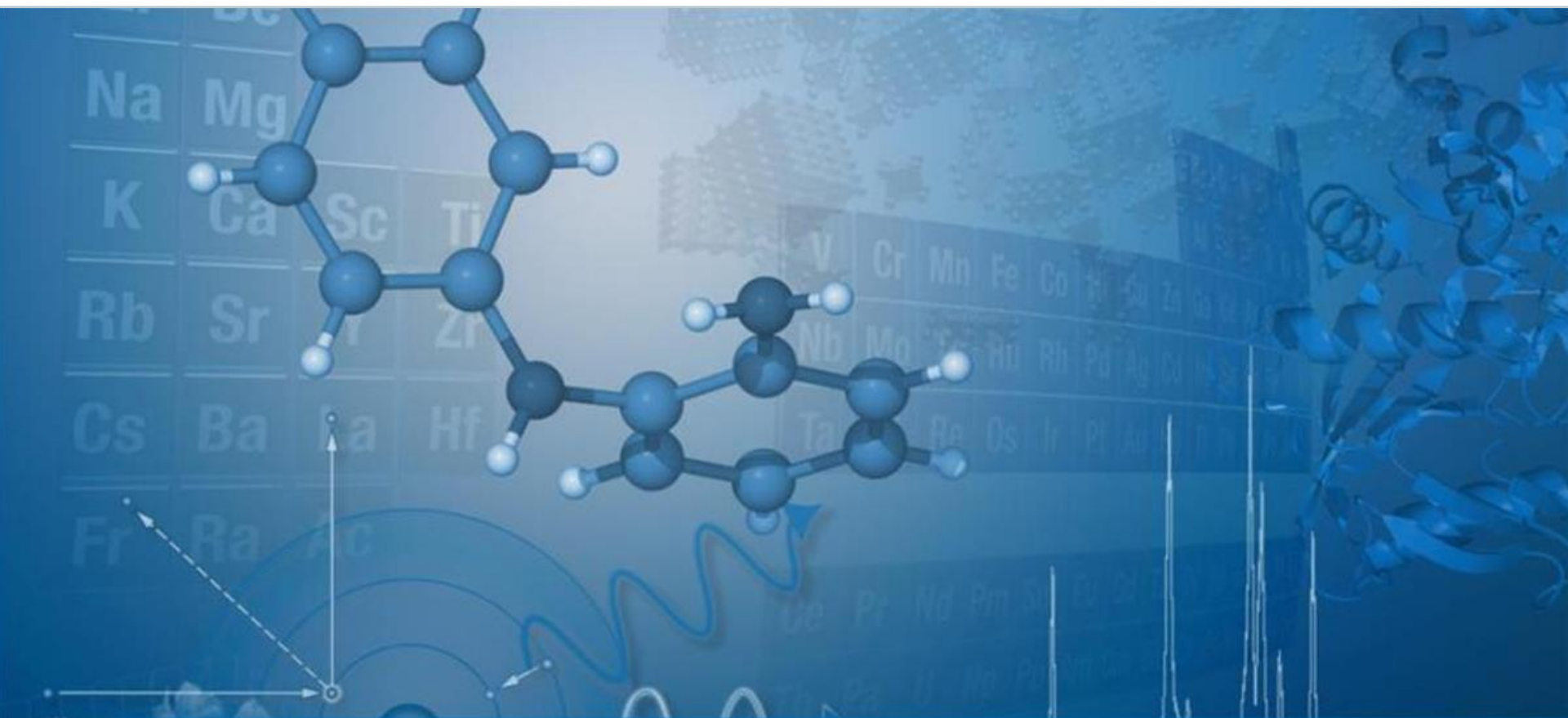


# Troubleshooting

Dr. Benjamin Görling



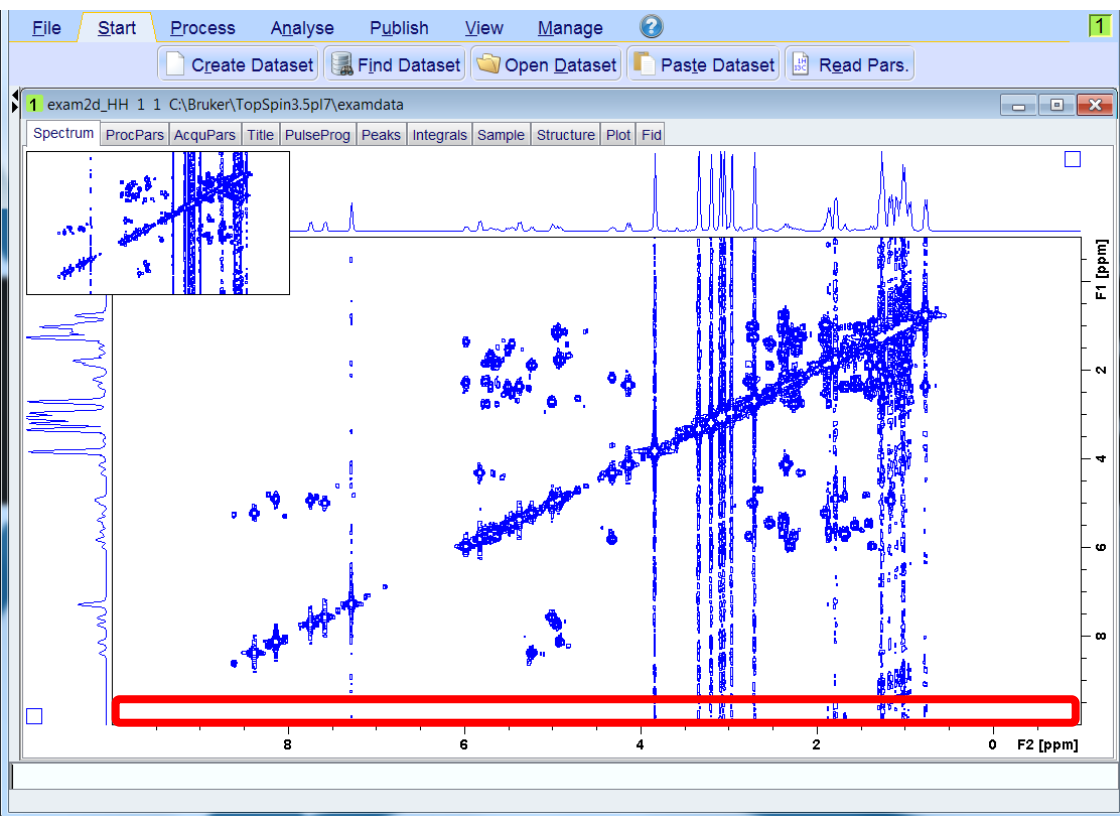
- **T1 noise**

- Many times 2D spectra reveal noise streaks either as a result of sample spinning modulations or modulations from very strong signals
- These are consistent in S/N horizontally and can be subtracted, leaving only true signal behind.

# T1 Noise Subtraction



1. Expand full vertical region of spectrum that contains only noise, no signals.



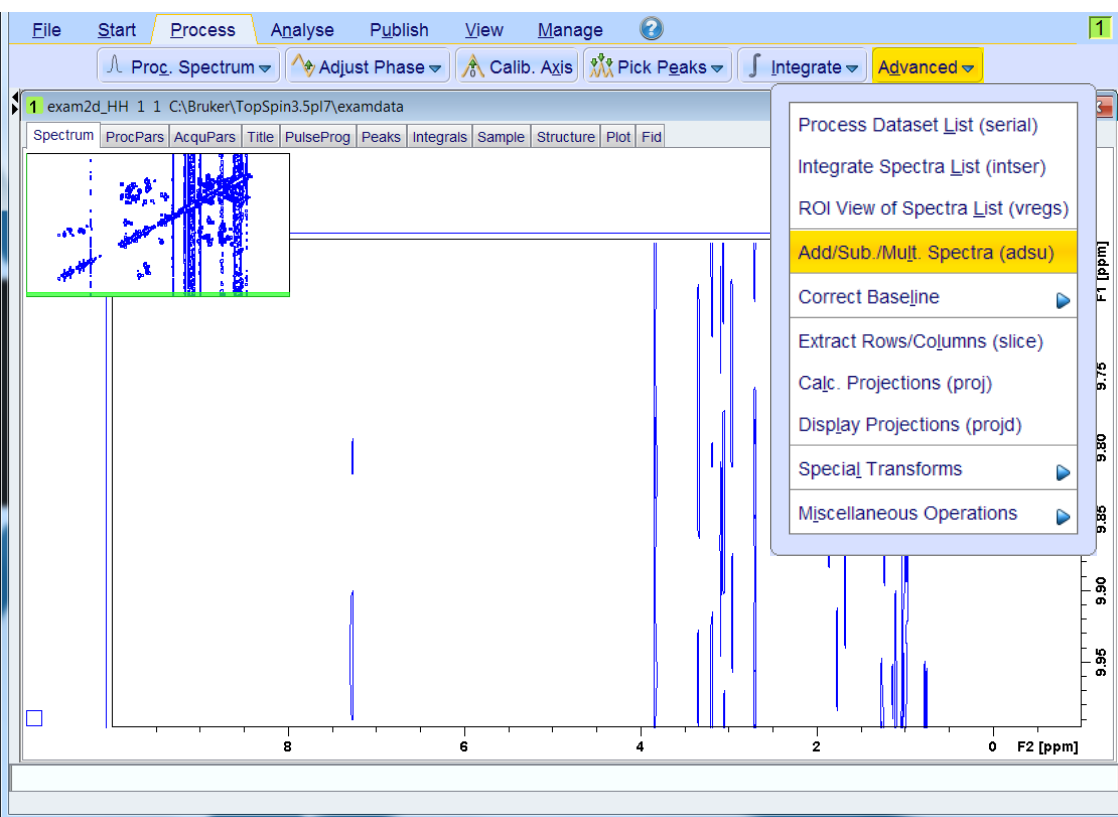
# T1 Noise Subtraction



The screenshot shows the Bruker software interface with the 'update' dialog box open. The dialog box has two instances, one in the foreground and one in the background. The foreground dialog box has the 'Update rows/cols from display' option selected and highlighted with a red box. The 'Required parameters' section shows the following values: Projection (sum) of = rows, Display projection = on 2D, First row/col = 1, Last row/col = 2048, Destination PROCNO = 999, and Disco reference col/row = 1. A red arrow points from the 'Last row/col' field in the foreground dialog to the 'Last row/col' field in the background dialog, which is set to 155.

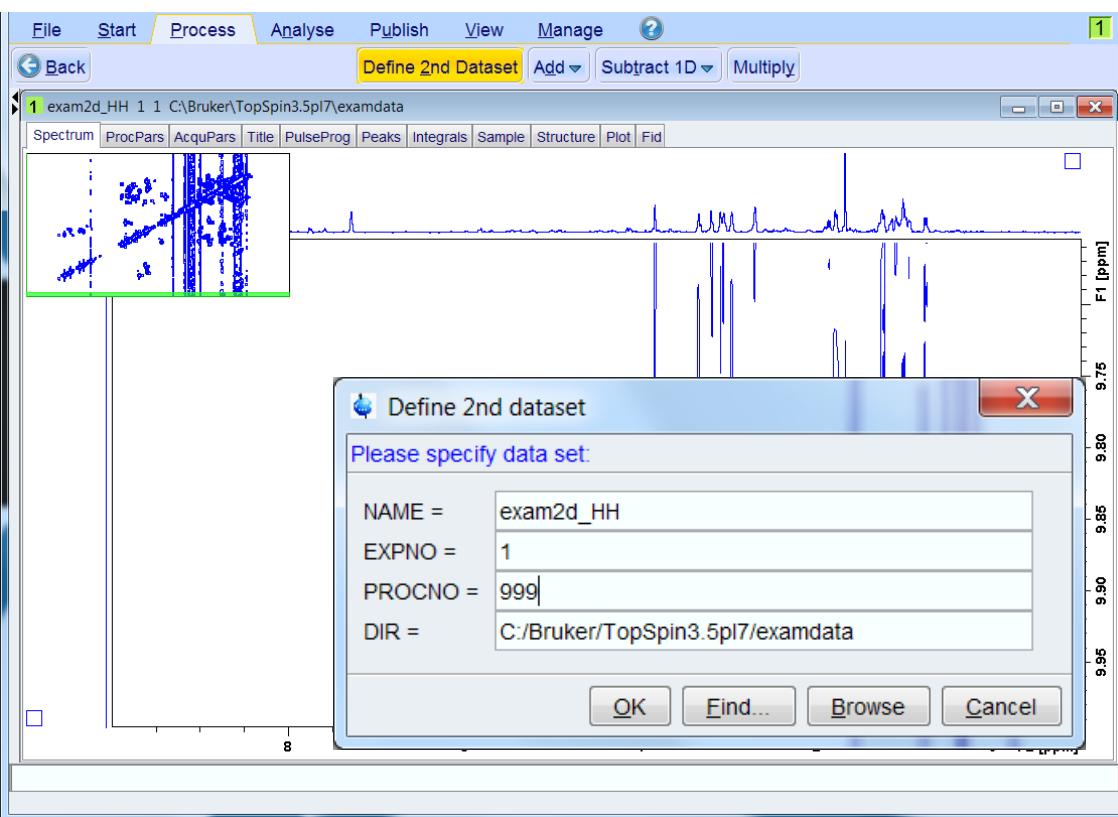
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
  - a. Select Update rows/columns from display
  - b. Click ok
  - c. Then Calculate Sum – make note of Destination PROCNO.
  - d. This is the 2<sup>nd</sup> dataset you will use to subtract from the 2D
  - e. Click ok

# T1 Noise Subtraction



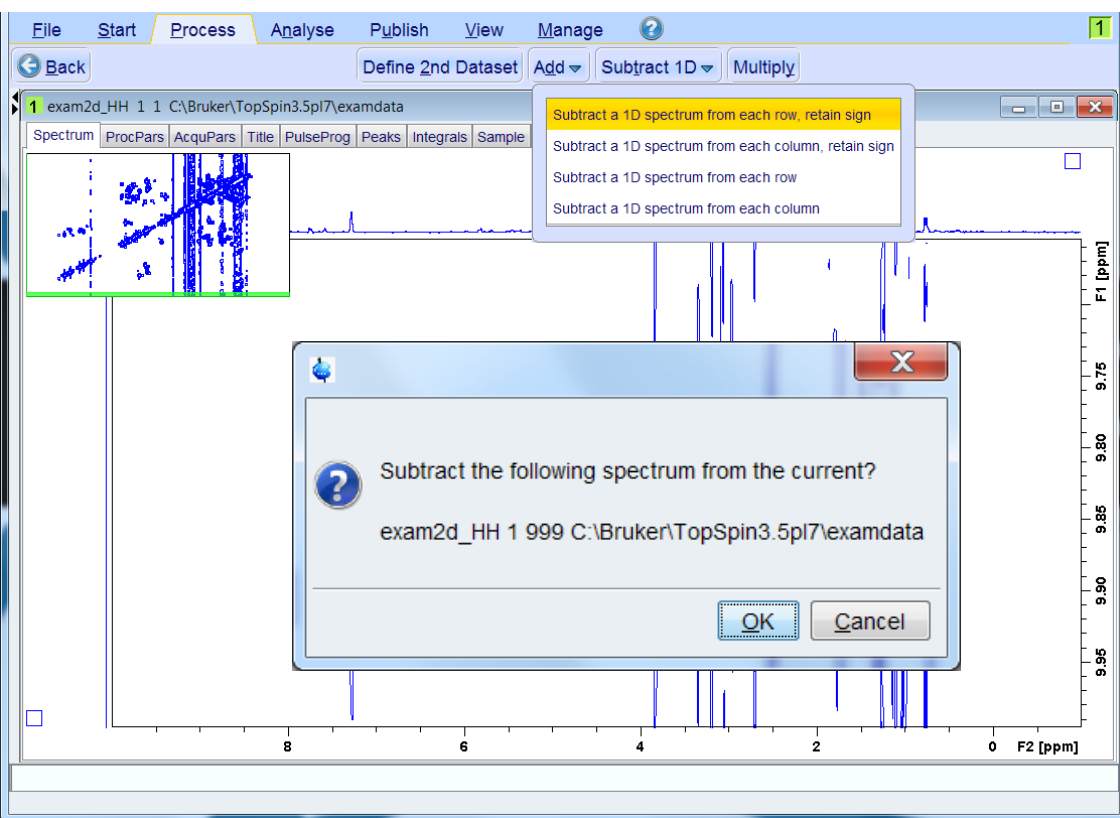
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. spectra

# T1 Noise Subtraction



1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. Spectra
  1. Define 2<sup>nd</sup> dataset you just made from previous slide

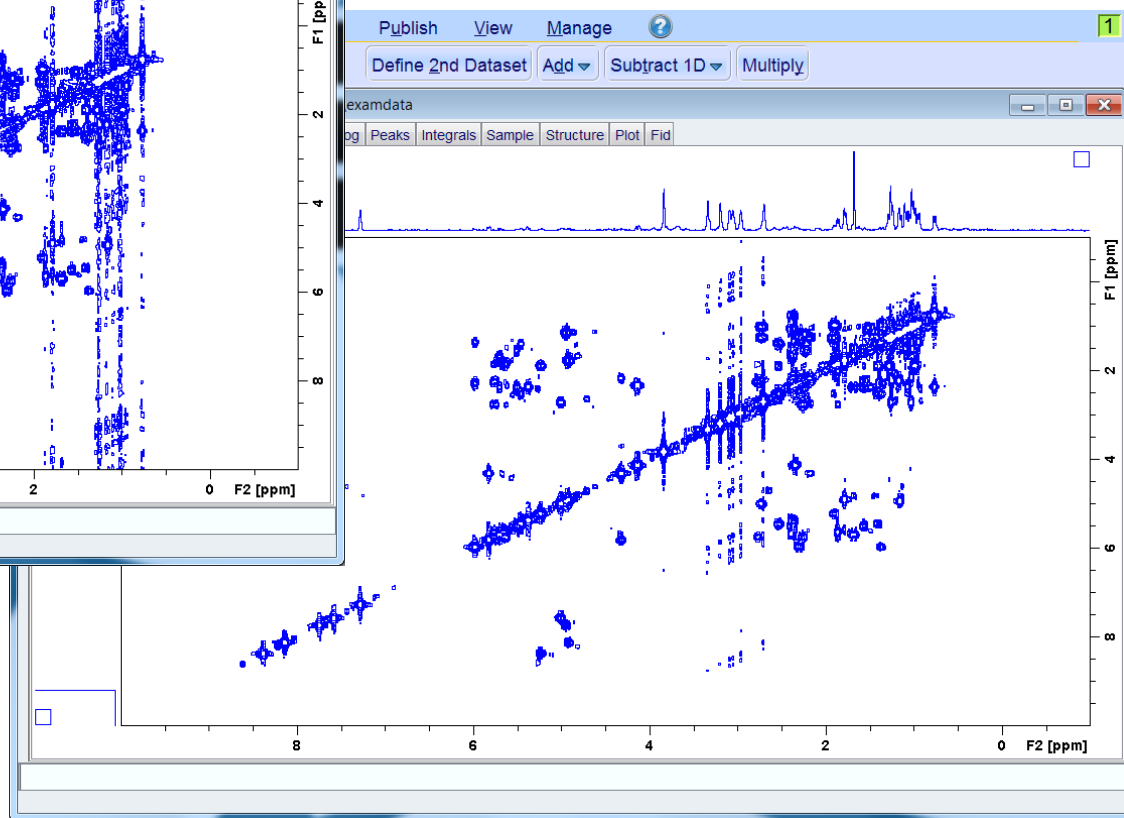
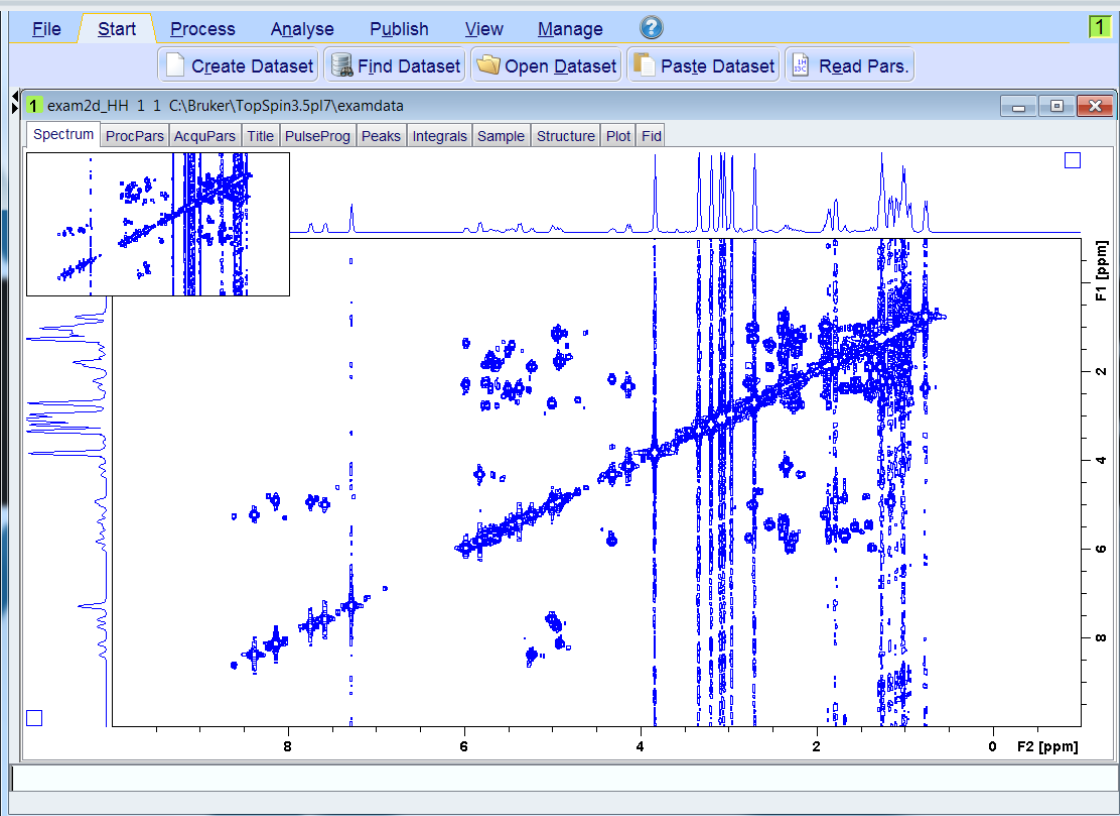
# T1 Noise Subtraction



