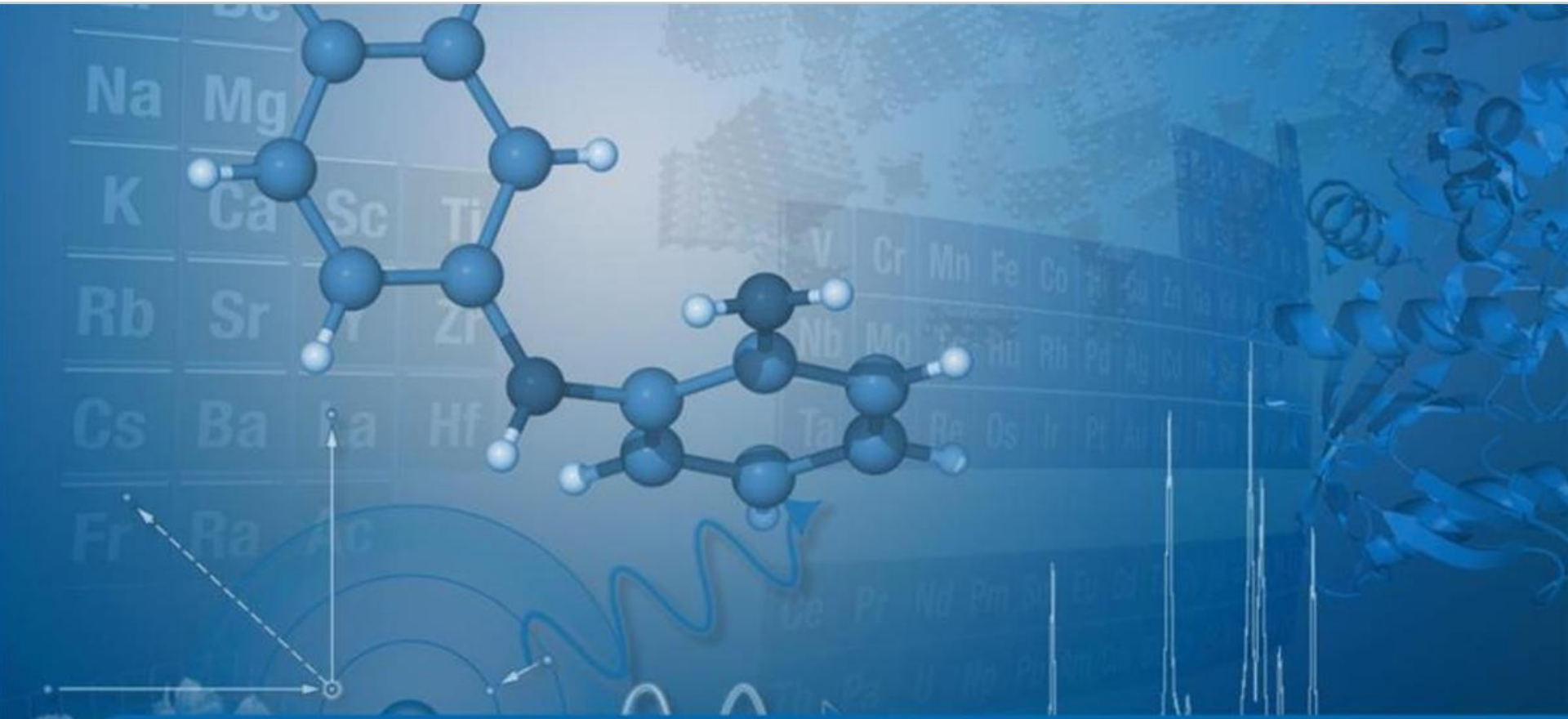
Troubleshooting

Dr. Benjamin Görling



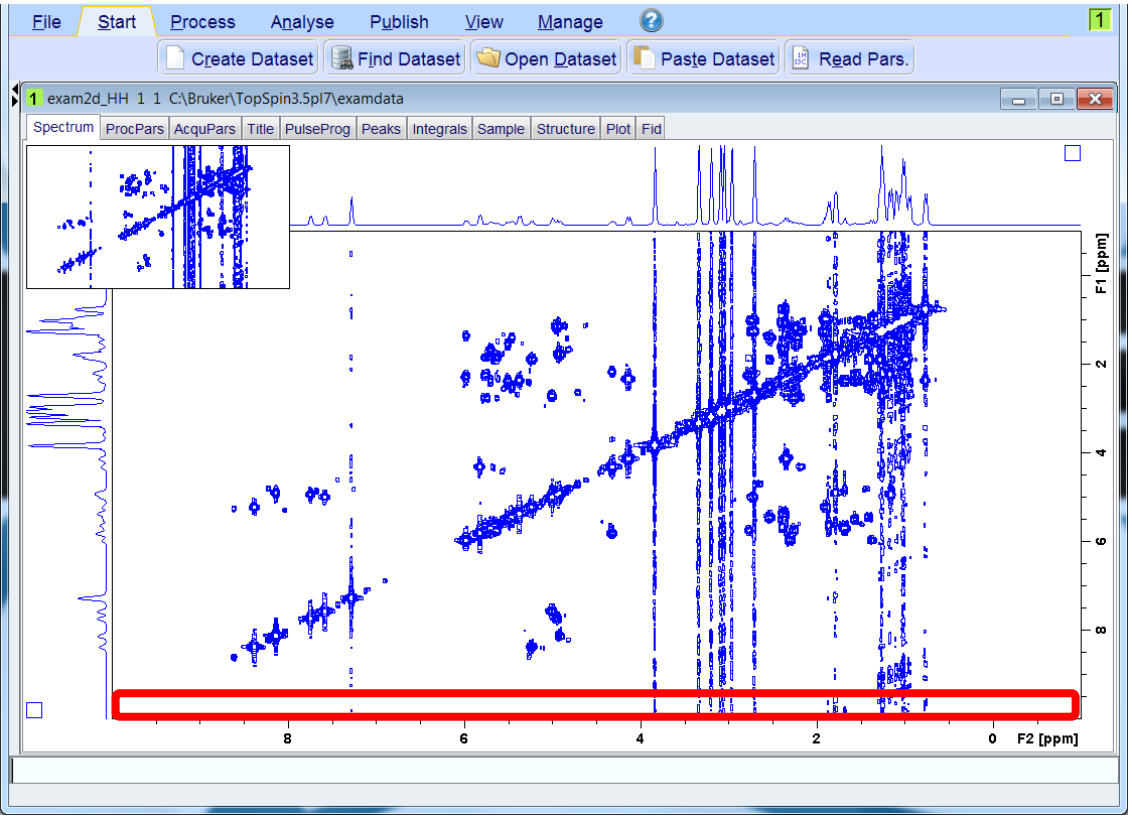
- **T1 noise**

T1 Noise Subtraction

- Many times 2D spectra reveal noise streaks either as a result of sample spinning modulations or modulations from very strong signals

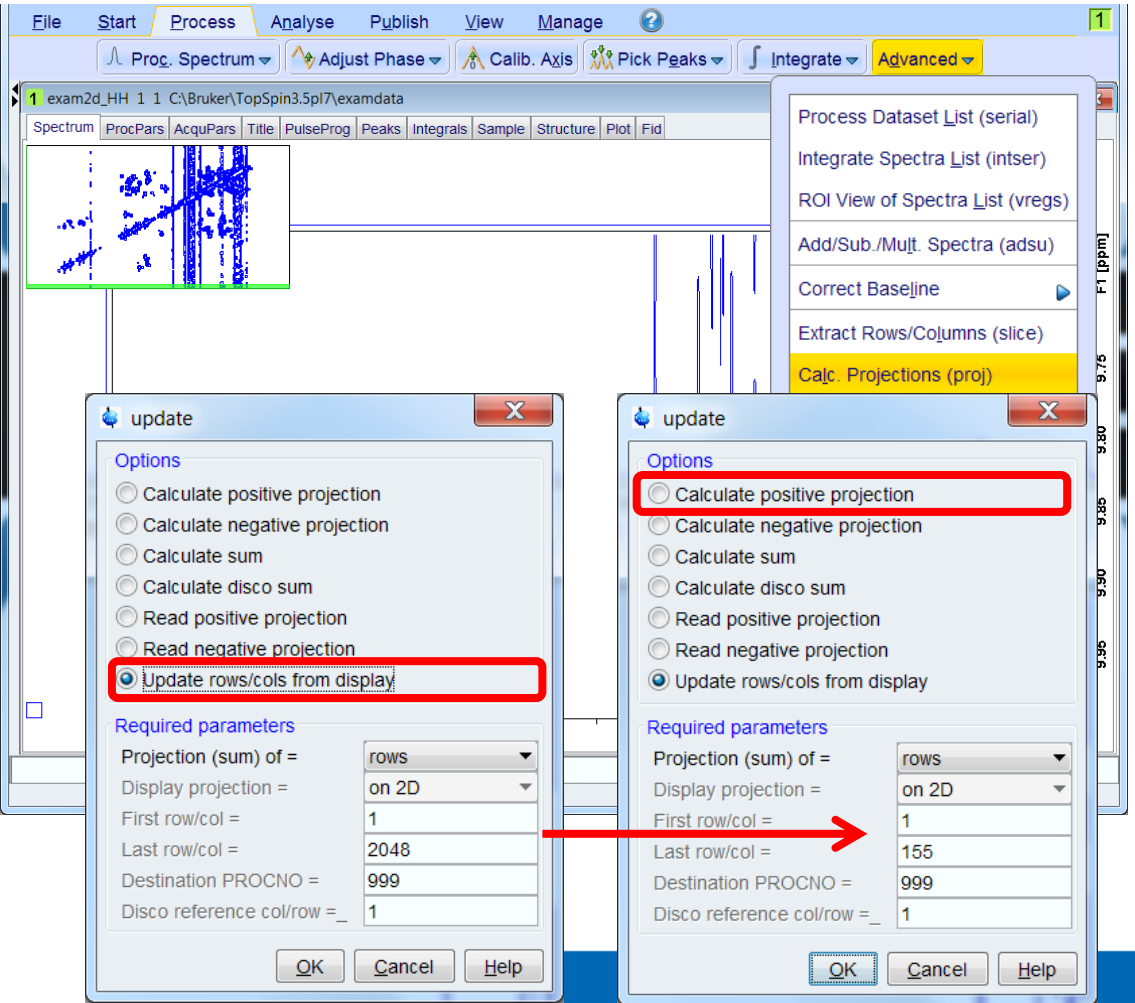
- These are consistent in S/N horizontally and can be subtracted, leaving only true signal behind.

