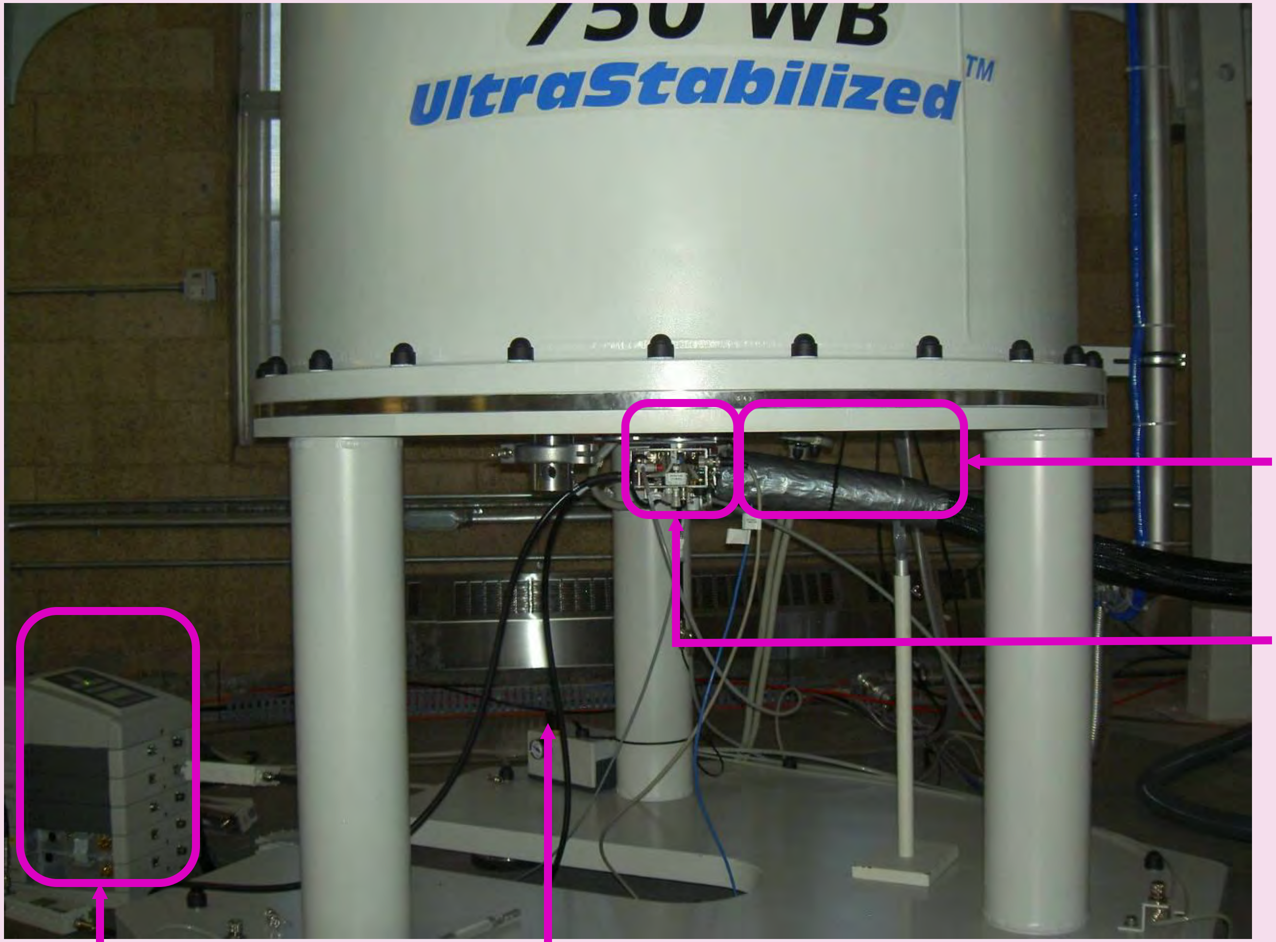
1H high resolution (solution) preamp

0-31P high resolution (solution) heteronuclear preamp (2H bandstop)

19F/1H high power (solids) preamp

0-31P high power (solids) preamp (with sideways plugin bandpass filters)

750wb magnet space



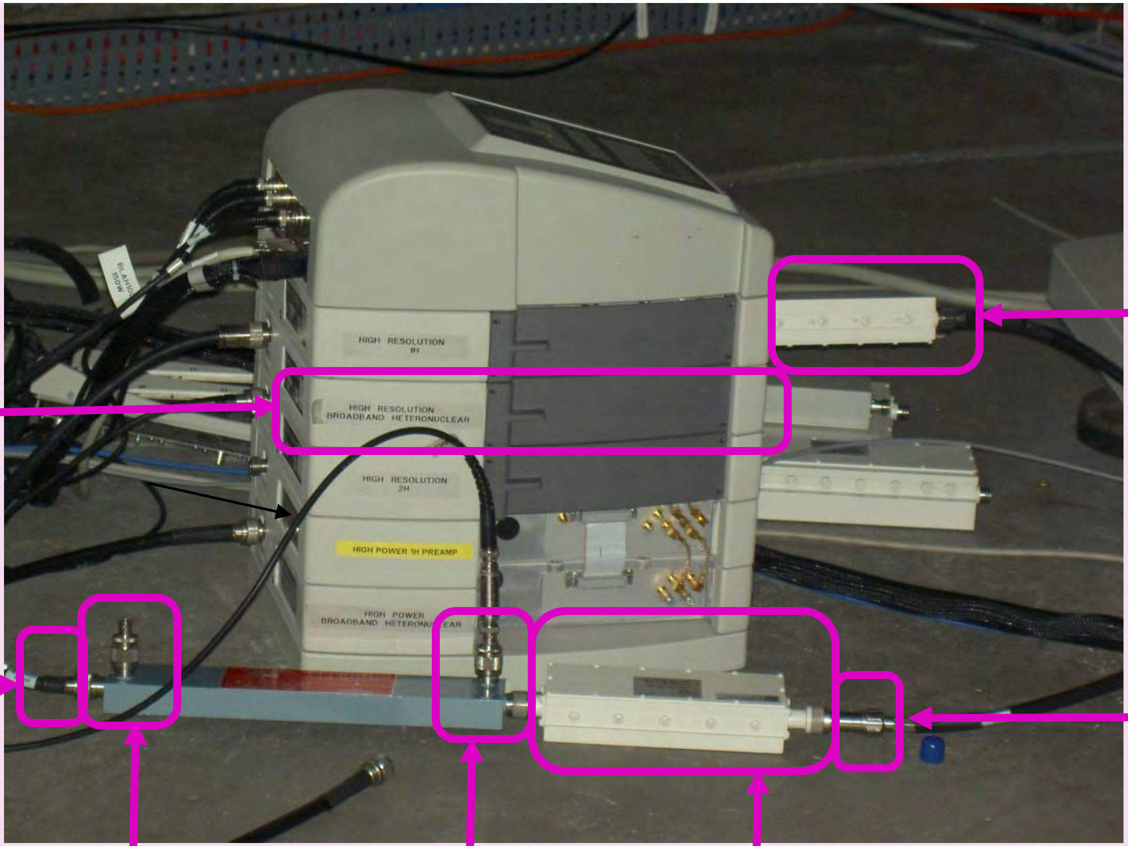
AirJet VT line

Probe box

Preamplifier (HPPR) tower

Trough with VT tubing
(next to the wall, behind the magnet)

Preamplifier tower



External filter (^{13}C)

Pre-amplifier slice (^{13}C)

From BLAH amplifier

Forward voltage to oscilloscope (^{1}H)

Reflected voltage to oscilloscope (^{1}H)

^{1}H external filter

To probe (^{1}H)

Magic Angle Spinning

4mm ZrO rotors	2-15kHz
3.2mm ZrO rotors	4-23kHz
3.2mm Saphire rotors	2-15kHz
1.3mm ZrO rotors	35-65kHz

Rotors should be well packed with a homogeneous sample.

Spacers should be centered.

Rotor caps should have snug fit and be in good condition.

A black mark should be drawn on the rotor bottom beveled sidewall. Make sure that the mark is fairly long. Some rotors have laser etched marks that are not bright enough – paint them over.

MAS controller is capable of starting spinning, maintaining spinning frequency within 2-5Hz, stopping spinning and ejecting a rotor.

Make sure that all air tubing quick connects are plugged in properly.
Make sure that MAS detector cable is plugged into the probe.

Rotor eject/insert on WB MAS probes

It is possible to eject and insert rotors in Bruker probes without removing a probe out of the magnet.

Before ejecting/inserting a rotor, turn air flow through the coil off.

Before ejecting a rotor, make sure that the transfer line is capped with a safety cap. However, don't close the safety cap all the way – leave a 1/8" opening to facilitate eject air flow.

.

To eject a rotor, press EJECT button on MAS controller. If a rotor doesn't eject, gently and slightly rotate (<5^0) and move up and down (2mm) the transfer line. Unplug and plug eject tubing quick connect into the transfer line.

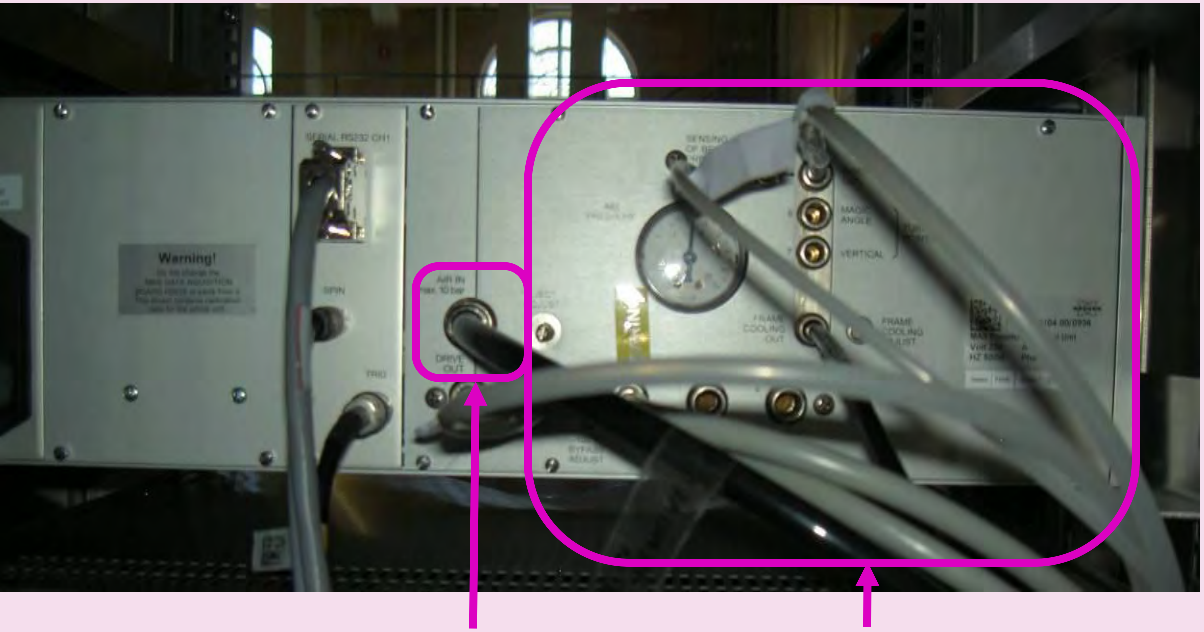
To insert a rotor, make sure that there is no rotor already inside the probe.
Turn the coil air flow off.
Drop a rotor into the transfer line with the cap pointing up.
Disconnect a 8mm gray tubing (shim stack purge line) from the quick connect joint on the shim stack brass ring and use this air flow to push rotor into the proper position inside the stator.
Start spinning before you turn air flow through the coil on.

If a rotor doesn't spin, eject and insert again.

MAS controller: front and back view

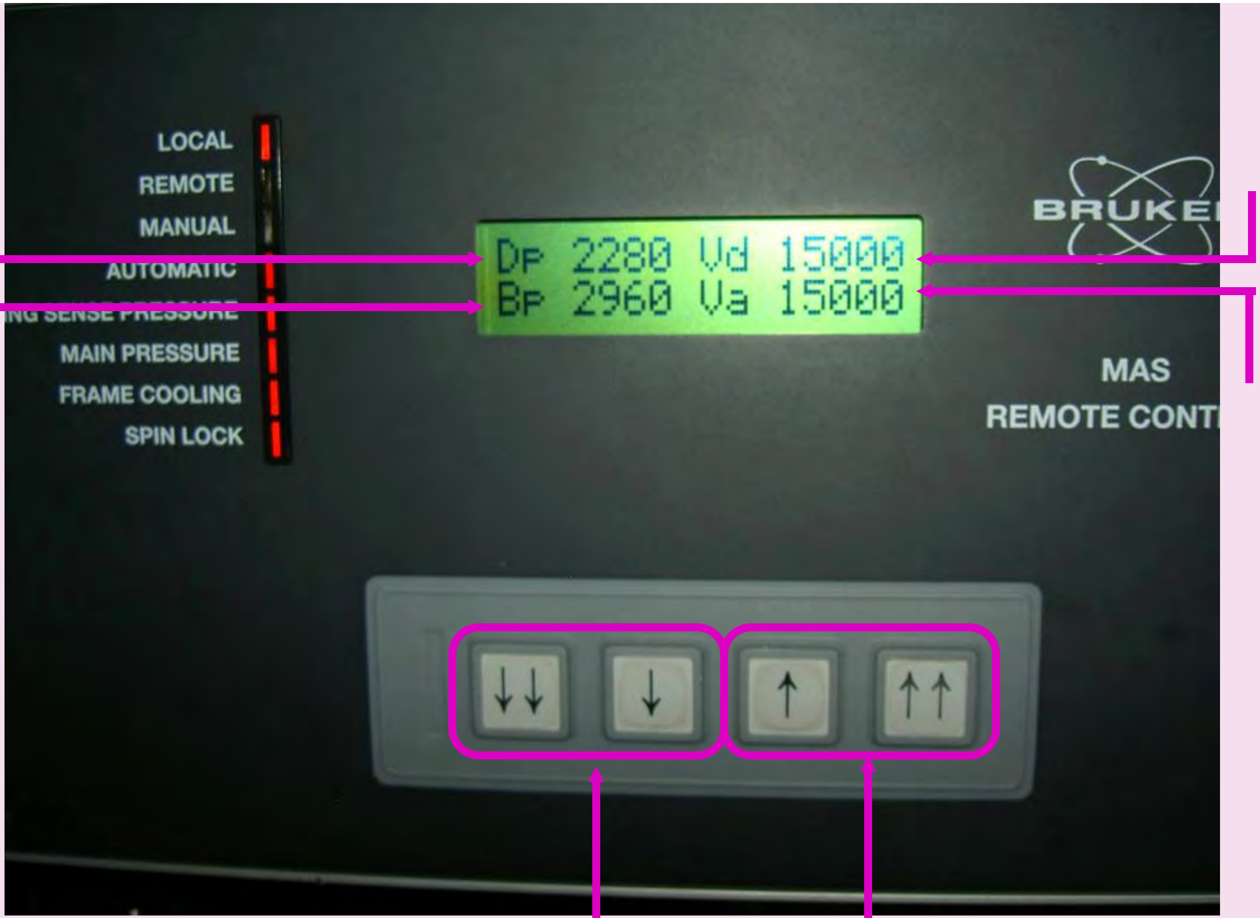
Comments:

When switching between SB and WB cable sets, don't pull air input cable.



Air input tubing **WB air tubing set**

MAS controller: front left



Dp:
drive pressure

Bp:
Bearing pressure

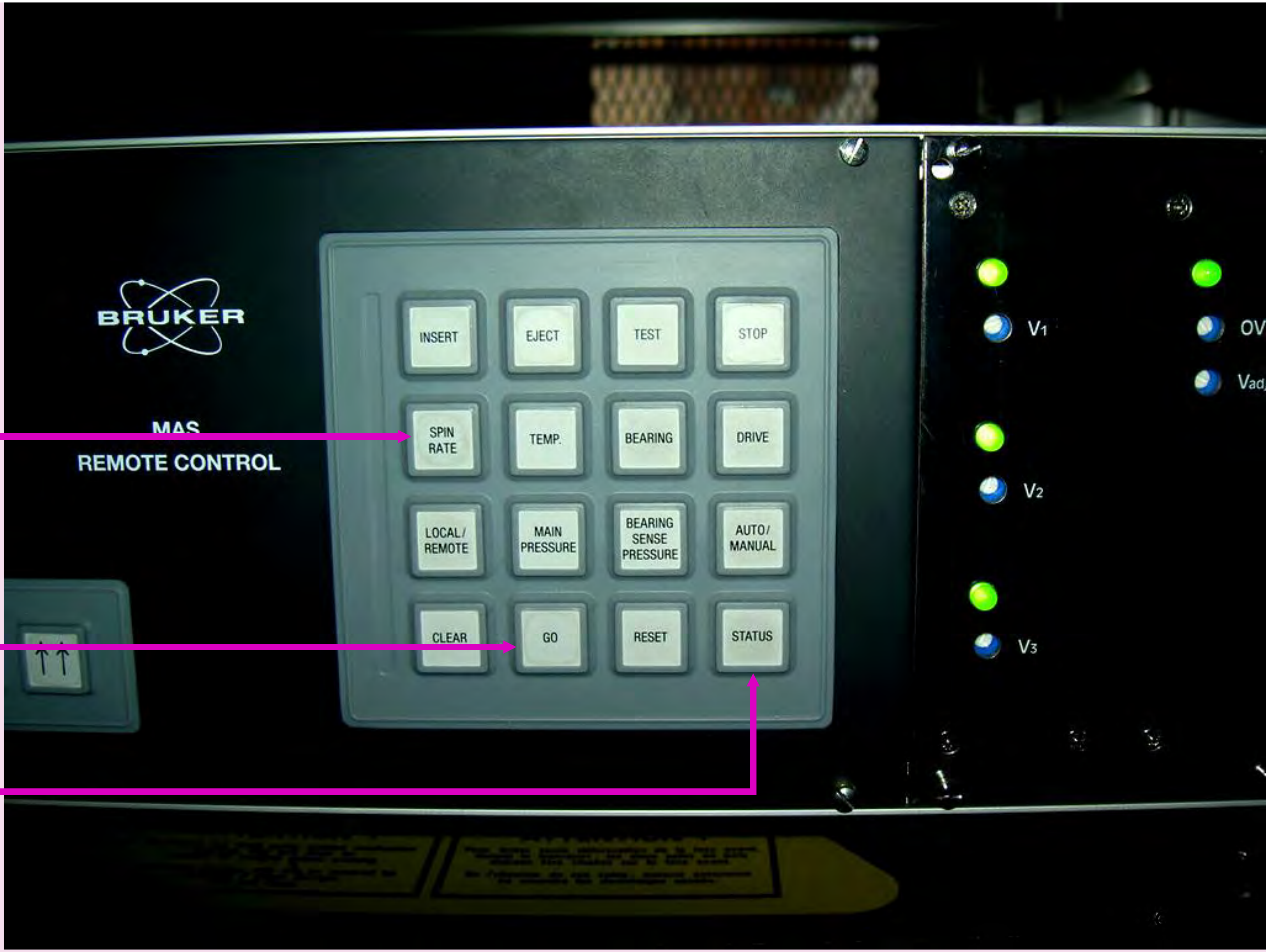
Vd: set spinning frequency

Va: effective spinning frequency

Rough decrement/increment adjust

Fine decrement/increment adjust

MAS controller: front right



Set spinning frequency

Start spinning

Adjust for rotor type and WB/SB

Solid state probes at NYSBC

Probe	Tuning range, MHz	RF fields, kHz	Comments
750MHz 4mm WB HXY	X:150-200 Y: 70-130	H/C/N: 100, 50, 40 DCP: 100/35/25	Good RF reliability. Unstable for VT DCP expts
750MHz 4mm WB HFX	H/F X: 40-220	H/X: 100, 50, 40	19F only 50kHz
750MHz 3.2mm WB HCN Efree and SB HCN	narrowband	H/C/N: 100, 60, 35 DCP: 100/35/25	The best probes to use for triple channel VT experiments
750MHz and 900 MHz 4mm HCND HRMAS	narrowband	H/C/N: 4us/6us/12us	
900MHz 4mm SB HCN Efree	narrowband	H/C/N: 100, 60, 35 DCP: 100/35/25	Good probe for triple channel VT expts
600MHz 3.2mm HCND and HCN DNP	narrowband	H/C/N: 100, 60, 35 DCP: 100/35/25	Testing/installation
900MHz 3.2mm SB HX	X:40-250	H/X: 80/50	Good for quadrupolar expts

750 WB HFX probe (1)

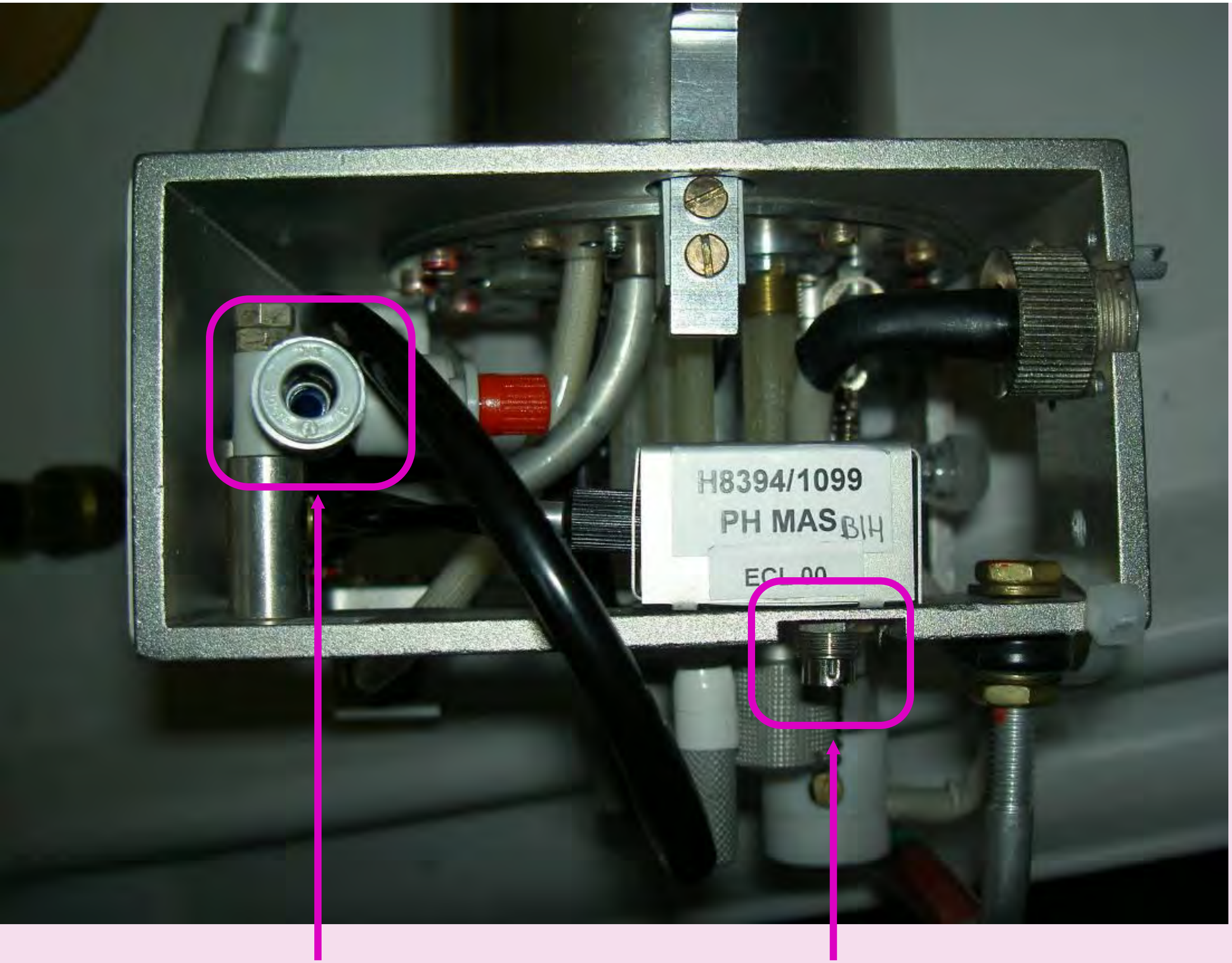
Comments:

Check that connections are tight and high power cables are screwed on all the way

Low Frequency (LF)
40MHz-220MHz channel

High frequency (HF)
H and/or F channel

750 WB HFX probe (2)



Frame cooling
quick connect

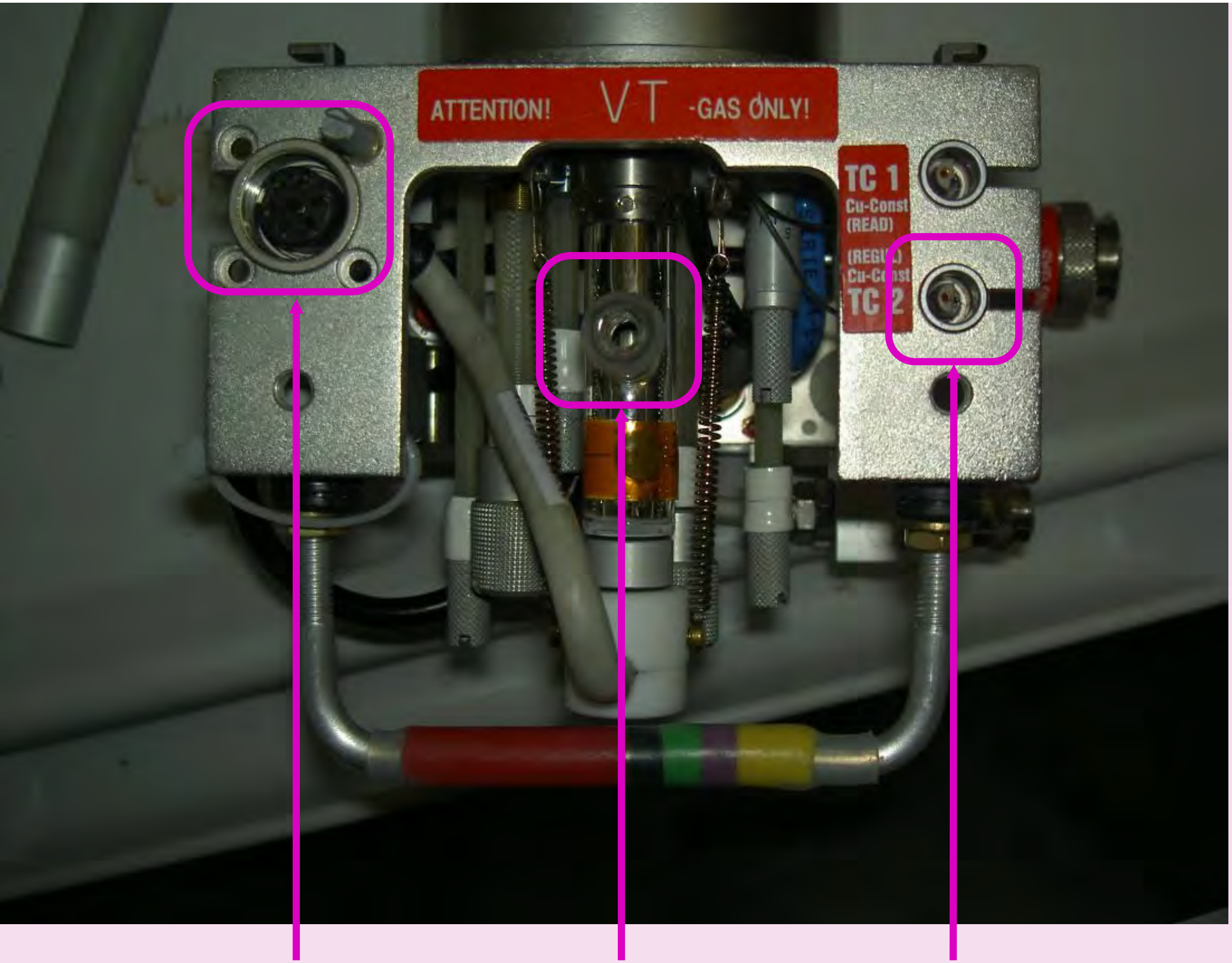
MAS cable
(three pins)