1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. Spectra
  1. Define 2<sup>nd</sup> dataset you just made from previous slide
  2. From the Subtract 1D pull-down menu, select 1<sup>st</sup> option
  3. Confirm with OK

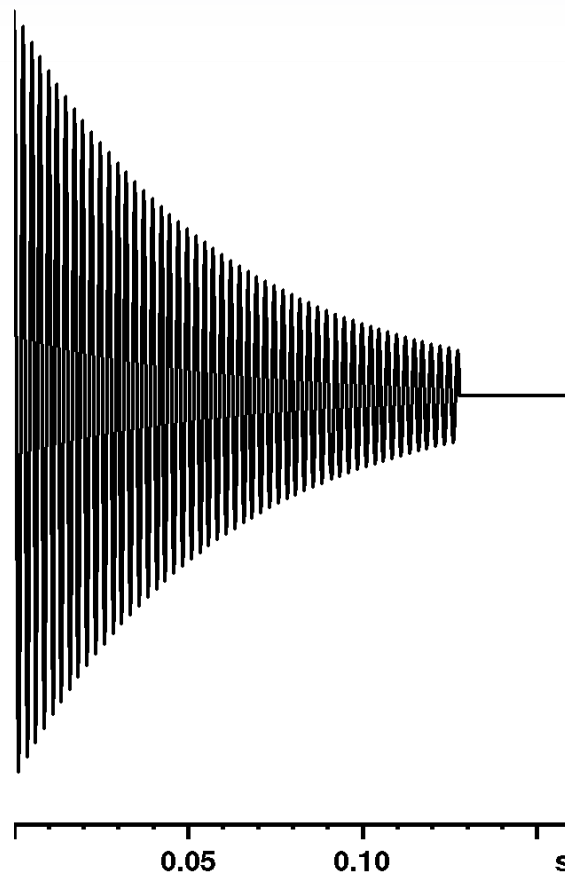
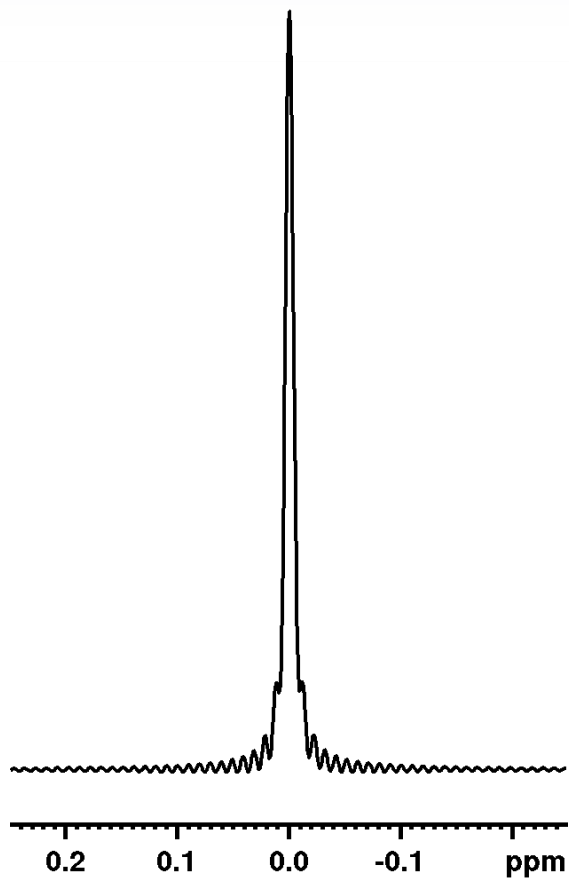


# T1 Noise Subtraction

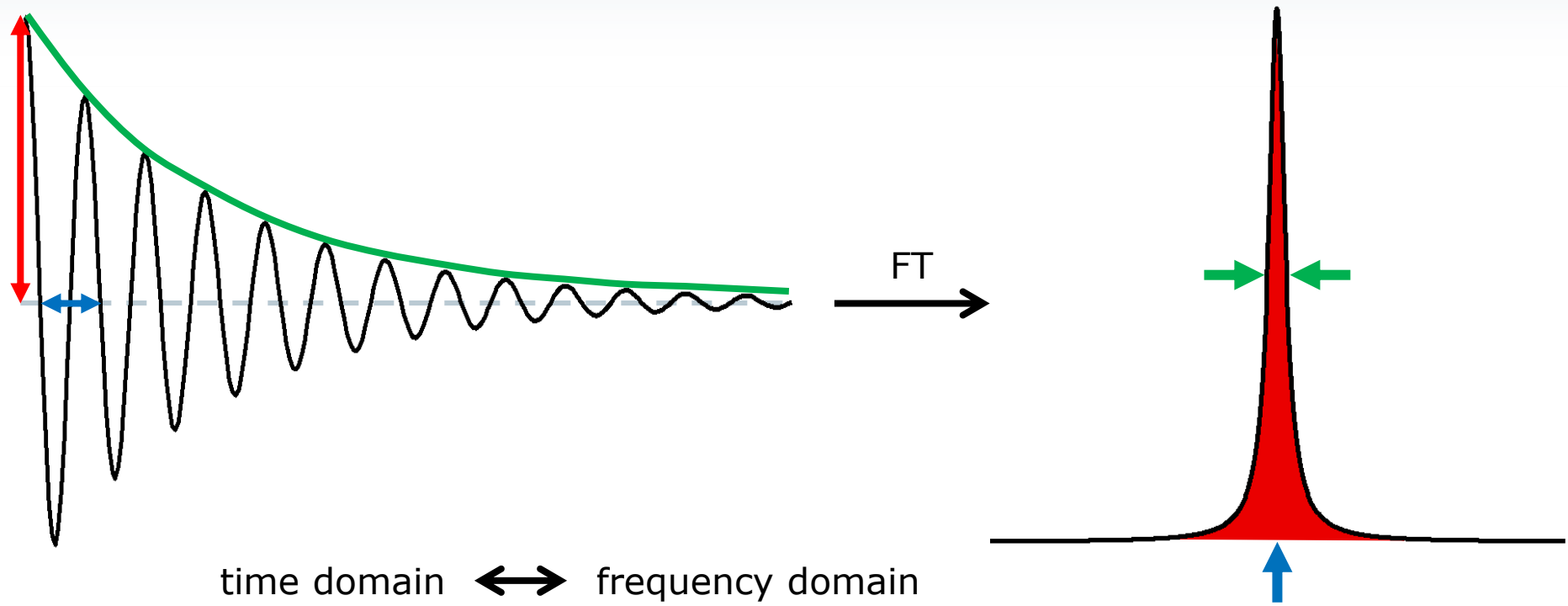


- **Truncation artifacts**

# Truncation artifacts



# Fourier transformation



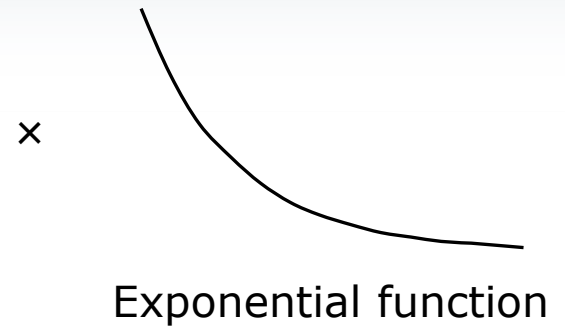
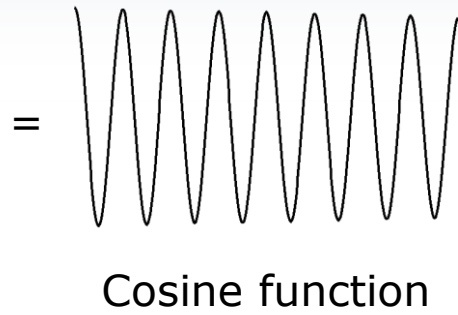
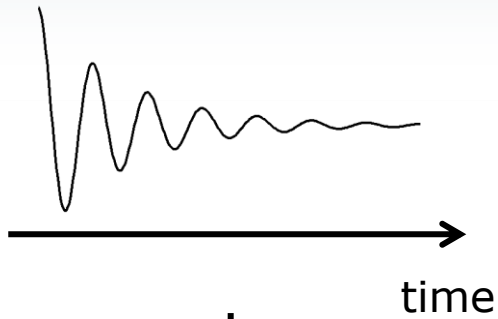
time domain  $\longleftrightarrow$  frequency domain

amplitude  $\longleftrightarrow$  integral

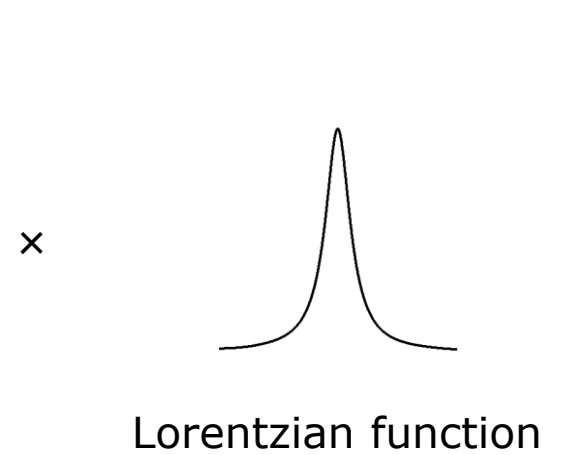
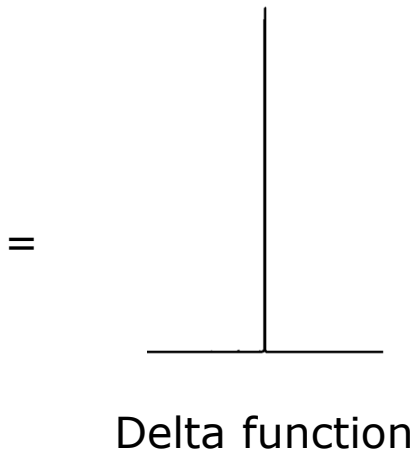
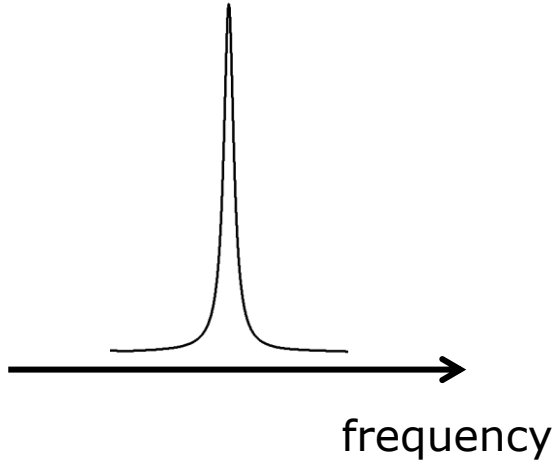
period  $\longleftrightarrow$  frequency

decay  $\longleftrightarrow$  lineshape

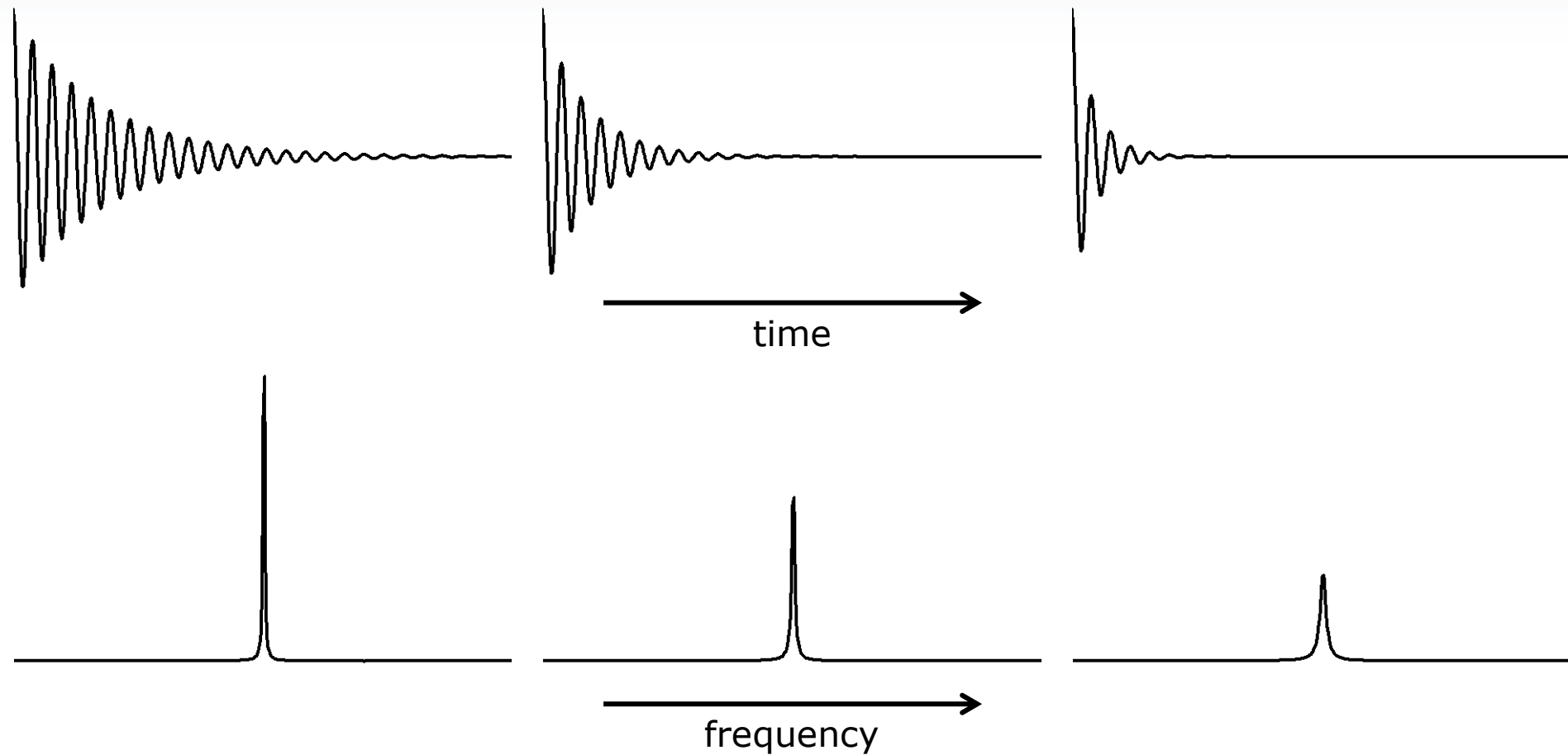
# Fourier transformation



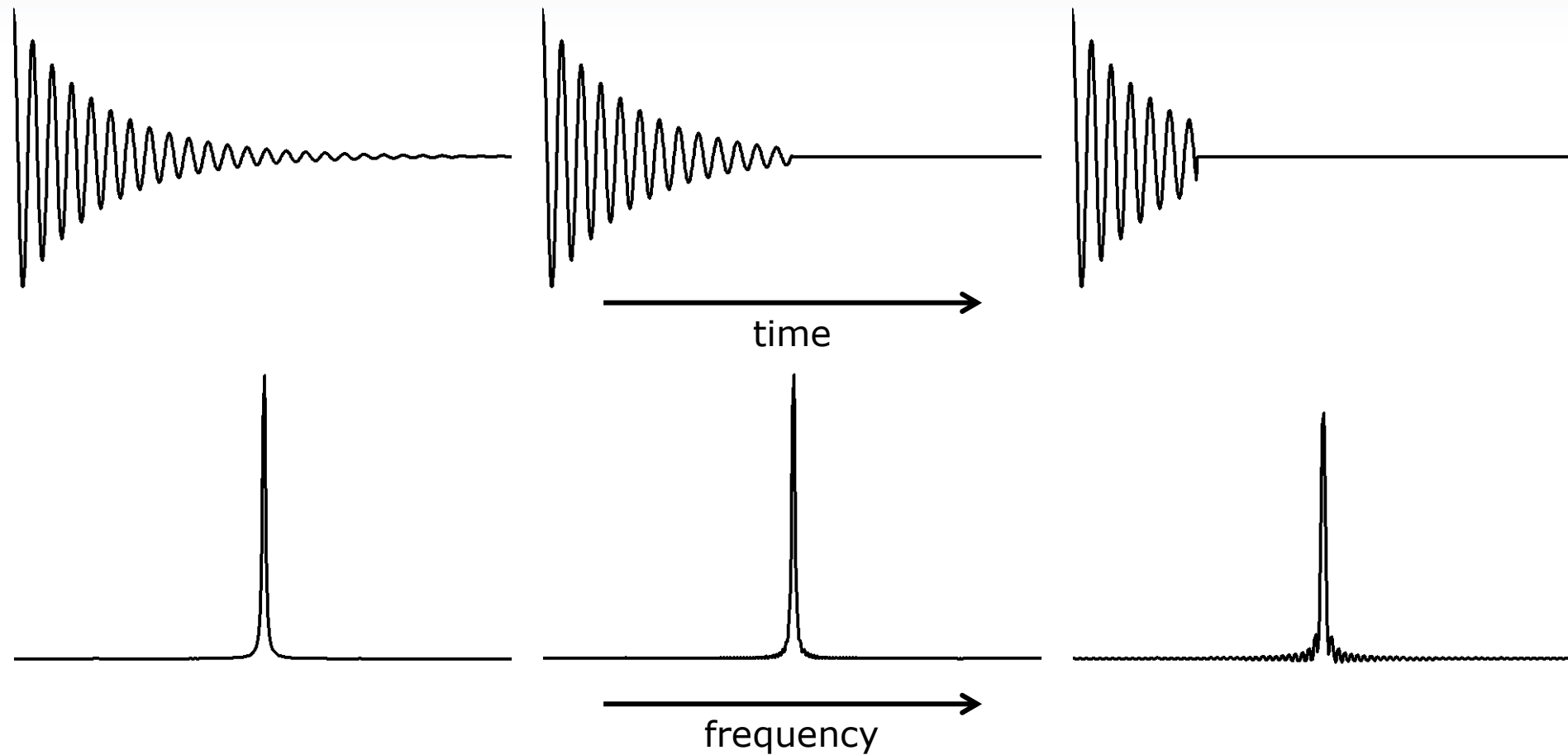
FT

A vertical arrow pointing downwards, indicating the direction of the Fourier transformation from the time domain to the frequency domain.