T1 Noise Subtraction



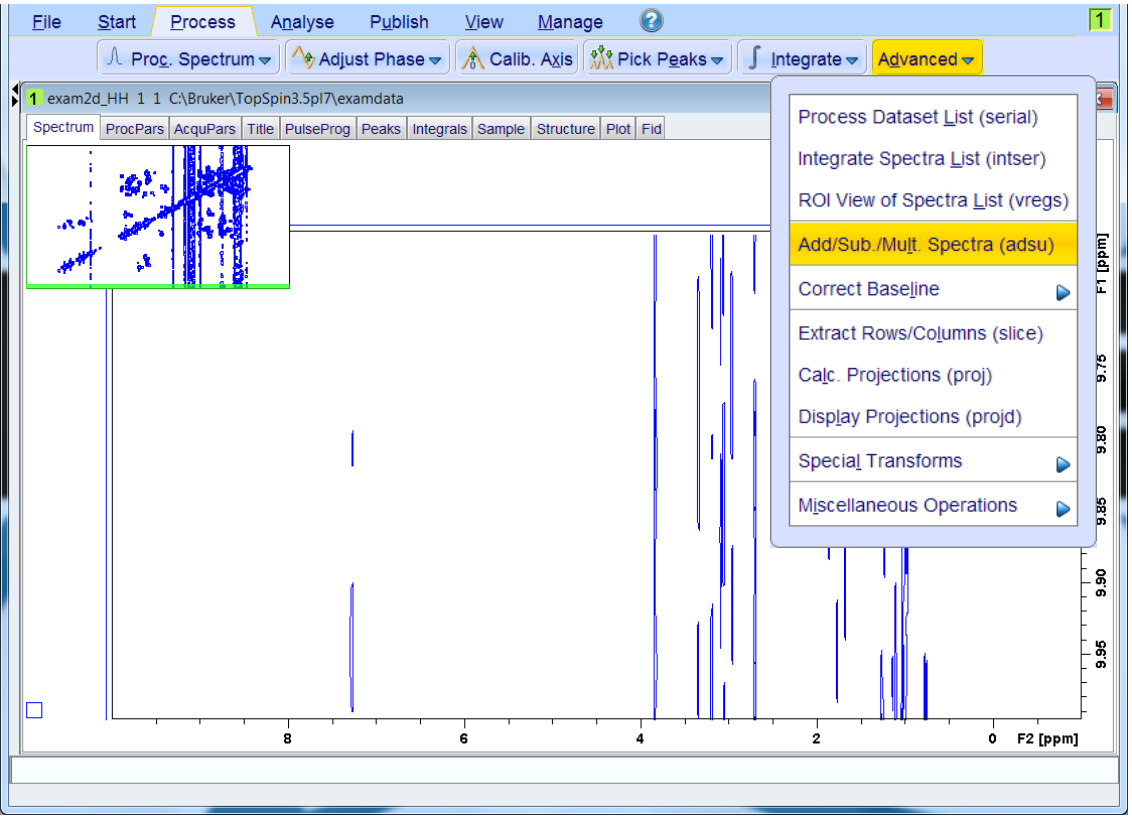
1. Expand full vertical region of spectrum that contains only noise, no signals.

T1 Noise Subtraction



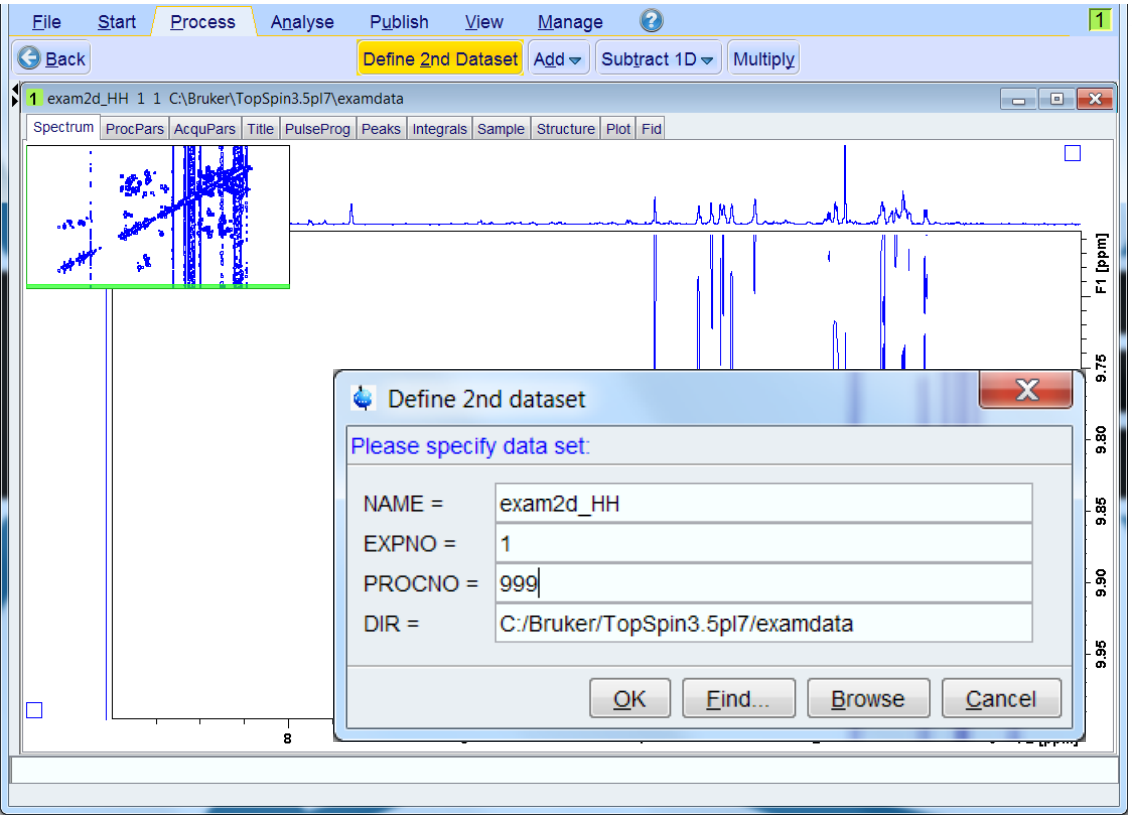
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
   a. Select Update rows/ columns from display
   b. Click ok
   c. Then Calculate Sum – make note of Destination PROCNO.
   d. This is the 2nd dataset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

T1 Noise Subtraction



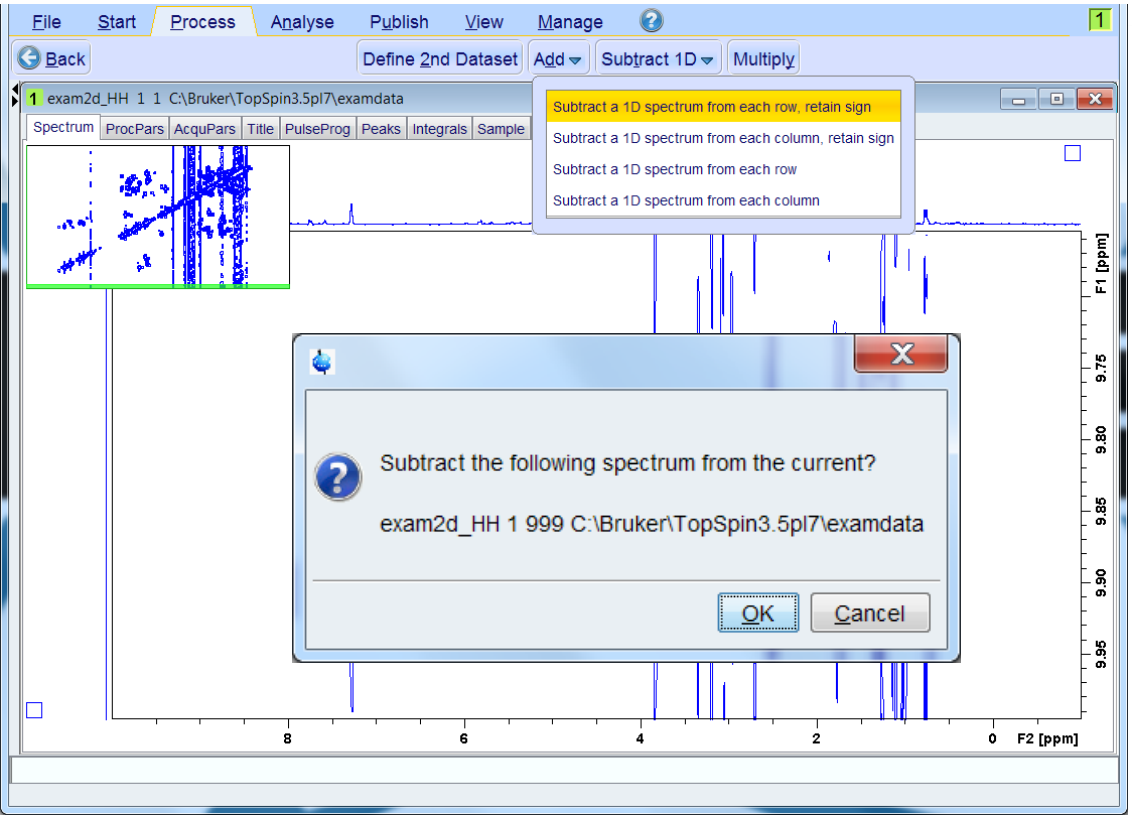
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. spectra

