Working at 700 MHz: from Theory to Practice

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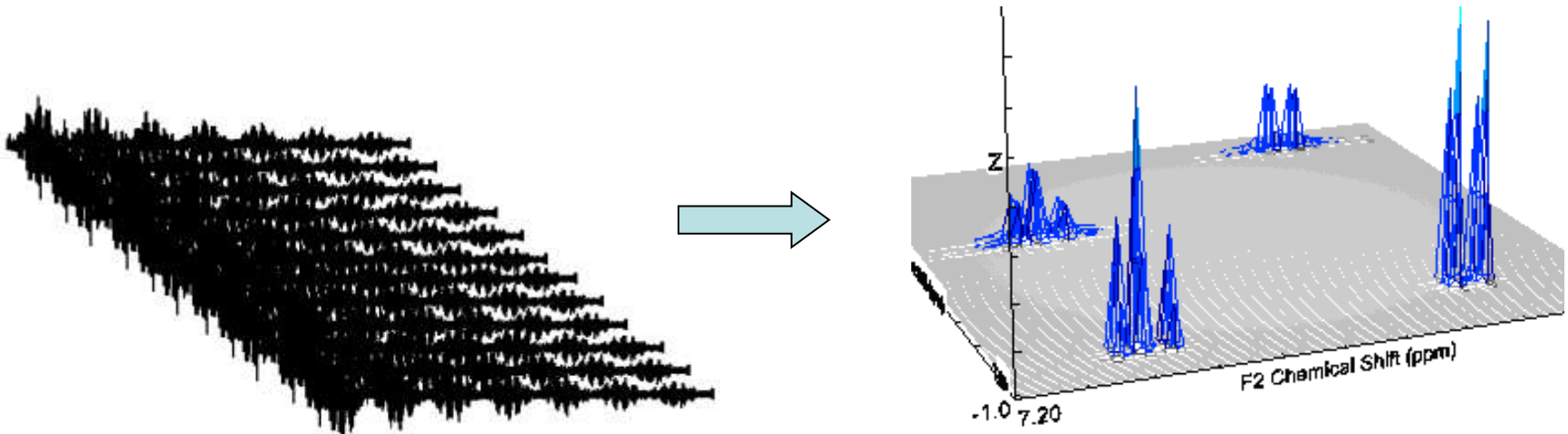
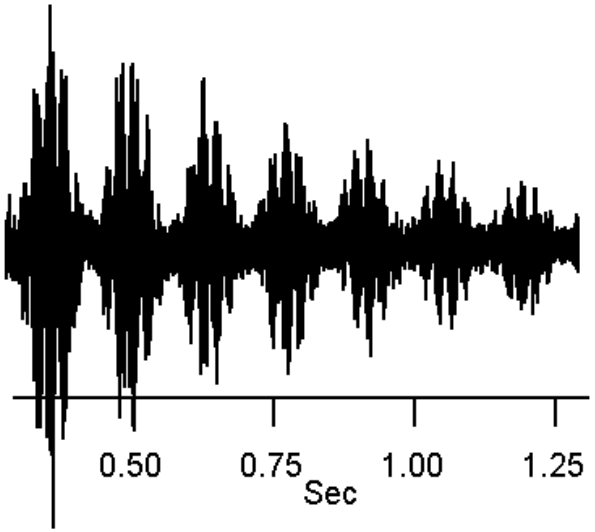


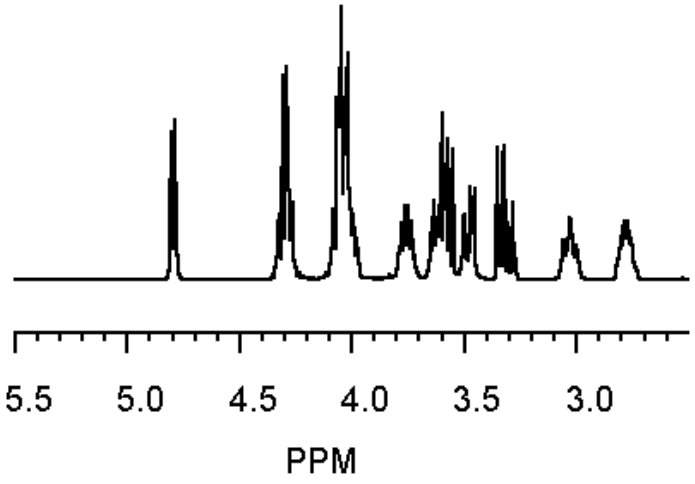

OXFORD
INSTRUMENTS
NMR 700
Active Shield

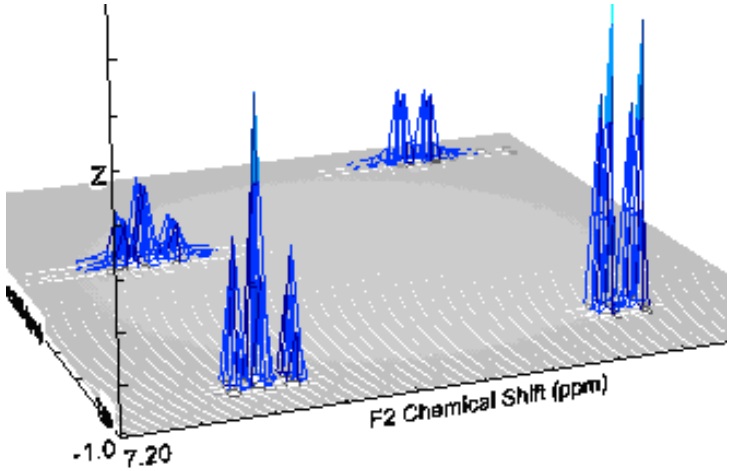
Quali parametri devo mettere???

Come devo trasformare???

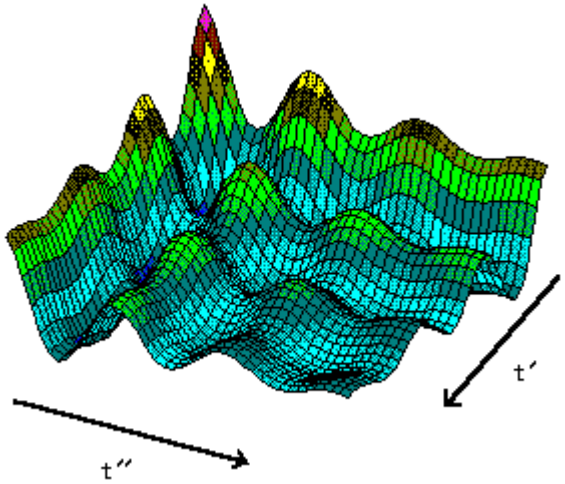
NMR Data Acquisition and Processing



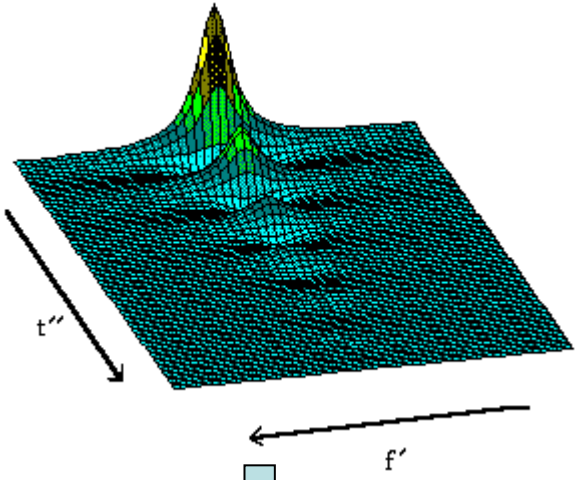




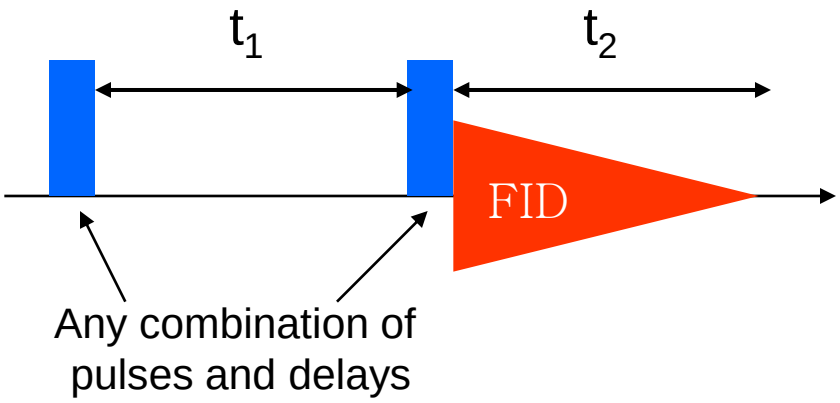
2D NMR

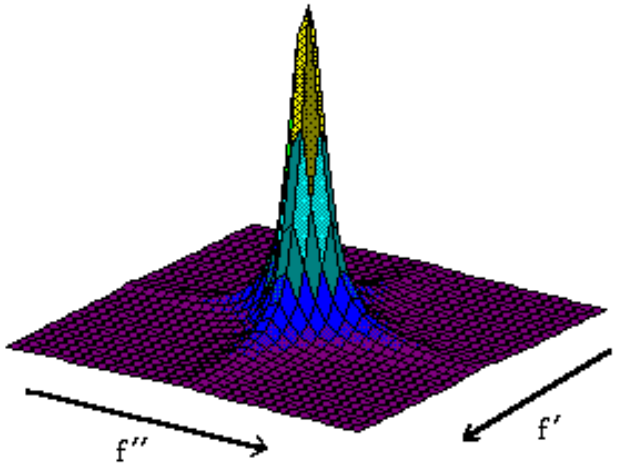


FT along t_2

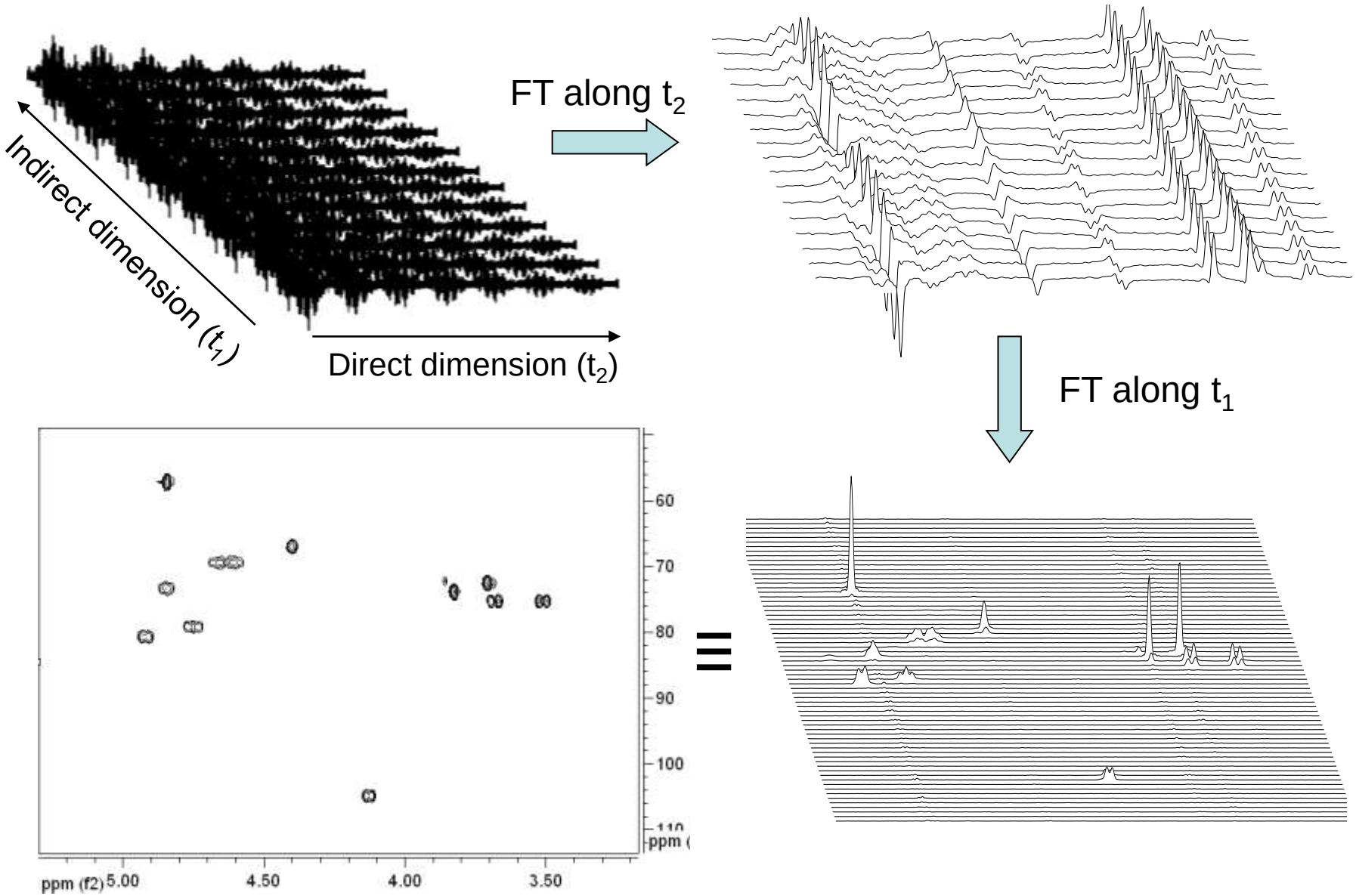


FT along t_1





2D NMR


FT along t_2
Indirect dimension (t_1)
Direct dimension (t_2)
FT along t_1
60
70
80
90
100
ppm (f2) 5.00
4.50
4.00
3.50

NMR Data Acquisition and Processing

- Digitalization
- Zero filling (digital resolution)
- Apodization (window functions)
- Linear prediction
- Fourier transform
- Phase correction
- Baseline correction
- Symmetrization

The Fourier Transform

Real FT:

$$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

the point at frequency ν of the spectrum

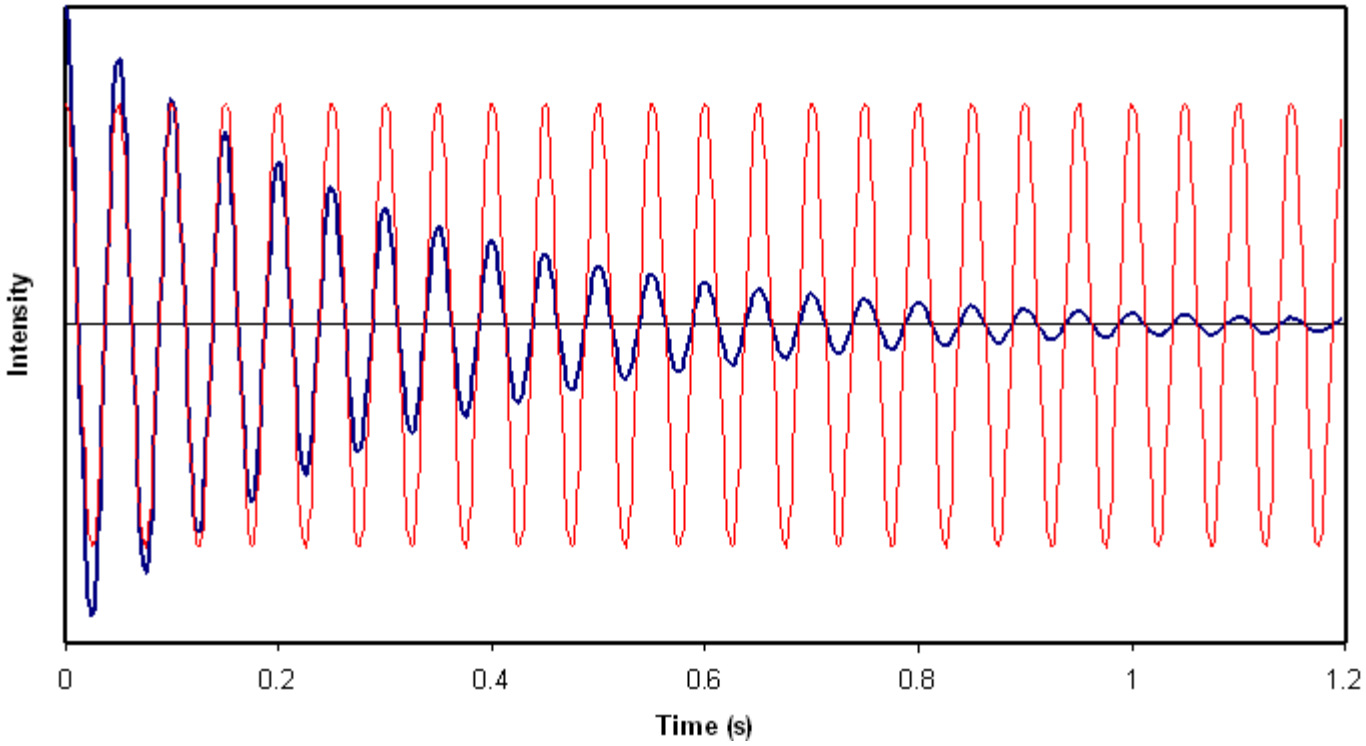
the FID

The Fourier Transform

Real FT: $$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

$f(t)$: 20 Hz

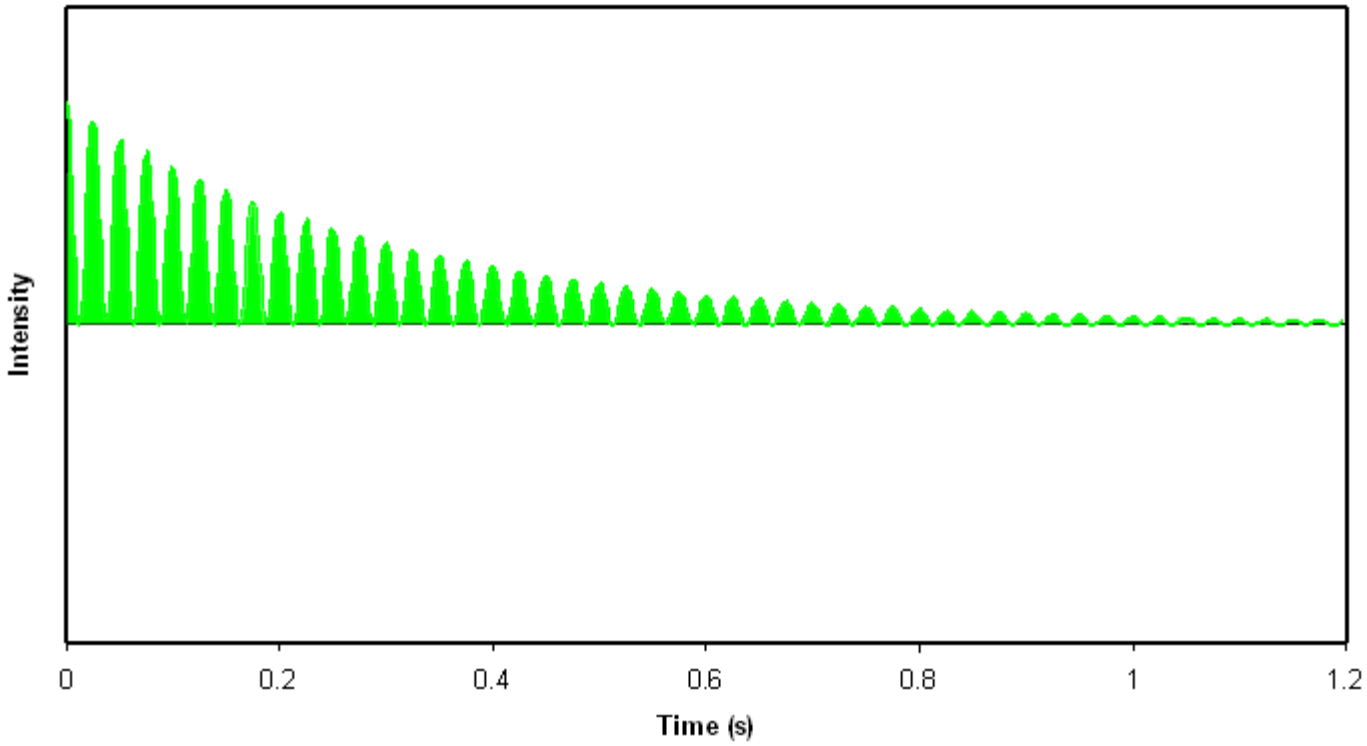
ν: 20 Hz



The Fourier Transform

Real FT: $$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

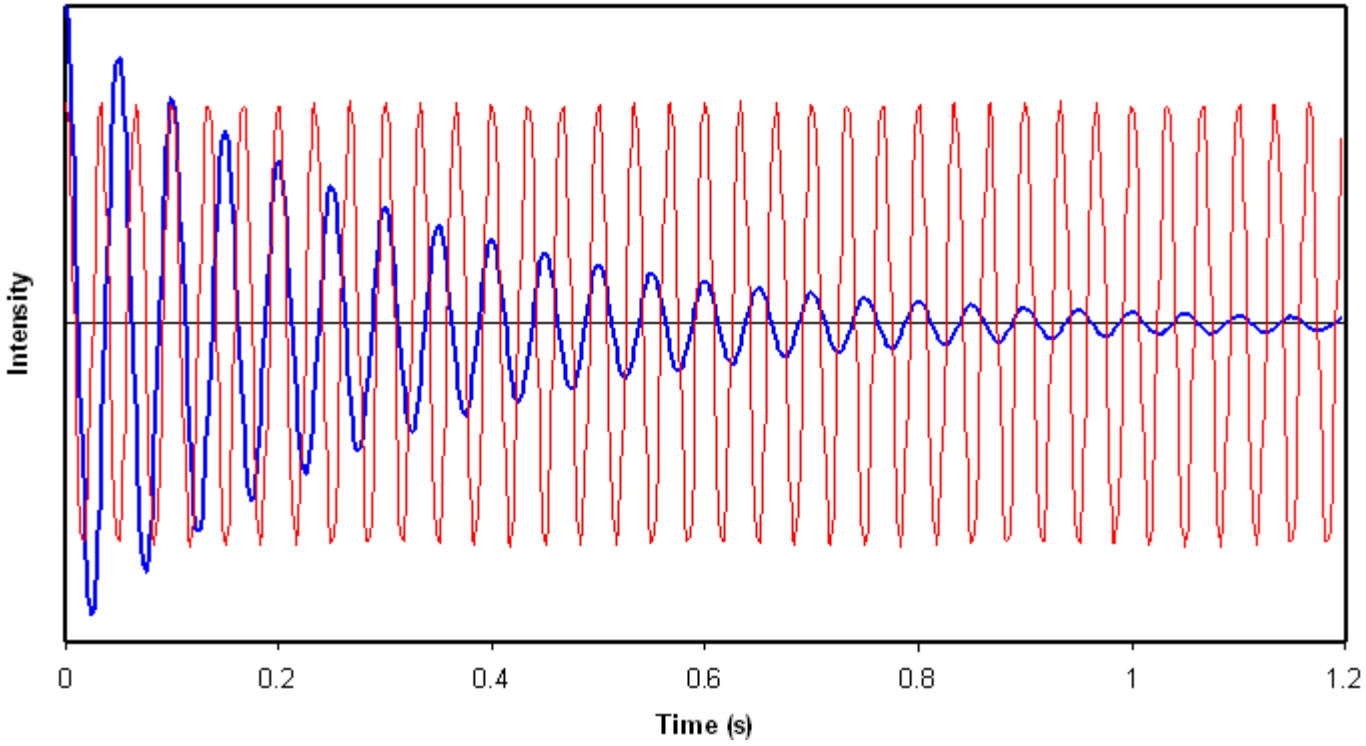
$f(t)$: 20 Hz ν: 20 Hz



The Fourier Transform

Real FT: $$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

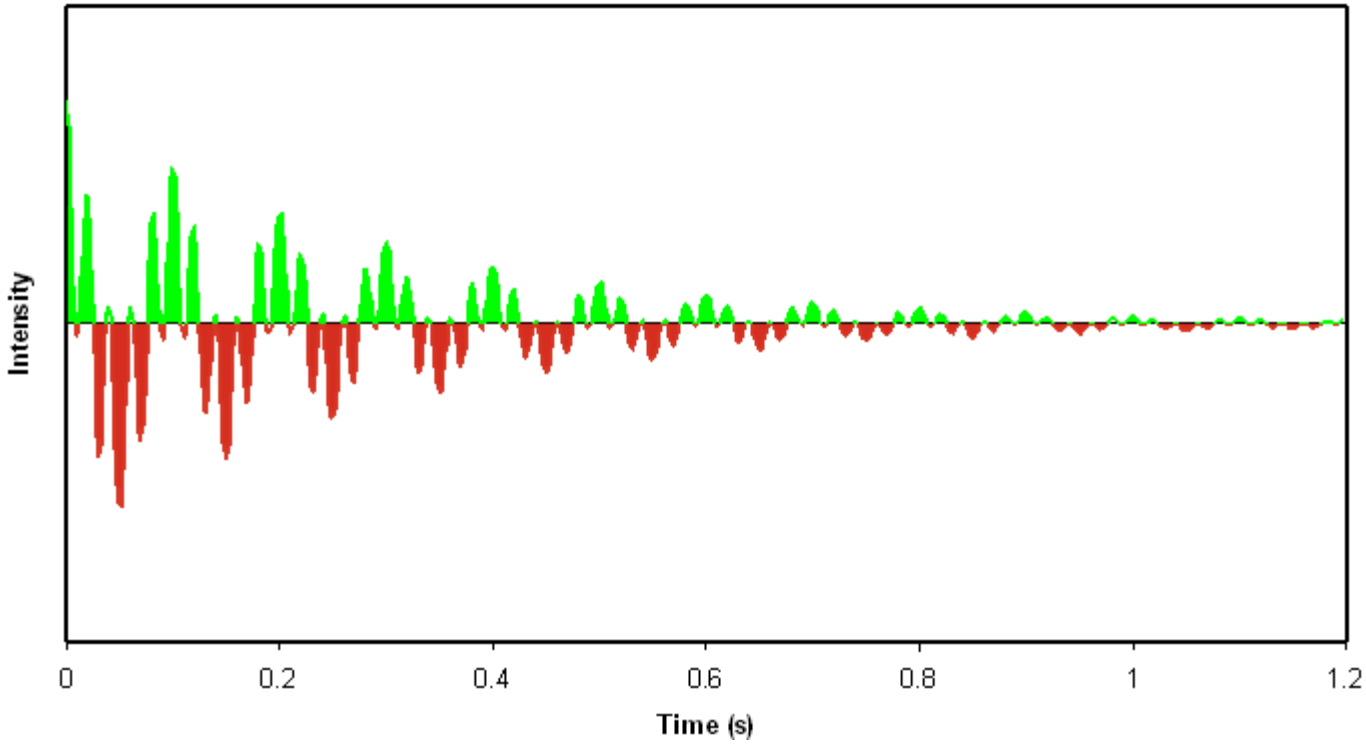
$f(t)$: 20 Hz $\qquad$ ν: 30 Hz



The Fourier Transform

Real FT: $$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

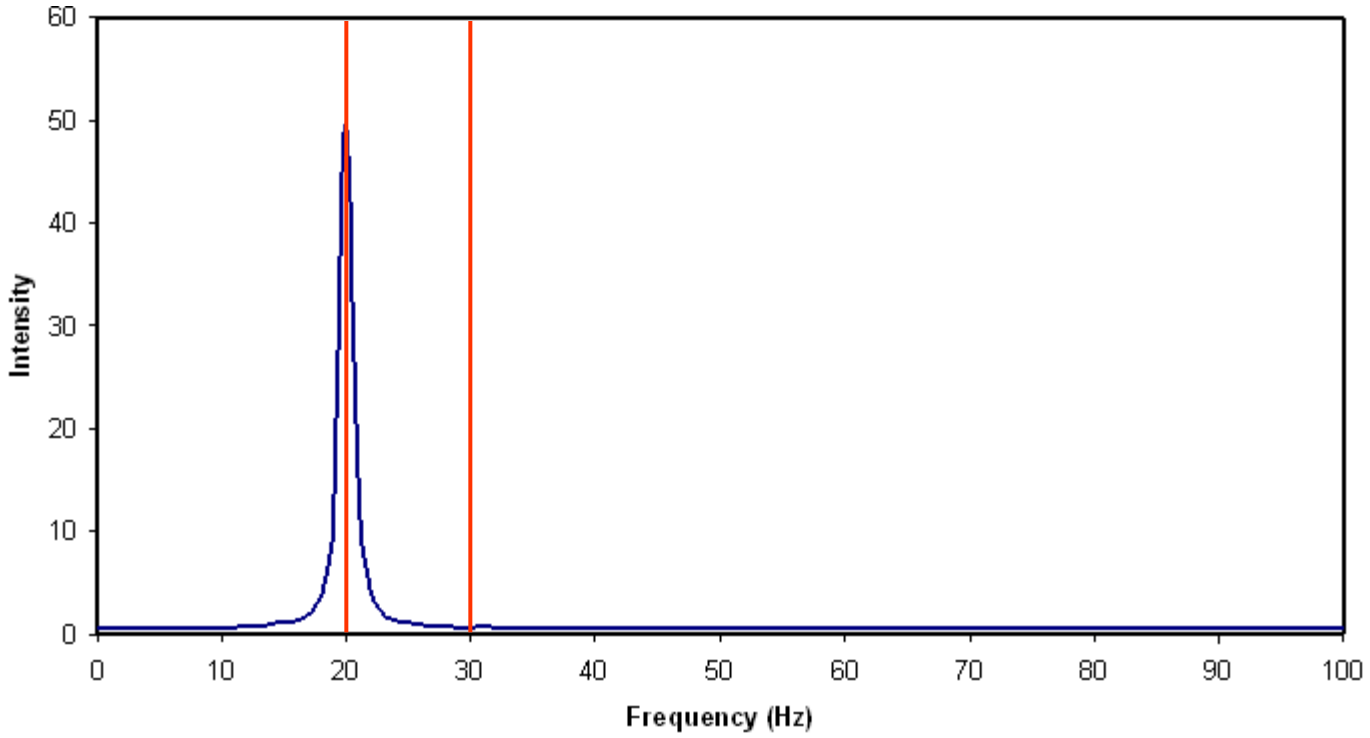
$f(t)$: 20 Hz ν: 30 Hz



The Fourier Transform

Real FT: $$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\mathrm{d}t$$

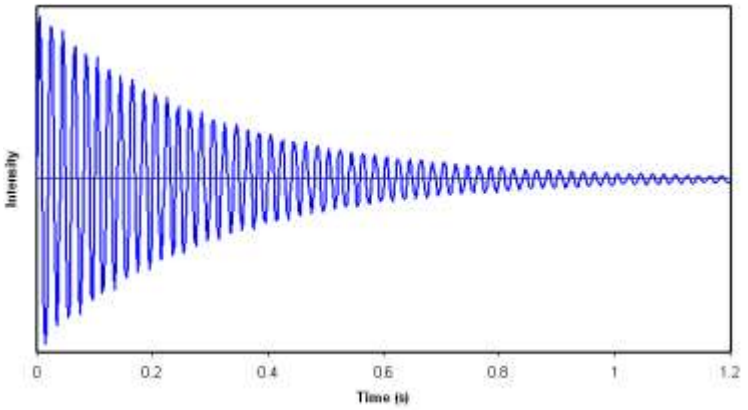
$f(t)$: 20 Hz



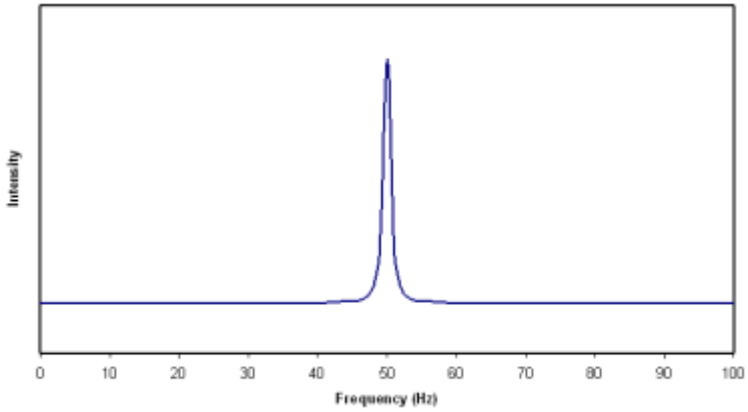
T_2^* and Linewidth

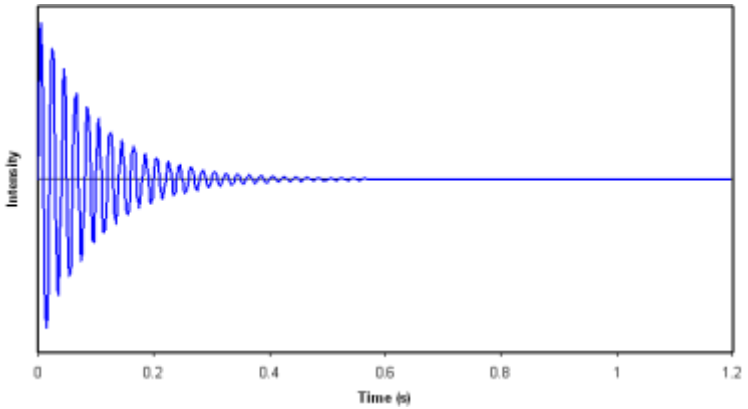
$$f(t) = \cos(2\pi\nu t) \cdot e^{-\frac{t}{T_2^*}}$$

$$\text{linewidth} \propto \frac{1}{T_2^*}$$

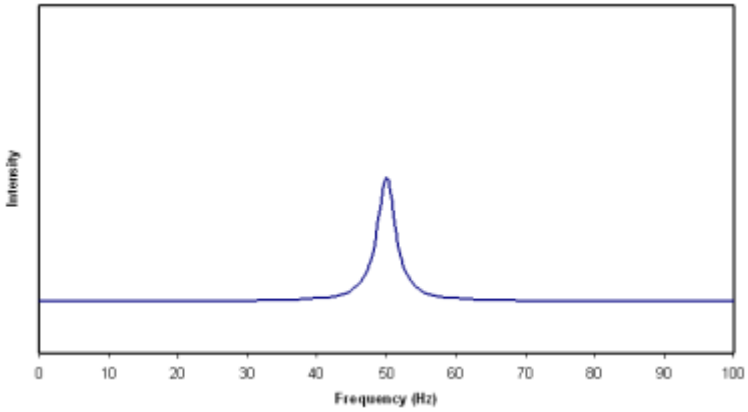


FT





FT



The Complex Fourier Transform

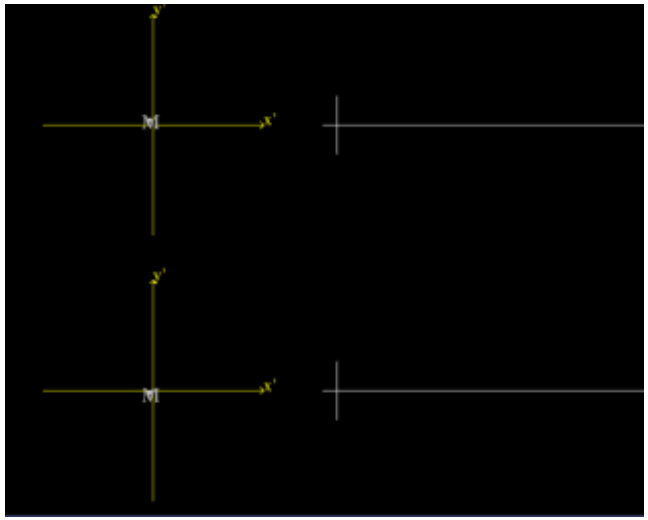
Real FT:

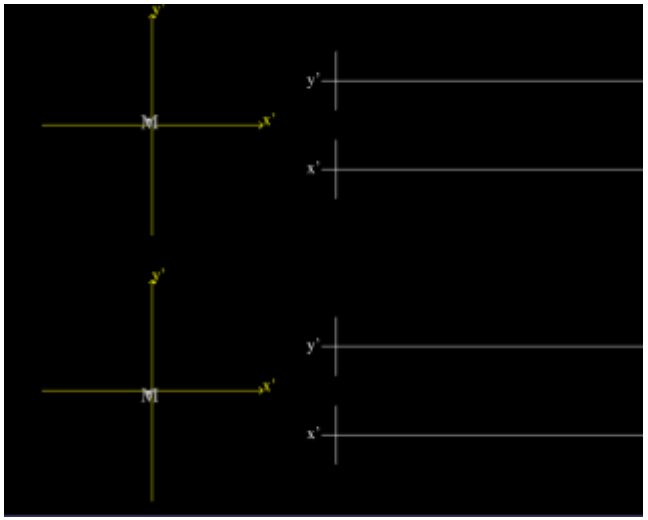
$$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot \cos(2\pi\nu t)\,\mathrm{d}t$$

Complex FT:

$$F(\nu) = \int_{-\infty}^{+\infty} f(t) \cdot e^{-i2\pi\nu t}\,\mathrm{d}t$$

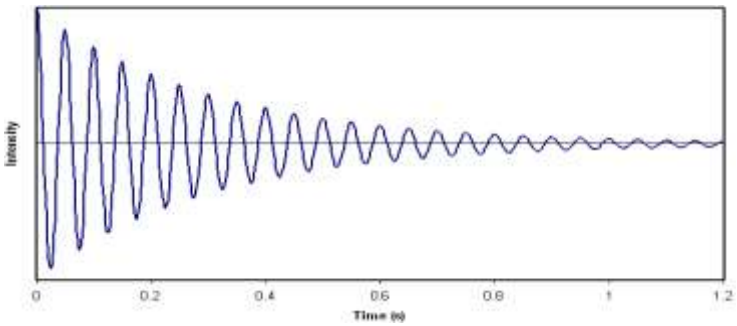
$$e^{-i2\pi\nu t} = \cos(2\pi\nu t) - i\sin(2\pi\nu t)$$





FT of complex NMR signal

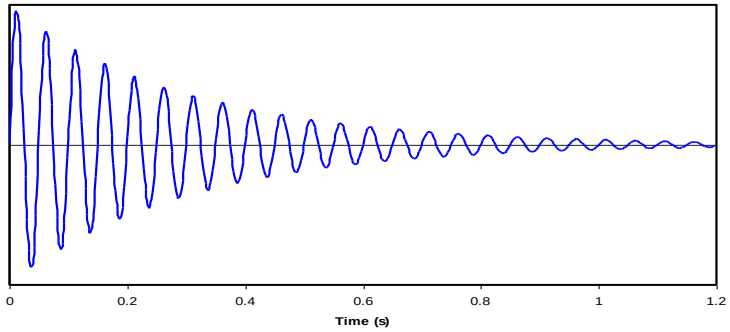
FT of complex NMR signal produces a complex spectrum, composed of real and imaginary parts. For a signal with initial phase $\phi = 0°$ the real part is the usual absorpion peak, while the imaginary part is a *dispersive lorentzian*.



real part

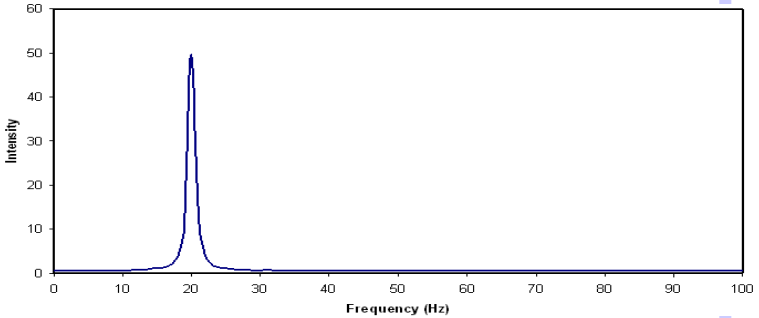
+

i



imaginary part

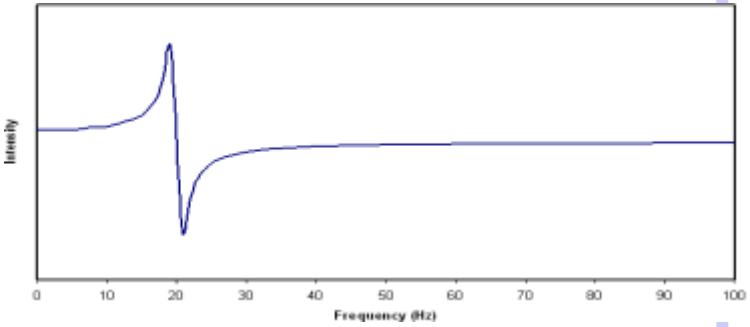
FT



real part

+

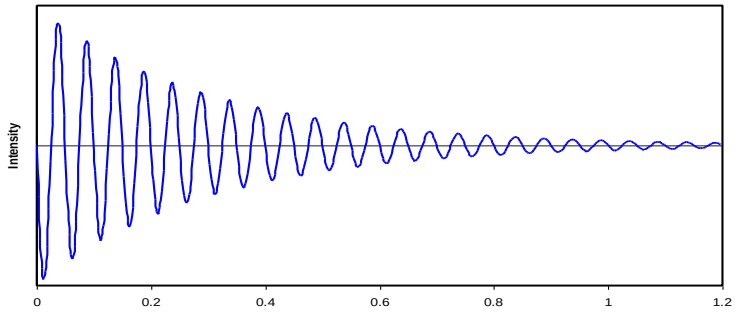
i



imaginary part

FT of complex NMR signal

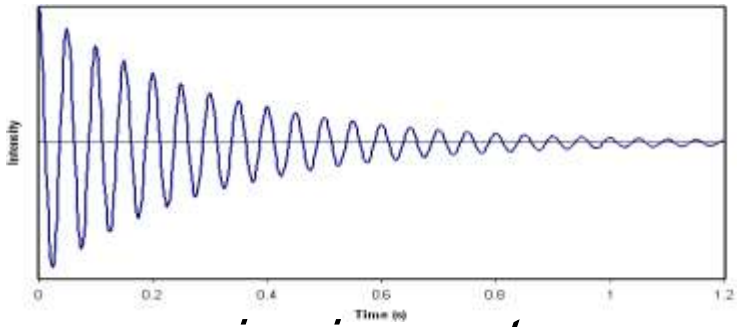
In contrast, if the initial phase is ϕ = 90°, the real part is dispersive peak, and the imaginary part is an absorpion peak.