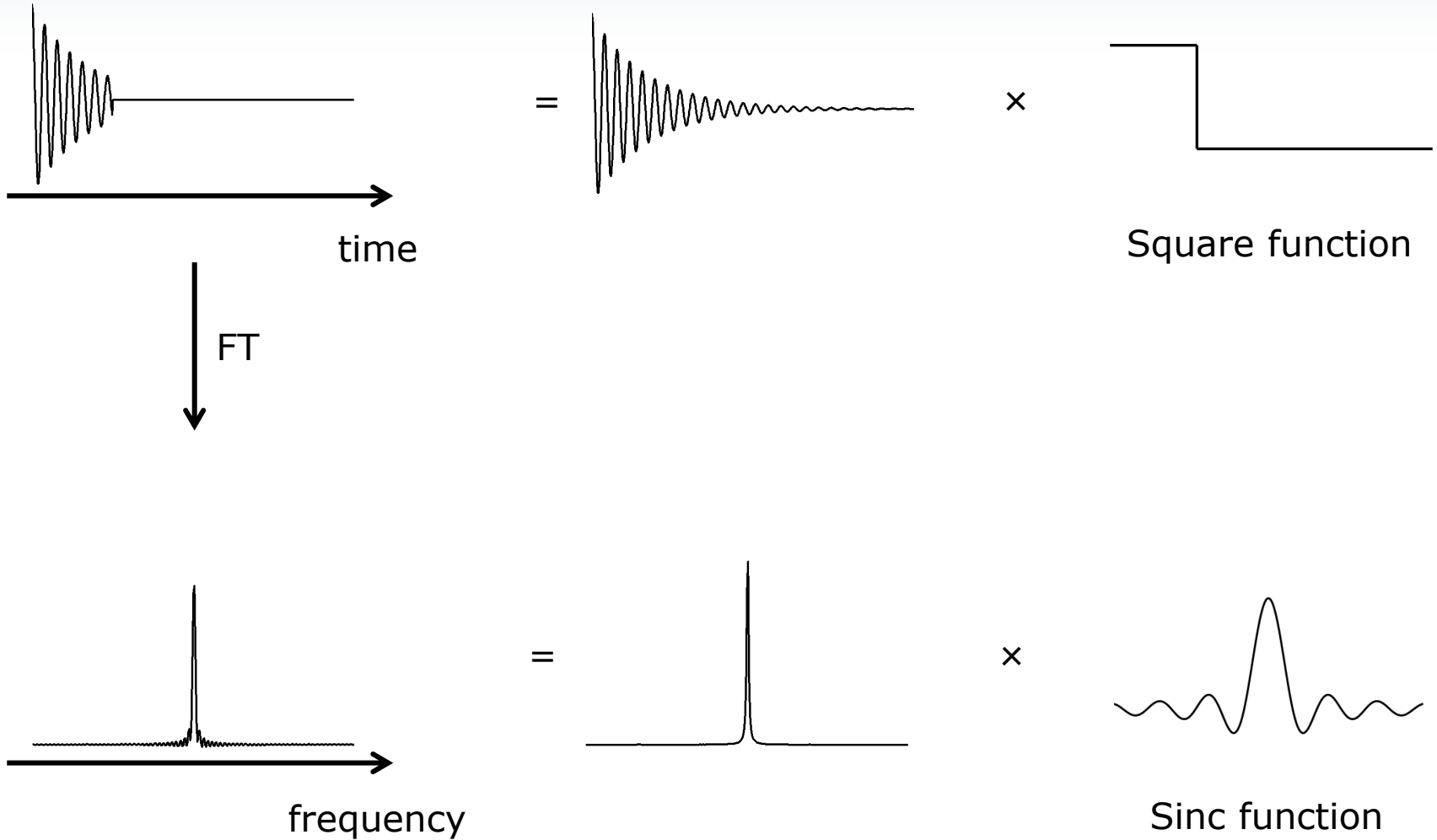
# Fourier transformation



# Fourier transformation

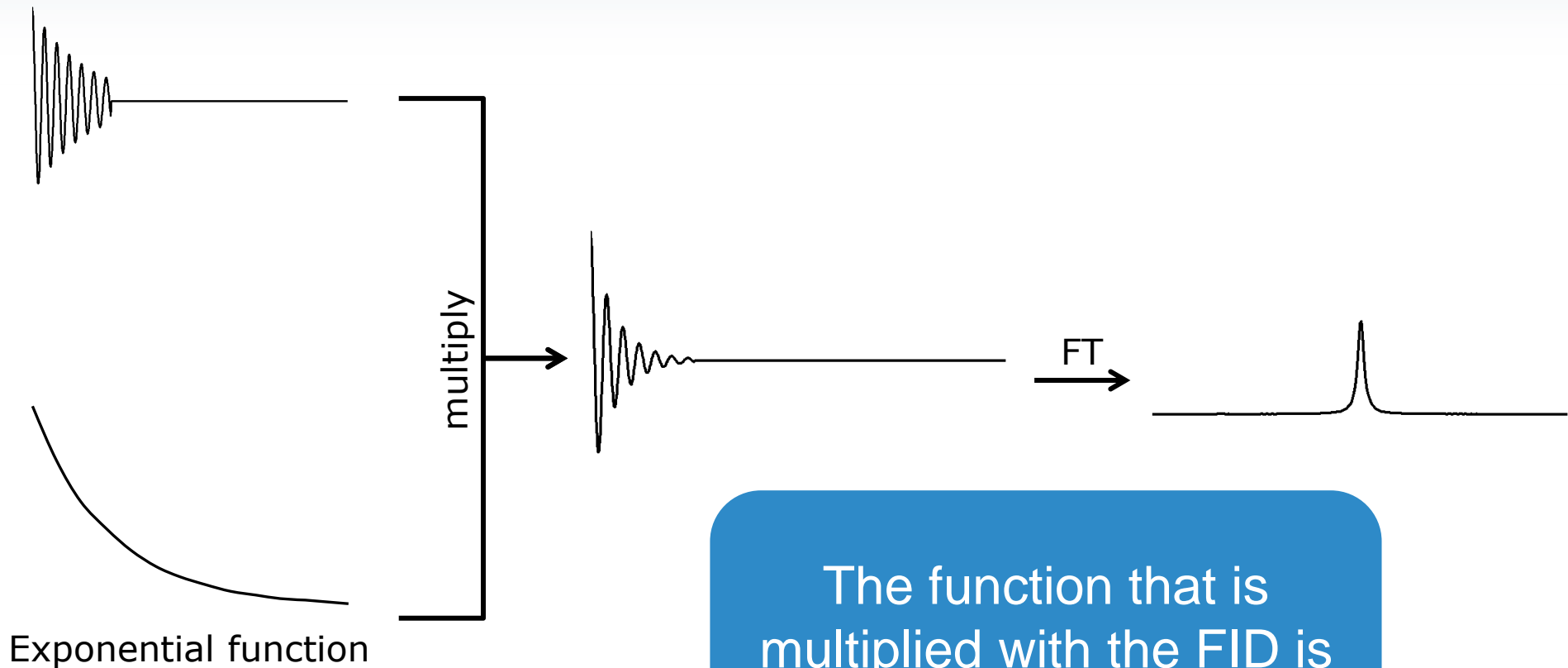


# Fourier transformation





# Fourier transformation



The function that is multiplied with the FID is called **window function**.

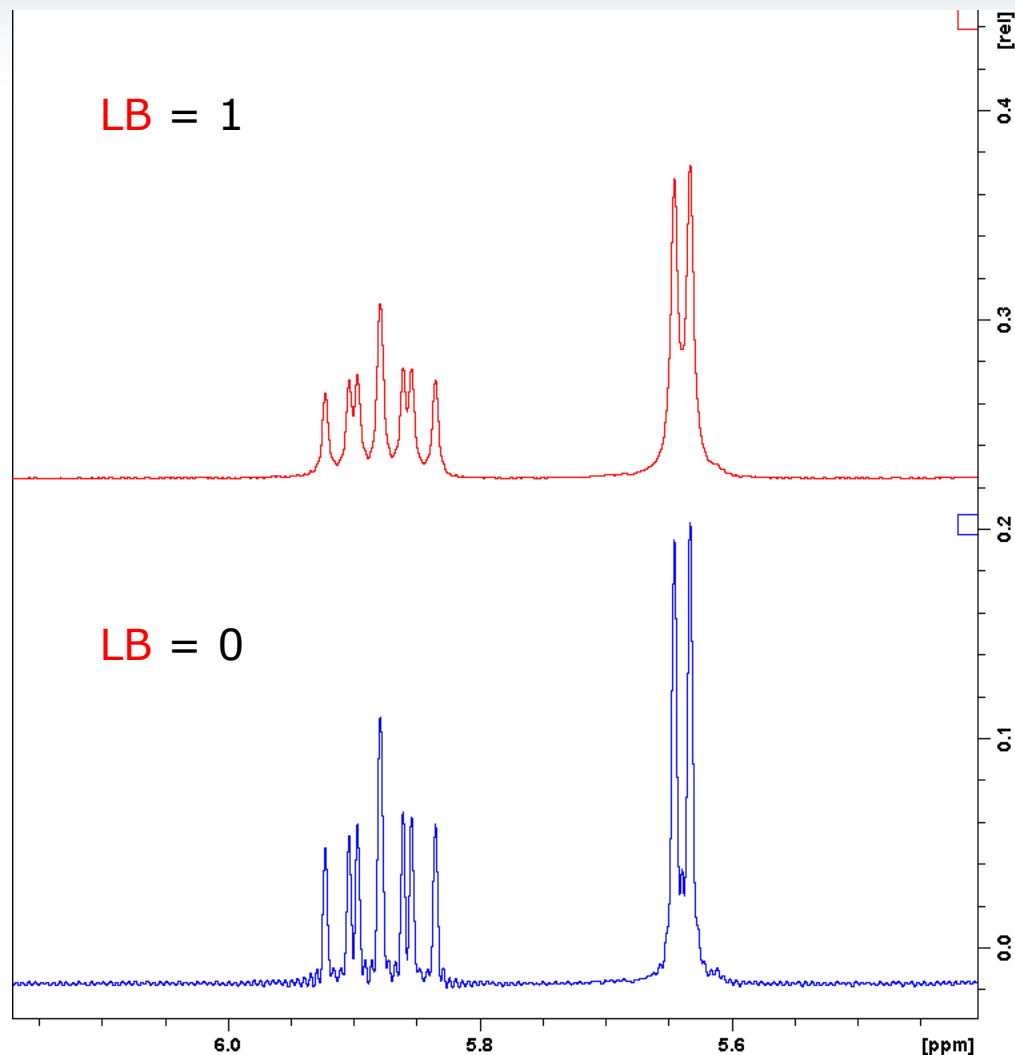
- **Window functions**

# Window functions

## EM



- Exponential multiplication
- Parameter: **LB**
- Value typically positive
- Forces FID to go to 0
  
- Broadening out signals and noise
  - Increase apparent S/N
  - BUT: at the cost of resolution

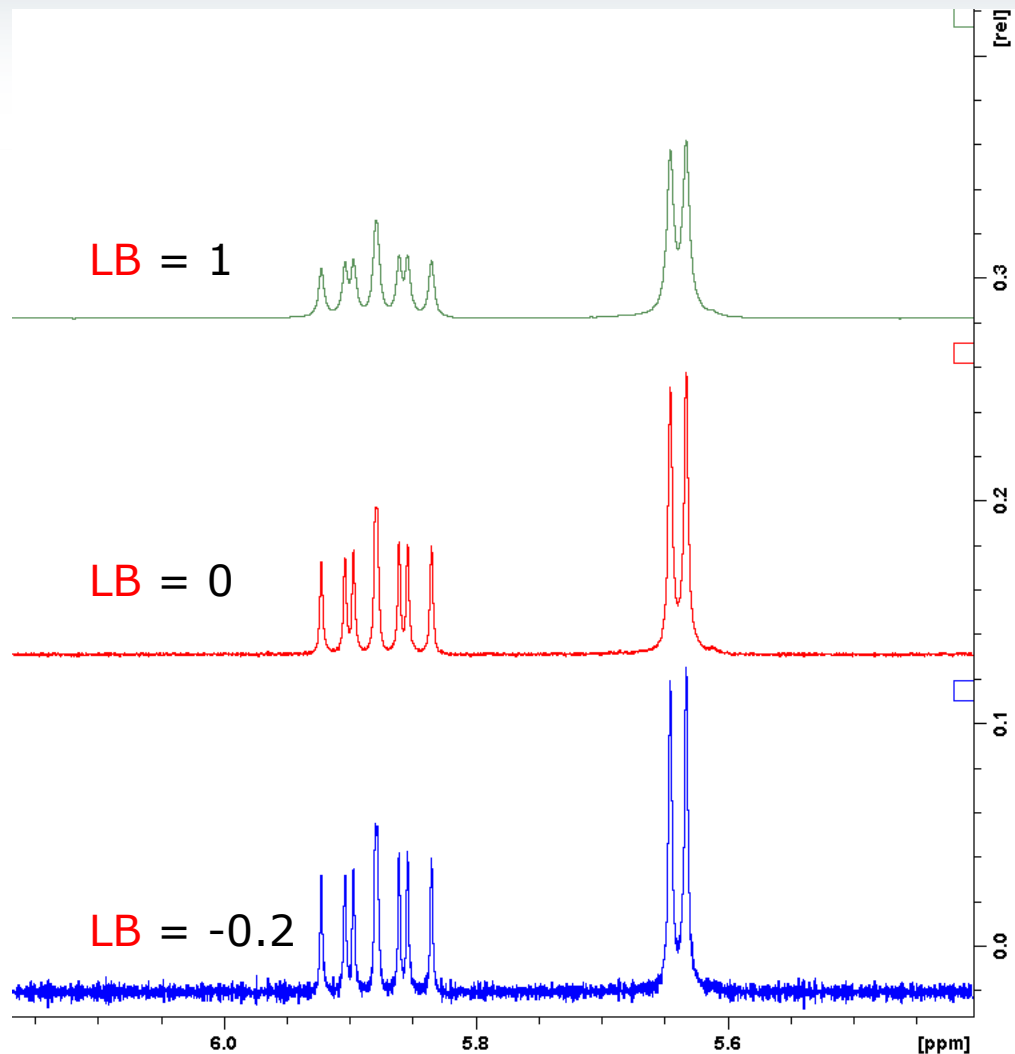
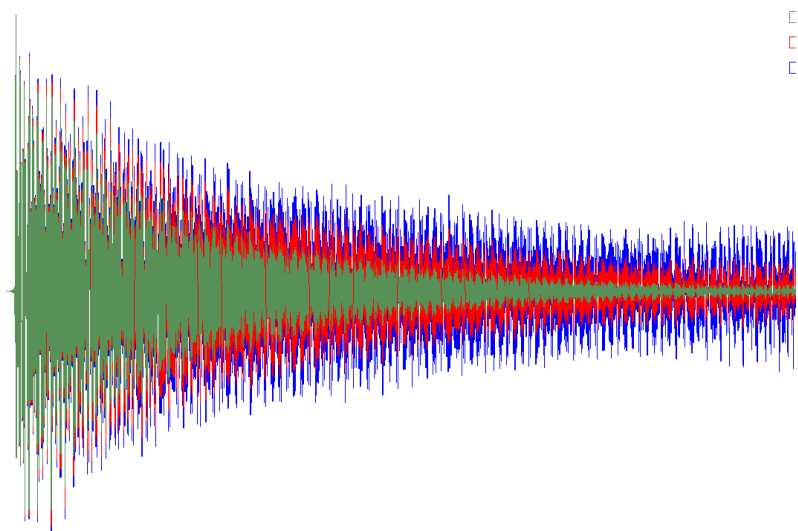