T1 Noise Subtraction



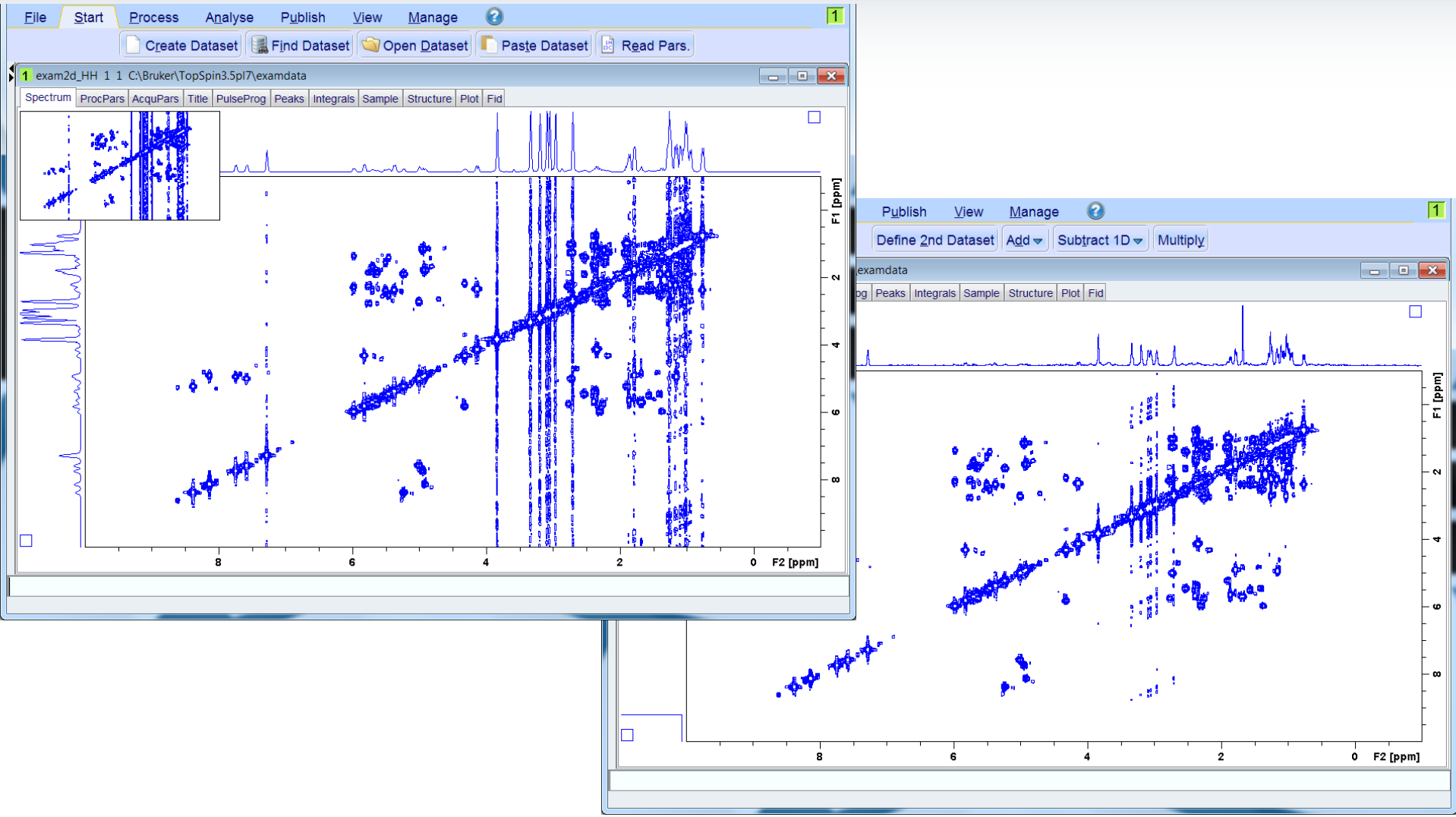
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. Spectra
    1. Define 2nd dataset you just made from previous slide

T1 Noise Subtraction



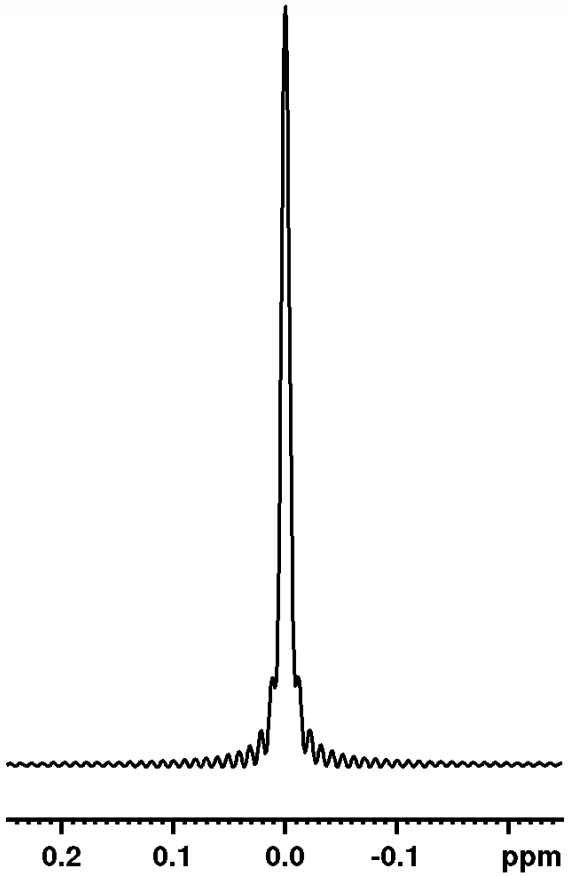
1. Expand full vertical region of spectrum that contains only noise, no signals.
2. In the Processing tab, under Advanced, select Calculate Projections.
3. In Processing tab, under Advanced menu, select Add/Sub/Mult. Spectra
    1. Define 2nd dataset you just made from previous slide
    2. From the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

