NMR course at the FMP:

Practical aspects

04.05.2009

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The program

The dynamic range

Solvent supression

Quadrature detection

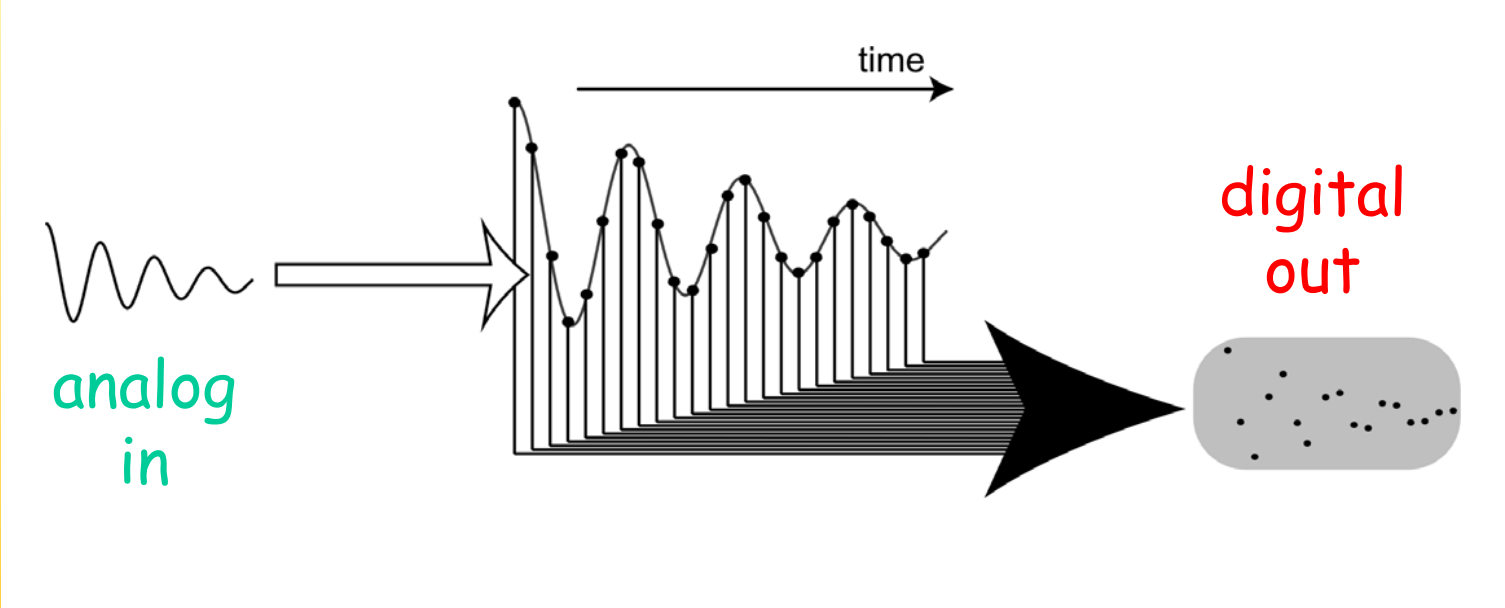
Folding/Aliasing

Phasing in the indirect dimensions

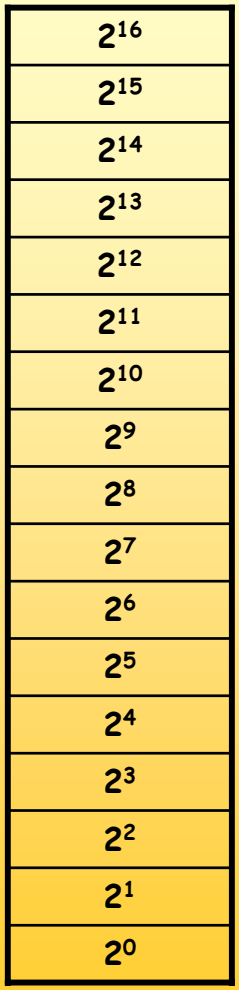
The „dynamic range“

The „dynamic range"

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



The „dynamic range"



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}

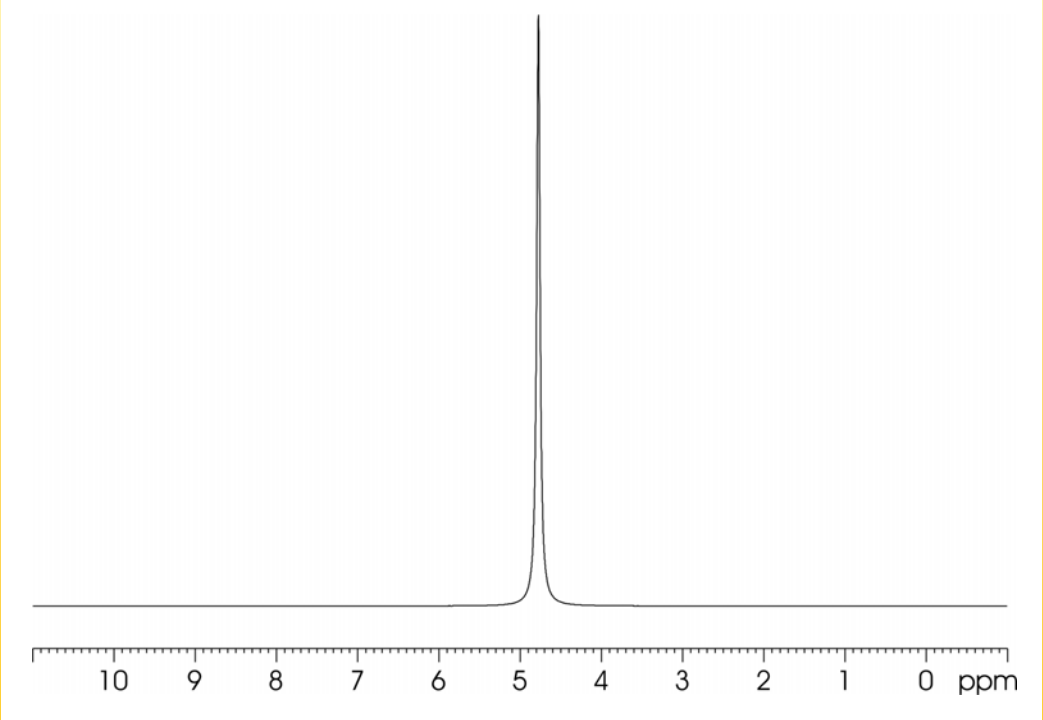
The concentration of the compound may be only 1 mM, i.e. in acqueus solution we have 55 000 times more solvenent than compound.

$2^{16} = 65536$

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

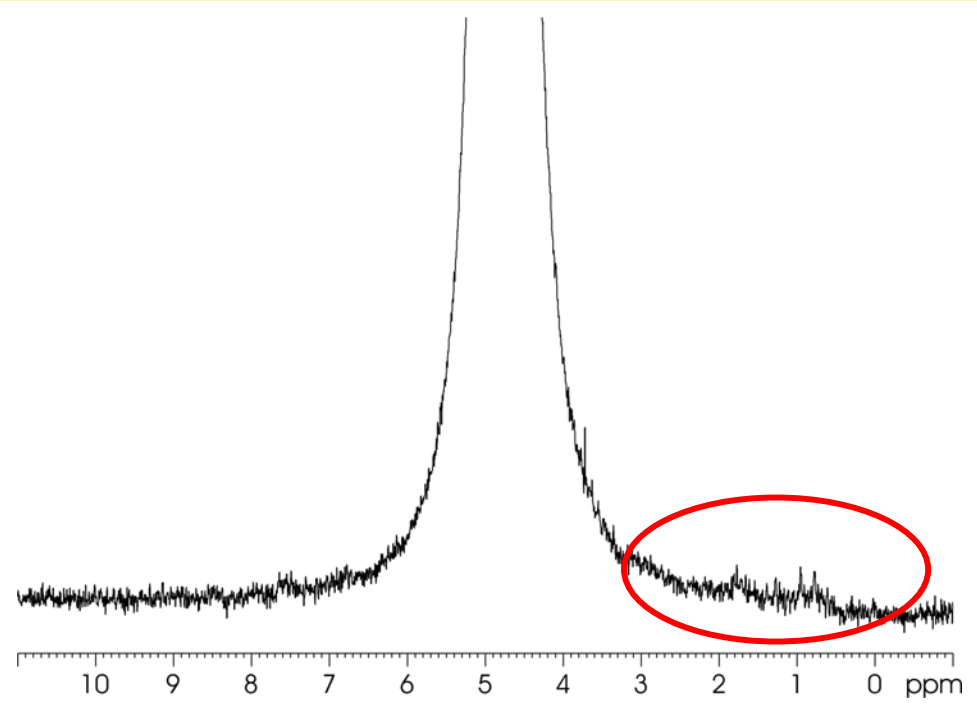
The „dynamic range"

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"

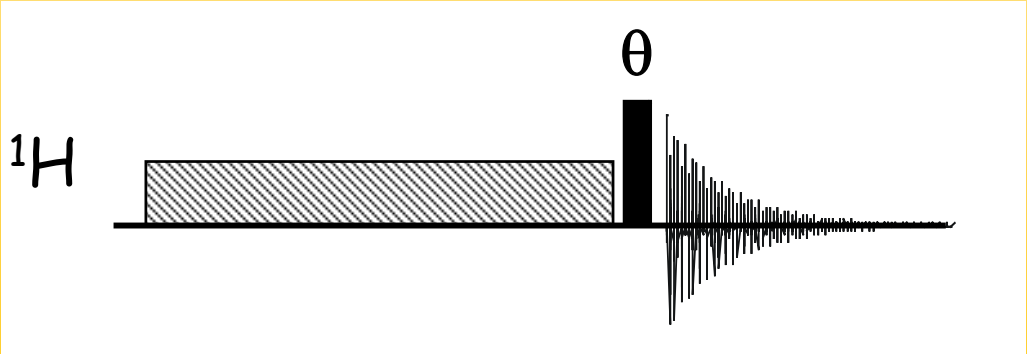
The solvent signal therefore needs to be removed from the spectrum.
One way is to deuterate the solvent. If DMSO is deuterated to 99.97 % that corresponds to a concentration of 4 mM, thats just $2^{2.}$

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

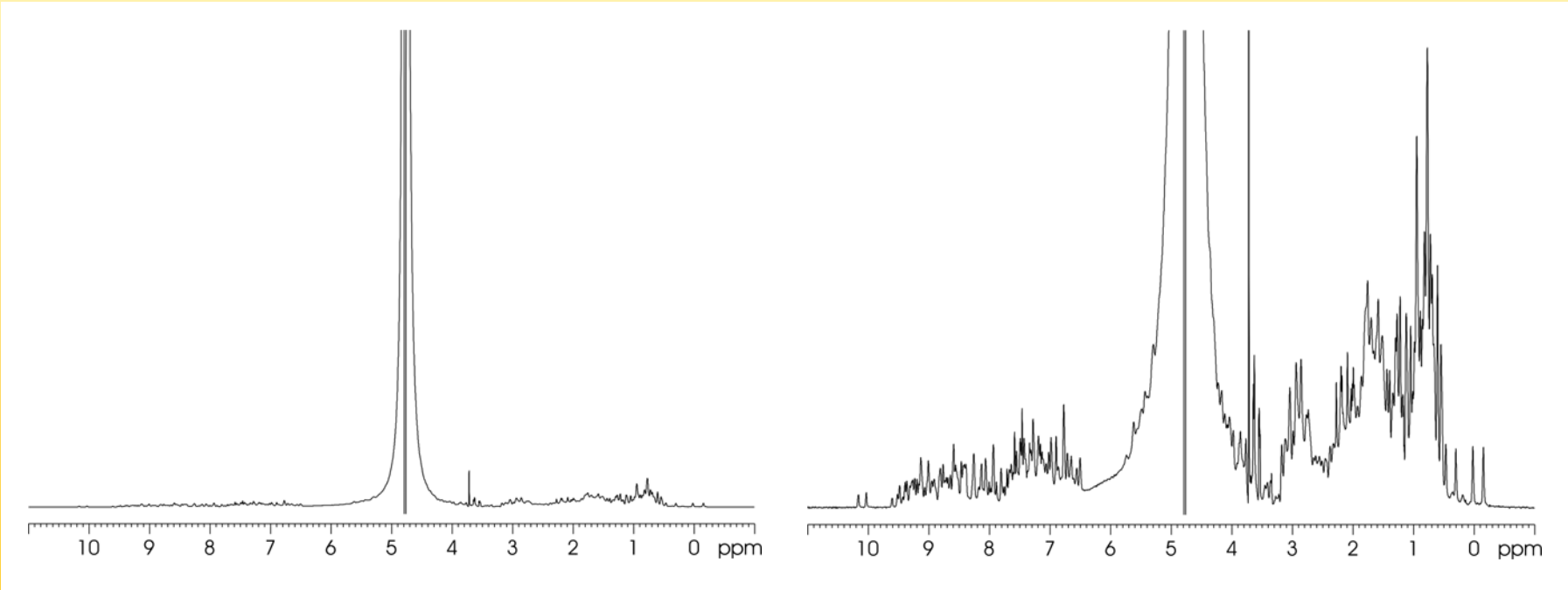
Solvent suppression

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

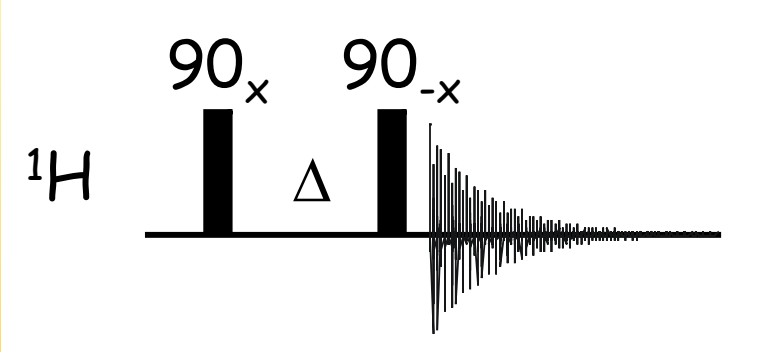
The residual signal can be rather large and broad in unfavorable cases