# Window functions

## EM



- Can increase apparent resolution as well
- BUT: at the cost of S/N

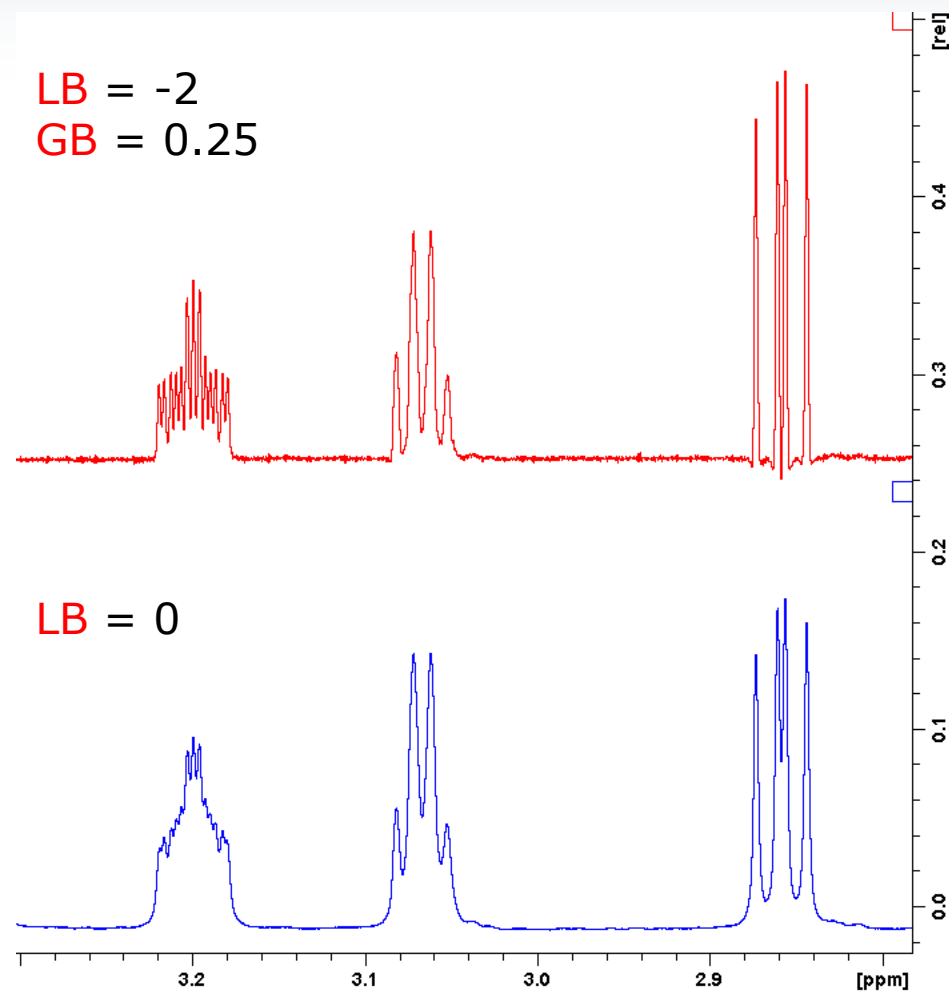


# Window functions

## GM



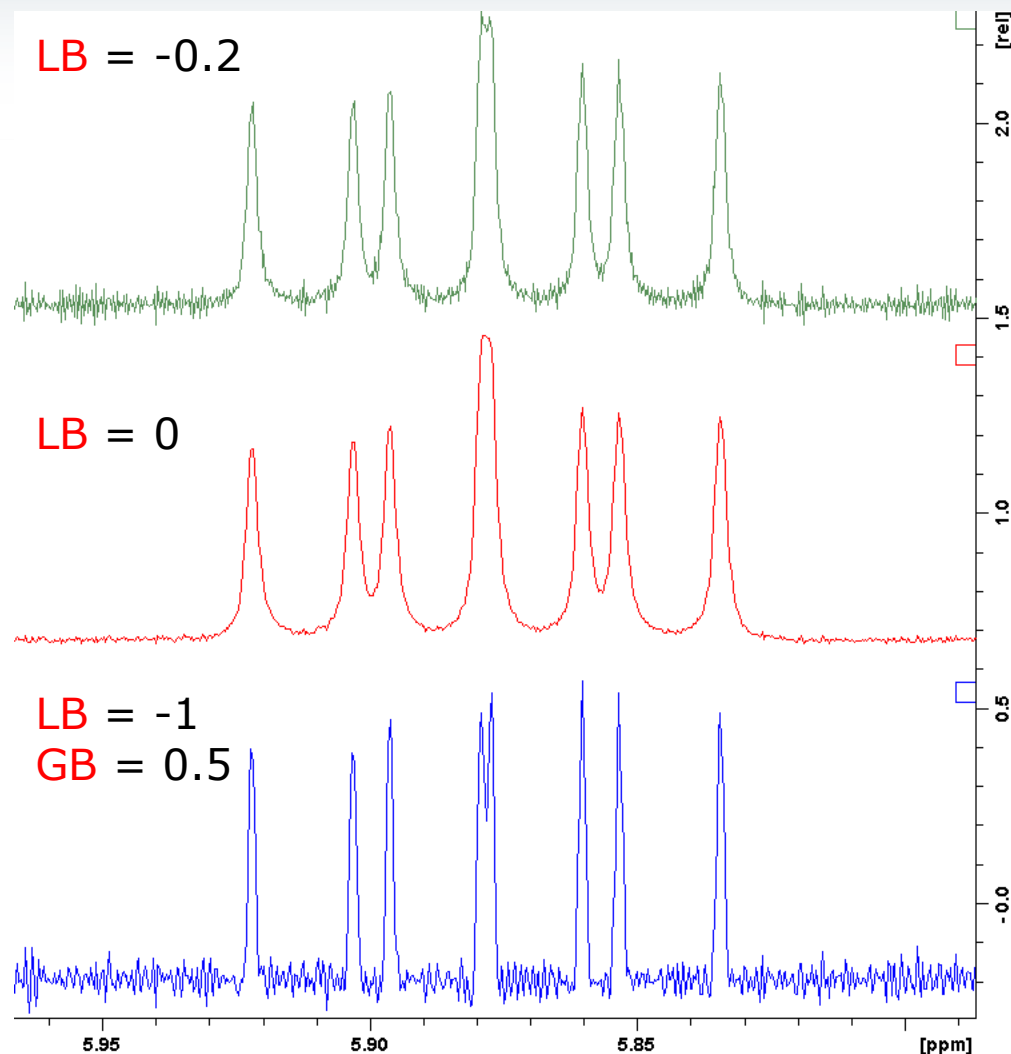
- Gaussian multiplication
- Parameters: **GB** and **LB**
- **LB** must be negative (typically  $LB = -(\text{half-width})$ )
- **GB** between 0 and 1
- processing with [**gfp**]
  
- Does decrease S/N a bit, but there is no method experimentally to increase resolution to this extent



# Window functions GM



- Gaussian multiplication
- Parameters: **GB** and **LB**
- **LB** must be negative (typically  $LB = -(\text{half-width})$ )
- **GB** between 0 and 1
- processing with [**gfp**]
  
- Does decrease S/N a bit, but there is no method experimentally to increase resolution to this extent

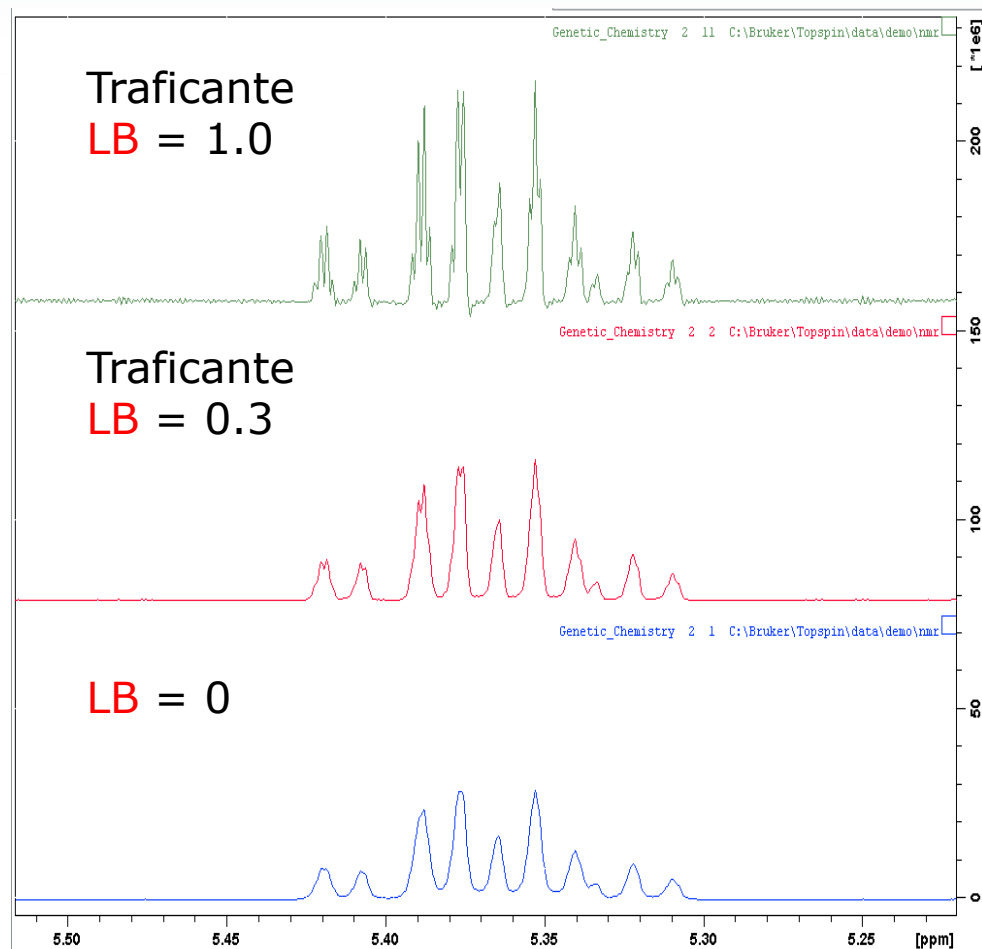


# Window functions

## TRAF



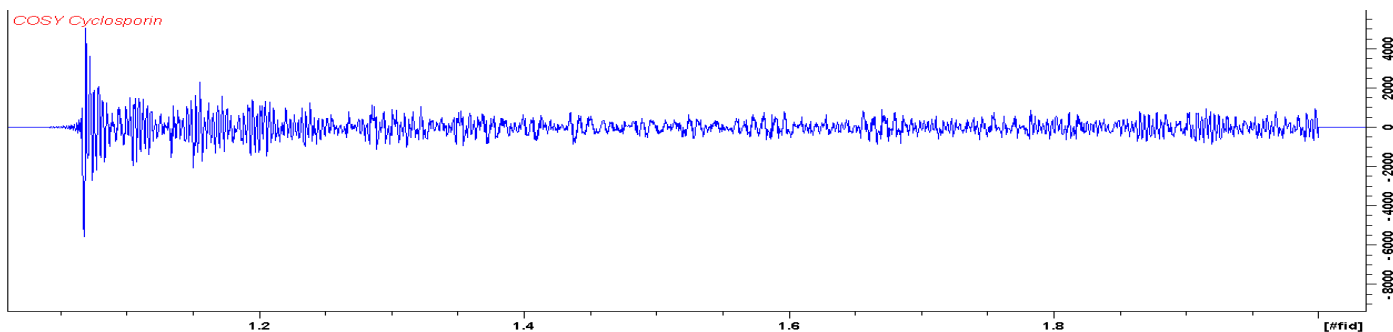
- This function is best applied to spectra with the correct acquisition time (AQ)
  - Best set to approximately 3 times longest T1
  - Signal is 1/3 FID; 2/3 noise
- traf function will increase resolution
- Parameter to modify is **LB** (range 0-1.0)



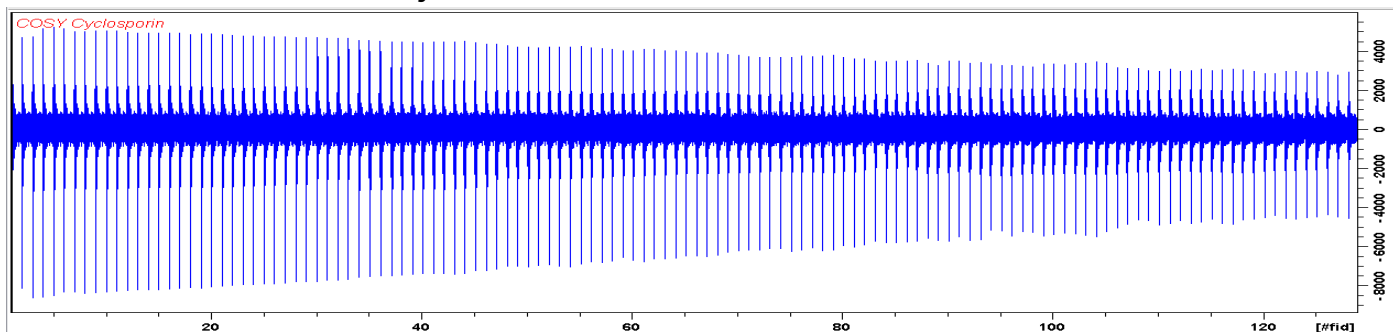
# Apodization for 2D spectra



- In 2D experiments:
  - Typical value for **TD** in F2 is 1k or 2k (this for a 1D proton that we normally acquire with 8k or 16k or more!)



- Typical value for **TD** in F1 is 128 or 256, resulting in a series of FIDs that show relaxation, just like an FID





# Apodization for 2D spectra

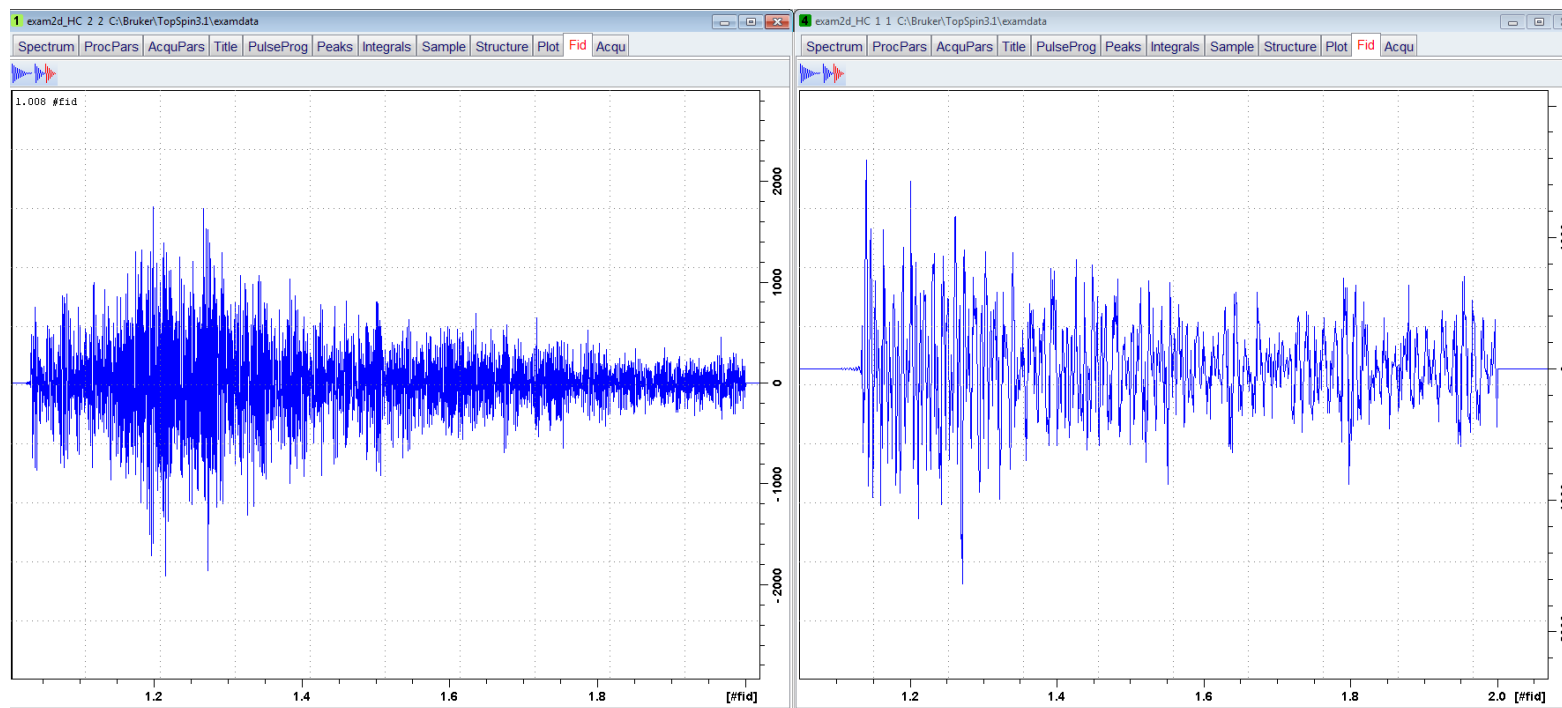


- All of this truncation requires stronger apodization than exponential multiplication
  - Application of a sine or cosine function helps more
  - This allows us to emphasize the beginning portion of the FID and greatly reduce the noise seen towards the end of the FID
- Window function is  $\text{sinn}$  or  $\text{qsinn}$ 
  - $\text{sinn}$  is sine multiplication
    - $$\text{sinn}(t) = \sin\left(\left(\pi - \left(\frac{\pi}{SSB}\right)\right)\left(\frac{t}{AQ}\right) + \frac{\pi}{SSB}\right)$$
  - $\text{qsinn}$  is sine the above function, squared
  - SSB values:
    - $SSB=0=1$  applies pure sine function
    - $SSB=2$  applies pure cosine function
    - $SSB>2$  applies a mix  $\text{sin/cos}$  function

# Step 1: evaluate the first FID



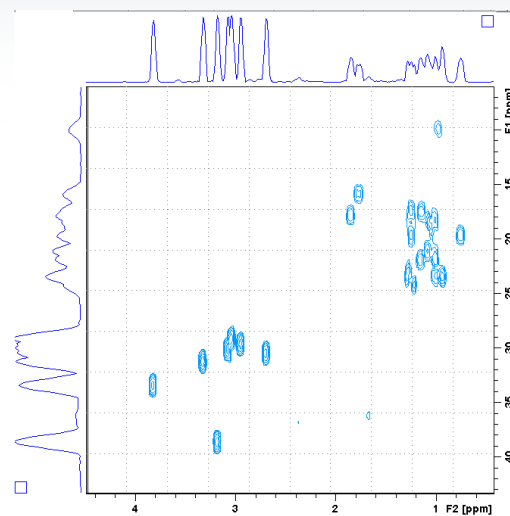
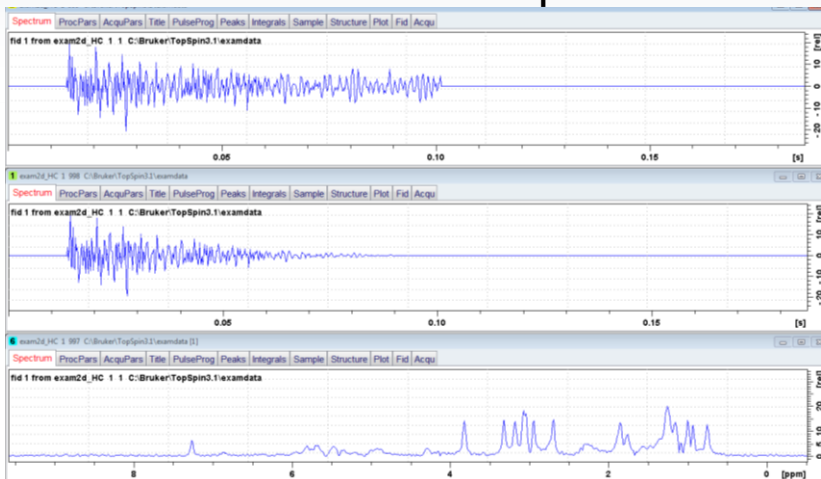
- On left is the 1<sup>st</sup> FID of an HMBC, note how signal builds from start, this uses the less common SSB=0,1 (these have the same effect)
- On right is the 1<sup>st</sup> FID of an HMQC. This looks more "normal" with max signal at beginning, and traditional decay. This uses SSB=2.