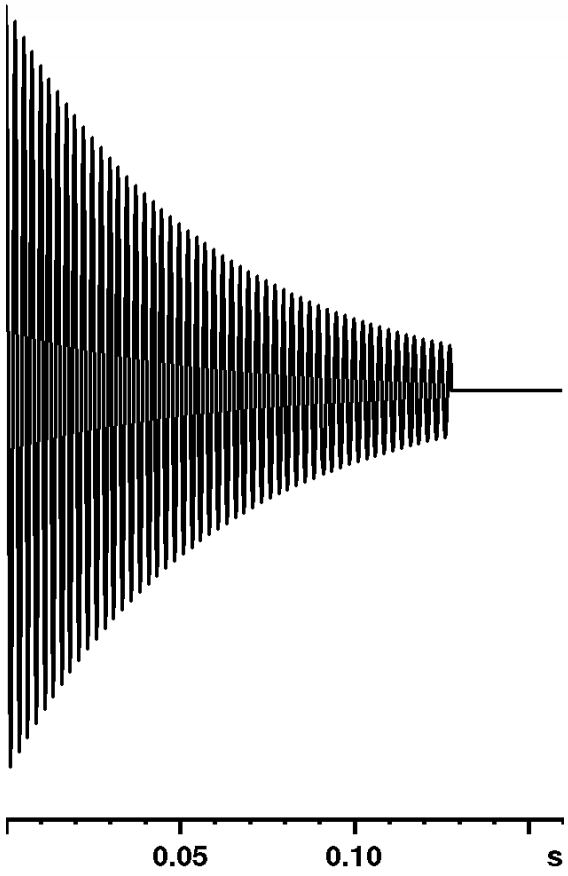
T1 Noise Subtraction



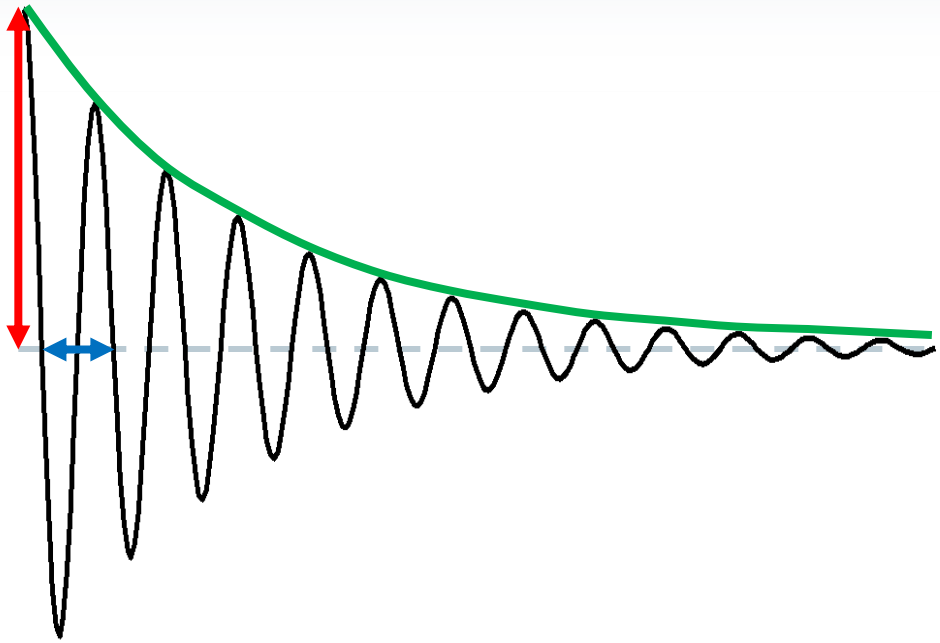
- **Truncation artifacts**

Truncation artifacts

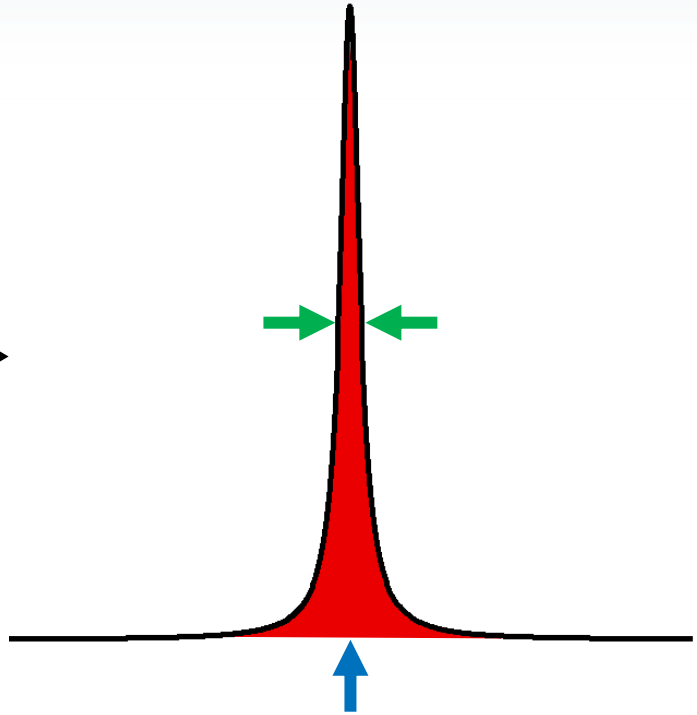




Fourier transformation



FT



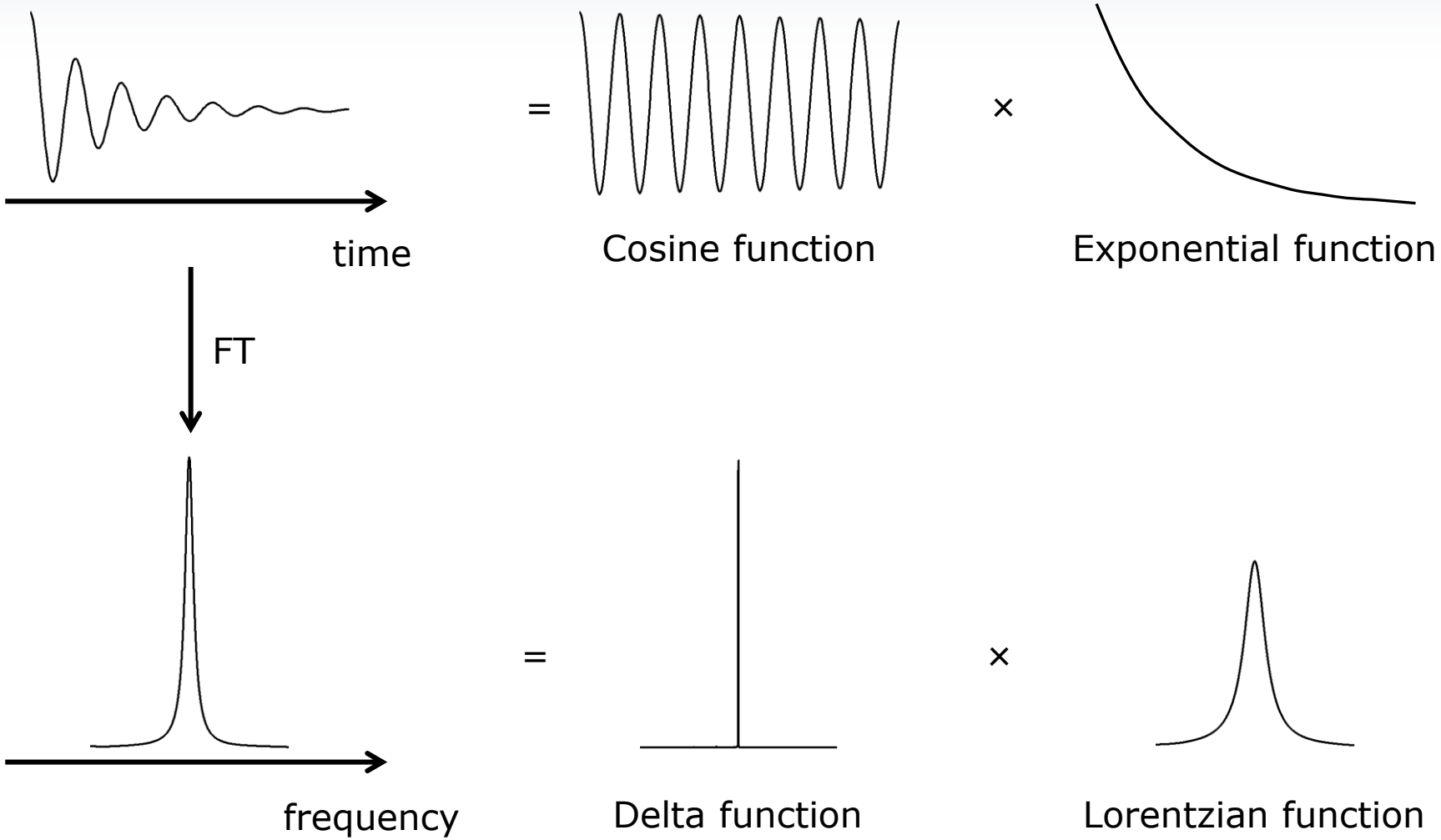
time domain ⟷ frequency domain

amplitude ⟷ integral

period ⟷ frequency

decay ⟷ lineshape

Fourier transformation



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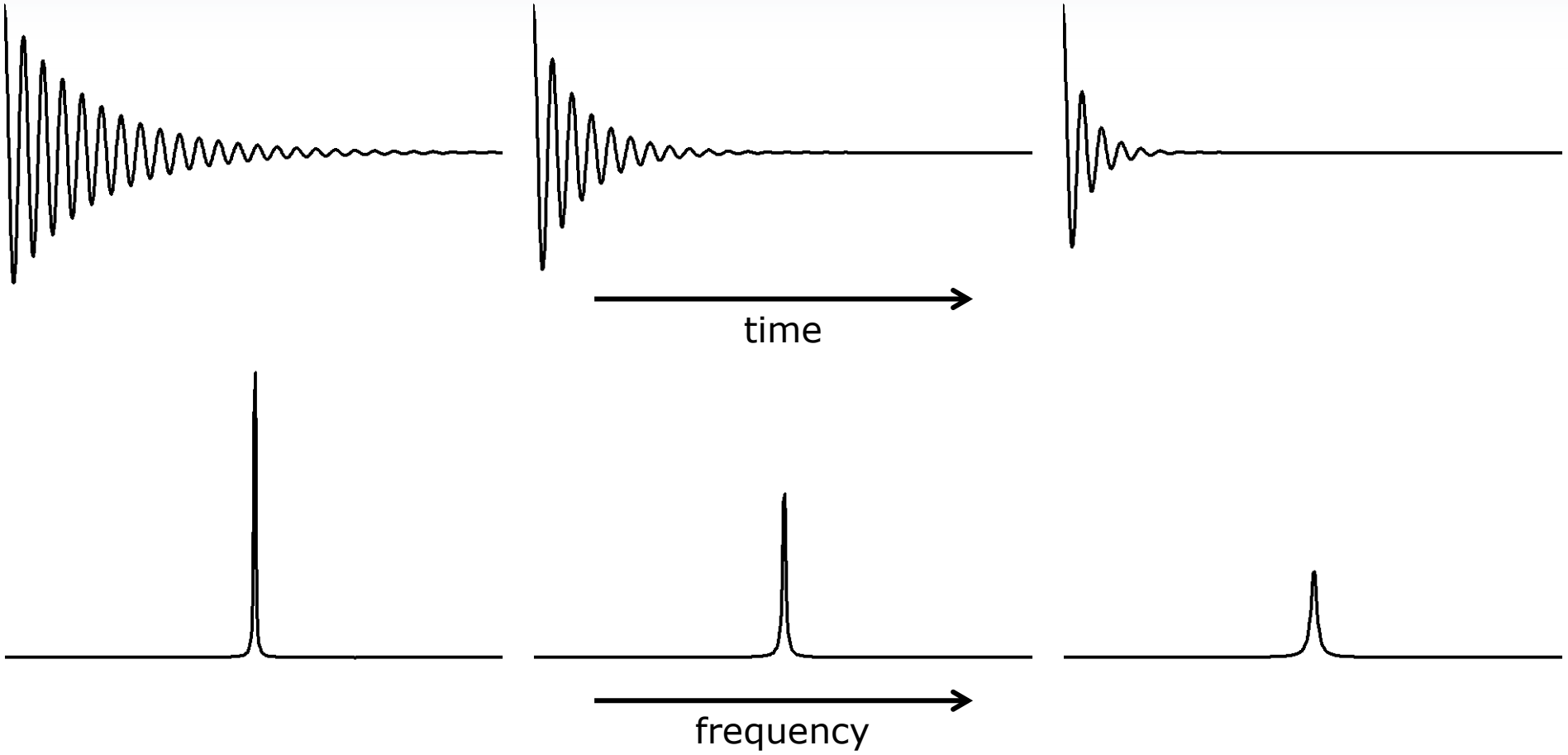