Comments:

Don’t forget
frame cooling

900MHz spectrometer:
make sure that you
Use MAS cable,
not VT heater cable,
they have similar three
pin connectors

750 WB HFX probe (3)



VT heater

VT air line connector

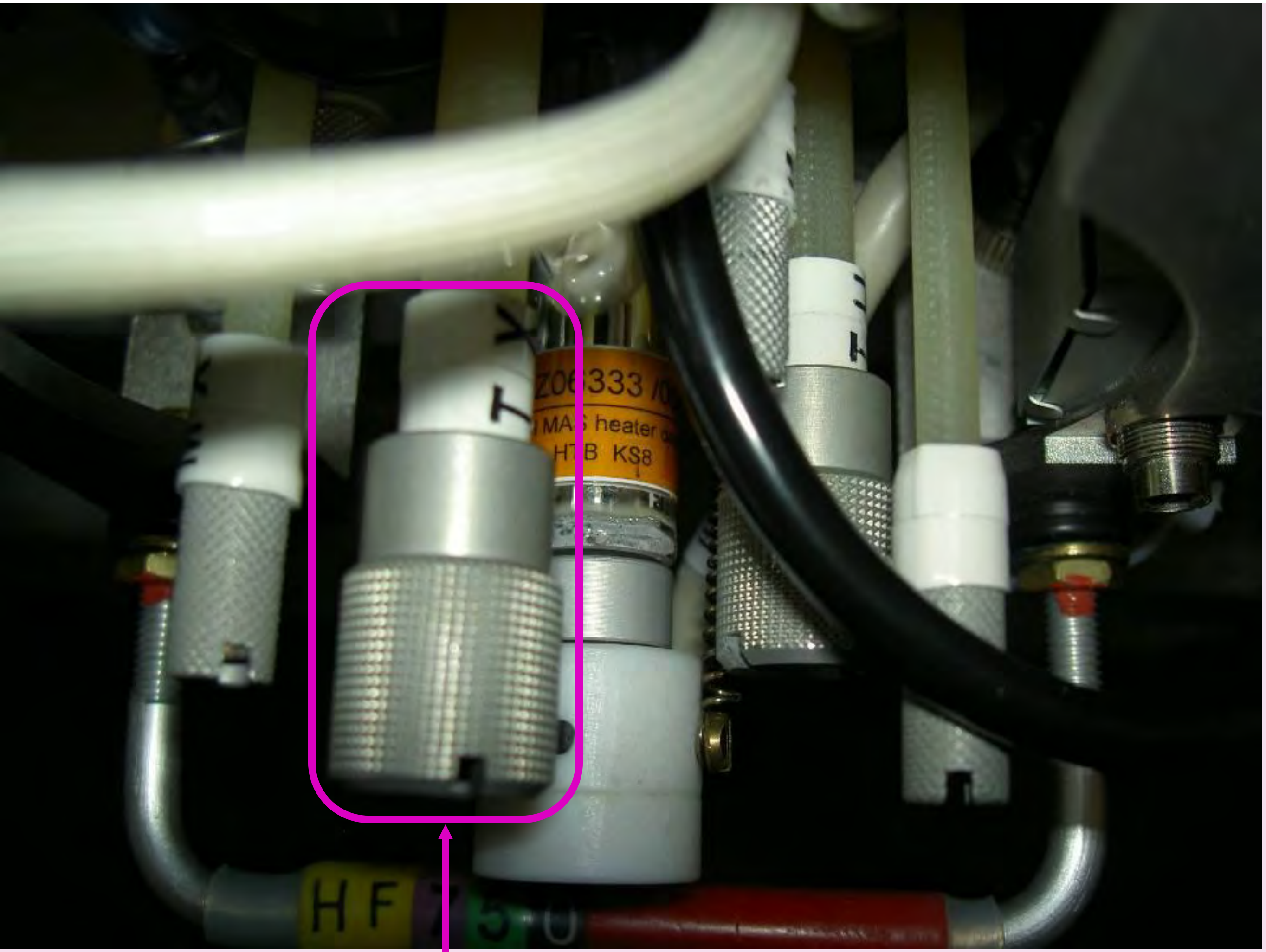
VT sensor

Comments:

Use the lower (TC2) VT sensor

Use **utmost** caution with glass ball joint connector, do not crack the glass dewar

750 WB HFX probe (4)



X channel tuning rod (TX)

Comments:

Before turning a tuning/matching rod make sure it’s the correct one

Do not accidentally turn magic angle adjust (unless you are adjusting magic angle)

Initial setup

Samples and Rotors: packing and spinning

Tuning and Matching using wobb (low power tuning)

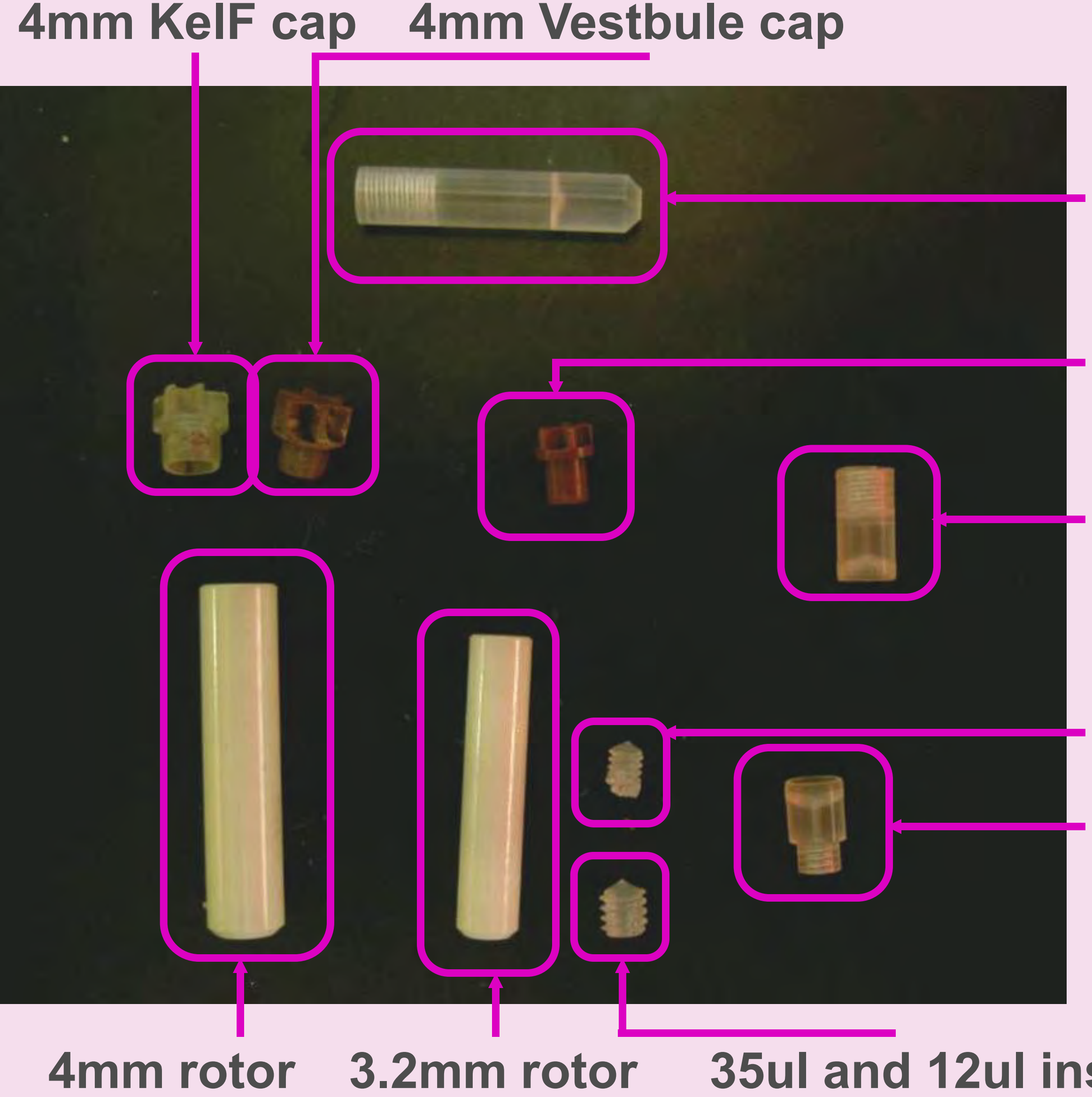
Tuning and Matching using oscilloscope (high power tuning)

Variable temperature and air flow

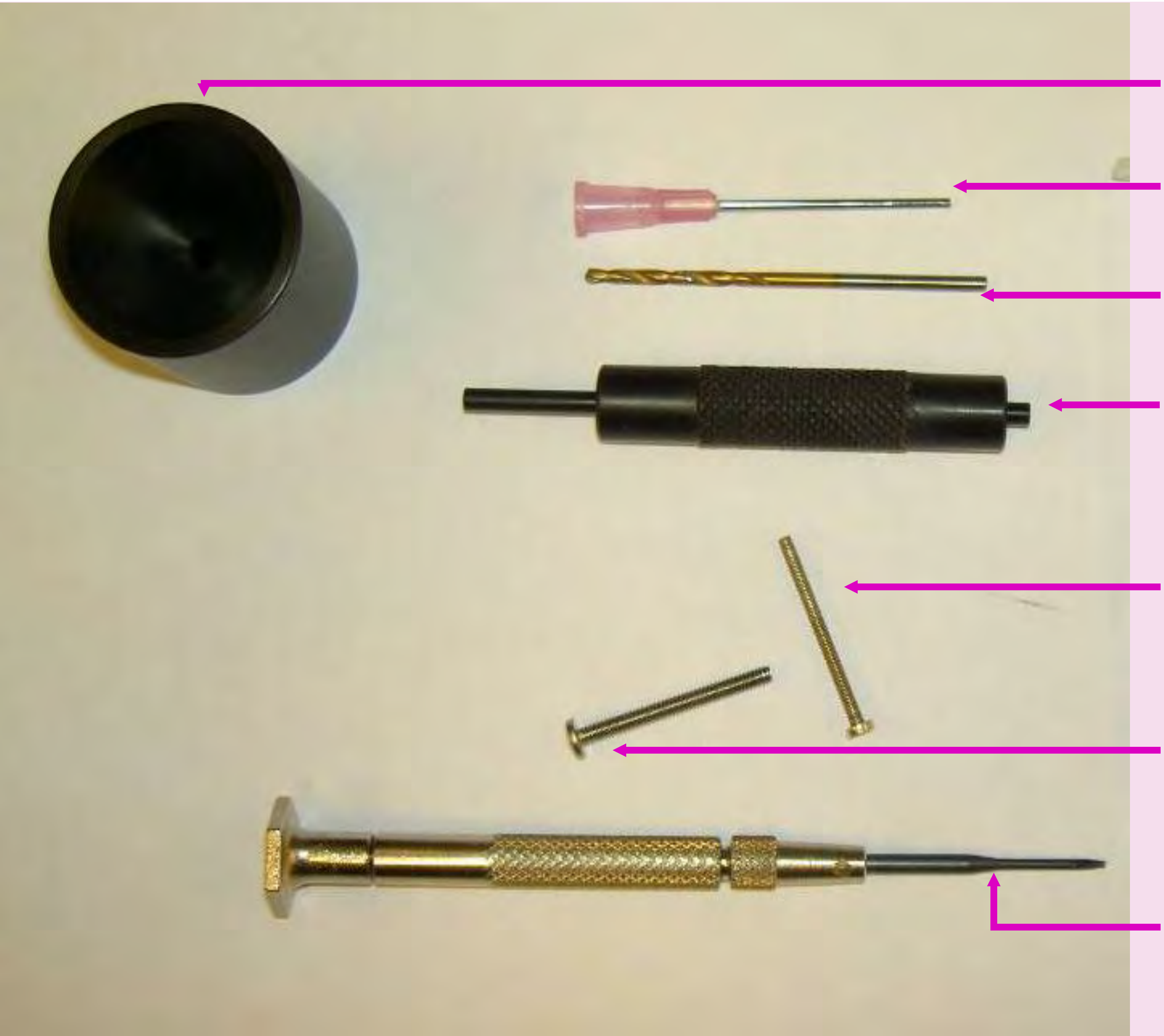
Bruker rotor/insert sets

Rotor type	Insert type	Active volume, µl	Active length, mm	B_1 homogeneity	Shimming
Fully drilled	none	100	10	50%	Solids: 4/12Hz HRMAS: n/a
	tube	35	5.5	70%	Solids: 3/10Hz HRMAS: 1.5/15/25
½ drilled	large	12	1.7	90%	Solids:1.5/4Hz HRMAS: 1.5/15/25
	small	38	???	>70%	Solids: 3/10Hz HRMAS:1.5/15/25
2/3 drilled	small	60	7.5	>70%	The same
Fully drilled or 2/3 drilled	Custom Center	35-40	5	90%	The same
Fully drilled 3.2mm	none	40ul		90% for 18-20ul	Solids: 4/12Hz

Bruker rotor/insert sets: view



Rotor (un)packing tools



4mm rotor holder

Flat bottom needle

Drill bit

Packing tool

12ul and 20ul insert bolt

60ul (small) insert bolt

Cap tool (pitchfork)

Packing rotors

Pack a rotor homogeneously.

Grind the sample uniformly

Center inserts

Don’t leave empty spaces

Make sure that the cap:

Is in all the way inside the rotor

Fits snug inside the rotor

Is in good condition (check the fins with the magnifying glass

Use only

ZrO (the same material as rotor) caps for high/low temperature experiments

Vestbule (brown plastic) caps for low temperature experiment.

tight fitting KelF (transparent plastic) caps for all other experiments

Power and voltage

Power (P) is proportional to voltage (V)
and current (I) in the circuit: $P=VI$

Accordingly to Ohm's law, $I=V/R$
where R is circuit resistance.

Substitution of the second equation into the first gives: $P=V^2/R$.

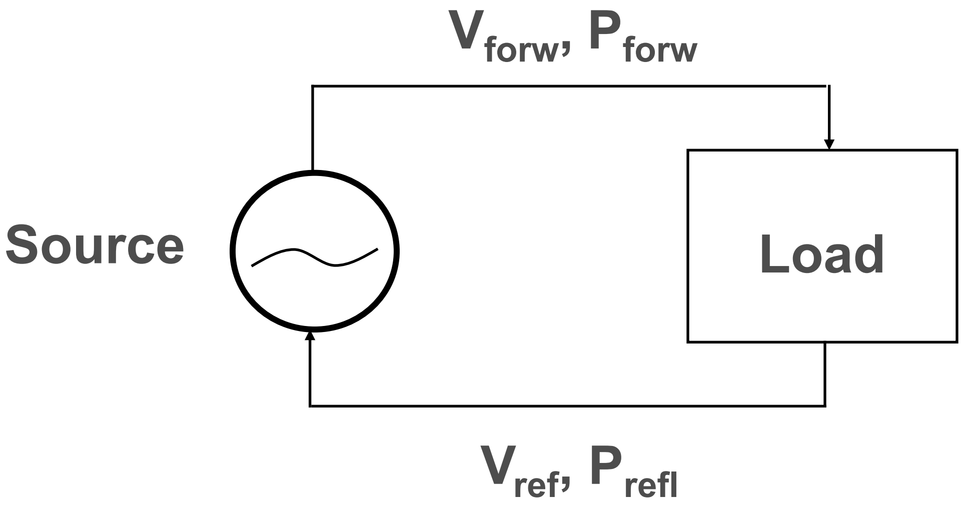
In an AC circuit, average voltage (V_{av})
is related to peak-to-peak voltage (V_{pp}) as: $V_{av}=V_{pp}/2*\mathrm{sqrt}(2)$
and complex impedance (Z) is used
instead of resistance (R).

Therefore, in an AC circuit power is related to voltage
as: $P = (V_{pp}/2*\mathrm{sqrt}(2))^2/Z = V_{pp}^2/8Z$.

Since impedance in all the NMR circuits is 50W, $P = V_{pp}^2/400$.

Tuning and matching(1) source and load

When a source sends power into a load, some power is transmitted (dissipated in the load) and some power is reflected back to the source

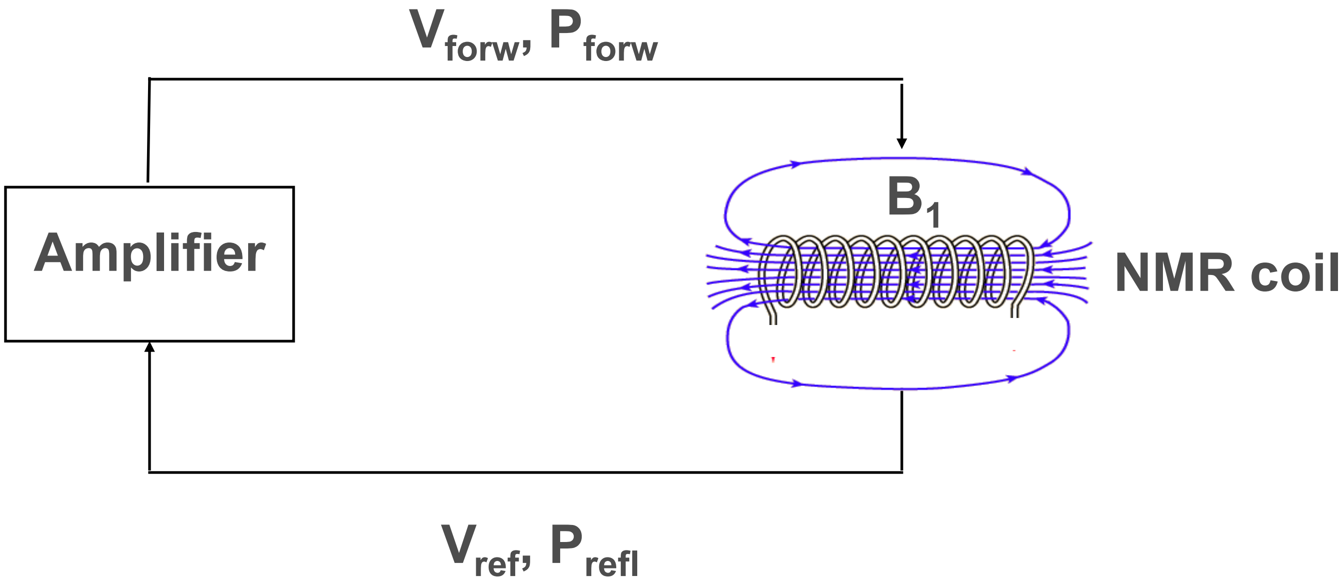


$Z_{source} = Z_{load} \Longrightarrow P_{refl} = 0$

Tuning and matching(2) source and load in NMR

In NMR, amplifier is a power source, probe is a load.
The B_1 field is generated by the power transmitted to the RF coil.
The objective is to minimize the reflected power.

$$P_{refl}\downarrow \;\Rightarrow\; B_1\uparrow$$



Tuning and matching(3) NMR probe

Source impedance is always 50Ω

NMR probe impedance can be tuned and matched to 50Ω
for a given frequency/range of frequencies

We use tuning and matching adjustments:
capacitors, coils, variable length transmission lines

Tuning and matching

If radiofrequency (rf) power is transmitted into a circuit, a part of it is reflected back
and a part of it is dissipated in the circuit.

To maximize the dissipation of the transmitted power in a probe, the probe impedance
at the rf frequency should be equal to the impedance of the outside circuit (50 Ohm).

It is achieved with tuning and matching capacitors.
The former adjusts the frequency of the circuit (tuning) and the latter optimizes the
power dissipation at this frequency (matching).

Rough tuning should be performed at low power (wobbling).

Precise tuning should be performed at high power (using oscilloscope.

Wobbing

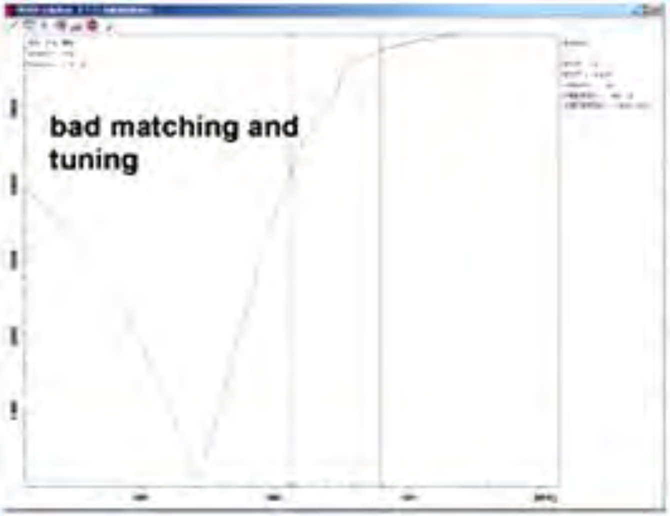
EDASP: make sure that EDASP pathway is correct.
Check the description in the manual.

To wobb X or Y channel, use the corresponding preamp.

To wobb ^{1}H, switch EDASP configuration to 100W -> ^{1}H LNA preamp and connect ^{1}H probe channel to LNA preamp. After wobbing, change EDASP and hardware configuration back to BLAH1000 -> directional coupler -> ^{1}H probe channel.

Wobb

Wobb goes from low to high frequency: 15N -> 13C -> 1H
Commands: *wobb high, wobb f1, wobb f2, wobb ext50*



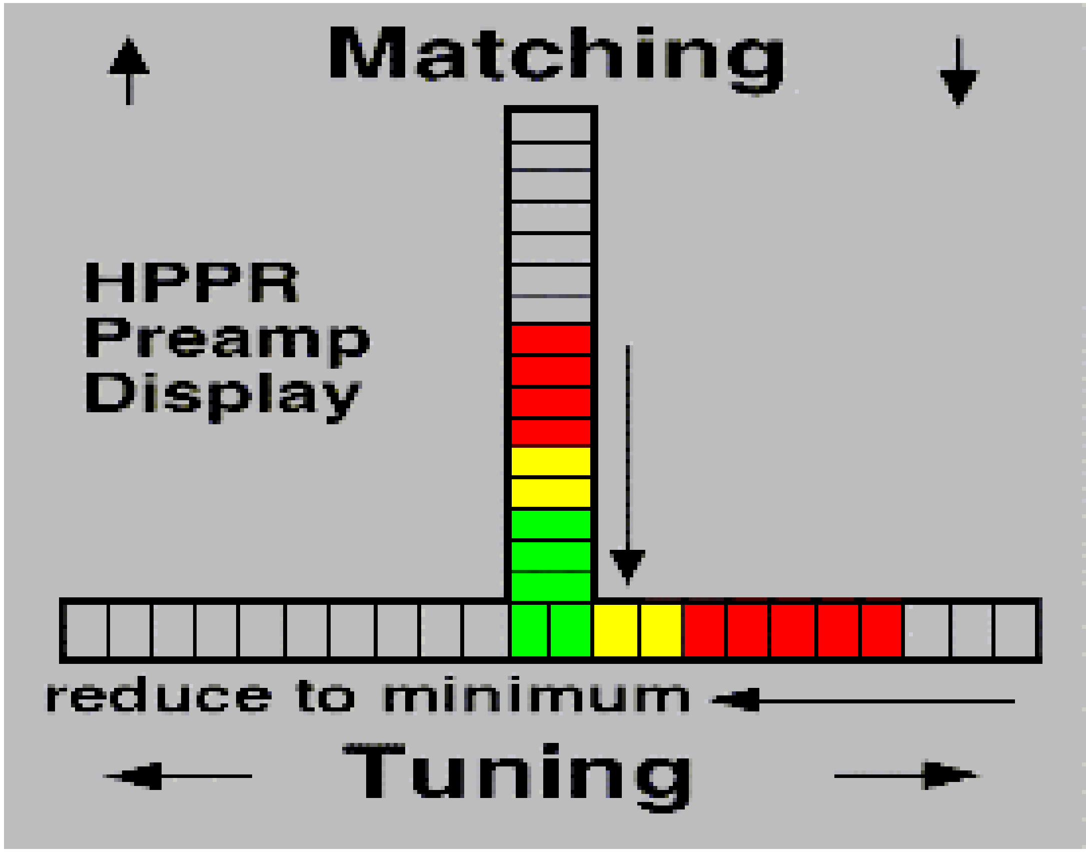
bad matching and tuning

bad matching, good tuning

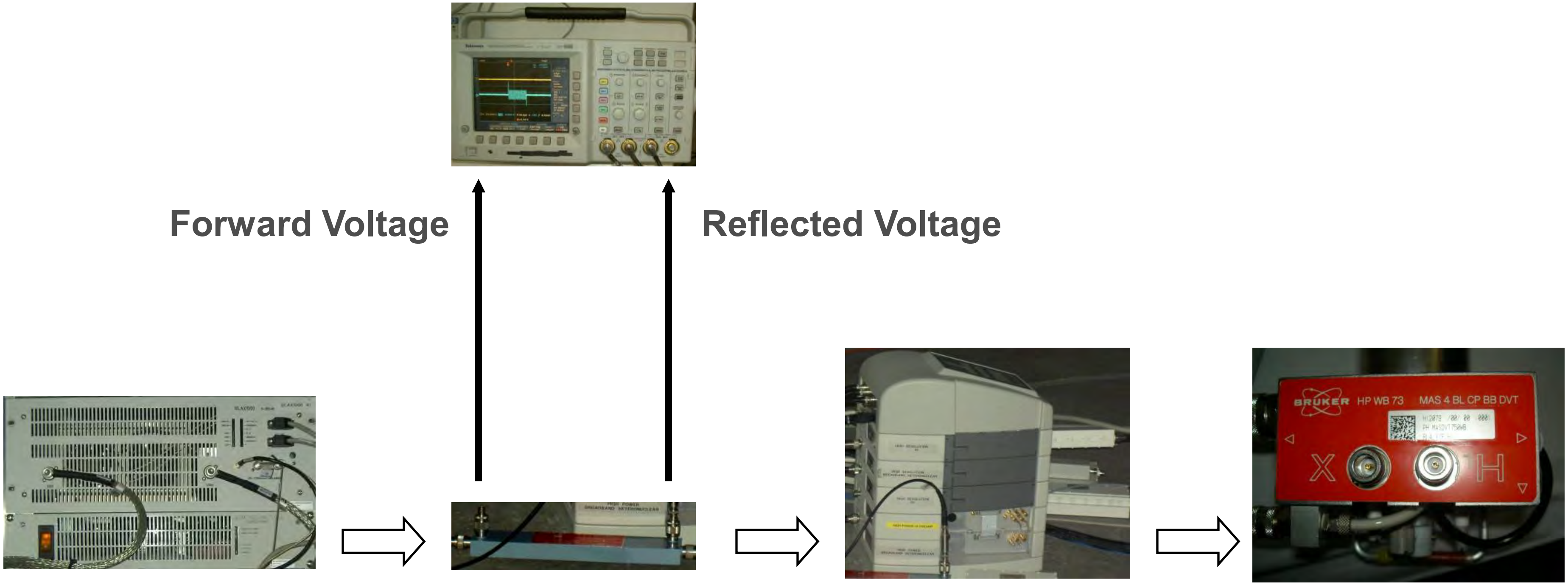
good matching, bad tuning

good matching, good tuning

Wobb with preamp rack



High power tuning: setup



Oscilloscope

Forward Voltage

Reflected Voltage

Transmitter
(amplifier)

Bi-directional coupler

Preamplifier
(HPPR)

Probe

Power goes from transmitter to probe via a bi-directional coupler. The coupler splits off 10% of voltage (1% of power) passing through it. This voltage can be measured via oscilloscope. The ratio of reflected to forward voltage is the most precise indicator of how well a probe is tuned.