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

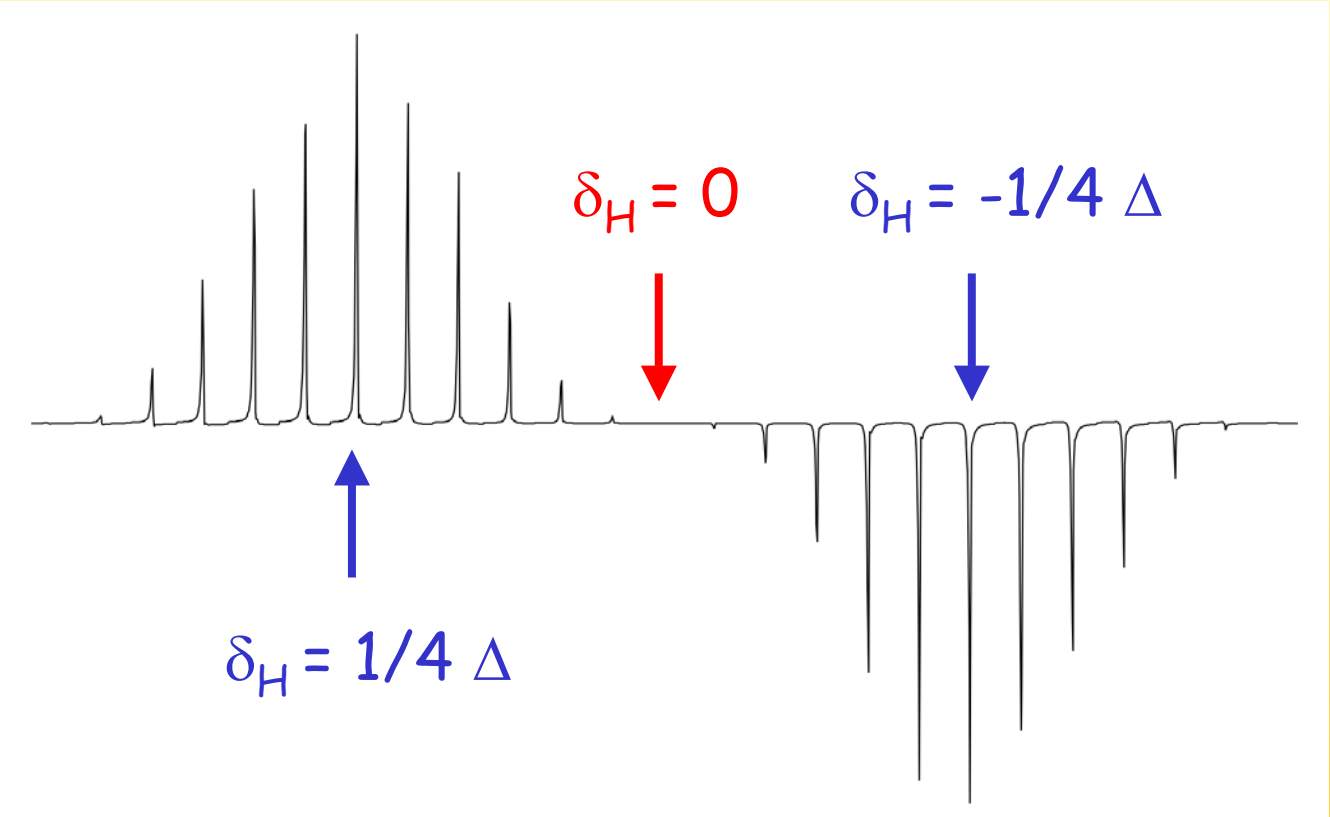
With Δ = 100 usec the maximum at 600 MHz is

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

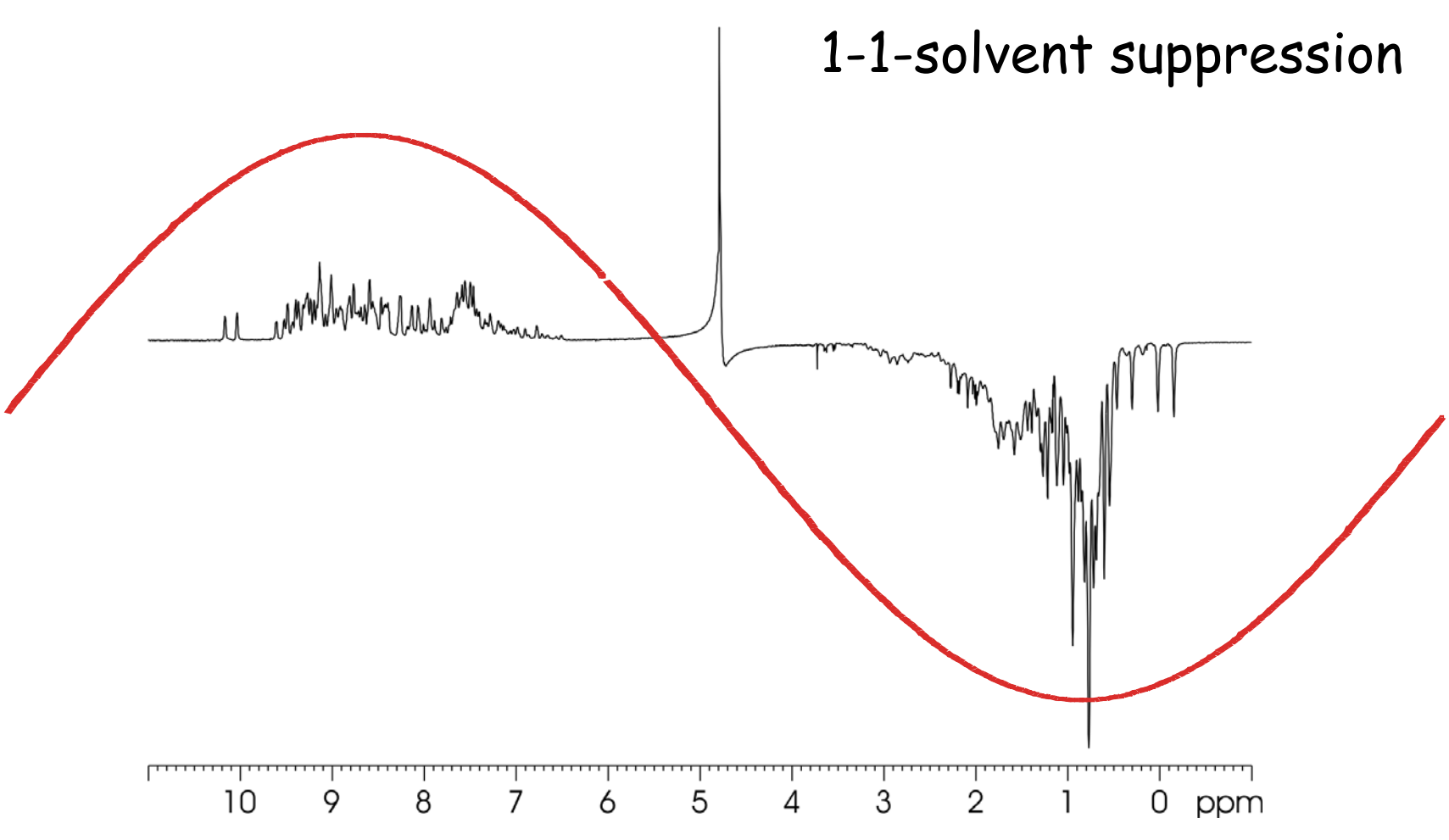
relative to the center of the spectrum

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

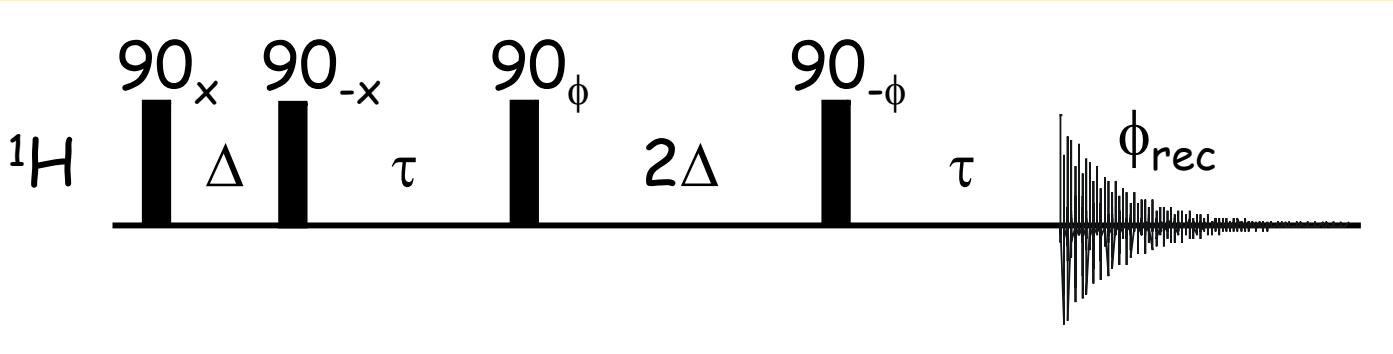
Disadvantage

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.

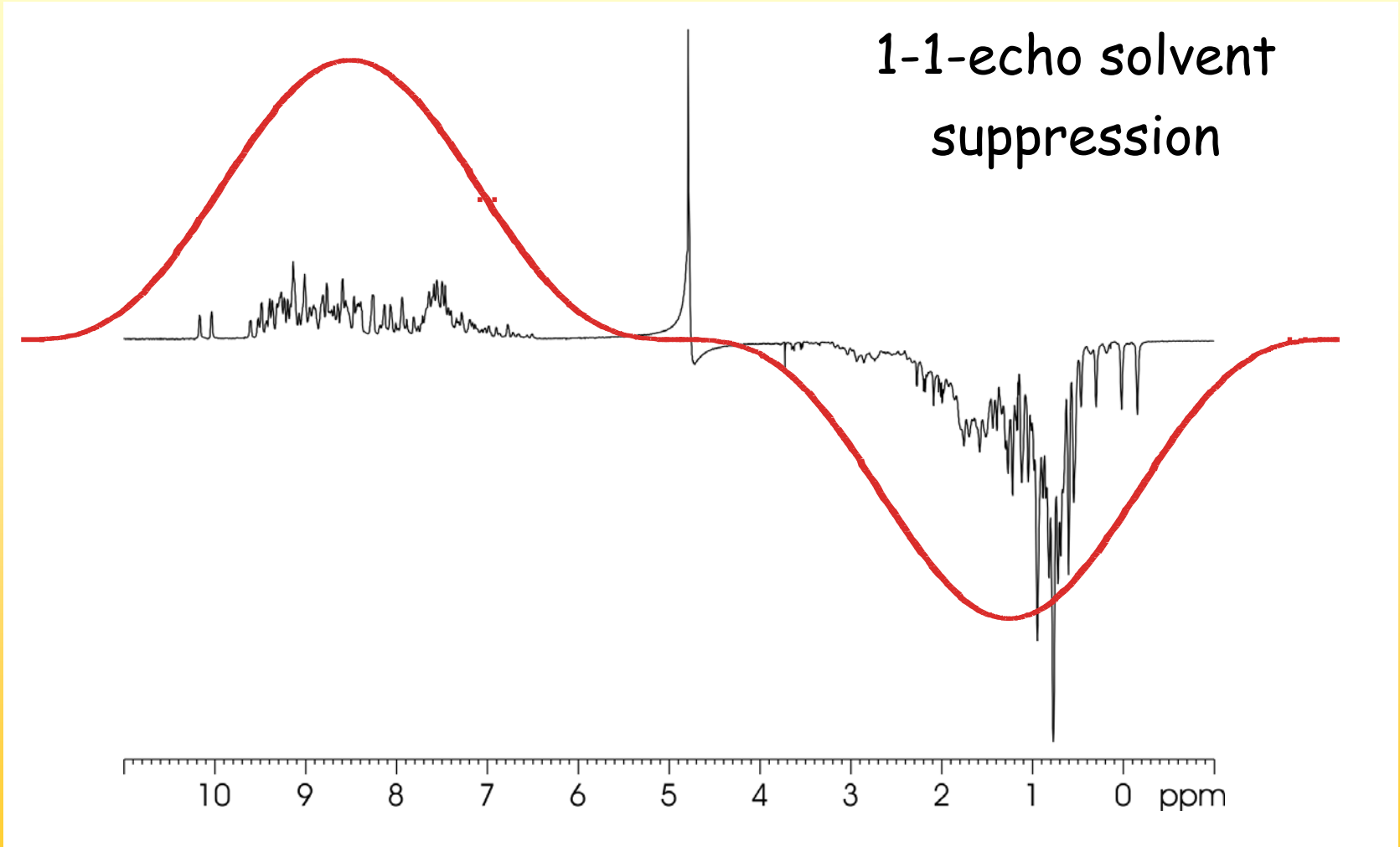


ϕ = x, y, -x, -y

ϕ_{rec} = +, -, +, -

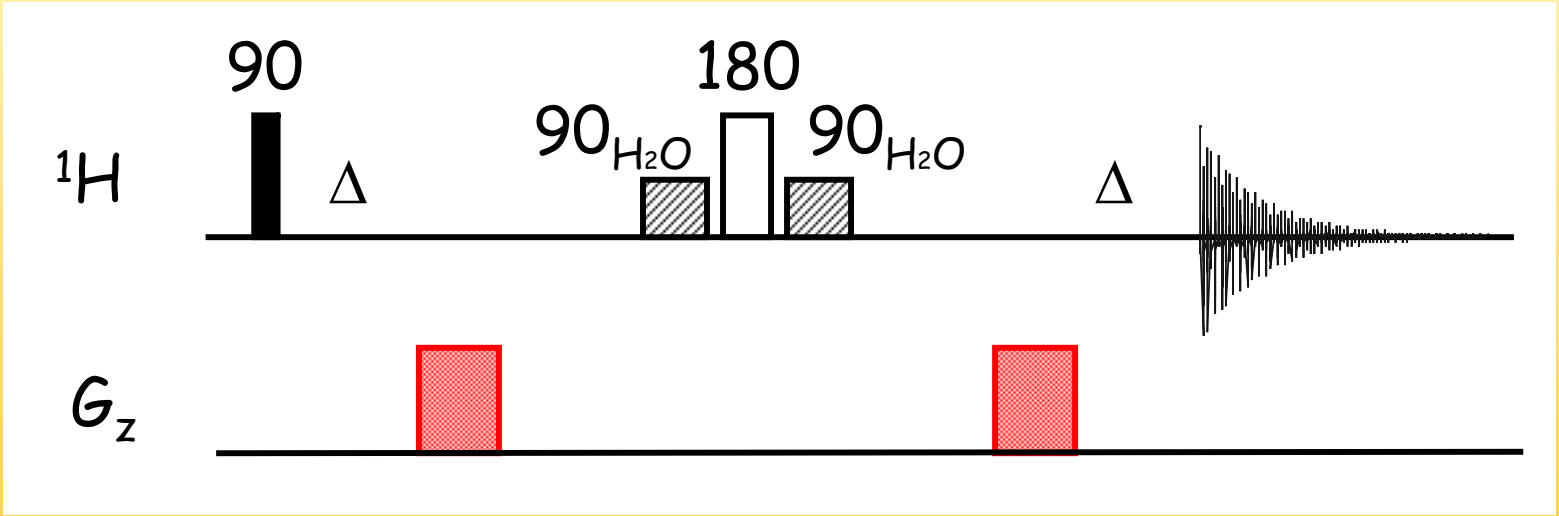
The sine is converted into a $(sine)^3$ with a much broader minimum