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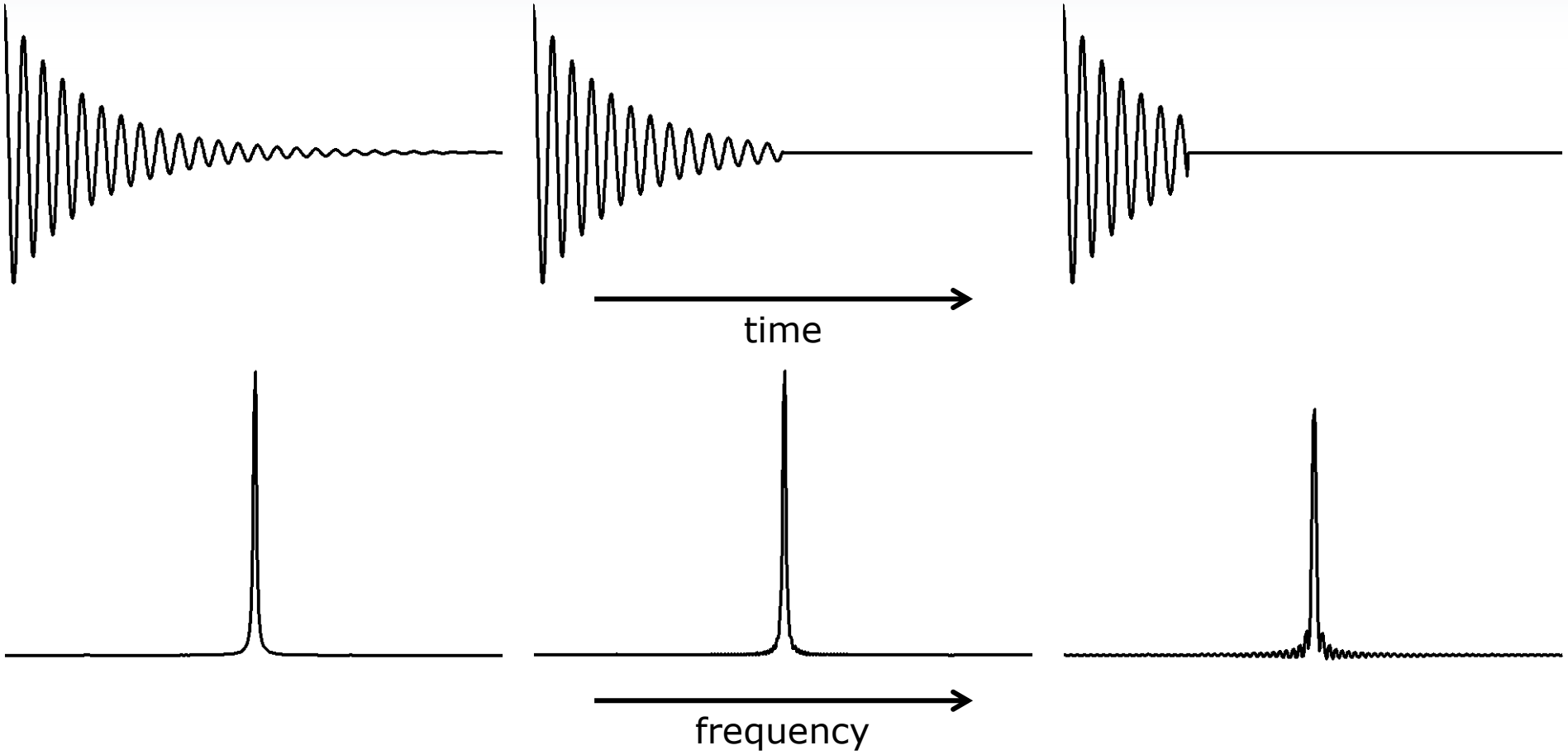

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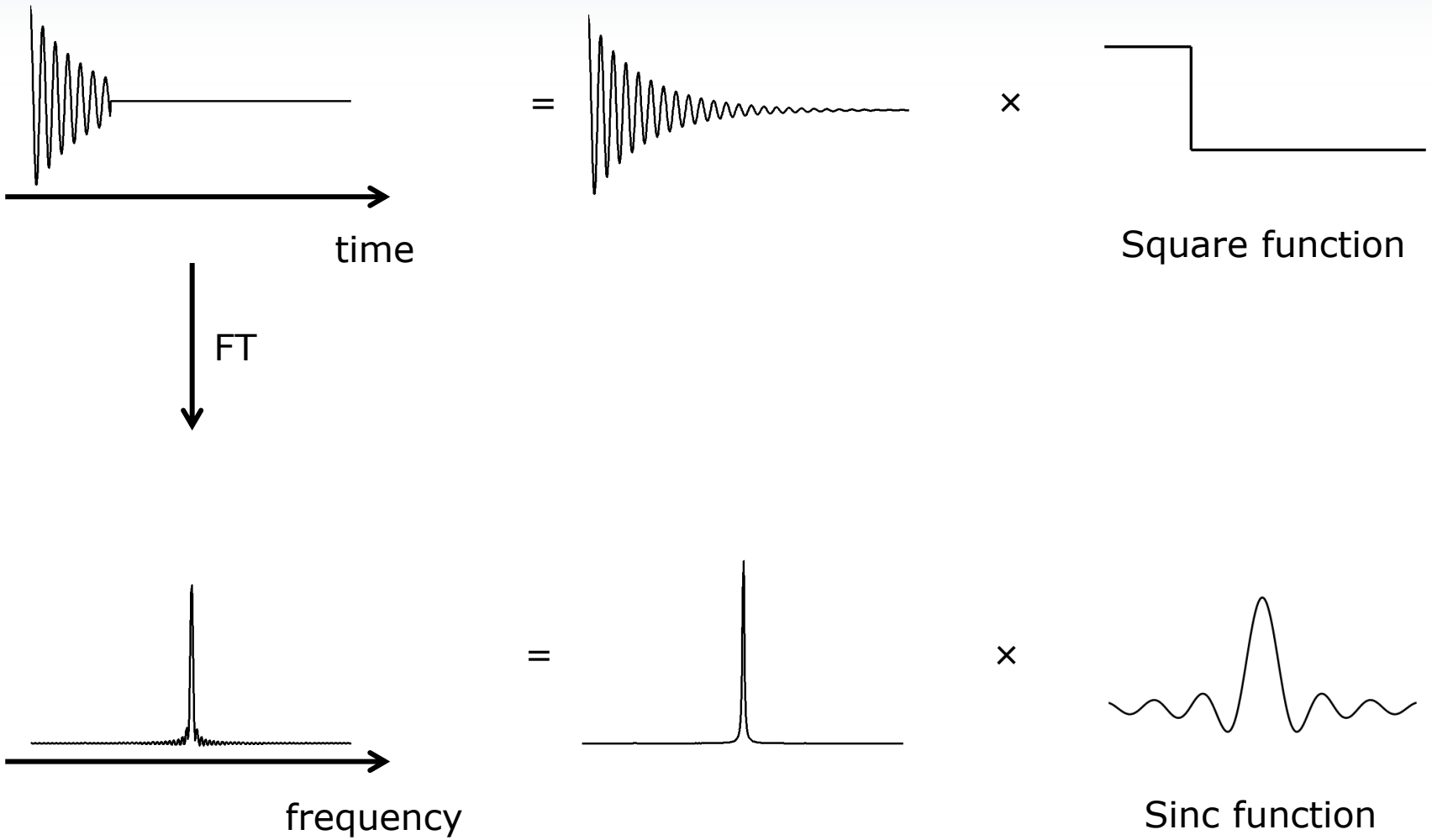

