NMR course at the FMP:

Basic concepts

spectrometer

02.03.2009

Peter Schmieder

AG Solution NMR

The program

Spectrometer components

Getting started

Doing a measurement

The dynamic range

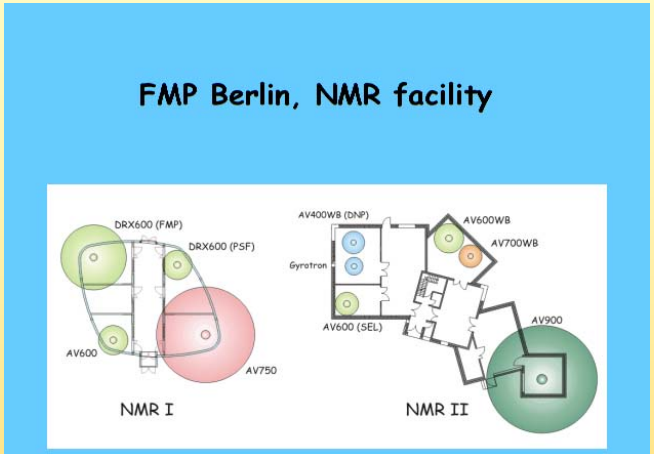
Solvent supression

Spectrometer components

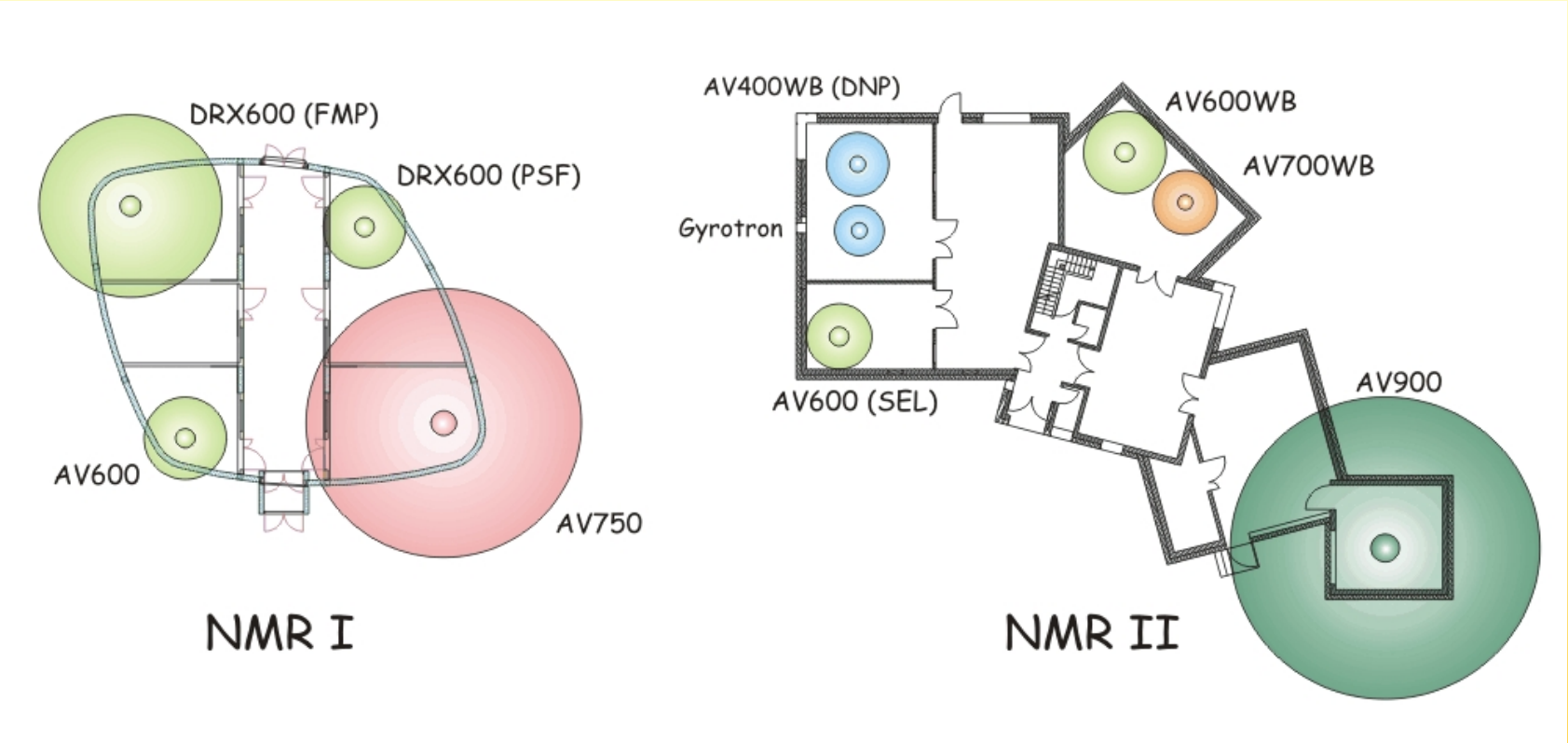
Spectrometer components

NMR I

NMR II

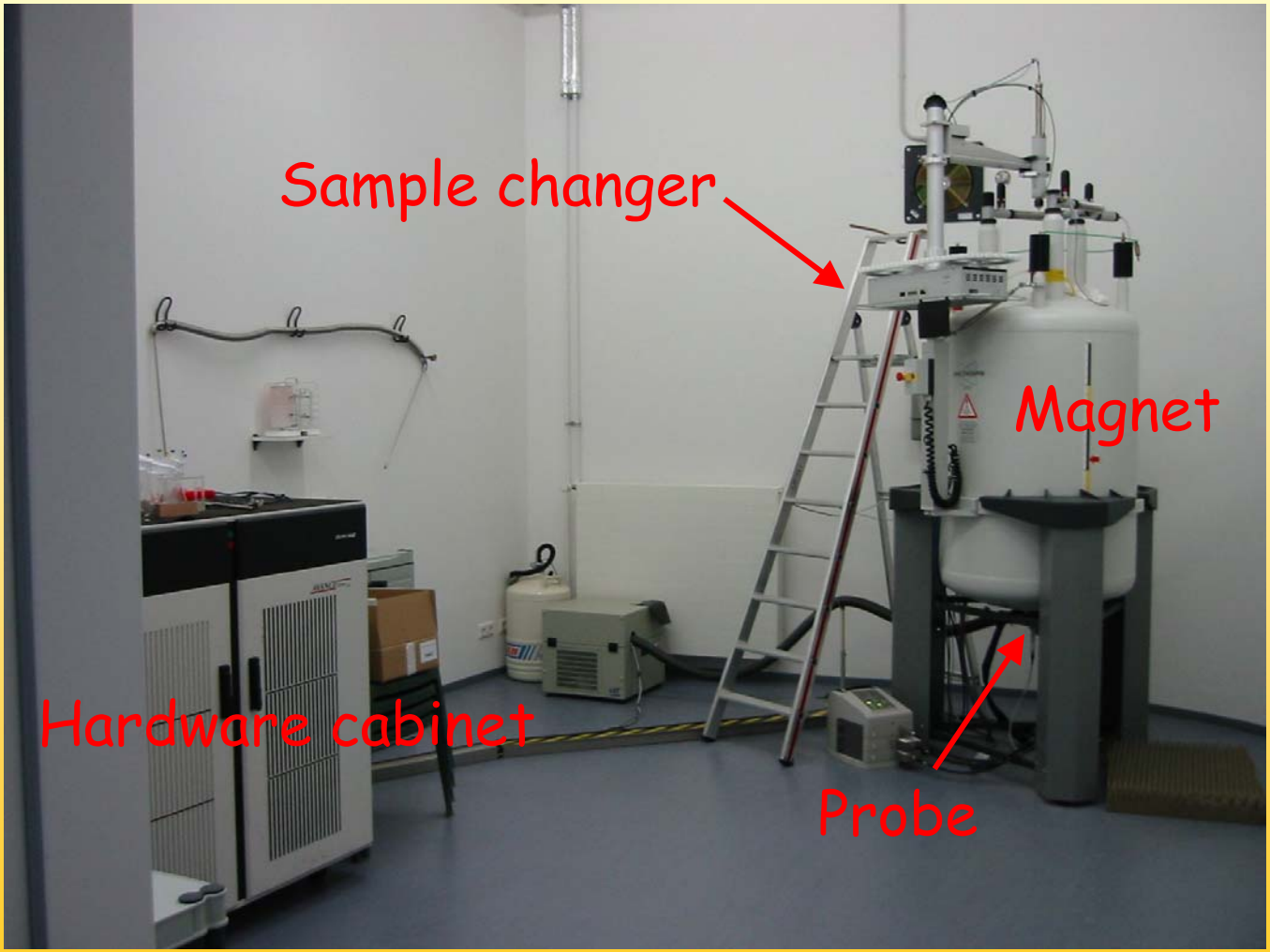


Spectrometer components



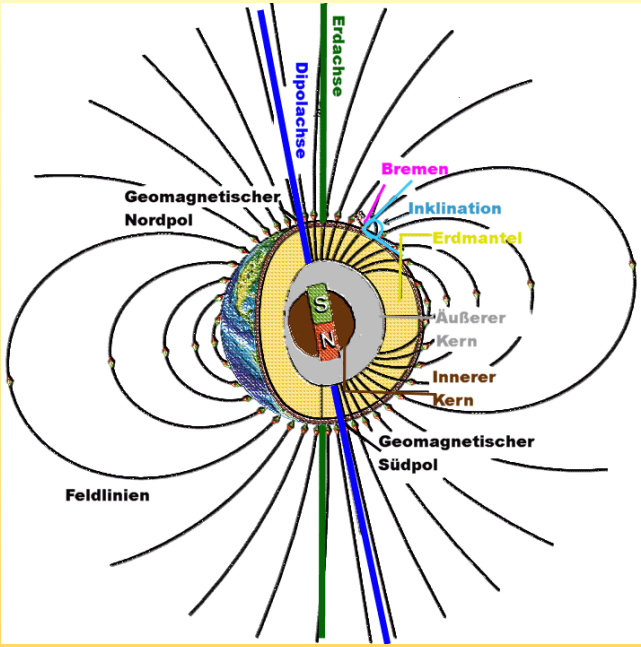
+ a AV 300 at the TRH and a AV400WB in the basement

Spectrometer components



Spectrometer components

Magnet

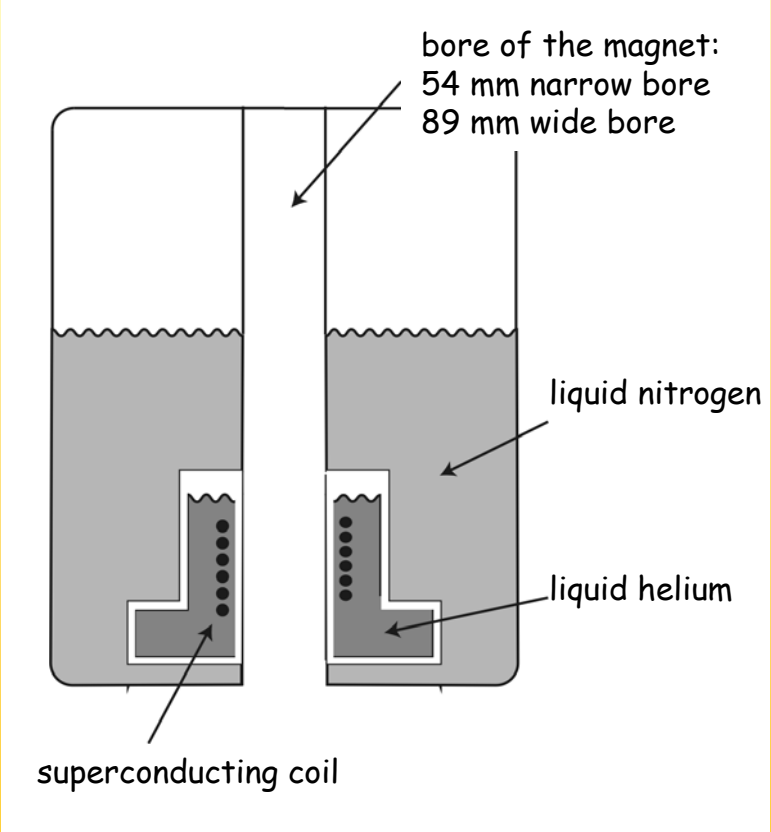


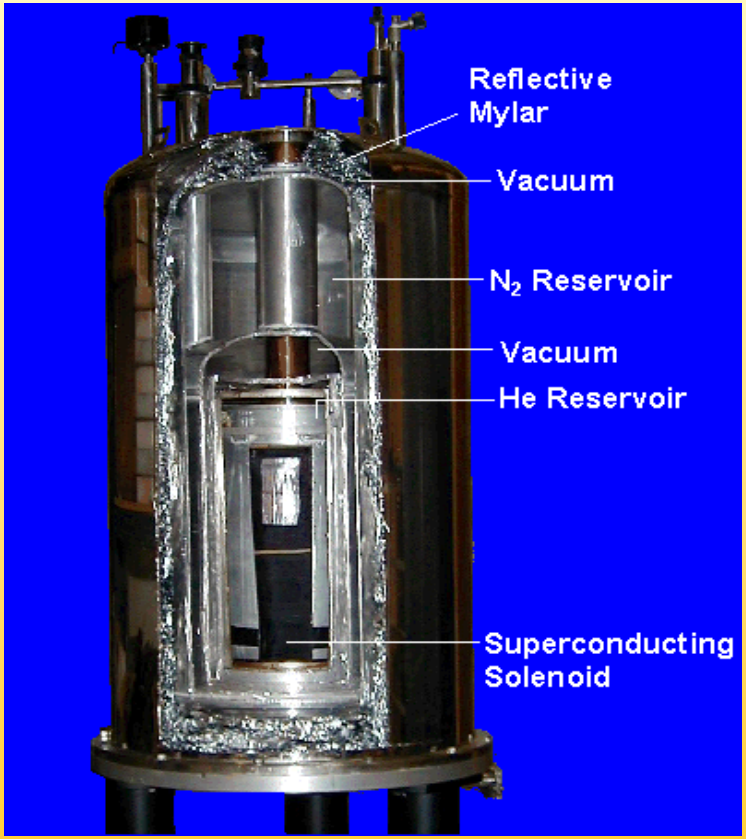
B_0 [Tesla]	ν_0 [MHz]
1.4	60
5.9	250
9.4	400
14.1	600
21.2	900

The earth magnetic field has a strength of 30-60 μT (0.3 –0.6 Gauss)