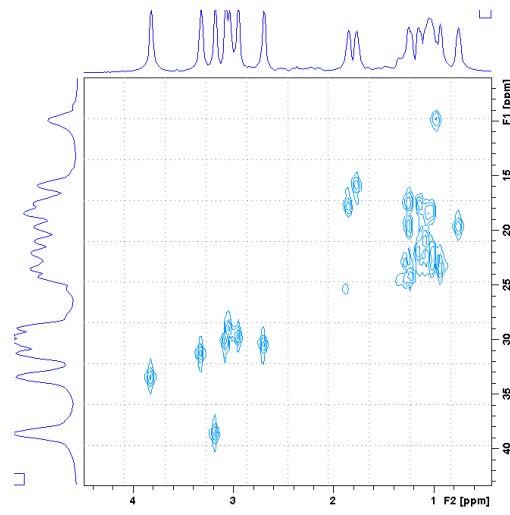
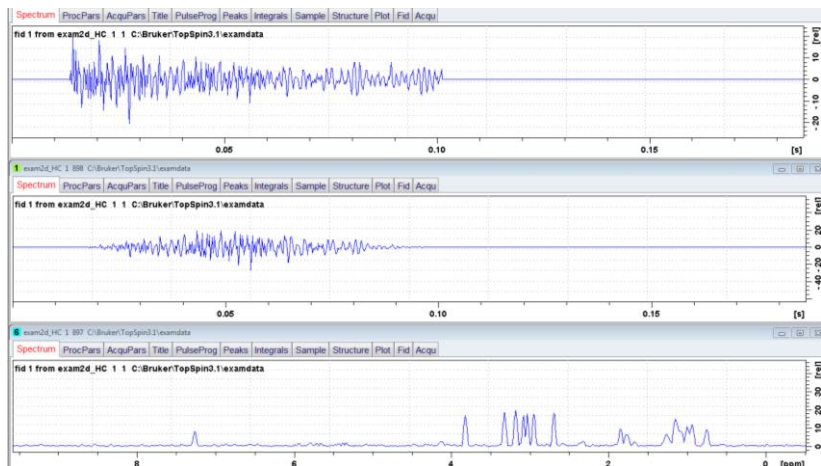
# Window function – HMQC



- HMQC with correct apodization



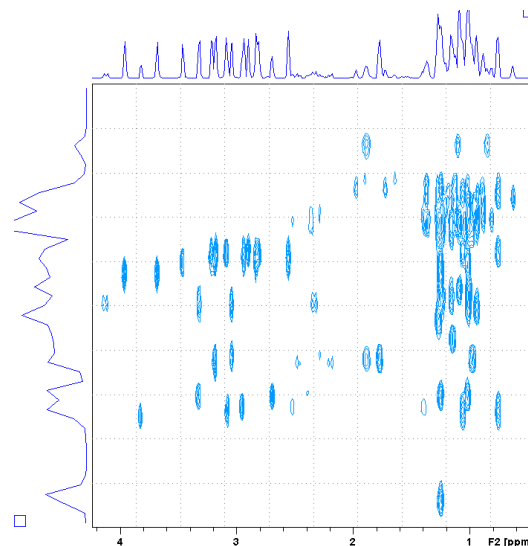
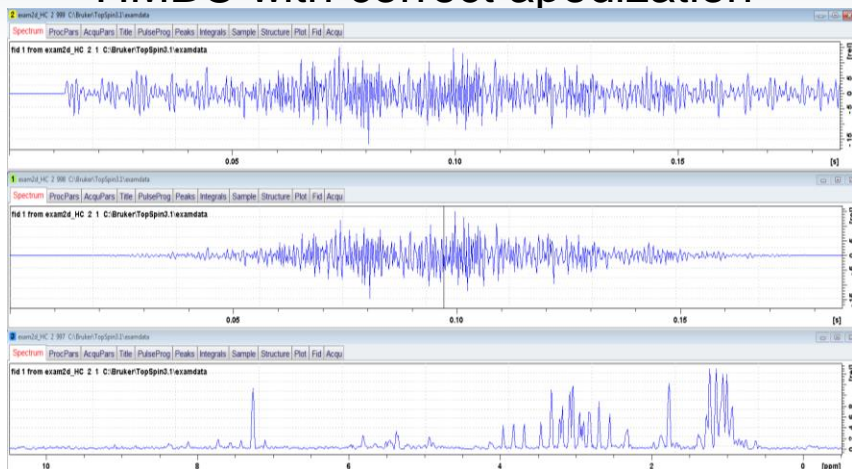
- HMQC with incorrect apodization



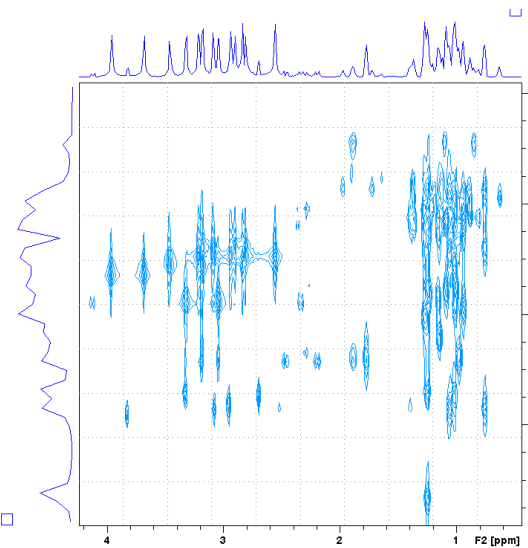
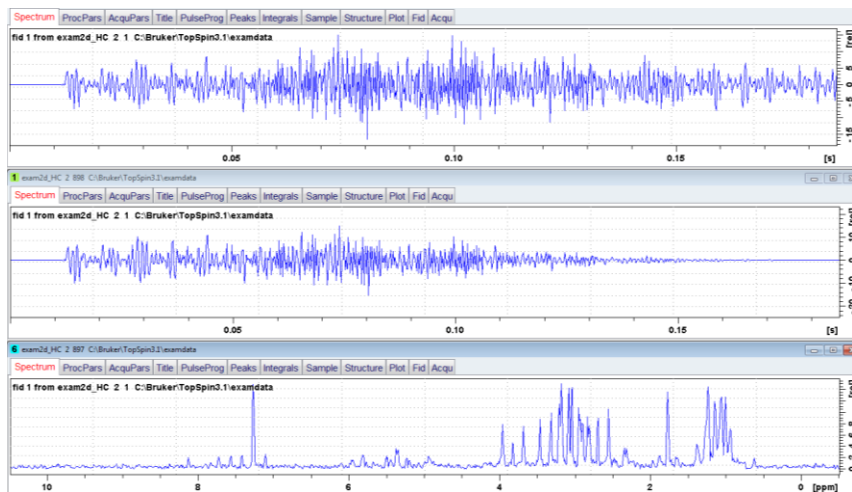
# Window function – HMBC



- HMBC with correct apodization



- HMBC with incorrect apodization

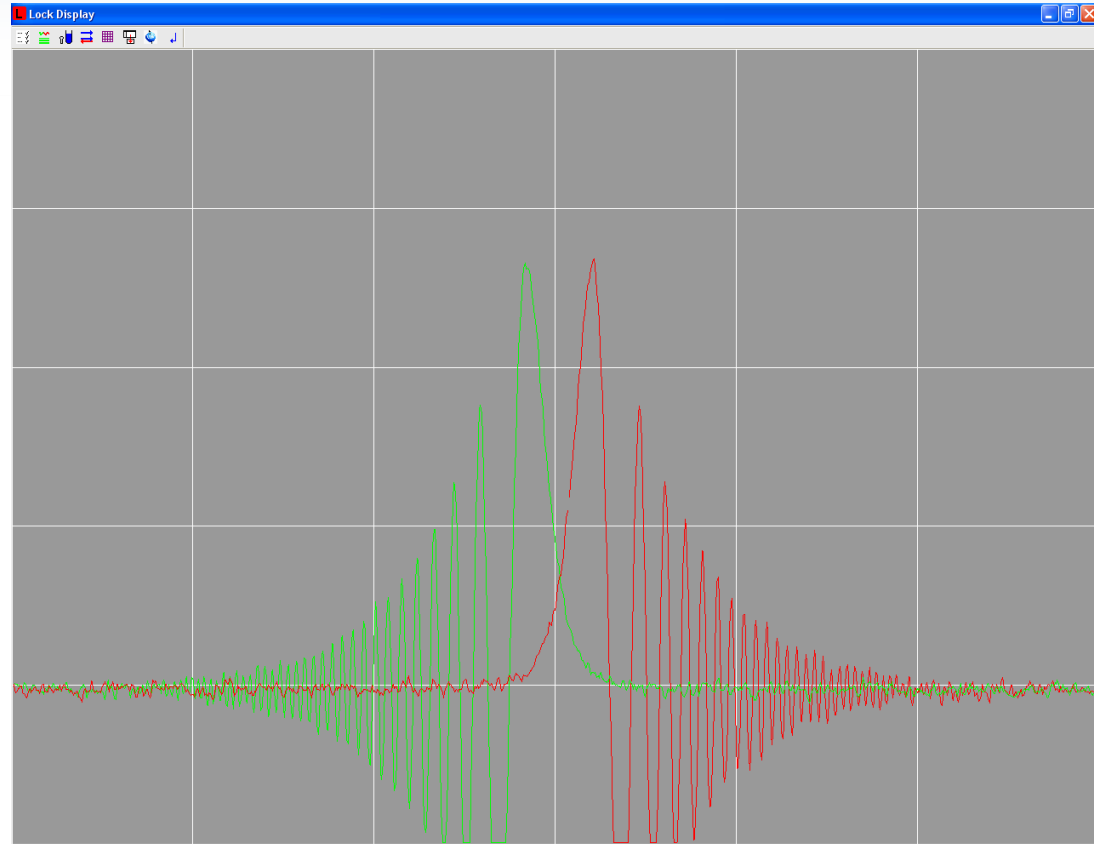


- **Lock phase**

# Lock phase



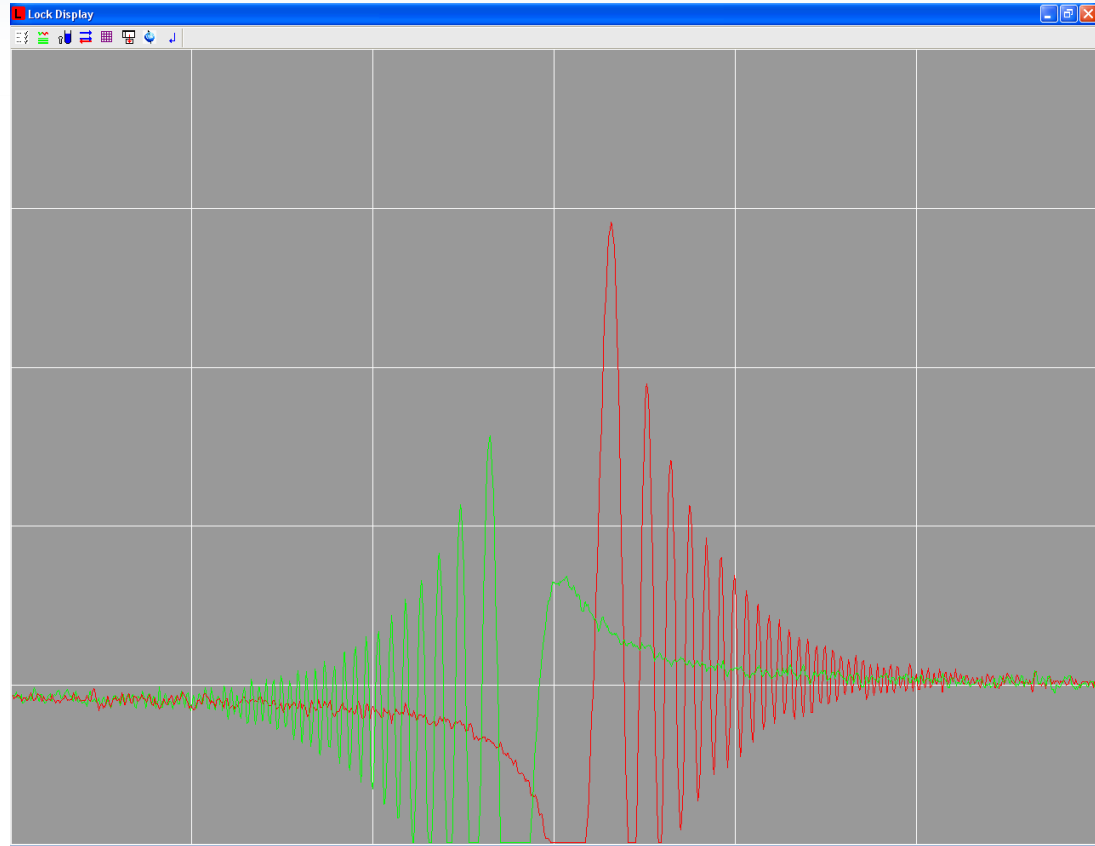
- That's what a lock should look like
- Lock phase set correctly



# Wrong lock phase



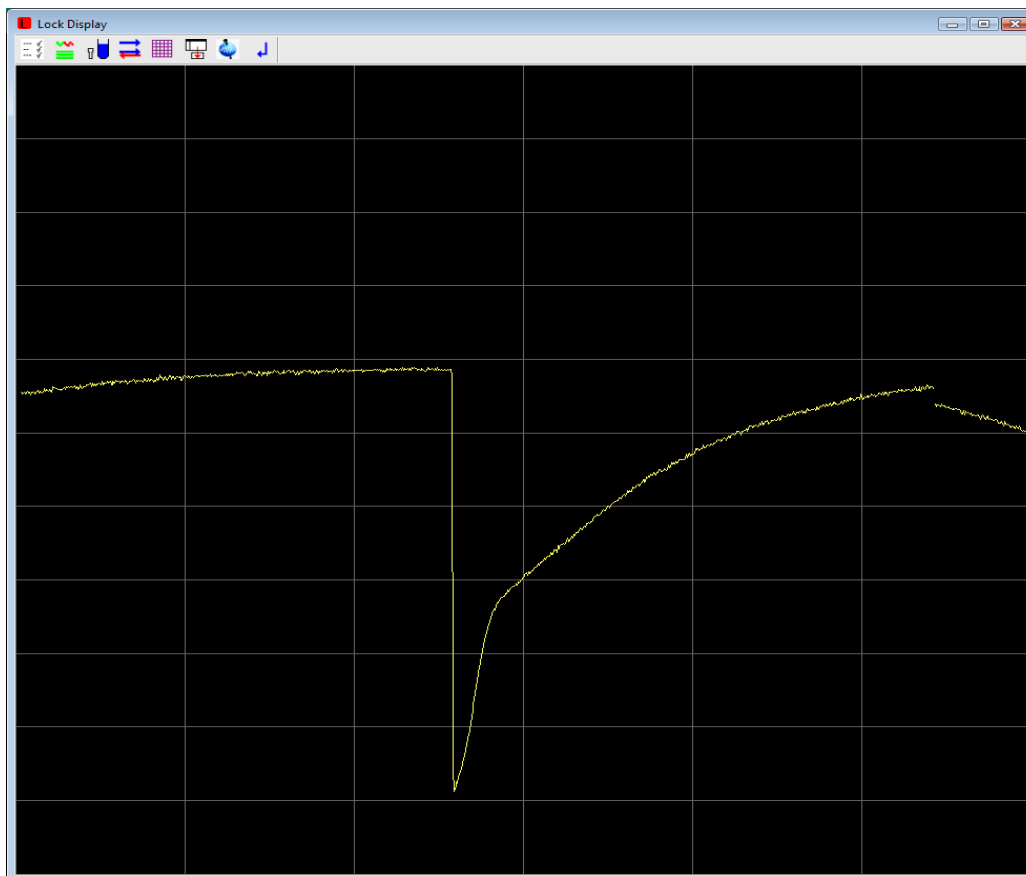
- Lock phase is off
- Locking might not be possible
- Artifacts can occur in spectra with gradients



# Wrong lock phase



- Lock phase off by  $30^\circ$
- Lock has a knee during gradient experiment

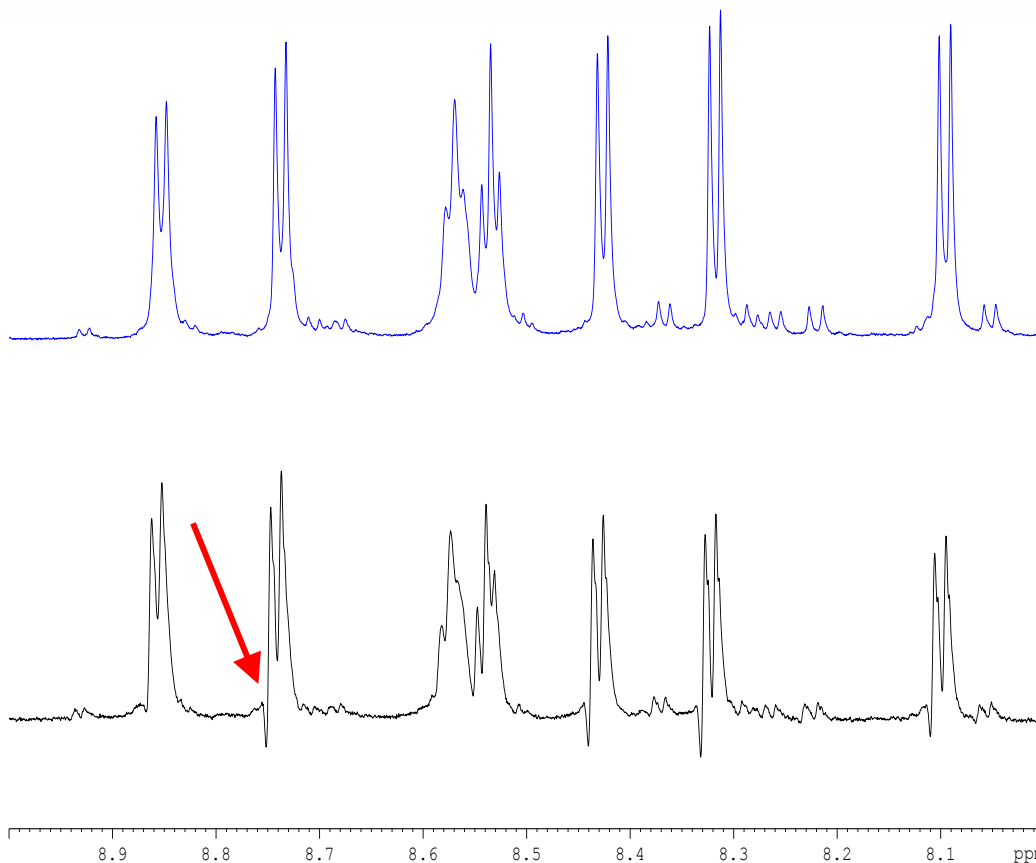




# Wrong lock phase



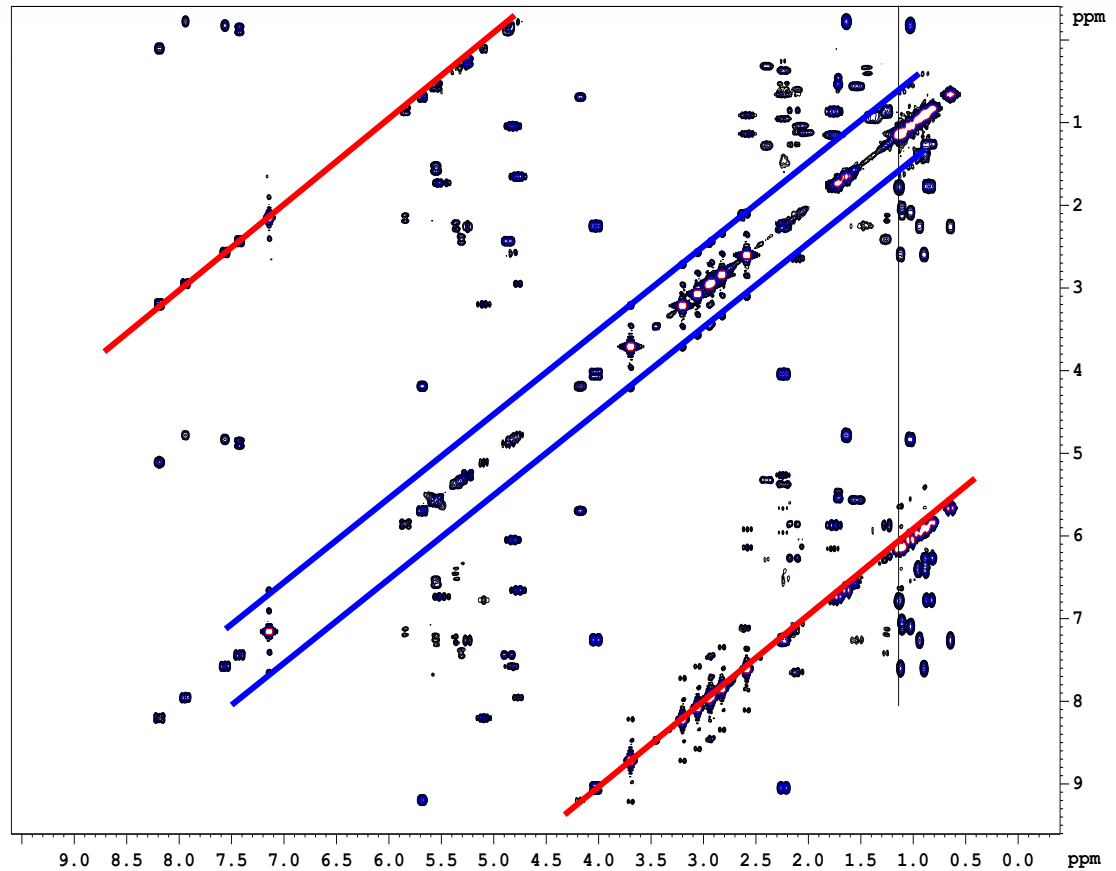
- WATERGATE
- Lock phase off by  $30^\circ$
- Artifacts can occur on the left or right side of the signals depending on offset (+/-)