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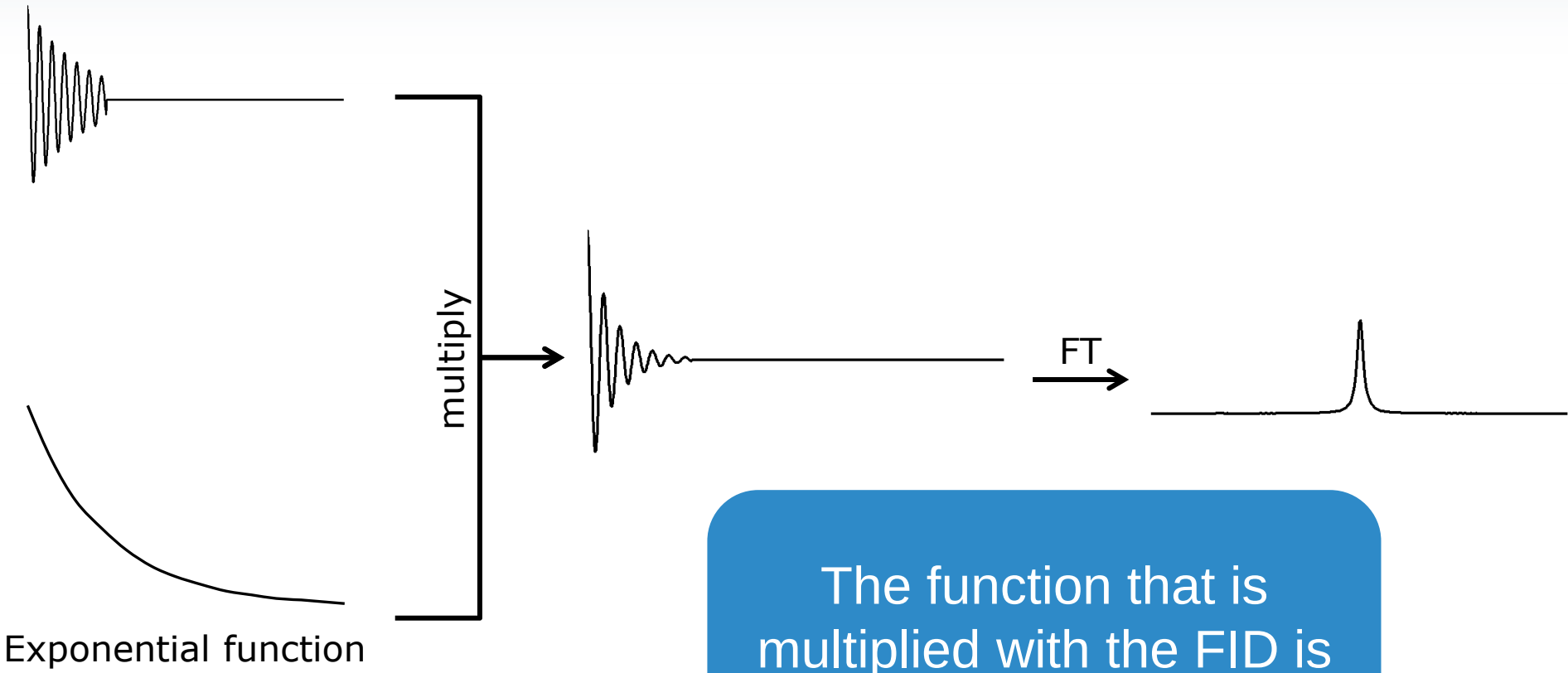


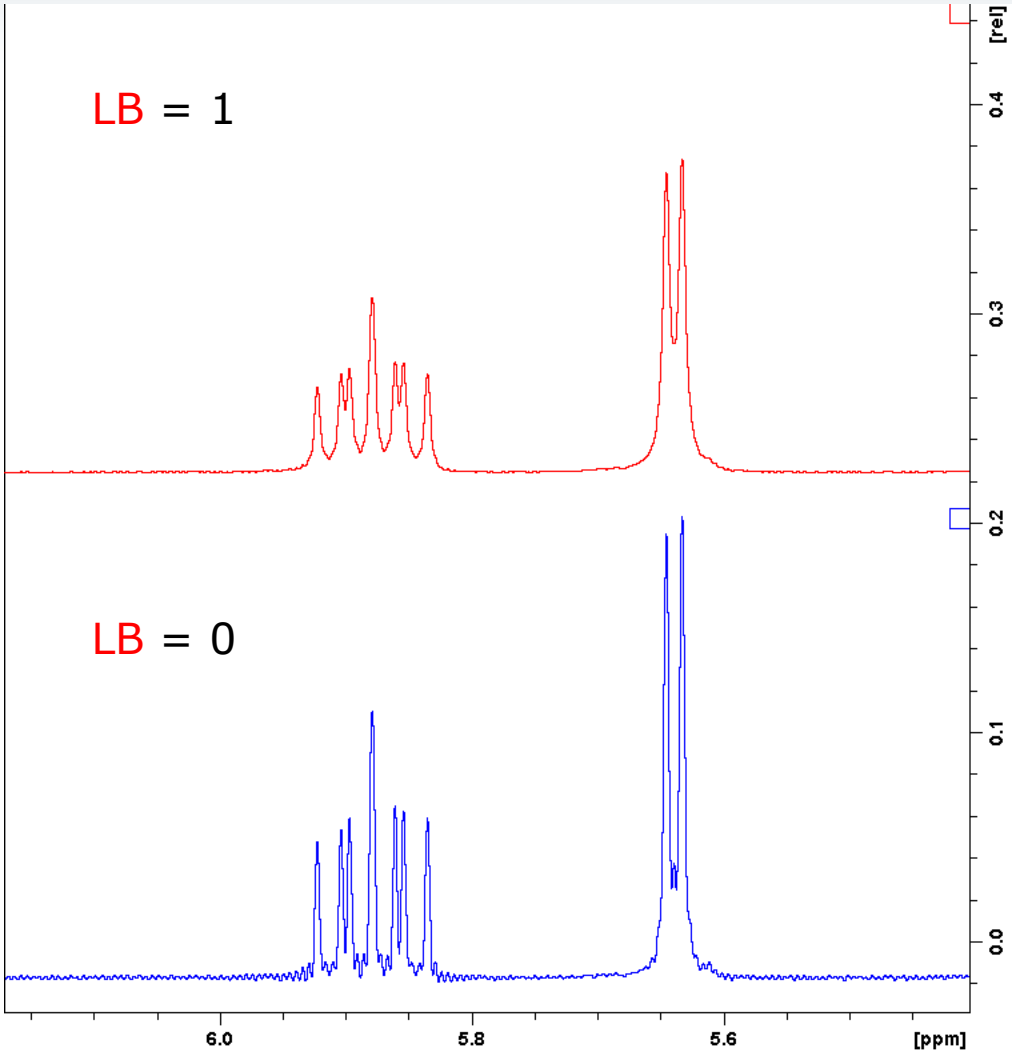
The function that is multiplied with the FID is called **window function**.

- **Window functions**

Window functions
EM

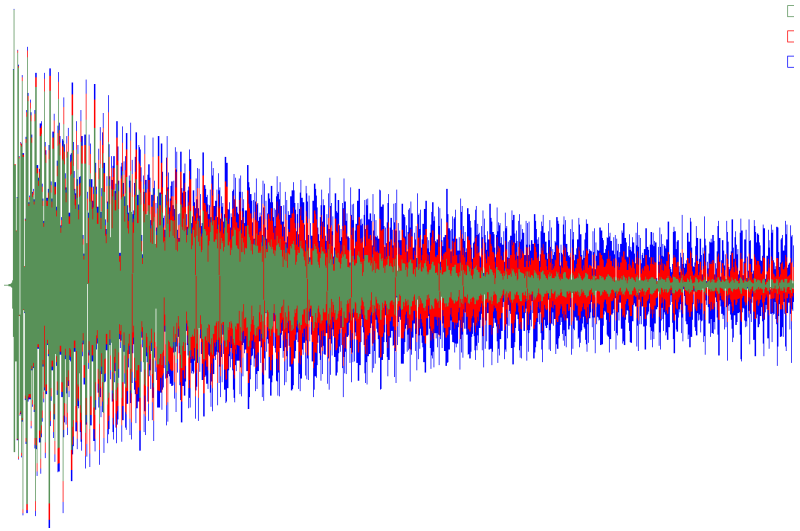
- Exponential multiplication
- Parameter: LB
- Value typically positive
- Forces FID to go to 0

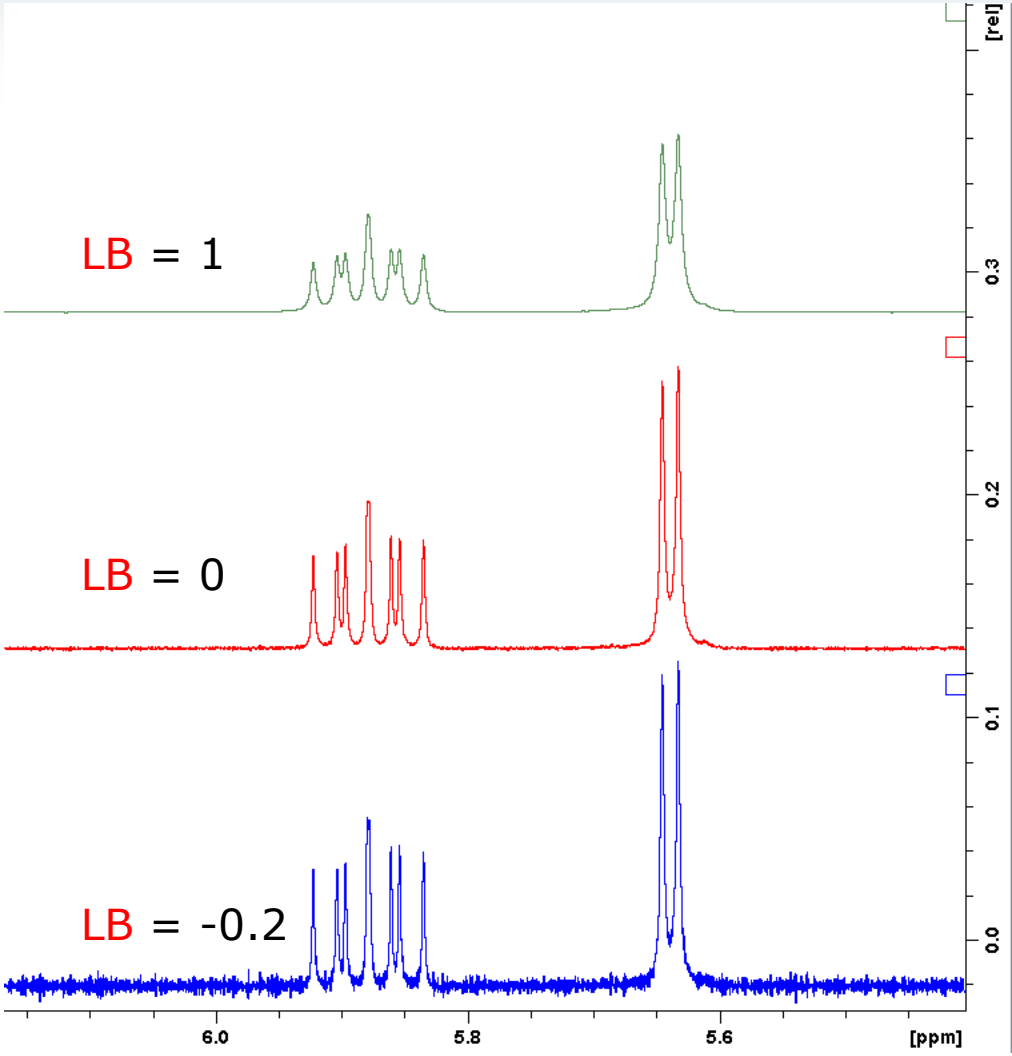
- Broadening out signals and noise
  - ➢ Increase apparent S/N
  - ➢ BUT: at the cost of resolution