Spectrometer components

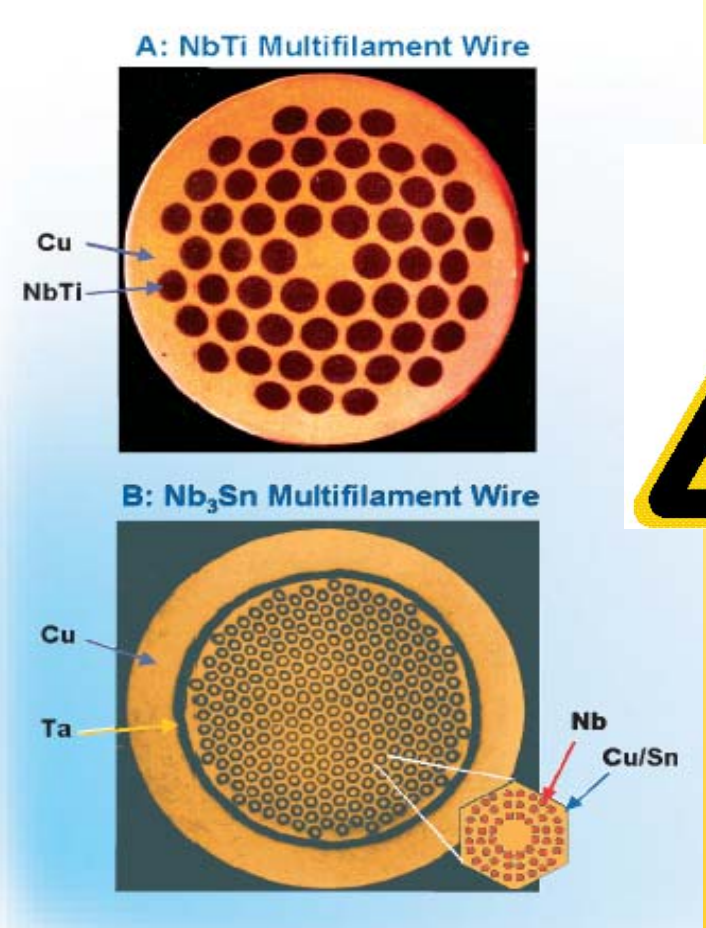
Magnet



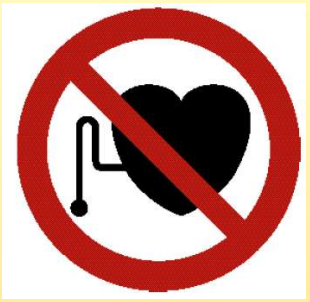


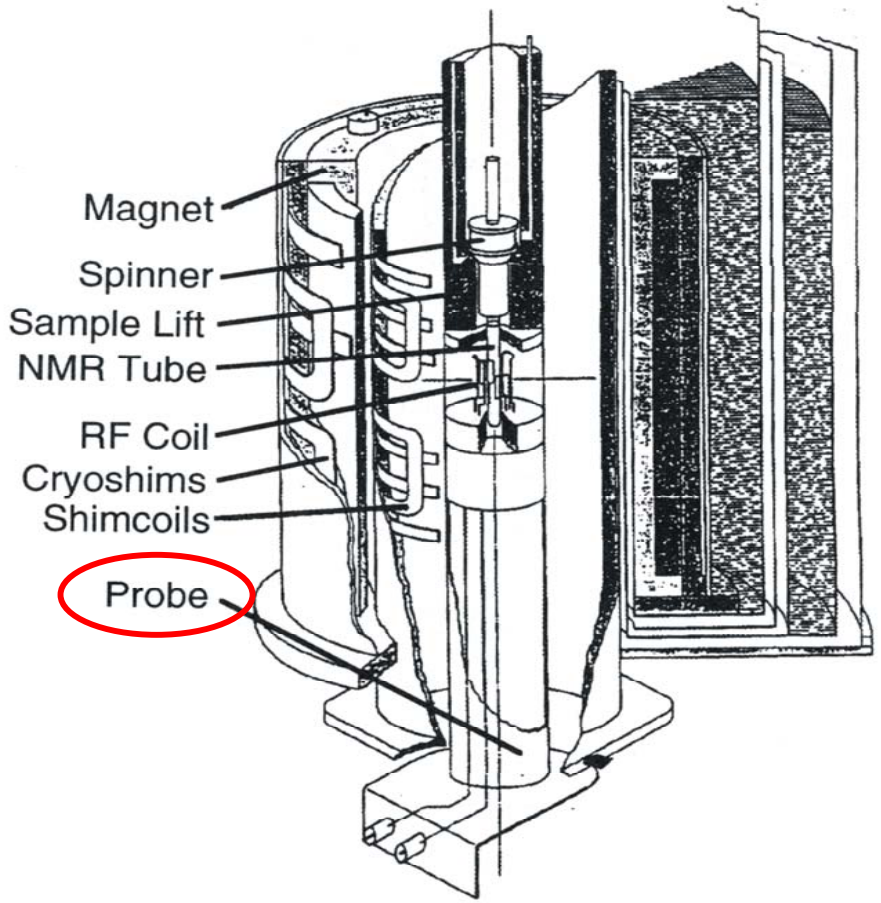
Spectrometer components

Magnets are fascinating but also dangerous



Spectrometer components

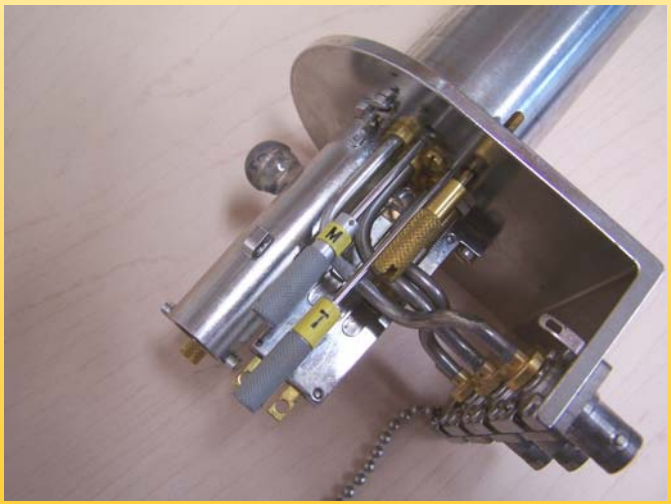




Spectrometer components

Cryo-probe

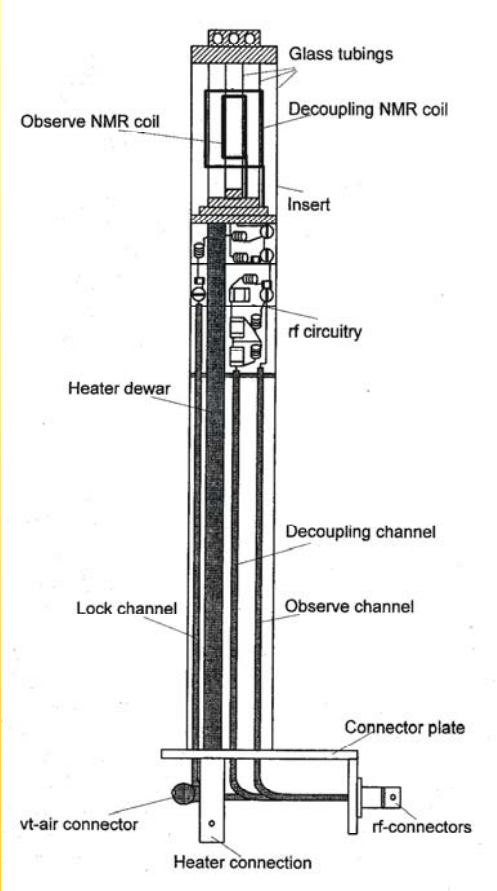
ATM-probe

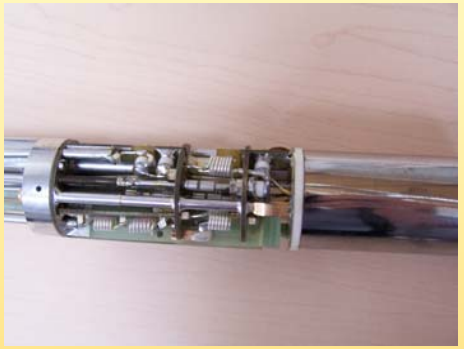


RT-probe

Spectrometer components

the probe

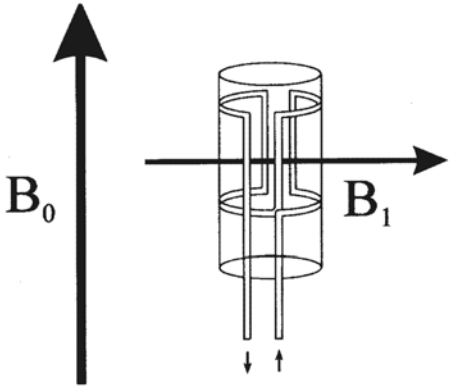




Spectrometer components

the probe

Helmholtz-Coil

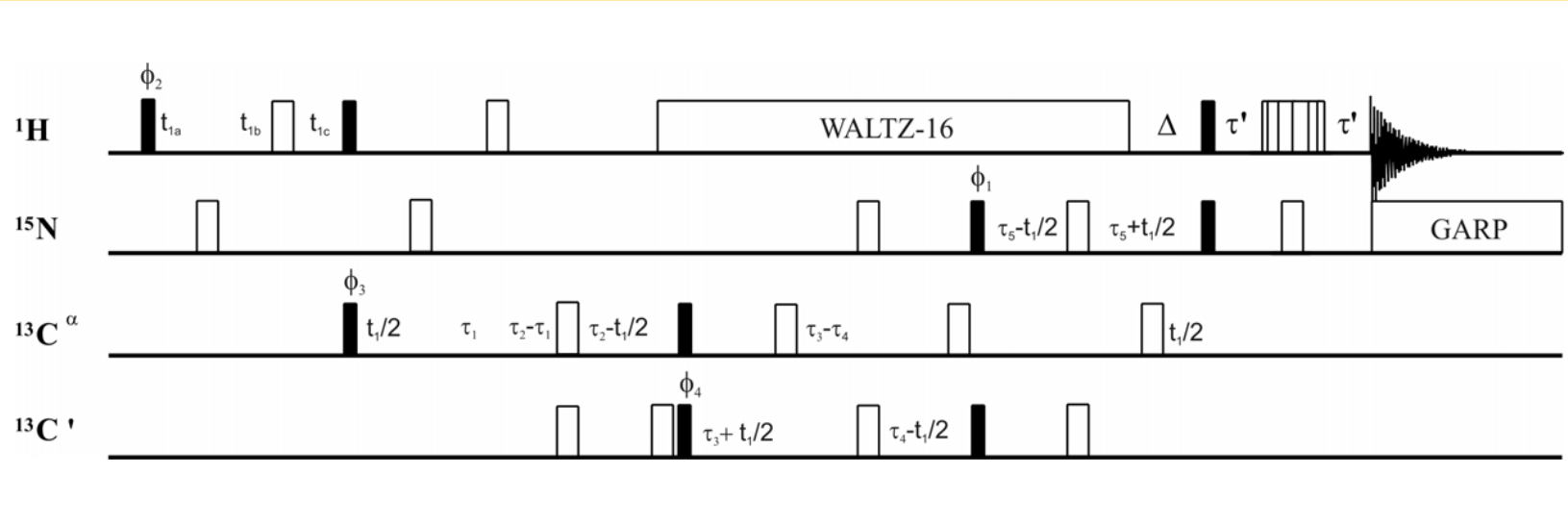


RF in
and out

Spectrometer components

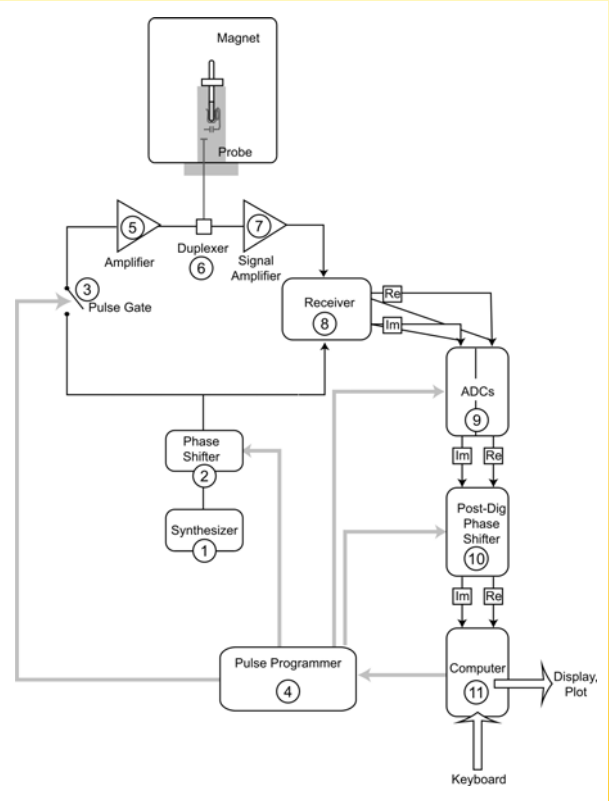
the electronics

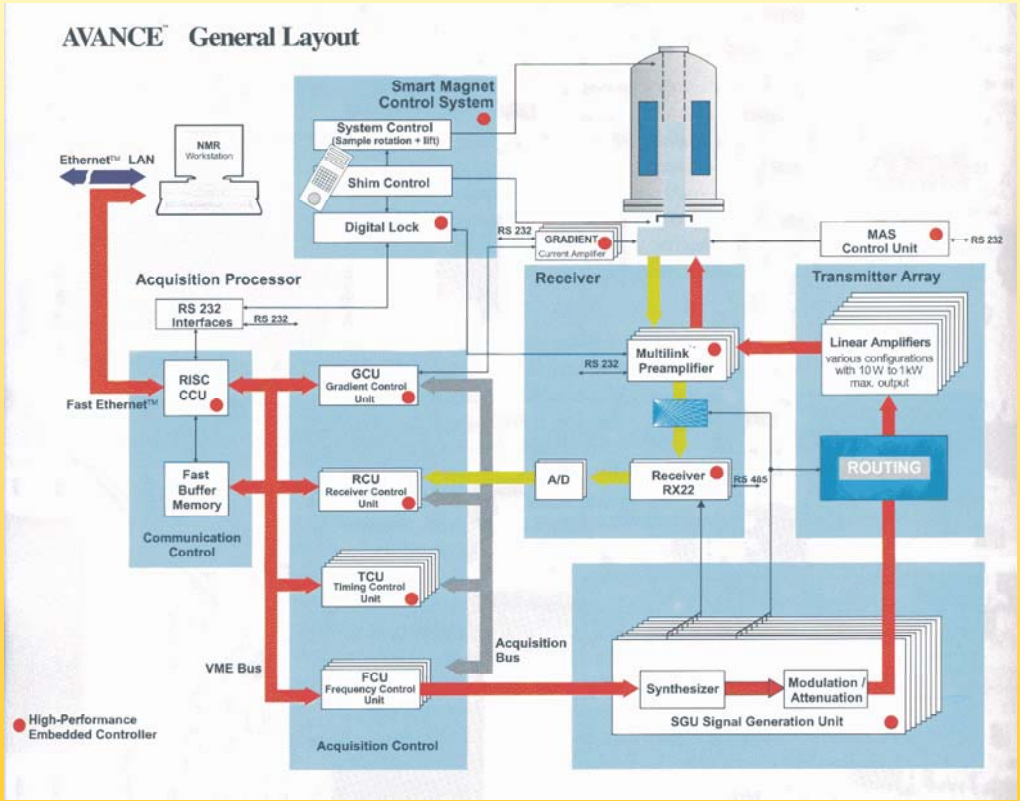
Since we want to do quite elaborate NMR experiments
we need hardware that is able to do them



Spectrometer components

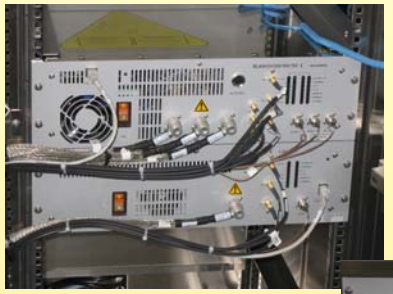
the electronics

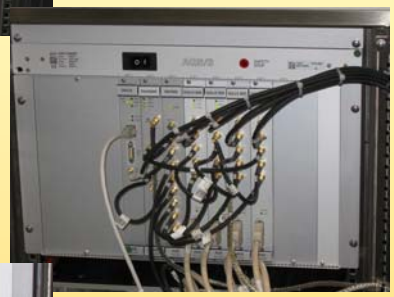


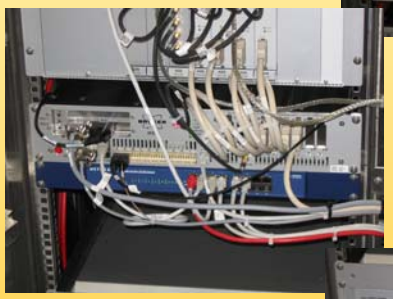


Spectrometer components

the electronics

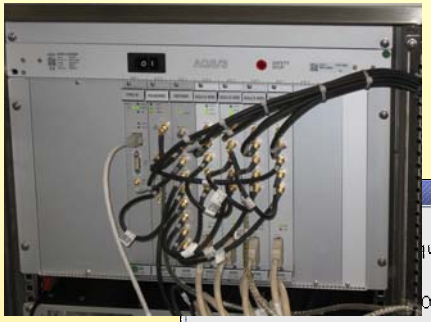


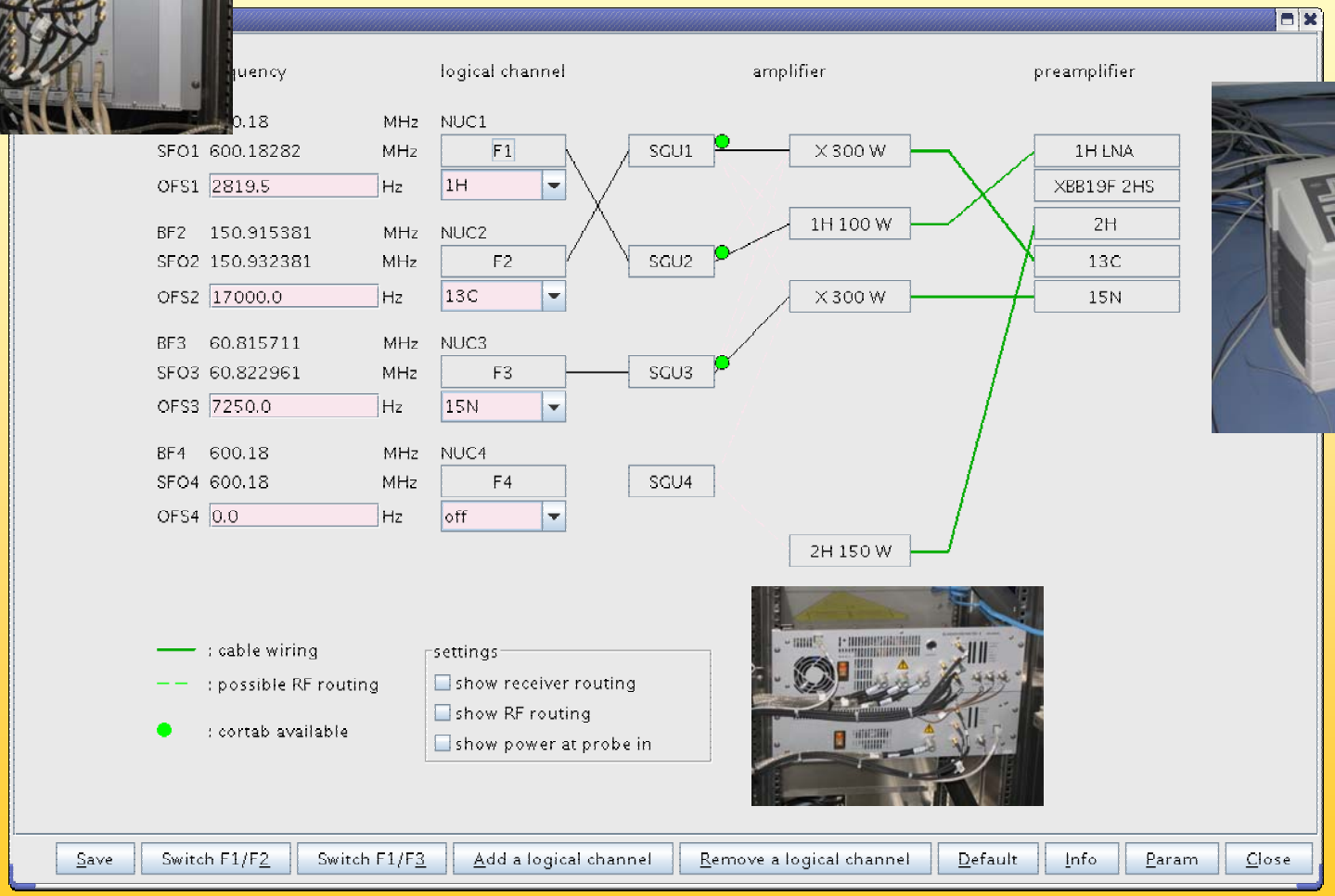




Spectrometer components

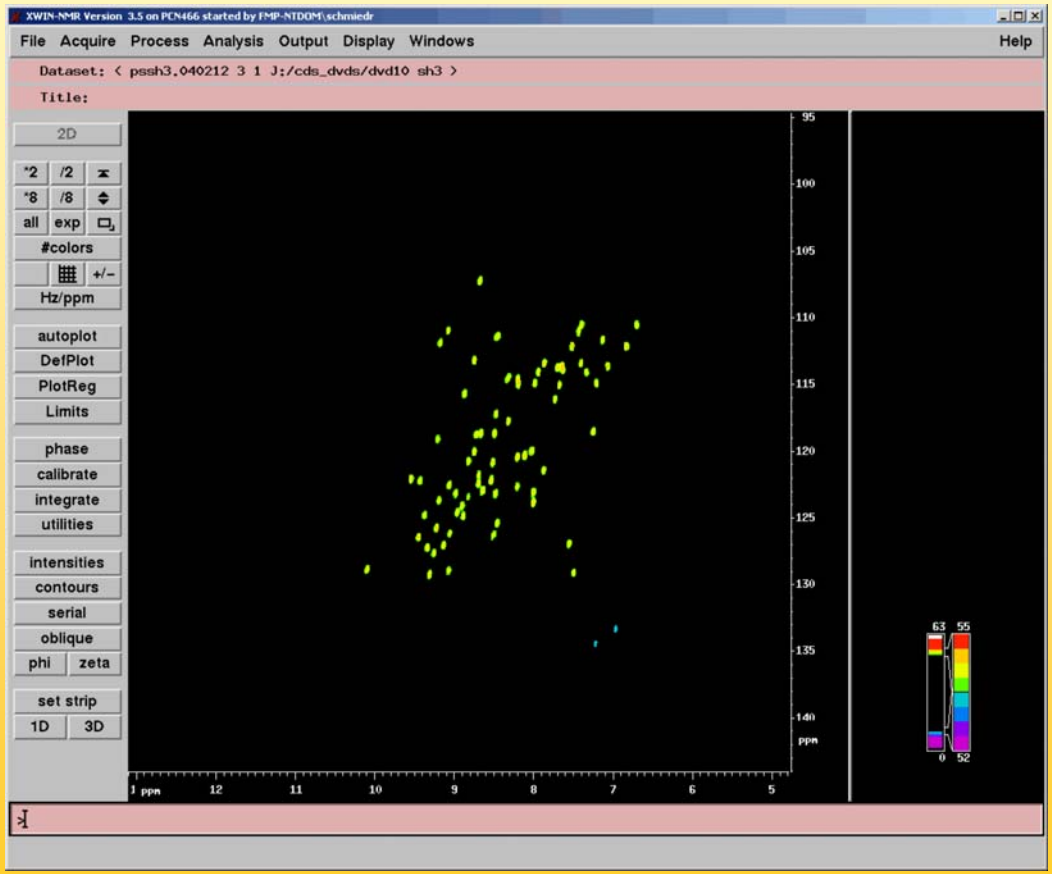
the electronics





Spectrometer components

software on a linux or (rarely) windows system




```
;zgdc
;avance-version (03/04/17)
;1D sequence with decoupling

#include <Avance.incl>

"d11=30m"

1 ze
  d11 pl12:f2
2 30m do:f2
  d11 cpd2:f2
  d1
  p1 ph1
  go=2 ph31
  30m do:f2 mc #0 to 2 F0(zd)
exit


ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1
```

Getting started

Getting started

Getting started

Adjusting the sample depth is important for good homogeniety

Depth is usually 20 mm on the more modern spectromter, it is still 18 (fmp600) and 19 (psf600) on older ones