Solvent suppression

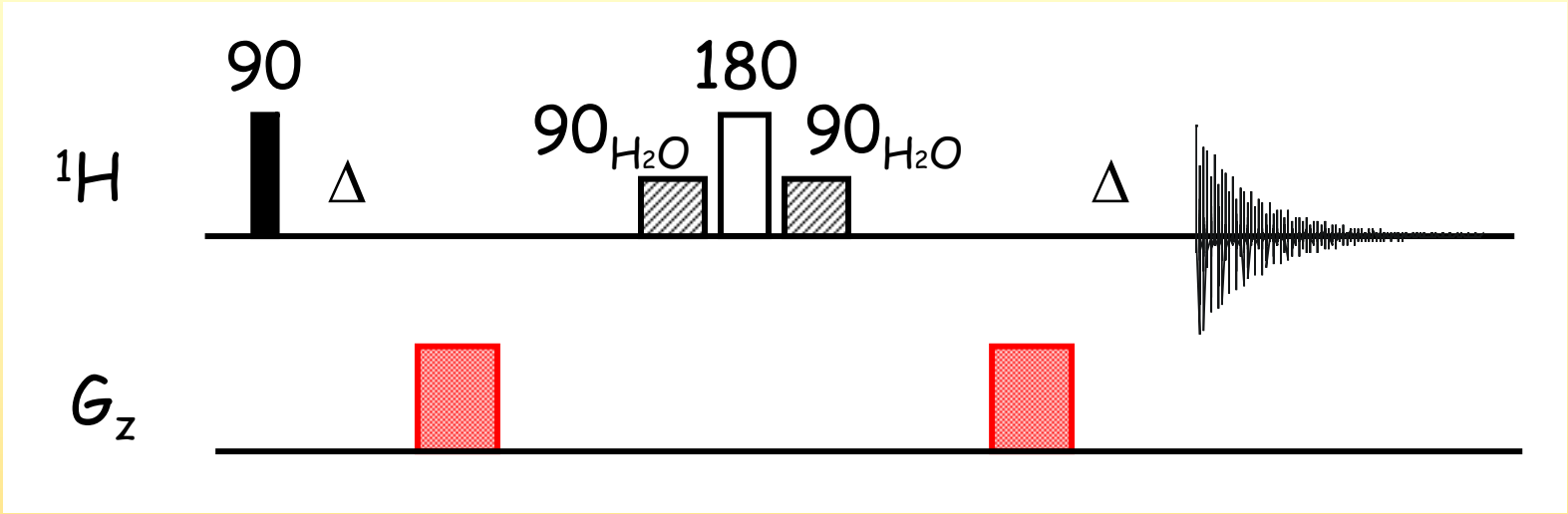


Solvent suppression

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

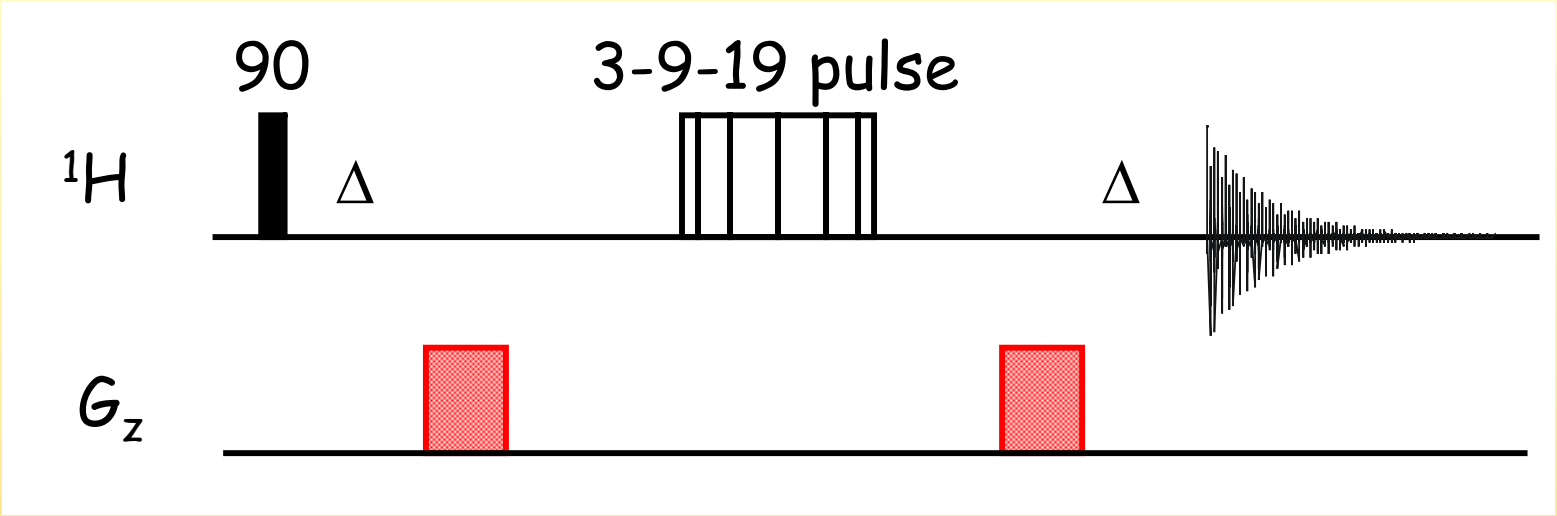


Solvent suppression



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

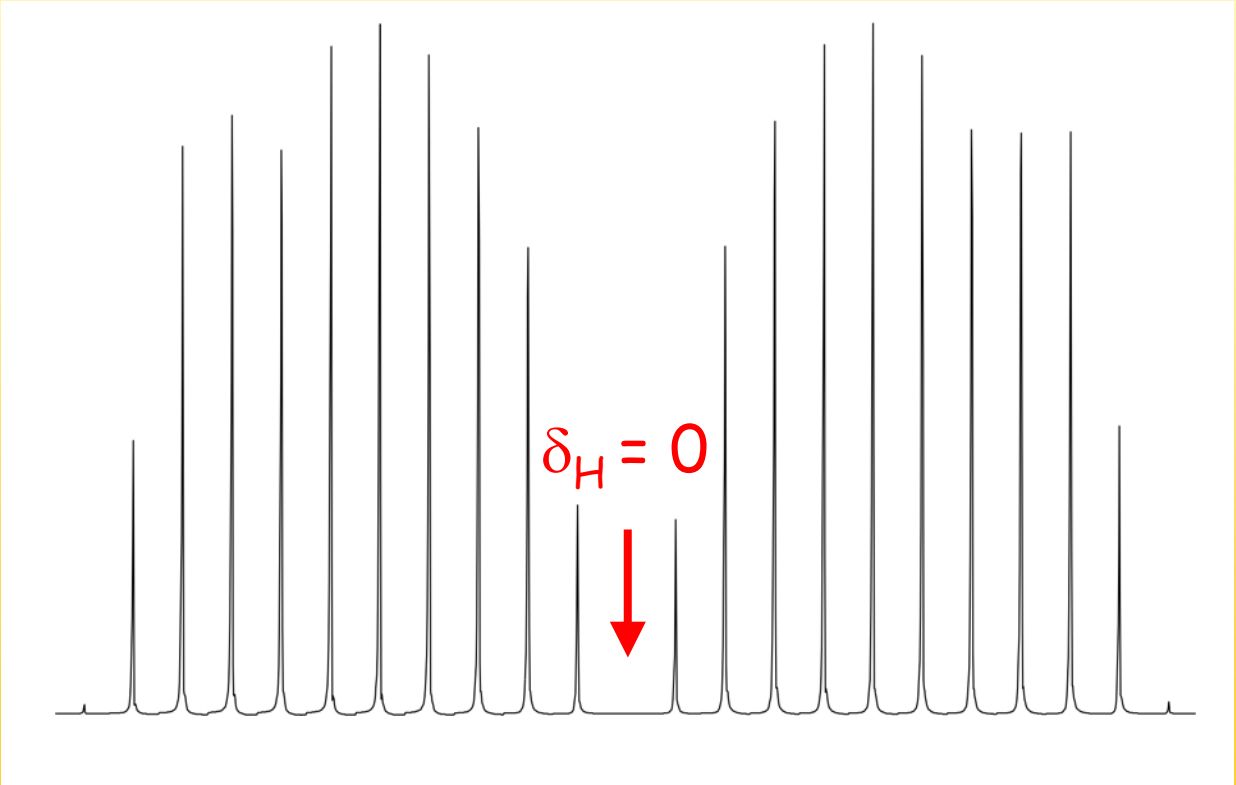


A 180° pulse will refocuss the effect of the gradients, a 360° pulse will have no effect and the water signal will be destroyed by the gradients.

Instead of the selective pulses we use a 3-9-19 pulse

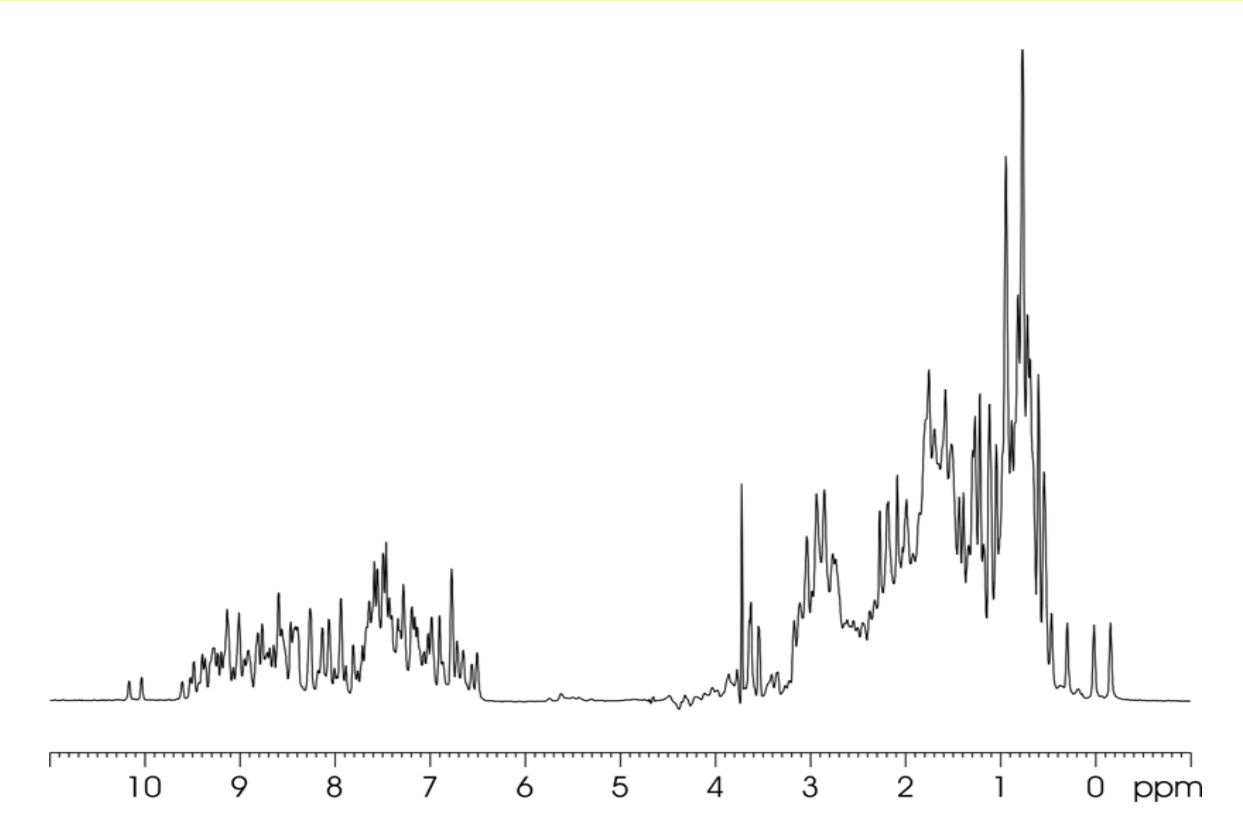
Solvent suppression

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.

Quadrature detection

Quadrature detection

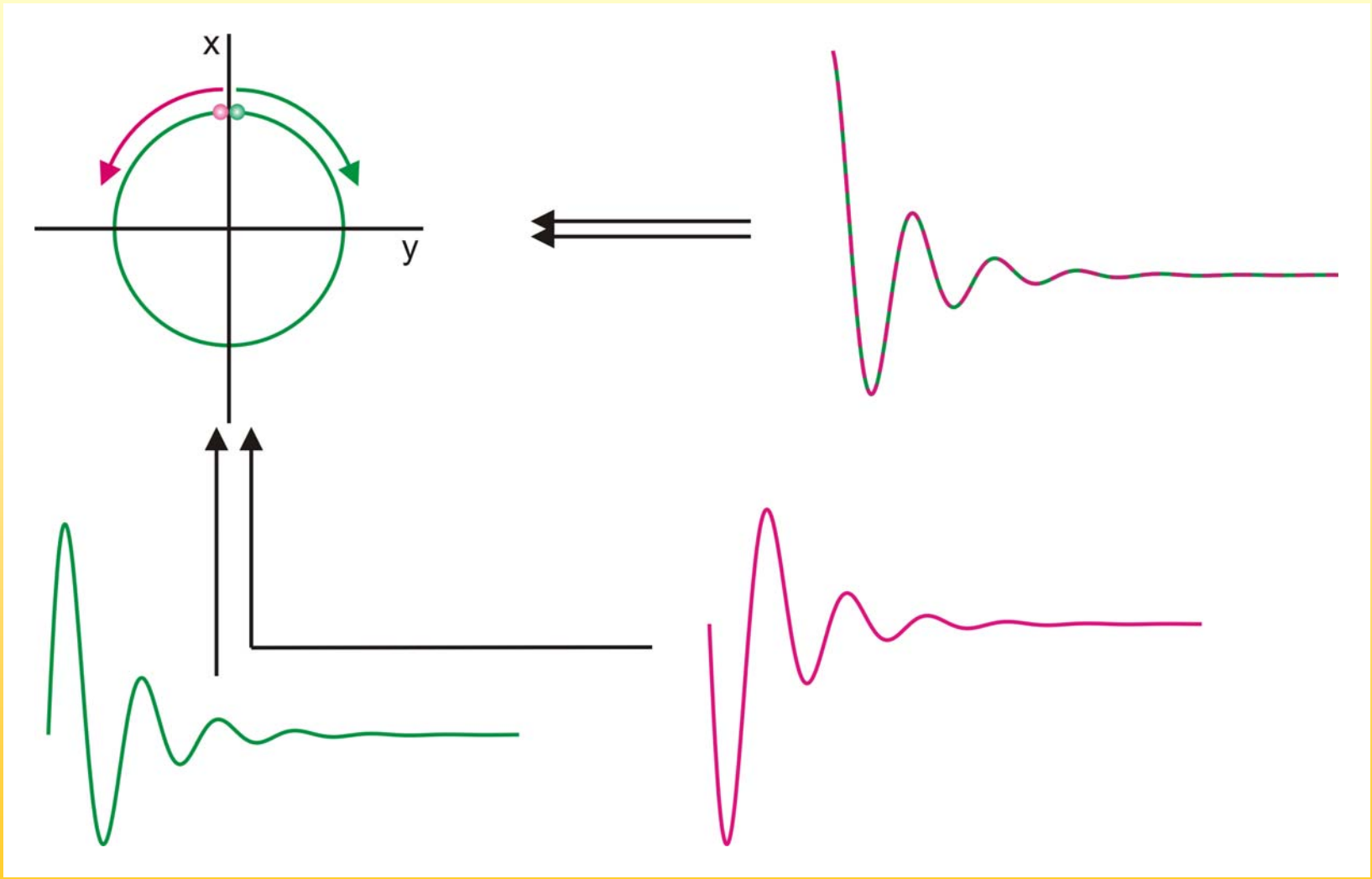
For practical reasons it is desirable to keep the spectral width as small as possible

This means that the center of the spectrum needs to be in the center of the signal region

And consequently the detection must be able to distinguish positive and negative frequencies or clockwise and counter-clockwise rotation relative to the center of the spectrum:

Quadrature detection

Quadrature detection



Quadrature detection

In formulas we can also see what happens if we have only a sine or a cosine

$$\exp(i\alpha) = \cos\alpha + i \sin\alpha$$

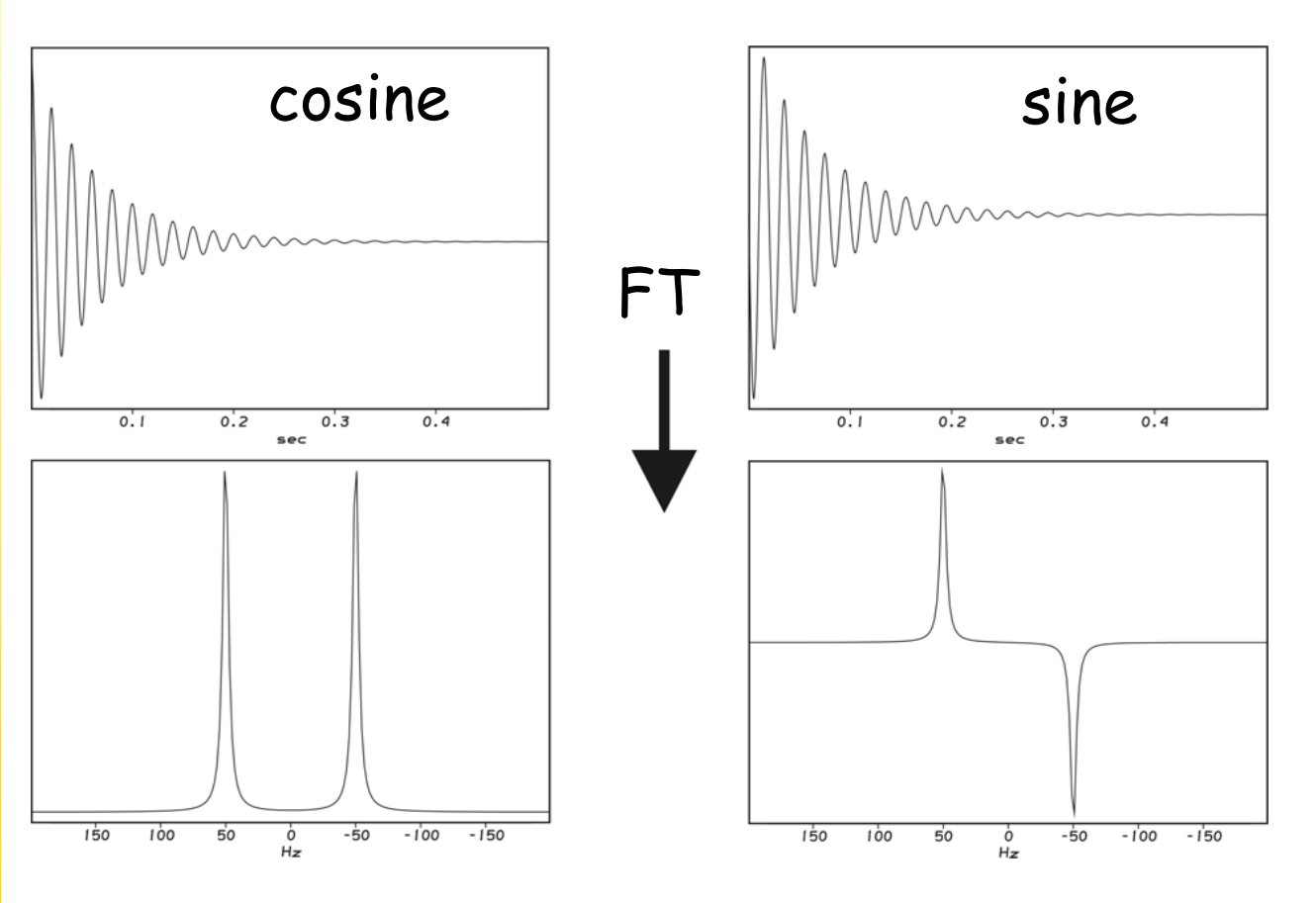
$$\exp(-i\alpha) = \cos\alpha - i \sin\alpha$$