real part

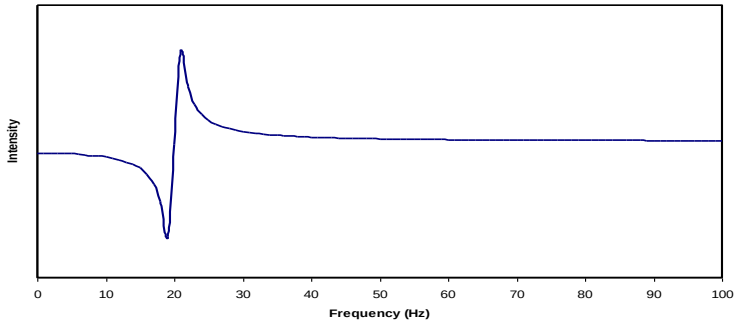
+

i



imaginary part

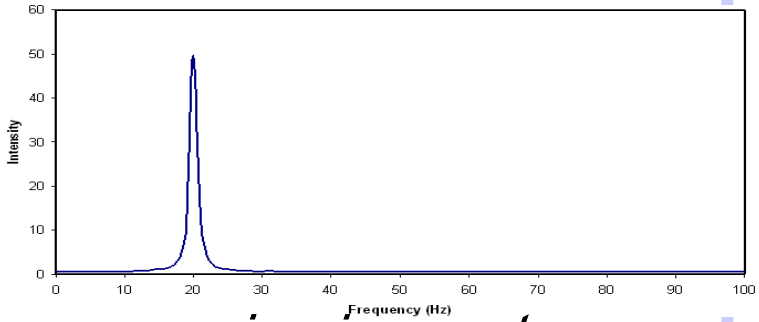
FT



real part

+

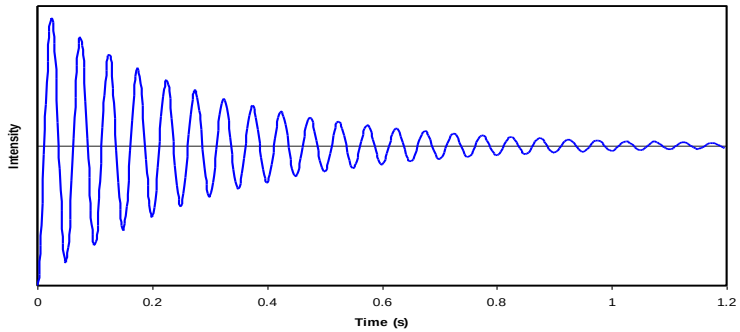
i



imaginary part

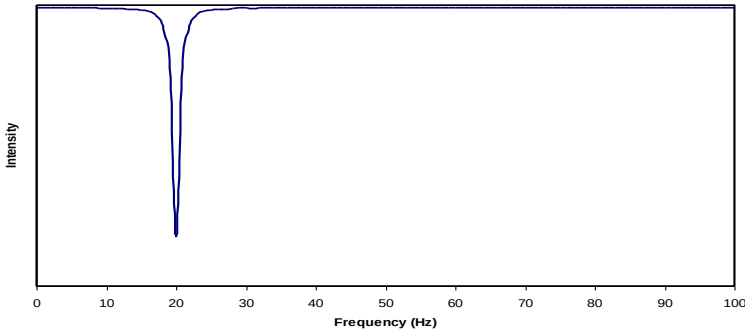
FT of complex NMR signal

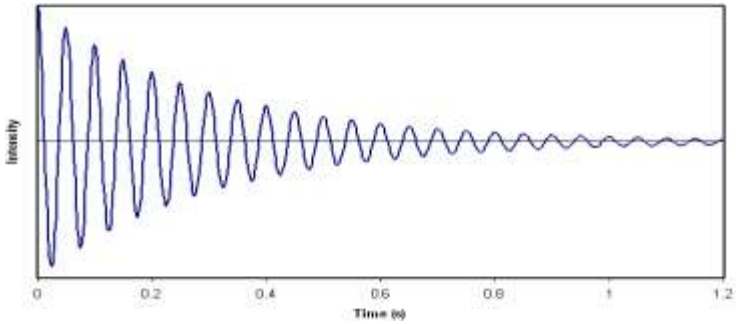
Finally, if the initial phase is ϕ = 180° the FID is the opposite of the FID for ϕ = 0° , and this also applies to the transformed spectrum (only the real part is shown here).



φ = 180°

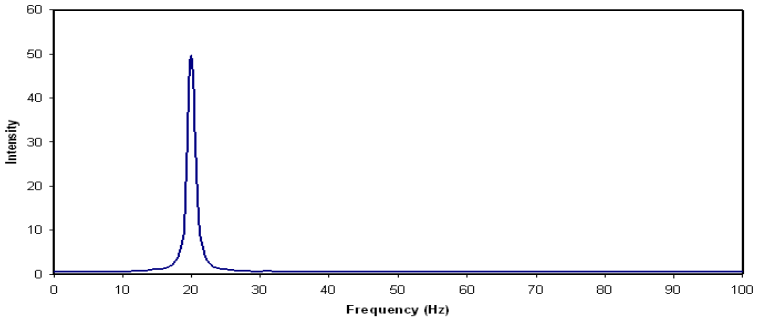
FT



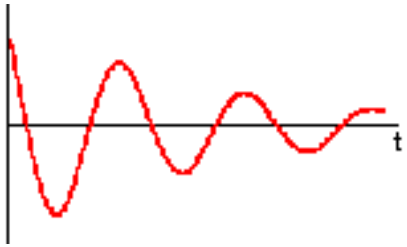


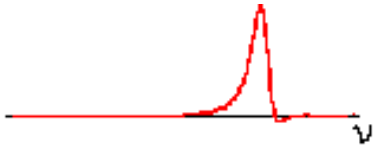
φ = 0°

FT



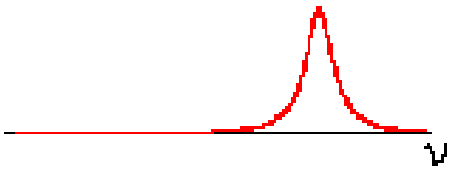
Phase in NMR signal





Re

Im

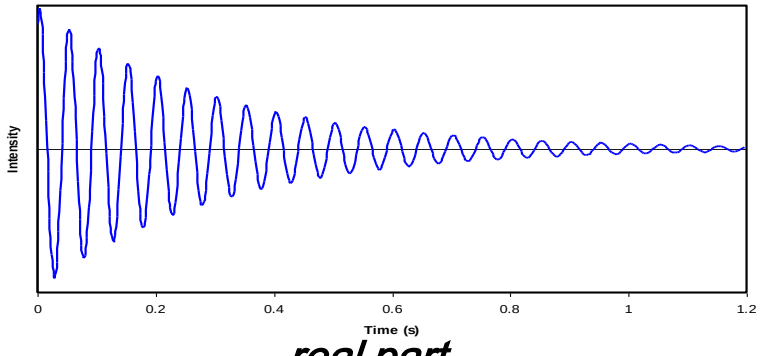


$$\begin{aligned} &\mathrm{Re}\cdot\cos(\varphi) \\ &+ \\ &-\mathrm{Im}\cdot\sin(\varphi) \end{aligned}$$

$$\mathrm{Re}' = \mathrm{Re}\cdot\cos(\varphi) - \mathrm{Im}\cdot\sin(\varphi)$$

Phase correction

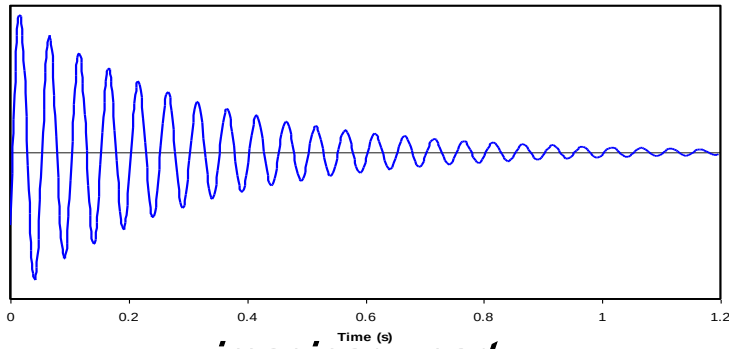
In practice, it is not necessary that the initial phase of the NMR signal is exactly 0°. If it is different from 0°, both the real and the imaginary parts are "mixtures" (more correctly, linear combinations) of absorption and dispersive peaks.



real part

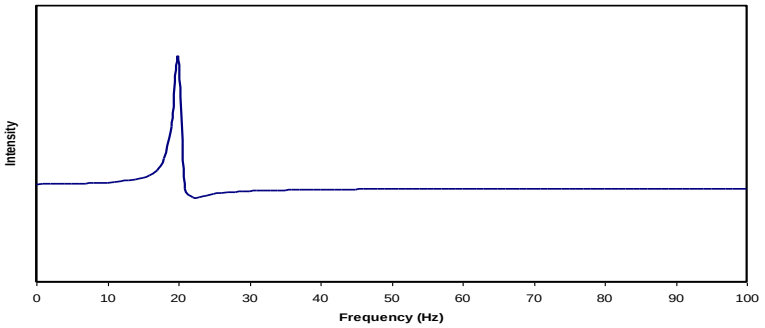
+

i



imaginary part

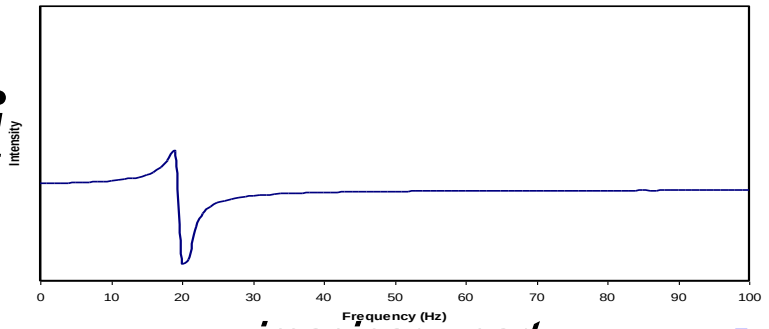
FT



real part

+

i



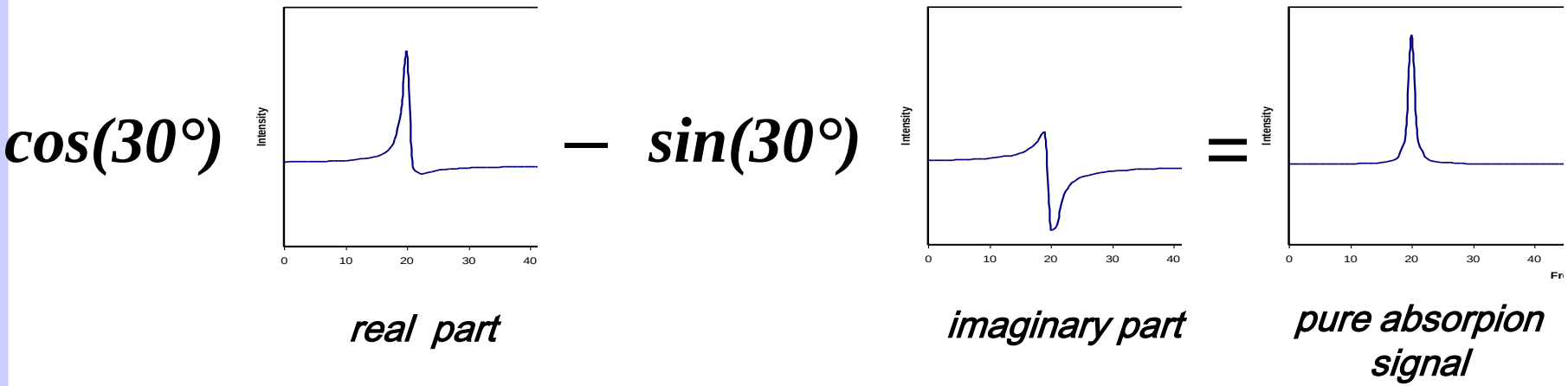
imaginary part

Phase correction

If we calculate a linear combination of the real and imaginary part:

$$\mathrm{Re} \cdot \cos(\theta) + \mathrm{Im} \cdot \sin(\theta)$$

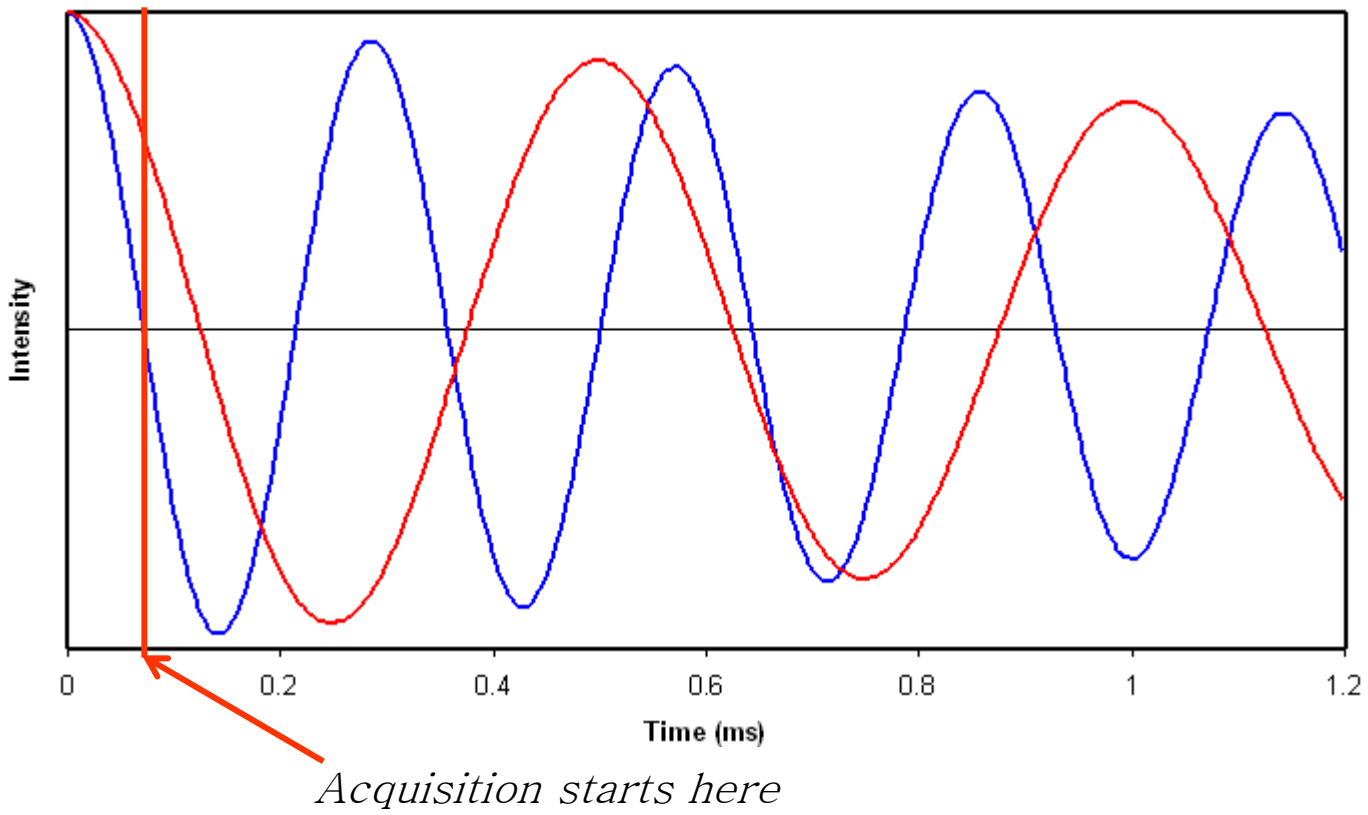
we can obtain a pure absorption specrtrum choosing a suitable θ parameter.



The θ parameter is the initial phase of the NMR signal, and is therefore an angle.

The most important contribution to phase error is a the difference between pulse rf and detection rf phases.

Phase error in the NMR signal

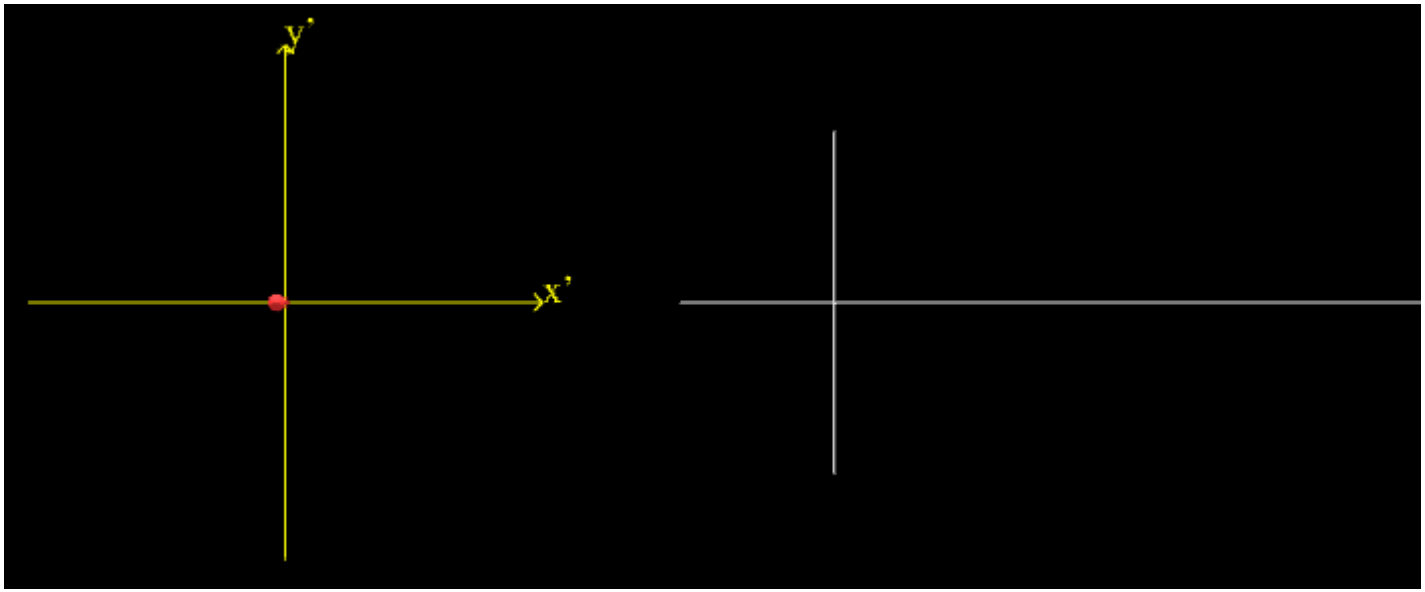


$$\mathrm{Re}' = \mathrm{Re}\cdot\cos(\theta) - \mathrm{Im}\cdot\sin(\theta) \qquad \theta = \theta_0 + \theta_1\cdot(\nu - \nu_0)$$

θ_0 is the zero-th order phase constant (Bruker: PHC0, Varian: rp)

θ_1 is the first order phase constant (Bruker: PHC1, Varian: lp)

Phase error in the NMR signal

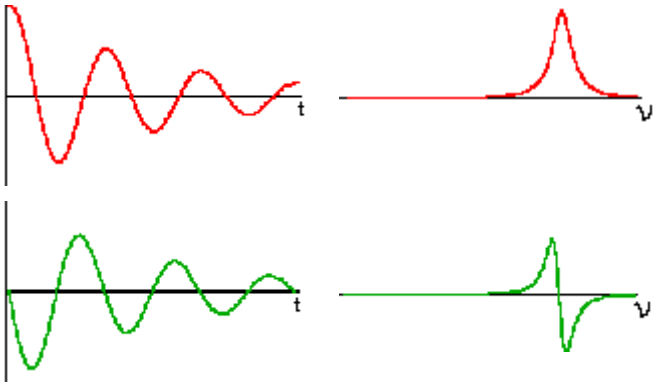


$$\mathrm{Re}' = \mathrm{Re}\cdot\cos(\theta) - \mathrm{Im}\cdot\sin(\theta) \qquad \theta = \theta_0 + \theta_1 \cdot (\nu - \nu_0)$$

θ_0 is the zero-th order phase constant (Bruker: PHC0, Varian: rp)

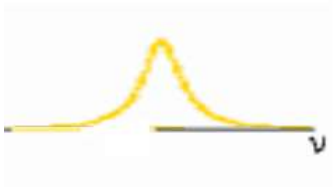
θ_1 is the first order phase constant (Bruker: PHC1, Varian: lp)