High power tuning: why

High power tuning is more precise than wobbling, particularly for higher frequencies.

If the isolation between channels is low (<15dB), the channels become coupled to each other and they have to be tuned simultaneously. It is possible only with the oscilloscope.

You should always monitor reflected voltage on the oscilloscope to make sure that no arcing is taking place.

If something is wrong with your hardware setup, parameter set, or pulse sequence, you can observe it on the oscilloscope.

High power tuning: how

Make sure that 20dB attenuators are plugged into the oscilloscope!
The oscilloscope input should not exceed 9V.

Use tuneHXY data set:
three channels pulsing simultaneously
d1=1s, aq=1ms, all pulses <= 1ms, power levels 6dB

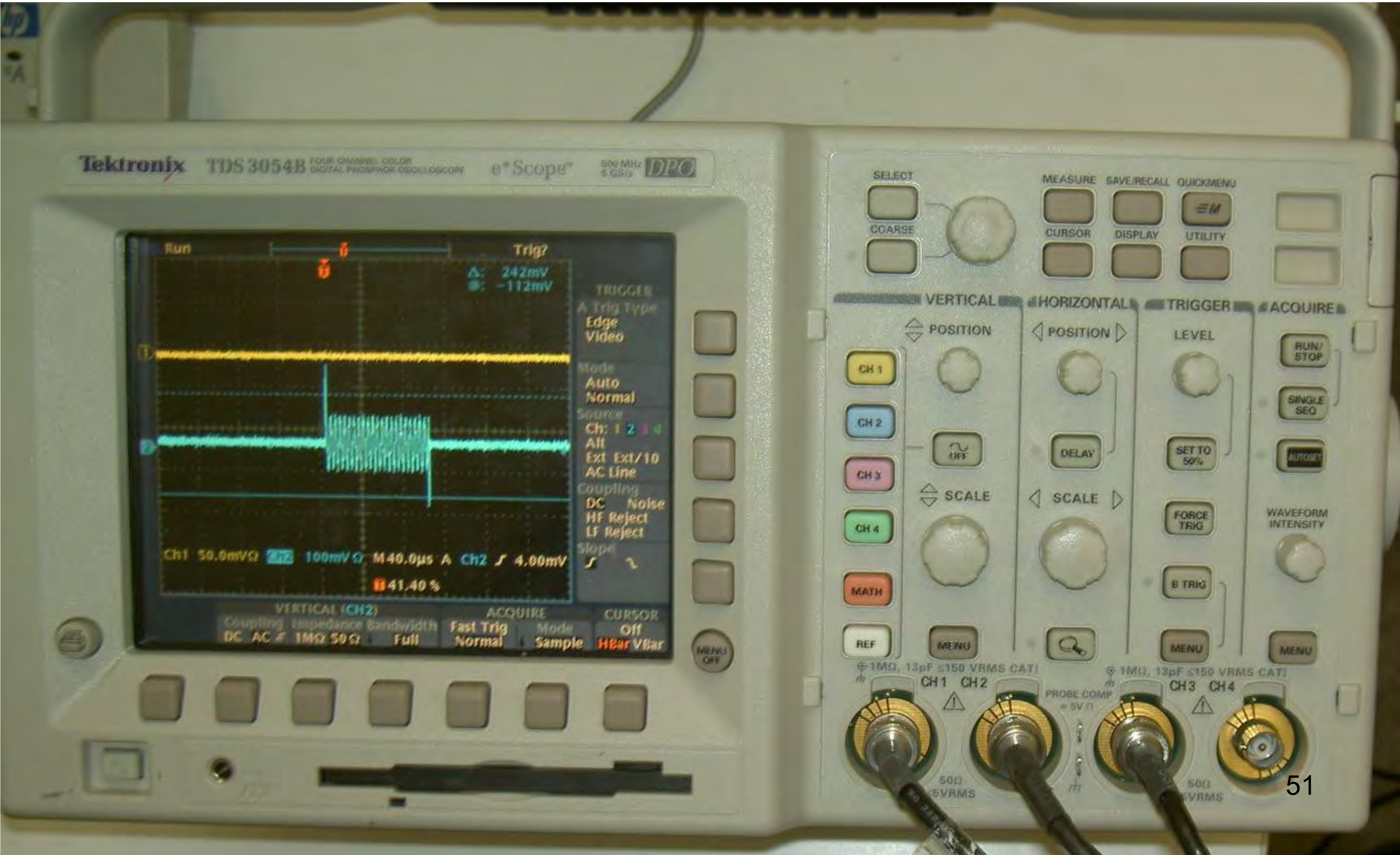
Measure forward peak-to-peak voltage

Measure reflected peak-to-peak voltage.

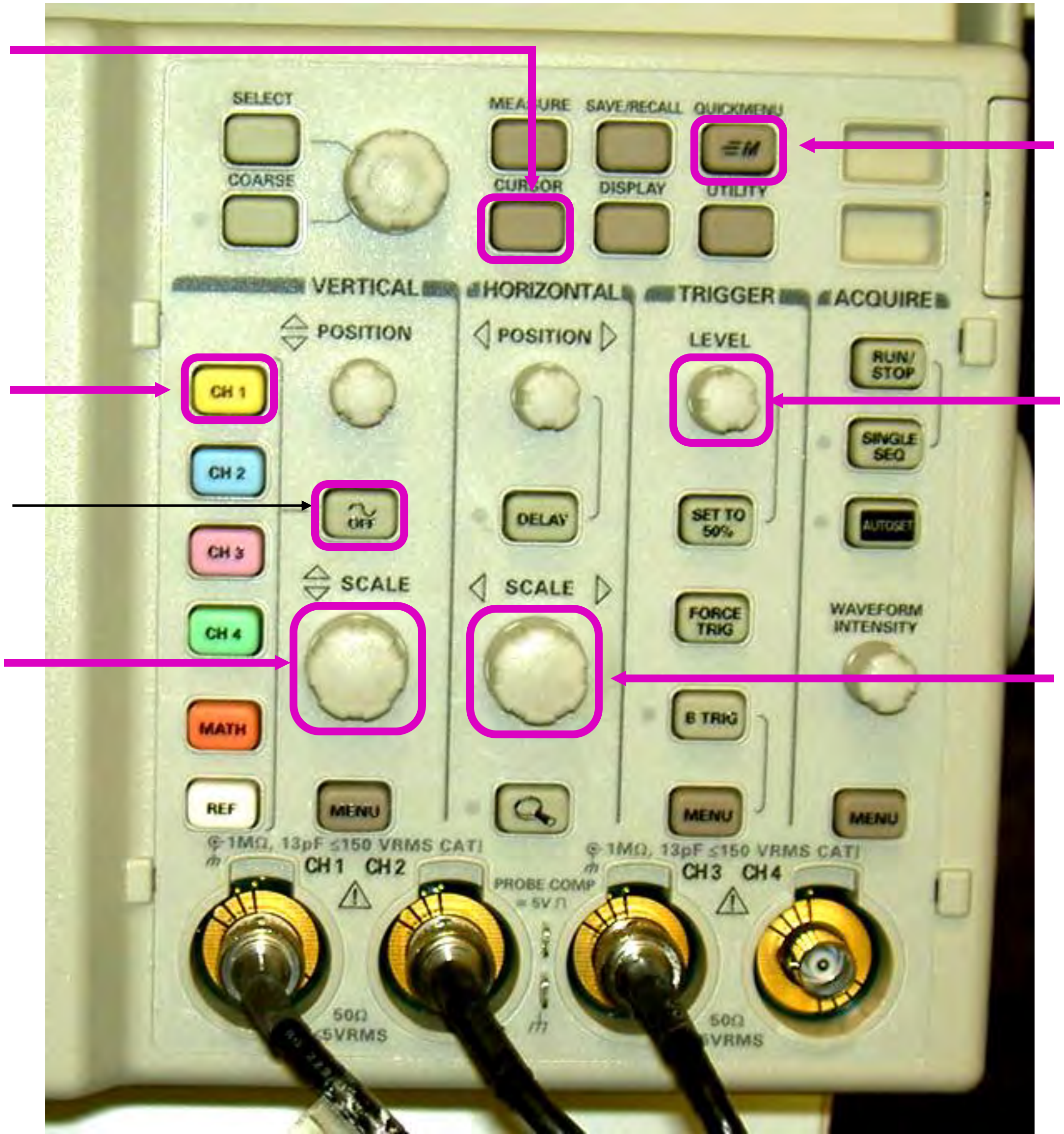
Minimize reflected voltage by adjusting tuning and matching knobs.
Overcouple to achieve better tuning/matching.

Aim for <5% reflected to forward voltage ratio.

Oscilloscope: front view



Oscilloscope: right side panel



Cursor menu

Cursor select

Cursor move

Channel on

Active channel off

Active channel
Amplitude scale

Quickmenu:
basic scope settings
(trigger, impedance)

Trigger amplitude
level

Active channel
Time scale

Oscilloscope: left side

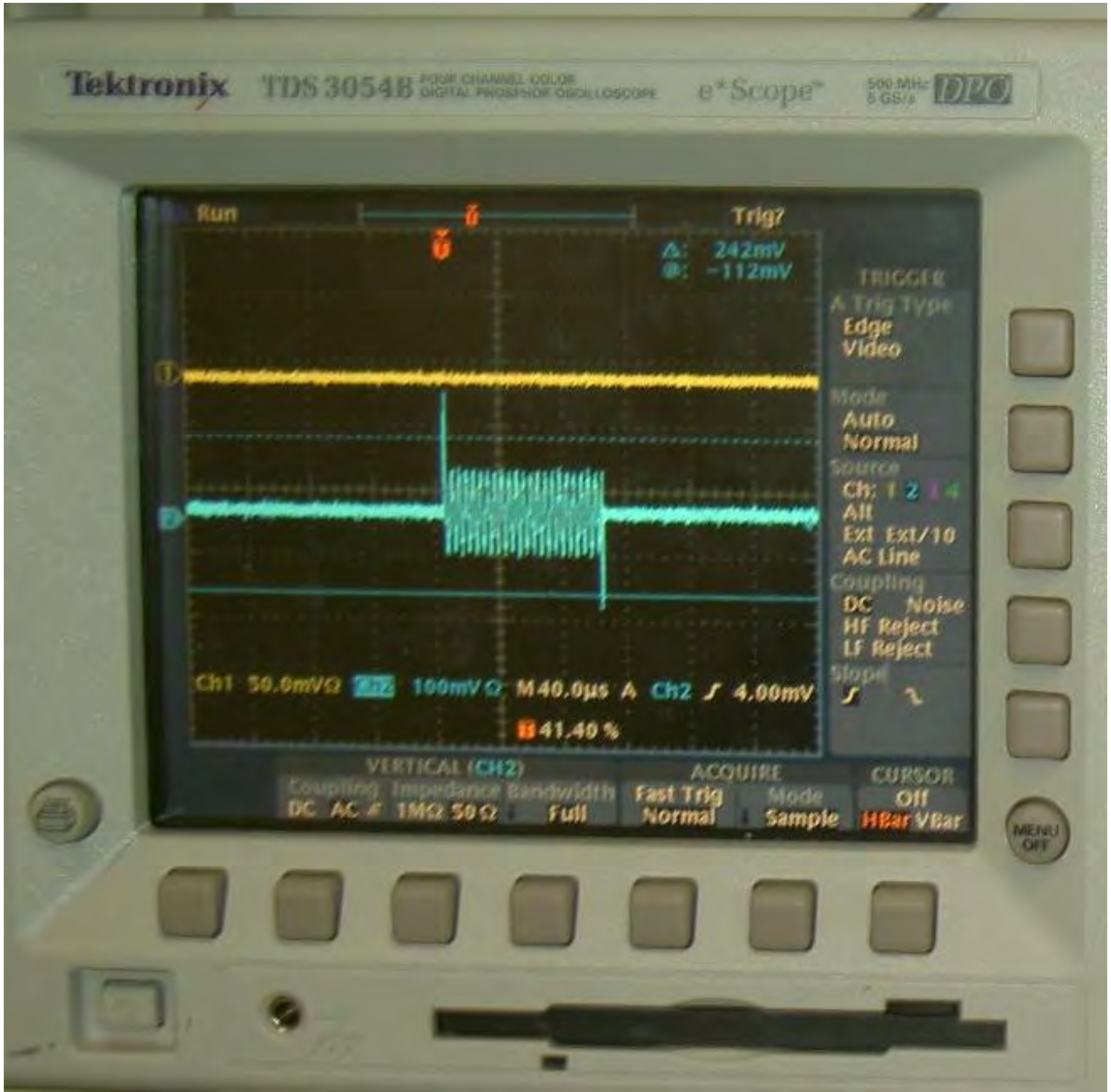
Signal

Channel 1 baseline

Channel 2 baseline

Active channel cursors

Time scale



Peak to Peak voltage between cursors

Q

Tr

Ty

M

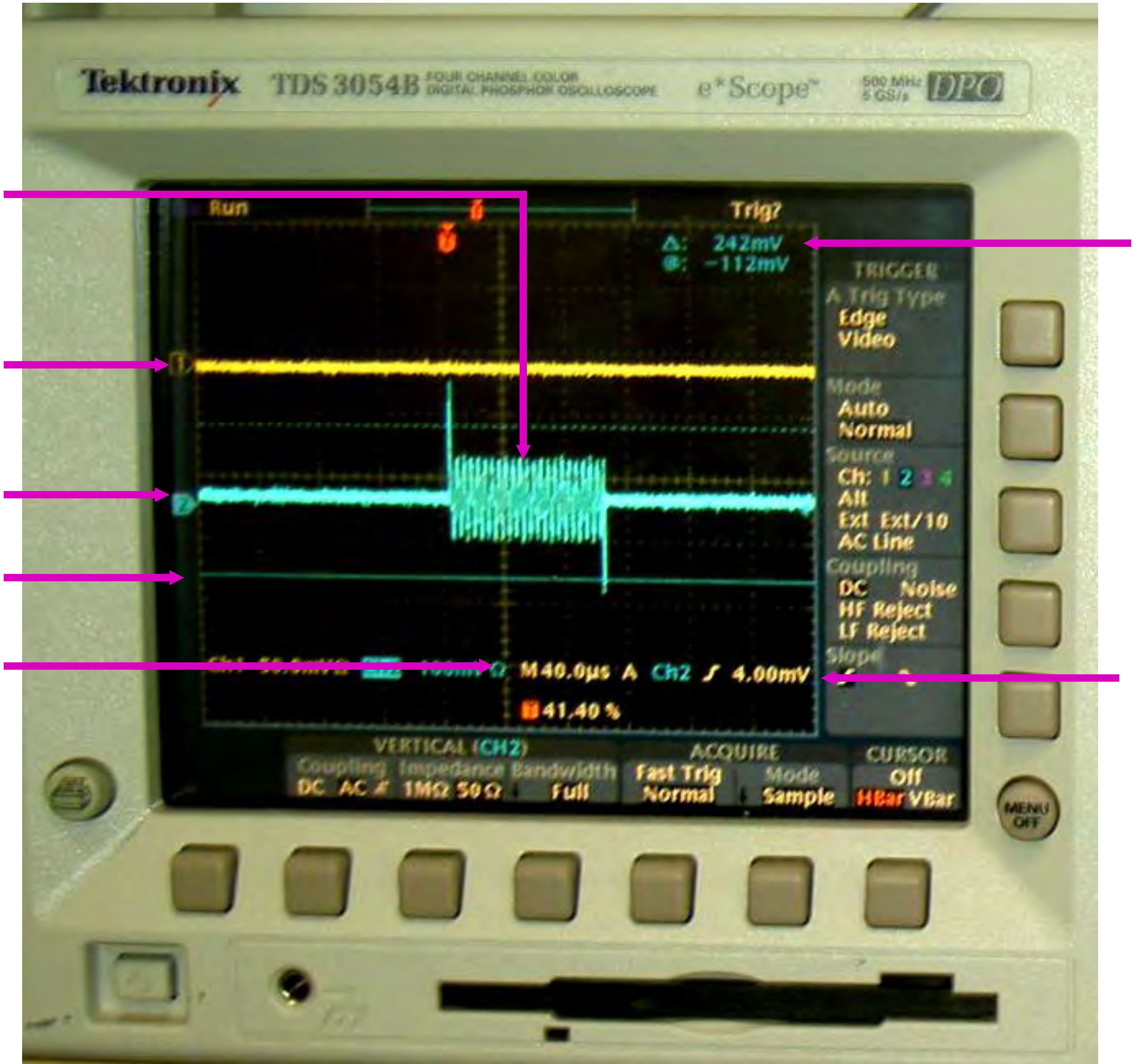
Se (in

D

50

Trigger level

Oscilloscope: left side



Signal

Channel 1 baseline

Channel 2 baseline

Active channel cursors

Time scale

Peak to Peak voltage between cursors

Trigger level

Variable temperature on 750MHz spectrometer

WB and SB CPMAS Bruker probes are capable of cooling/heating a spinning rotor within +100/-100C temperature range.

A rotor is cooled/heated by a separate gas flow running through the center of the stator.

The gas flow is regulated by BVT3000 unit located in the spectrometer console.

A temperature sensor reads gas temperature inside the stator.

Use EDTE command to set gas flow and temperature from the console.

VT gas is delivered to the stator via an evacuated glass dewar.

Heating coil is located at the bottom of the dewar.

AirJet Cooler is used to cool the input gas (2cfm @ -85C input temperature).

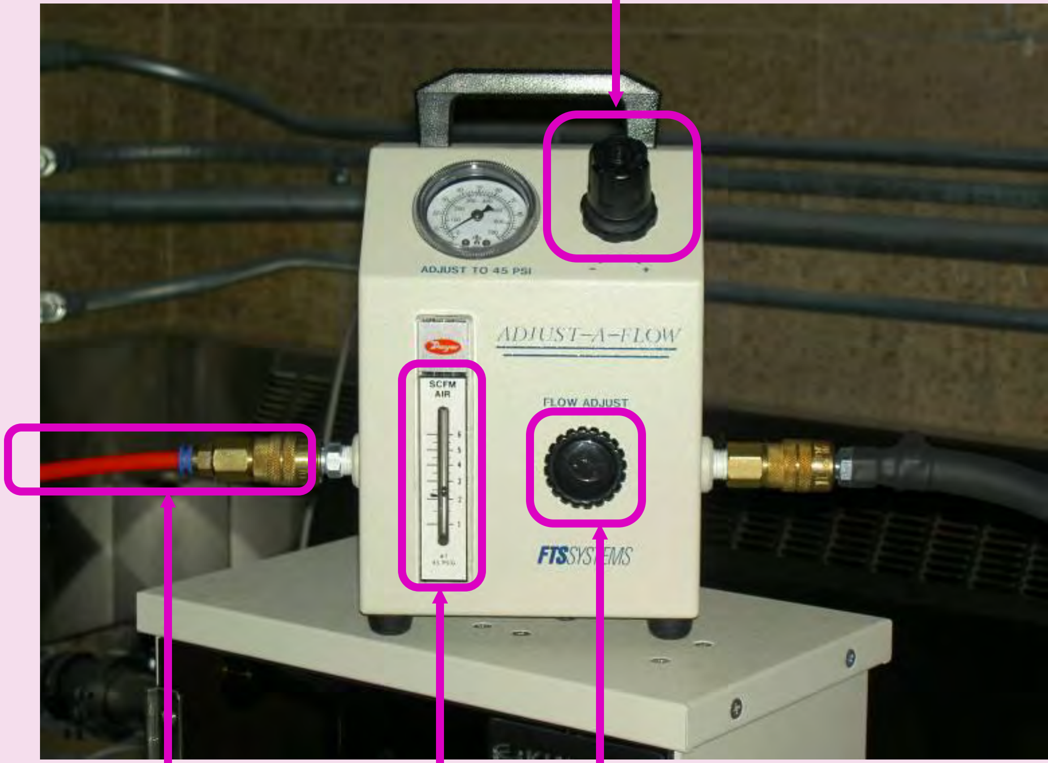
Effective temperature inside a rotor depends on spinning frequency and gas flow.

NMR thermometer (lead nitrate or methanol) can be used to calibrate sensor and sample temperature.

Cooling setup at 750MHz spectrometer

Heater/controller

Cooler



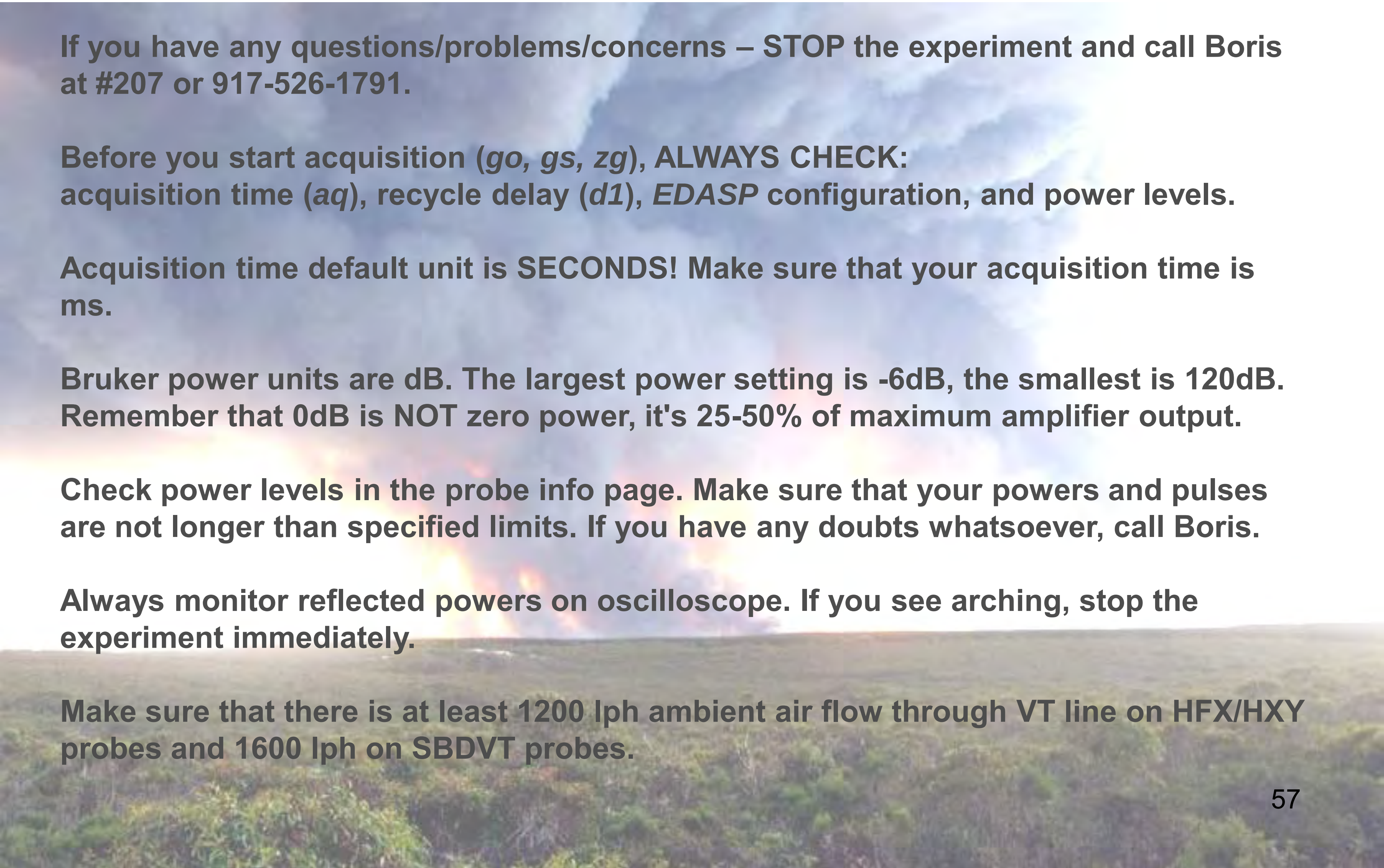
Pressure regulator:
(always open)

Air input
from console

Manual
air flow
meter

Flow
regulator

STOP and READ this



If you have any questions/problems/concerns – STOP the experiment and call Boris at #207 or 917-526-1791.

Before you start acquisition (*go, gs, zg*), ALWAYS CHECK:
acquisition time (*aq*), recycle delay (*d1*), *EDASP* configuration, and power levels.

Acquisition time default unit is SECONDS! Make sure that your acquisition time is ms.

Bruker power units are dB. The largest power setting is -6dB, the smallest is 120dB. Remember that 0dB is NOT zero power, it's 25-50% of maximum amplifier output.

Check power levels in the probe info page. Make sure that your powers and pulses are not longer than specified limits. If you have any doubts whatsoever, call Boris.

Always monitor reflected powers on oscilloscope. If you see arching, stop the experiment immediately.

Make sure that there is at least 1200 lph ambient air flow through VT line on HFX/HXY probes and 1600 lph on SBDVT probes.