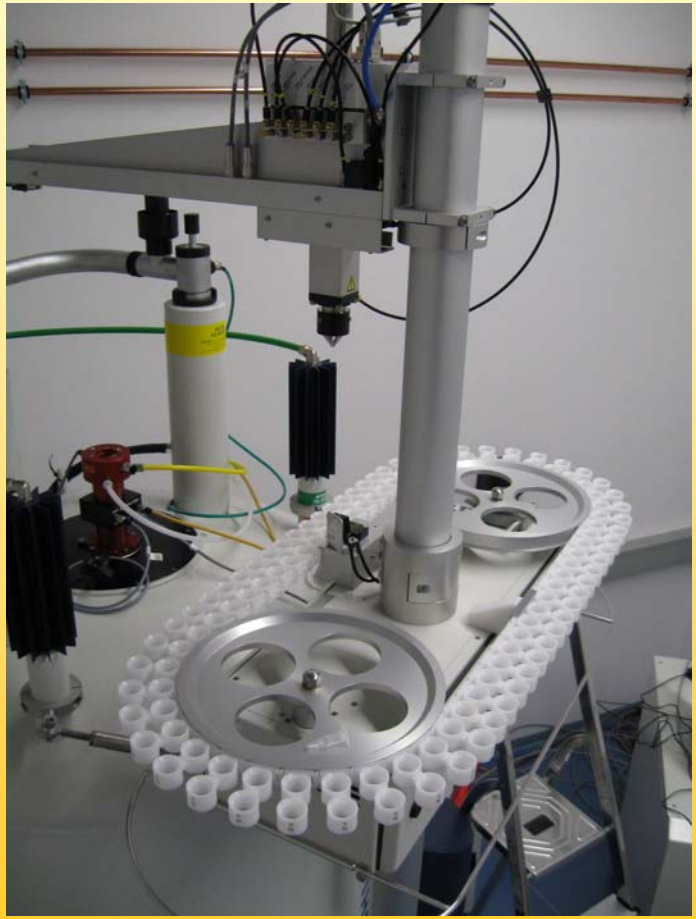
Getting started

NMR experiment can be run in different ways

- Routine analysis is usually done in full automation, using a sample changer and "ICON-NMR"
- Screening is also done using a sample changer and ICON-NMR but with a little more effort to prepare the run
- "Normal" measurements are done "by hand" with several steps from putting in the sample to the actual start

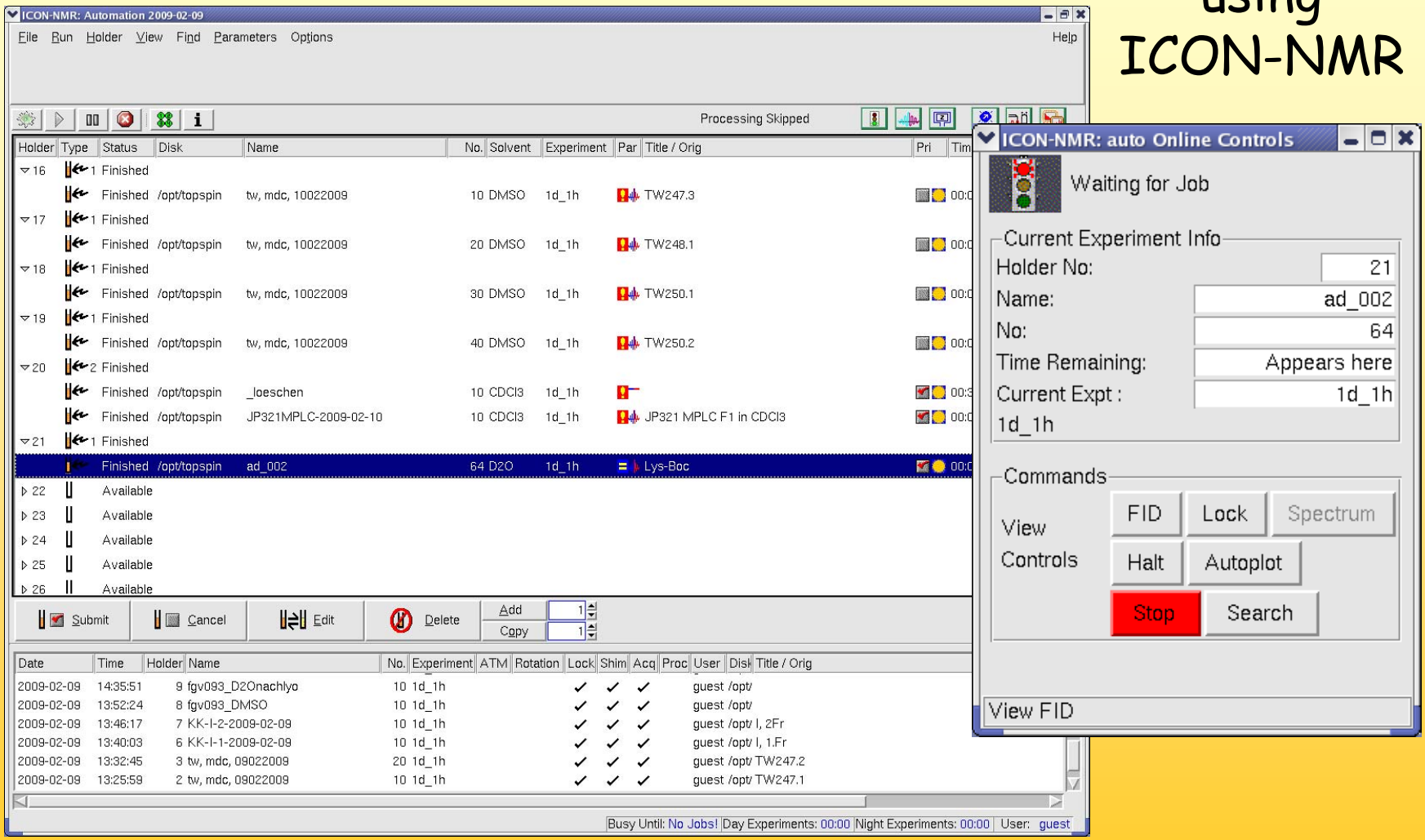
Getting started

Sample changer



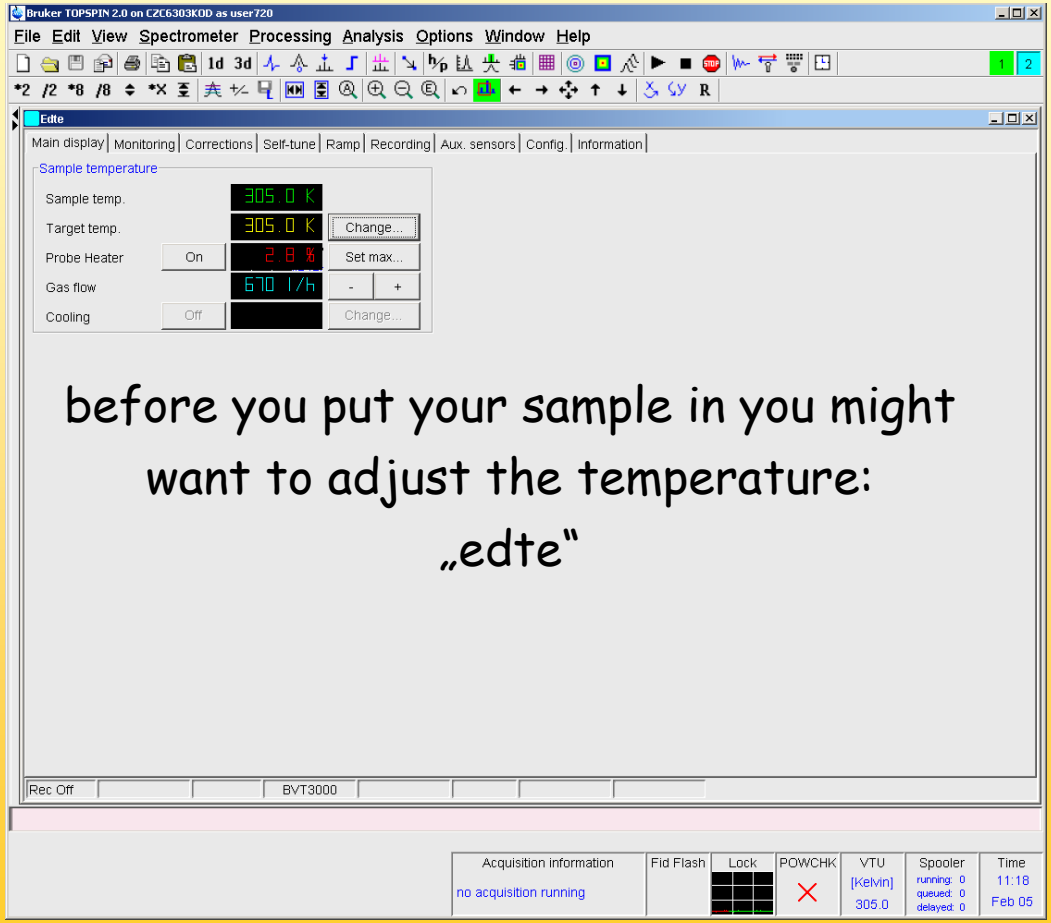
Getting started

using ICON-NMR



Getting started

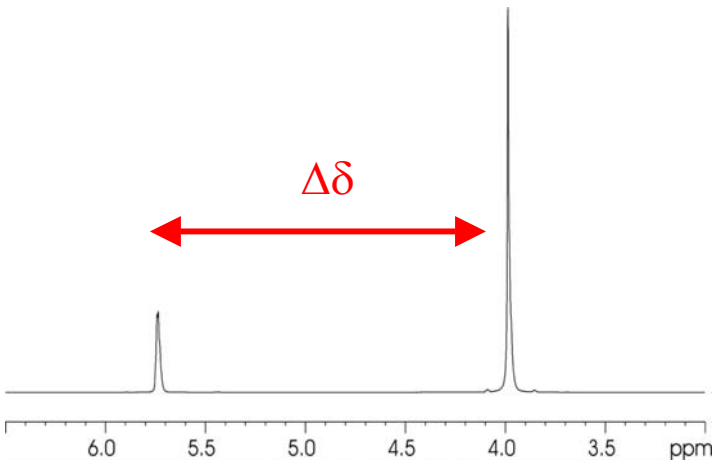
adjusting the temperatur

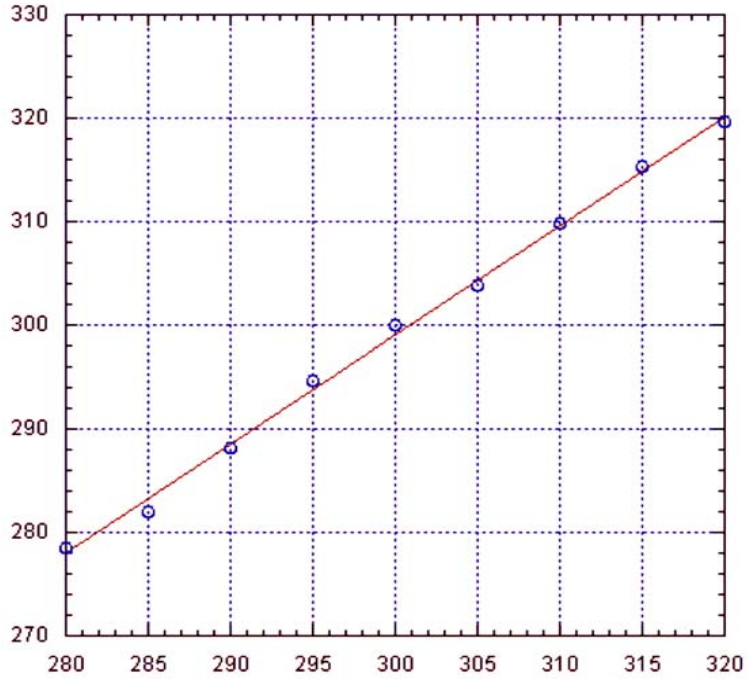


before you put your sample in you might want to adjust the temperature:

„edte"

Getting started





$\Delta\delta = \delta(OH) - \delta(CH_3)$

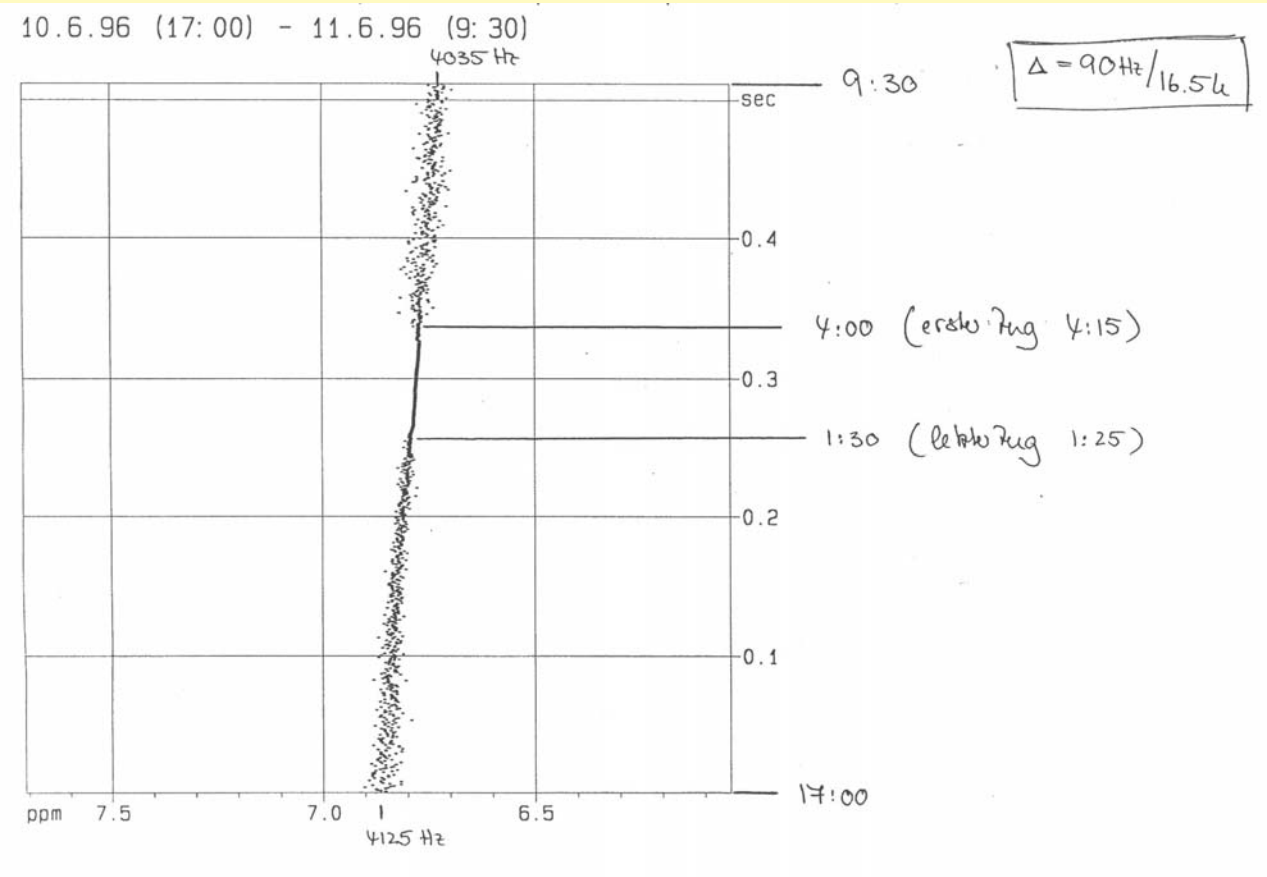
$T_{real} = -17.726 + 1{,}0582 \times T_{BVT}$

$T_{real}\,[K] = 403 - 29.53\Delta\delta - 23.87\,(\Delta\delta)^2$

300 K at BVT is 299.76 K

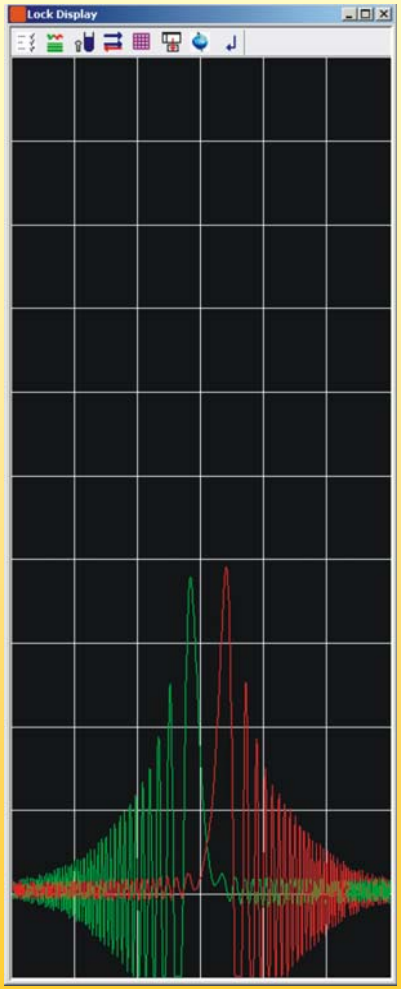
Getting started

the lock



Getting started

the lock



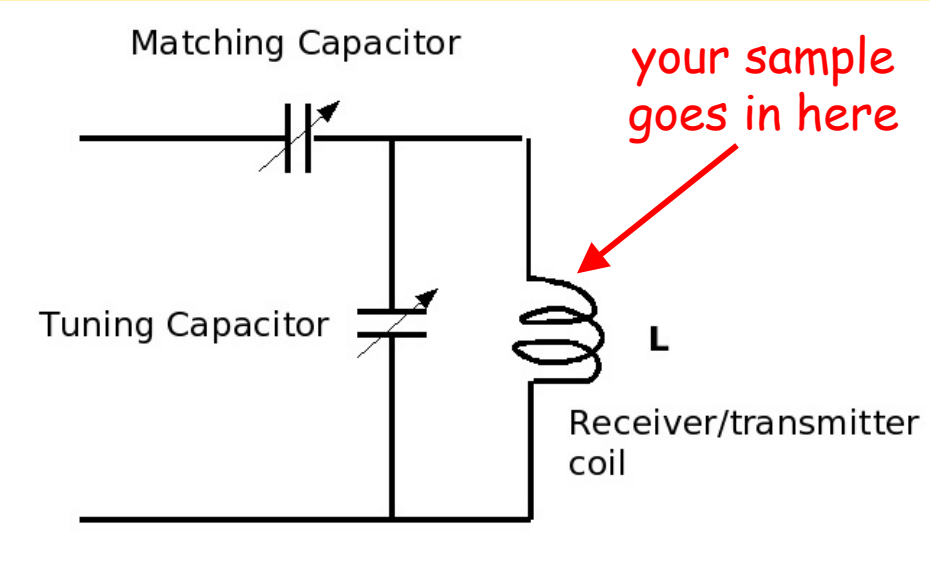
Solvents table

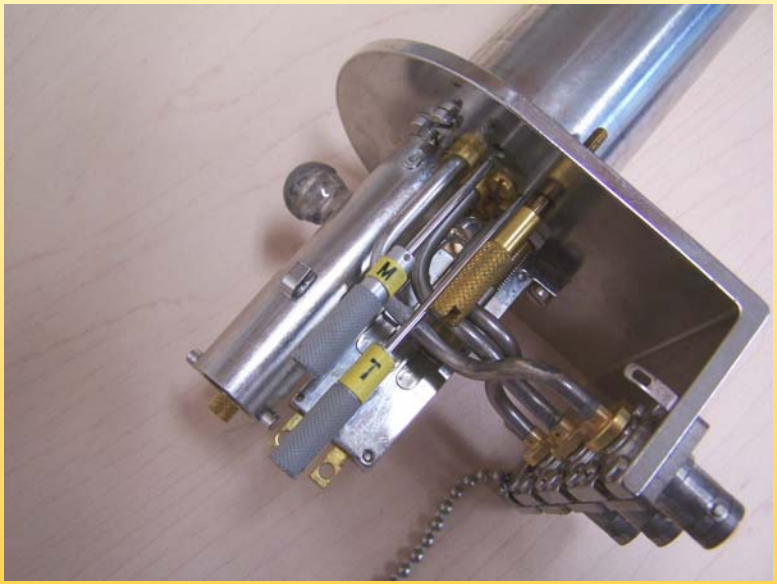
Solvent	Description
Acetone	acetone-d6
C6D6	benzene-d6
CD2Cl2	methylenechloride-d2
CD3CN	acetonitrile-d3
CDCl3	chloroform-d
CH3CN+D2O	HPLC Solvent (Acetonitril/D2O)
D2O	deuteriumoxide
DEE	diethylether-d10
Dioxane	dioxane-d8
DME	dimethylether-d6
DMF	dimethylformamide-d7
DMSO	dimethylsulfoxide-d6
EtOD	ethanol-d6
H2O+D2O	90%H2O and 10%D2O
H2O+DMSO	Evotec special
HMPT	hexamethyphosphotriamid
MeOD	methanol-d4
MeOH+D2O	HPLC Solvent (Methanol/D2O)
Pyr	pyridine-d5
THF	tetrahydrofurane-d4
Tol	toluene-d8