Magnitude-mode spectra

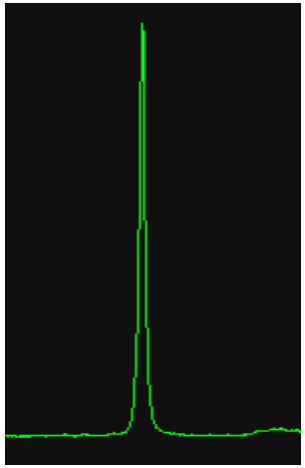


Re

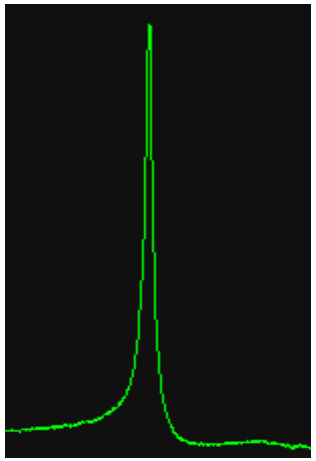
Im



$(Re^2 + Im^2)^{1/2}$



Absorption signal

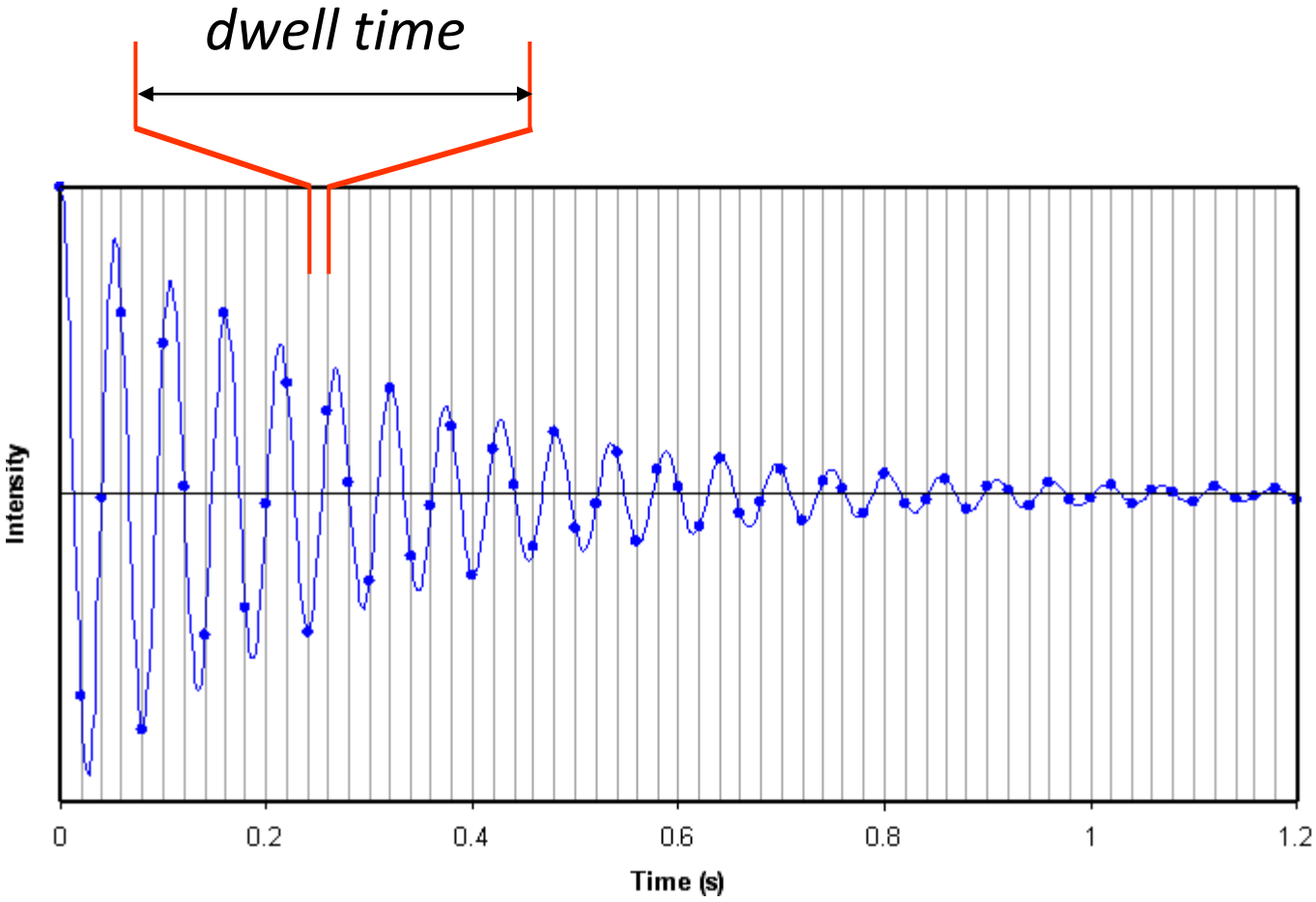


Magnitude signal

The discrete Fourier transform

Real DFT:

$$FT(\nu) = \sum_{k=0}^{N-1} A_k \cdot \cos(2\pi\nu \cdot k \cdot \Delta t)$$



Fast Fourier Transform

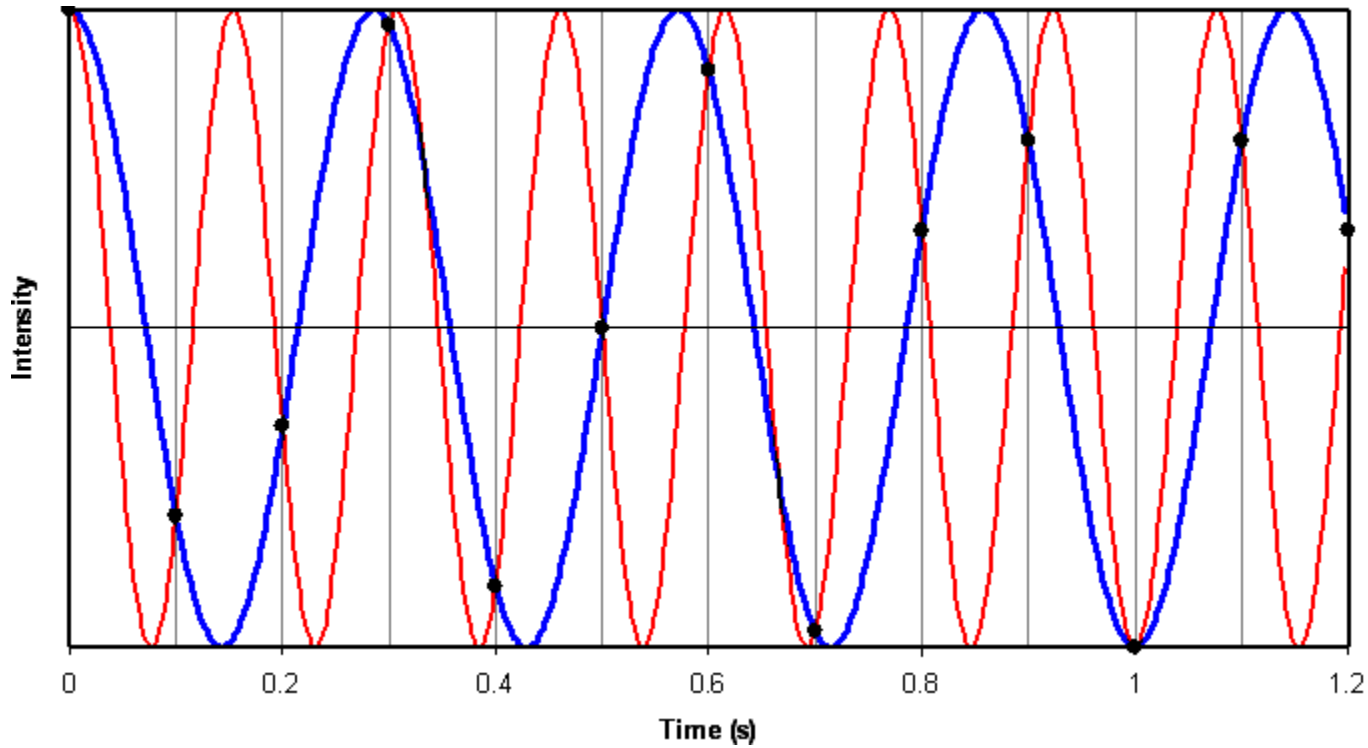
Discrete Fourier Transform requires that:

- the signal is measured at fixed time intervals
- all the points are included in the calculation

The (fast) algorithm used to perform DFT, requires that:

- the transformed spectrum has the same number of points as the original FID
- this number of points is a power of 2 (256, 512, 1024, 2048, 4196, …..)

Digitalization: the Nyquist Sampling Theorem



sampling frequency = 2 · SW

(but sampling frequency = SW with quadrature detection)

Bruker params:

F2: SW, DW

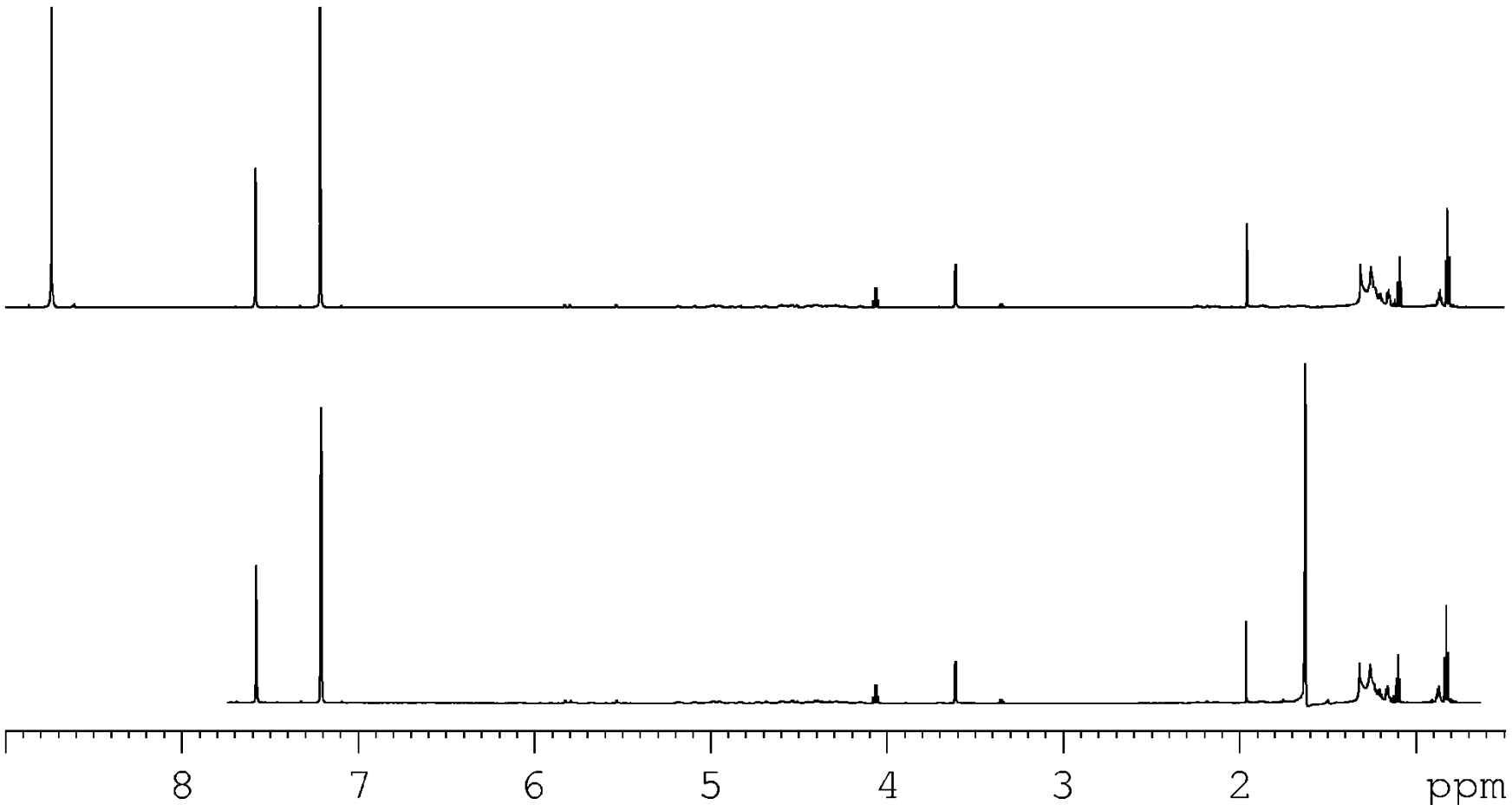
F1: SW, IN0 (or INF1)

Varian params:

sw, dw

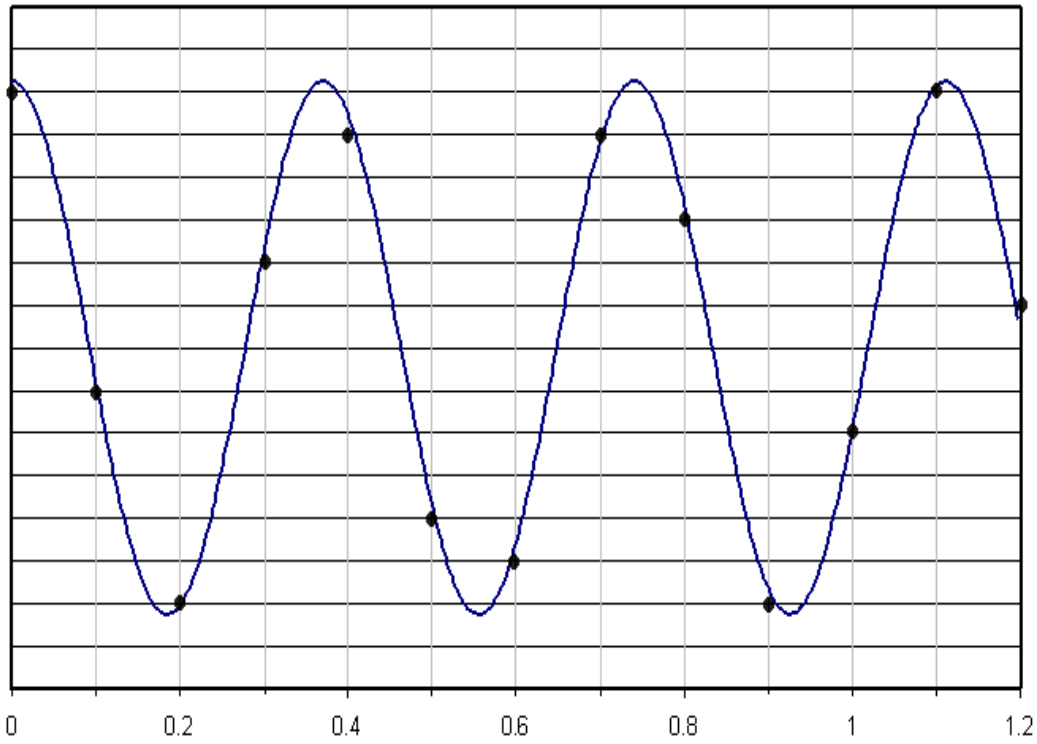
sw1, d2

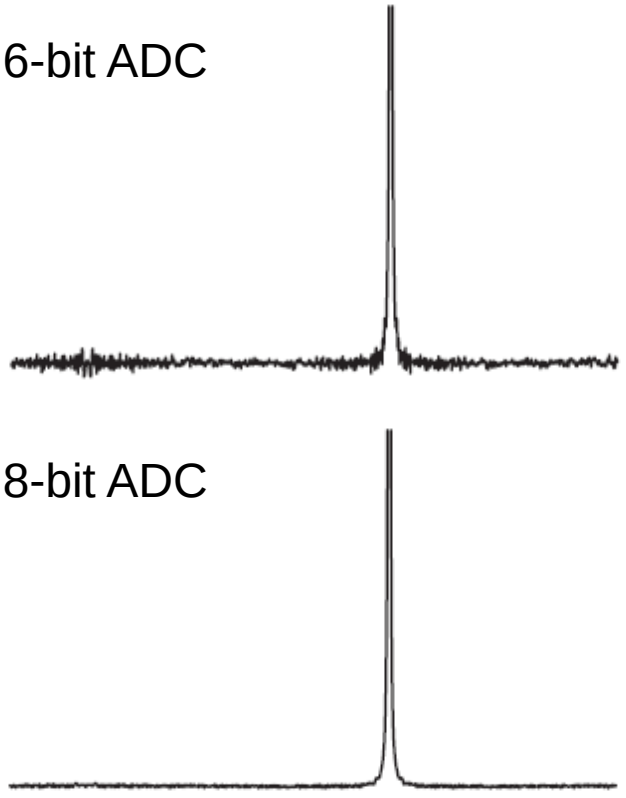
Digitalization: Folded Peaks



Folded (aliased) peaks in the direct dimension

Quantization noise



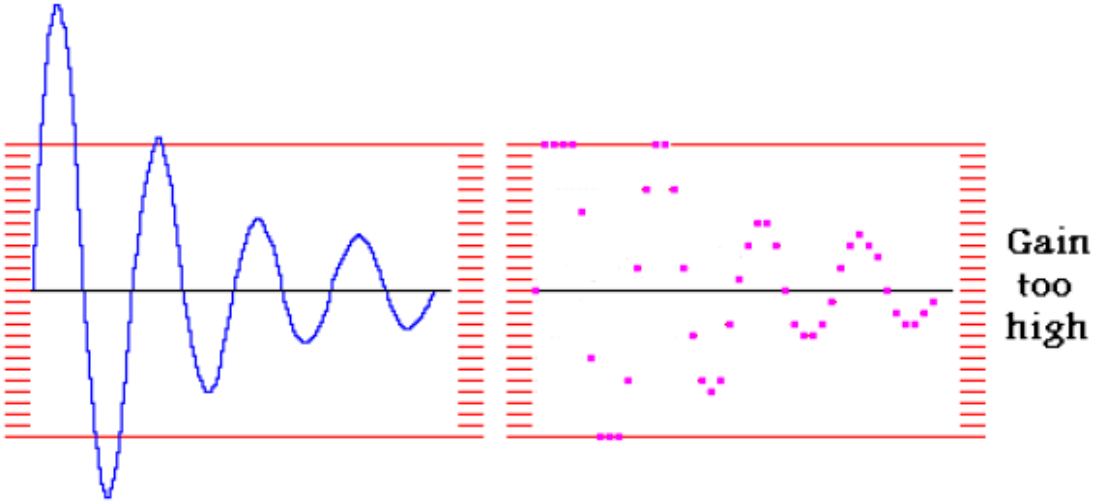


The accuracy by which the intensity of the signal can be measured is limited by the resolution of the ADC

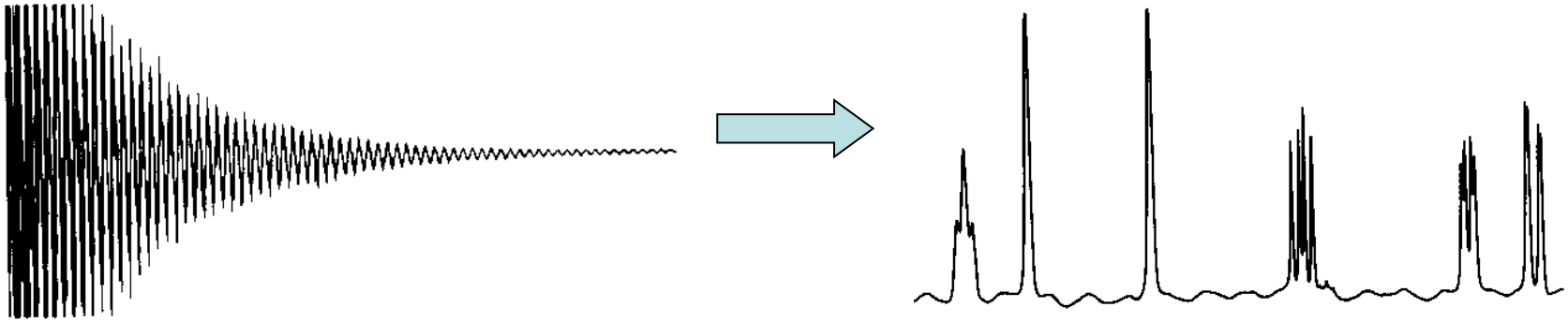
This results in noise, which is called quantization noise

Most high-resolution spectometers have a 16-bit ADC = 65536 levels

ADC overflow



If the gain is too high, the most intense points of the FID are clipped to the maximum ADC value. A “clipped” FID generates a spectrum with distorted baseline



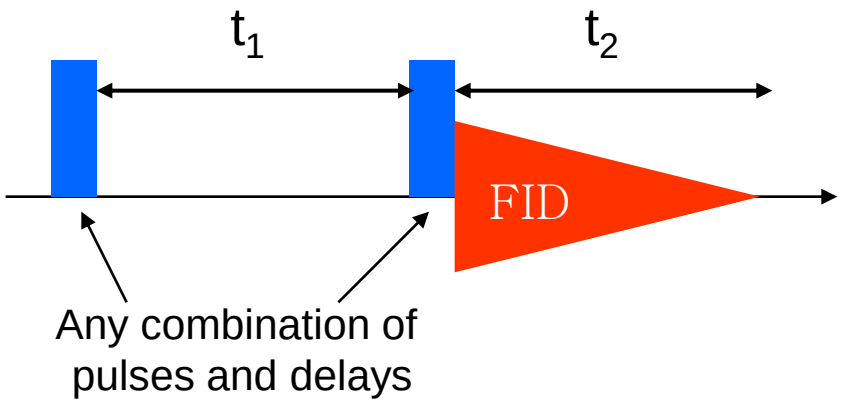
Bruker params:

RG

Varian params:

gain

2D NMR: sampling the indirect dimension



Sampling frequency along t_2 is the actual sampling speed

Sampling frequency along t_1 is determined by the increment of t_1 between subsequent experiments (Δt_1)

$$\text{sampling frequency} = 2 \cdot SW = 1/(\Delta t_1)$$

Bruker params:

F2: SW, DW

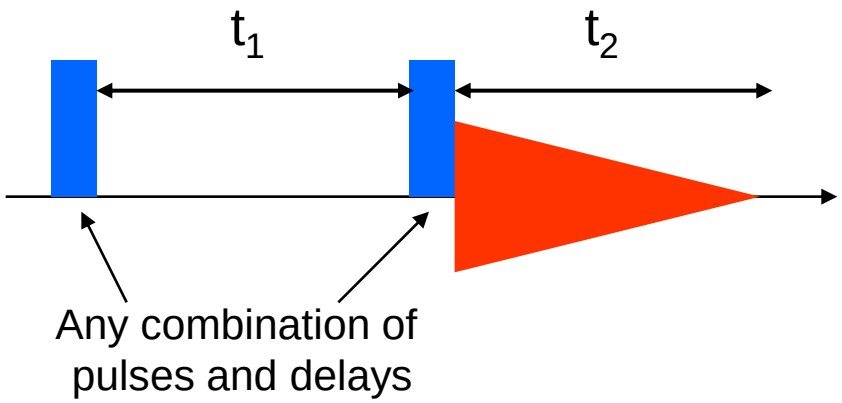
F1: SW, IN0

Varian params:

sw, dw

sw1, d2

Digitalization: indirect vs. direct dimension



Sampling frequency along t_2 is determined by the increment of t_1 between subsequent experiments