STOP and READ some more

Always use the following configuration for CPMAS experiments:

SGU1 – BLAX1000 #1 – bidirectional coupler - high resolution broadband preamp -13C bandpass filter – probe for 13C
SGU1 – BLAX1000 #1 – bidirectional coupler - high power broadband preamp – filter – probe for any other nucleus
except 1H and 15N
SGU2 – BLAH1000 – bidirectional coupler – 1H bandpass filter – probe for 1H
(bypass the preamp if you are not observing 1H)
SGU3 – BLAX1000 #2 – bidirectional coupler - high power broadband preamp – 15N bandpass filter – probe for 15N

Always use the following configuration for HRMAS experiments:
SGU1 – BLAX300 #1 - high resolution broadband preamp - probe for 13C
SGU2 – BLAH150 -1H high resolution preamp – probe for 1H
SGU3 – BLAX1000 #2 - high power broadband preamp - probe for 15N

13C, 1H and 15N are cortab'd – the corresponding amplifier outputs are linear for this nuclei. However, the other nuclei are not cortab'd. The power output at the other nuclei frequencies will be slightly higher and non-linear (within 20%).

Use 1H high resolution preamp to wobb proton.

If you are running a VT experiment, make sure that shim stack/sample transfer line are purged properly (see VT section).

Magic angle

Spinning a sample at the magic angle Q = 54.7 ($1-3\cos^2 Q = 0$) attenuates anisotropic interactions (CSA, dipolar, partially quadrupolar)

It is the only way to achieve high resolution spectra in solid samples

Setting up magic angle precisely and easily is very important

^{79}Br in KBr is convenient system to set a magic angle on

KBr is cheap and stable

^{79}Br has high natural abundance, and its gyromagnetic ratio is very close to that of 13C

It is possible to set magic angle using KBr very quickly with 0.5^0 precision

For higher precision other systems should be used

Setting up magic angle on KBr

Load a KBr sample

Set up a zg experiment with 79Br nucleus on channel 1
(or, better, pick it up from “bitin” directory)

Wobble:
if the tuning dip is anywhere close to the cursor, don’t bother wobbling

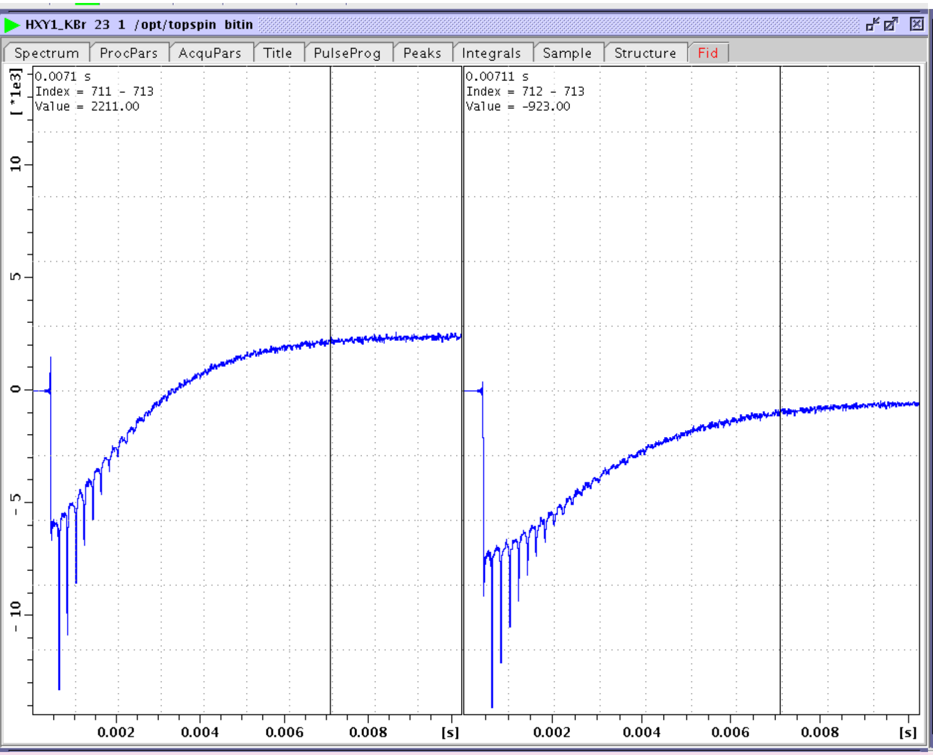
Run a 1 scan zg experiment, ft and apk the spectrum,
set the O1 carrier exactly on resonance

Measure the first spinning side band/center band ratio

If it’s less than 1:10 adjust magic angle
start acquiring in gs mode.

You should be able to see a train of rotationary echoes (next slide) on FID.
Very (!) slowly adjust magic angle to maximize rotational echoes.

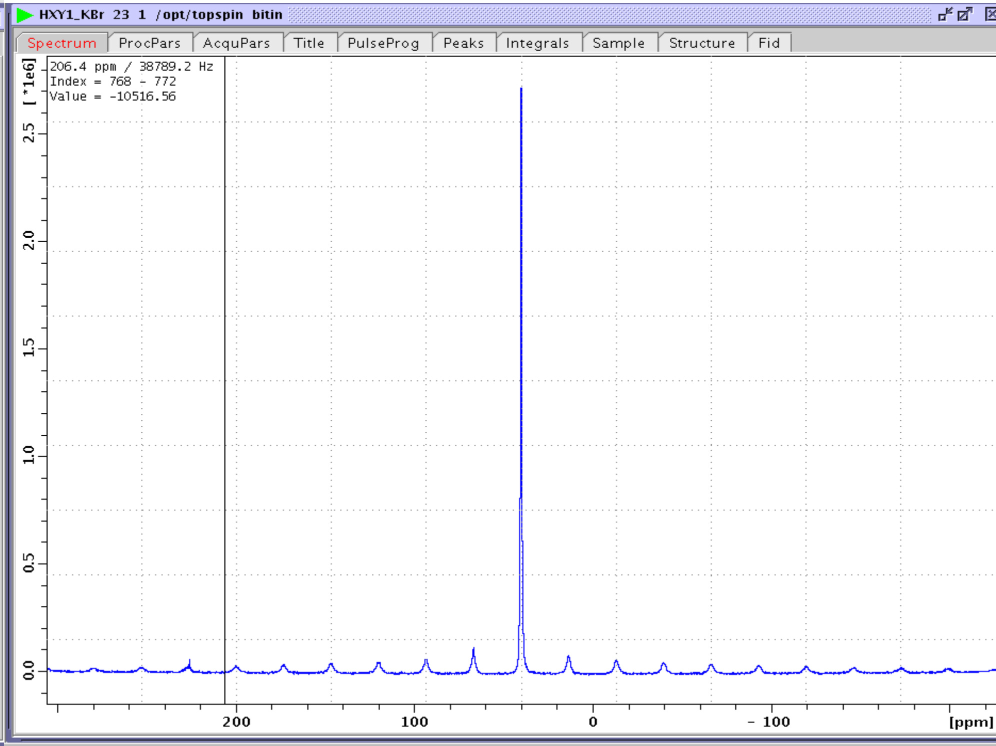
Setting up magic angle on KBr: off magic angle



HXY1_KBr 23 1 /opt/topspin bitin

0.0071 s
Index = 711 - 713
Value = 2211.00

0.00711 s
Index = 712 - 713
Value = -923.00



HXY1_KBr 23 1 /opt/topspin bitin

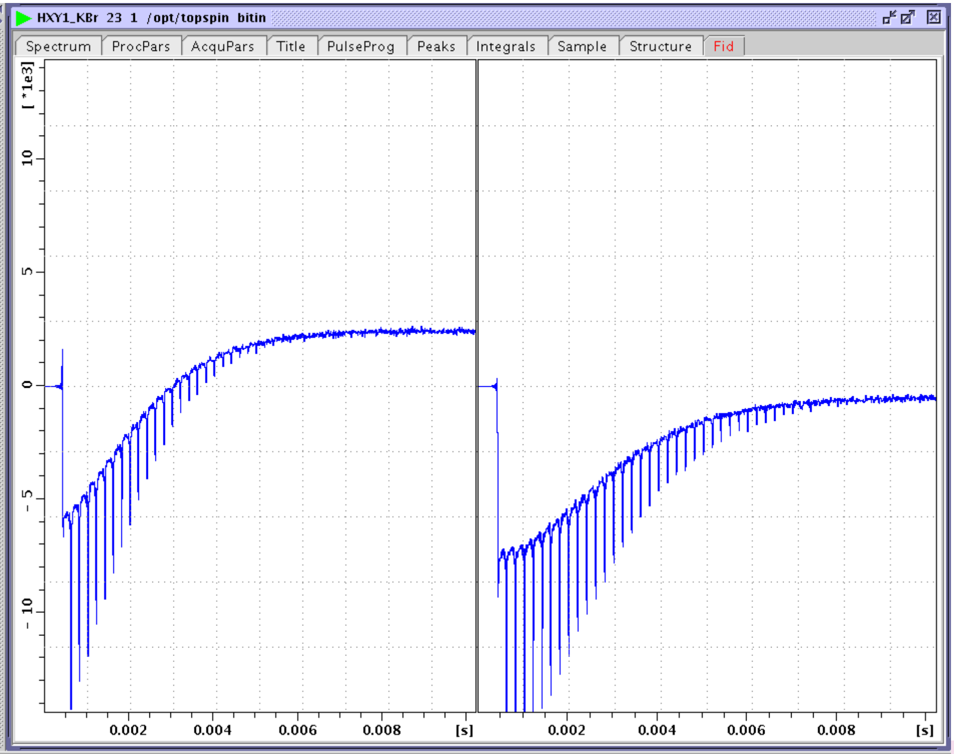
206.4 ppm / 38789.2 Hz
Index = 768 - 772
Value = -10516.56

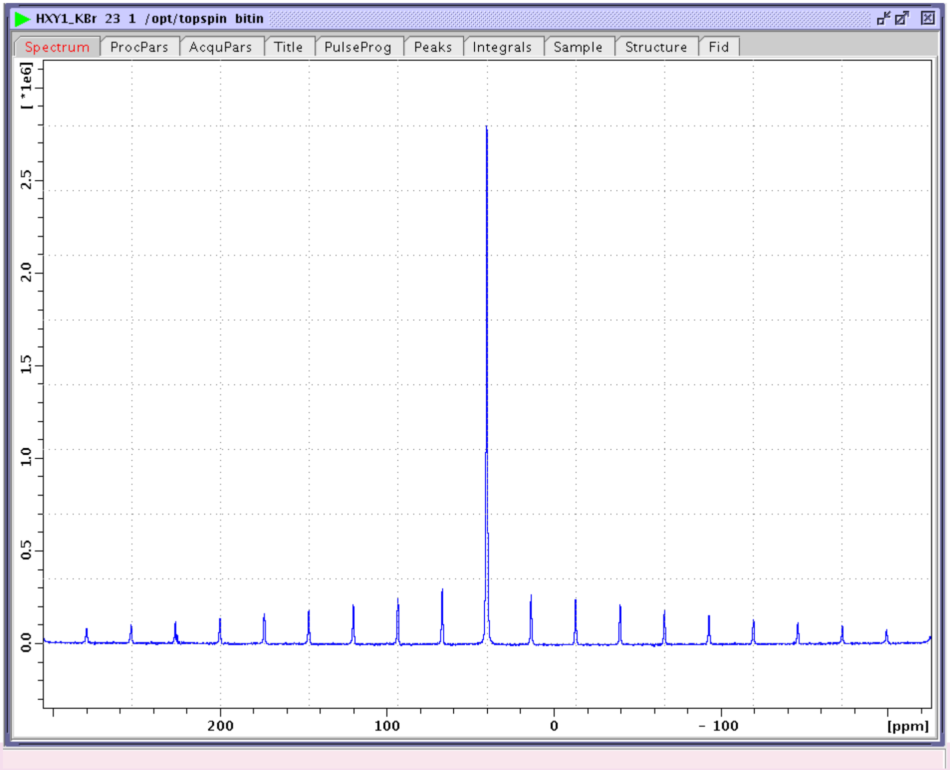
The carrier frequency should be set exactly on resonance to obtain FID as shown above.

Clearly the first spinning side band/center band ratio is worse than 1:10 and the rotationary echo pulse train is too short (it should last 3-4ms)

Slowly adjust magic angle rod to achieve this (next slide)

Setting up magic angle on KBr: on magic angle





Slowly adjust magic angle rod to achieve the FID shown above. Now, the rotationary echo pulse train extends for 3-4ms and the first sideband/centerband ratio is 15%

Power handling of solid state probes

All solid state probes arc sooner or later..
No NMR spectroscopists are satisfied with probes' power handling capabilities..

Bruker WB HXY and HFX probes are spec'd for:
1H/13C/15N: 120kHz@50ms, 50kHz@10ms, 50kHz@10ms in single channel mode.

We test the probes with a double cross polarization experiment:
1H/15N 60kHz/50kHz@3ms followed by 1H/13C/15N 100kHz/25kHz/35kHz@8ms followed by 1H 120kHz@1ms with 3s recycle delay
Do not exceed 80% of this total power input.

Always track reflected power on the oscilloscope.

Always test an adjusted/new pulse program on oscilloscope at 20dB lower power levels.

Make sure that your power levels are correct (remember 0dB is not 0% power!!!)

For longer decoupling times, decrease decoupling field proportionally

Power and voltage

Power (P) is proportional to voltage (V) and current (I) in the circuit:

$$P = VI$$

Accordingly to Ohm's law, where R is circuit resistance.

$$I = \frac{V}{R}$$

Substitution of the second equation into the first gives:

$$P = \frac{V^2}{R}$$

In an AC circuit, average voltage (V_{av}) is related to peak-to-peak voltage (V_{pp}) as:

$$V_{av} = \frac{V_{pp}}{2\sqrt{2}}$$

and complex impedance (Z) is used instead of resistance (R).
Therefore, in an AC circuit power is related to voltage as

$$P = \left(\frac{V_{pp}}{2\sqrt{2}}\right)^2 \frac{1}{Z}$$

Since impedance in all the NMR circuits is 50W,

$$P = \frac{V_{pp}{}^2}{400}$$

Power, W	0.1	1	10	50	100	500	1000
Voltage, V_{pp}	6	20	60	140	200	450	630

Pulses, powers and dB units

Traditionally, attenuation of power and voltage in electrical circuits is measured in dB units.

We say that power is attenuated by N dB if it is decreased by a factor of $10^{N/10}$.
We say that voltage is attenuated by N dB if it is decreased by a factor of $10^{N/20}$.

The table below relates voltage and power attenuation for several dB values.

dB	*1*	*3*	*6*	*10*	*20*
Power attenuation by	0.26	2	4	10	100
Voltage attenuation by	0.12	0.4	2	3.16	10

Evidently, it is easiest to measure power in 3 or 10dB steps and voltage in 6 or 20dB steps.

Pulses, powers and fields in TOPSPIN

RF field is proportional to voltage and to square root of power.

$$B_1 \propto V, \quad B_1 \propto \sqrt{P}$$

Bruker software uses dB as a power unit.

-6dB - maximum amplifier output,

$$P_{\max}, \quad V_{\max}, \quad B_{1\max}$$

0dB – typically used amplifier output

$$\frac{P_{\max}}{4}, \quad \frac{V_{\max}}{2}, \quad \frac{B_{1\max}}{2}$$

120dB – zero power/voltage/rf field

Use command *pulse* to calculate powers/pulses/fields in Topspin:

Measure pulse/field at a certain power level
Switch to a zg data set (i.e. tuneh) and enter experimentally measured pulse/field values.
Use pulse command to calculate pulse length/field at a needed power level or vice versa

Composite pulse decoupling

There is never enough decoupling power in the solid state NMR of 1H abundant samples.

Continuous wave (CW) decoupling is both less efficient and less broadband than composite pulse decoupling (CPD)

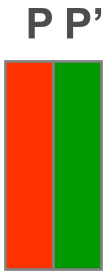
There is a number of CPD schemes in solid state NMR.
TPPM, XiX, and SPINAL are, probably, the most popular decoupling schemes.

The table below lists their advantages and drawbacks.

CPD	Advantages	Drawbacks
TPPM	The most efficient CPD for <100kHz 1H field	Pulse length and, sometimes, flip angle have to be optimized
SPINAL	The most efficient CPD for >100kHz 1H field	Less efficient than TPPM for low/medium 1H fields
XiX	Fairly efficient for all decoupling fields. Doesn't have to be optimized	Less efficient than TPPM and SPINAL

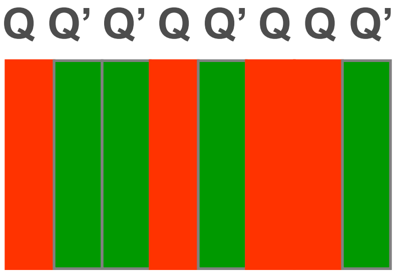
Composite pulse decoupling (2)

TPPM



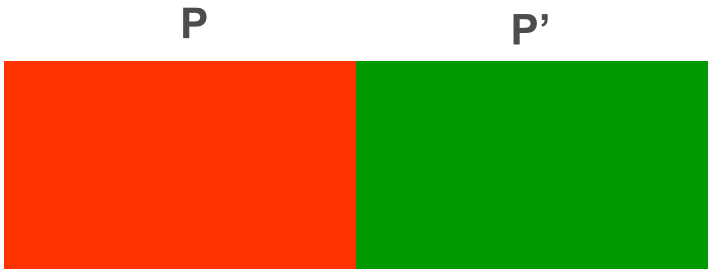
P = 165 (θ), P’ = 165 ($-\theta$), $\theta = 10^0$-50^0

SPINAL 64



Q = 165(-10) 165(10) 165(15) 165(-15)X
X165(20) 165(-20) 165(15) 165(-15)

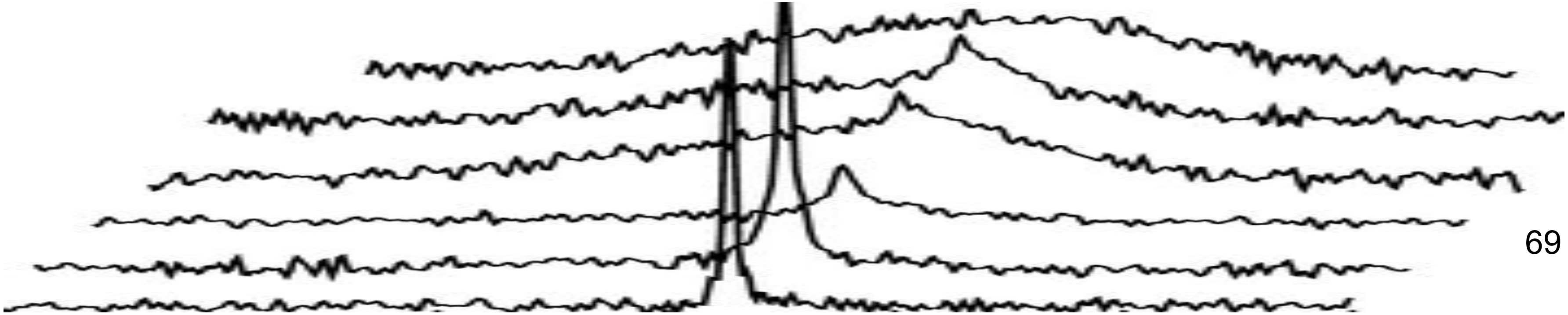
XiX



P = 2.85 *τ_r, τ_r – rotor period.

Lineshape broadening in SSNMR

Sample type	Interaction	What to do about it
Crystalline and ^{1}H abundant	^{1}H-^{1}H homogeneous component in ^{1}H-X dipolar coupling	Decouple as hard as you can
Inhomogeneous and/or amorphous	Inhomogeneous line broadening	Nothing. Recrystallize it. Study another system.
Not ^{1}H abundant	Not ^{1}H - X dipolar coupling	Use medium strength ^{1}H decoupling field.
Paramagnetic/high unpaired electron density/etc	Very short T_2	Not much you can do about it.



Why CPMAS

CPMAS is the most often used 1D experiment in the world of biological NMR.
Majority of 13C and 15N observe experiments start with a CP.

In a ^{1}H abundant spin system, ^{1}H – X CP increases sensitivity due to
higher ^{1}H gyromagnetic ratio γ
shorter ^{1}H T_1 relaxation time.

CP is very easy to set up on a standard sample or on a system of interest.

Be aware

The spin system should be ^{1}H abundant and the 1H – X distance should be fairly short

High molecular mobility may attenuate dipolar coupling to the point when polarization transfer rate becomes too slow.

Relaxation rate in spin locking frame T_{1r} is generally fairly slow. However, presence of unpaired electrons or other relaxation pathways may substantially compromise CP efficiency.

Cross polarization

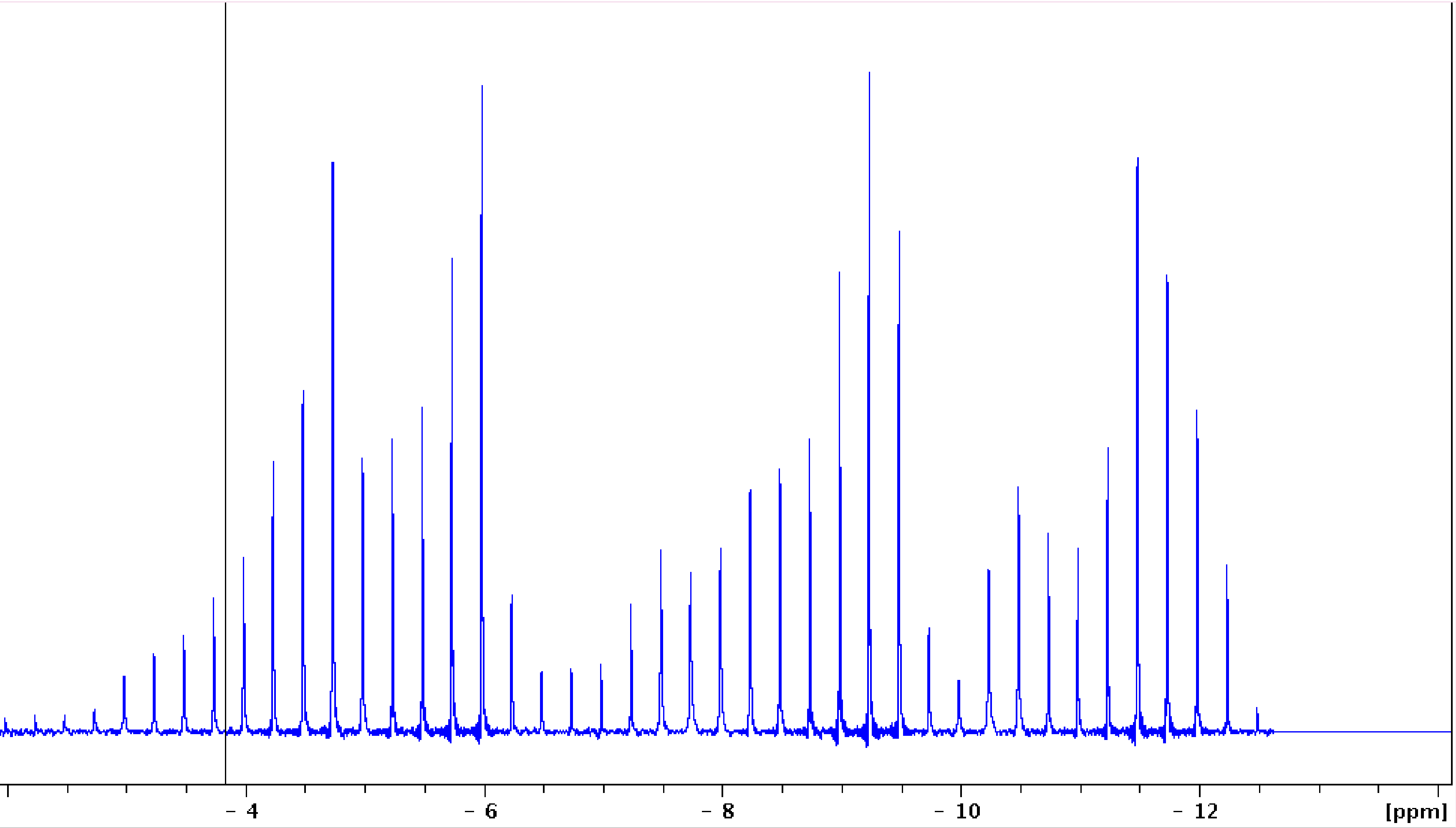
If in a IS spin system,
I magnetization is locked in the transverse plane with a field ω_I
and $\omega_I = \omega_S$ (Hartmann Hahn matching condition)

Then polarization is transferred from spin I to spin S at a rate proportional to the effective IS dipolar coupling.
and magnetization enhancement equal to
γ_I/γ_s

If a sample is spinning at ω_r spinning frequency, then Hartmann Hahn matching condition changes to:
$\omega_I = \omega_S + n\ \omega_r$, where n=+/-1,2

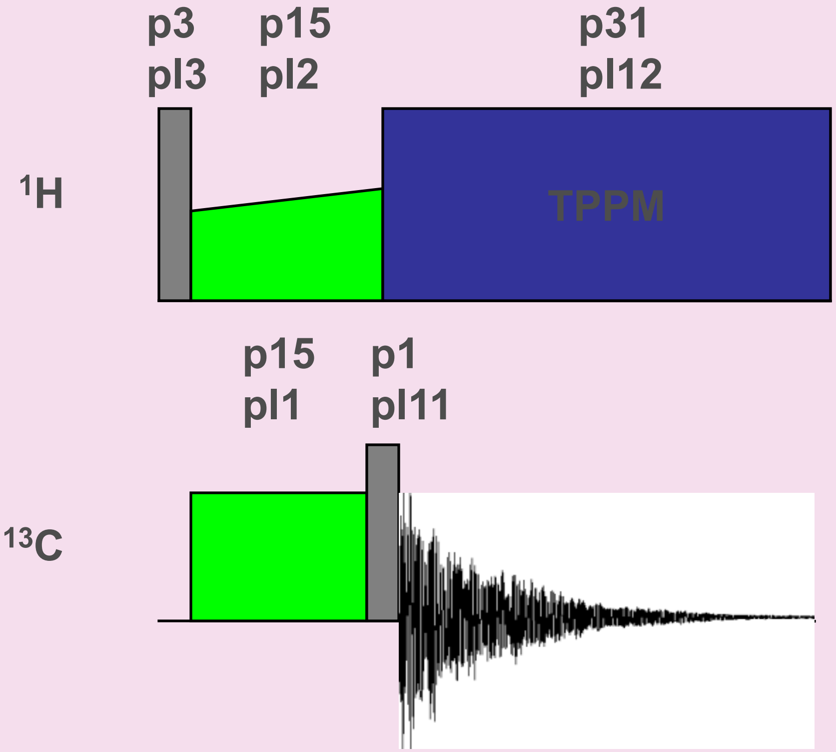
Also, in a cross polarization experiment, while S nucleus is observed, relaxation time scale is determined by T_1 of I nucleus.