OK Cancel

Getting started

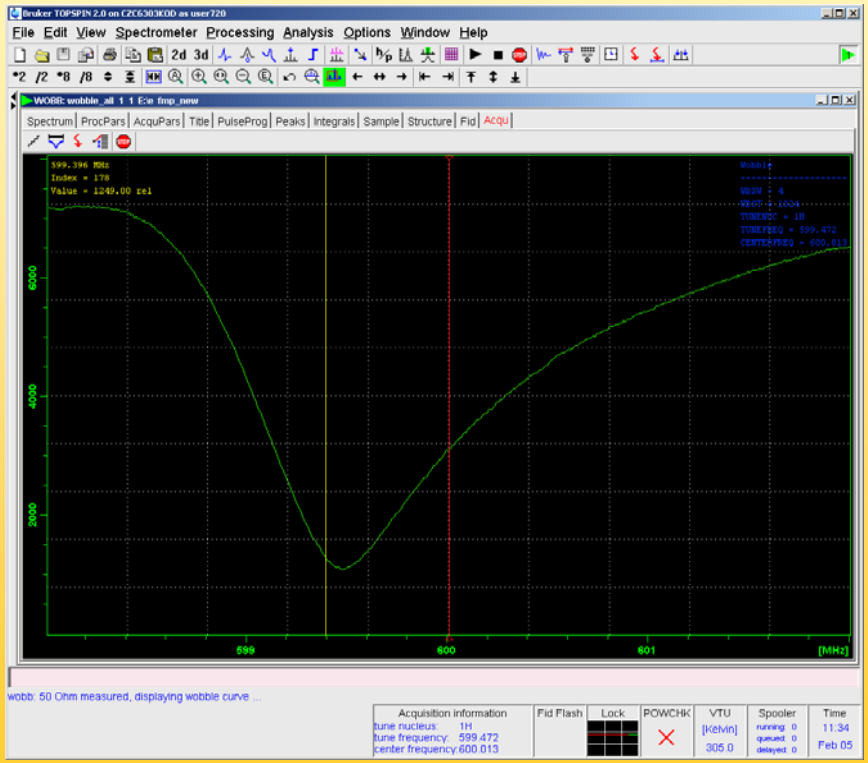
tuning and matching

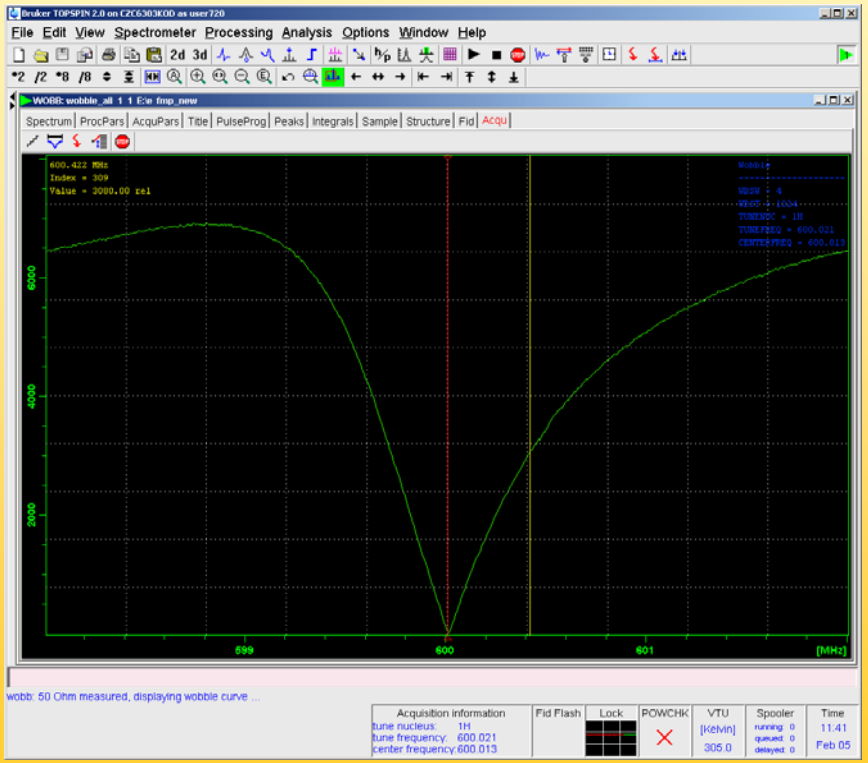




Getting started

tuning and matching
the wobbeling procedure





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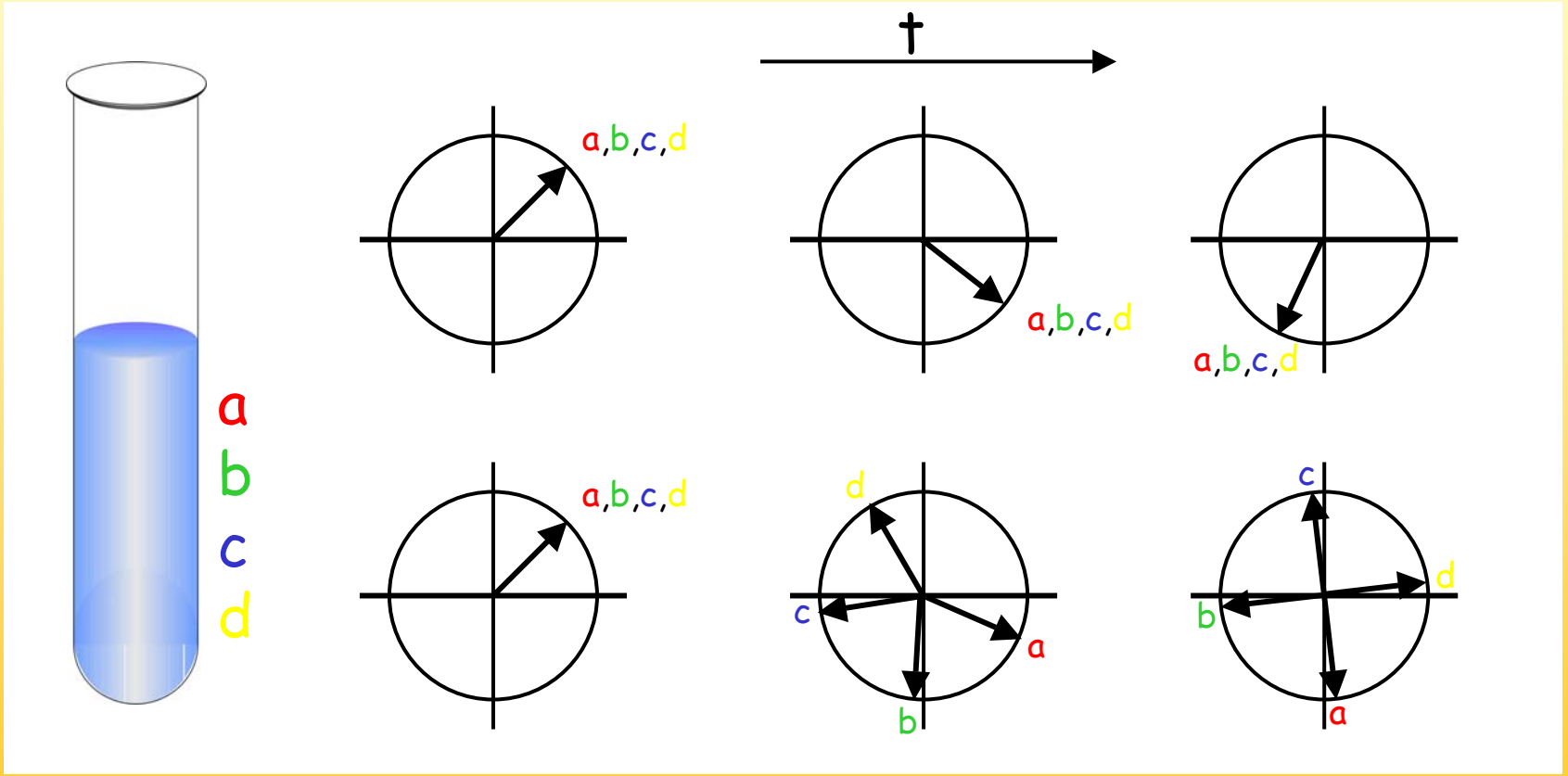
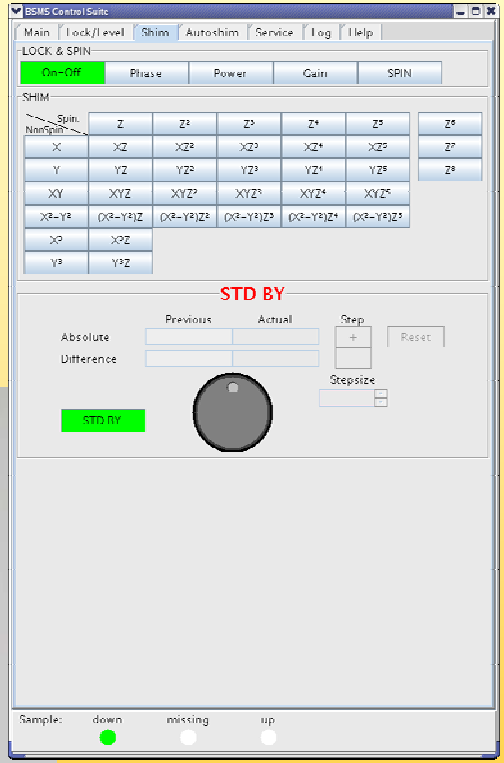
Getting started

shim and homogeniety



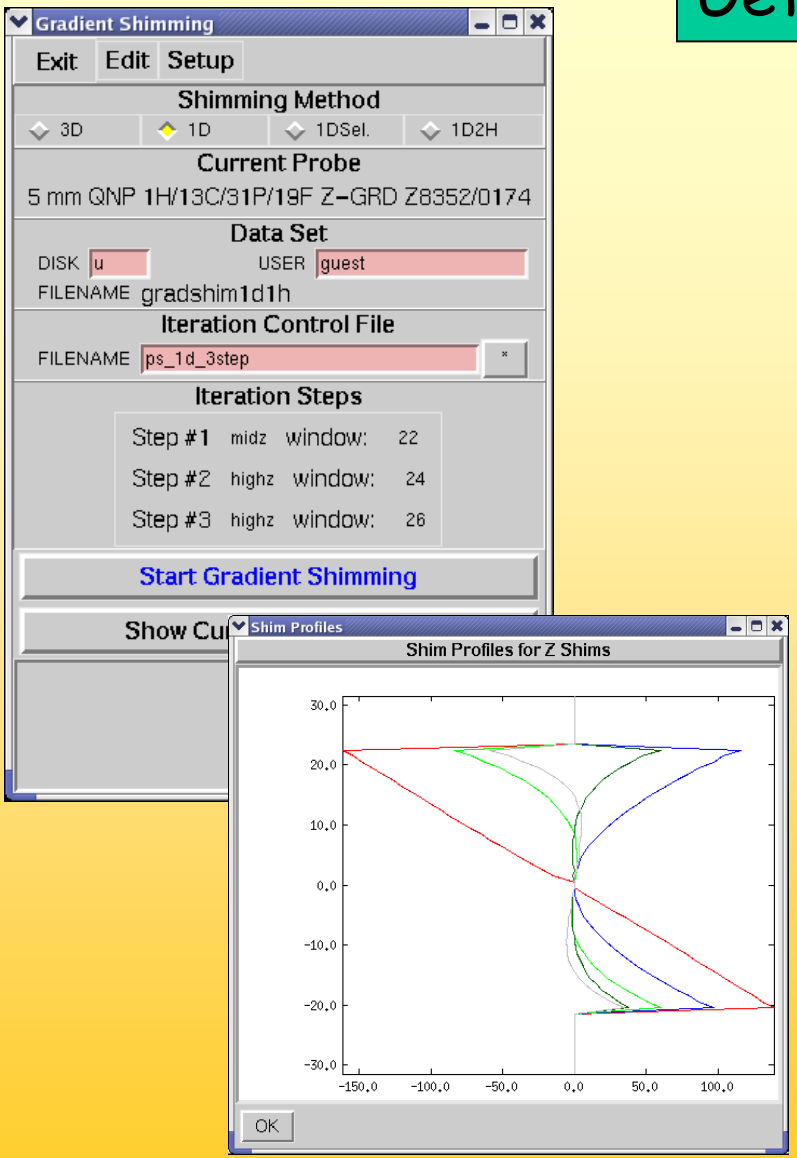
Getting started

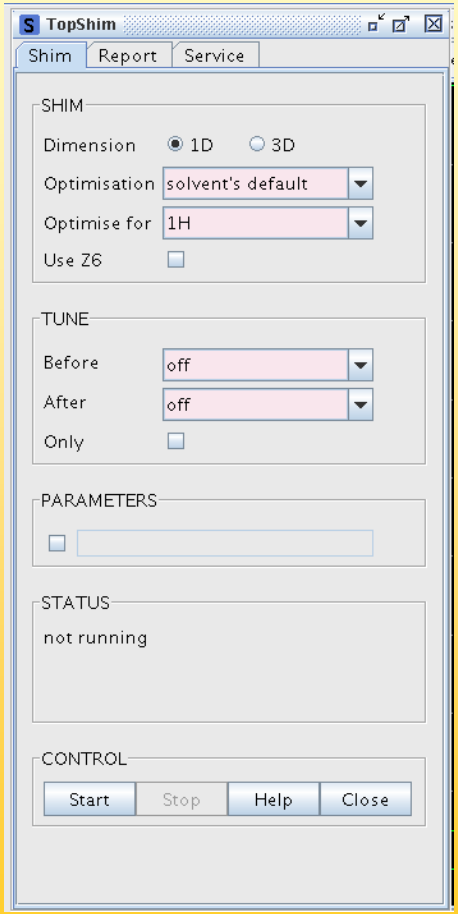
shim by hand

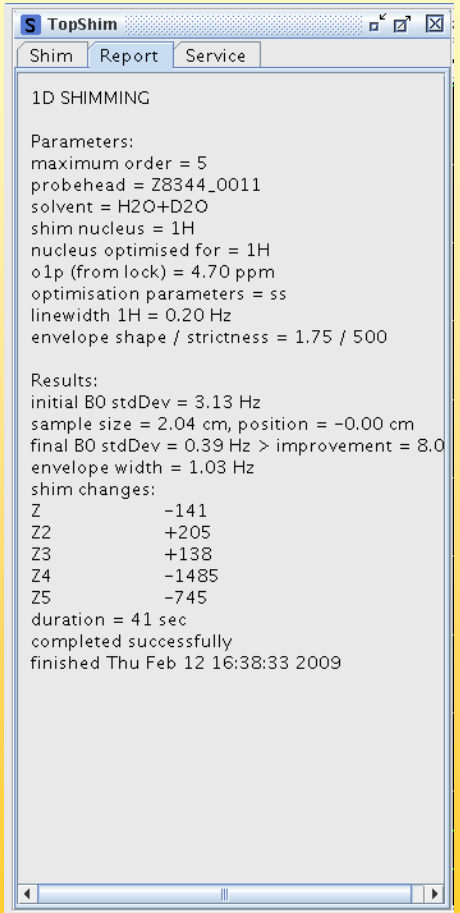


Getting started

automated shimming







Doing a measurment

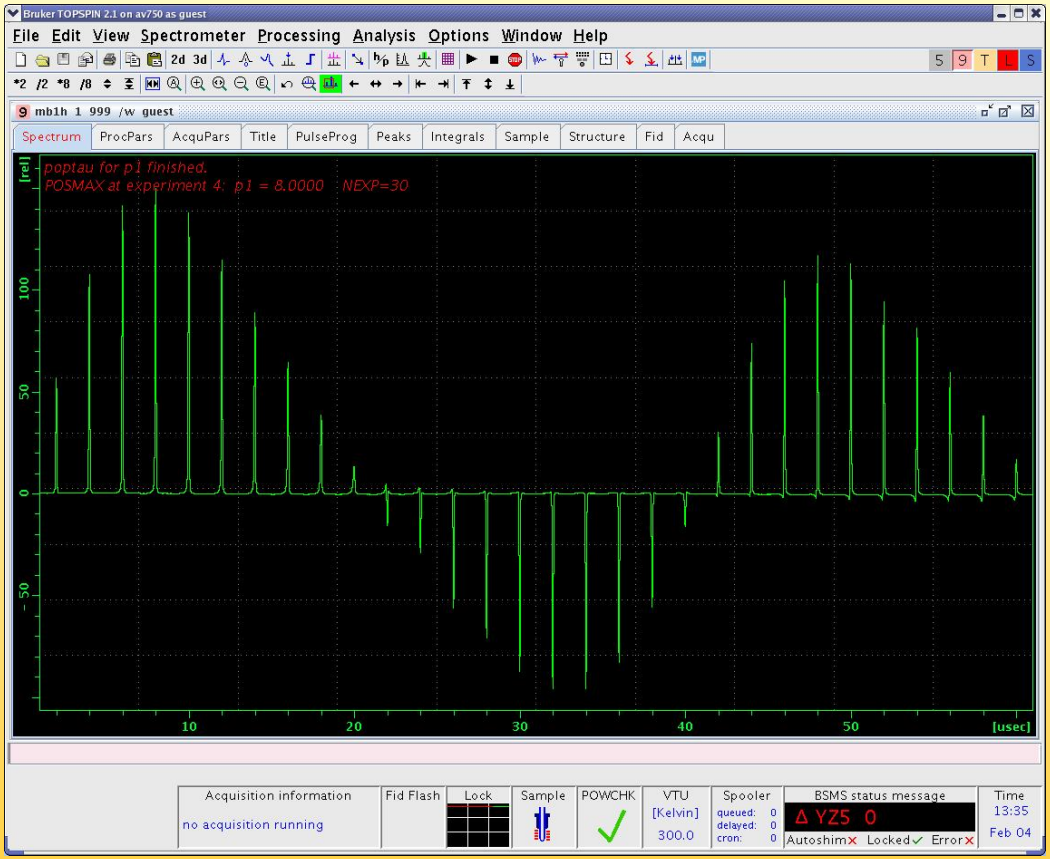
Doing a measurment

When working in organic solvent, you now have to determine a pulse and then you can get going using the „mf“, “getprosol”, “plop” and “rga” commands

When working in water then you have to optimize the solvent suppression also

Doing a measurment

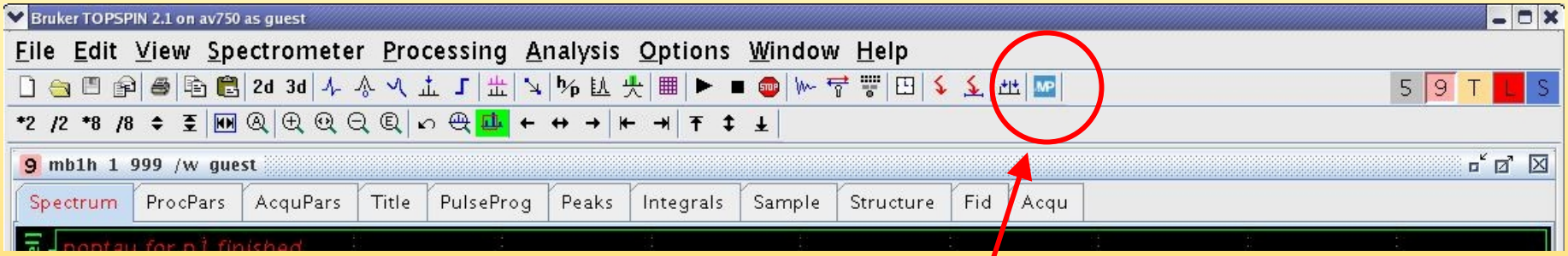
determining a ^{1}H-pulse in organic solvent



heteronuclear pulses are best determined on a separate sample, this is done regularly and can be loaded via the PROSOL mechanism