$$\cos\alpha = \exp(i\alpha) + \exp(-i\alpha)$$

$$\sin\alpha = \exp(i\alpha) - \exp(-i\alpha)$$

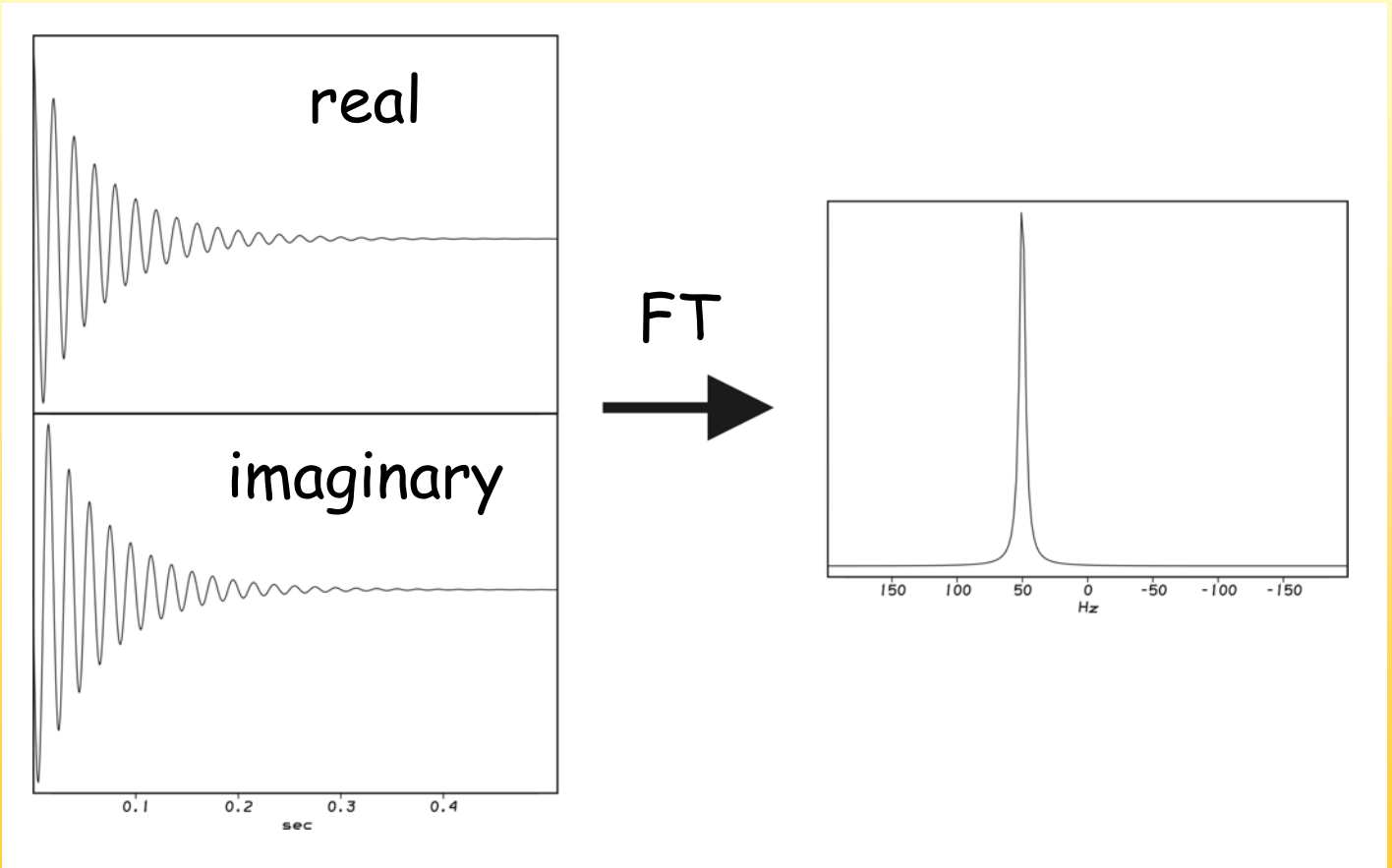
Quadrature detection

We get two signals each



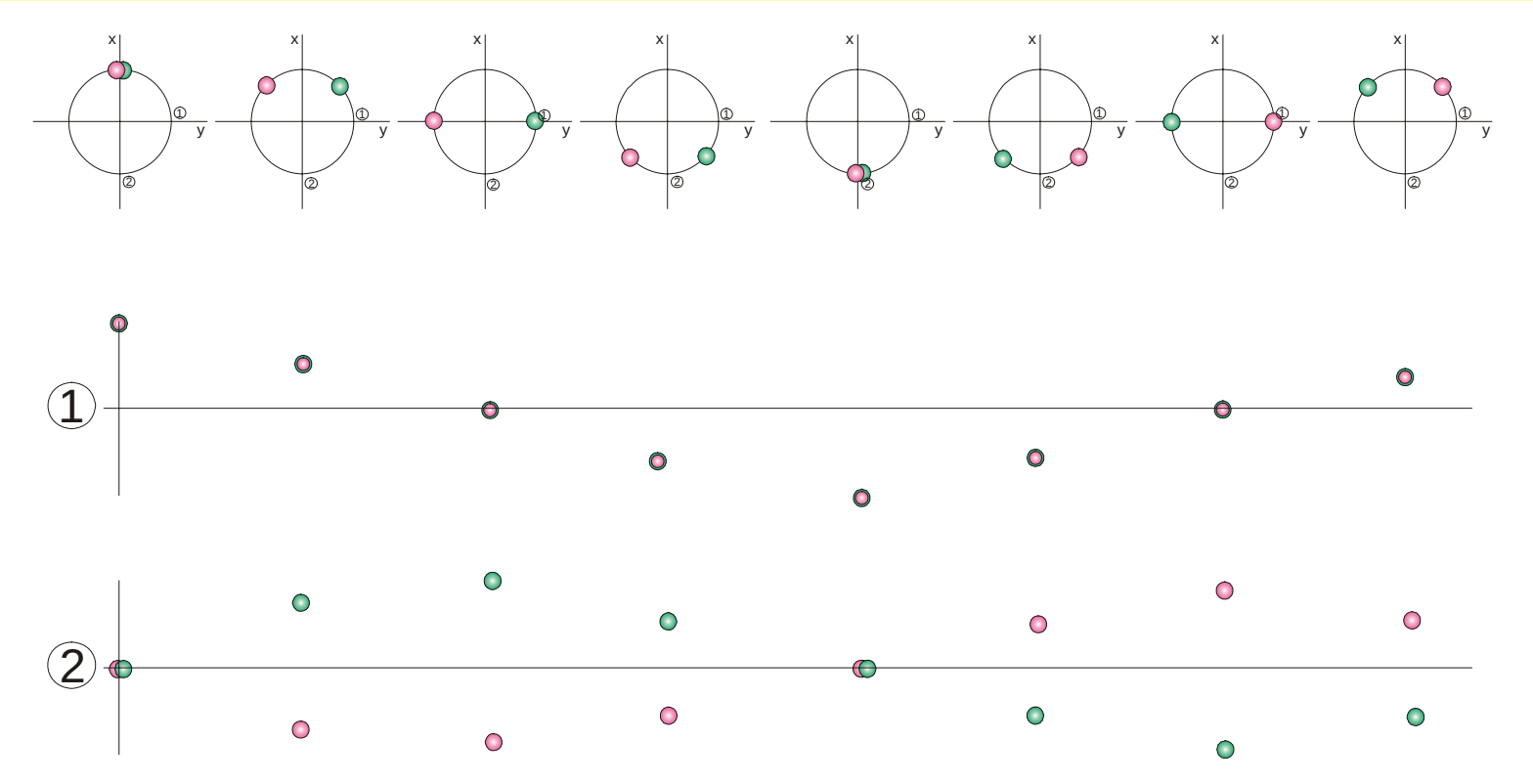
Quadrature detection

But if we collect two signals we can distinguish the sign



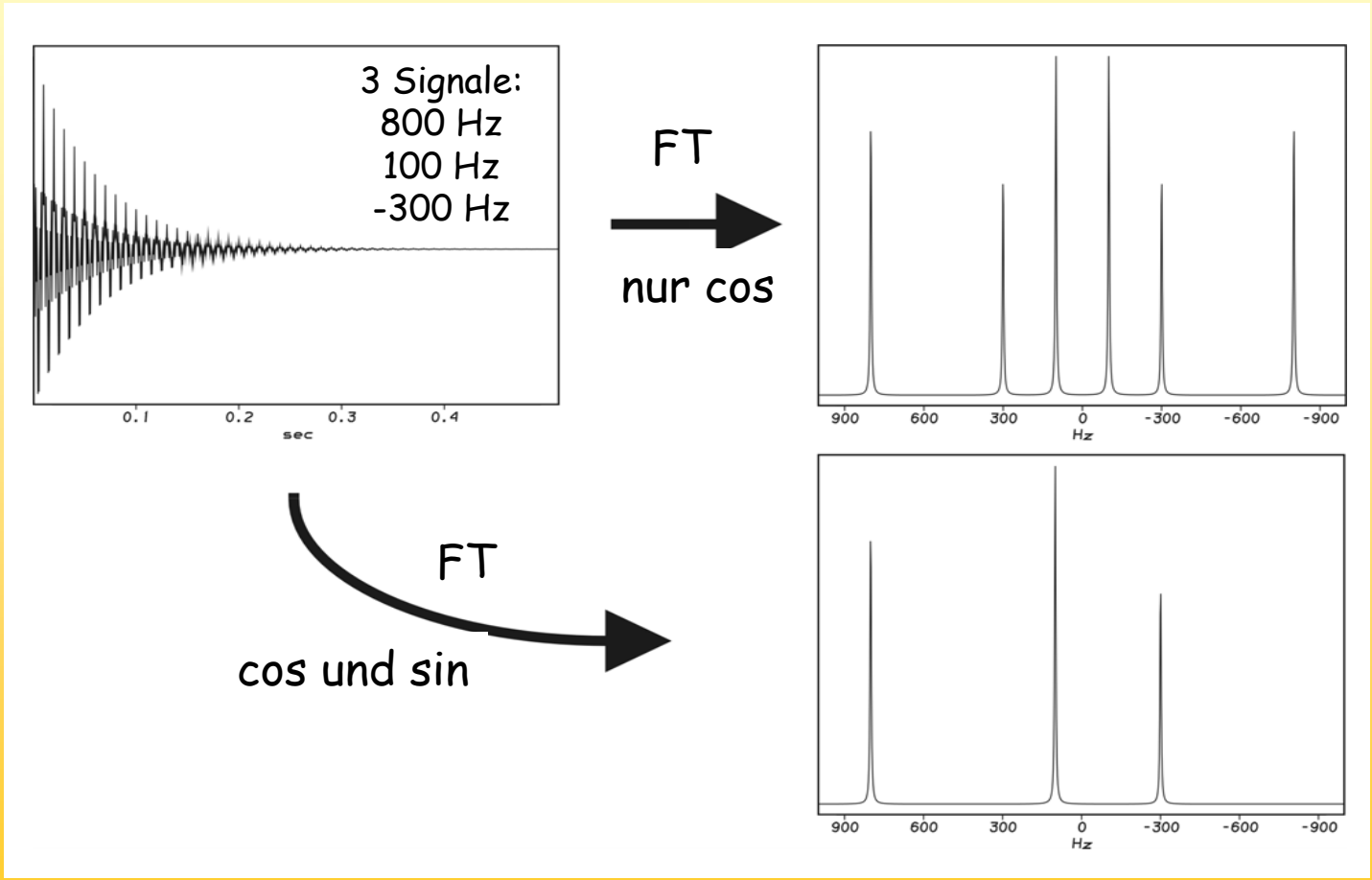
Quadrature detection

The easiest way to accomplish that are two ADCs, that are phase-shifted by 90°



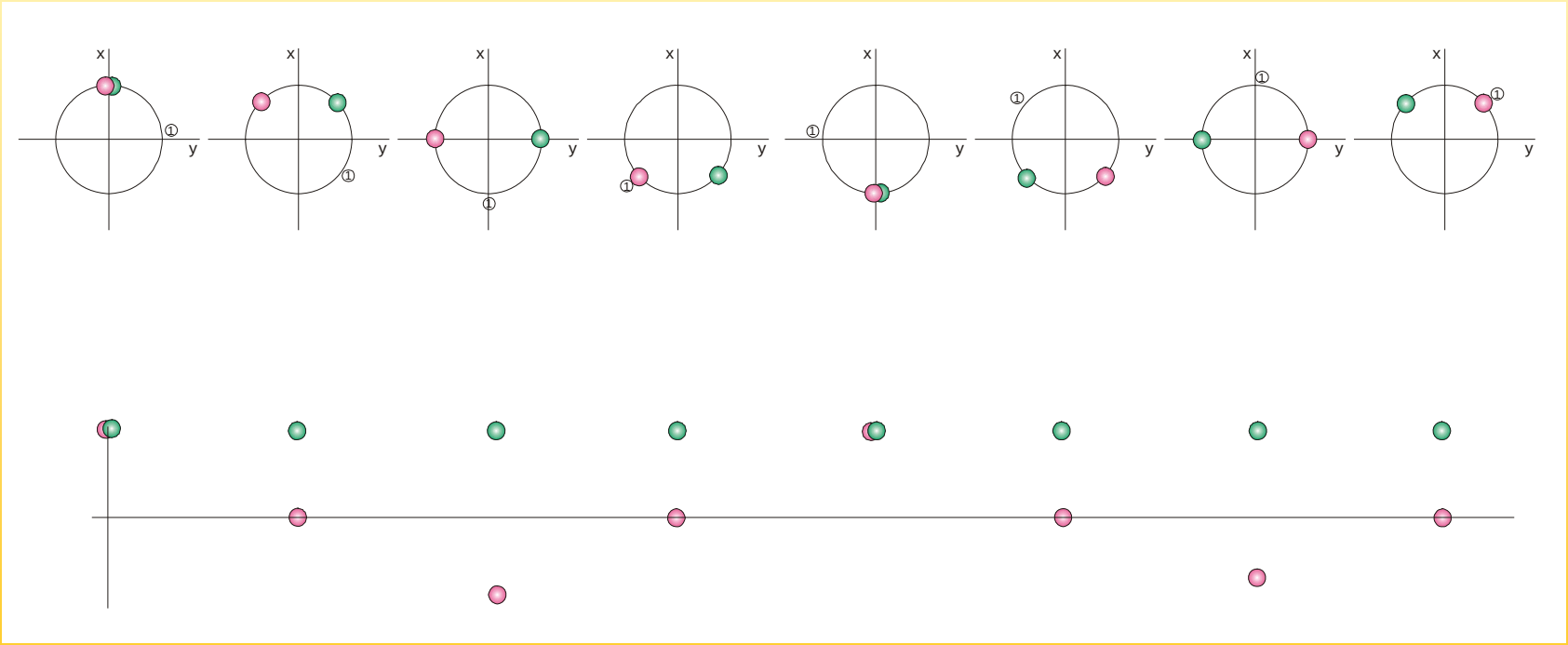
Quadrature detection

That way we can distinguish the signs of the signals



Quadrature detection

Since two ADCs were expensive some spectrometers used the „Redfield-trick", in which the phase of the receiver is changed from data point to data point



Quadrature detection

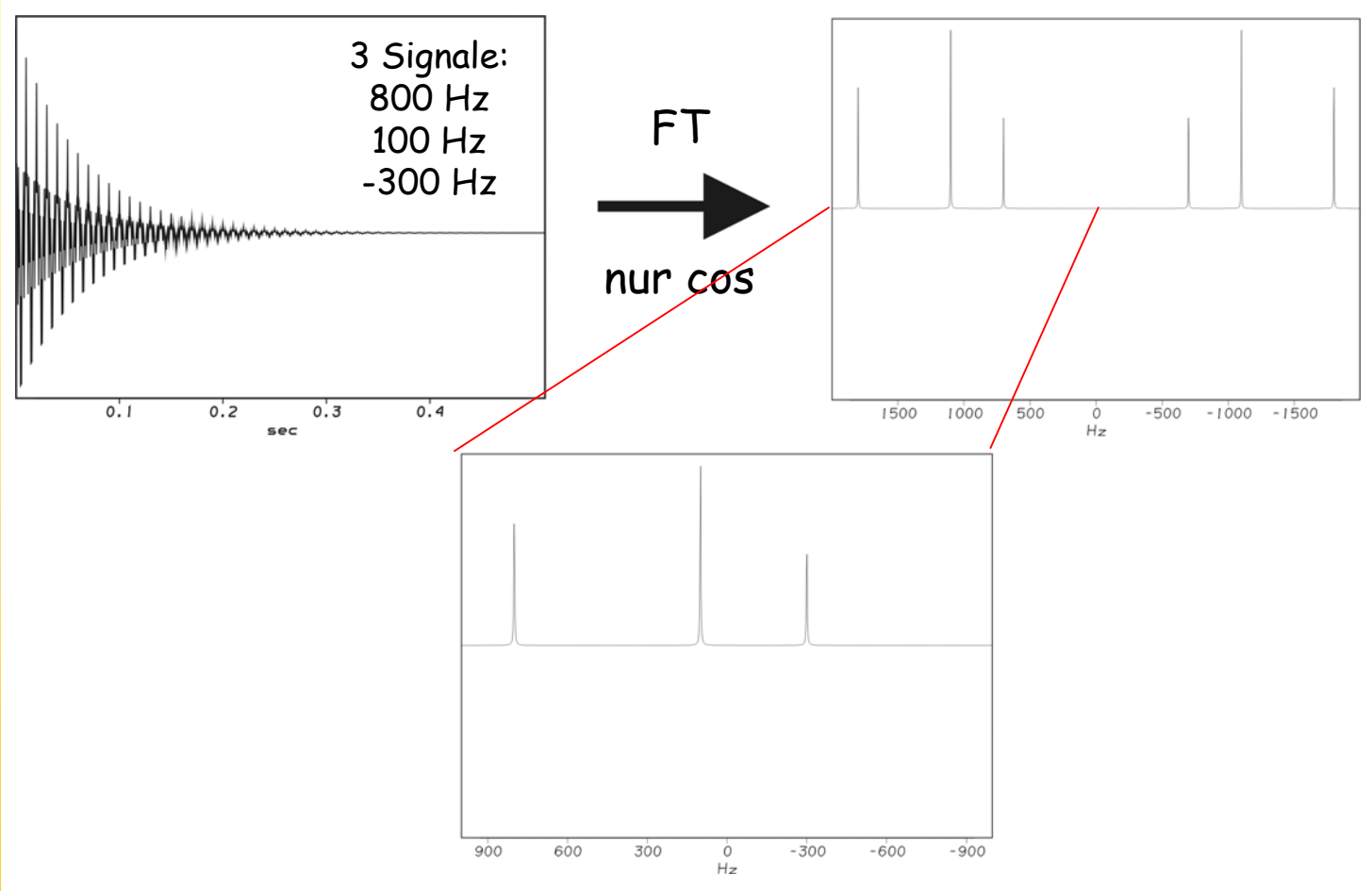
One edge of the spectrum appears to have the frequency zero, the other one to have twice the frequency.

Since in addition the sampling rate is doubled all frequencies can be detected and all have the same sign.

The FT results in a symetric spectrum, but the signals do not overlap any more.

Quadrature detection

In the end one half of the resulting spectrum is discarded



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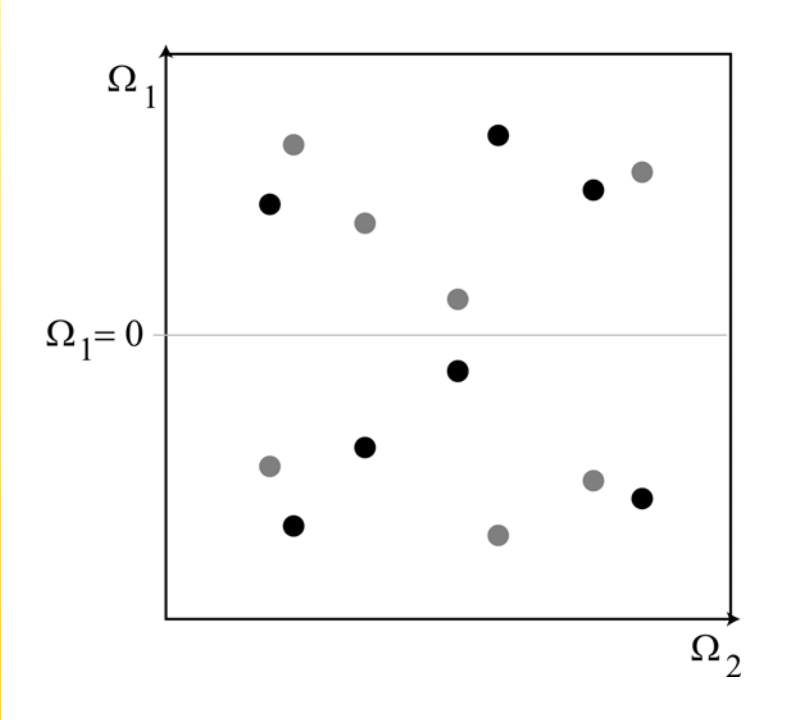
AG Solution NMR

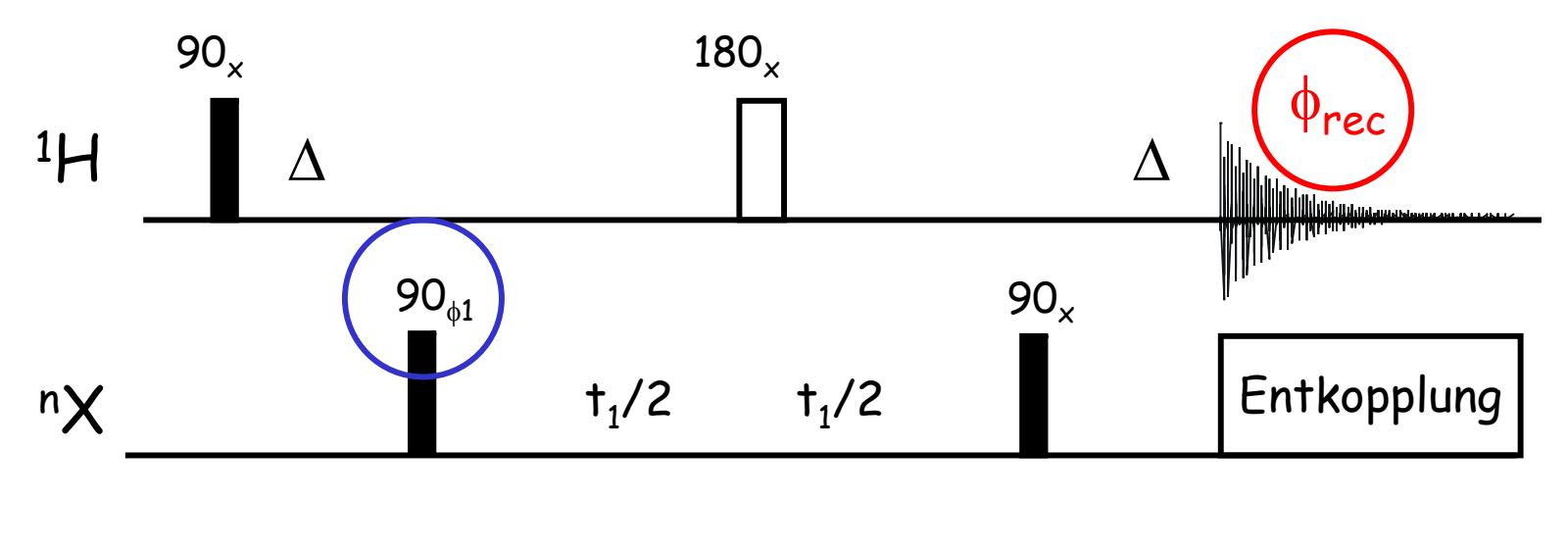
Quadrature detection

In the detection dimension quadrature detection is not an issue any more, but of course the same problem arises in the indirect dimensions



Quadrature detection

Quadrature detection in F1



1.FID: ϕ_1 = x, -x
2.FID: ϕ_1 = y, -y

1.FID: ϕ_{rec} = +, -
2.FID: ϕ_{rec} = +, -

Quadrature detection

There are several methods for quadrature detection

Methode	Phase des Präparations pulses	Empfänger-phase	Art der Fouriertrans-formation	Position der Axialpeaks
Redfield	x (t_1 + 0*Δ)	x	reell	Zentrum
	y (t_1 + 1*Δ)	x		
	x (t_1 + 2*Δ)	-x		
	y (t_1 + 3*Δ)	-x		
TPPI	x (t_1 + 0*Δ)	x	reell	Rand
	y (t_1 + 1*Δ)	x		
	-x (t_1 + 2*Δ)	x		
	-y (t_1 + 3*Δ)	x		
SHR	x (t_1 + 0*Δ)	x	komplex	Zentrum
	y (t_1 + 0*Δ)	x		
	x (t_1 + 2*Δ)	x		
	y (t_1 + 2*Δ)	x		
TPPI-States	x (t_1 + 0*Δ)	x	komplex	Rand
	y (t_1 + 0*Δ)	x		
	-x (t_1 + 2*Δ)	-x		
	-y (t_1 + 2*Δ)	-x		

Quadrature detection

In addition there is the echo/antiecho method that is particularily important since quadrature detection is also achievable by gradients.