Hartmann Hahn matching – finger pattern



Hartmann Hahn matching condition in adamantane at SF = 5kHz.
Keeping ^{13}C field @ 50kHz and varying ^{1}H field in 0.25dB steps

Setting up CPMAS experiment



Pulse Sequence:	cp90.itin
SF (spinning frq)	10kHz
aq (acquisition time)	15ms
sw (sweep width)	350ppm
o1p (13C carrier offset)	100ppm
o2p (1H carrier offset, optimize)	5/+5ppm
d1 (recycle delay)	3-5s
pl1 (13C CP field pl)	50kHz, 0/-3dB
pl2 (1H CP field pl, optimize)	8/1dB
pl12 (1H decoupling) (~0dB)	100k
p1/pl11 (pulse/power level to calibrate 13C)	
P15 (spin locking time, optimize)	0.5-5ms

For spin lock, use 20-50% ramp for 1H and maximum 13C field.

Why glycine

While often enough it’s possible to set up a CP experiment on the sample of interest, generally it’s still better to use a model compound.

The model compound should be
cheap, stable, reasonably well crystallized.

The model compound should have
short enough (2-5 sec) T_1 and long enough (>5ms) T_{1r}.

The model compound should have
a CH2 group (for decoupling optimization)
a C(O) group (for MA check/optimization)
an NH3 group for N15 CP optimization.

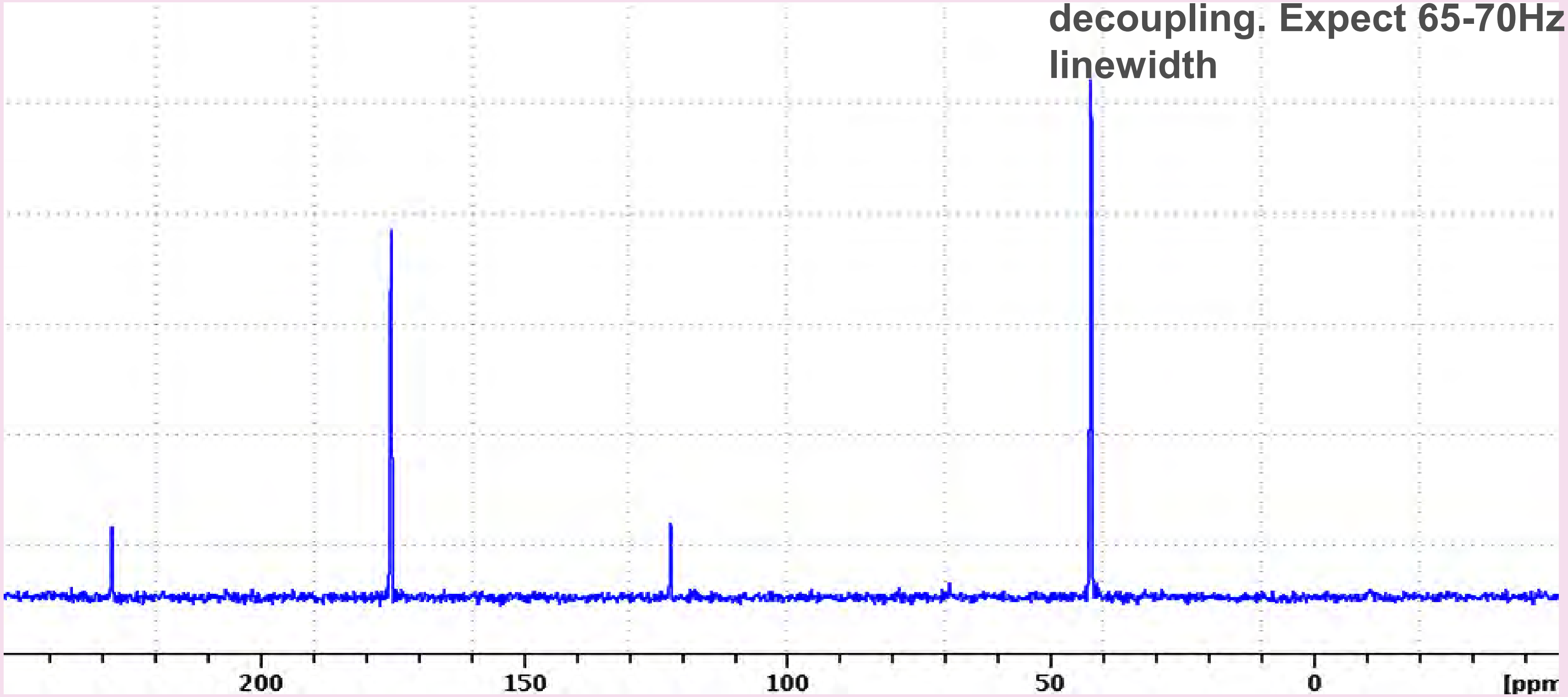
n/a Glycine satisfies all these parameters.

Alternatives: NAV, other aminoacids.

Setting up CP: n/a glycine

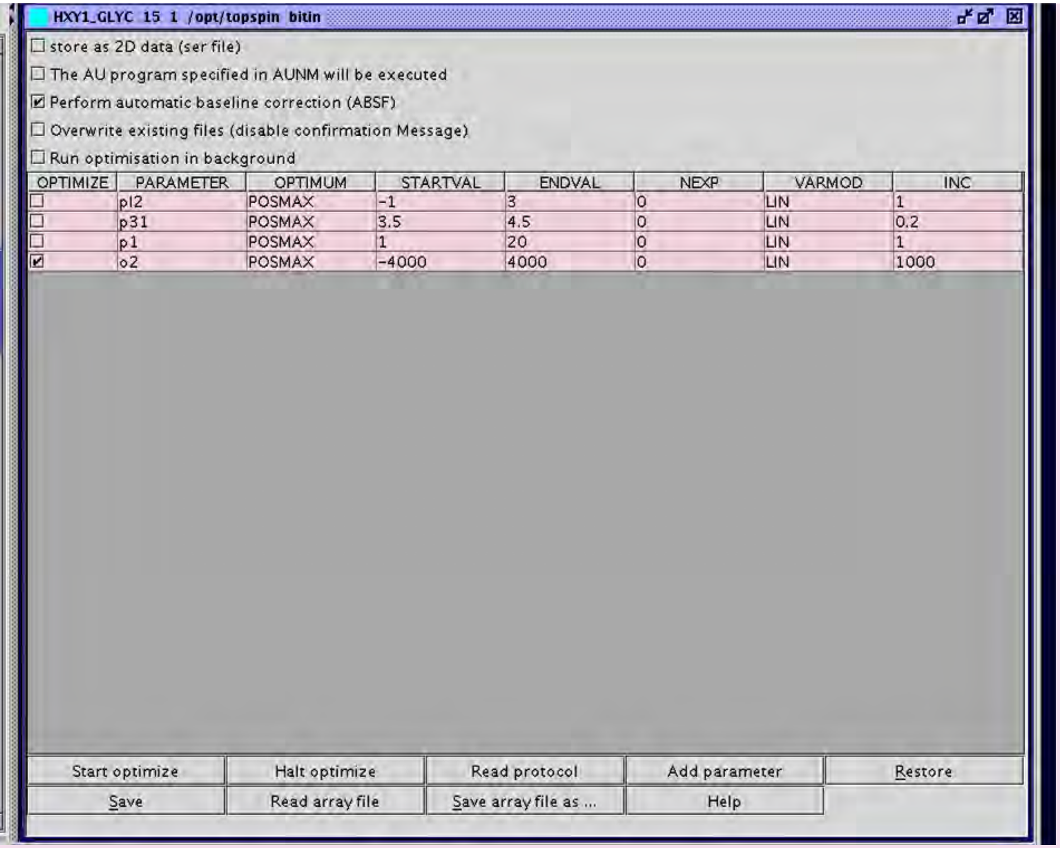
C(O) peak:
lineshape indicates MA.
Expect 65-70Hz linewidth.

α-C peak. HH homonuclear coupling makes CH_2 group the most demanding for decoupling. Expect 65-70Hz linewidth



50ul centerpacked n/a glycine spectrum taken on 750MHz spectrometer.
CP with TPPM decoupling.
SF=10kHz, ns=4, ^{13}C rf field 50kHz, ^{1}H rf field 120kHz. S/N = 170 (a-C).

Optimizing CPMAS: popt



HXY1_GLYC 15 1 /opt/topspin bitin

☐ store as 2D data (ser file)
☐ The AU program specified in AUNM will be executed
☑ Perform automatic baseline correction (ABSF)
☐ Overwrite existing files (disable confirmation Message)
☐ Run optimisation in background

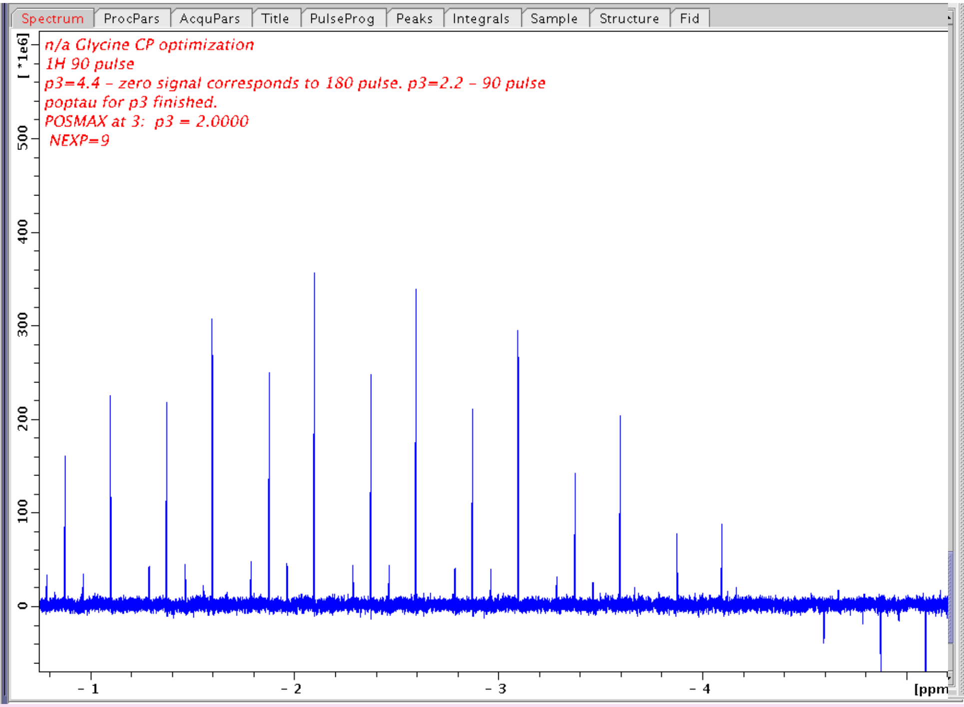
OPTIMIZE	PARAMETER	OPTIMUM	STARTVAL	ENDVAL	NEXP	VARMOD	INC
☐	pl2	POSMAX	-1	3	0	LIN	1
☐	p31	POSMAX	3.5	4.5	0	LIN	0.2
☐	p1	POSMAX	1	20	0	LIN	1
☑	o2	POSMAX	-4000	4000	0	LIN	1000

#999, 998, 997, etc processed data

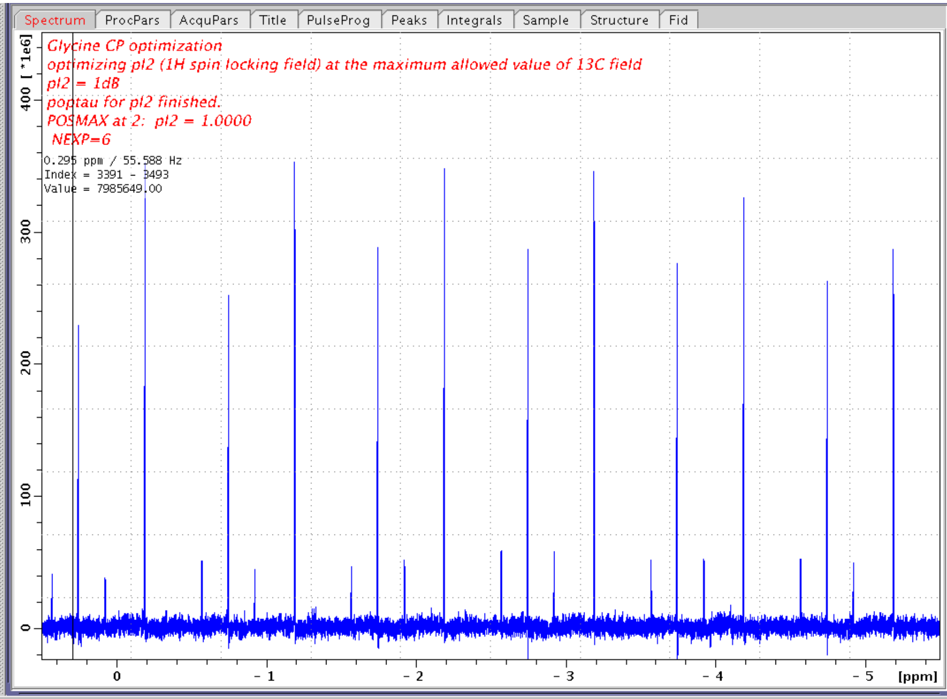
#899,898, 897, etc 2D data

Use popt macro to optimize the parameters:
Run a zg experiment and select a proper spectral window (right cursor button, dpl1)
Make sure that you are in processed experiment #1 and type popt command.
Enter a parameter you would like to optimize.
Decide whether or not you need to keep a 2D file
Start optimization.

Optimizing CPMAS: ^{1}H



n/a Glycine CP optimization
1H 90 pulse
p3=4.4 - zero signal corresponds to 180 pulse. p3=2.2 - 90 pulse
poptau for p3 finished.
POSMAX at 3: p3 = 2.0000
NEXP=9



Glycine CP optimization
optimizing pl2 (1H spin locking field) at the maximum allowed value of 13C field
pl2 = 1dB
poptau for pl2 finished.
POSMAX at 2: pl2 = 1.0000
NEXP=6

```
0.295 ppm / 55.588 Hz
Index = 3391 - 3493
Value = 7985649.00
```

Using popt to optimize 1H parameters:

Set ^{1}H fields fairly low (<3 db) and measure p3 (1H 90).

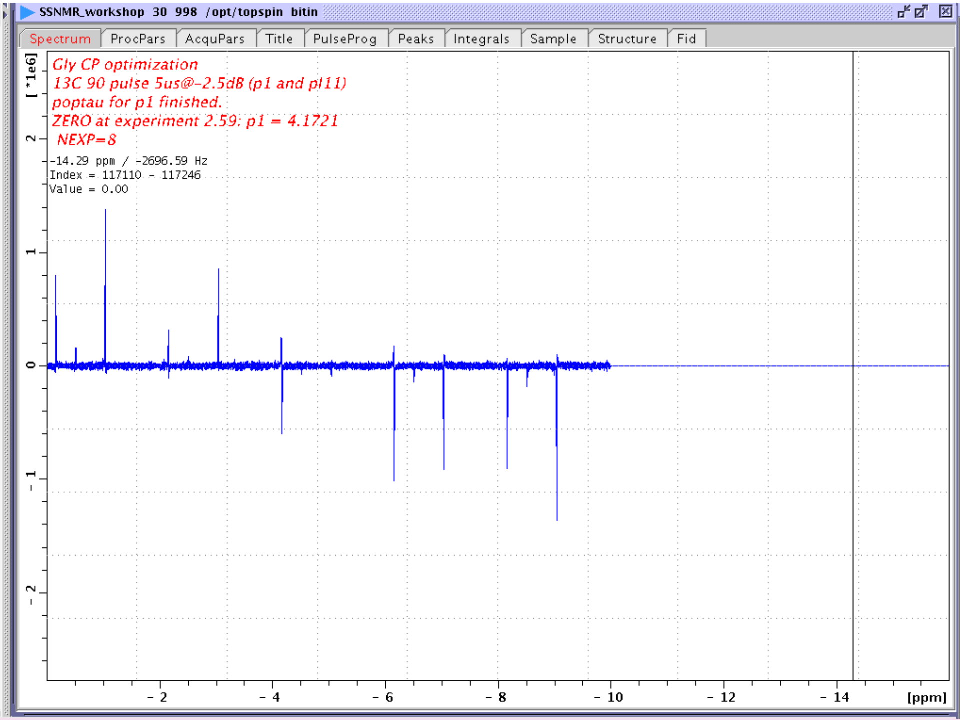
It's more reliable to measure ^{1}H 180 – zero signal.

Once you know what ^{1}H field is, optimize pl2 (^{1}H spin locking field).

Pl2 optimal value may be different for C(O) and CH$_2$.

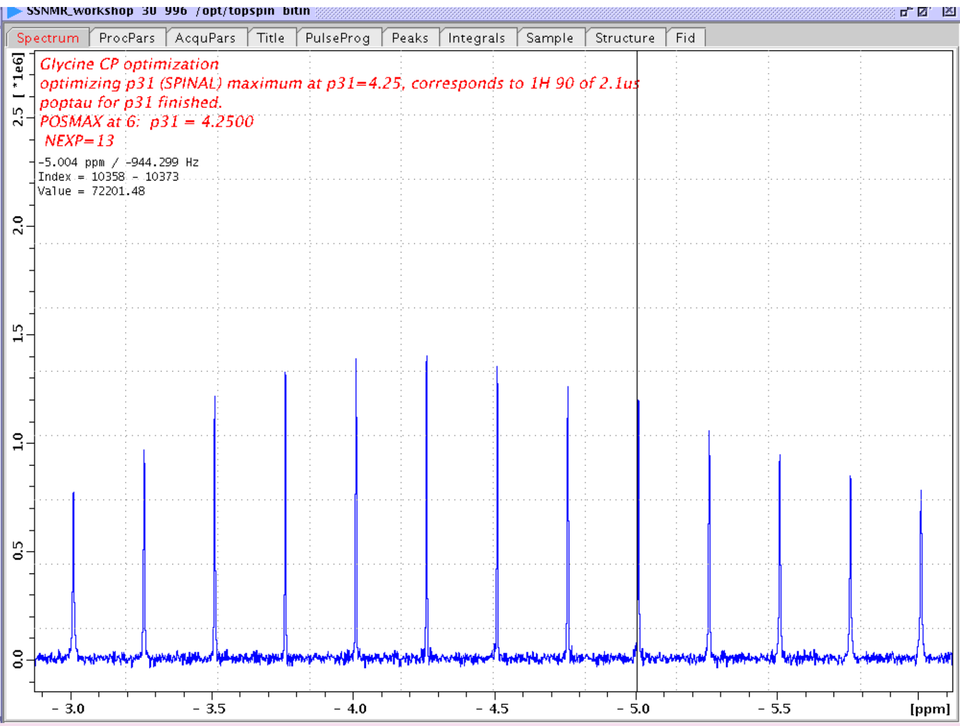
Ramping ^{1}H field will shift optimal pl2 and make CP matching conditions less sensitive.

Optimizing CPMAS: more parameters



Gly CP optimization
13C 90 pulse 5us@-2.5dB (p1 and pl11)
poptau for p1 finished.
ZERO at experiment 2.59: p1 = 4.1721
NEXP=8

```
-14.29 ppm / -2696.59 Hz
Index = 117110 - 117246
Value = 0.00
```



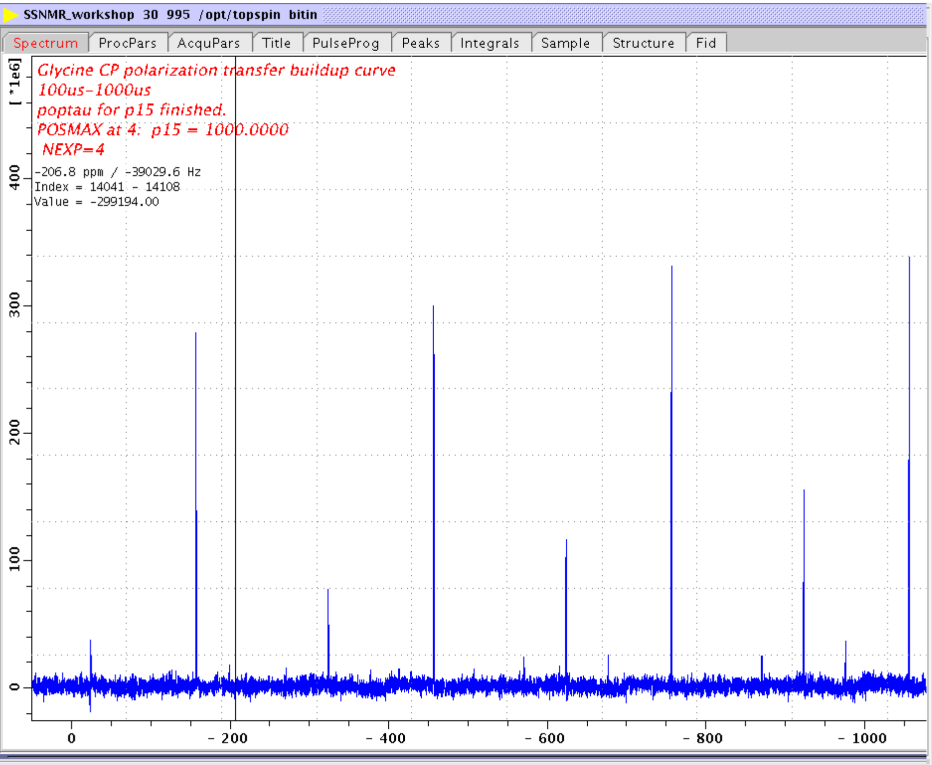
Glycine CP optimization
optimizing p31 (SPINAL) maximum at p31=4.25, corresponds to 1H 90 of 2.1us
poptau for p31 finished.
POSMAX at 6: p31 = 4.2500
NEXP=13

```
-5.004 ppm / -944.299 Hz
Index = 10358 - 10373
Value = 72201.48
```

Measure ^{13}C field using p1/pl11
(optional ^{13}C 90 pulse)
Set the carrier on CH_2 peak (40ppm).
Set pl11 to approximately 50kHz (-3dB – 0dB)
Run popt with p1 varying from 1 to 10us
Zero signal (crossing) corresponds to 90 pulse.

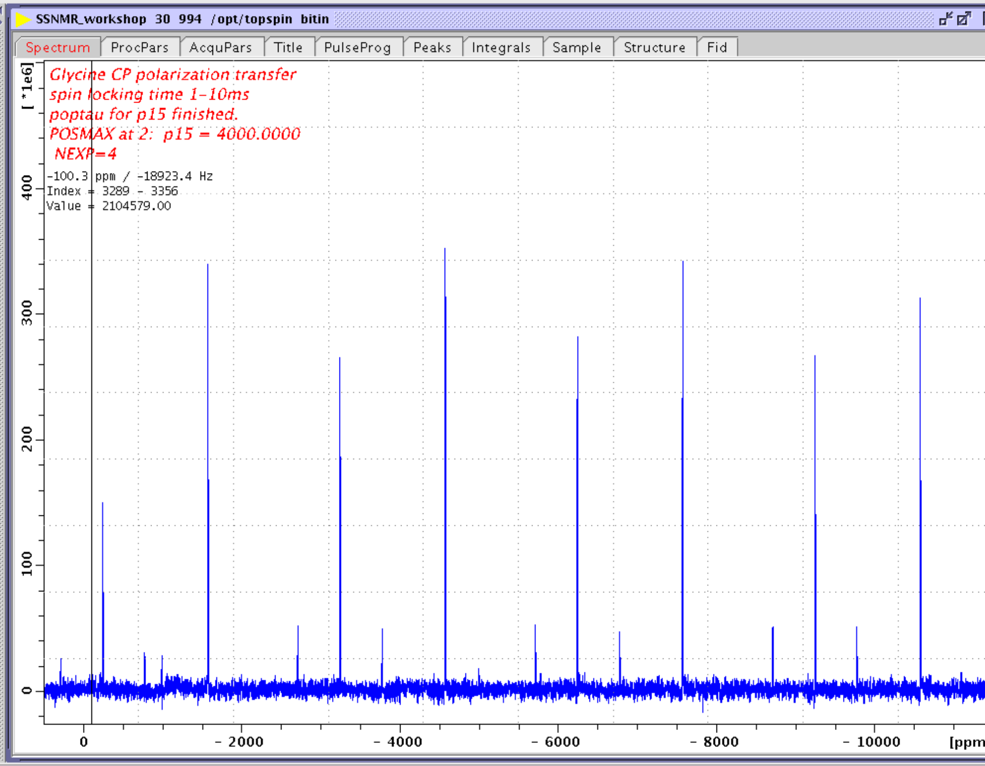
Use popt to optimize TPPM decoupling.
Zoom in on CH_2 peak
Set pl12 to approximately 100kHz
(or lower for sensitive samples).
Run popt for p31 +/- 25% of ^{1}H 180 pulse.
Optionally: optimize o2 (^{1}H carrier offset)
in the range +/- 3000Hz.

CP polarization transfer dynamics



Glycine CP polarization transfer buildup curve
100us-1000us
poptau for p15 finished.
POSMAX at 4: p15 = 1000.0000
NEXP=4

```
-206.8 ppm / -39029.6 Hz
Index = 14041 - 14108
Value = -299194.00
```



Glycine CP polarization transfer
spin locking time 1-10ms
poptau for p15 finished.
POSMAX at 2: p15 = 4000.0000
NEXP=4

```
-100.3 ppm / -18923.4 Hz
Index = 3289 - 3356
Value = 2104579.00
```

Optimal CP time depends on two parameters: polarization transfer rate and T_{1r}

Above, in a crystalline sample with reasonably slow relaxation rates (glycine), optimal polarization transfer time is 1ms for directly bonded ^{13}C (CH_2) and 6ms for 2 bond removed ^{13}C (C(O)).

Optimizing CP on a real sample

Optimize CPMAS on glycine or NAV.

In a conductive sample, effective ^{1}H and ^{13}C fields may be significantly lower (by 10-30%) for the same power levels.
Nevertheless, do not increase power levels beyond recommended.

A good qualitative indication of sample conductivity is the ramping of reflected power.
A conductive sample heats up during decoupling pulse and tuning/matching changes.
It causes reflected power to change during the decoupling pulse – ramp.

If a sample is conductive, increase starting pulse widths somewhat and start optimization. Follow glycine optimization procedure.

If a sample is sensitive to heating (protein), start with very low decoupling power level (3-4dB down). You can always increase it later – better than baking a protein.

If a sample is mobile, keep spin locking time low – 0.5-1ms.

Use bigger ramp for large volume samples.

Optimizing CP on a real sample: more

If you can't see any signal with parameters fairly close to standard,
quickly run pl2 optimization in 1dB steps.

If you still can't see any signal, try running direct observation with decoupling (hpdec).