Doing a measurment

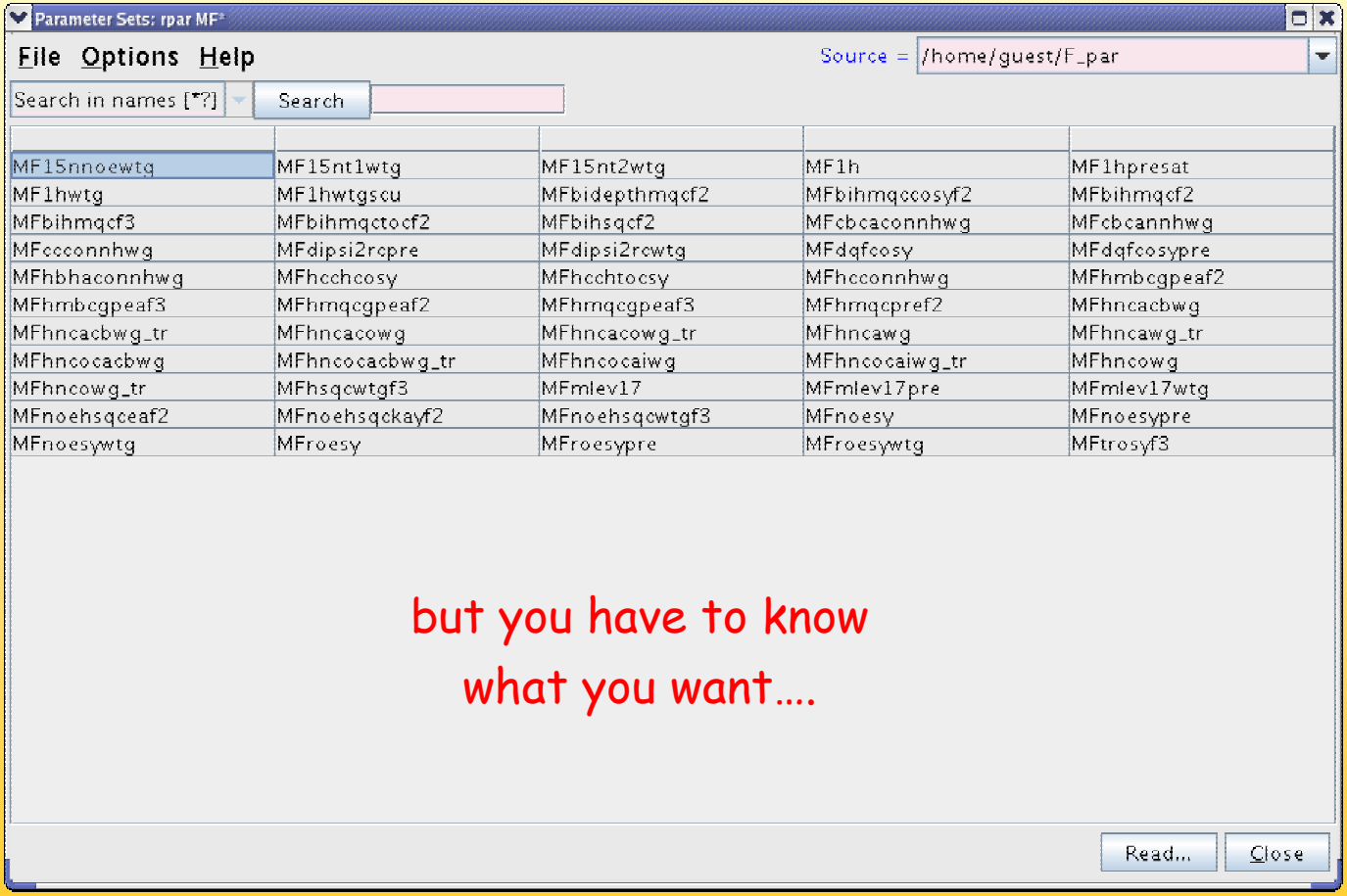
Once you have your pulse, you can use the "mf" setup mechanism by either typing "mf" or



by clicking on the little FMP-button (if availible)

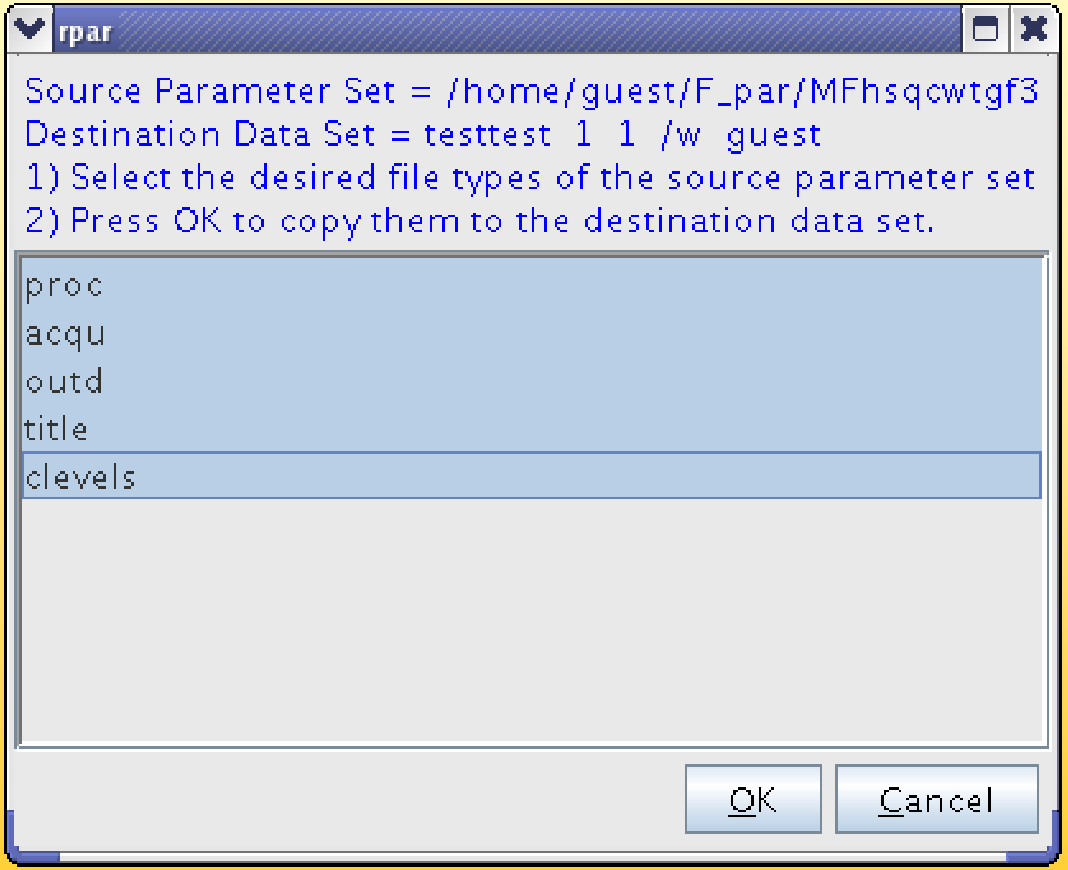
Doing a measurment

Then you can choose an experiment…



Doing a measurment

...and load the parameters



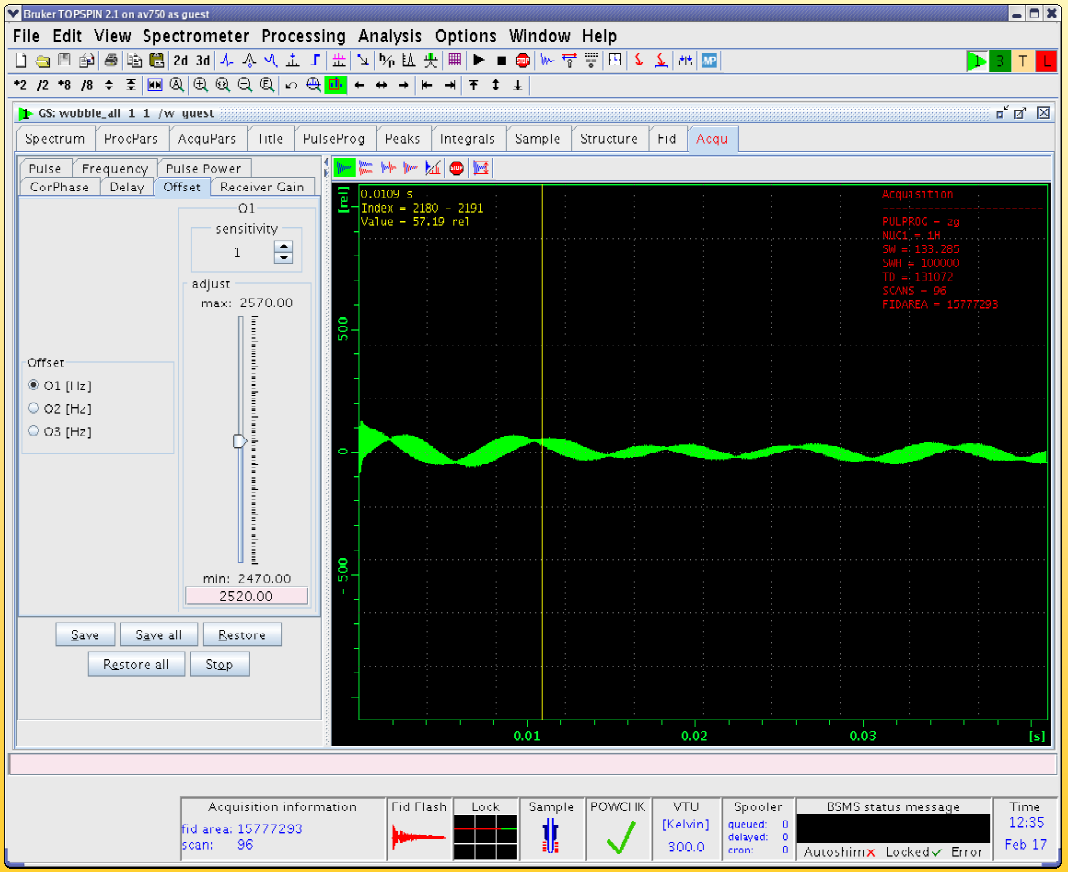
Doing a measurment

You load the parameters, then you type "getprosol" to load the current values for the hetero-pulses and powerlevels and for the gradients

After having done that you can repeat that for all the experiments you want to do and transfer the proton pulse, power level and the o1 by using "plop"

Doing a measurment

In water you have to check for the water frequency by doing a presaturation experiment in the "gs"-mode



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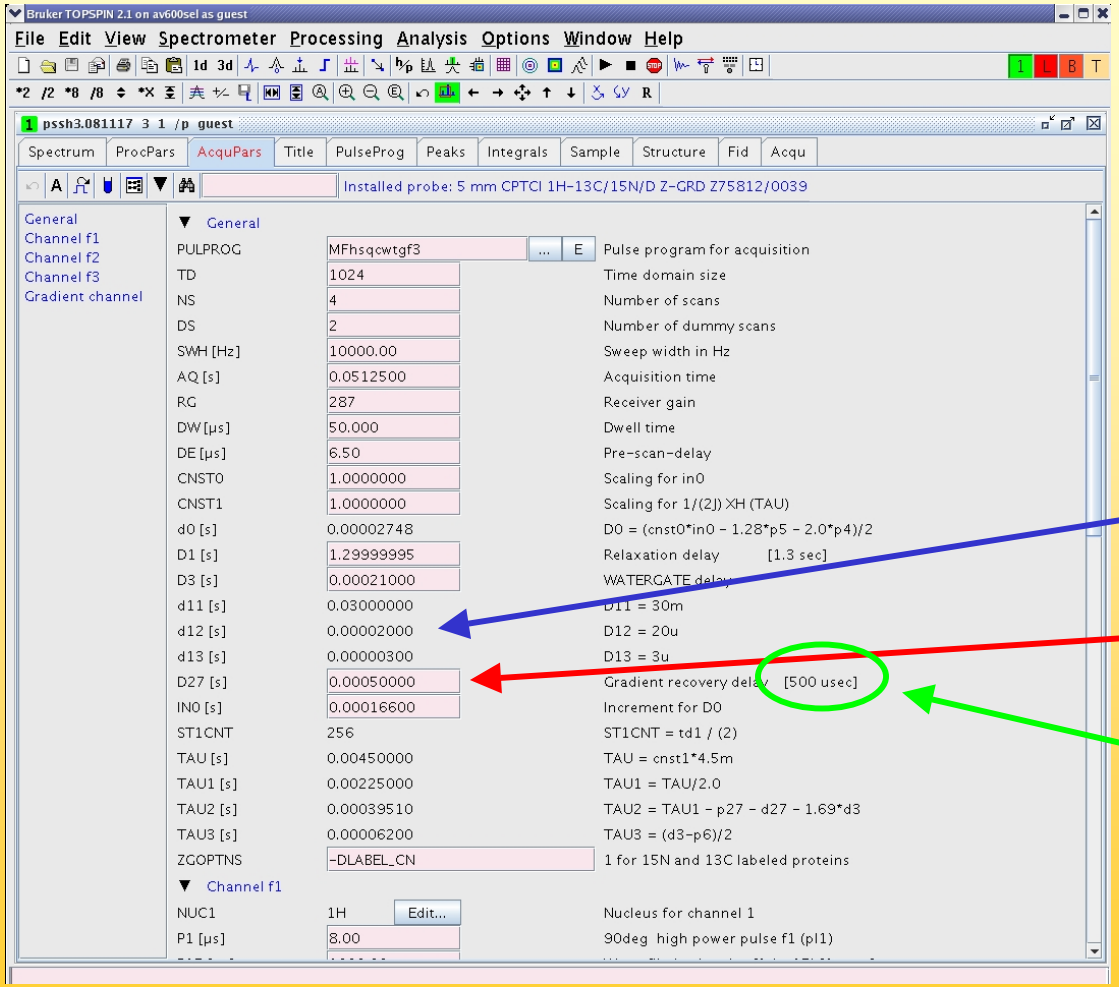
Doing a measurment



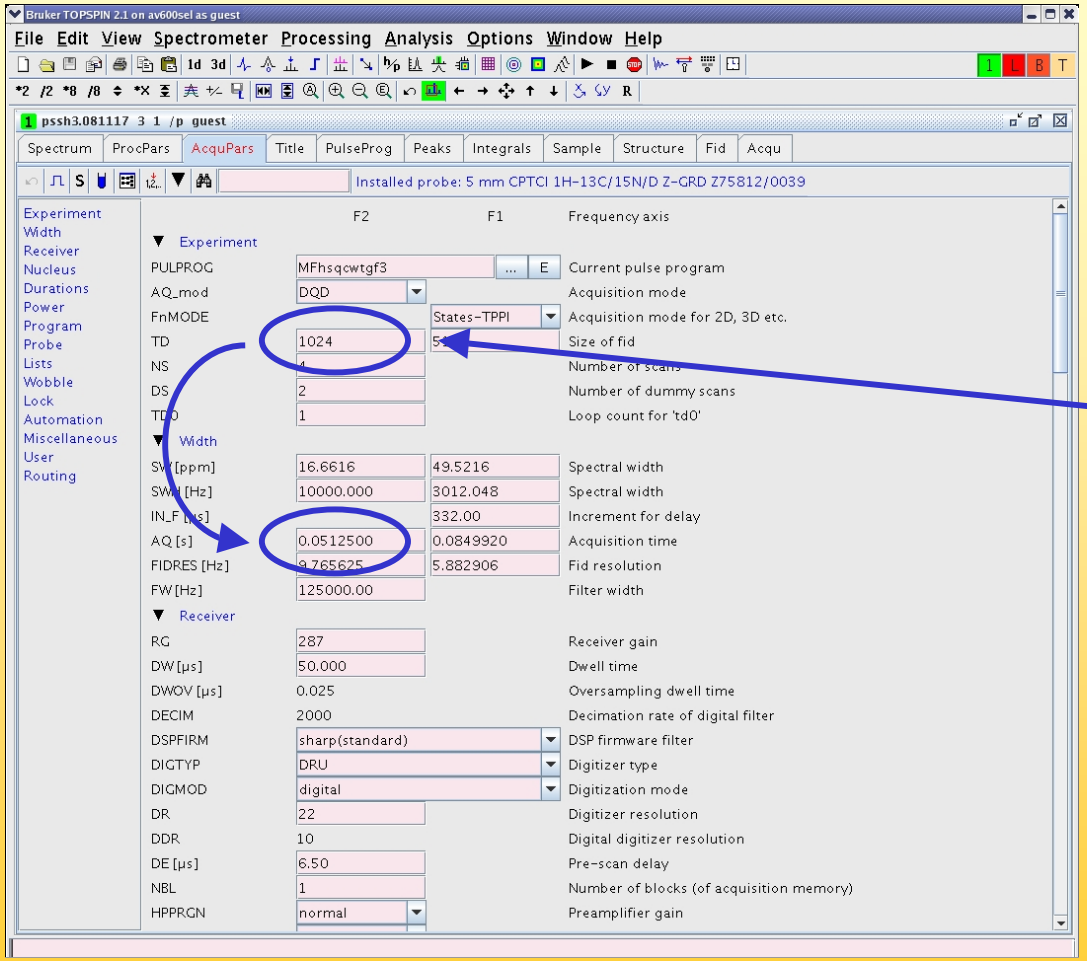
More advanced or less trusting users will take a look at the parameters using "ased"

some have fixed values

some can be changed

but hints for good values are given

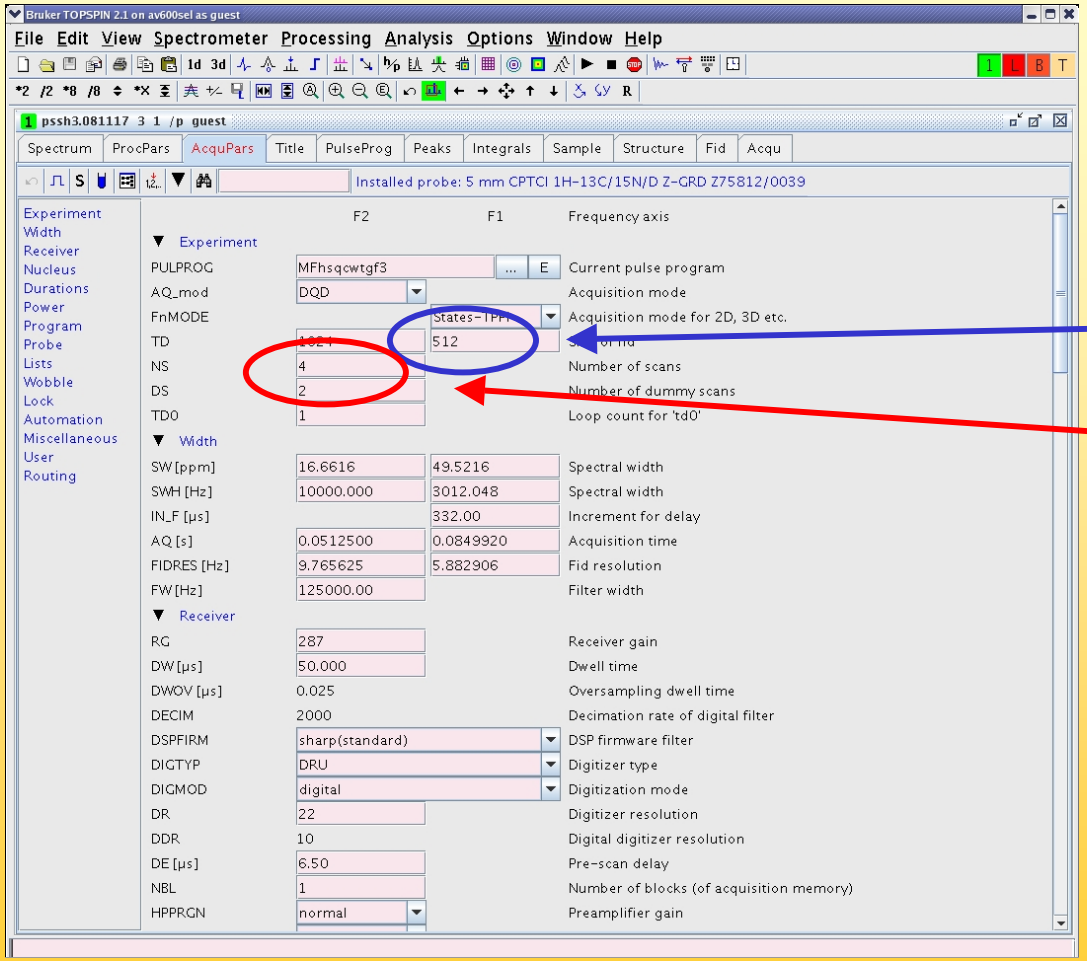
Doing a measurment



One important parameter is the number of points in the acquisition dimension since it determines the resolution: TD[F2]

But in case of heteronuclear experiments it has to be choosen with some consideration !