Window functions
EM

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

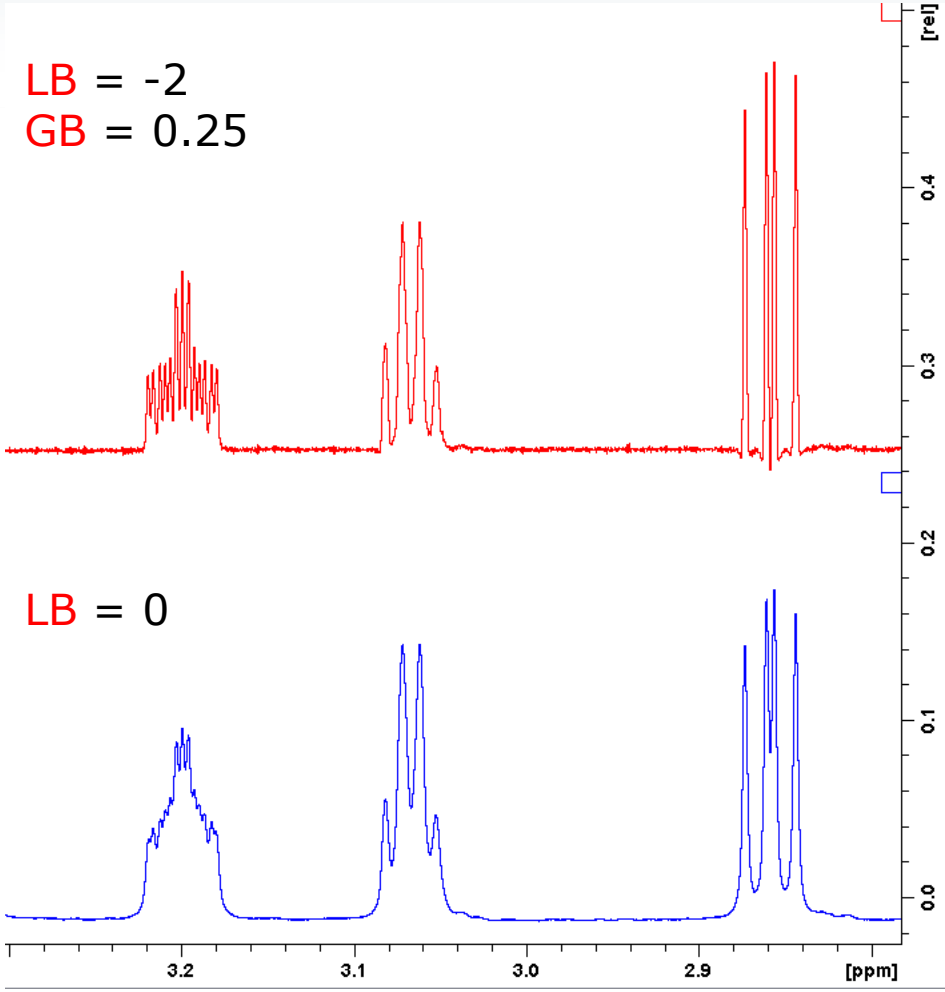




Window functions
GM

- Gaussian multiplication
- Parameters: GB and LB
- LB must be negative (typically LB =-(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



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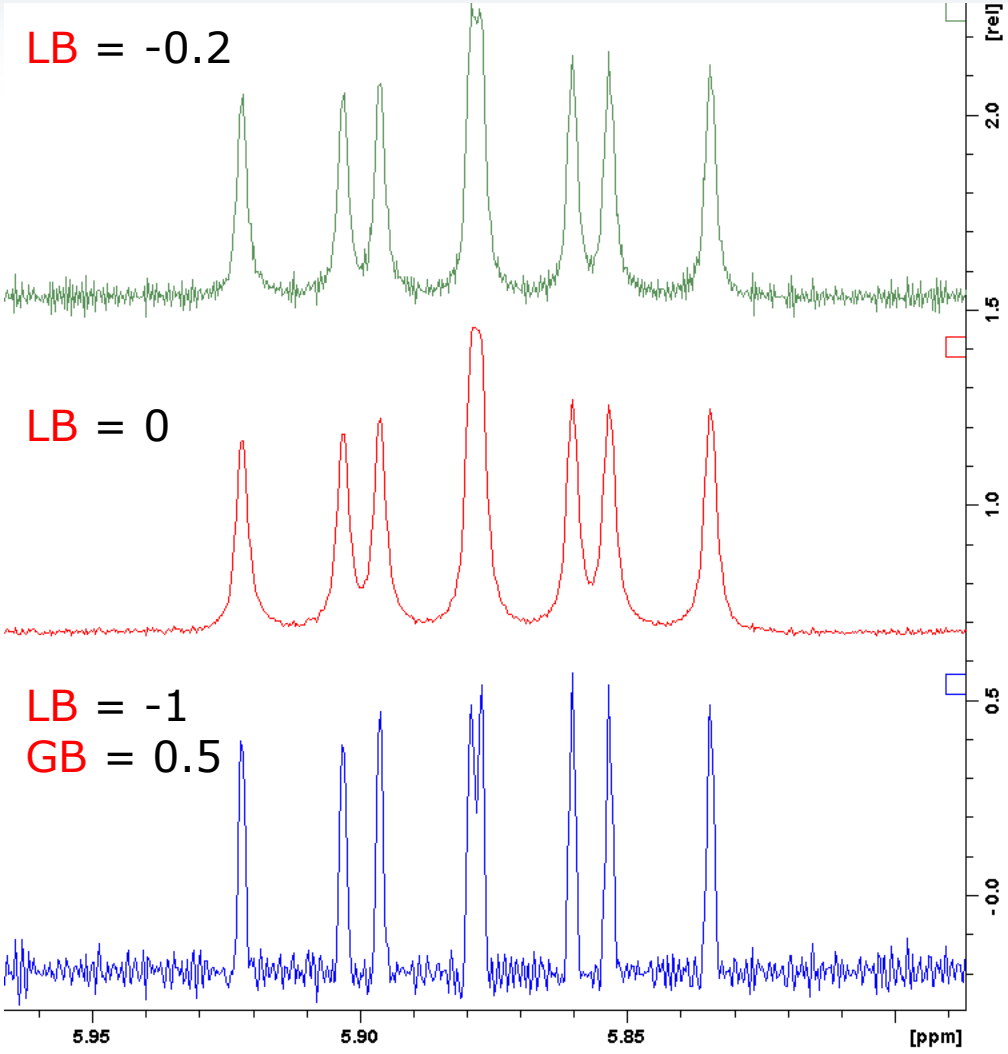
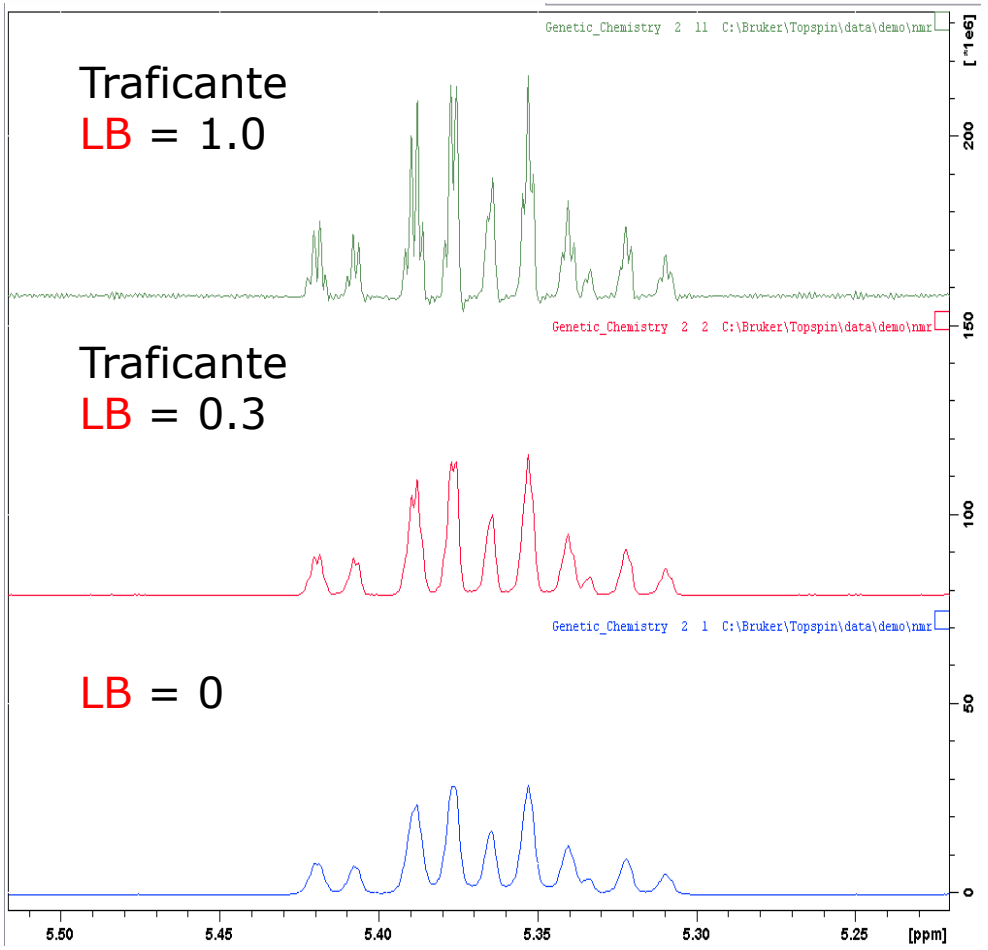