It requires some additional data treatment but in essence is the same as recording complex points

Folding/aliasing

Folding/Aliasing

There are limits of the frequencies that can be detected by a digitizing ADC. We have the Nyquist-frequency

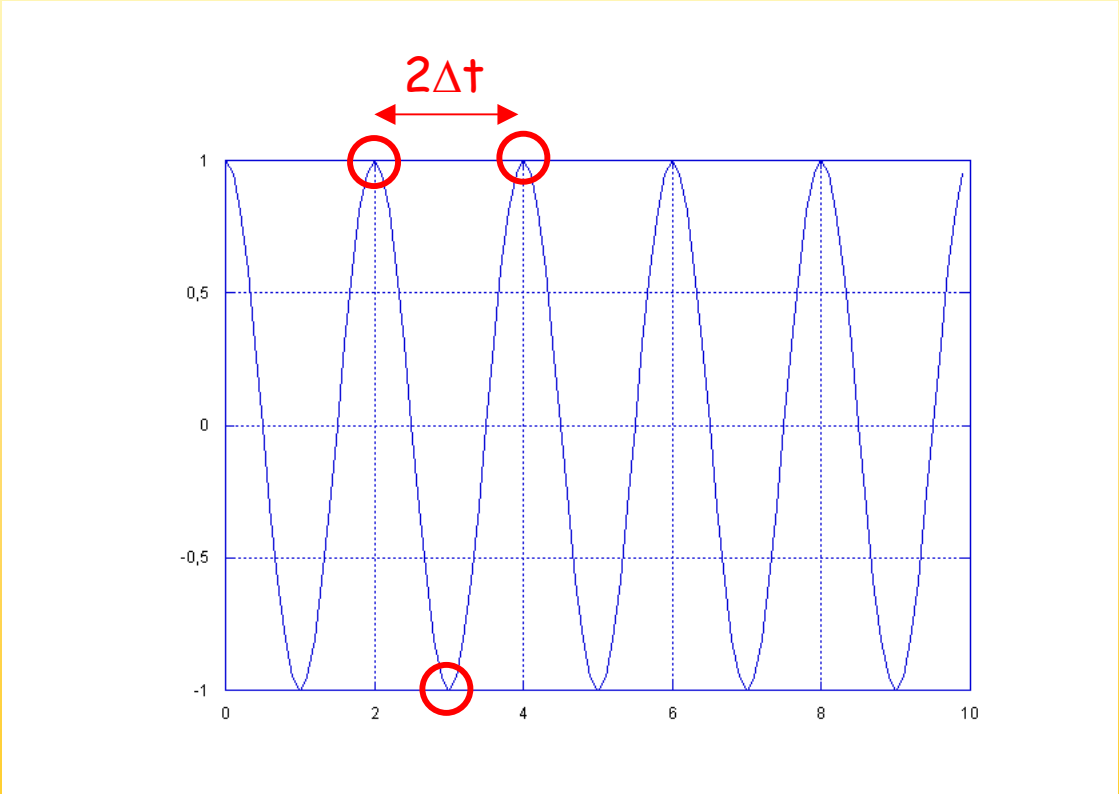
$$f_n = 1/(2\Delta t)$$

which is the largest frequency, that can still be detected if the time between datapoints is Δt. Since we are in the center of the spectrum and can detect $+f_n/-f_n$ we have a range of frequencies, the spectral width („SW")

$$SW = 2 * f_n = 1/\Delta t$$

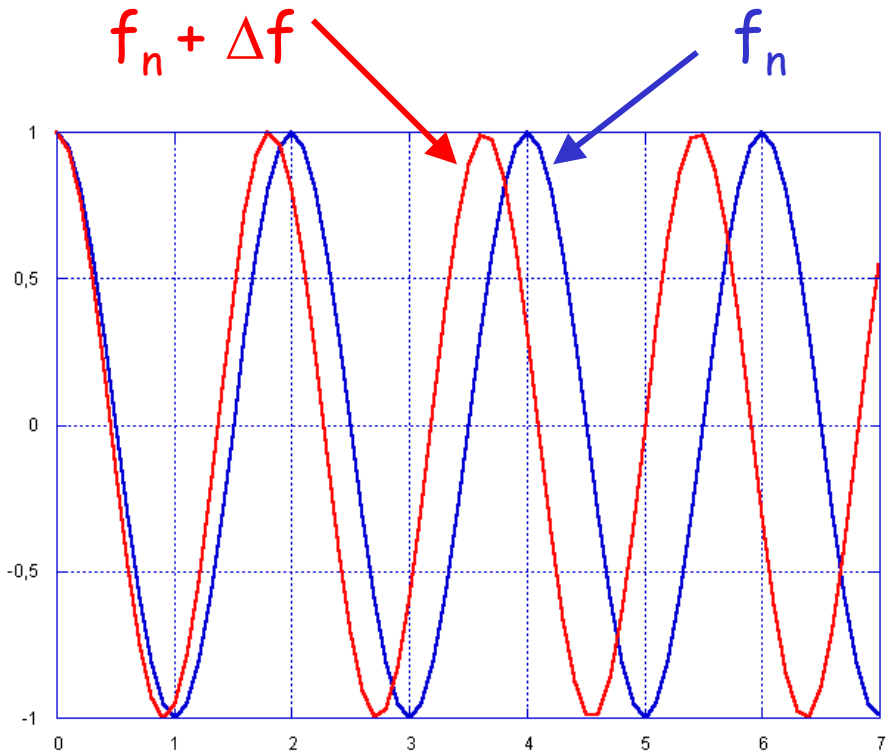
Folding/Aliasing

It means that we need three data points to detect a full period of the oscillation



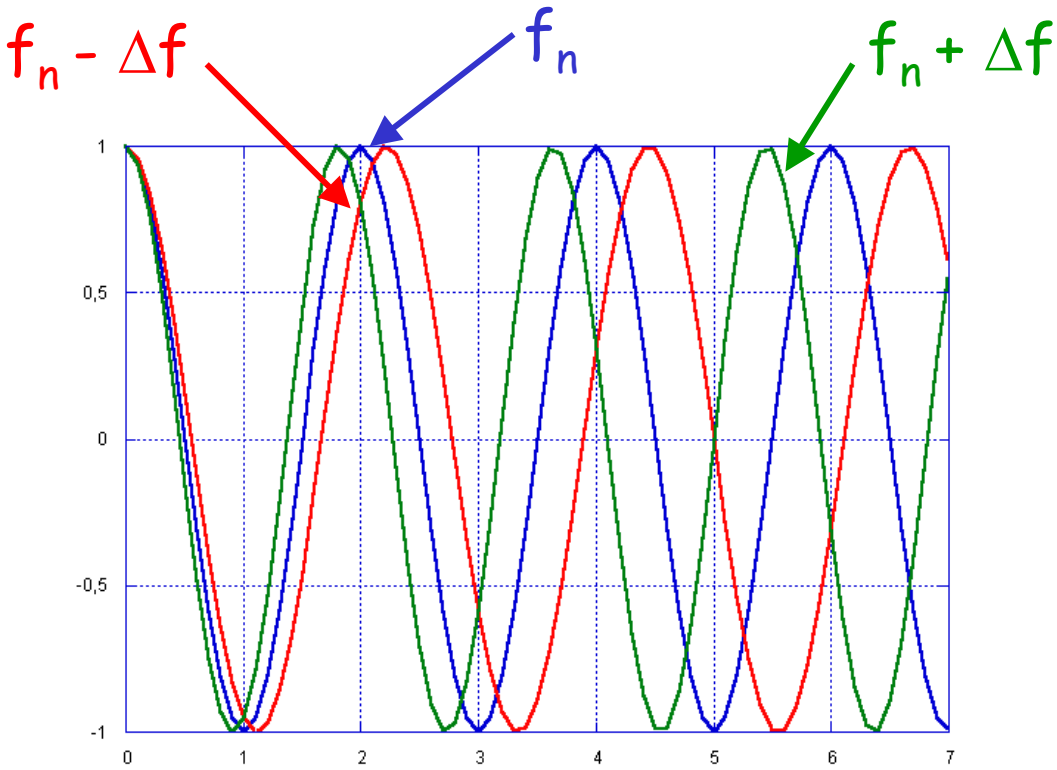
Folding/Aliasing

What happens if a frequency is larger than f_n ?



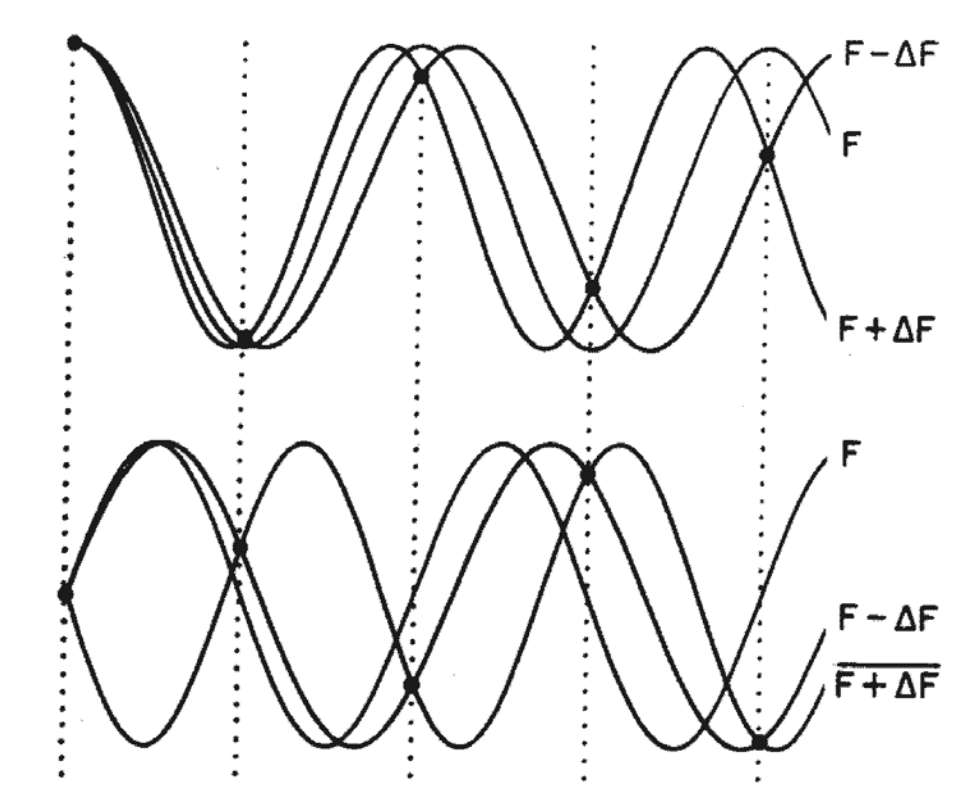
Folding/Aliasing

It simply appears lower !



Folding/Aliasing

This has different consequences for the two types of quadrature detection we have seen



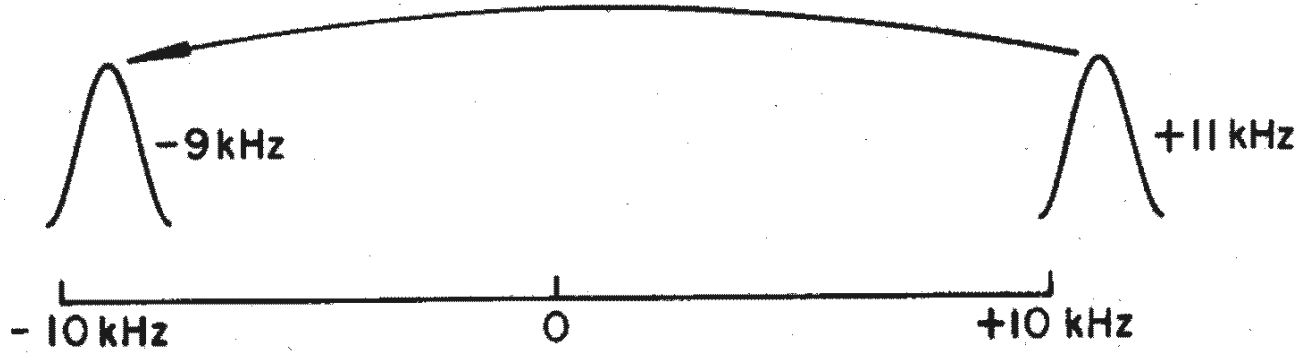
Only cosine
„Redfield Trick"

cosine and sine
„true" quadratur
detection

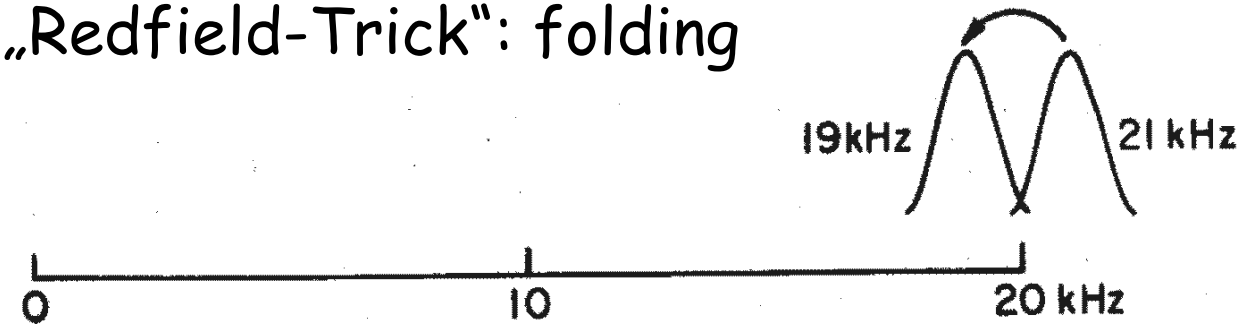
Folding/Aliasing

In one case we fold, in the other we alias

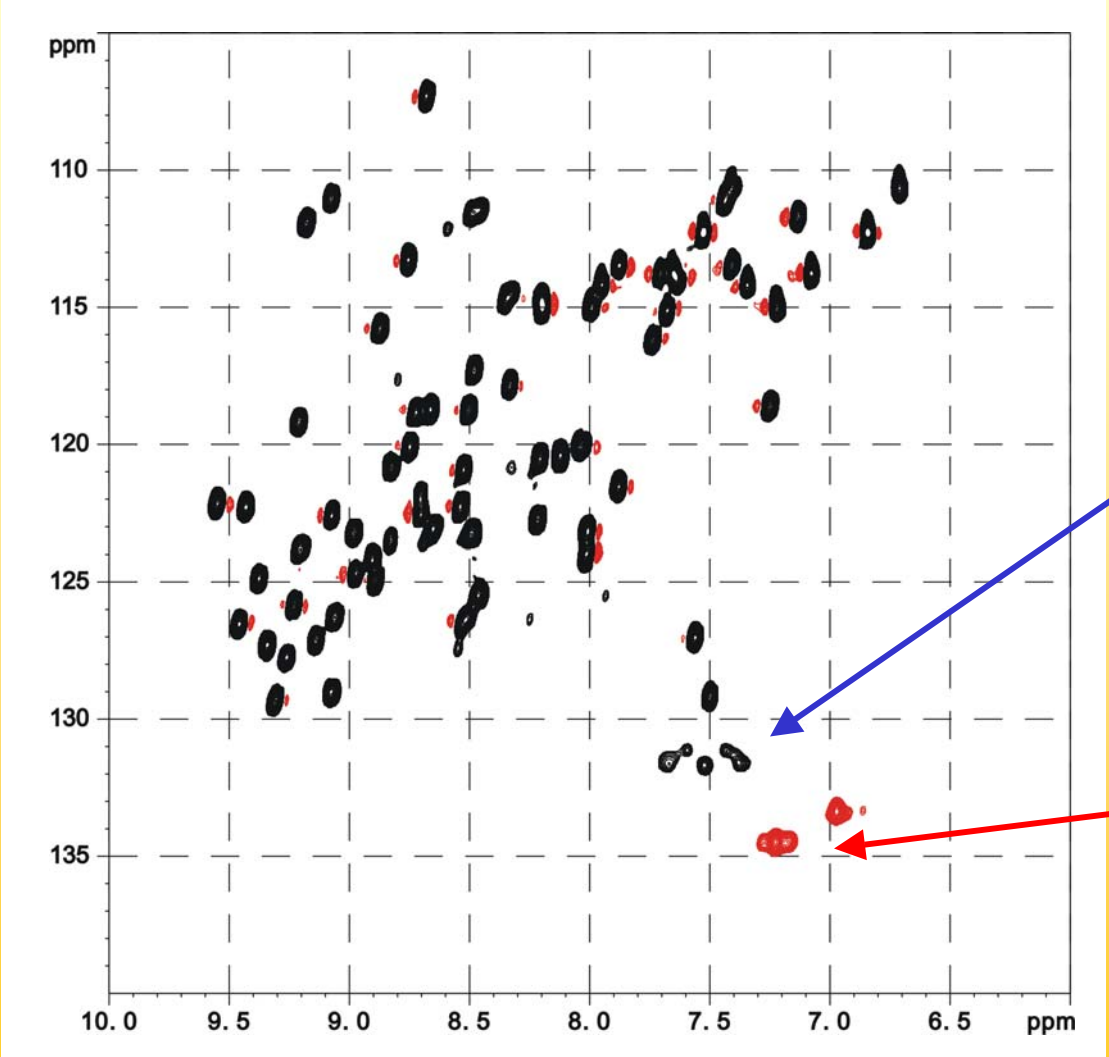
„true" quadratur detection: aliasing



„Redfield-Trick": folding



Folding/Aliasing



HSQC with 50 ppm SW in ^{15}N

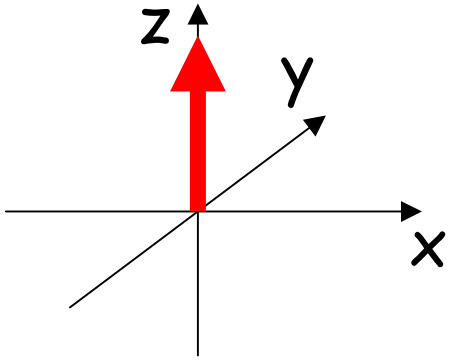
Lys side chains aliased twice (from 30 ppm)