If you still can't see any signal, put glycine back in and make sure that everything works properly.

If you can see hpdec signal, measure T_1 and T_2.
If T_2 of the sample is very short (<5ms), the chances are that
T_{1r} is also too short for a successful polarization transfer.
Then, you will have to use hpdec.

Do you have protons in your sample?
Try ^{1}H direct observe just to make sure that you indeed have protons.
It's pointless to try ^{1}H-X CP in proton poor environment.

If you have a low density proton non-mobile sample with large ^{1}H-X distances,
try long spin locking times – 5-10ms with large (50%) ramp.

Shimming

Typical NMR linewidth is 1-100Hz
Typical B_0 static magnetic field is 100-900MHz

Therefore B_0 field homogeneity should be 1ppb
(less than 0.1-5Hz)

Superconductive magnet coil has ~0.1ppt homogeneity in the center (sweet spot)

Extra homogeneity is achieved by:
superconductive/cryogenic shims
room temperature shims

Adjustable rf current runs through a 3D set of coils.
Resulting small magnetic fields are used to homogenize the main magnetic field

Superconductive shims are more powerful but they are not adjustable:
They bring linewidth down to 100Hz

A set of ~30 room temperature shims is used to bring linewidth down to 1-5Hz.

Shimming in SSNMR

In solid state NMR, shimming is performed by optimizing
FID/FT signal lineshape of a rotating sample.

Typically, lineshape specs are
2-5Hz full width half height (FWHH) and 7-15Hz width at 10% height.

Generally, only few shims are needed to achieve lineshape specs.
Either ZY plane shims or ZX plane shims should be used.
Probes are usually designed so that the rotor is aligned in either ZY or ZX plane.

First order shims optimize FWHH: z, x2-y2, z2, xy(?) and
second order shims optimize a foot/shoulder of the signal lineshape: z2,z3, z4

Shimming in SSNMR: a perfect sample

In the solid state NMR, shimming is performed by optimizing
FID/FT signal lineshape of a rotating sample.

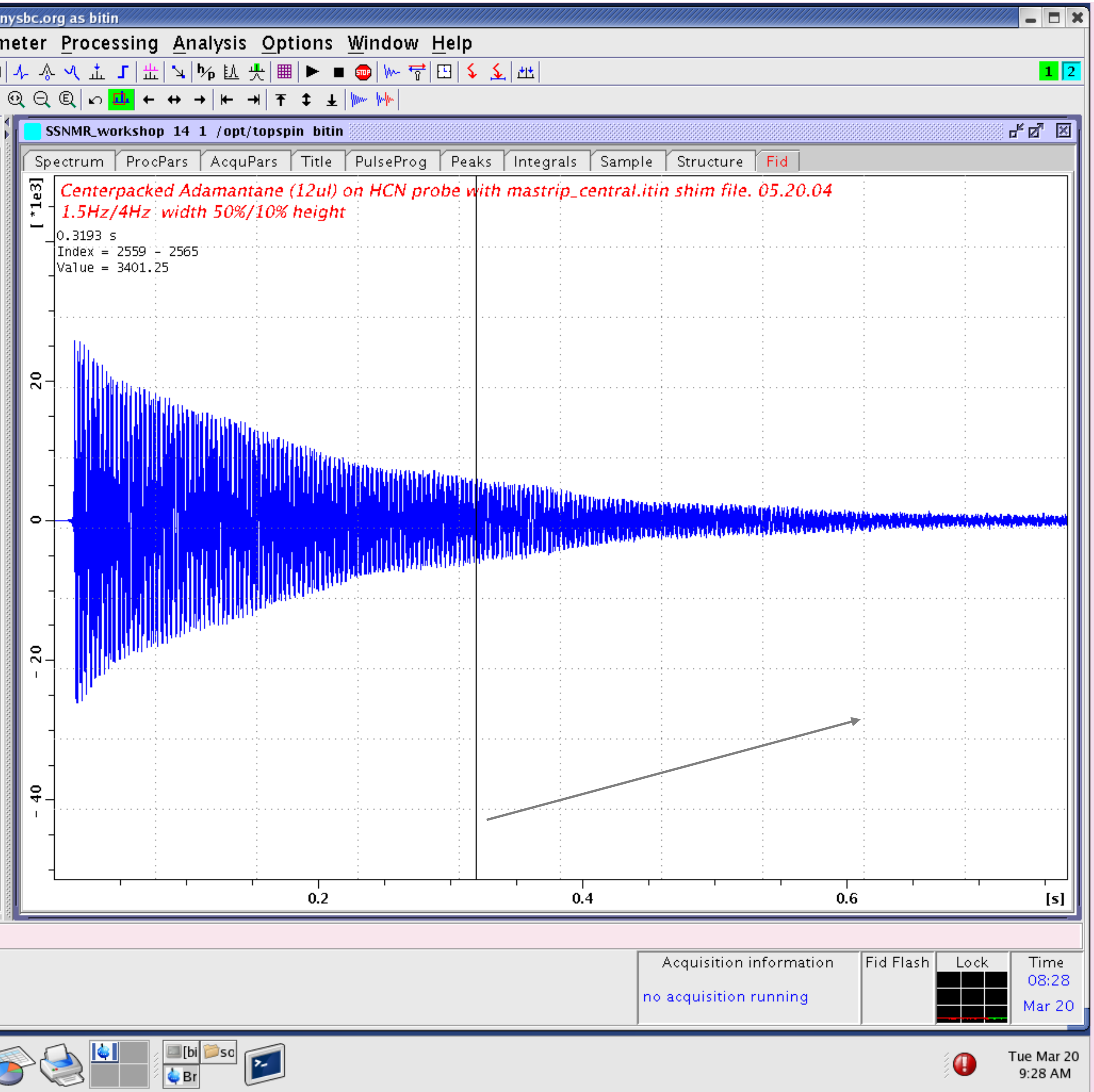
Typically, lineshape specs are 2-5Hz full width half height (FWHH).
FID is 200-500ms long, therefore decoupling has to be very low power (25kHz or less)

The sample should be highly ordered, have high signal (CP),
short relaxation time and small HC effective coupling.

ADAMANTANE

Adamantane is a unique compound.
Molecular motion attenuates CH coupling dramatically.
25kHz TPPM decoupling is sufficient.
Adamantane is highly ordered and natural linewidth is less than 1Hz.
Adamantane has short (<3s) ^{1}H T_1.

Shimming on adamantane: FID



Centerpacked Adamantane (12ul) on HCN probe with mastrip_central.itin shim file. 05.20.04
1.5Hz/4Hz width 50%/10% height

0.3193 s
Index = 2559 - 2565
Value = 3401.25

Acquisition information
no acquisition running

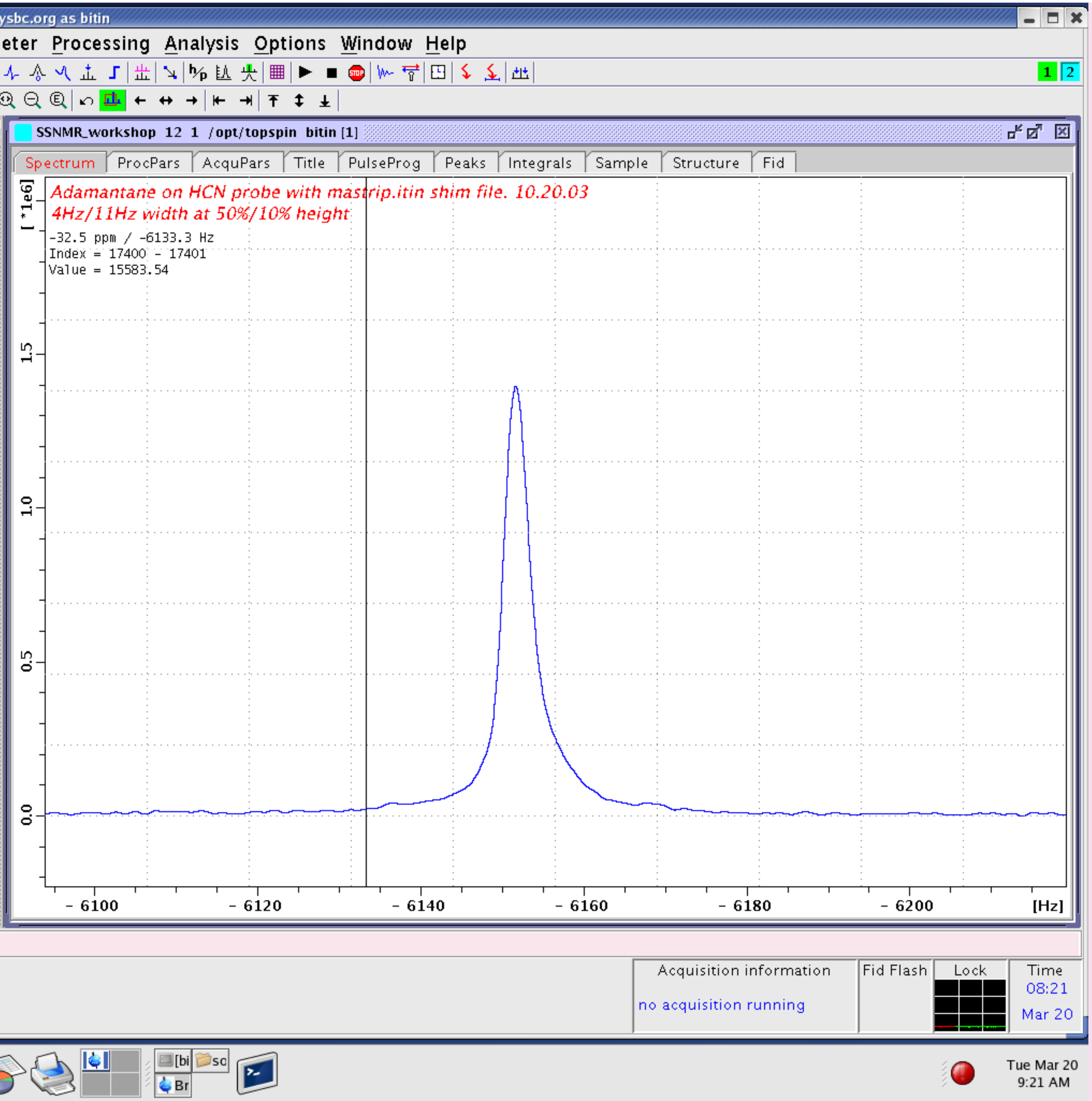
Time
08:28
Mar 20

This is an FID of a well shimmed small center packed rotor

The total FID length is 1s – that done just to show off,
don’t decouple more than 1/2s.

The beginning of FID looks fairly exponential – the foot will be small

Shimming on adamantane: FT, 60ul



Adamantane on HCN probe with mastrip.itin shim file. 10.20.03
4Hz/11Hz width at 50%/10% height

```
-32.5 ppm / -6133.3 Hz
Index = 17400 - 17401
Value = 15583.54
```

Acquisition information

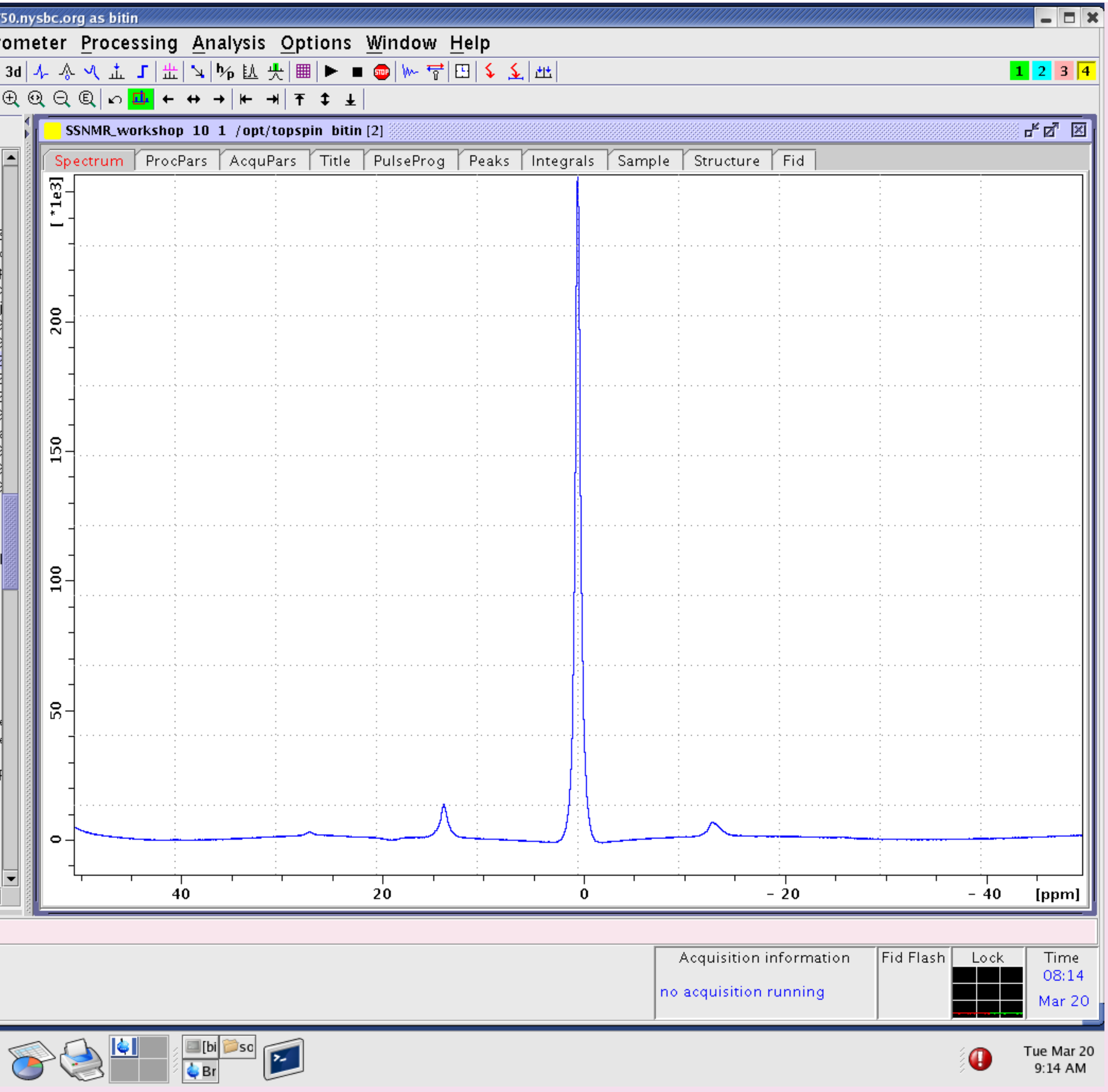
no acquisition running

This is more typical sample volume and shimming lineshape: 60ul

Full width half height: z, x2-y2, z2 shims

The foot:
z2, z3, z4 shims
Use z4 to minimize asymmetric foot.
Use z3 to minimize symmetric linewidth at the bottom

Adamantane ^{1}H spectrum



In adamantane, ^{1}H homonuclear dipolar coupling is attenuated sufficiently to observe ^{1}H signal and check ^{1}H pulses/powers directly.

Imperfections

Nothing is perfect

Pulse sequence commands take time
(typically few nanoseconds time scale)

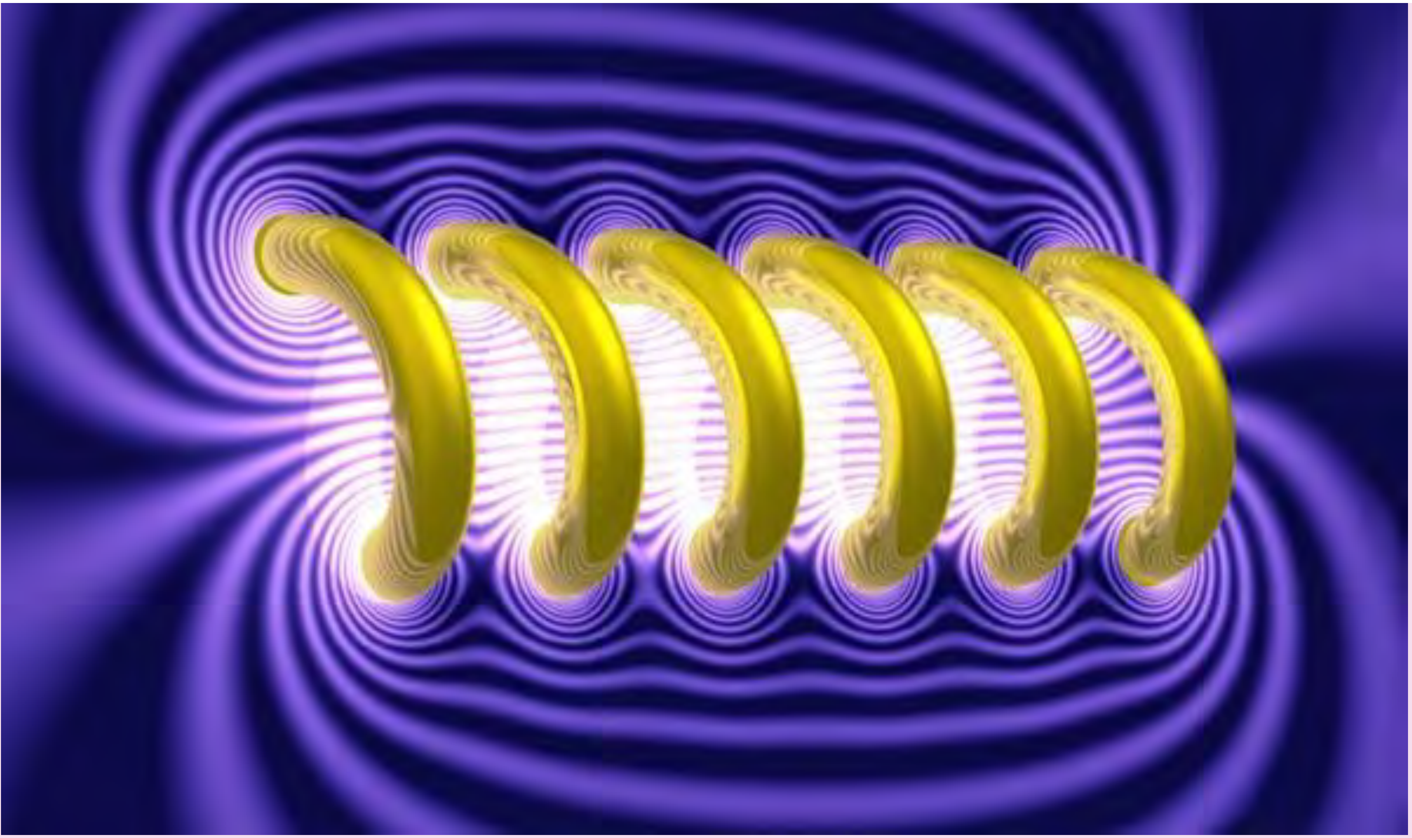
Console electronic components need time to start and stop pulsing

Hard rectangular pulses are not infinitely short.
Neither are they rectangular.
Rise and fall times are approximately 200ns.
Make sure that you take that into account when measuring fields.

Long pulses are long and magnetization evolves during them.

Amplifiers and probe need at least 4-5us to quiet down after the last pulse before the acquisition can start.

B_1 homogeneity

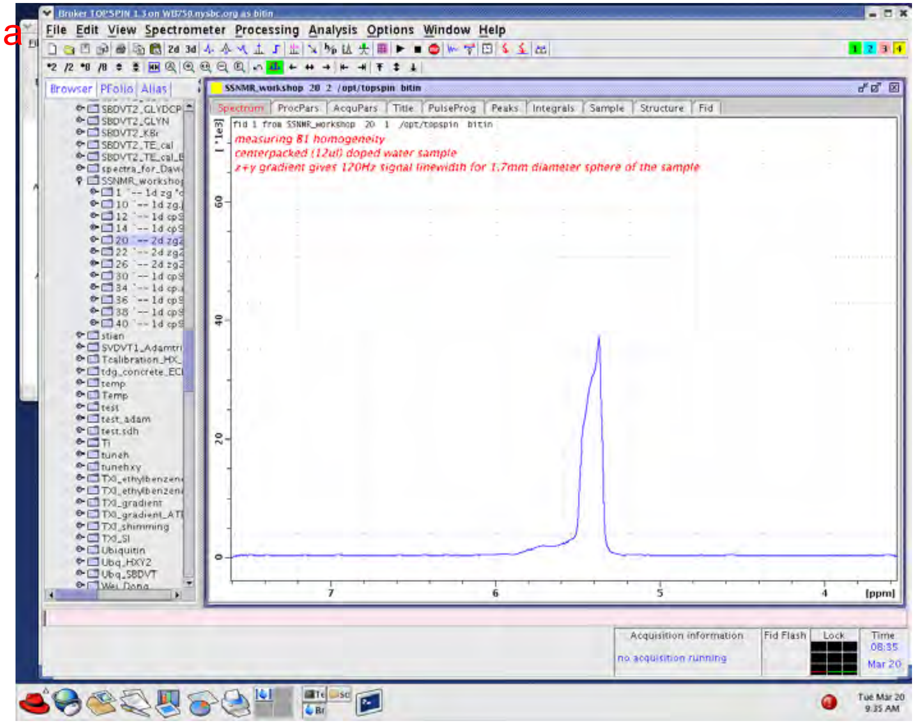


In an infinite length solenoid coil B_1 is the same throughout the coil

In a limited length solenoid B_1 decreases towards the ends of the coil

Tapering a coil (tightening outside turns) may improve B_1 homogeneity somewhat

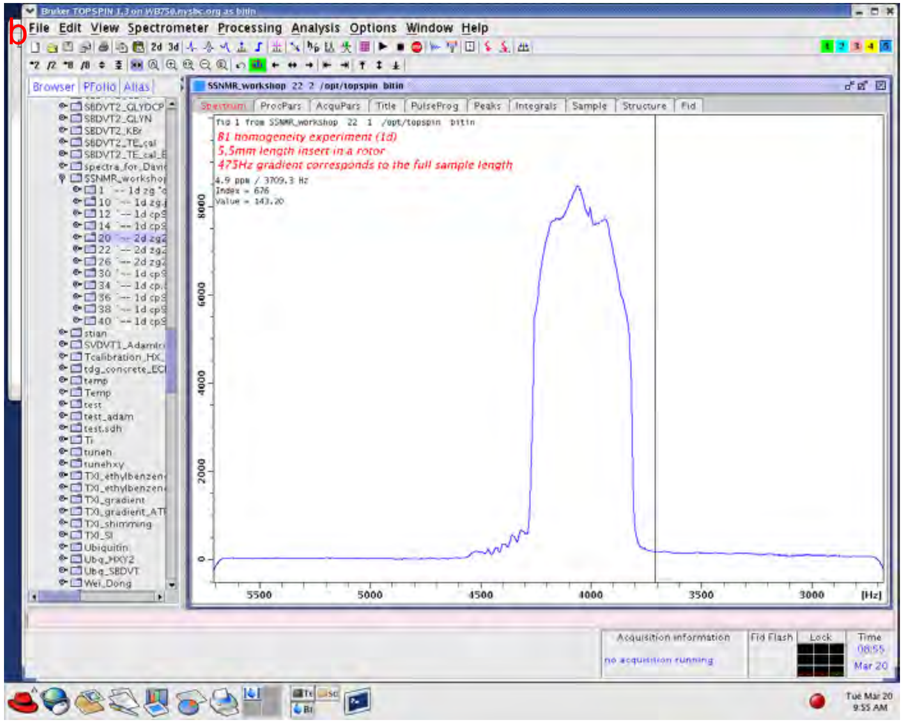
B_1 homogeneity



SSNMR_workshop 20 2 /opt/topspin bitin

fid 1 from SSNMR_workshop 20 1 /opt/topspin bitin

measuring B1 homogeneity
centerpacked (12ul) doped water sample
z+y gradient gives 120Hz signal linewidth for 1.7mm diameter sphere of the sample



SSNMR_workshop 22 2 /opt/topspin bitin

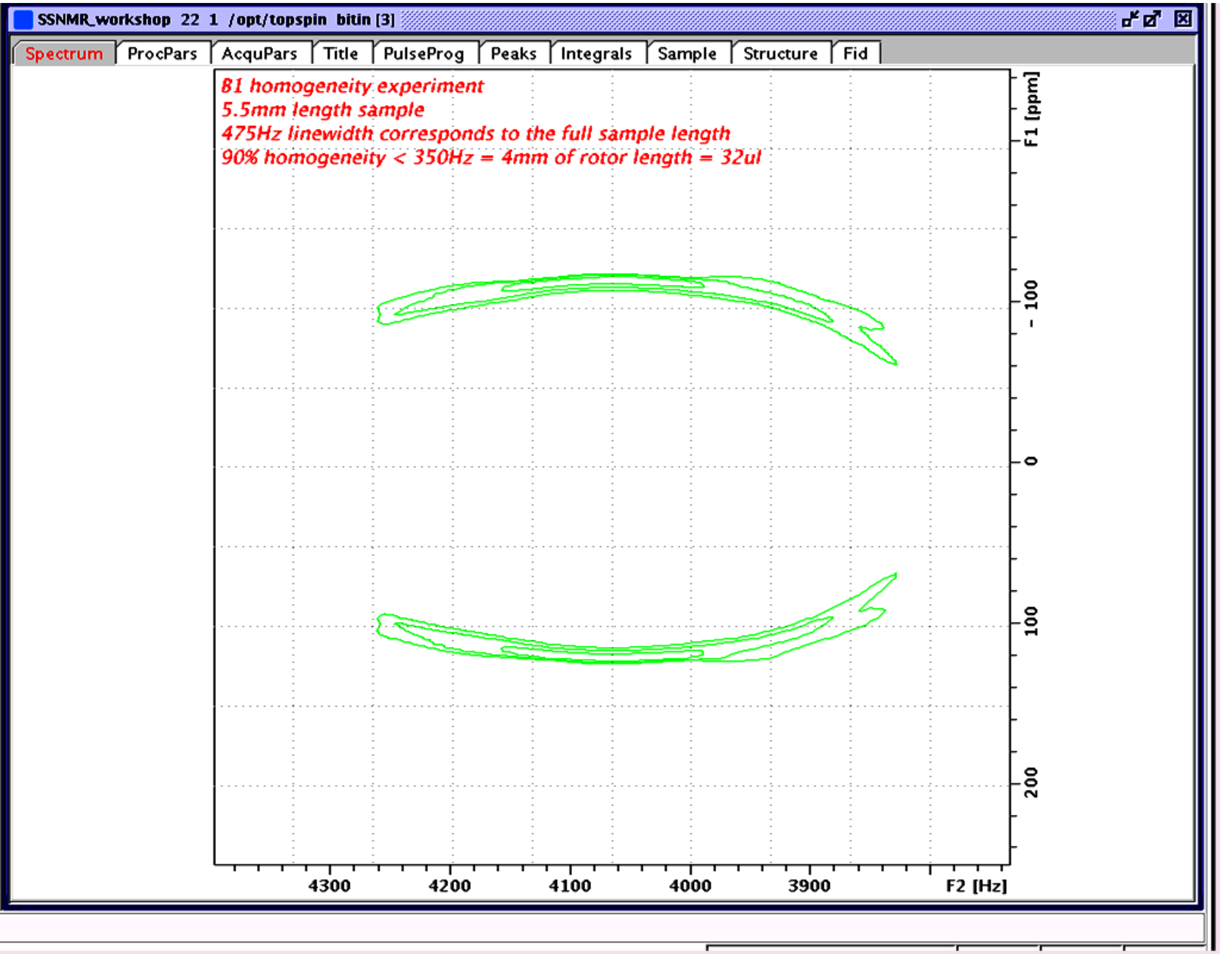
fid 1 from SSNMR_workshop 22 1 /opt/topspin bitin