Doing a measurment



Two parameters that are related to each other are

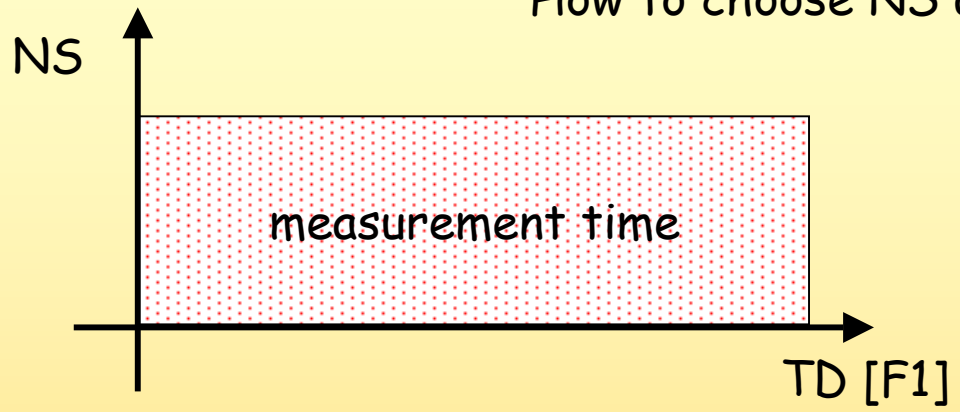
TD[F1]

and

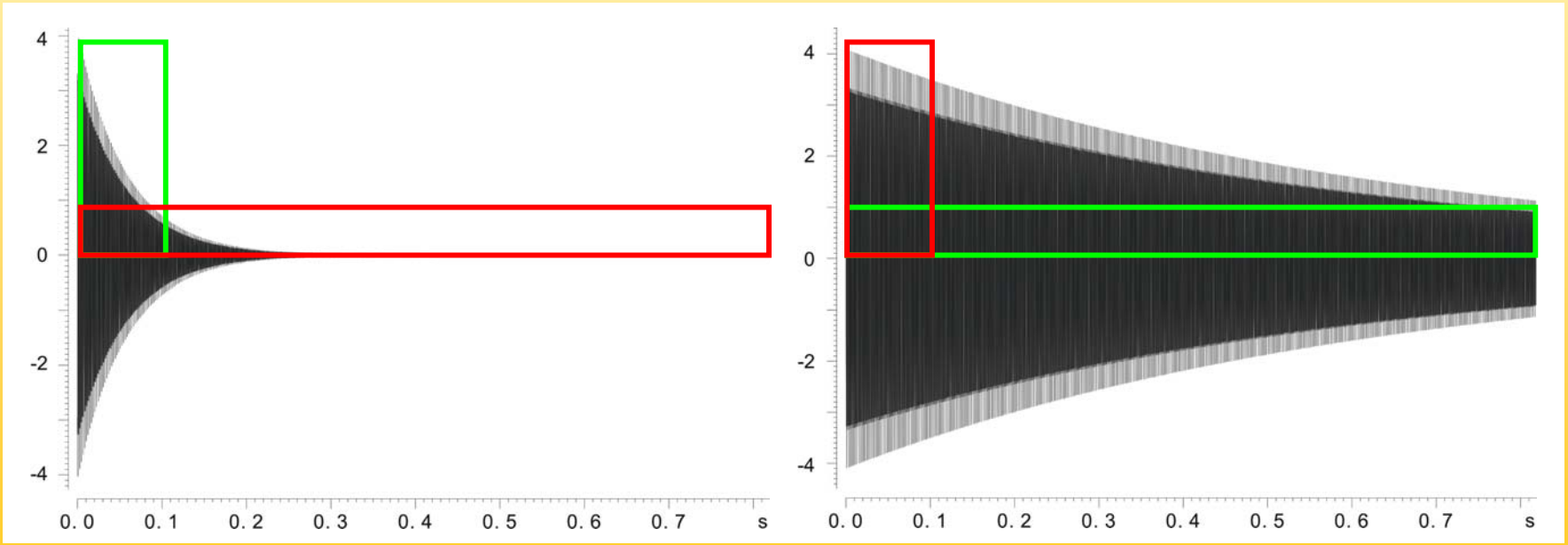
NS

Doing a measurment

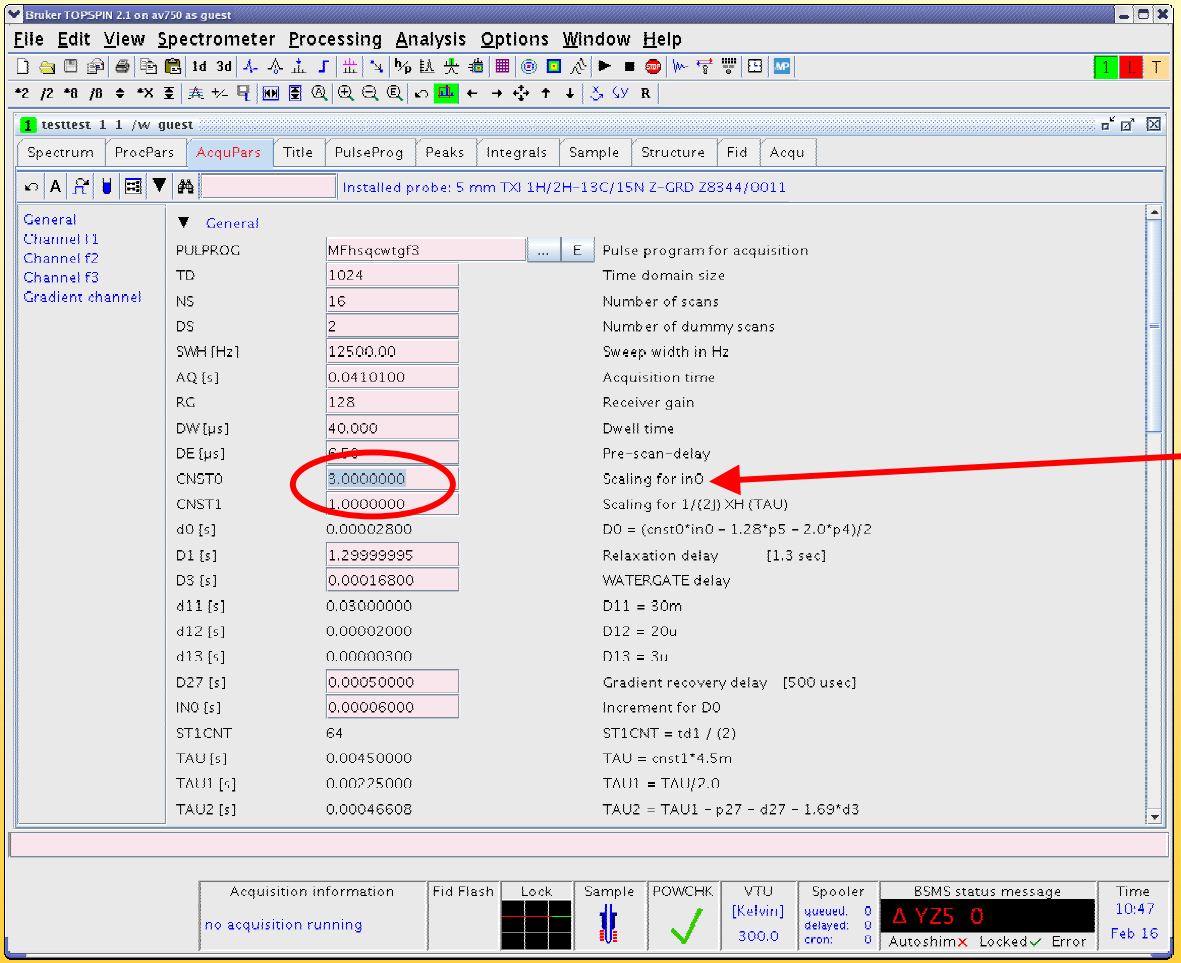
How to choose NS and TD[F1]



a large TD [F1] provides good resolution but S/N has to be considered

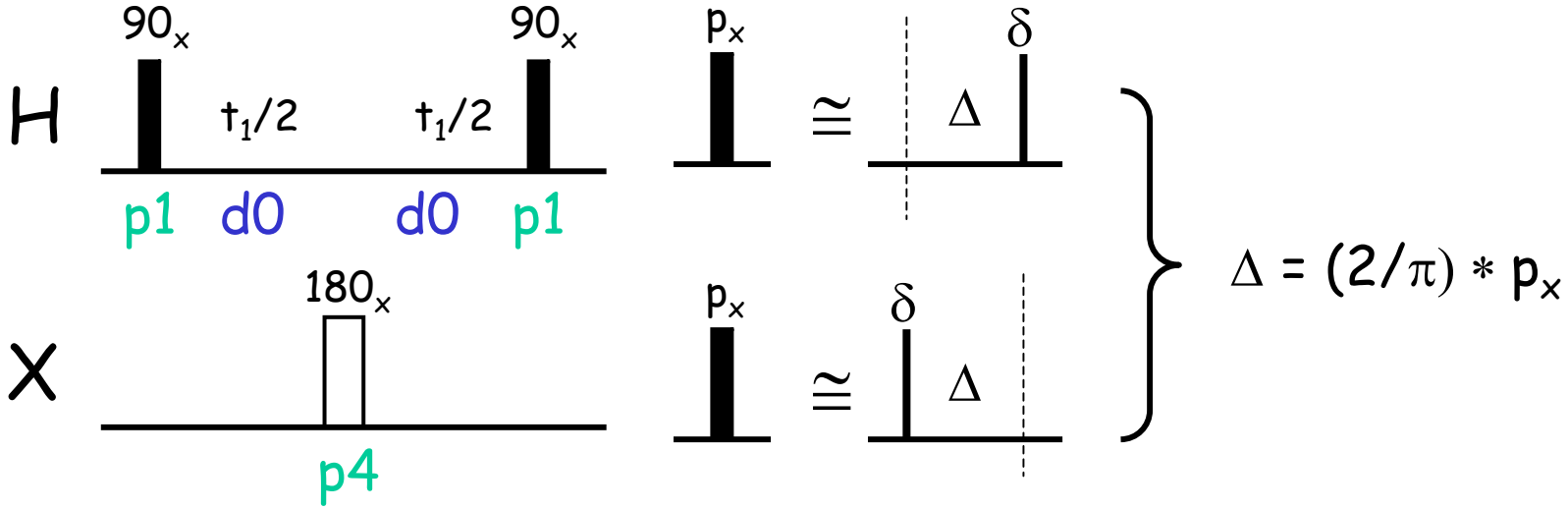


Doing a measurment



Another important parameters influences the phase correction in the indirect dimension cnst0 or cnst10

Doing a measurment



"d0 = (n*in0 - 1.28*p1 - p4) /2"

phc1 = -180°*[(2*d0 + 1.28*p1 + p4)/in0]

phc0 = $-\frac{1}{2}$*phc1

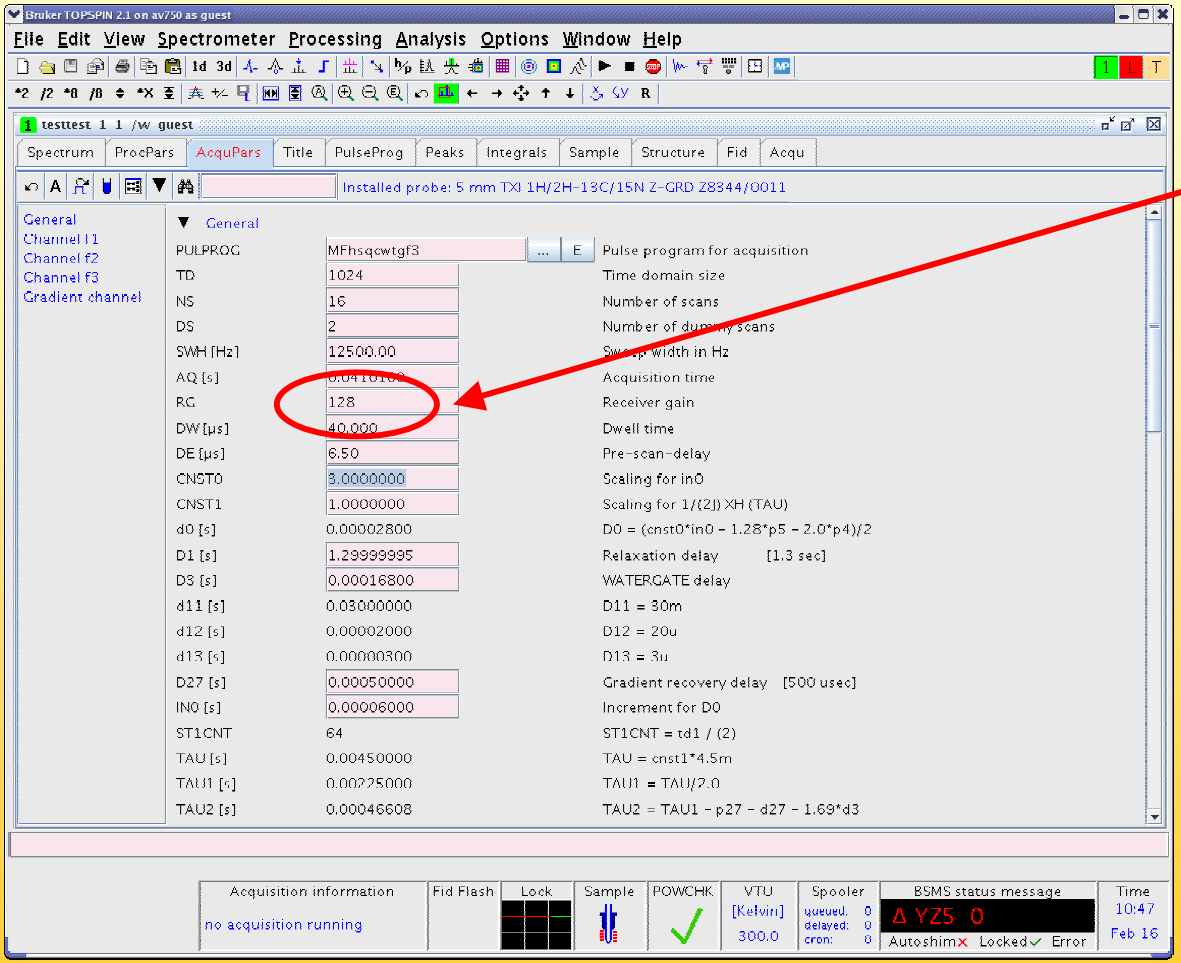
Doing a measurment

DW [µs]	40.000		Dwell time
DE [µs]	6.50		Pre-scan-delay
CNST0	1.0000000		Scaling for in0
CNST1	1.0000000	!	Scaling for 1/(2J) XH (TAU)
d0 [s]	50000000.0000...		D0 = (cnst0*in0 - 1.28*p5 - 2.0*p4)/2
D1 [s]	1.29999995		Relaxation delay [1.3 sec]
D3 [s]	0.00016800		WATERGATE delay
d11 [s]	0.03000000		D11 = 30m
d12 [s]	0.00002000		D12 = 20u

If not choosen properly it will have strange effects !

DW [µs]	40.000		Dwell time
DE [µs]	6.50		Pre-scan-delay
CNST0	3.0000000		Scaling for in0
CNST1	1.0000000	!	Scaling for 1/(2J) XH (TAU)
d0 [s]	0.00002800		D0 = (cnst0*in0 - 1.28*p5 - 2.0*p4)/2
D1 [s]	1.29999995		Relaxation delay [1.3 sec]
D3 [s]	0.00016800		WATERGATE delay
d11 [s]	0.03000000		D11 = 30m
d12 [s]	0.00002000		D12 = 20u

Doing a measurment

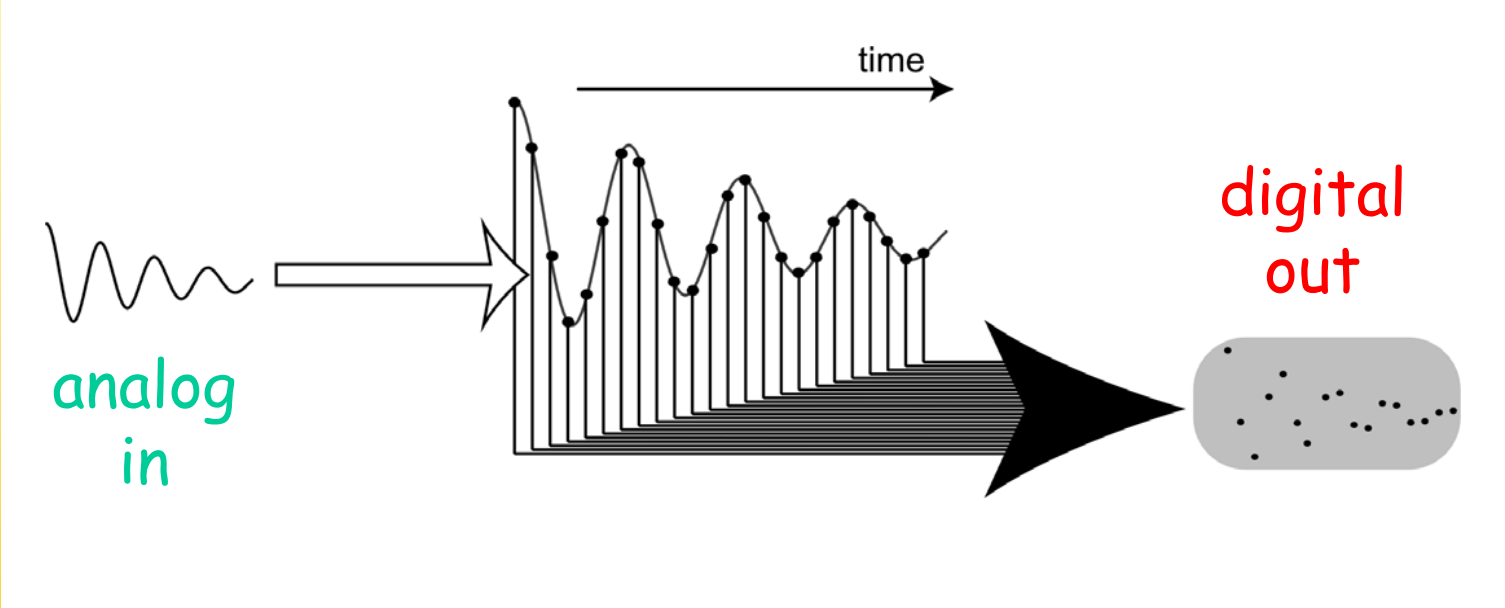


Finally the receiver gain has to be adjusted to the signal. This can be done automatically ("rga") which sometimes does not work that convincingly. On older machines it is possible to adjust it after inspecting the FID

The „dynamic range“

The „dynamic range"

After detection by the NMR coil the signal has to be digitized to be processed on the computer



The „dynamic range"

2^{16}
2^{15}
2^{14}
2^{13}
2^{12}
2^{11}
2^{10}
2^{9}
2^{8}
2^{7}
2^{6}
2^{5}
2^{4}
2^{3}
2^{2}
2^{1}
2^{0}

The ADC (Analog-Digital-Converter) in a modern spectrometer has 16 to 18 bit. The receiver that records the signal that is subsequently digitized needs to be adjusted to the strongest signals, it has to fit to the largest bit.

The „dynamic range"

The largest signal will be that of the solvent, since it has the highest concentration.