# Gradient-COSY



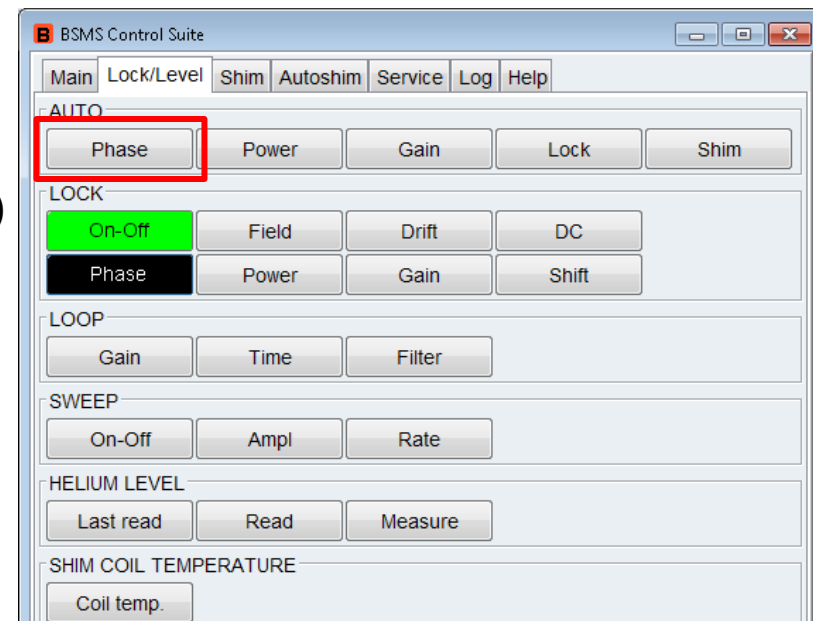
- COSYGPQF:
- Lockphase wrong by ca.  $40^\circ$



# Lock phase



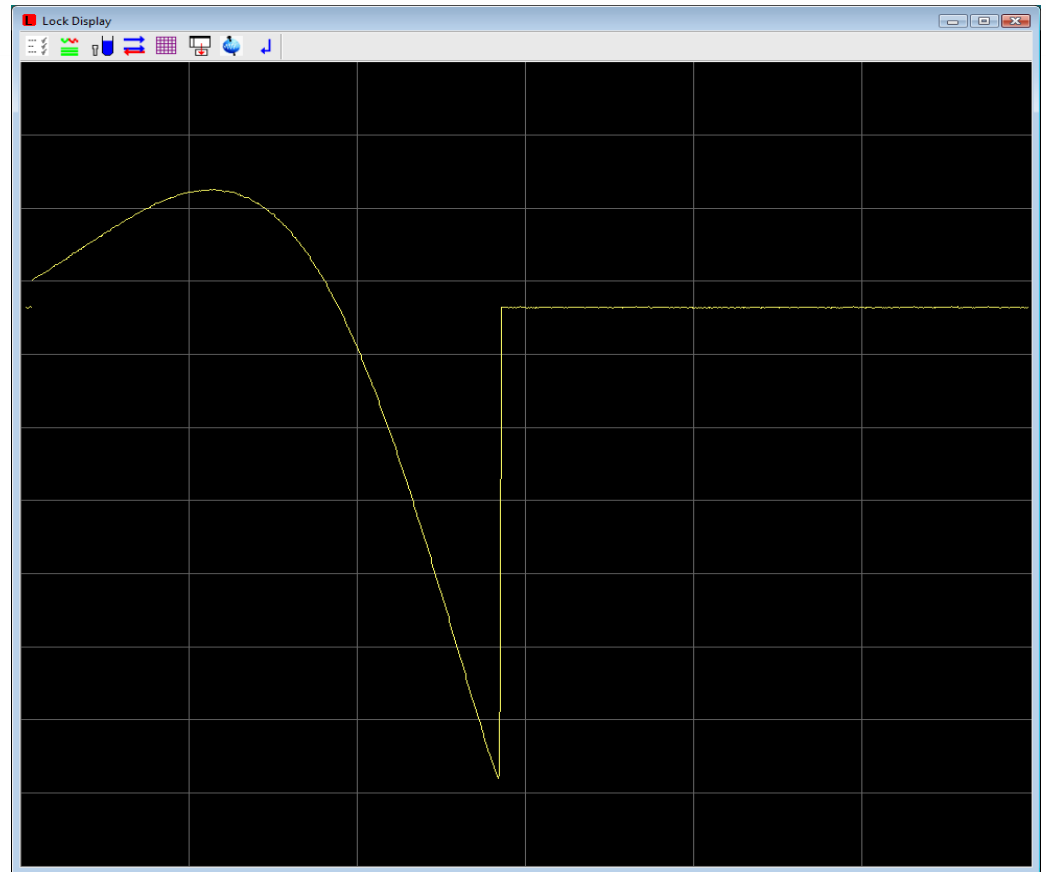
- Manual:
  - Always adjust on locked sample (shimmed & non-saturated)
  - Optimize lock level or check lineshape of sharp line (dip at signal base) in gs mode with a gradient sequence
- Software:
  - loopadj (AU)
  - Tune during Topshim ([**topshim tunea**])
  - Autophase on BSMS display



# Lock power



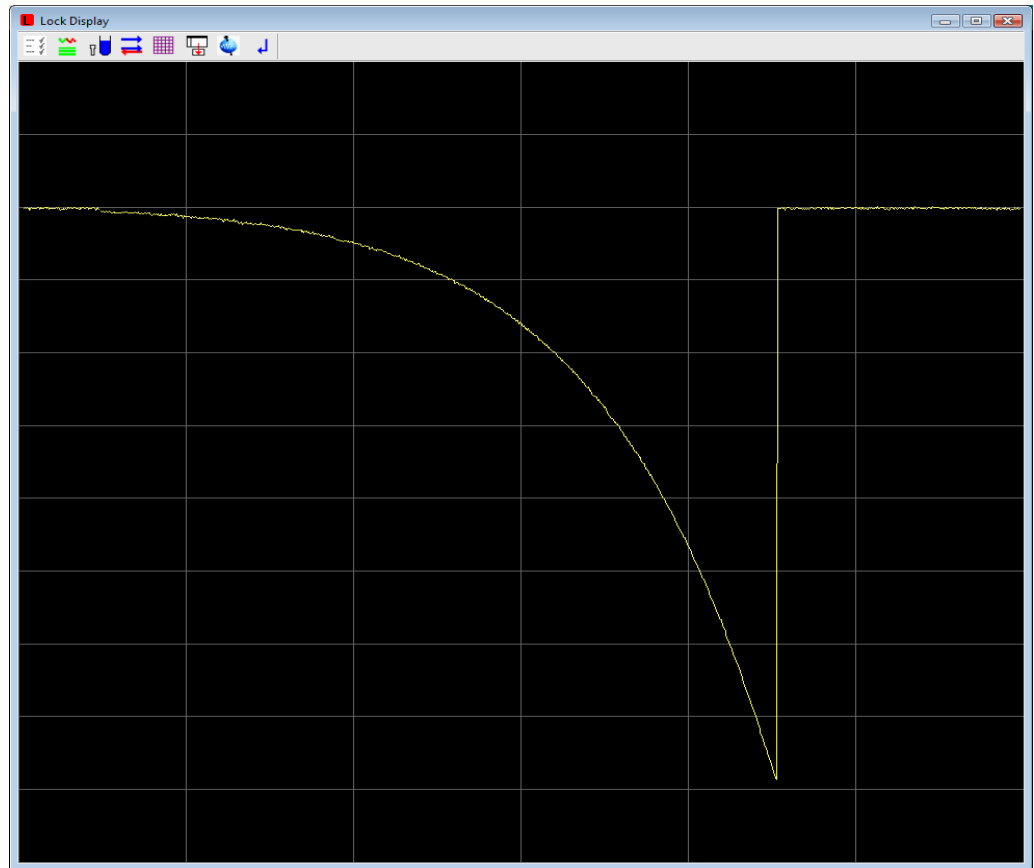
- «overshoot» after gradient
- Lock power too high



# Lock power



- Lock power set correctly



# Lock power



- «overshoot» after gradient
  - Lock power too high
1. Reduce lock power
  2. Press «STD BY»
  3. Reduce power
  4. Press «Reset»
  5. If «overshoot» is still visible restart with 1.

The screenshot shows the BSMS Control Suite software interface. The 'Lock/Level' tab is selected, and the 'Power' button is highlighted in black. The interface includes sections for AUTO, LOCK, LOOP, SWEEP, HELIUM LEVEL, and SHIM COIL TEMPERATURE. The 'Power [dBm]' section displays a table with 'Previous' and 'Actual' values, and a 'Step' control.

	Previous	Actual	Step
Absolute	-20.0	-20.0	+
Difference	0.4	0.0	-

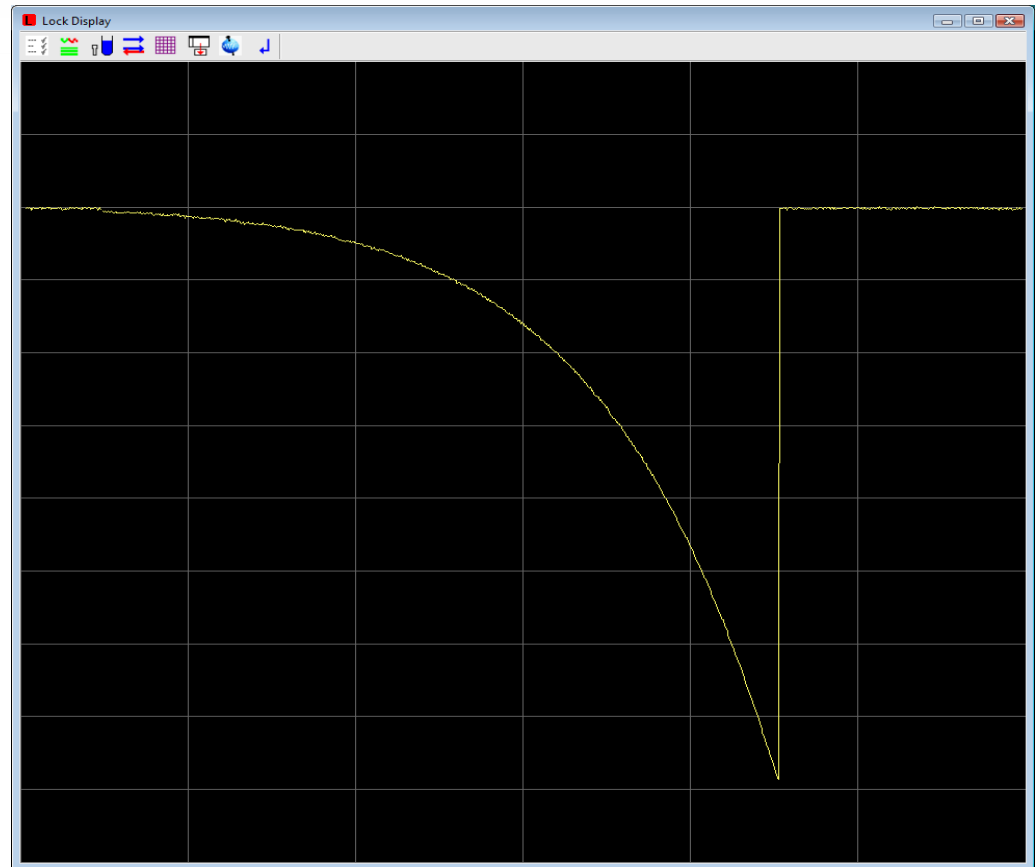
Stepsize: 0.1

Buttons: STD BY, Reset

# Lock power



- «overshoot» after gradient
  - Lock power too high
1. Reduce lock power
  2. Press «STD BY»
  3. Reduce power
  4. Press «Reset»
  5. If «overshoot» is still visible restart with 1.



Lock power optimized

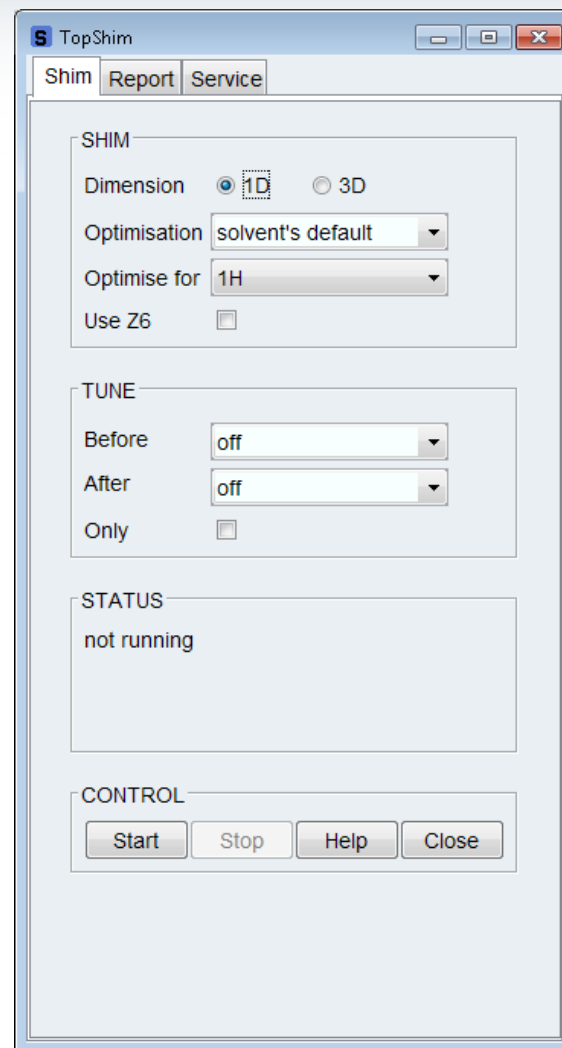
- **TopShim**



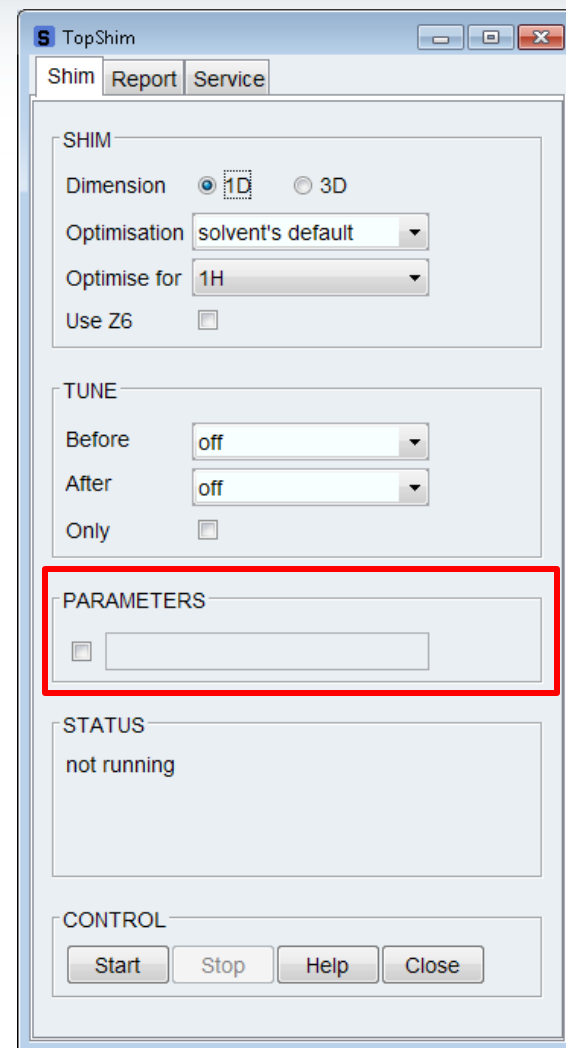
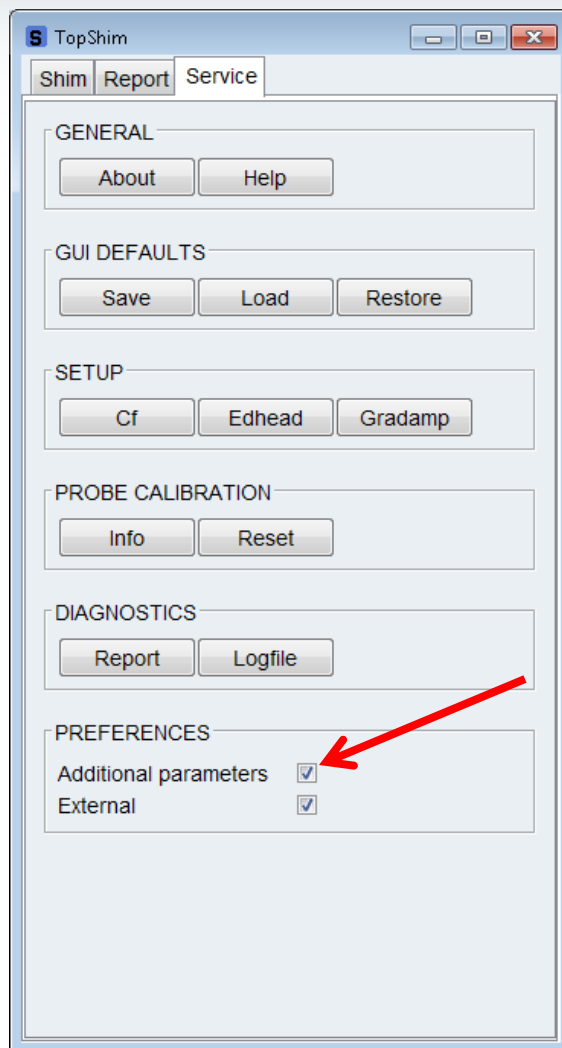
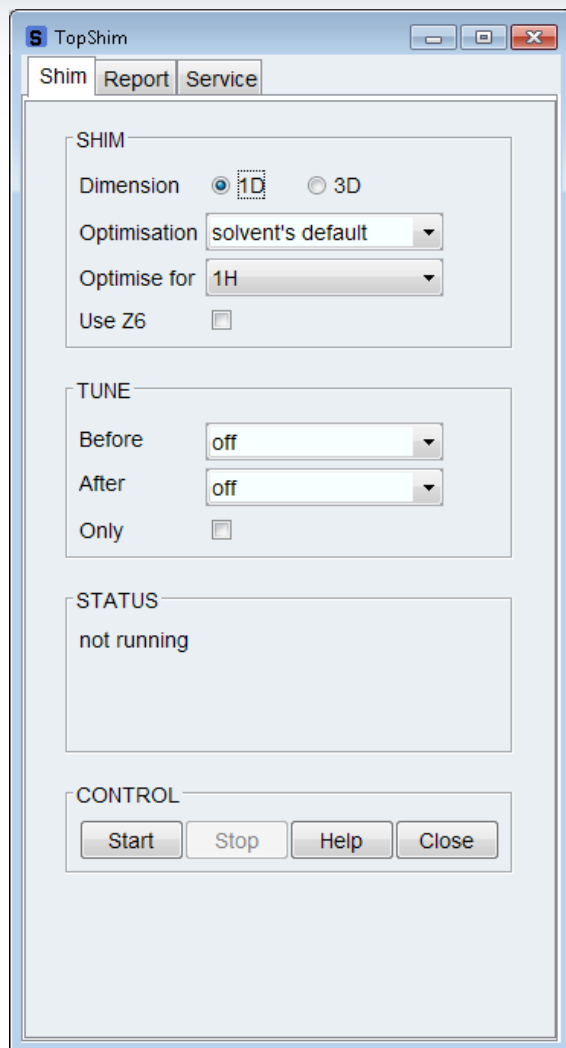
# TopShim GUI



- Can be opened with command  
[**topshim gui**]
- Allows several options



# TopShim GUI – Additional Parameters



- **ordmax=** Sets the maximum total order of shim functions (default = 5)  
ordmax=3 limits shimming to Z-Z3 (1mm and 1.7mm probes)  
[**topshim ordmax=8**] (SmartProbe)
- **1H or 2H** Explicitly sets shimming nucleus
- **lockoff** Enables shimming with system unlocked
- **o1p=** Explicitly sets excitation frequency in PPM  
[**topshim 1H lockoff o1p=2.49**] (DMSO-h<sub>6</sub>)
- **selwid=** Enables selective excitation of a bandwidth expressed in ppm  
Useful when shimming on a solvent with multiple signals  
[**topshim o1p=1.93 selwid=0.5**] (CD<sub>3</sub>CN+D<sub>2</sub>O)
- **durmax=** maximum duration per 1D field map acquisition (expressed in seconds)  
default = 7

- **rga** force receiver gain optimization before shimming  
[*topshim rga durmax=60*]
- **tune\*** also shim on the lock before and/or after gradient shimming  
(tuneb shims X,Y,Z,XZ,YZ before running gradient shimming)  
[*topshim tuneaz*] (shims Z after running gradient shimming)
- **shigemi** Used to eliminate unreliable data at axial Shigemi tube walls  
when 1D shimming
- **zlim** sets the range in cm in the Z direction used for shimming  
[*topshim shigemi zrange=-0.8,0.8*] ('short' Shigemi)
- **plot** Saves data after completion in <TopSpin\_home>/data/topshimData

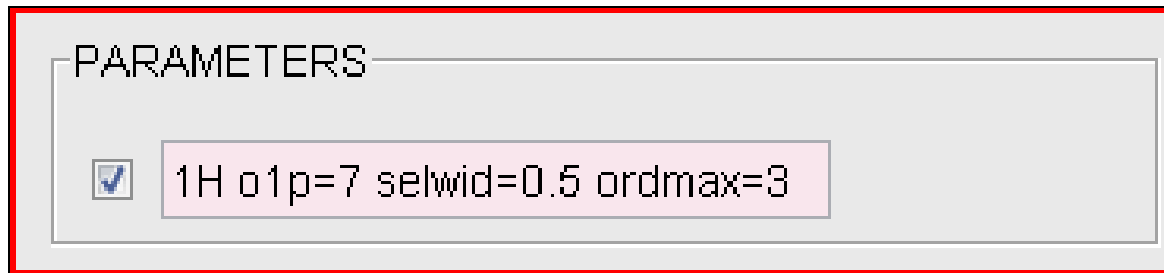
... read about more in the Topshim manual!