Direct dimension (t_2):

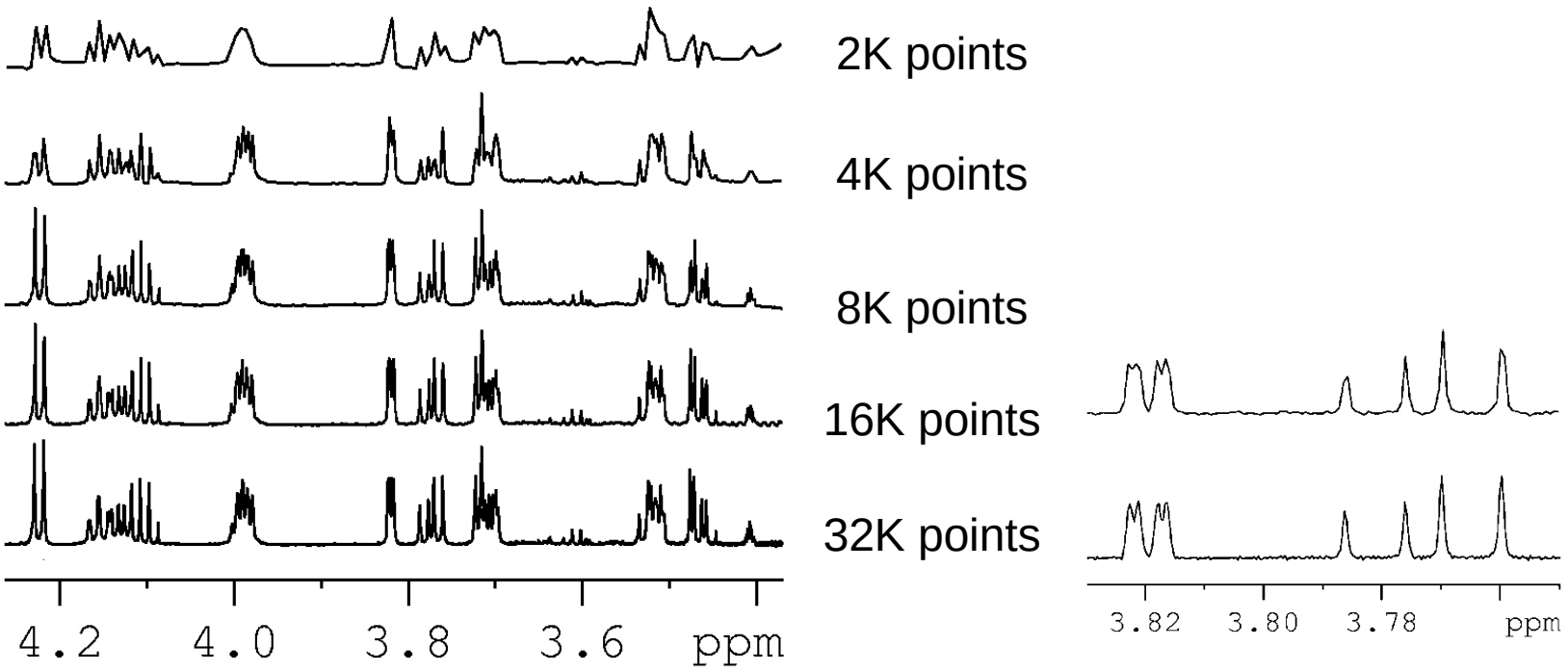
- sampling can be as fast as desired at (almost) no price
- oversampling is standard
- t_2 can be as long as desired at (almost) no price

Indirect dimension (t_1):

- any increase in sampling speed results in more FIDs to acquire
- any increase in t_1 results in more FIDs to acquire
- oversampling is generally impractical

Digital resolution

Digital resolution is the distance in Hz between two contiguous points in the specrum



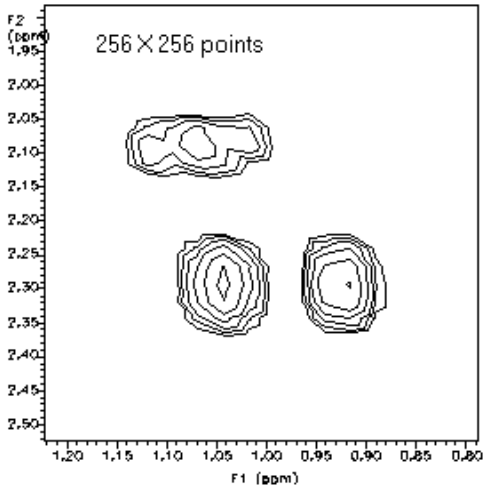
It depends on t_2, i.e. how long the FID is acquired

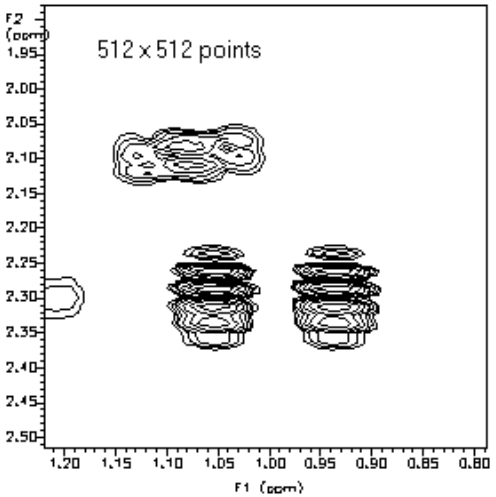
In 1D NMR usually $t_2 >> T_2^*$

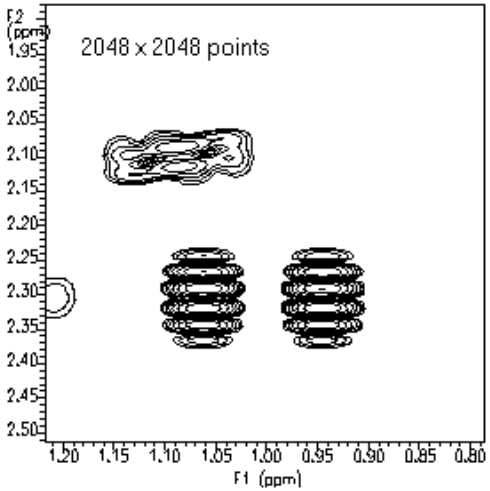
This is not always true in 2D NMR (both for t_2 and t_1)

Digital resolution in 2D NMR

To avoid huge data matrices (t_2) and to reduce the duration of the experiment (t_1), digital resolution is usually kept low in 2D NMR







Bruker params:

F2: TD, AQ, SI, SW

F1: TD, SI, IN0

Varian params:

np, at, fn, sw

ni, fn1, sw1

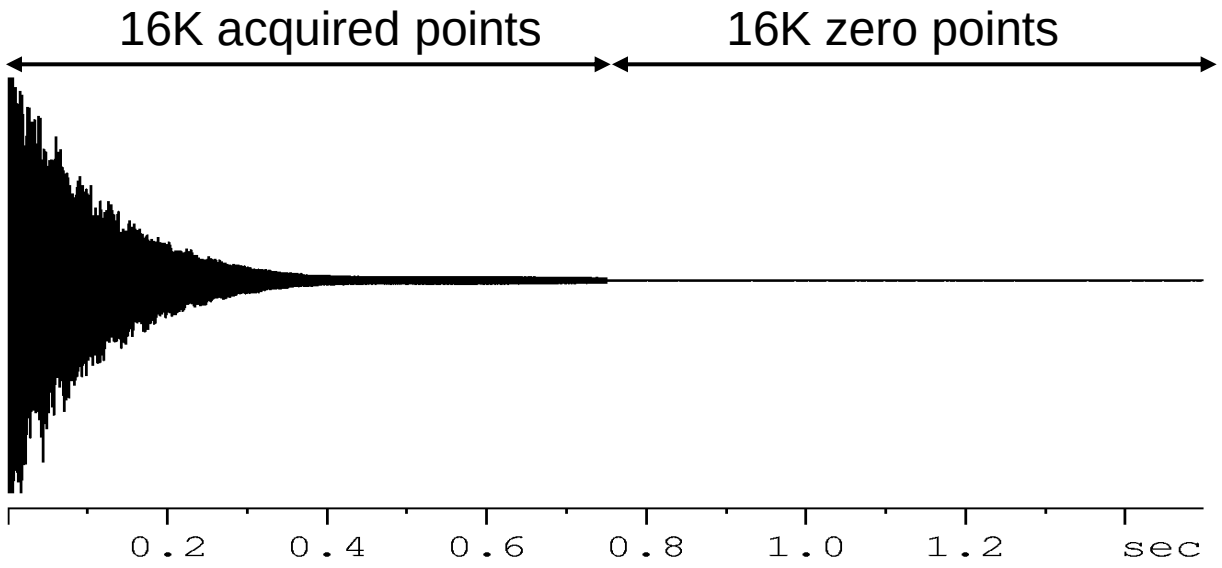
Zero filling

There is no reason to acquire the FID after the signal is decayed to zero

However, it is useful to have a digital resolution higher than the actual resolution of the spectrum (which depends on T_2^*)

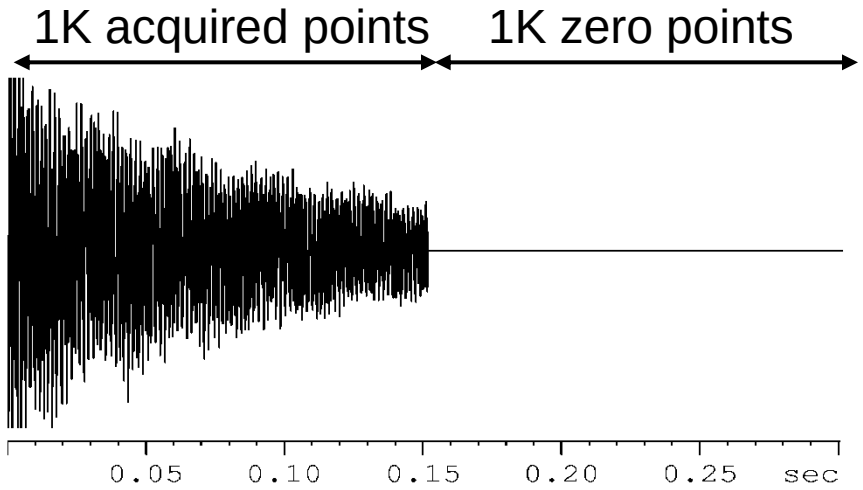
The FFT algorithm requires that the transformed spectrum has the same number of points as the original FID

The solution is to append zero points at the end of the FID before FFT

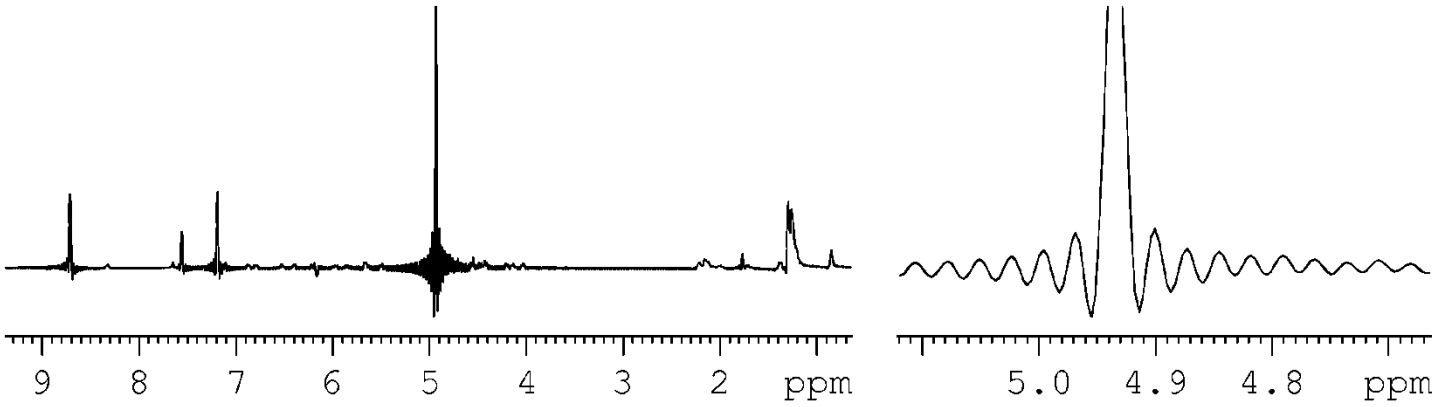


Truncated FID

In 2D NMR, usually the FID is *not* decayed to zero at the end of t_2 (*truncated* FID).

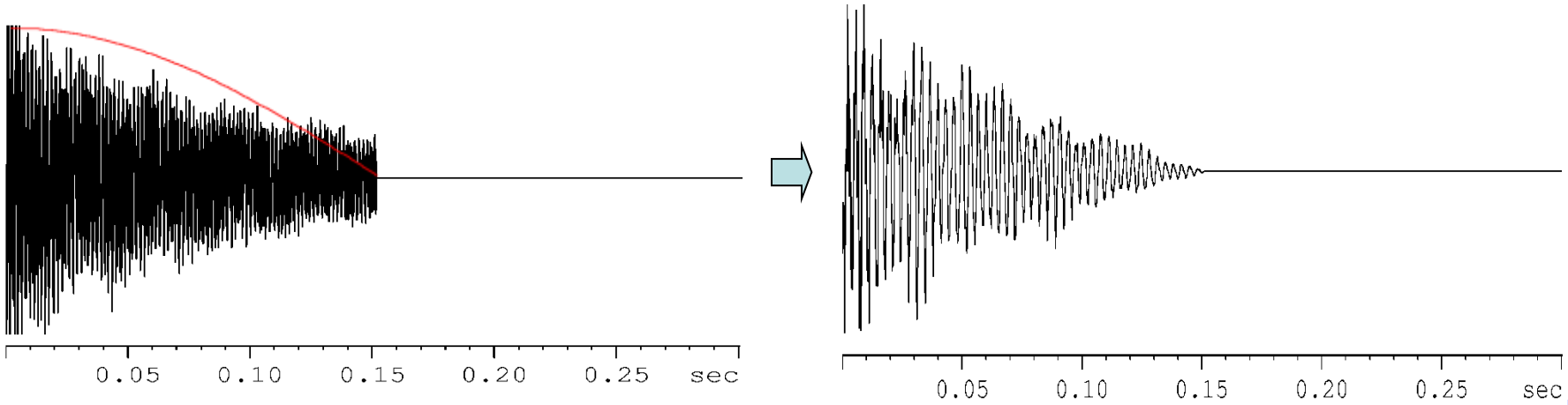


In the FT of a truncated FID, all signals have *wiggles*

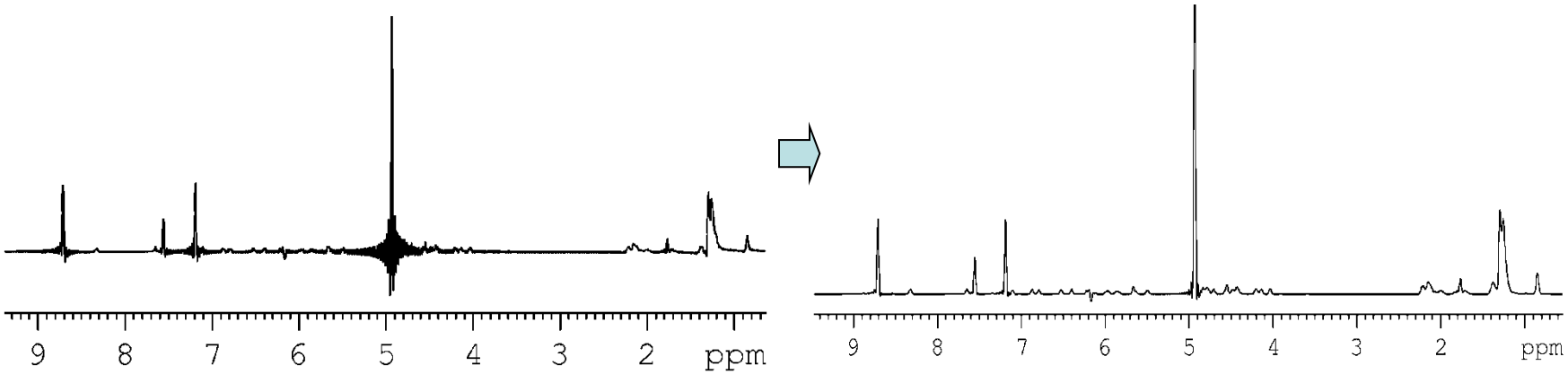


Apodization

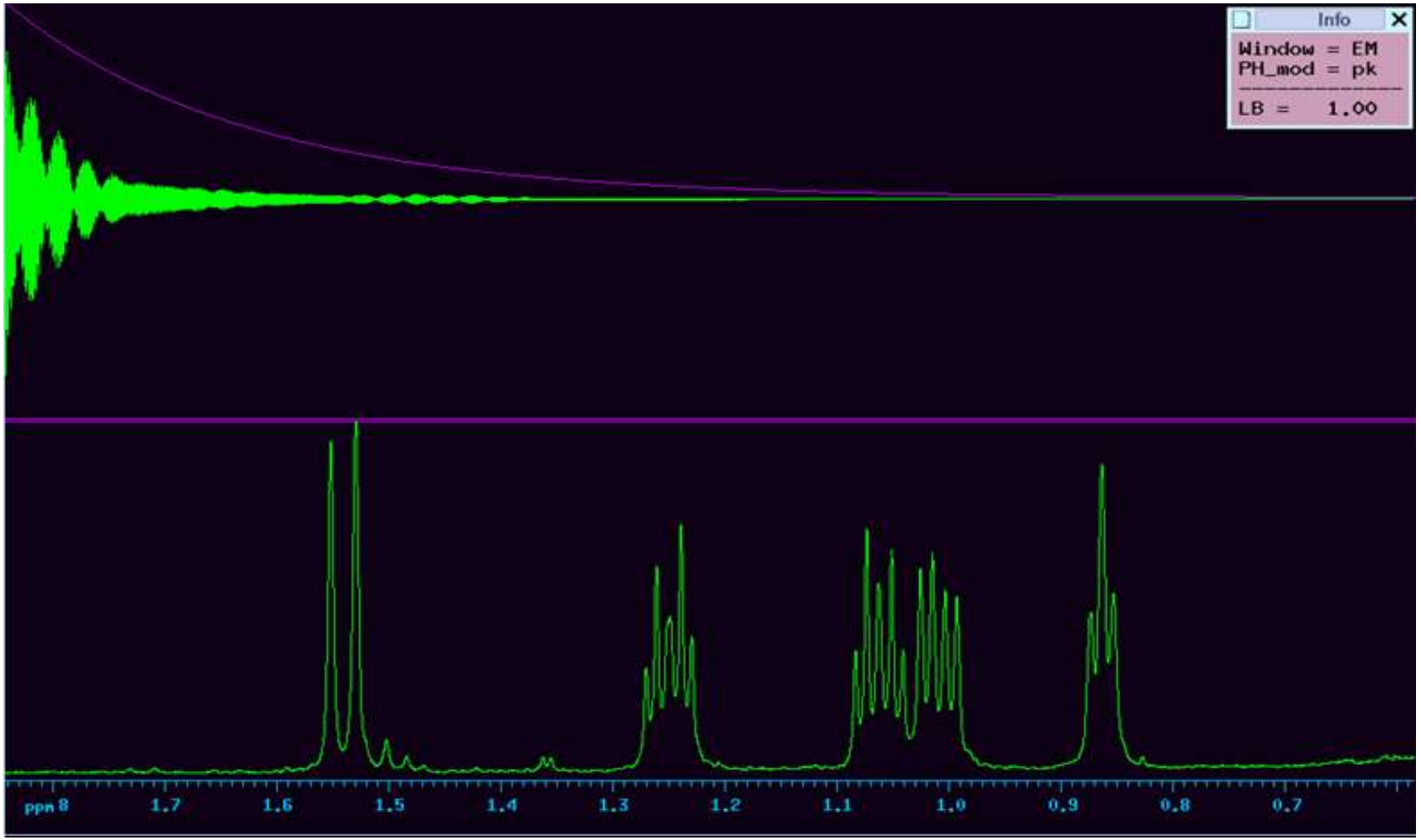
The reason for the wiggles is the step in the FID. If the step is removed, wiggles disappear



This is done by multiplying the FID for a function (here a cosine bell) called *window function*