B1 homogeneity experiment (1d)
5,5mm length insert in a rotor
475Hz gradient corresponds to the full sample length

4.9 ppm / 3709.3 Hz
Index = 676
Value = 143.20

[redacted]

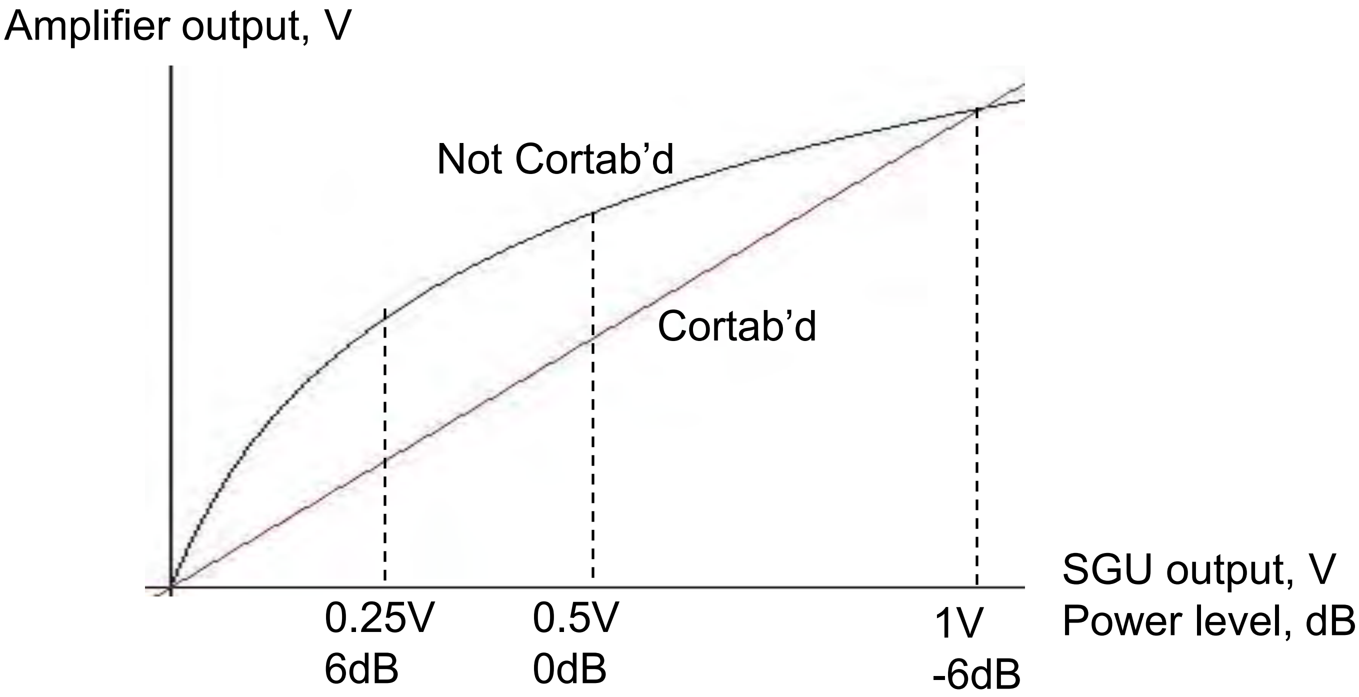
B_1 homogeneity: 2D ^{1}H field map



B1 homogeneity experiment
5.5mm length sample
475Hz linewidth corresponds to the full sample length
90% homogeneity < 350Hz = 4mm of rotor length = 32ul

2D nutation experiment on a 60ul/5.5mm doped water sample.
The field map along rotor axis demonstrates 90% B_1 homogeneity for ~ 3.5mm length/35ul sample volume.
B_1 field drops off dramatically towards the ends of a rotor.

Amplifier linearity



SGU output is always linear

Amplifier output is non linear, particularly between 0 and -6dB

Cortab (correlation table) adjusts SGU output so that amplifier output is linear

Delays in pulse program and acquisition parameters

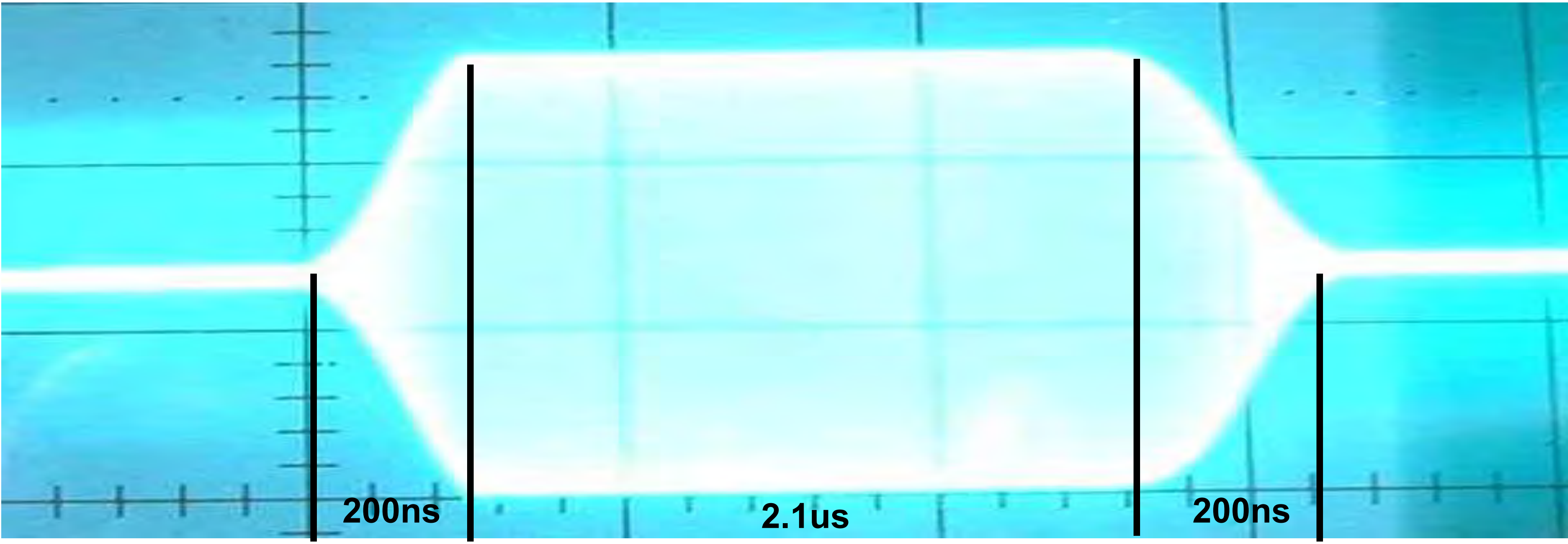
```
1u fq=cnst21:f2

10u pl12:f2                         ;preselect pl2 drive power for F2
trigg
p3:f2 ph1                           ;proton 90 pulse
0.3u
(p15 ph2):f1 (p15:spf0 pl2 ph10):f2    ;contact pulse
1u cpds2:f2                         ;use cpdprg2=tppm15, SPINAL64 or XiX
d0
0.5u do:f2
(p1 pl11 ph3):f1 (1u do):f2
(mixing pl14 ph11):f2
(p1 ph5):f1 (1u cpds2):f2
```

Every frequency and power level switch takes time.

It is also safer to have some delays before and after composite pulses.

Always wait at least 4-5us before starting acquisition (dead time or d6).

Amplifier rise time

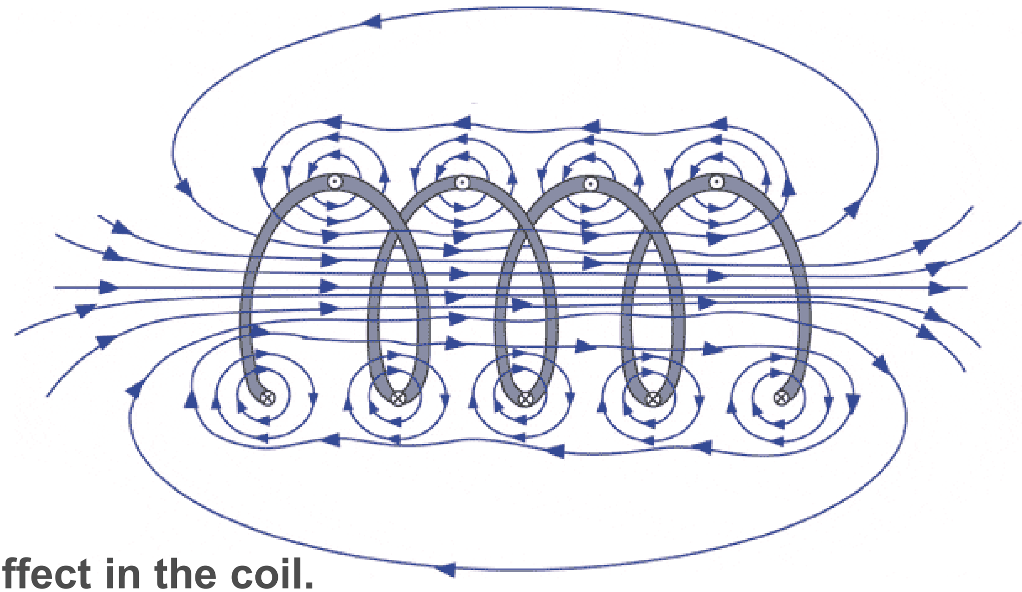


A typical Bruker or Varian (AMT) amplifier has approximately 200ns rise and fall times.

Therefore an experimentally measured 2.5us 90^0 pulse is, in fact, roughly 2.2-2.3us real pulse (corresponding to 110-114kHz instead of 100kHz field)

Use 360^0 pulse to measure field, not 180^0 – the relative error is smaller.

Ringdown



An NMR sample coil is not an ideal system.

A high power pulse generates a ringdown effect in the coil.

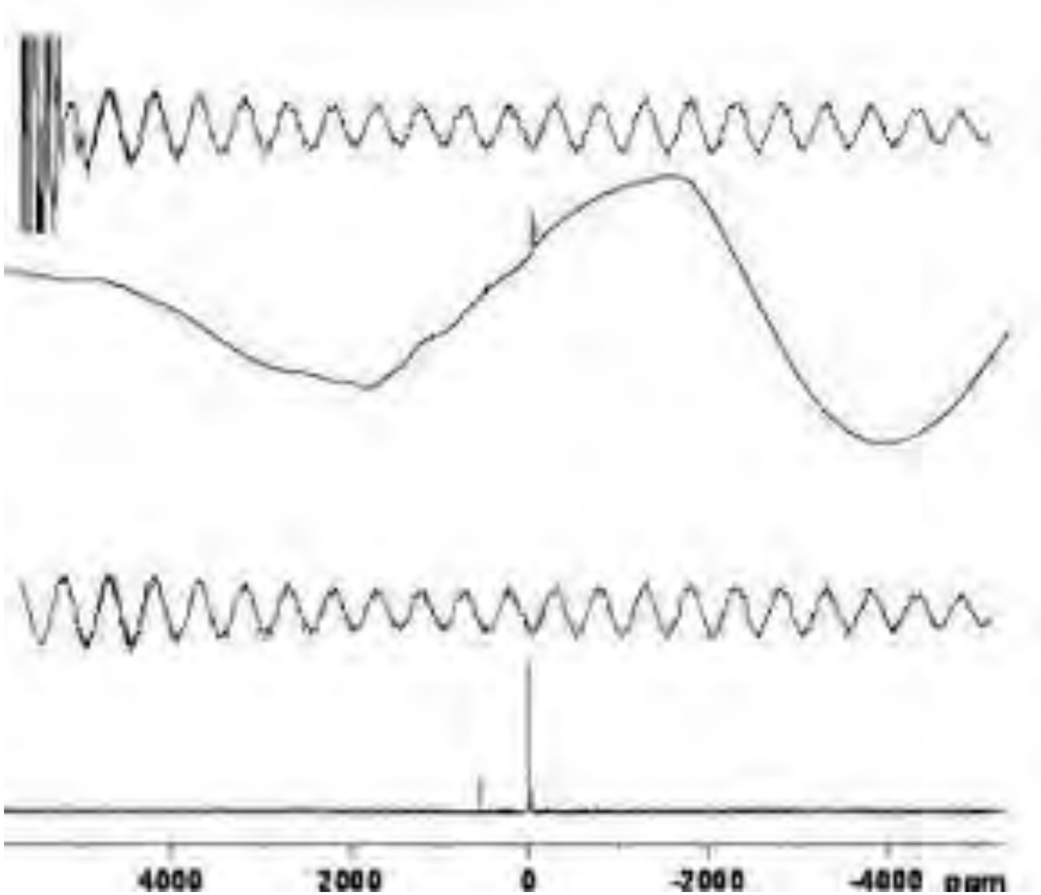
After the end of the pulse, residual currents keep running in the coil generating magnetic fields.

Ringdown time scale is proportional to probe efficiency (Q).
Ringdown time scale is inversely proportional to Larmor frequency.

Ringdown effect is usually
negligible above 300MHz: ringdown time<5us
very strong below 100MHz: ringdown time>20us

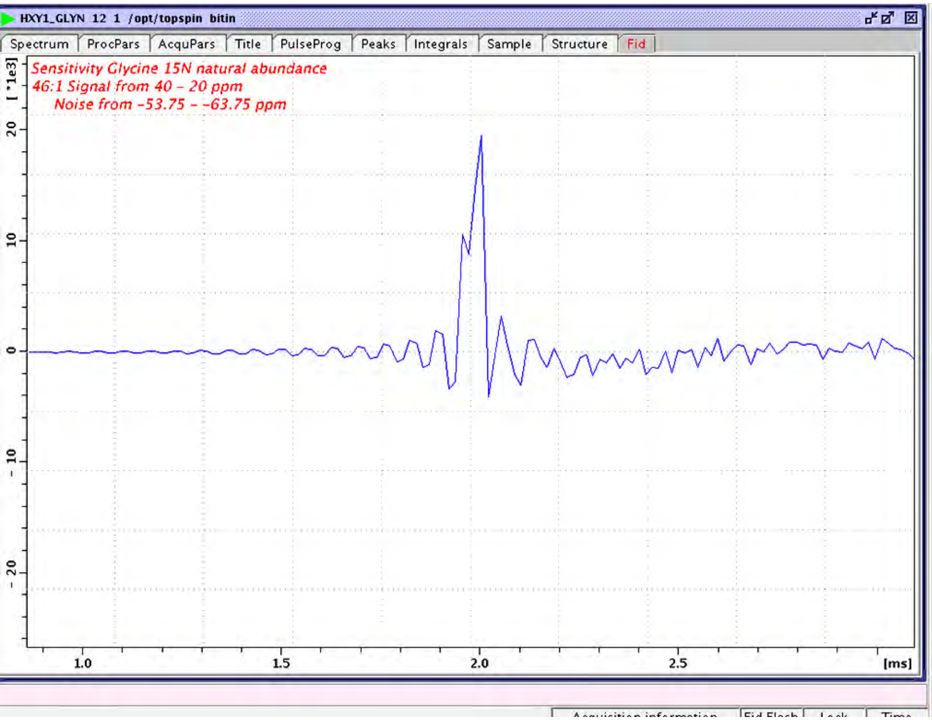
If signal lineshape is broad enough that 20us of FID is important,
direct excitation signal will be distorted by ringdown effect.

Ringdown spectra

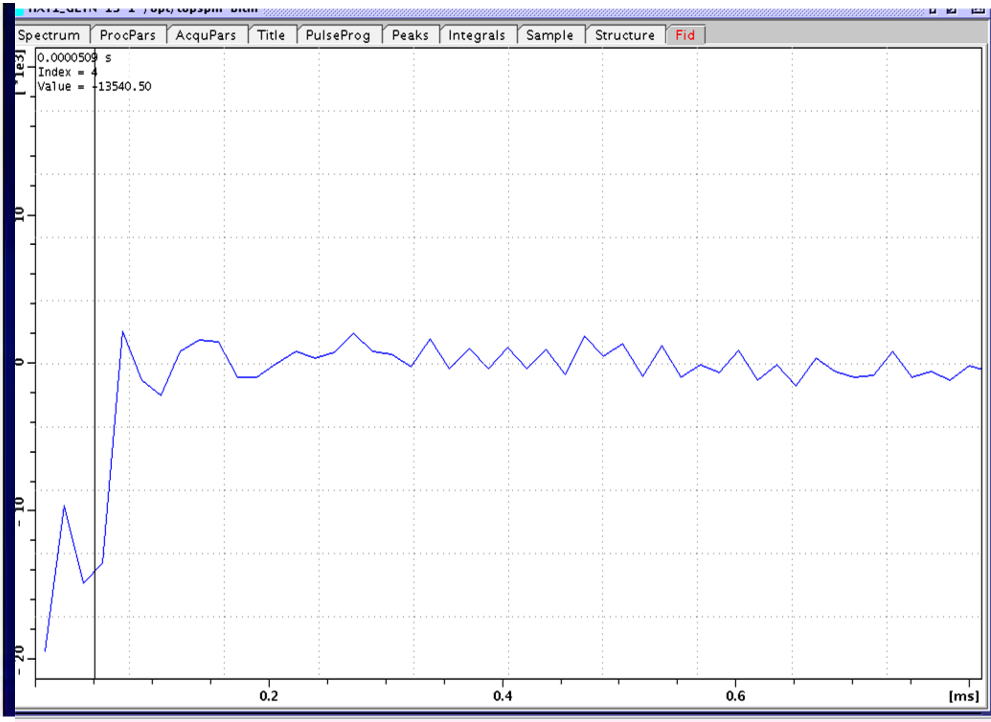


First several points of FID have abnormally high intensity – this is ringdown.
FT transform causes baseline roll.
Since the signal is fairly narrow, FID can be linearly predicted backwards or shifted to the left without any problem.

Convdta and left shift



Sensitivity Glycine 15N natural abundance
46:1 Signal from 40 – 20 ppm
Noise from -53.75 – -63.75 ppm



```
0.0000509 s
Index = 4
Value = -13540.50
```

If the ringdown time scale is much smaller than the FID time scale, it is possible to edit the beginning section of FID.

Use *convdta* command to convert Bruker digital FID into analog FID.

Set datmod = raw. Set nsp (number of set points) to the minimal number of points needed to eliminate ringdown

Use ls (left shift) command to shift FID to the left by nsp points. Process the resulting FID.

Linear Prediction (1)

Linear prediction (lp) is a method to construct FID points either forward or backward based on the existing FID section.

Lp forwards is used in 2D experiments to construct FID in the indirect dimension

Lp backward is used to counteract distorting effects of ringdown in the beginning of FID.

Linear prediction is tricky!

Linear Prediction (2)

For linear prediction backward:

Define ME_mod = lpr or lpc

Set TD_OFF to number of points in the beginning of FID you'd like to predict

Set TD_EFF to the number of points in FID you'd like to use for prediction

Set NCOFF to a "reasonable number"

For linear prediction forward for a indirect dimension 2D FID:

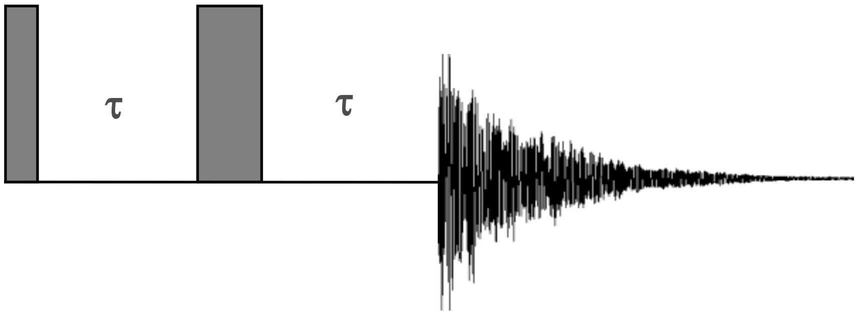
Define ME_mod = lfc or lfr

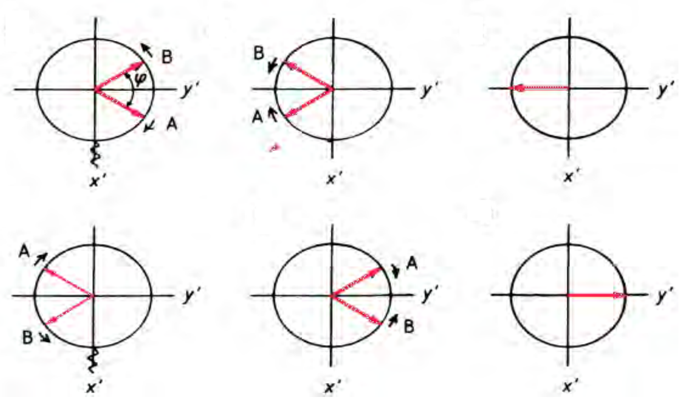
Set TD_EFF to the number of points you collected

Set LPBIN to the total number of points you would like FID to have

Set NCOFF to a "reasonable number"

Hahnecho (1)





**If ringdown time is long enough on FID time scale,
then neither left shift nor linear prediction would work reliably.**

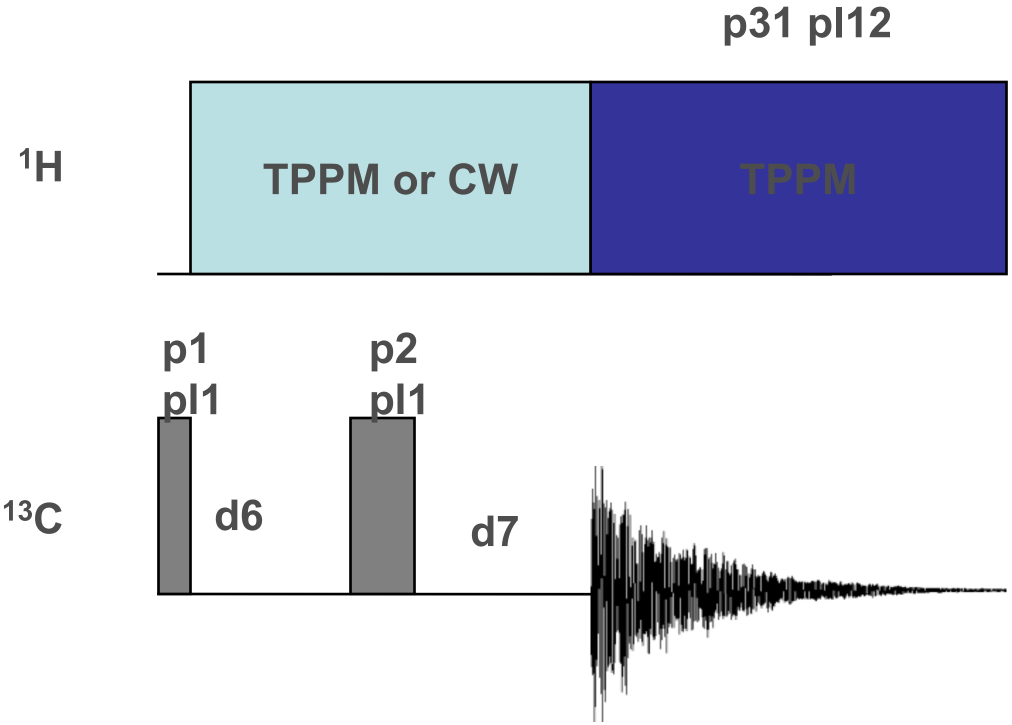
Hahnecho or solidecho refocusing scheme bypasses this problem.