Type [*help topshim*] from the Topspin command line.

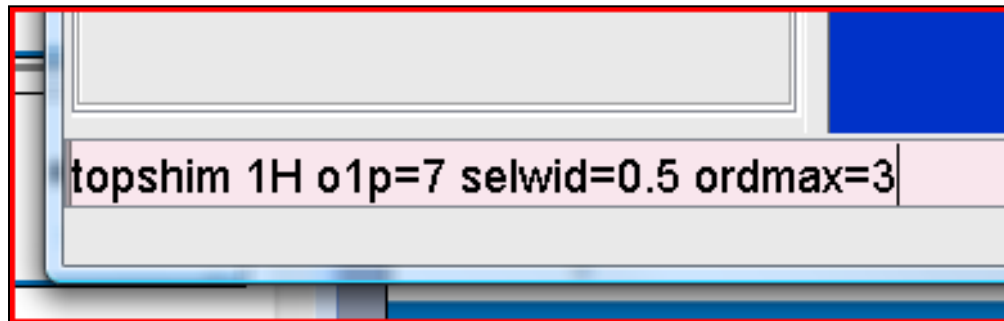
# Command Line Arguments



- Command line arguments
  - Multiple arguments can be used simultaneously



- Similarly, these arguments can be executed directly from the topspin command line.



- Can be used in macros as well

# Topshim Tips

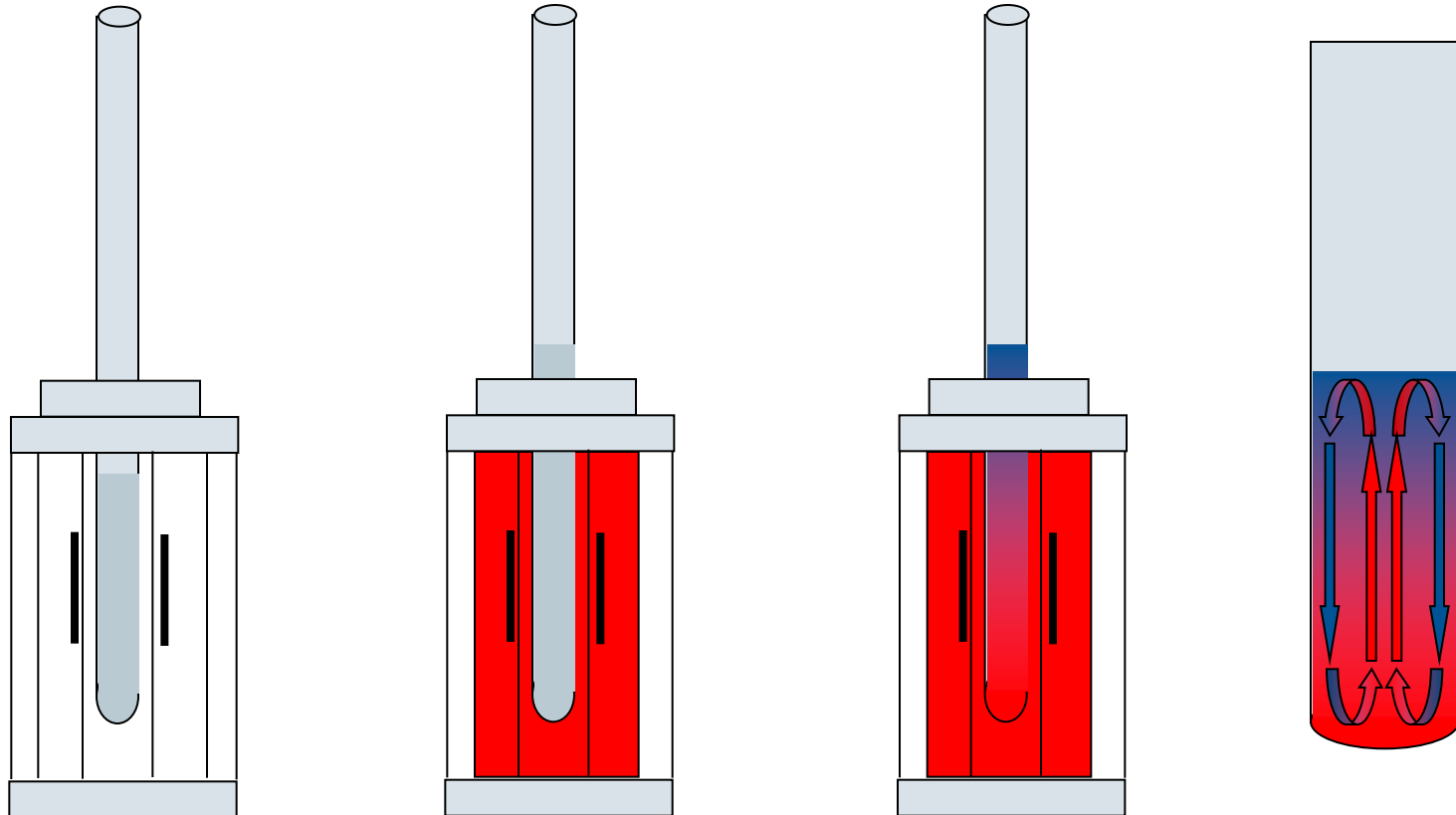


- Check  $^2\text{H}$  pulse length
- Poor initial field homogeneity
  - Shims can be so bad that topshim cannot get data good enough to use for the calculation.
  - Try running topshim with sample spinning.
  - Try [**topshim tuneb**] to improve initial homogeneity.
- S/N too low
  - Try running [**topshim durmax=30**], 60, or even 120
  - Argument rga can also be added, [**topshim rga durmax=30**]
- Convection in a sample may cause topshim to fail.
  - Low viscosity solvents like chloroform and acetone are particularly susceptible.
  - Can manifest itself as "too many points lost during fit".
  - Use [**topshim convcomp**] to run topshim with convection compensation. > TS 2.1pl5.

# Topshim Tips



- Convection and temperature gradients
- Raising temperature + filling height too high = problems



# Convection Compensation



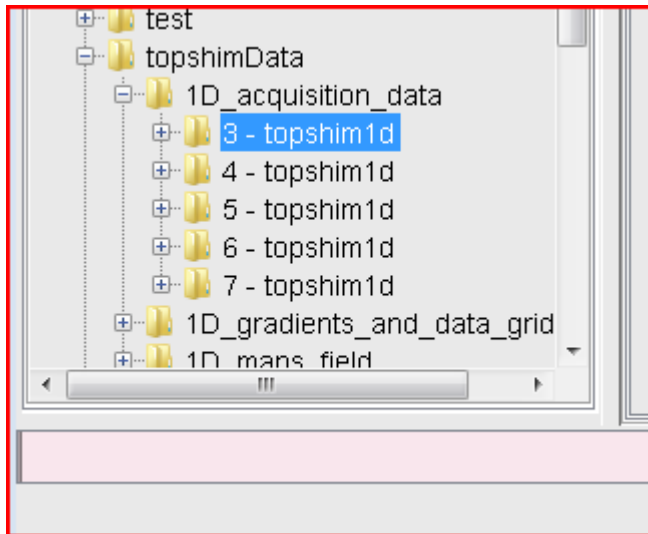
- In order to eliminate / reduce convection:
  - Sample filling height  $\leq$  40 mm
  - Increase VT gas flow (if possible)
  - Spin sample (if possible)
  - Lower sample temperature (if possible)
  - Use solvent with higher viscosity (if possible)
  - Use shigemi or smaller sample diameter



# Topshim Tips



- Troubleshooting Tips
  - Topshim deletes data immediately after execution. The command [***topshim plot***] can be used to save topshim data.

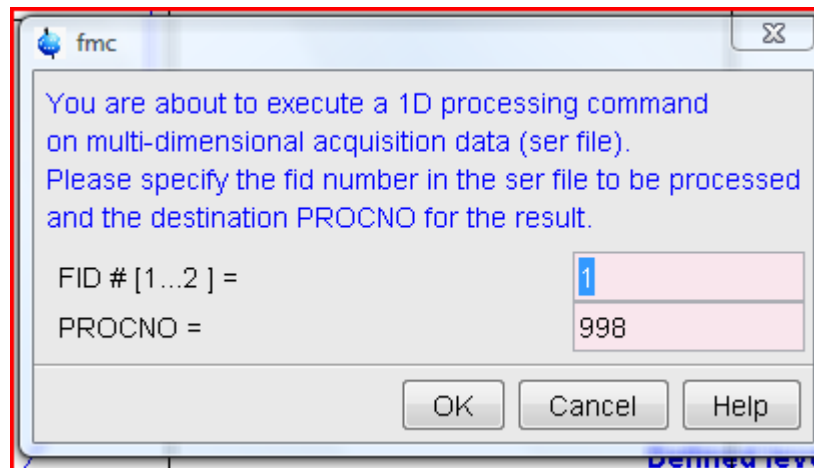


- Data located in **<topspin\_home>**\data\topshimData

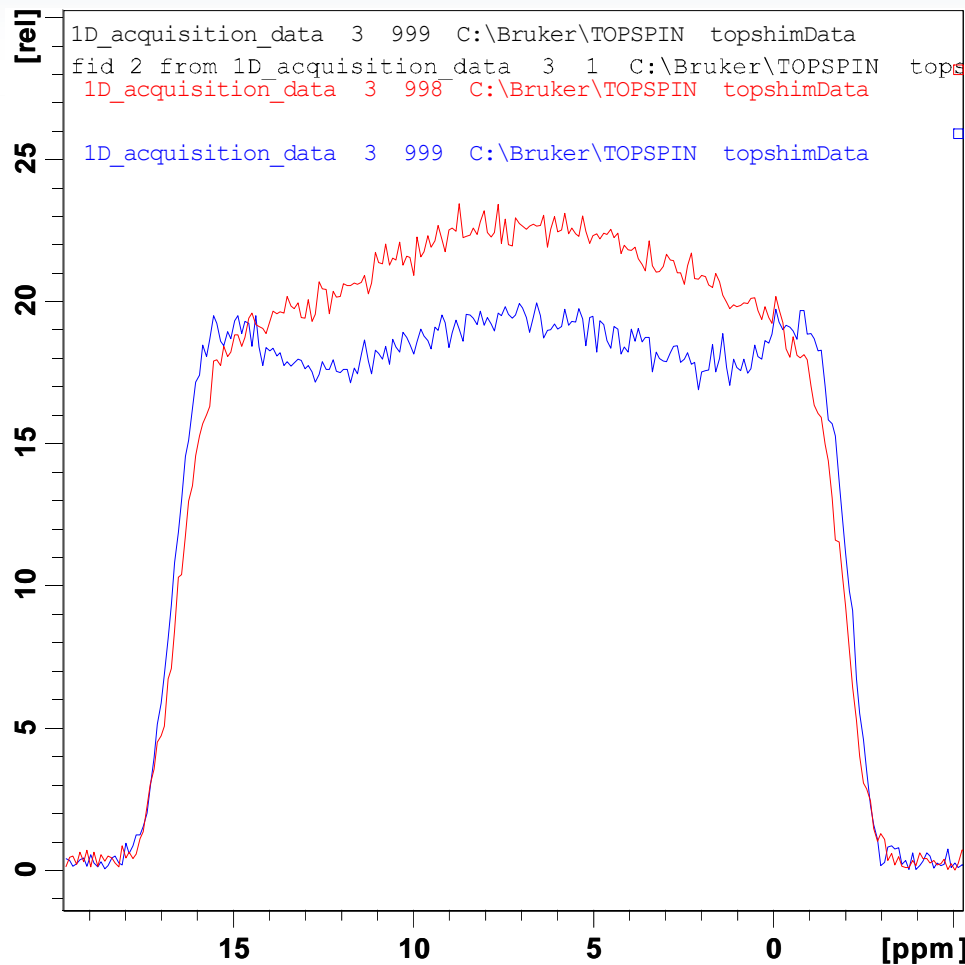
# Topshim Tips



- Troubleshooting Tips
  - Data is stored in serial file. Long echo time in FID 1 and short echo time in FID 2. Process with [**fmc**]



# Topshim Tips



- Profiles should have similar intensity. If long echo time spectrum is  $<0.5$  then there may be homogeneity issues.
- For homogeneity issues try
  - [***topshim tuneb***]
  - [***topshim 3d***]
- For 2H, check pulse length
- Type [***help topshim***] in Topspin command line to open topshim manual

- **.info files**

# How to choose a pulse program



I want to measure an HSQC!

hsqc2htrfpgpphtcwg3d

! hsqc\*]

hsqct1etf3gpsi3d

158 pulse p

ns

hsqcetf3gpro

hsqcdietgpsisp3d.2

hsqcdietgpsisp3d.2

hsqcetgp

hsqcctetgpsisp

hsqcdietgpsisp

hsqcetgpipjcsp

hsqcphpr

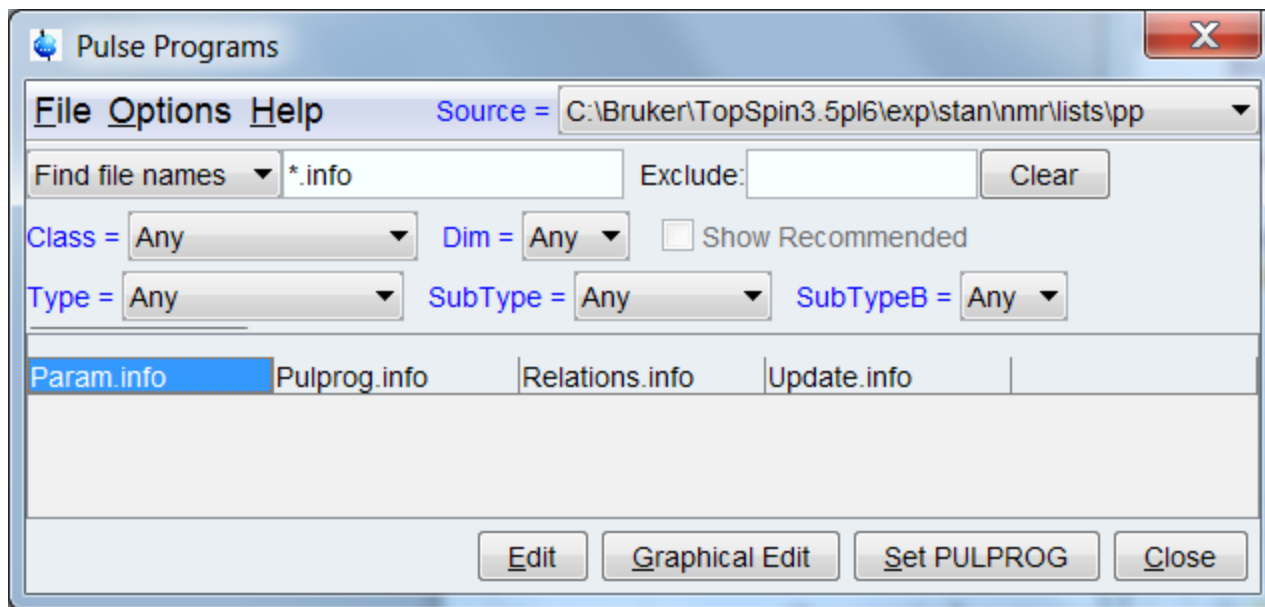
# .info files



- .info files can be found in the pp folder  
<topspinhome>\exp\stan\nmr\lists\pp

- [**edpul \*.info**]

- Available file:
  - Param.info
  - Pulprog.info
  - Relations.info
  - Update.info



# Param.info and Relations.info



```
Param.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
1 ;Param.info
2 ;avance-version (16/07/28)
3 ;
4 ;The following convention is used
5 ;and loop counters throughout the
6 ;
7 ;$CLASS=HighRes Info
8 ;$COMMENT=
9
10
11 ;p10 :
12 ;p11 : f1 channel - power level fo
13 ;p12 : f2 channel - power level fo
14 ;p13 : f3 channel - power level fo
15 ;p14 : f4 channel - power level fo
16 ;p15 : f5 channel - power level fo
17 ;p16 : f6 channel - power level fo
18 ;p17 : f7 channel - power level fo
19 ;p18 : f8 channel - power level fo
20 ;p19 : f1 channel - power level fo
21 ;p110: f1 channel - power level fo
22 ;p111: f1 channel - power level fo
23 ;p112: f2 channel - power level fo
24 ;p113: f2 channel - power level fo
25 ; or f2 channel - power level fo
26 ;p114: f2 channel - power level fo
```

```
Relations.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
1 ;Relations.info
2 ;avance-version (16/07/28)
3 ;
4 ;$CLASS=HighRes Info
5 ;$COMMENT=
6
7
8 ;The following convention is used for power levels, pulses, delays
9 ;and loop counters in the different relation files for prosol:
10 ;
11 ;all = default + lcnmr + triple + triple2 + triple_c + triple_na
12 ;triple* = triple + triple2 + triple_c + triple_na
13 ;!__ = except
14 ;
15 ;prosol par.      rel. file      pulseprogram parameter
16 ;
17 ;DE              all              de
18 ;D_grad          all              d16: delay for homospoil/gradient recovery
19 ;
20 ;PW90 (F1)       all              p0 :
21 ;PW90 (F1)       all              p1 : f1 channel - 90 degree high power pulse
22 ;PW90 (F1)       all(!triple_c)    p27: f1 channel - 90 degree pulse at p18 (3-9-19 watergate)
23 ;PW90*2 (F1)     all              p2 : f1 channel - 180 degree high power pulse
24 ;PW90 (F2)       all              p3 : f2 channel - 90 degree high power pulse
25 ;PW90*2 (F2)     all              p4 : f2 channel - 180 degree high power pulse
26 ;PW90 (F2)       all              p21: f2 channel - 90 degree high power pulse
```