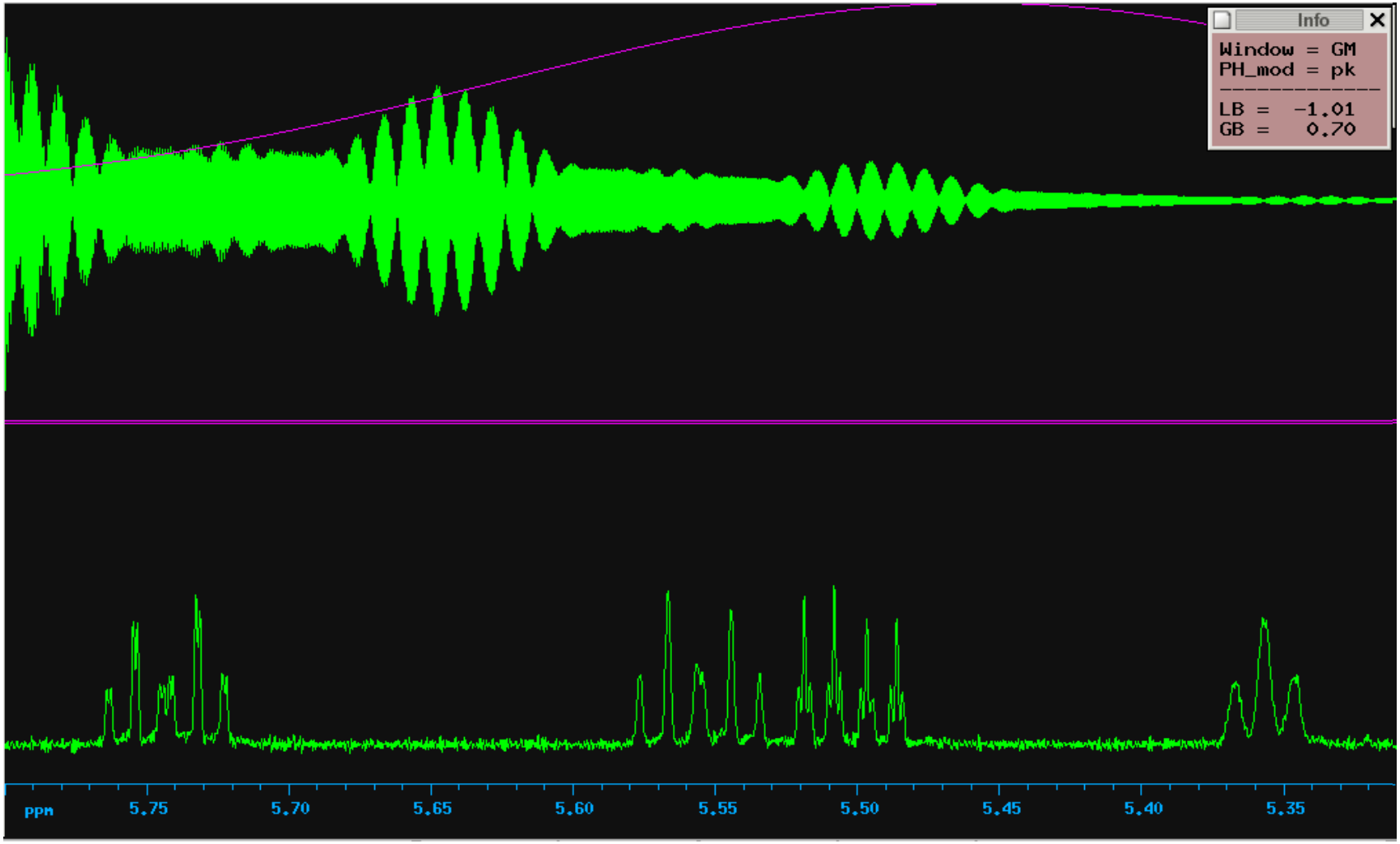


Windows Functions: Exponential



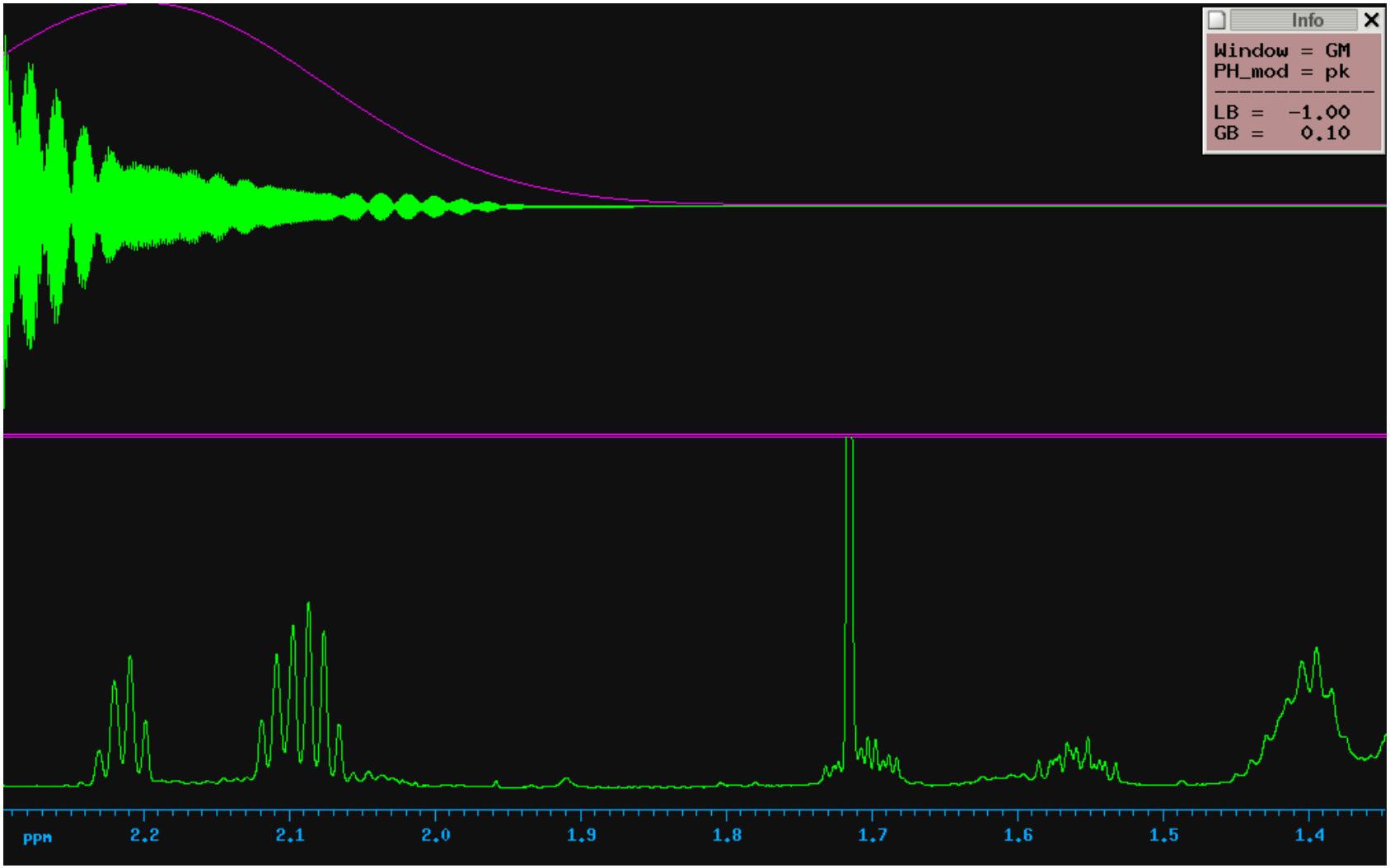
	Bruker params:	Varian params:
F2:	LB	lb
F1:	LB	lb1

Windows functions: Gaussian

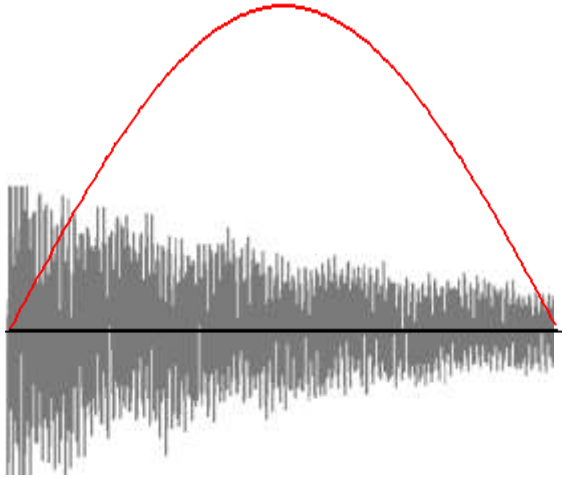


	Bruker params:	Varian params:
F2:	LB, GB	lb, gf
F1:	LB, GB	lb1, gf1

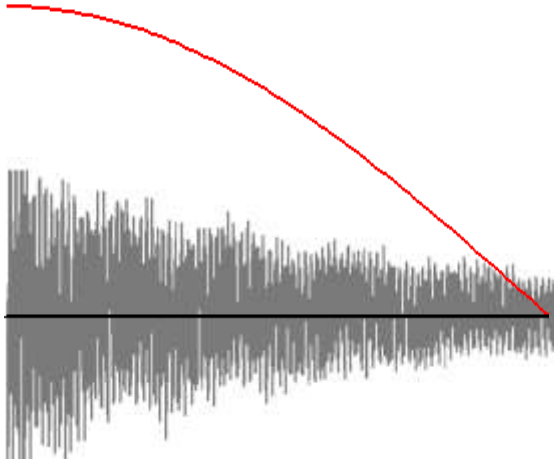
Windows Functions: Gaussian



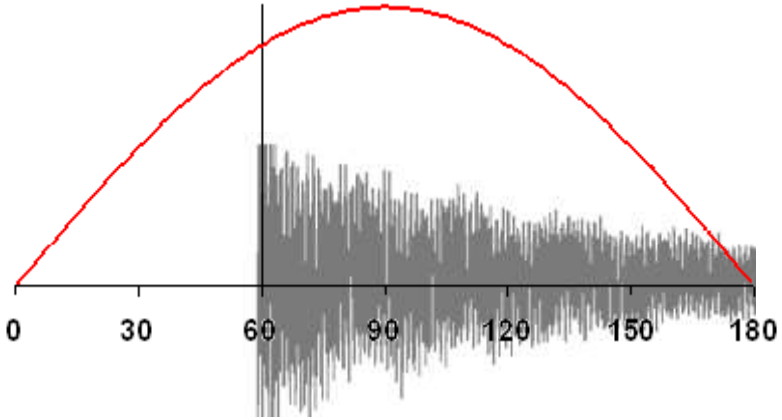
Windows Functions: Sine and Cosine



sine

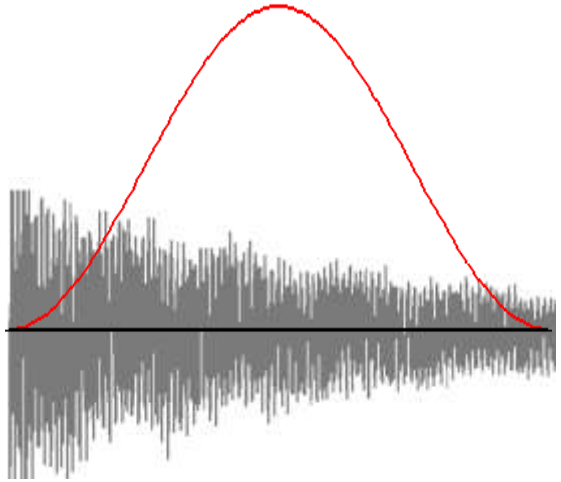


cosine
(90° shifted sine)

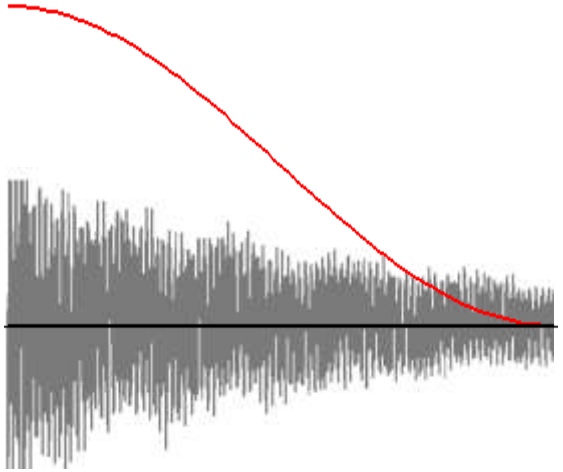


60° shifted sine

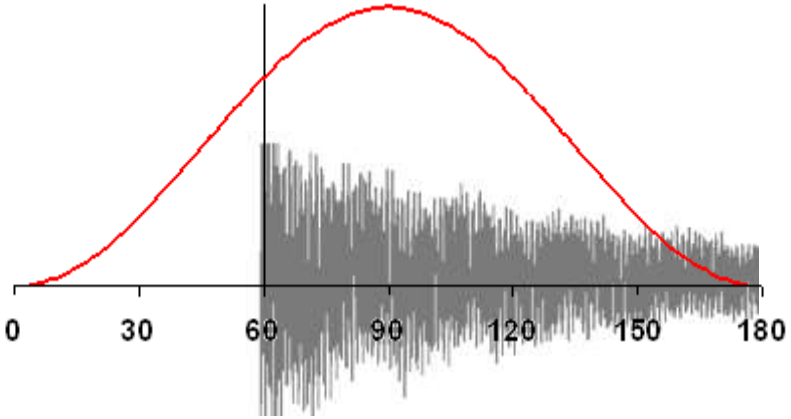
Windows Functions: Sine and Cosine



square sine

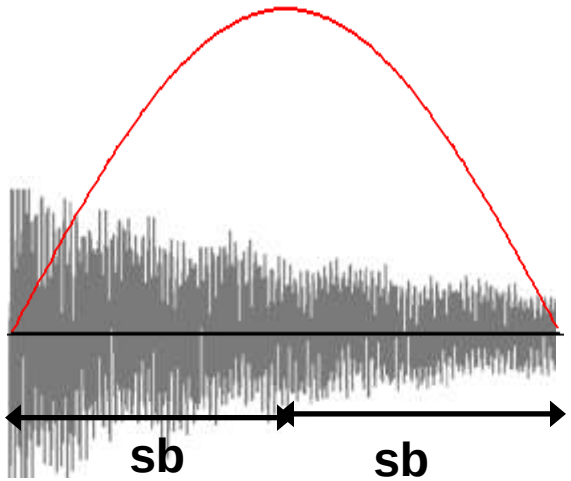


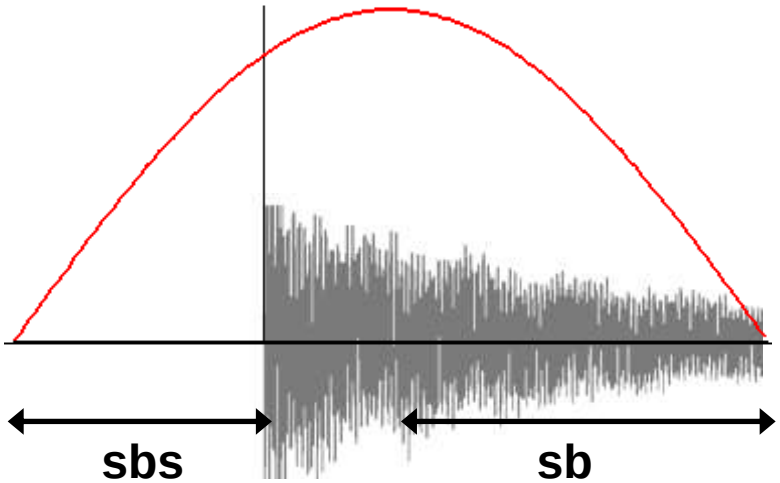
square cosine
(90° shifted square sine)