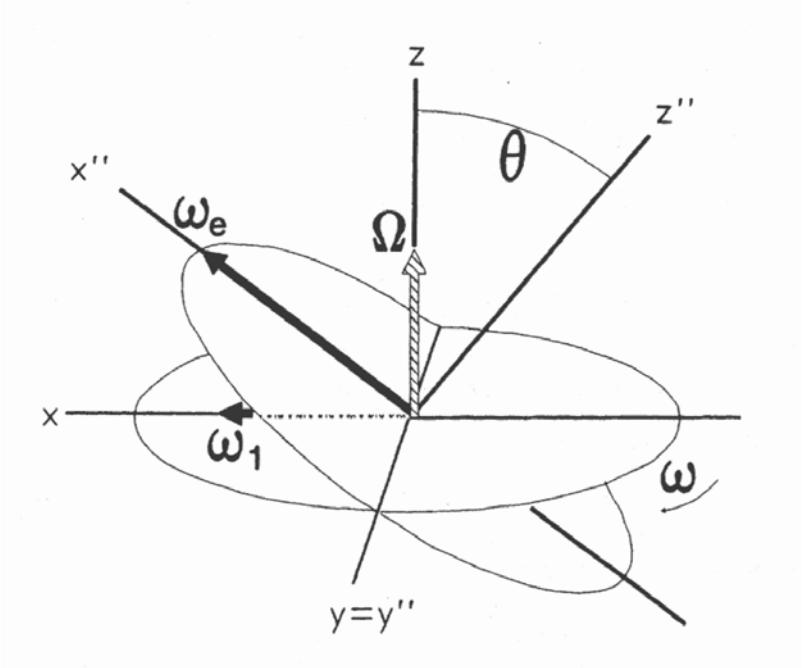
Arg side chains aliased once (from 85 ppm)

Phasing indirect dimensions

Phasing in indirect dimensions

One important aspect of the phasing in the indirect dimension is the phase created by the finite length of pulses





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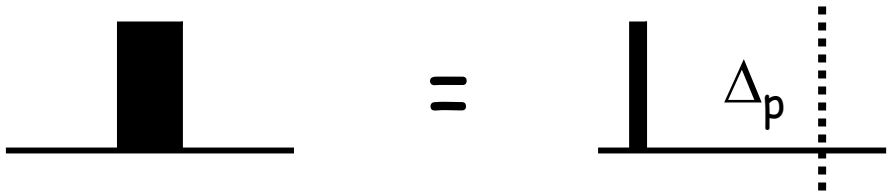
AG Solution NMR

Phasing in indirect dimensions

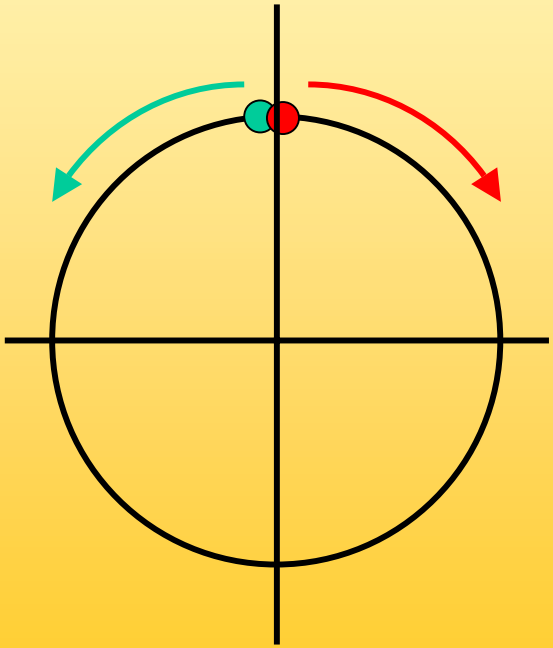
It can be calculated by a somewhat lengthy but in fact not very complicated calculcation of which we will only inspect the result

$$\Delta_p = 1/\omega_1 = (2/\pi) * \tau_p = 0.64 * \tau_p$$



Phasing in indirect dimensions

A phase correction in the indirect dimensions does only result from chemical shift evolution during the time $(t_1)_0$ between the pulses flanking the evolutiuon time for the first time point



right/left end of the spectrum moves to an angle

$$\phi = 2\pi * (t_1)_0 * SW/2$$

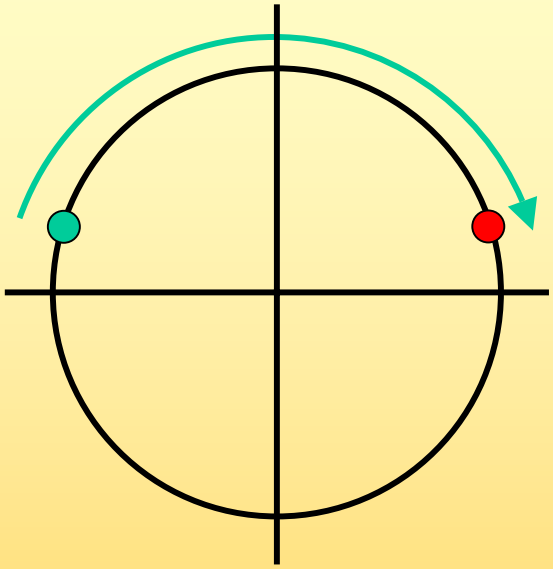
assuming

$$(t_1)_0 = 1 / 2 * SW$$

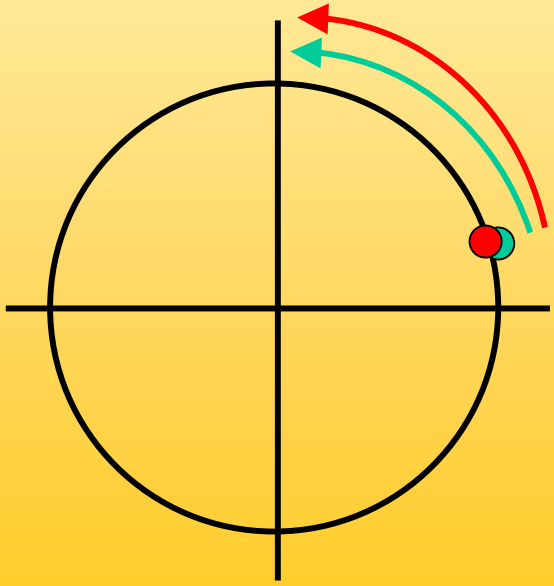
we get

$$\phi = \pi / 2$$

Phasing in indirect dimensions



The first order phase correction moves the left end to the right end so that all signals have the same phase, for $(t_1)_0 = 1 / 2 *$ SW we get phc1 = -180°

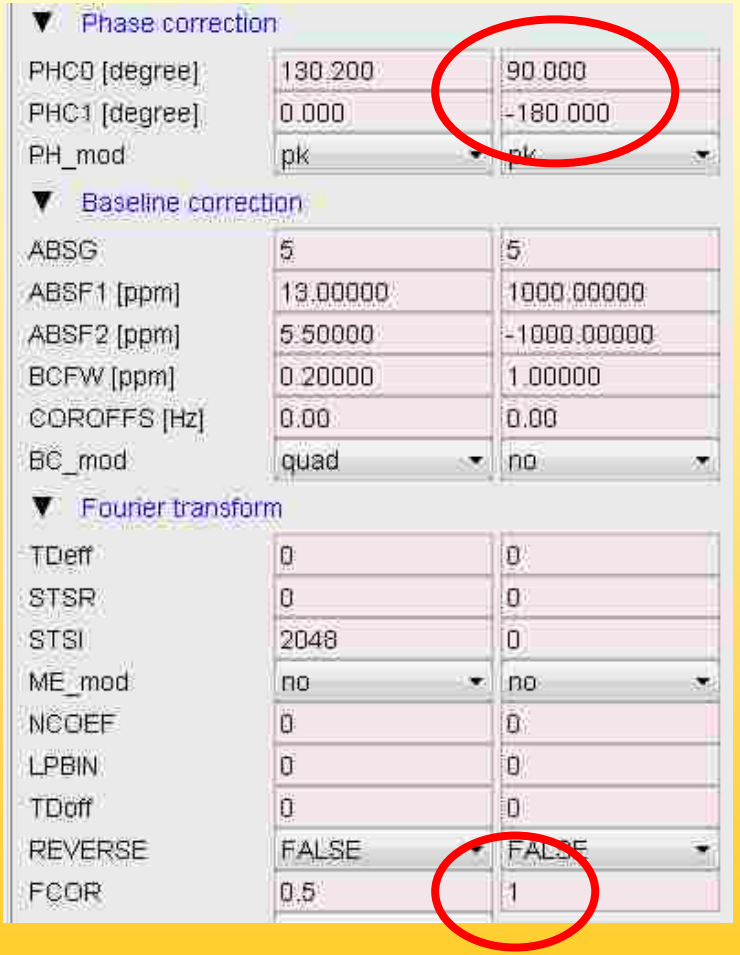


The zero order moves both back to zero were they would have been for $(t_1)_0 = 0$
That's why phc0 = -1/2 phc1 !

Phasing in indirect dimensions

Two scenarios are desirable

1. phc1 = 0°, phc0 = 0°,
   first point multiplied by 0.5
   folded/aliased peaks have
   same sign as unfolded
2. phc1 = -180°, phc0 = 90°,
   first point multiplied by 1
   folded/aliased peaks have
   opposite sign as unfolded



Phasing in indirect dimensions

If that is not possible

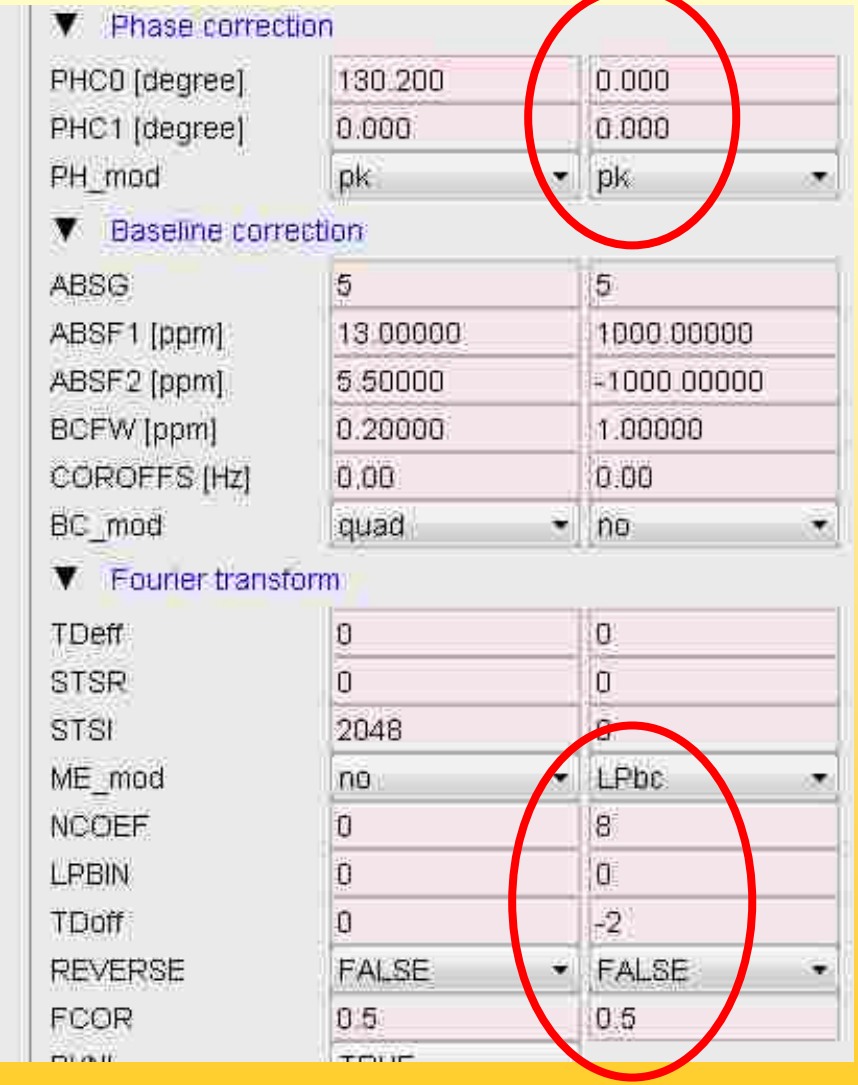
try to achive

phc1 = n * (-360°)

phc0 = n * 180°

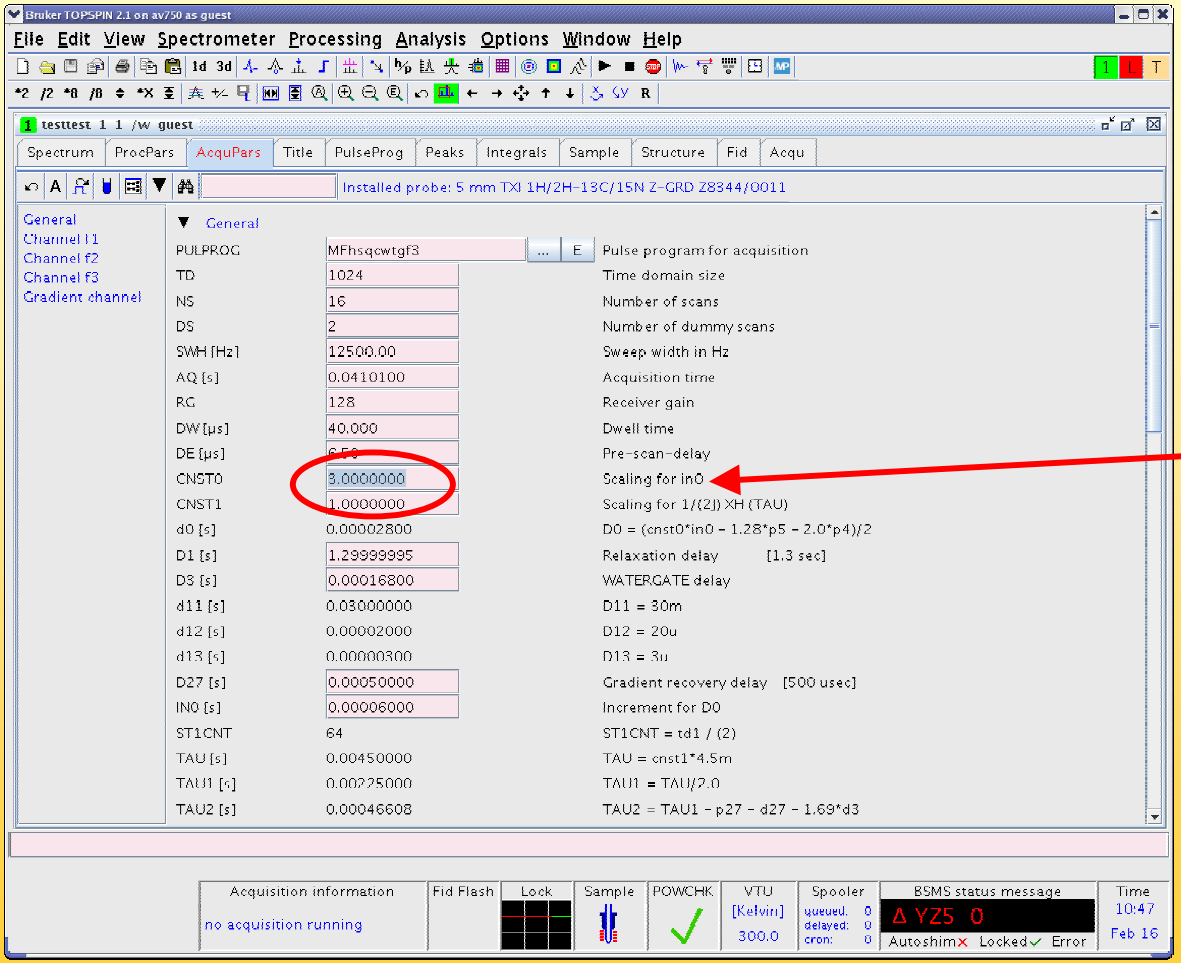
and use linear prediction

to get back to 1. or 2.