**A magnetization is allowed to evolve in transverse plane for either:
rotor synchronized time period or as short time period as possible**

**Then, refocusing 180^0 (dipolar, hahnecho) or 90^0 (quadrupolar, solidecho) pulse is applied.
After the same delay, the magnetization is more or less refocused.
Since FID is collected after a substantial delay, ringdown is no longer an issue.**

Homogeneous interactions (homonuclear dipolar coupling) are not refocused

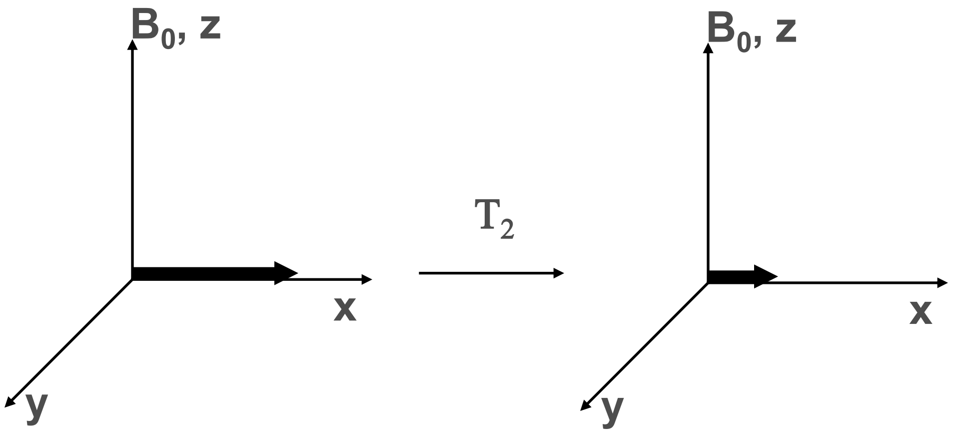
Hahnecho (2)



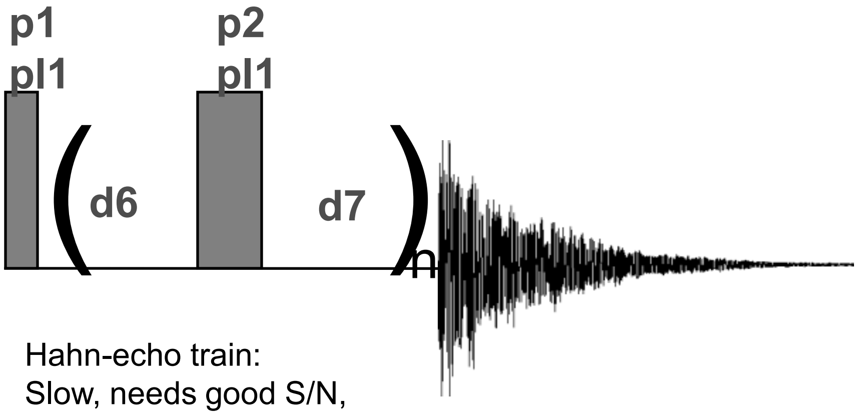
hahnecho.itin

90 pulse	p1
180 pulse	p2
defocussing delay $n\tau_r$	d6
refocussing delay $n\tau_r$-p2/2	d7
Decoupling	p31 pl12

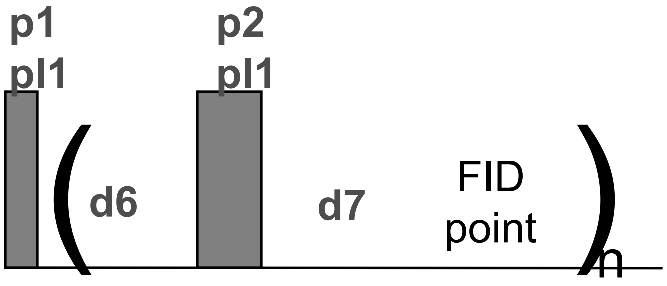
T_2 measurement



$$I(t) = I_0 e^{-\frac{t}{T_2}}$$

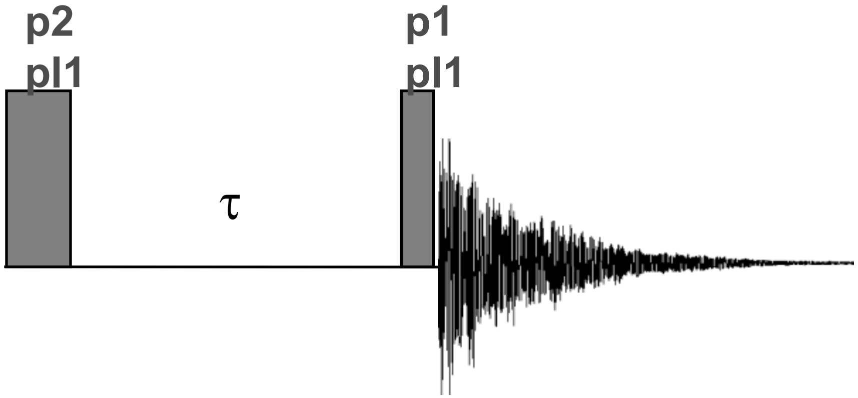


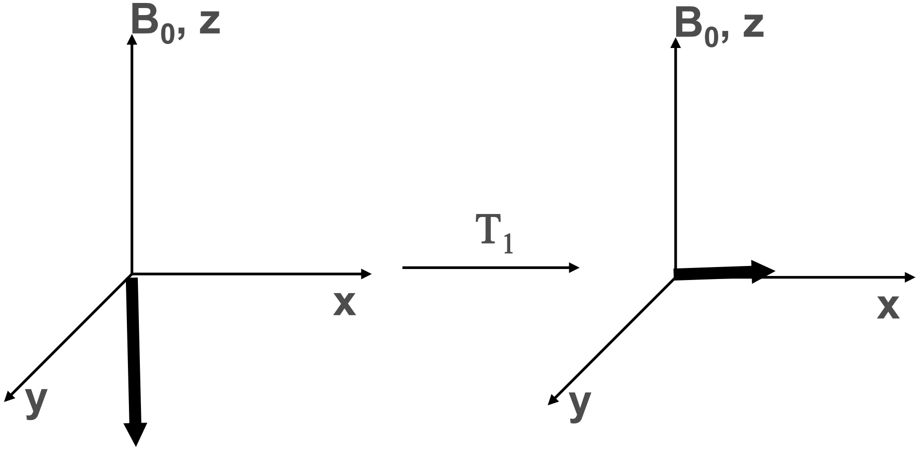
Hahn-echo train:
Slow, needs good S/N,
precise



CPMG:
quick, works for low S/N,
has T1 error/contribution

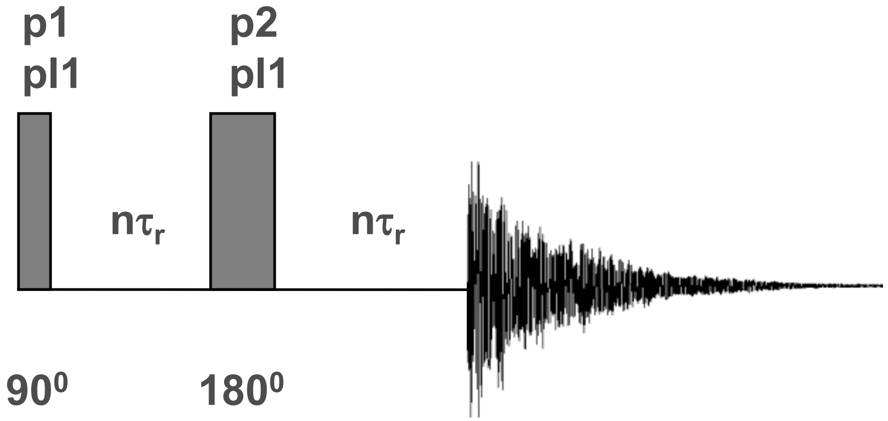
T_1 measurement (inversion recovery)



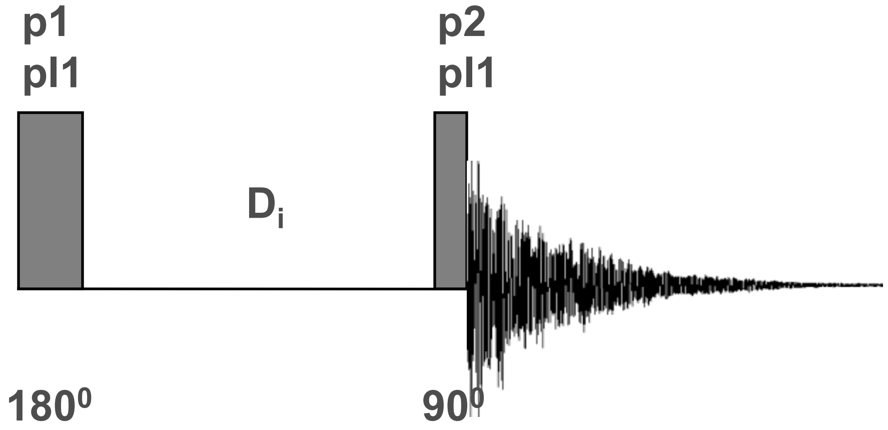


$$I(t) = I_0\left(1 - e^{-\frac{t}{T_1}}\right)$$

Measuring T_1 and T_2



Hahnecho can be used as a pseudo 2D experiment to measure T2.
The signal intensity can be fit as a function of n
(remember: rotor synchronized)



Inversion recovery experiment is used to measure T1. *D* array consists of exponentially increasing delay values D_i i.e. (1ms, 4ms, 16ms, 64ms, …). Signal intensity can be fit as a function of delay

Double Cross Polarization

Double cross polarization is one of the most widely used heteronuclear correlation experiments in solid state NMR.

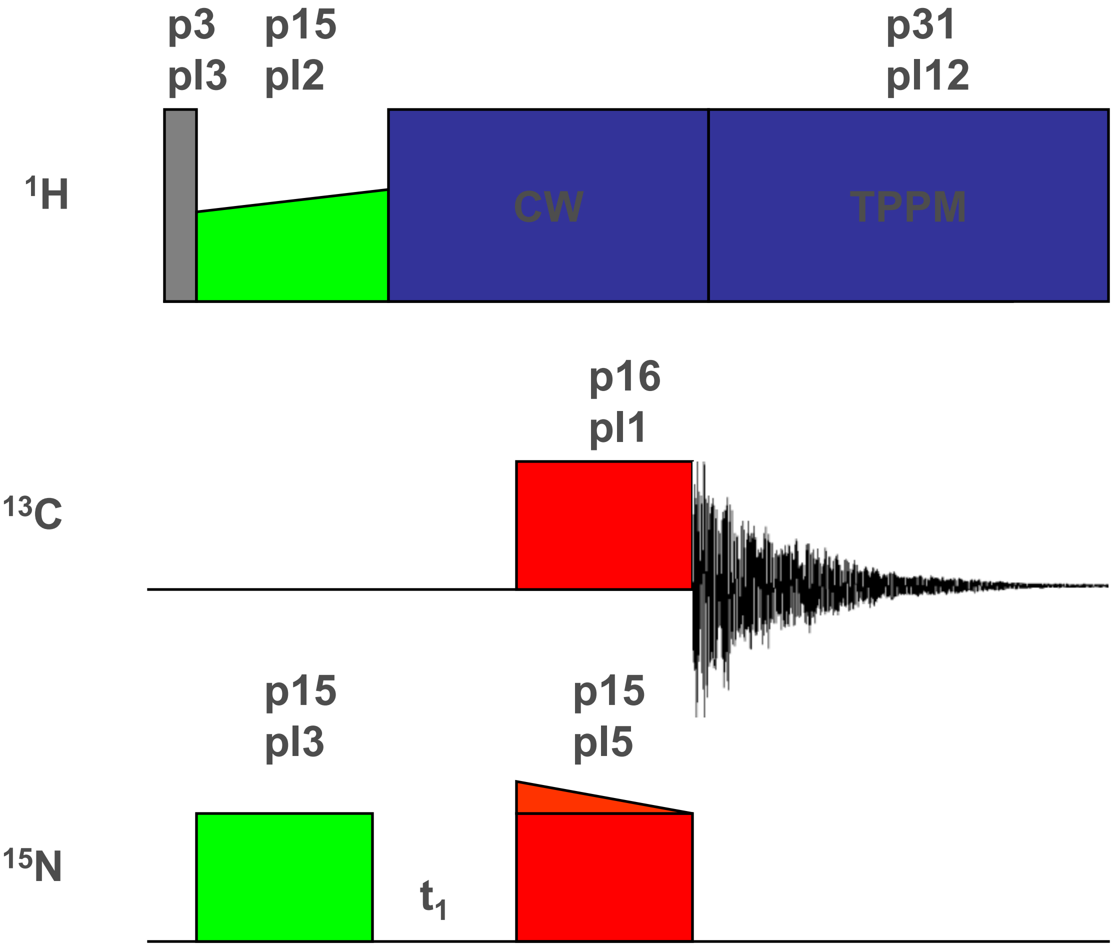
DCP is robust, easy to set up and effective way to achieve heteronuclear polarization transfer.

Typical DCP experiment used in an HCN spin system consists of:
^{1}H/^{15}N cross polarization
followed by evolution t_1 time period to evolve ^{15}N chemical shift term,
followed by ^{15}N/^{13}C cross polarization,
followed by ^{13}C acquisition.

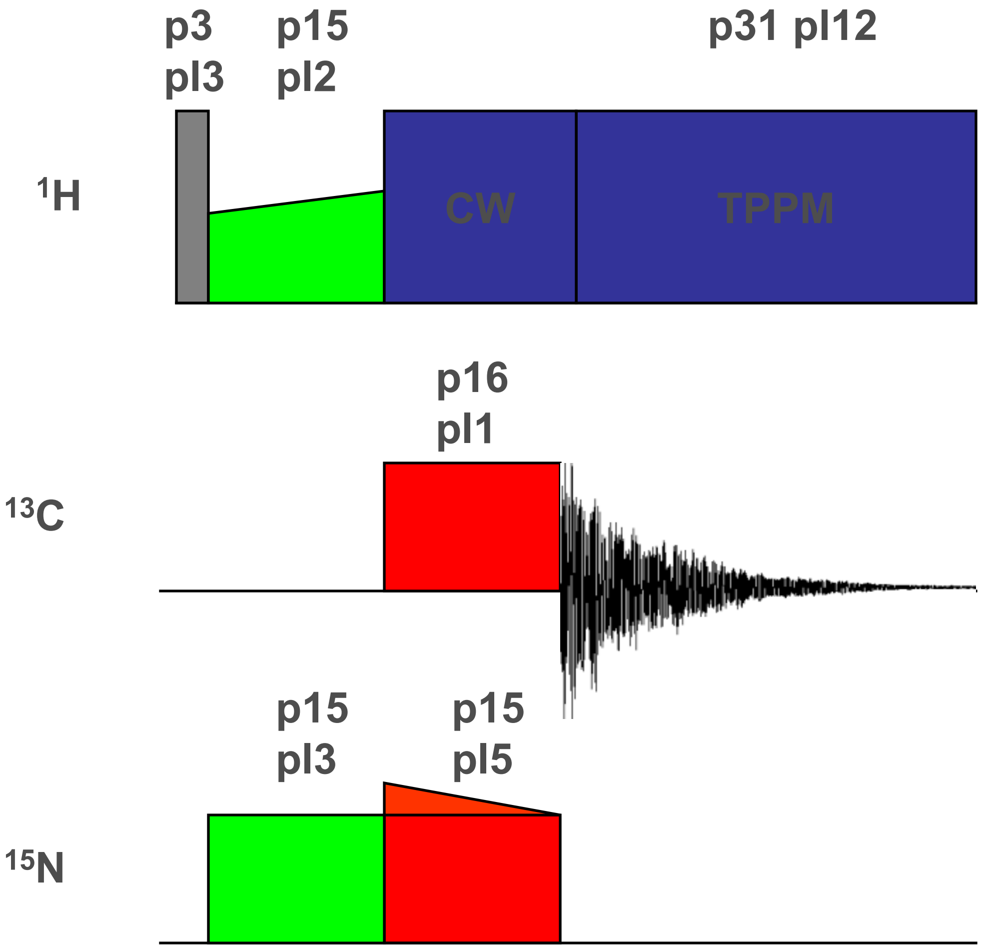
1D DCP experiment on a model compound can be used to optimize all power/pulse parameters.

U-gly (or another amino acid) is a good compound for DCP optimization.

2D DCP pulse sequence



Setting up DCP



Pulse Sequence:	cp90.itin
SF (spinning frq)	10kHz
aq (acquisition time)	15ms
sw (sweep width)	350ppm
o1p (13C carrier offset)	100ppm
o2p (1H carrier offset, optimize)	-5/+5ppm
d1 (recycle delay)	3-5s
pl1 (13C CP field pl)	50kHz, 0/-3dB
pl2 (1H CP field pl, optimize)	8/1dB
pl12 (1H decoupling)	100kH (~0dB)
p1/pl11 (pulse/pl to calibrate 13C)	

DCP: optimizing parameters

DCP is a very demanding pulse sequence in terms of power handling.

Start with a model compound (u-gly)

Optimize 1H/15N cross polarization, and measure 15N/13C fields using CP90 experiment.

Use 1H decoupling field during $^{15}N/^{13}C$ CP such that $\omega_H > 2.5\omega_C$
Otherwise, polarization leaks from ^{13}C spin bath into 1H spin bath.

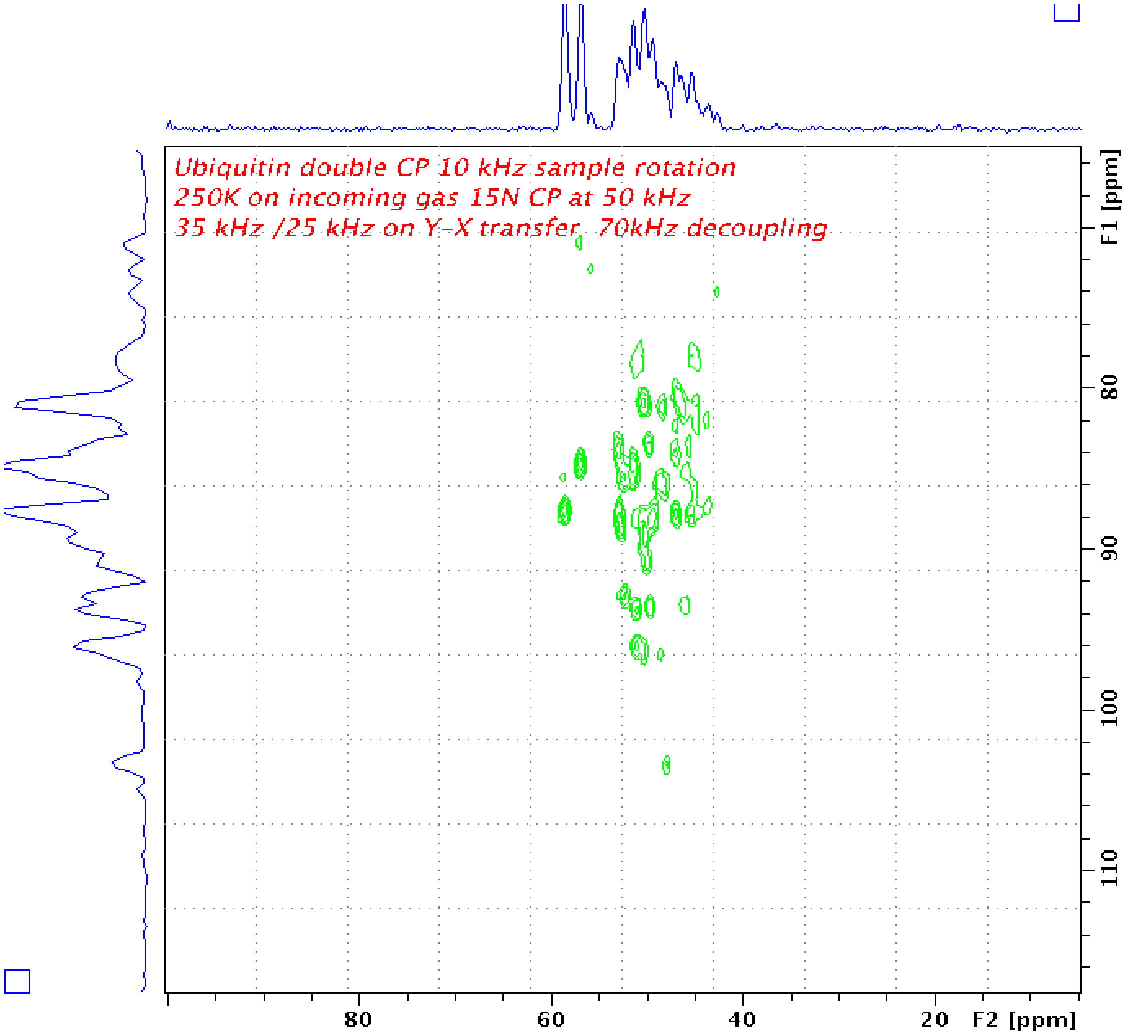
Make sure that $\omega_C =/ n\omega_{r,}$ n=1, 2, 3(?)
To achieve higher efficiency of 15N/13C polarization transfer

Within the limits described above, choose proper fields for 1H/15N/13C section
(for instance 80kHz/35kHz/25kHz at 10kHz spinning frequency)

Use 10-20% linear or tangential ramp for DCP.

For a set ^{13}C DCP power level, optimize ^{15}N pl starting with calculated values.

Ubiquitin 2D DCP spectrum



Ubiquitin 50ul, 4mm rotor, HXY probe, 750MHz spectrometer, 75kHz 1H decoupling, 35kHz ^{15}N field, 25kHz ^{13}C field, ns = 16, t=-30C

13C-13C correlation – RAD/DARR/PDSD

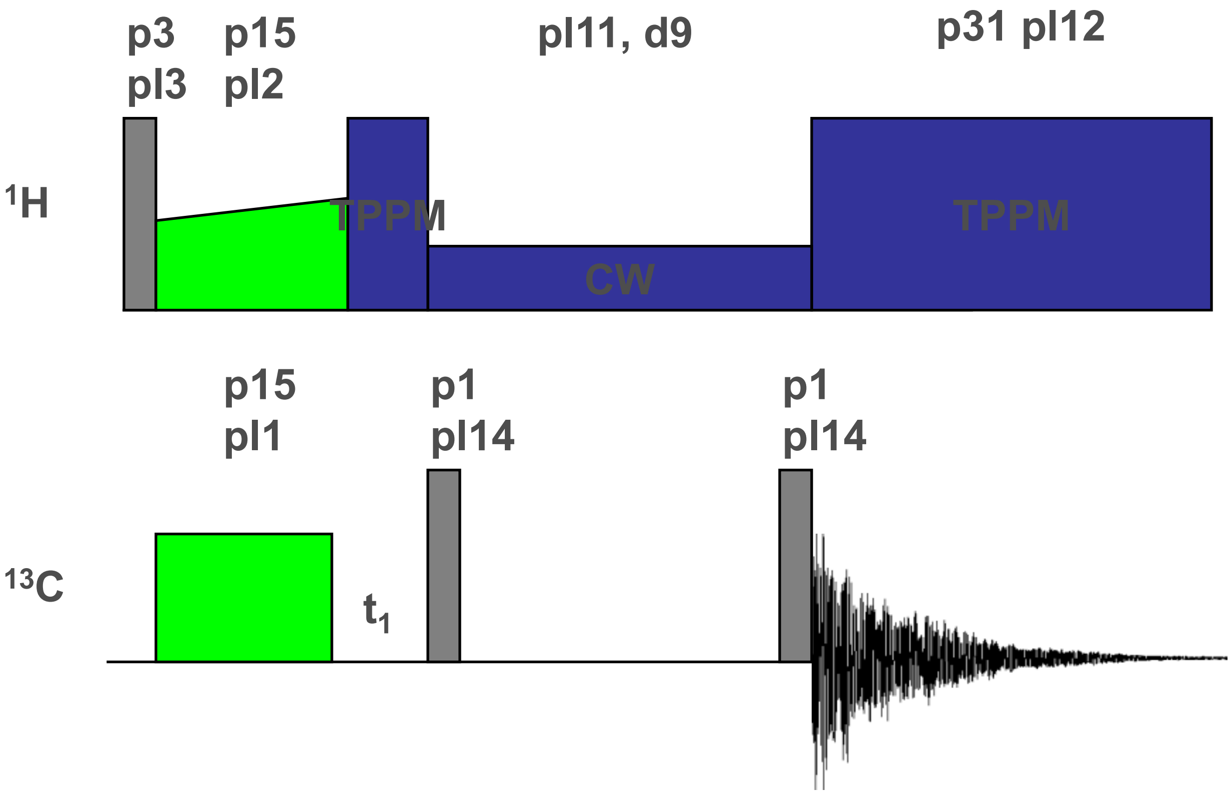
There are numerous 13C-13C correlation methods in solids.
We are not going to discuss them here.

RAD/DARR/PDSD set of experiments

easy to implement
not hardware demanding
Work well in a u-labeled system
Recover all interactions within 4+ A

non-specific
can't measure distance

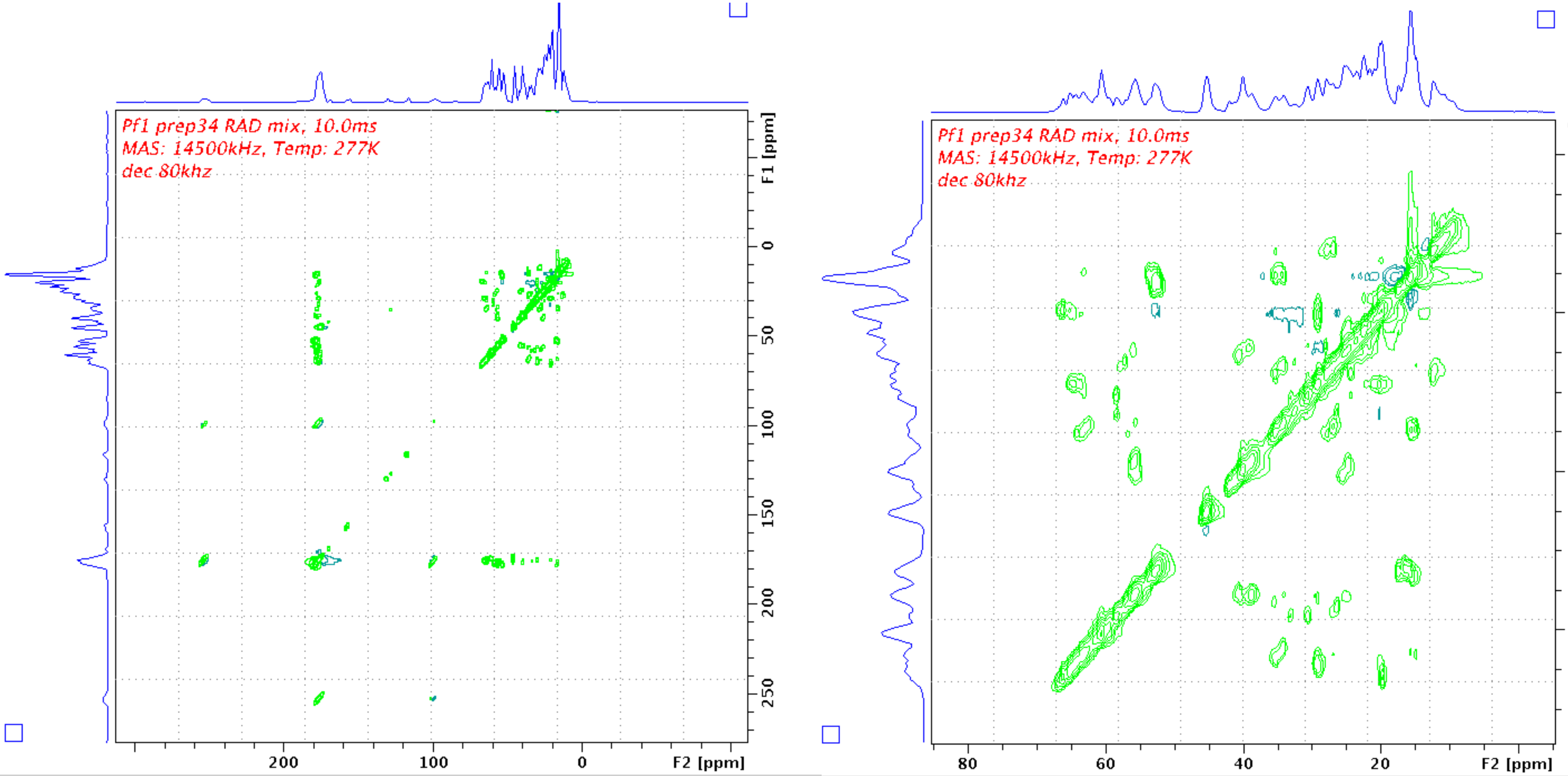
RAD/DARR pulse sequence



Radmix.lorieau

13C 90 pulse	p1
13C 90 pl	pl14
1H 90 pulse	p3
1H 90 pl	pl3
Spin locking time	p15
Spin locking 1H pl	pl2
Spin locking 13C pl	pl1
Low power CW spin diffusion field	pl11
Low power CW spin diffusion delay	d9
TPPM decoupling (=SF)	pl12

Pf1 DARR spectrum



Pf1, 4mm rotor, HXY probe, 750MHz spectrometer, 80kHz 1H decoupling, 50kHz ^{13}C field

3D DCP-DARR pulse sequence