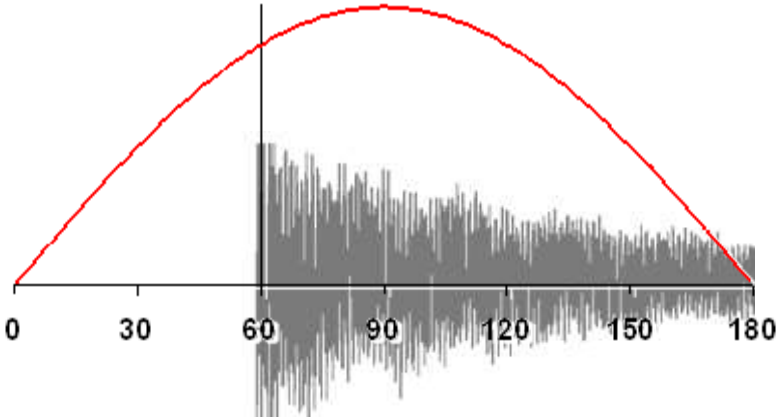


60° shifted square sine

Windows Functions: Sine and Cosine



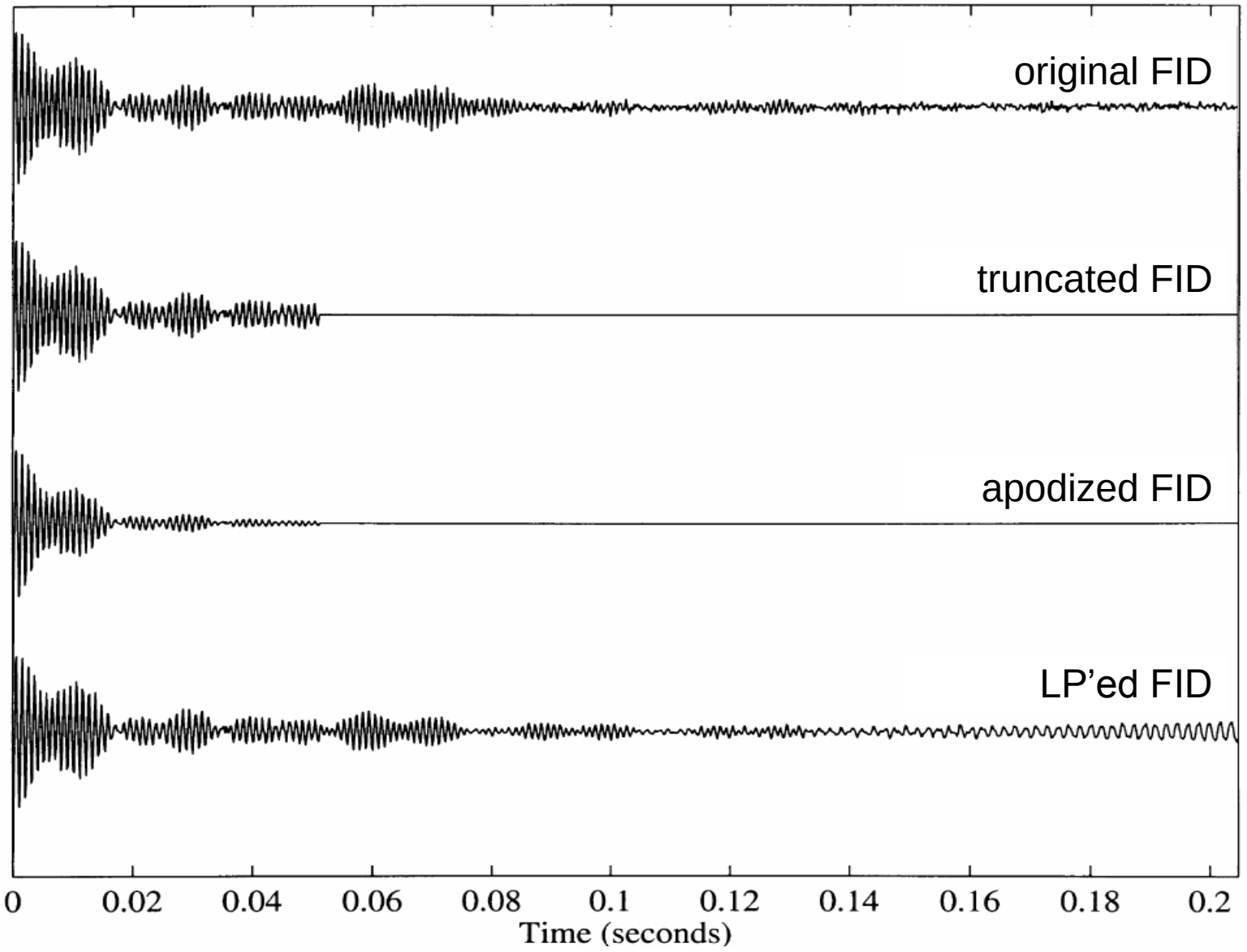




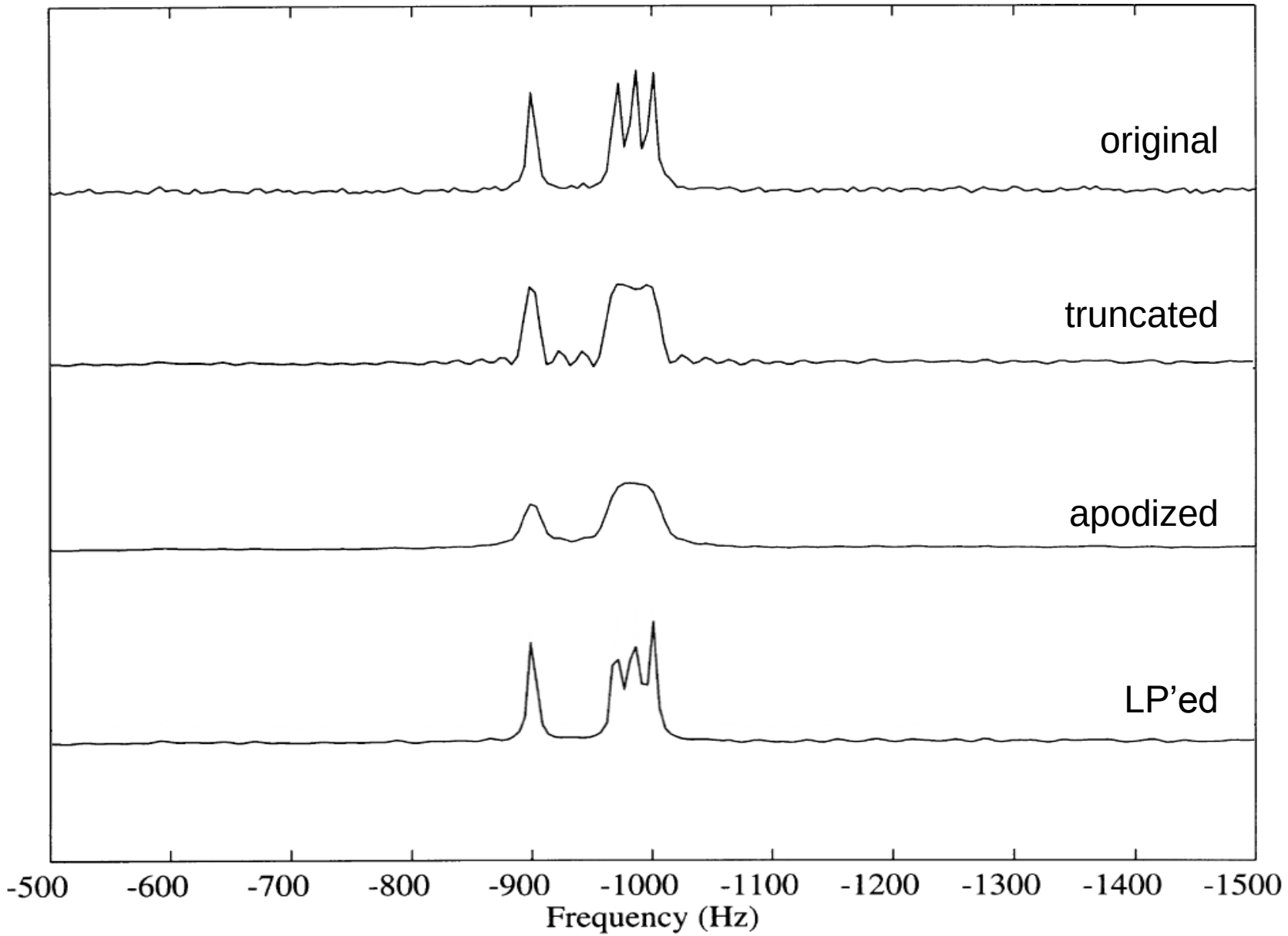
if SSB < 2 $\alpha = 0°$

else $\alpha = \frac{180°}{SSB}$

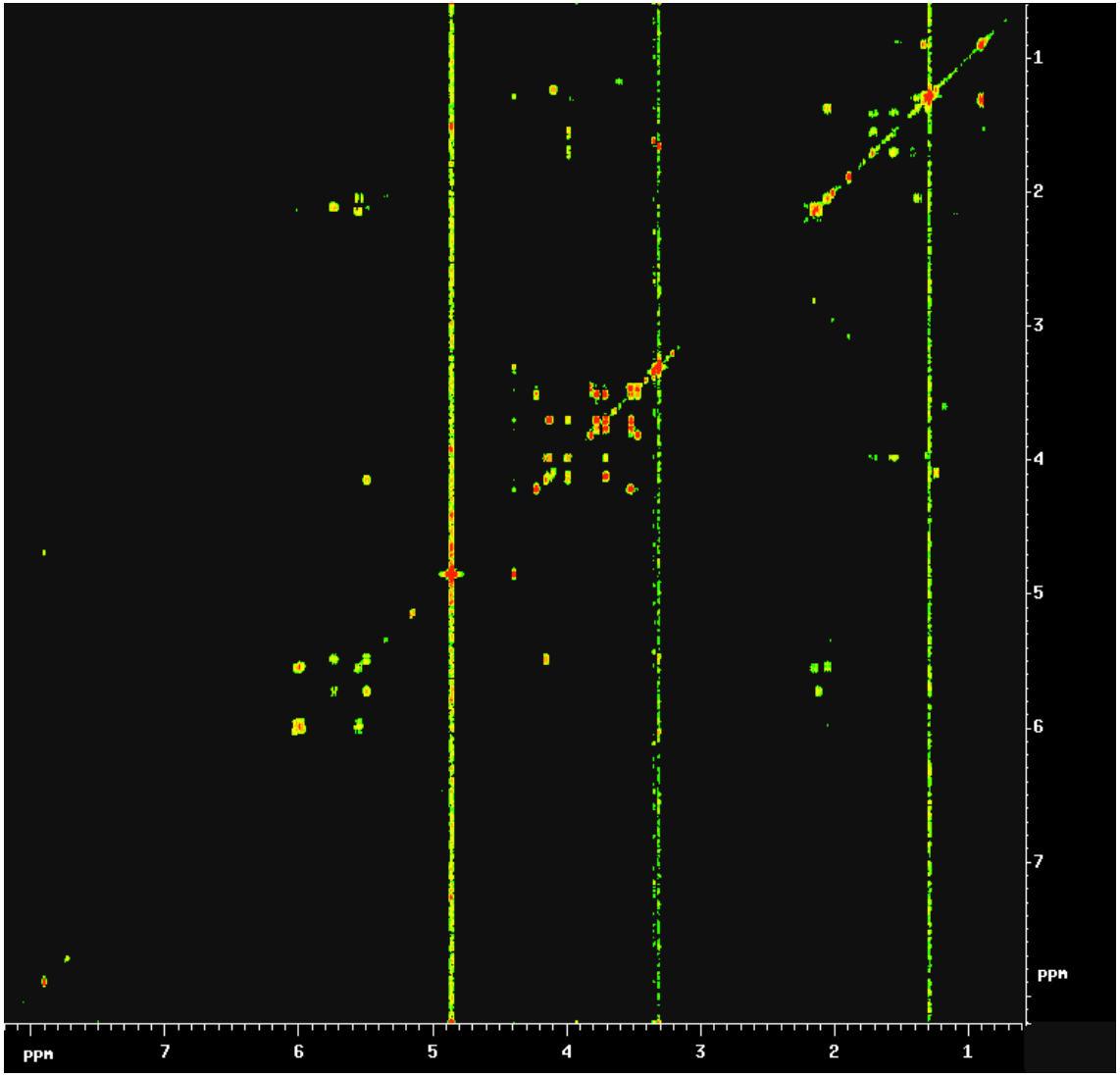
Linear Prediction



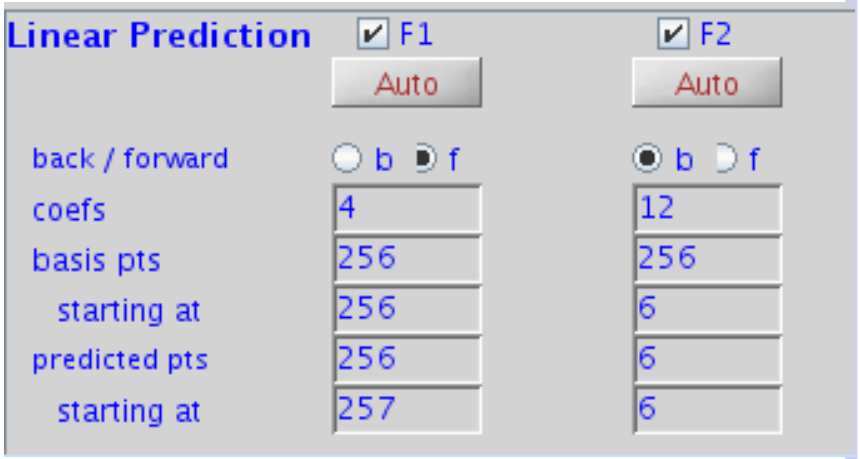
Linear Prediction

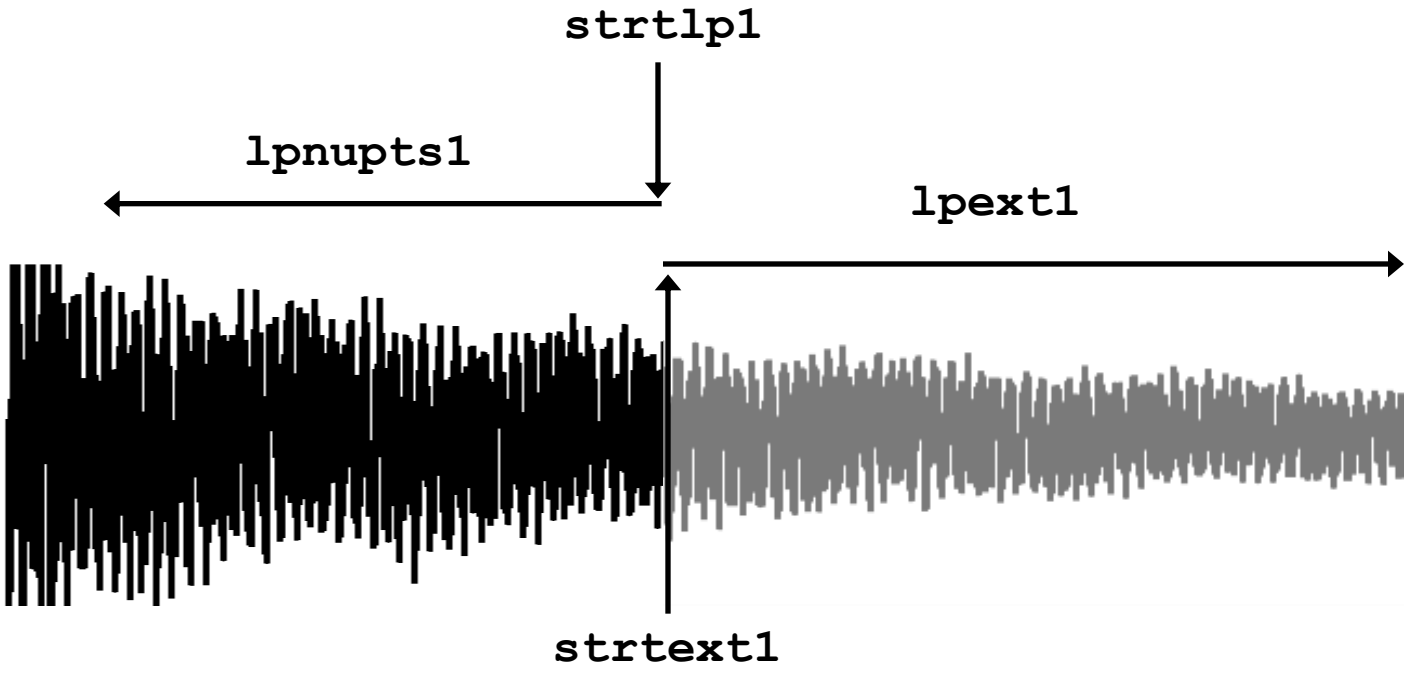


Magnitude-Mode 2D Spectra: Sine



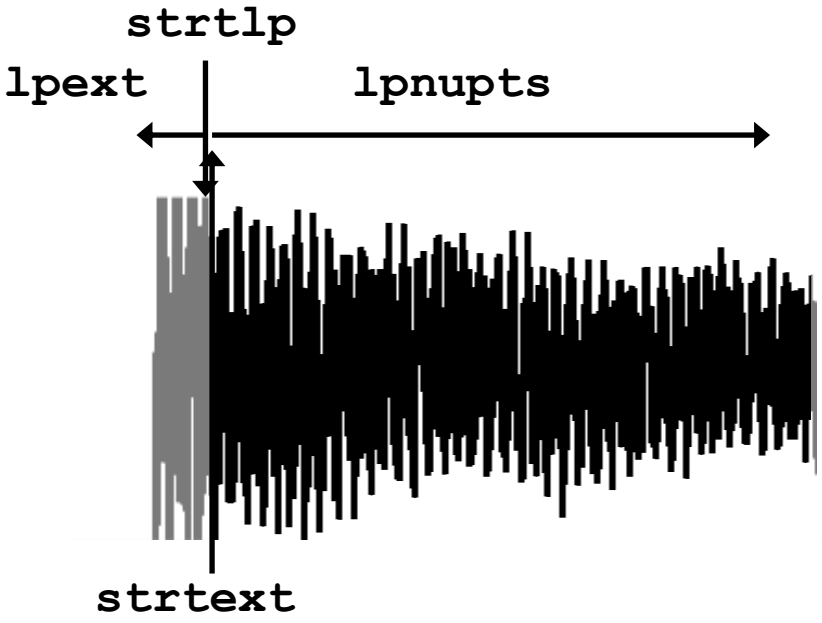
Forward linear prediction





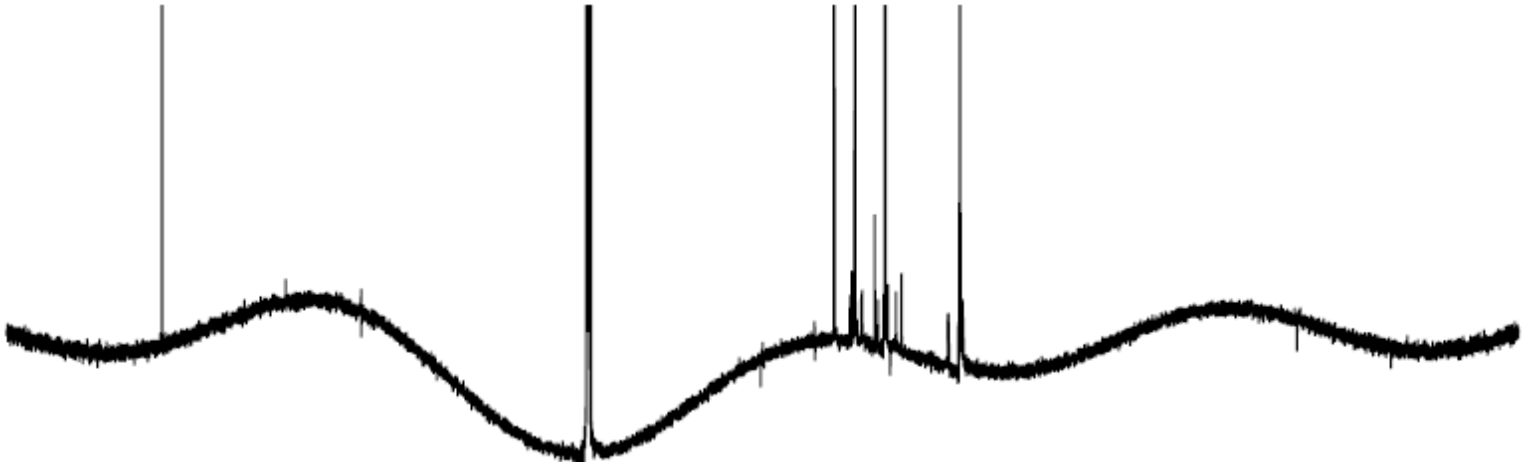
Backward linear prediction

Linear Prediction	F1	F2
	Auto	Auto
back / forward	b f	b f
coefs	4	12
basis pts	256	256
starting at	256	6
predicted pts	256	6
starting at	257	6

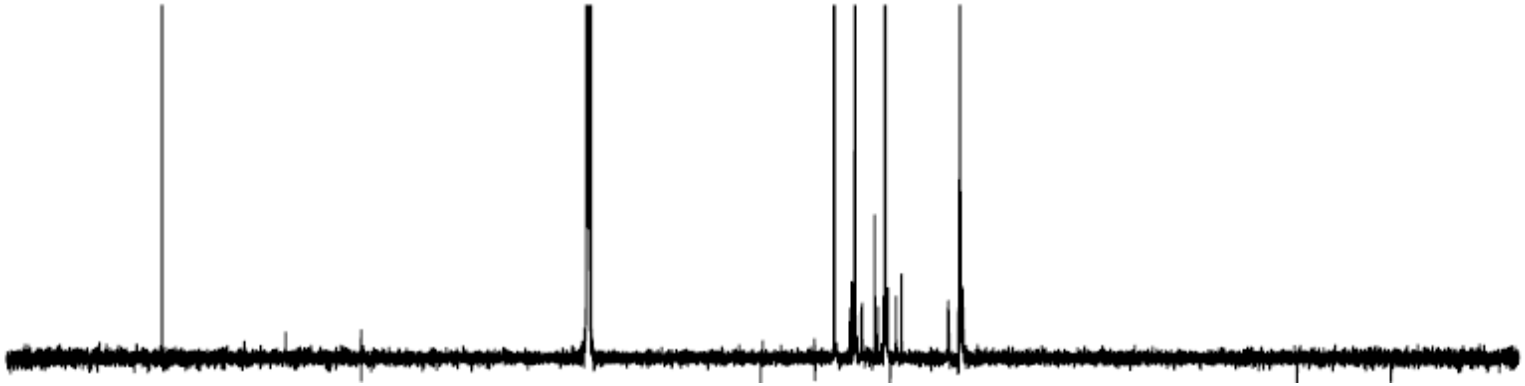


Backward Linear Prediction

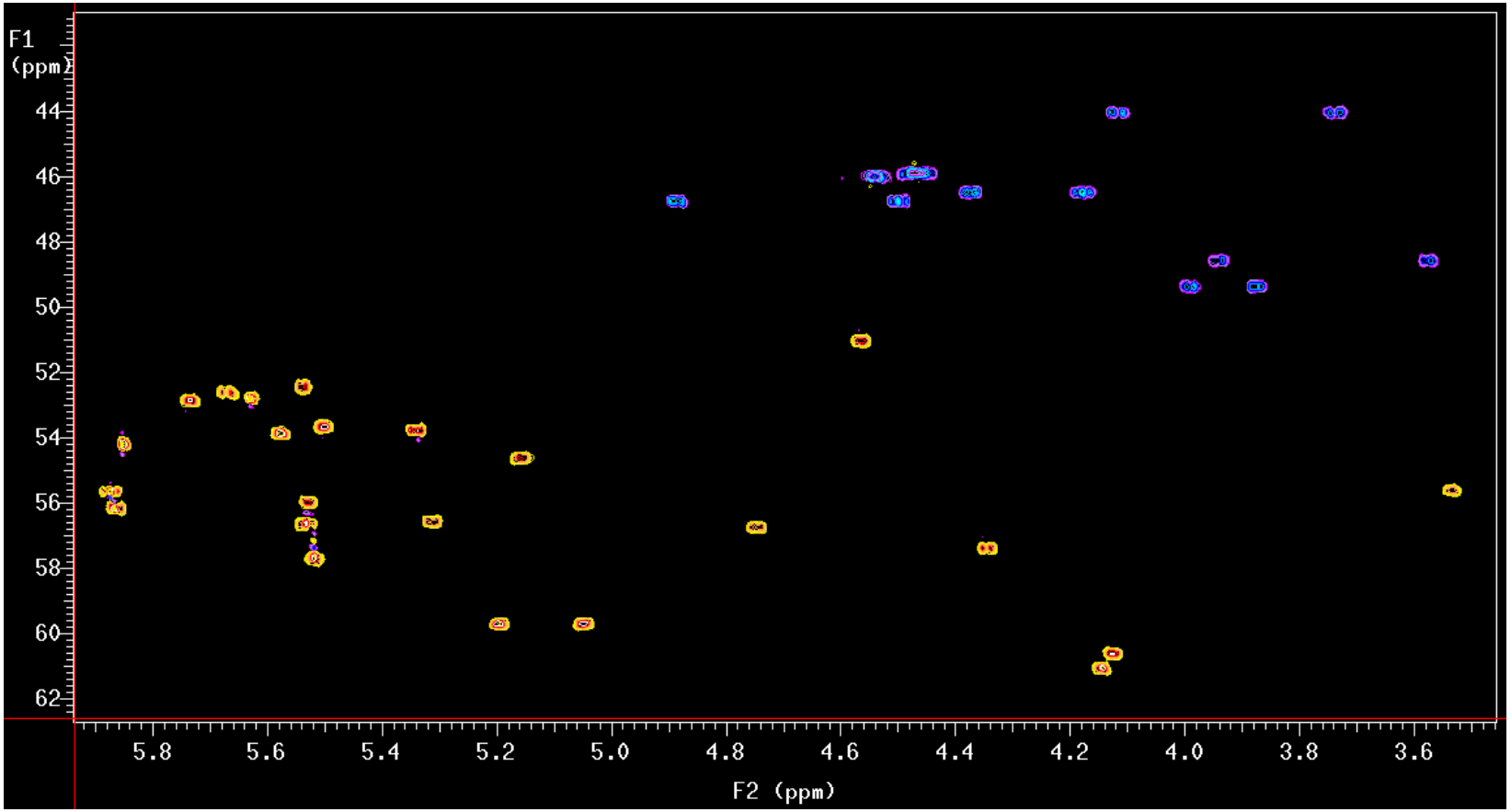
A few corrupted points at the beginning of the FID can cause extensive baseline distortion