Peter Schmieder
AG Solution NMR

Phasing in indirect dimensions



The parameters cnst0 or cnst10 that we have already seen are in fact the "n"

Phasing in indirect dimensions

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

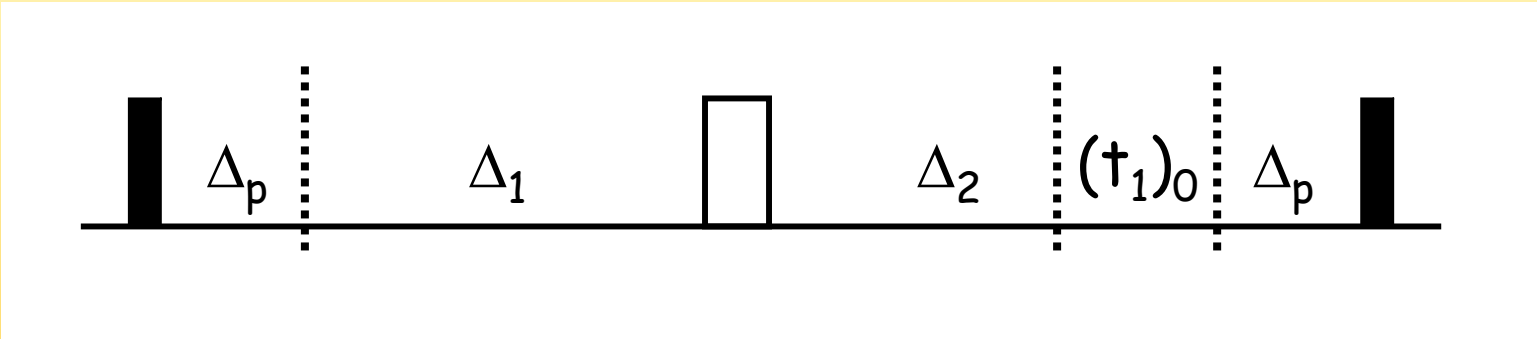
$1.28 = 2 * 2/\pi$

The puls sequences will force you to choose them

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

Phasing in indirect dimensions

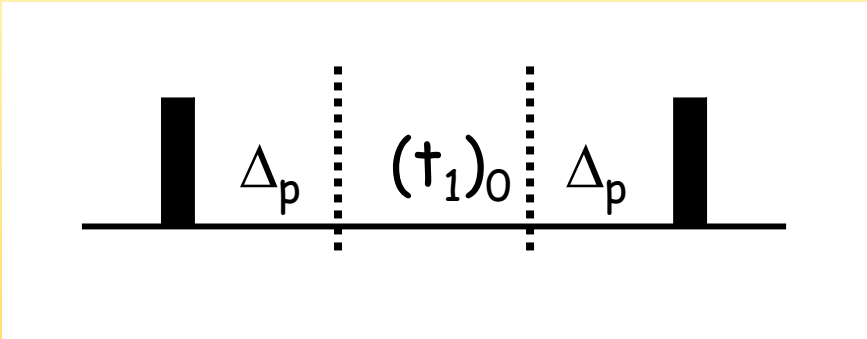
The first scenario (phc1 = 0°, phc0 = 0°) can only be achieved if a 180° pulse is present in the pulse sequence



The 180° pulse refocusses the delays Δ_p and if Δ_1 is set to be $\Delta_1 = \Delta_2 + (t_1)_0$ then no chemical shift can evolve and no phase is necessary

Phasing in indirect dimensions

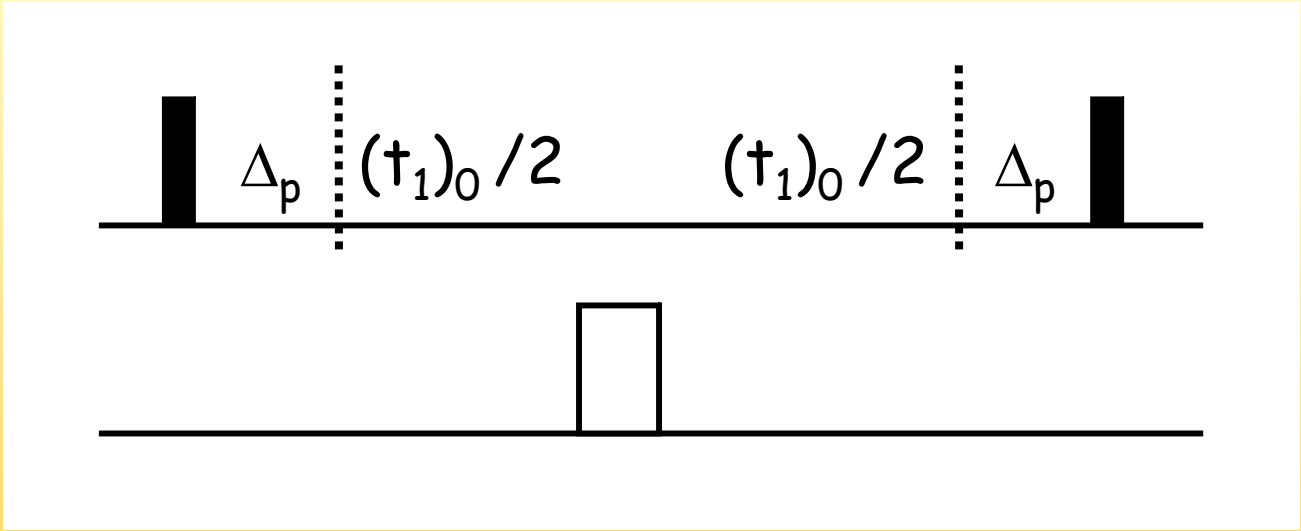
In homonuclear spectra the second scenario is relatively easy to achive



$$(t_1)_0 = 1 / 2 * SW - 2*\Delta_p = in_1/2 - 1.28 * \tau_p$$

Phasing in indirect dimensions

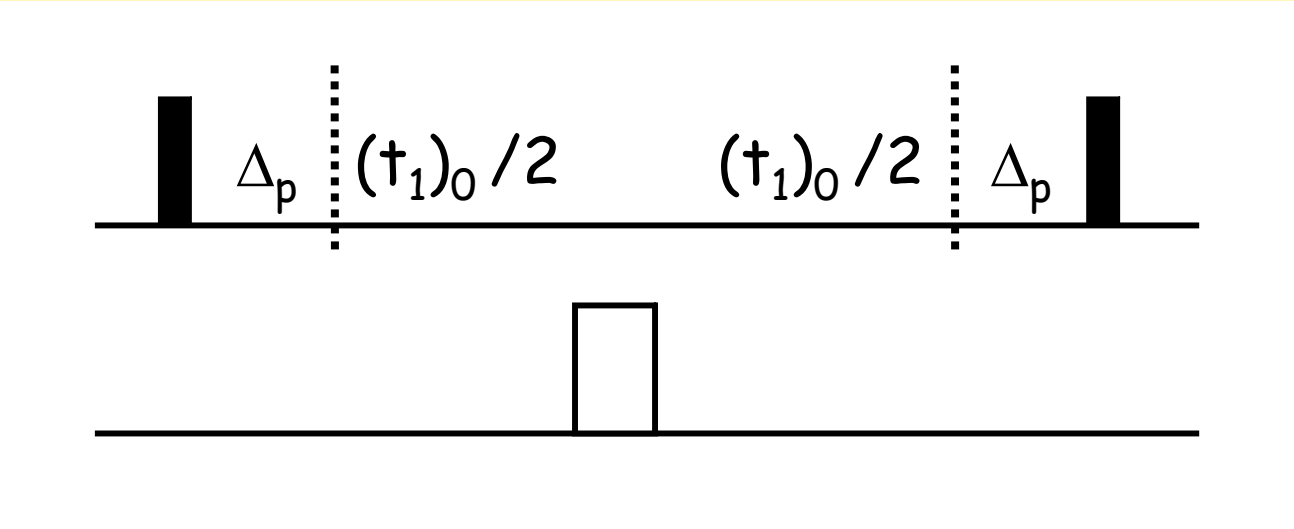
In heteronuclear spectra it might be a bit more difficult



$$(t_1)_0 = 1 / 2 * SW - 2 * \tau_p(X) - 2 * \Delta_p$$
$$= in_1/2 - 2 * \tau_p(X) - 1.28 * \tau_p(H)$$

Phasing in indirect dimensions

If that is still not enough we can go further and rely on back-prediction



$$(t_1)_0 = \text{cnst1} * 1 / 2 * SW - 2 * \tau_p(X) - 2 * \Delta_p$$
$$= \text{cnst1} * in_1/2 - 2 * \tau_p(X) - 1.28 * \tau_p(H)$$

Phasing in indirect dimensions

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

!

$1.28 = 2 * 2/\pi$

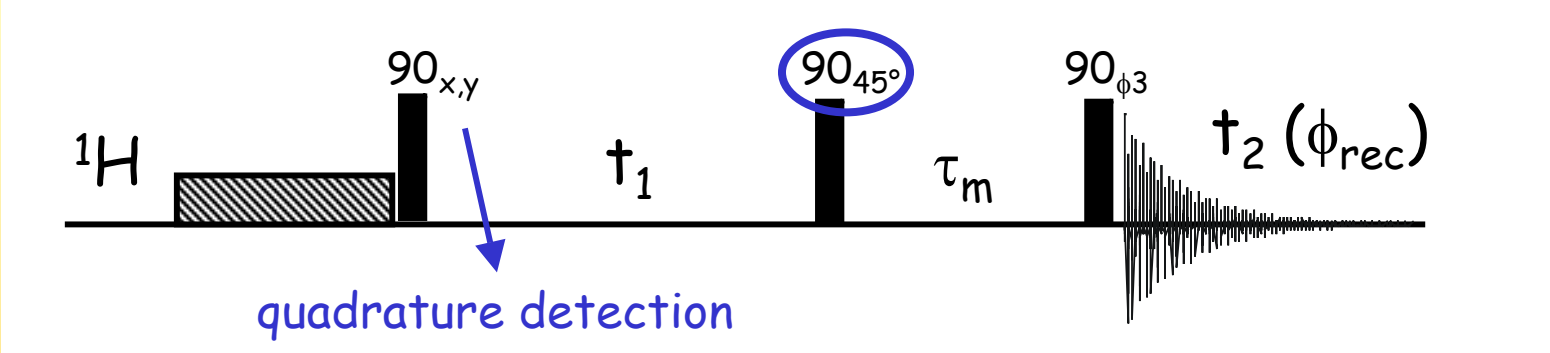
Recognize the fomula ?

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

!

Phasing in indirect dimensions

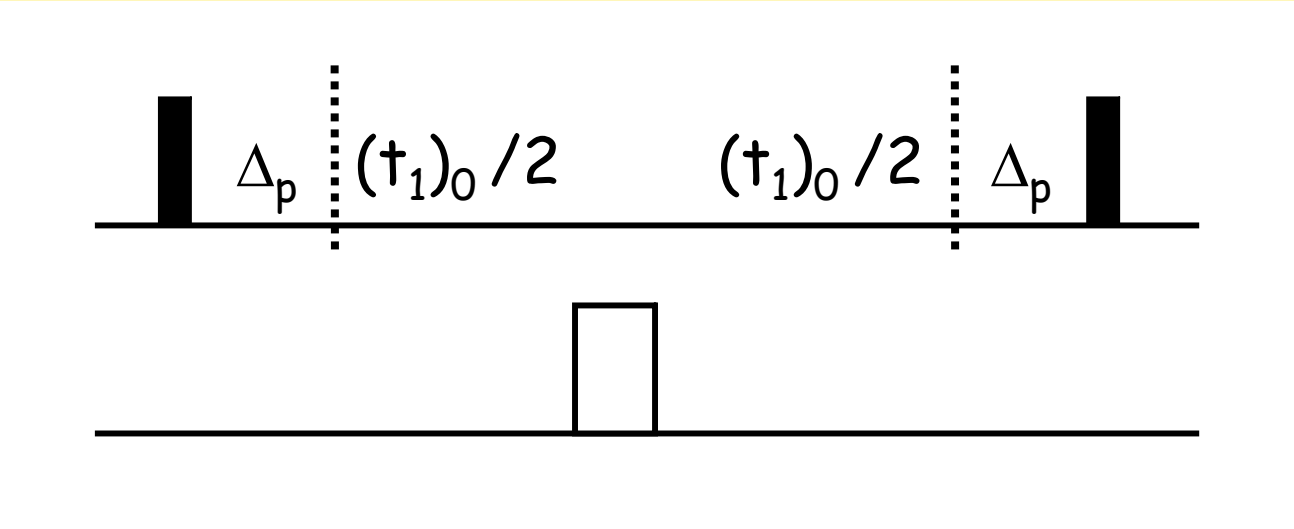
Of course there are exceptions to those rules



Sometimes care has to be taken that the water does not cause unfavorable effects: To avoid that water behaves differently in real and imaginary FID the phase of the second pulse is shifted: Then the phase is 135°/-180°

Phasing in indirect dimensions

The other is the "Bloch-Siegert Shift" or "z-rotation"



If all pulses hit the same typ of nucleus (because they are selective pulses), then the 180° pulse causes a zero-order phase correction

That's it

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