H_2O (18 g/mol), density 1.0, 55 mol/ltr

$CHCl_3$ (119 g/mol), density 1.5, 12 mol/ltr

DMSO (78 g/mol), density 1.1, 14 mol/ltr

The „dynamic range"

2^{16}
2^{15}
2^{14}
2^{13}
2^{12}
2^{11}
2^{10}
2^{9}
2^{8}
2^{7}
2^{6}
2^{5}
2^{4}
2^{3}
2^{2}
2^{1}
2^{0}

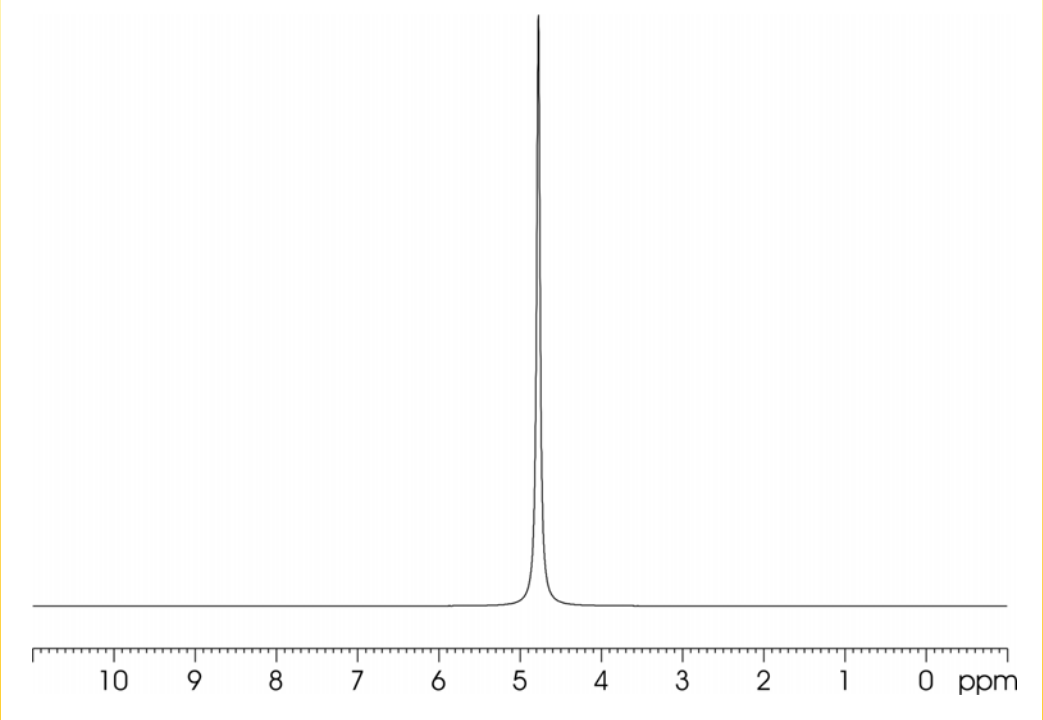
The concentration of the compound may be only 1 mM, i.e. in acqueus solution we have 55 000 times more solvenent than compound.

$2^{16} = 65536$

Good digitization of the solvent will then mean that the compound is similar to the noise in the lowest bit.

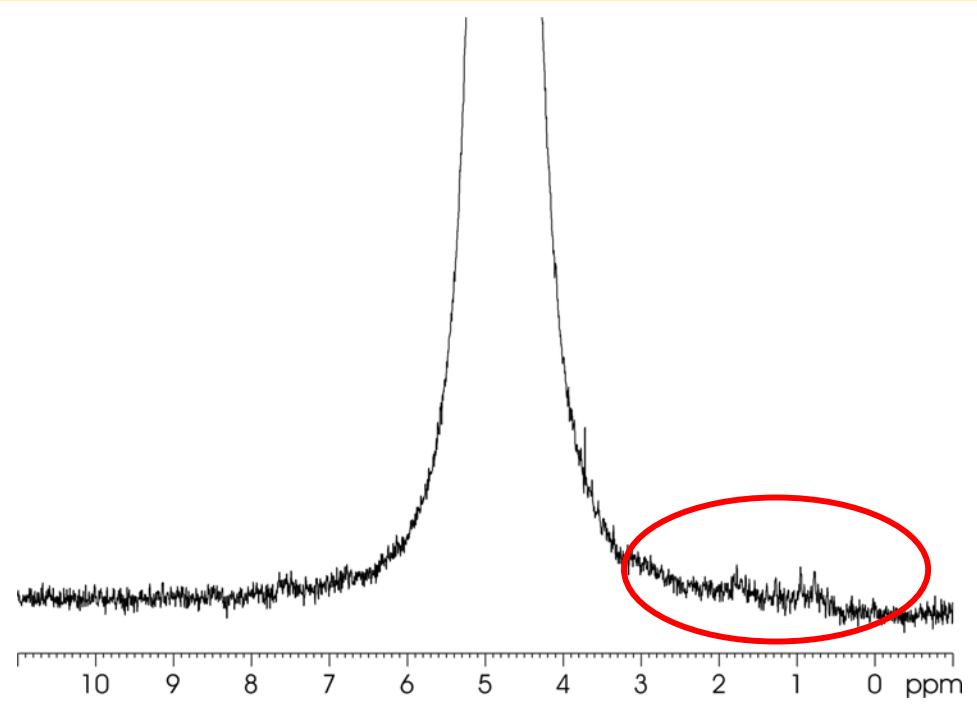
The „dynamic range"

As a consequence a protein is hardly visible in a spectrum in aqueous solution



The „dynamic range"

The protein is there, but hardly distinguishable from noise



The „dynamic range"

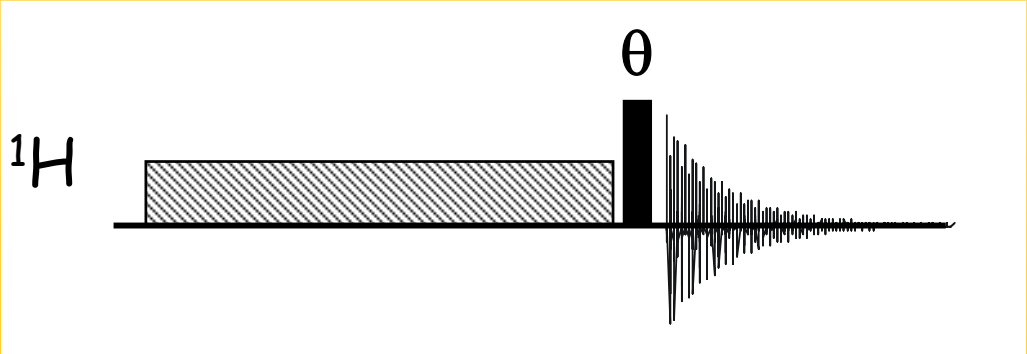
The solvent signal therefore needs to be removed from the spectrum.
One way is to deuterate the solvent. If DMSO is deuterated to 99.97 % that corresponds to a concentration of 4 mM, thats just $2^{2.}$

That does only work if there are no exchanging protons. $CHCl_3$ can be replaced by $CDCl_3$ but H_2O can not be replaced by D_2O without loosing the exchangable protons

Solvent suppression

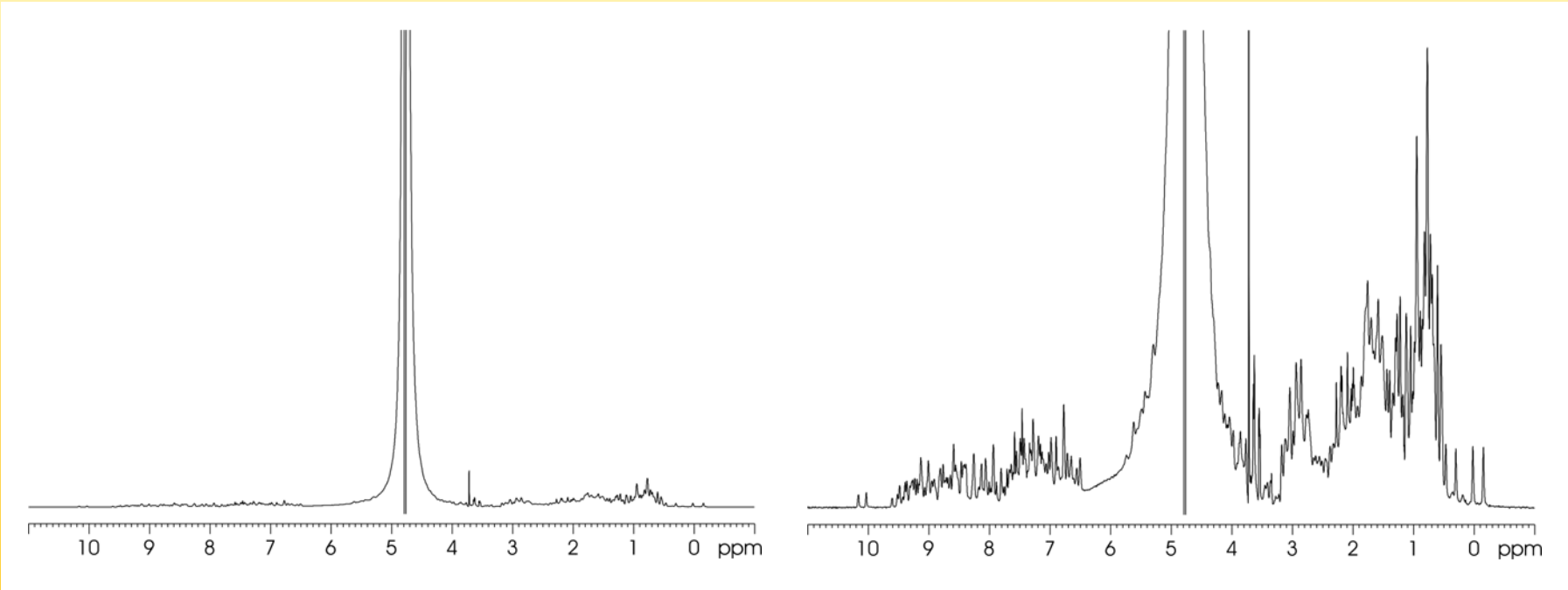
Solvent suppression

The simplest and most robust solvent suppression is by „presaturation". At the onset of the experiment a long and weak and therefore selective pulse is given to the solvent signal.This requires the spectrometer frequency to match the frequency of the solvent, i.e. the center of the spectrum has to coincide with the solvent frequency.



Solvent suppression

The solvent signal is not completely suppressed but the dynamic range problem is overcome



Solvent suppression

Advantage

The method can be combined with any NMR experiment

The 90°-pulse does not have to be known

Disadvantage

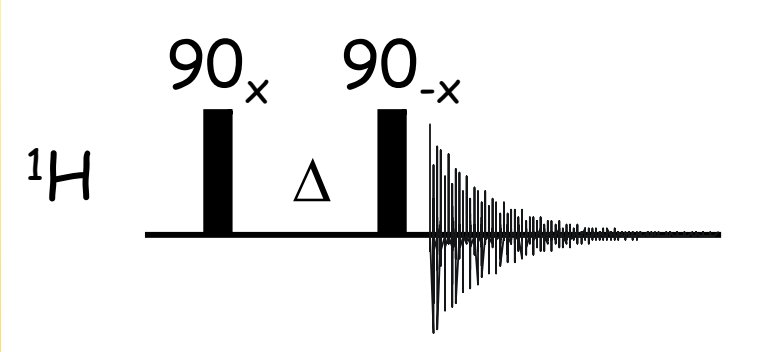
The residual signal can be rather large and broad in unfavorable cases

The saturation is transferred to exchangeable protons

Signals under the solvent are completely removed from the spectrum, also in 2D

Solvent suppression

An experiment that does not need presaturation is the 1-1-sequence, which is quite simple.



Solvent suppression

As with presaturation the center of the spectrum is placed on the solvent resonance. This resonance does therefore not have a chemical shift relative to the center of the spectrum and does not move during Δ.

Then the second pulse simply reverses the effect of the first, the solvent is flipped back to the z-direction and does not give a signal. This is independent on the value of Δ !

Solvent suppression

Which signals appear depends on the value for Δ and on the chemical shift δ_H of the resonances. A maximum is where the resonances have traveled 90° during the time Δ

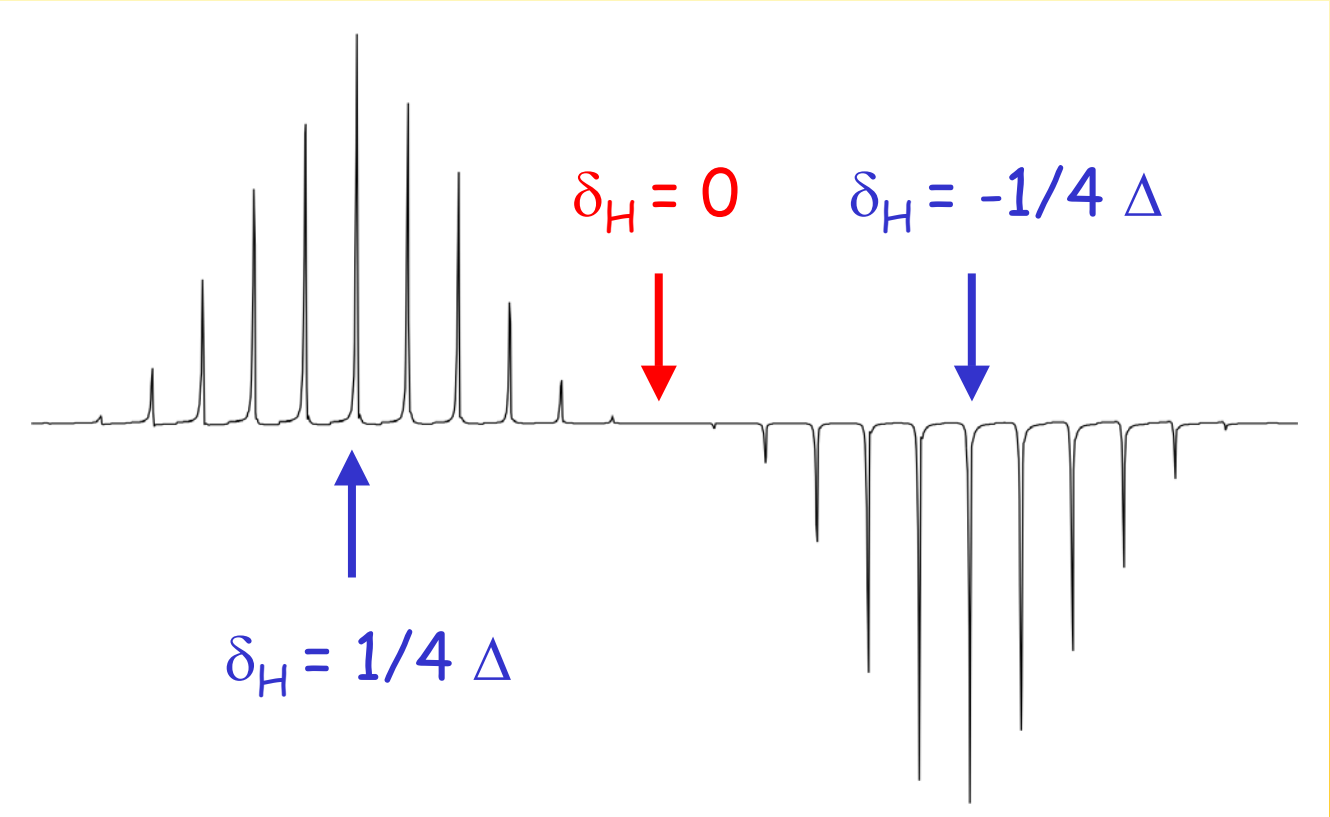
With Δ = 100 usec the maximum at 600 MHz is

$\delta_H\Delta = \frac{1}{4}$, $\delta_H = 1/4\Delta$, i.e. δ_H = 2500 Hz = 4.1 ppm

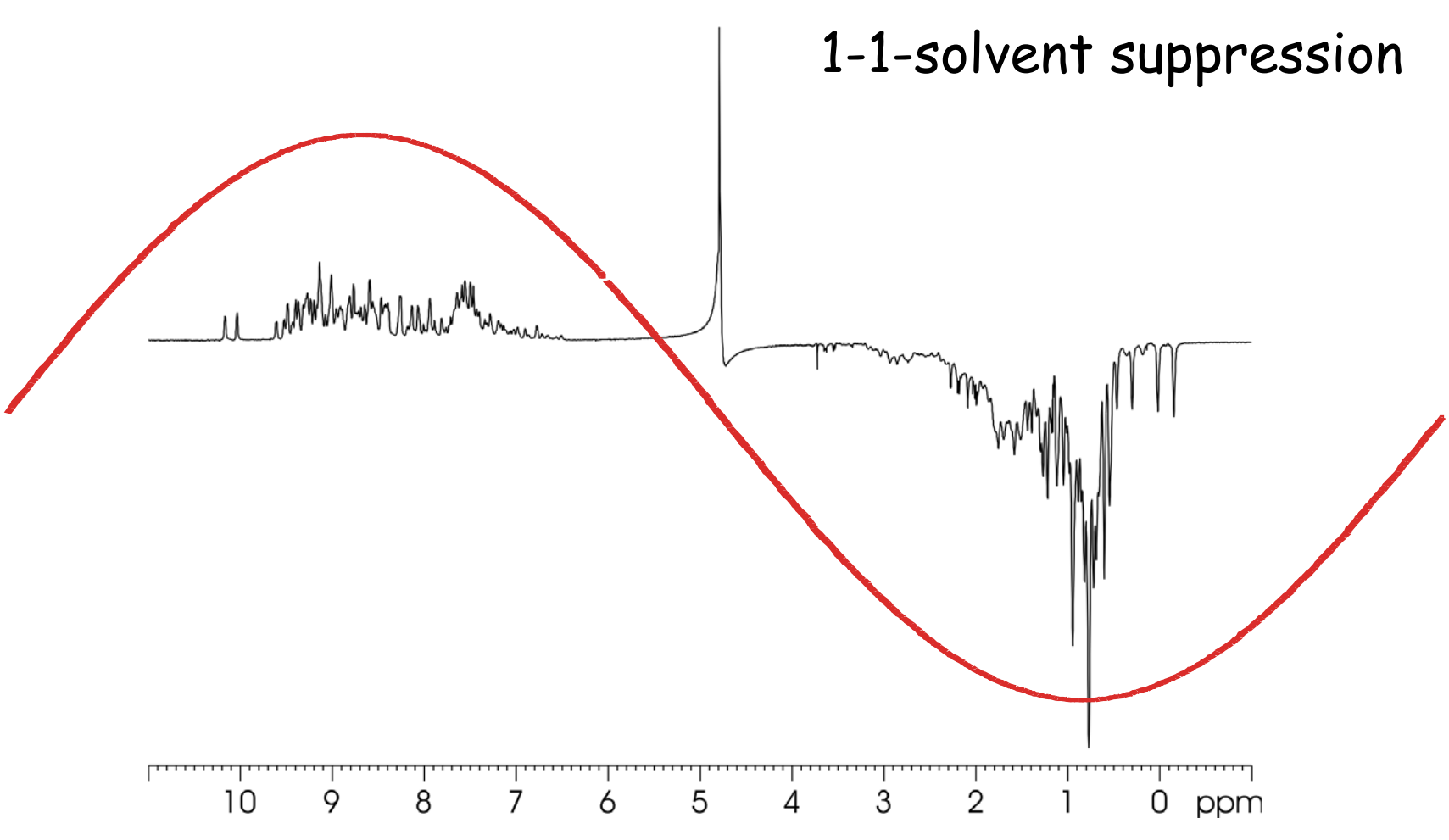
relative to the center of the spectrum

Solvent suppression

The excitation profile can be determined experimentally



Solvent suppression



Solvent suppression

Advantage

The method avoids disturbances of the exchanging protons

It can be combined with many important experiments

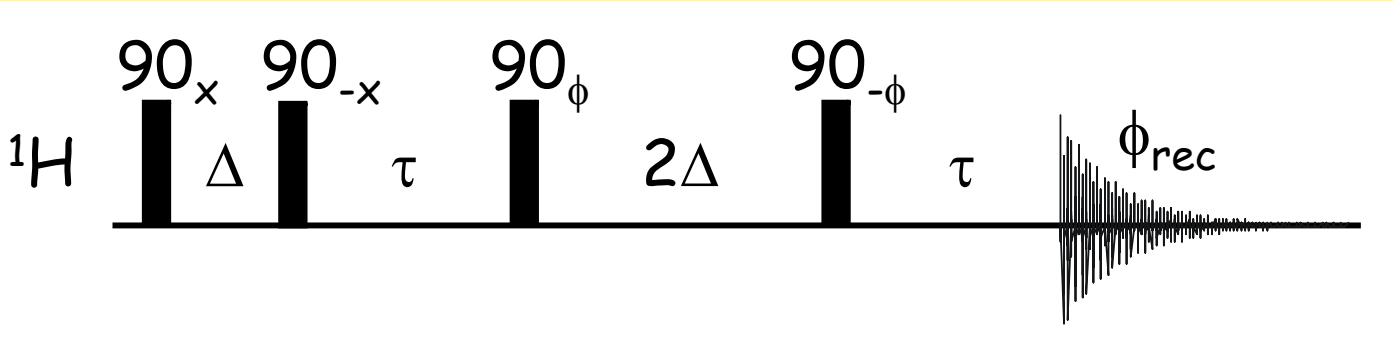
Disadvantage

In some cases the residual solvent signal can be quite big.