# Update.info



```
Update.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
1 ;Update.info
2 ;avance-version (16/08/01)
3 ;
4 ;difference to previous versions
5 ;
6 ;$CLASS=HighRes Info
7 ;$COMMENT=
8
9 *****
10 differences to 16/07/26
11
12 added          dr_tocsy_hetcor          dual receive
13
14 *****
15 differences to 16/06/03
16
17 added          dr_hc_fc_hmbc           dual receive
18                dr_hc_fc_hmqc
19                dr_hc_fc_hsqc
20                dr_pansy_cosy
21
22 *****
23 differences to 16/04/06
24
25 corrected      trosytf3gpsi.2         timing (else case)
26
```



# Pulprog.info



```
Pulprog.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
1 |;Pulprog.info
2 |;avance-version (13/08/21)
3 |;
4 |;$CLASS=HighRes Info
5 |;$COMMENT=
6 |
7 |
8 |;For a pulseprogram the first chara
9 |;sometimes more) specify the type o
10 |;NOESY etc.. Further properties of
11 |;indicated by a two-character code,
12 |;in alphabetical order. For 2D exper
13 |;phase sensitive or echo-antischo)
14 |;decoupling is assumed to be defaul
15 |;but not for homonuclear ones (exce
16 |;In case of redundant information s
17 |;ommitted.
18 |;
19 |;The two-character codes used are t
20 |
21 |
22 |ac accordion type experiment
23 |ad using adiabatic spinlock
24 |ar experiment for aromatic resi
25 |at adiabatic TOCSY
26 |bi with bird pulse for homonuc
```

```
Pulprog.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit Set PULPROG
23 |ad using adiabatic spinlock
24 |ar experiment for aromatic residues
25 |at adiabatic TOCSY
26 |bi with bird pulse for homonuclear J-decoupling
27 |bp using bipolar gradients
28 |cc cross correlation experiment
29 |cn C13 and N15 dependent information in different indirect dimensions
30 |co with COSY transfer
31 |cp with composite pulse
32 |ct constant time
33 |cv convection compensated
34 |cw decoupling using cw command
35 |cx using CLEANEX_PM
36 |dc decoupling using cpd command
37 |df double quantum filter
38 |di with DIPSI mixing sequence
39 |dh homonuclear decoupling in indirect dimension
40 |dw decoupling using cpd command only during wet sequence
41 |dq double quantum coherence
42 |ea phase sensitive using Echo/Antiecho method
43 |ec with E.COSY transfer
44 |ed with multiplicity editing
45 |es excitation sculpting
46 |et phase sensitive using Echo/Antiecho-TPPI method
47 |fb using f2 - and f3 - channel
48 |fd using f1 - and f3 - channel (for presaturation)
1 : 1
```

phase sensitive using echo/antiecho-method

basic experiment name

sensitivity improved

hsqc ed et gp si sp

with multiplicity editing

using a shaped pulse

using gradients

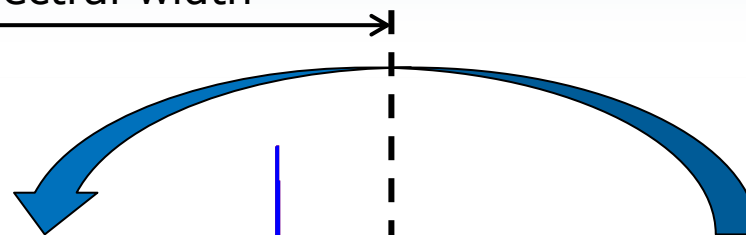
- **Folding**

# Folding



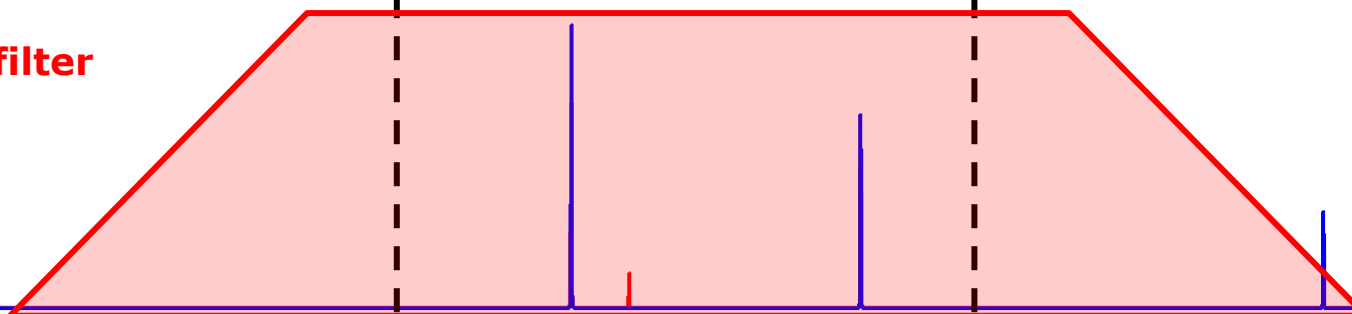
spectral width

no filter

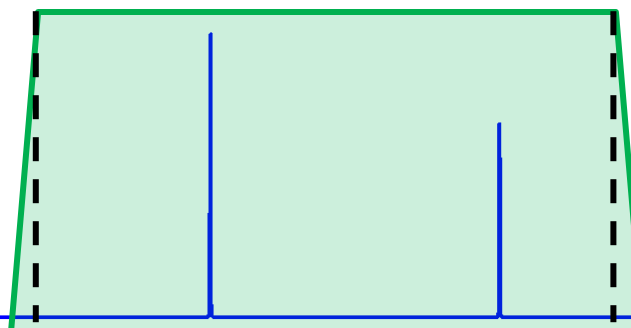


folded signal

analog filter



digital filter



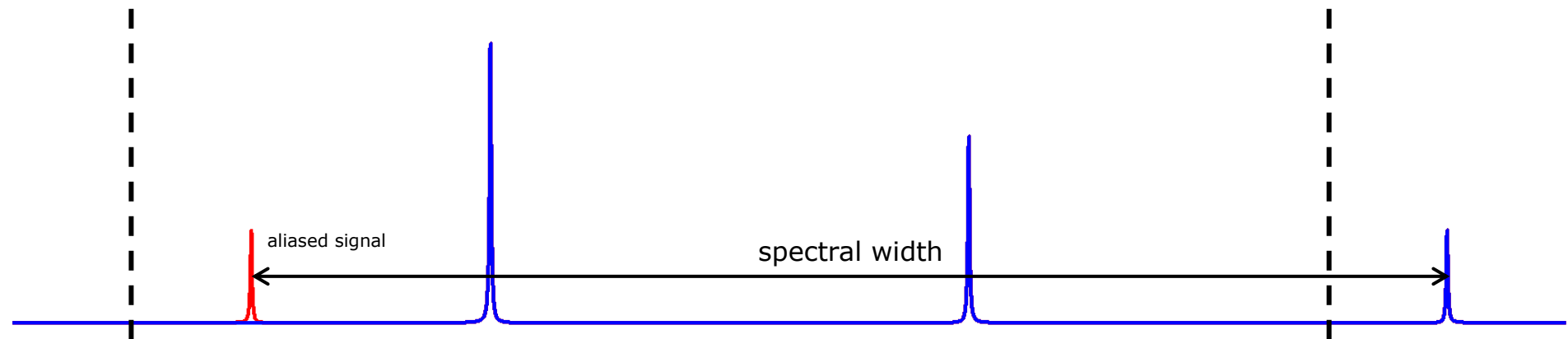
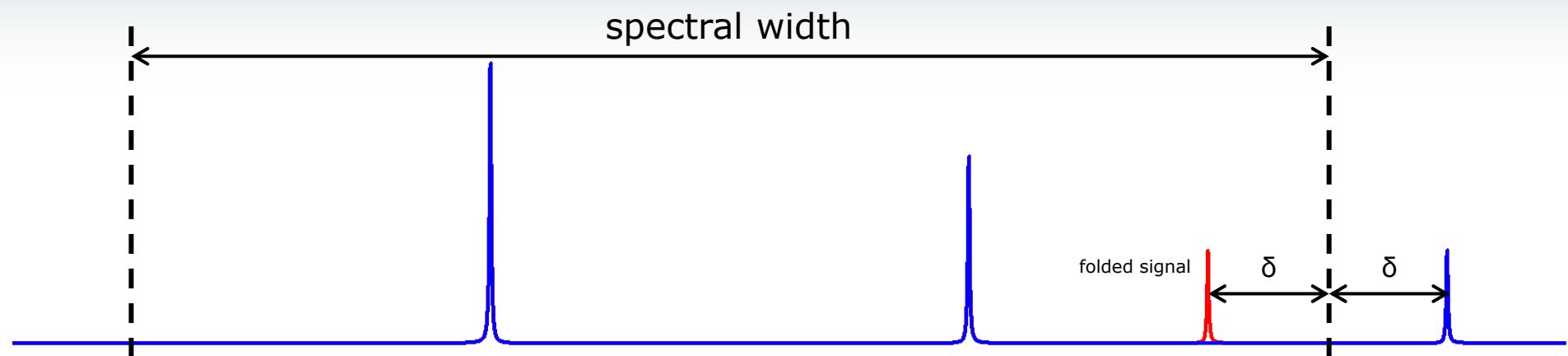
Folding is not a problem for 1D spectra in modern spectrometers due to very efficient filters.

BUT

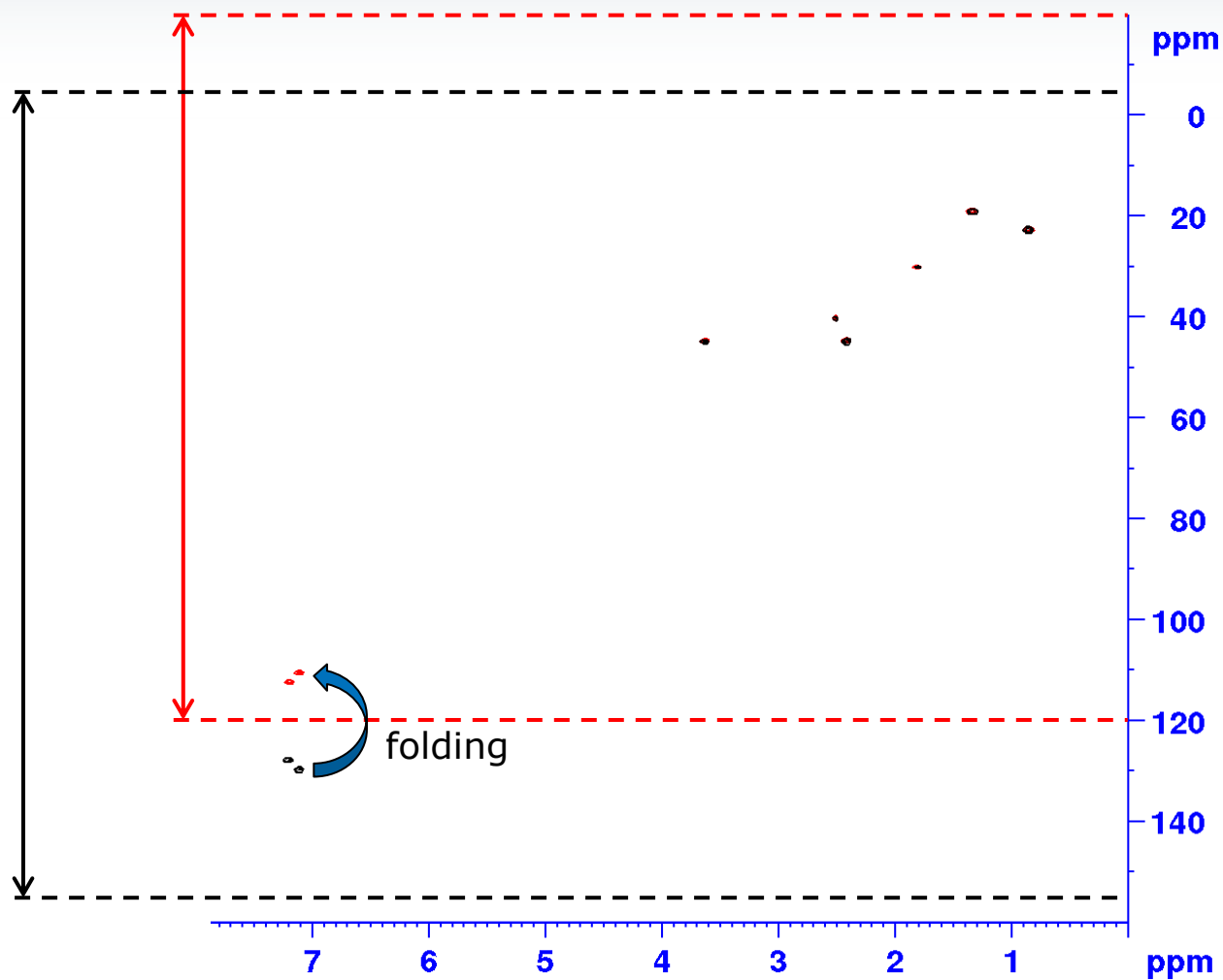
There are no filters in the second dimension!

Folding will occur in 2D spectra if experimental parameters are not set well!

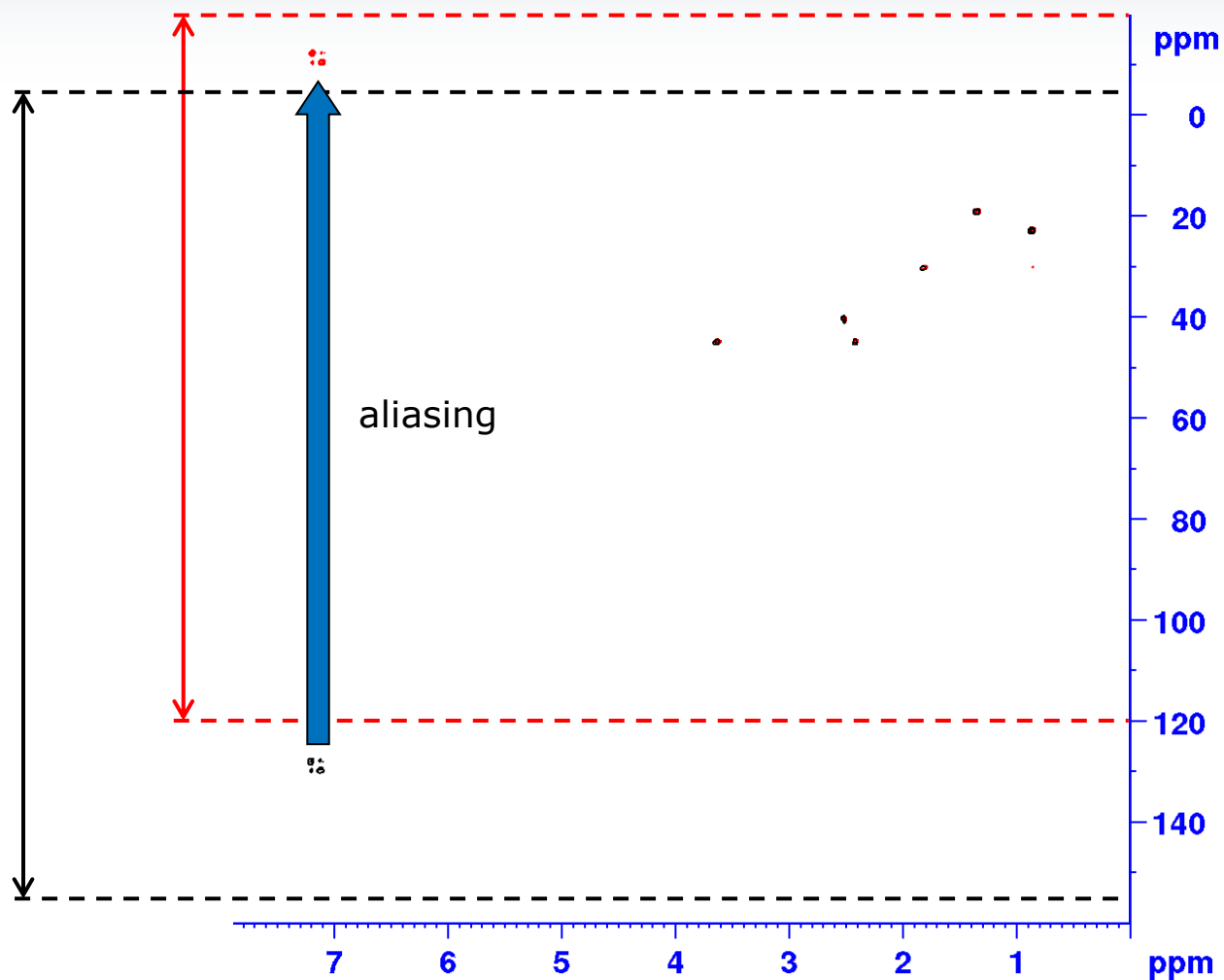
# Folding and aliasing



# Folding and aliasing



# Folding and aliasing





# How to find out if a peak is folded or aliased?

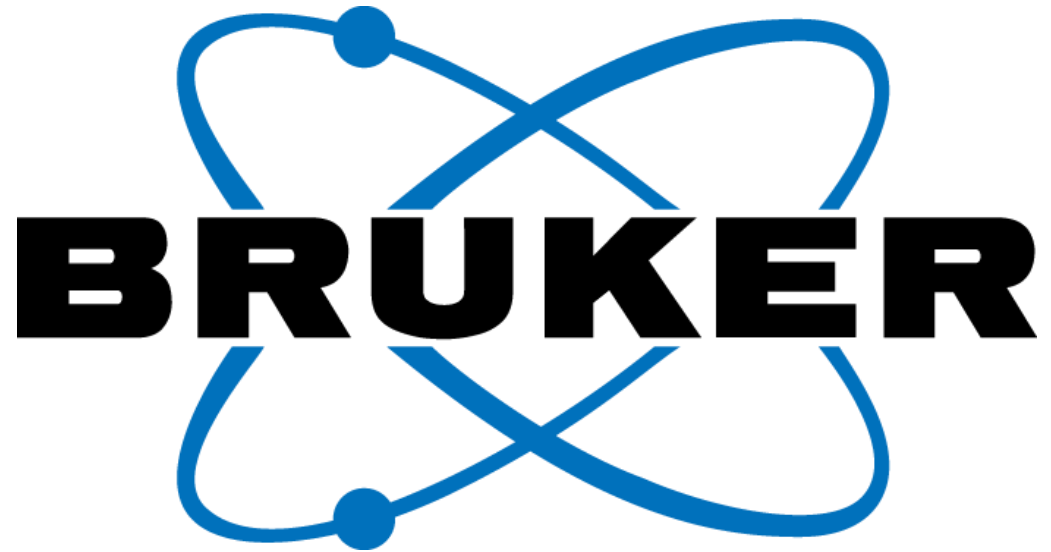


**folded**

**aliased**

**O1  
changed  
SW  
kept**

**SW  
changed  
O1  
kept**



[www.bruker.com](http://www.bruker.com)