Backward linear prediction can be used to replace this points

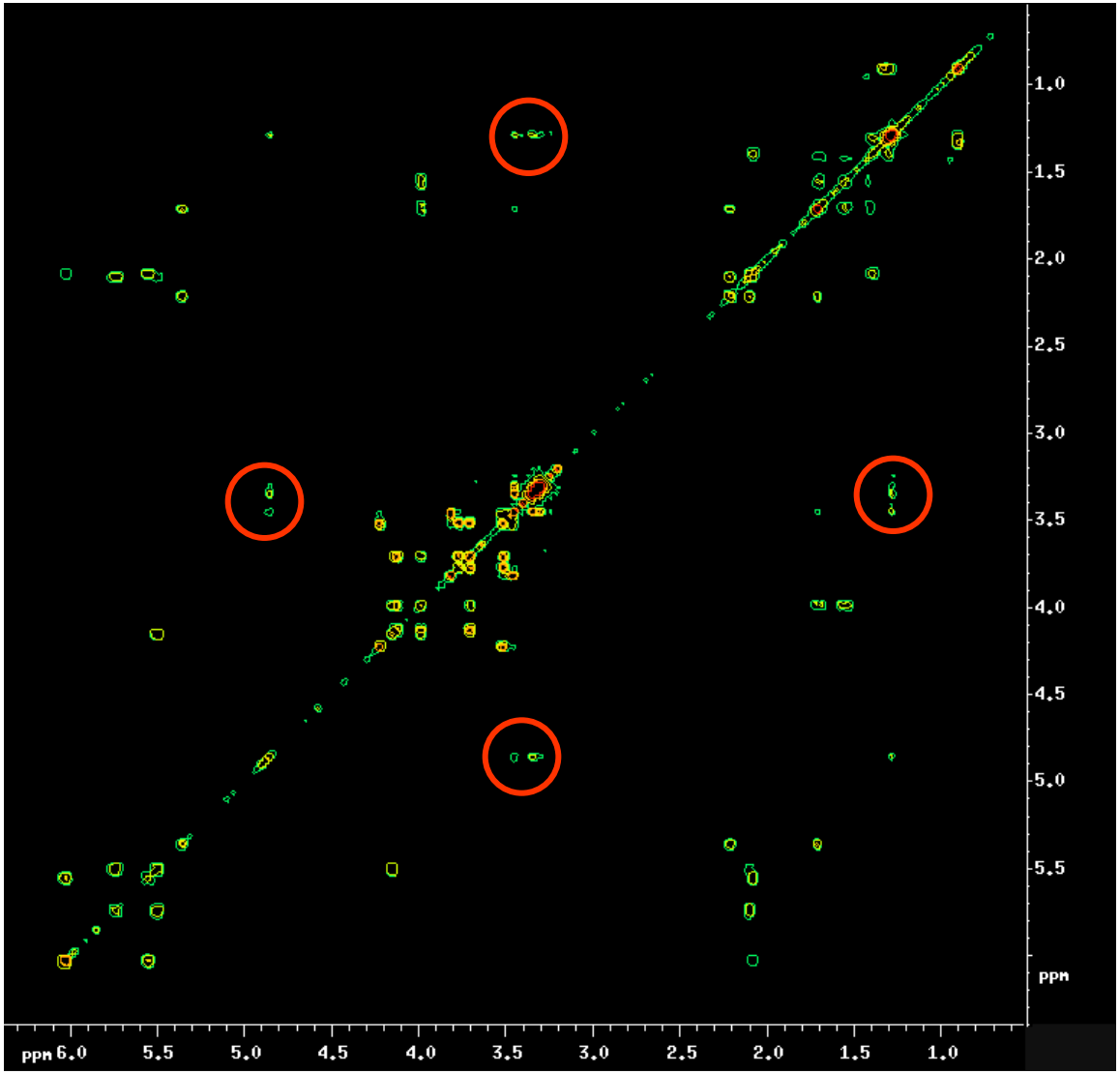


Linear Prediction

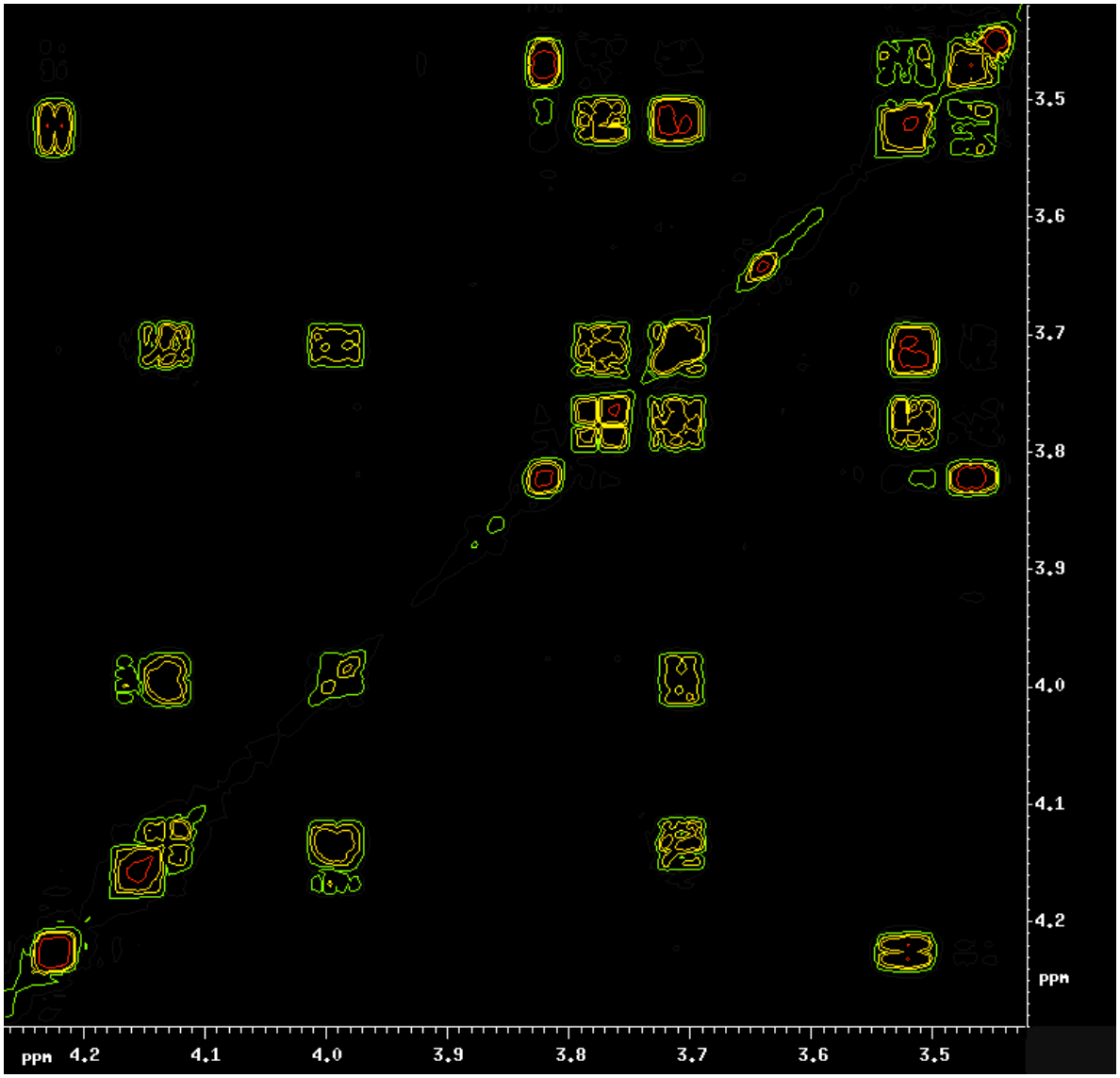


ni = 256, 3× LP, t_1 = 0.107 s, 90° shifted sine, null at t_1 = 0.428 s

Symmetrization



Symmetrization



Suggested parameters for COSY

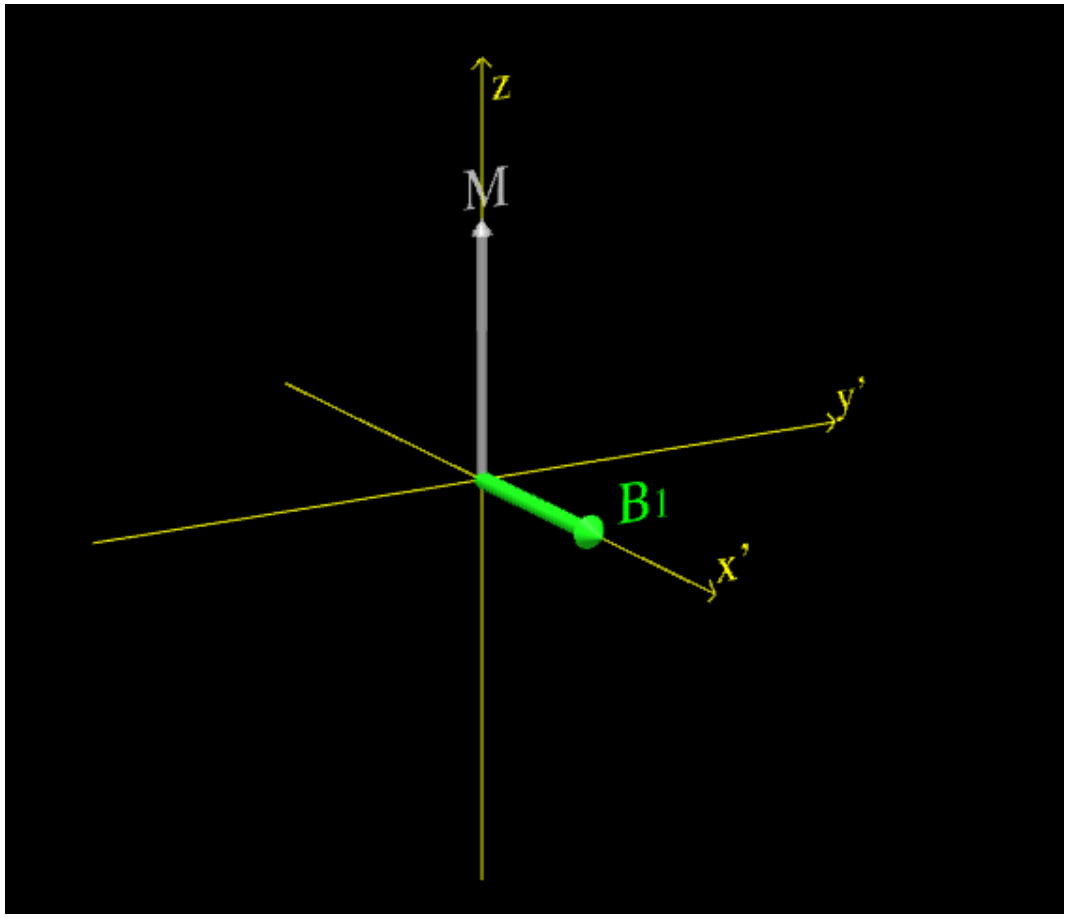
		Varian	*Bruker*
sequence	the simplest	COSY	cosyqf
	(magnitude mode)	gCOSY	cosygpqf
points acq. (F2)	1-4k	np (**)	TD (F2)
points acq. (F1)	512-1k	ni	TD (F1)
scans	1-4 (with gradients)	nt	NS
	8*n (w/o gradients)		
window funct. (F2)	square sine	sb=–at/2	QSIN
		sbs=0	SSB=0
window funct. (F1)	square sine	sb1=–ni/(sw1*2)	QSIN
		sbs1=0	SSB=0
linear prediction	NO!		
Tranf.d size	same in F1 and F2,	fn	SI (F2)
	1K-4k	fn1	SI (F1)
symmetryzation	with care	foldt	sym

Suggested parameters for TOCSY

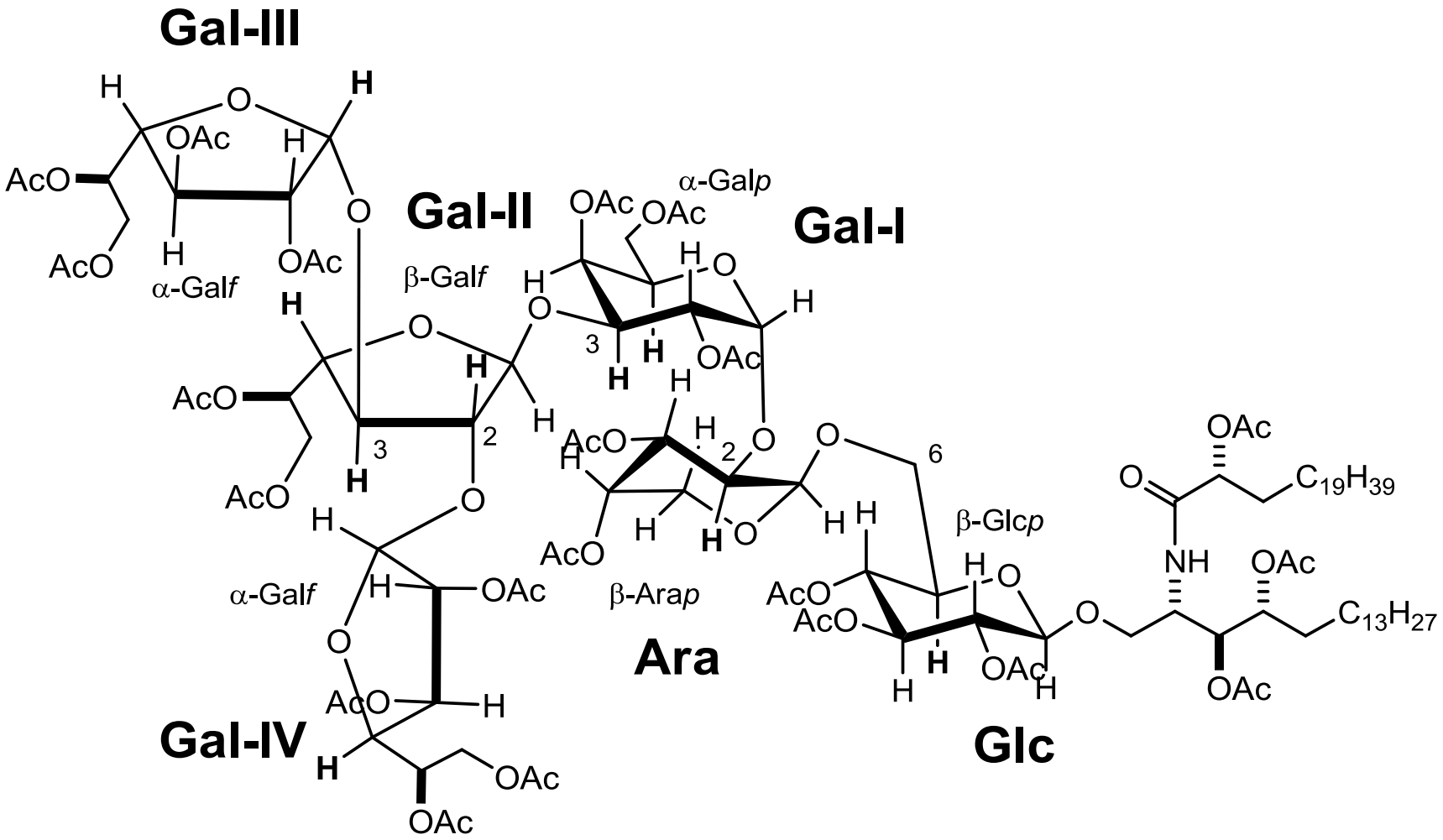
		Varian	*Bruker*
sequence	zq filtered!	zTOCSY	dipsi2gpphzs
		sspul='n'	
points acq. (F2)	4k-32k	np (*)	TD (F2)
points acq. (F1)	256-1k	ni	TD (F1)
mixing time	100 ms	mixT	d9
spinlock power	<8000 Hz	spinlockT (*)	p6, pl10 (*)
scans	8*n	nt	NS
window funct. (F2)	square cosine	sb=–at/2	QSIN
		sbs=sb	SSB=2
window funct. (F1)	square cosine	sb1=–ni/sw1	QSIN
		sbs1=sb1	SSB=2
linear prediction	yes (F1)		
transformed size	2-32k	fn	SI (F2)
	512-2k	fn1	SI (F1)
symmetryzation	no		

B_1 strength

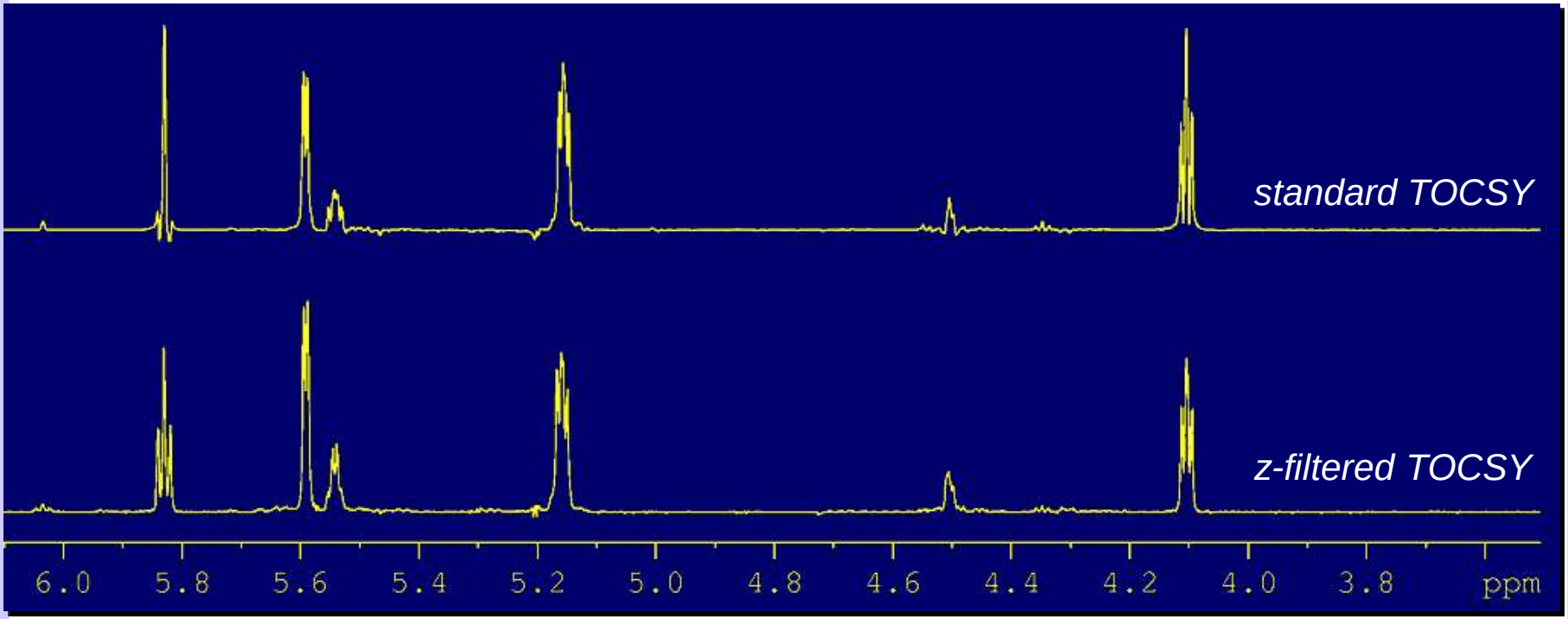
$$B_1(\mathrm{Hz}) = \frac{1{,}000{,}000}{4 \cdot \mathrm{PW90}(\mu s)}$$



Vesparioside B



Zero-quantum filtered TOCSY (zTOCSY)



M. J. Thrippleton, J. Keeler, Angew. Chem. Int. Ed. **2003**, **42**, 3938-3941

Suggested parameters for ROESY

		Varian	*Bruker*
sequence		ROESY	roesyph
points acq. (F2)	1k-4k	np (*)	TD (F2)
points acq. (F1)	256-1k	ni	TD (F1)
mixing time	200-600 ms	mixR	p11
spinlock power	2000-4000 Hz	spinlockR (*)	pl11
scans	8*n	nt	NS
window funct. (F2)	square cosine	sb=−at	QSIN
		sbs=sb	SSB=2
window funct. (F1)	square cosine	sb1=−ni/sw1	QSIN
		sbs1=sb1	SSB=2
linear prediction	yes (F1)		
transformed size	1-4k	fn	SI (F2)
	512-2k	fn1	SI (F1)
symmetryzation	no		

ROESY e TOCSY

When diagonal peaks are positive:

	cross peak
TOCSY	+
ROESY	–
chemical exchange	–
Zero-quantum	antiphase

but also

ROESY→ROESY	+
TOCSY→ROESY	+

Suggested parameters for HSQC

		Varian	*Bruker*
sequence	the most recent available (gradients, editing, echo-antiecho, adiabatic pulses...)		
points acq. (F2)	1k-2k	(at < 150 ms)	TD (F2)
points acq. (F1)	256-512	ni	TD (F1)
scans	1*n	nt	NS
J_{CH}	145 Hz	j1xh	cnst2
window funct. (F2)	square cosine	sb=−at	QSIN
		sbs=sb	SSB=2
window funct. (F1)	square cosine	sb1=−ni/sw1	QSIN
		sbs1=sb1	SSB=2
linear prediction	yes (F1)		
transformed size	1k-4k	fn	SI (F2)
	512-2k	fn1	SI (F1)

Suggested parameters for HMBC

		Varian	*Bruker*
Sequence		gHMBCAD	hmbcetgpnd
	(gradients, editing, adiabatic pulses, F1 phase		sensitive)
points acq. (F2)	1k-4k	np	TD (F2)
points acq. (F1)	256-1k	ni	TD (F1)
scans	1*n	nt	NS
J_{CH}	8 Hz	jnxh	cnst13
window funct. (F2)	square sine	sb=–at/2	QSIN
		sbs=0	SSB=0
window funct. (F1)	square cosine	sb1=–ni/sw1	QSIN
		sbs1= –sb1	SSB=2
linear prediction	yes (F1 only)		
transformed size	1k-4k	fn	SI (F2)
	512-2k	fn1	SI (F1)