The 90°-pulse has to be determined first

Solvent suppression

To solve the problem with the small minimum in the sine curve the 1-1-echo sequence was created.

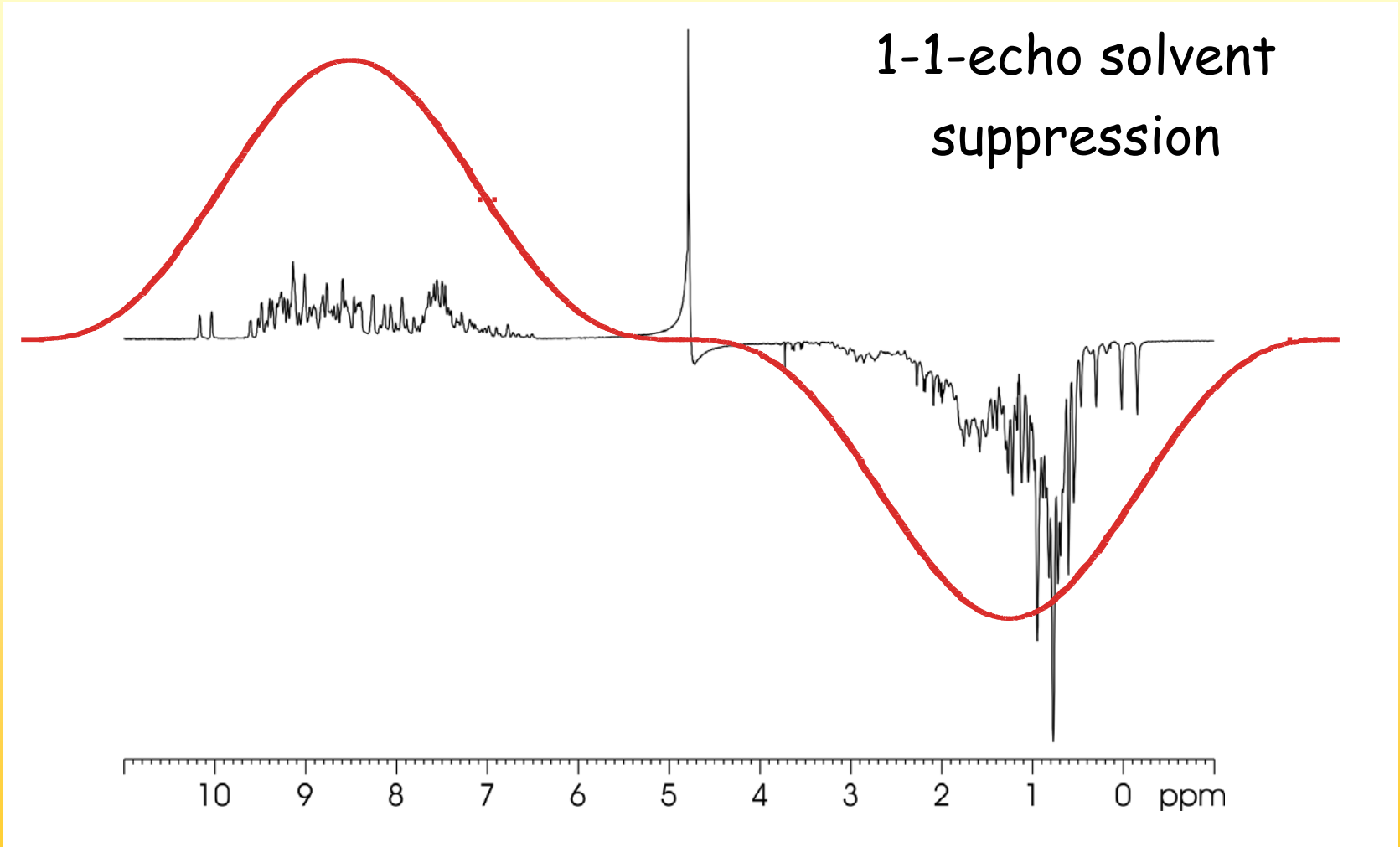


ϕ = x, y, -x, -y

ϕ_{rec} = +, -, +, -

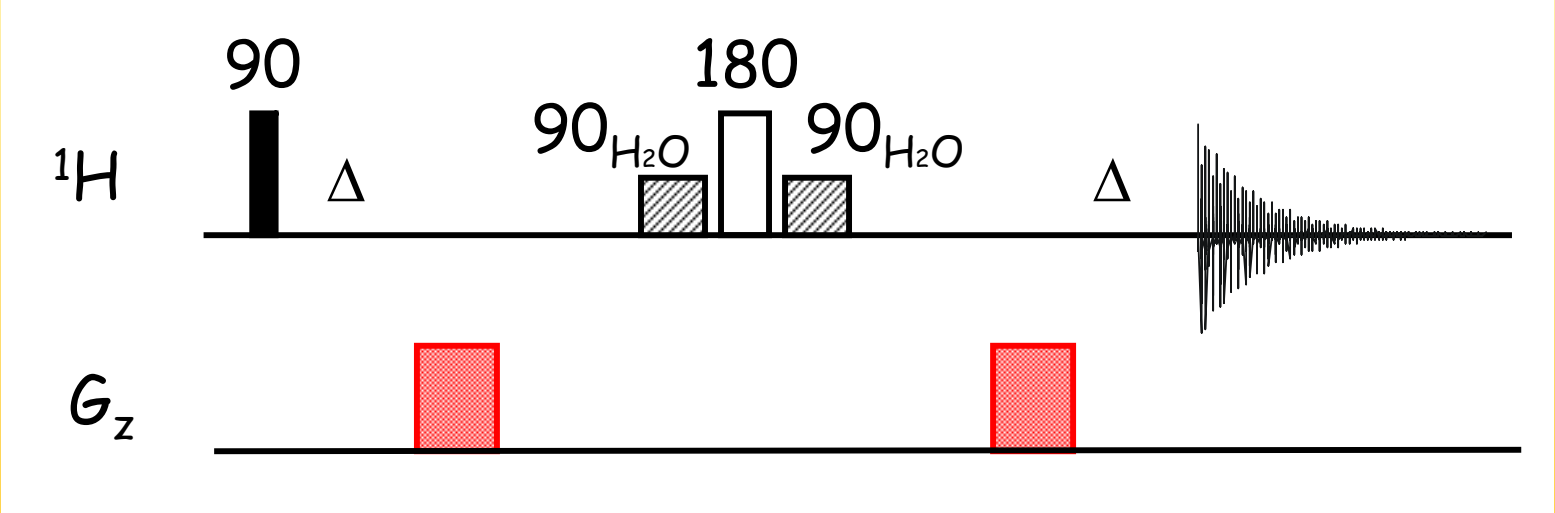
The sine is converted into a (sinus)^3 with a much broader minimum

Solvent suppression



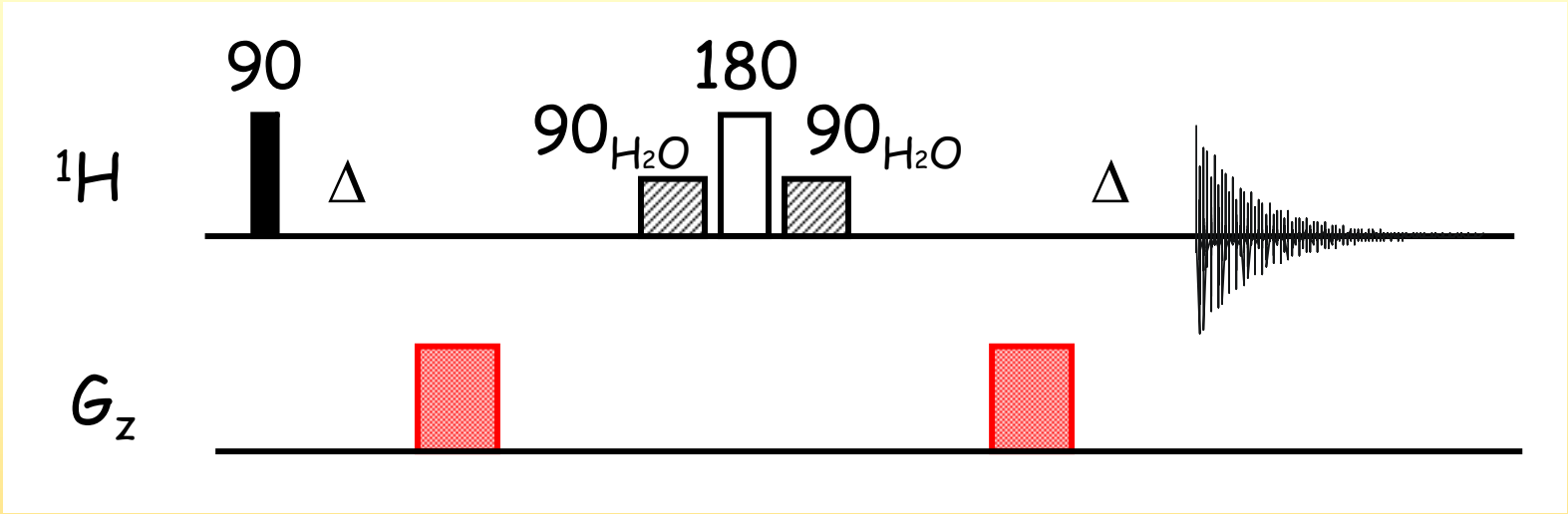
Solvent suppression

An experiment that offers very good solvent suppression is the WATERGATE-Sequence



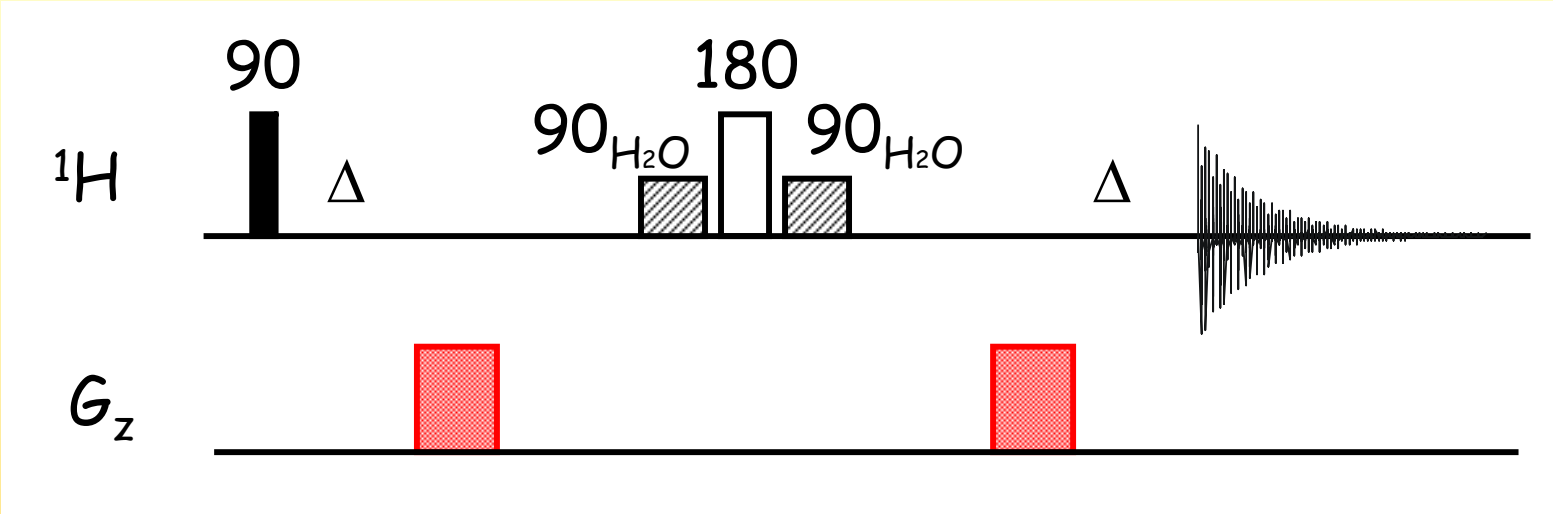
Peter Schmieder
AG Solution NMR

Solvent suppression



The two selective pulses at the water frequency add up to a 360°-pulse with the hard 180° pulse, all signals that are not hit by the selective pulses will experience a 180° pulse

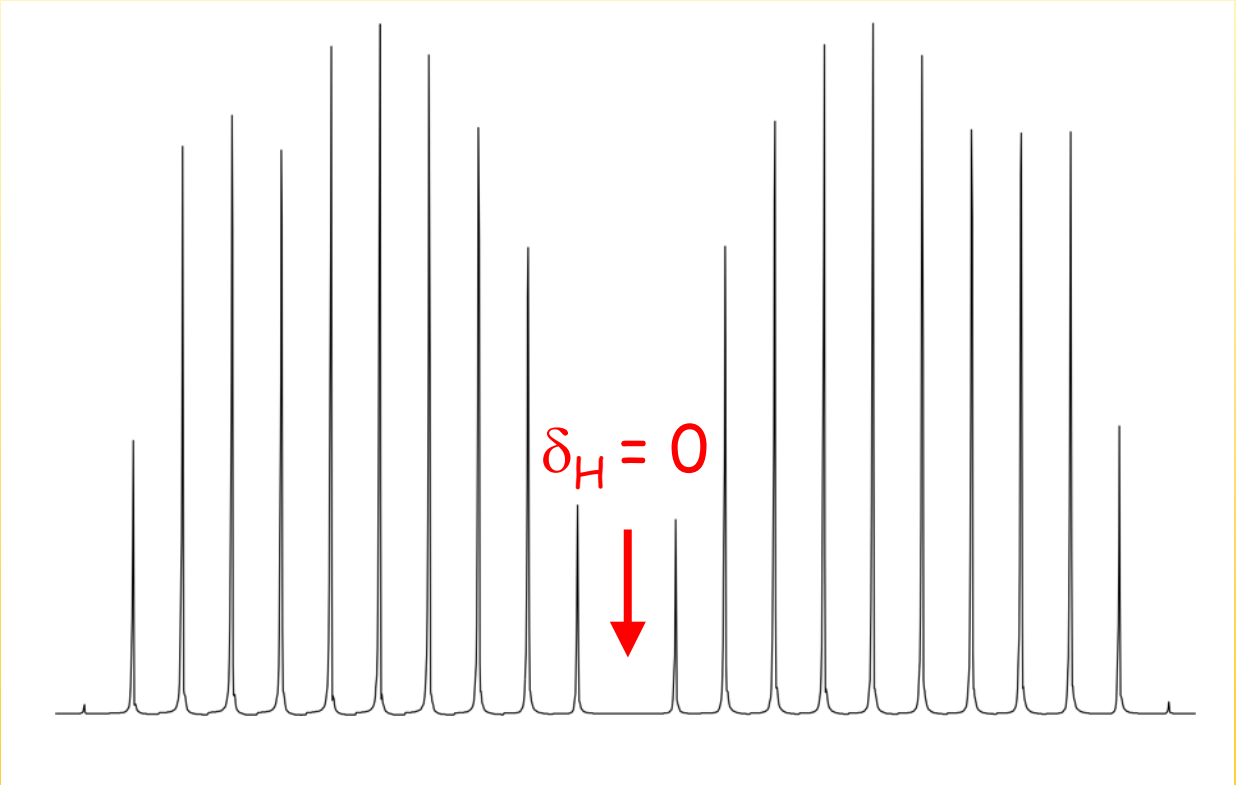
Solvent suppression



A 180° pulse will refocuss the effect of the gradients, a 360° pulse will have no effect and the water signal will be destroyed by the gradients

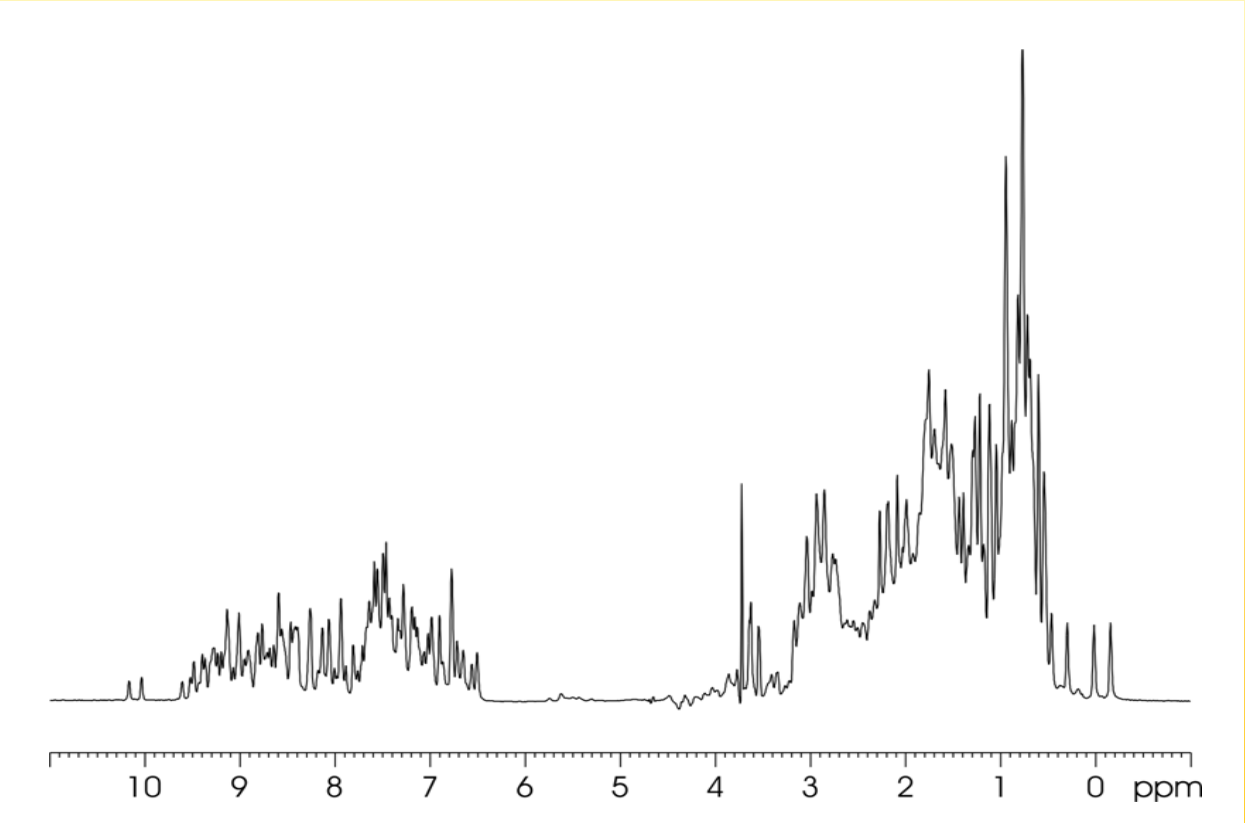
Solvent suppression

The excitation profile depends on the choice of the selective pulses



Solvent suppression

The excitation profile is chosen so that the desired signals have good intensity



Solvent suppression

All these techniques assume that only one signal from the solvent is present.

Should there be several signals (e.g. in LC-NMR) suppression can be more complicated but is still possible.

That's it

www.fmp-berlin.de/schmieder/teaching/selenko_seminars.htm