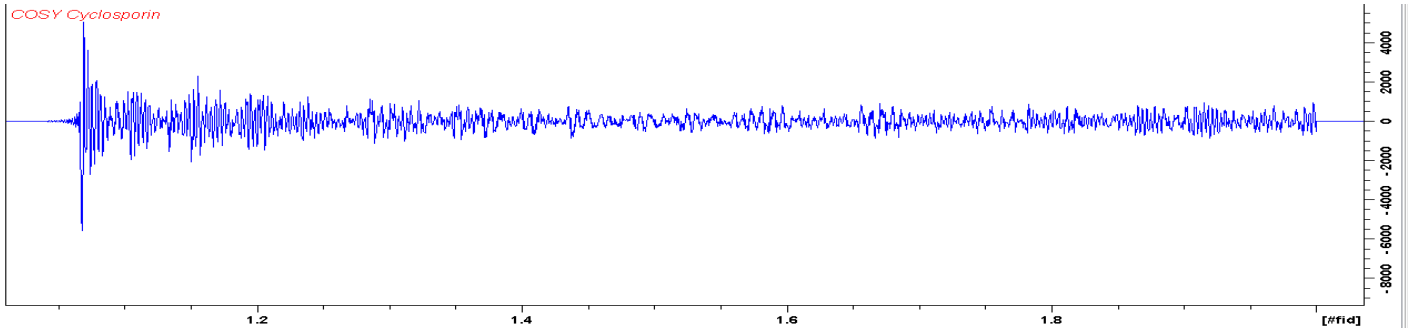
Window functions
TRAF

- 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)

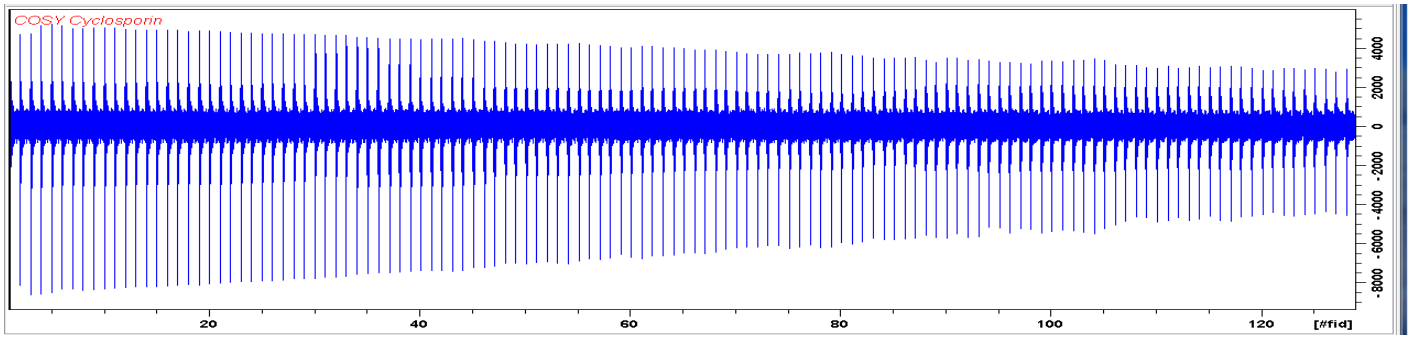


Apodization for 2D spectra

- 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

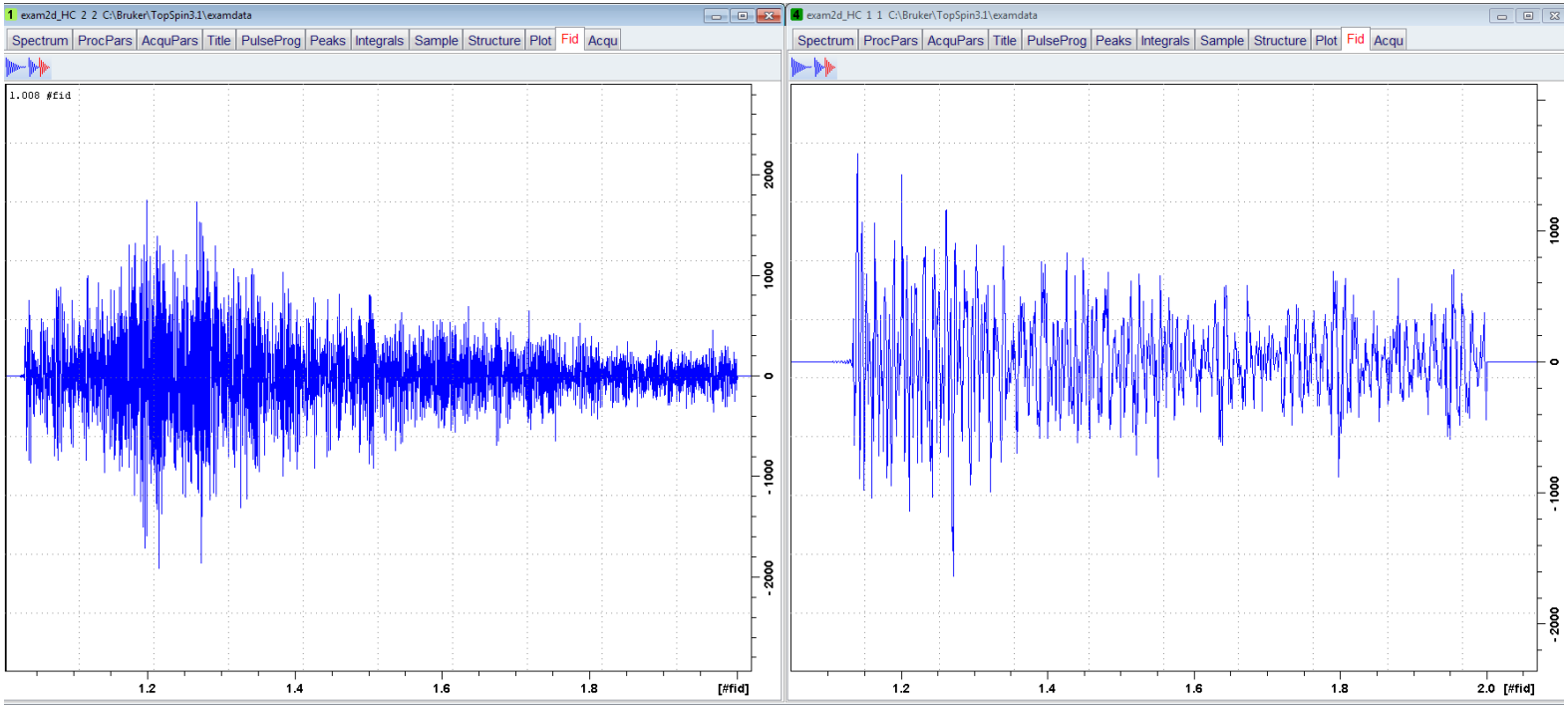


Apodization for 2D spectra

- 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 sinm or qsin
  - sinm is sine multiplication
    - $sinm(t) = \sin\left(\left(\pi - \left(\frac{\pi}{SSB}\right)\right)\left(\frac{t}{AQ}\right) + \frac{\pi}{SSB}\right)$
  - qsin 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 sin/cos function

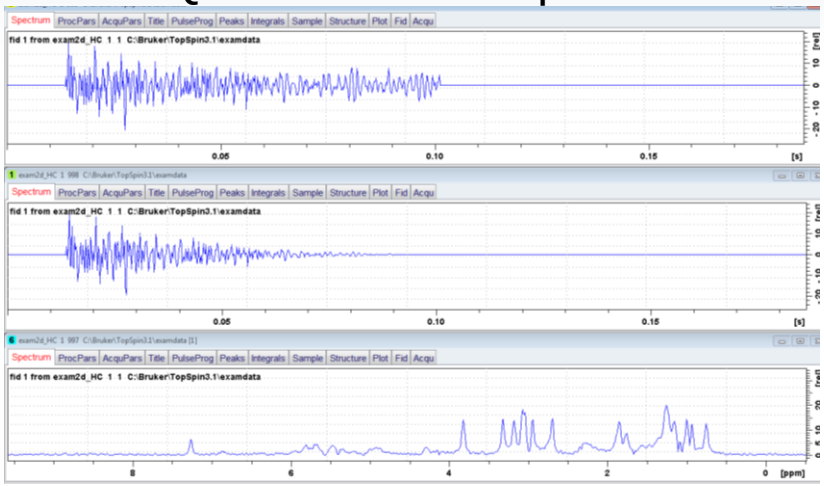
Step 1: evaluate the first FID

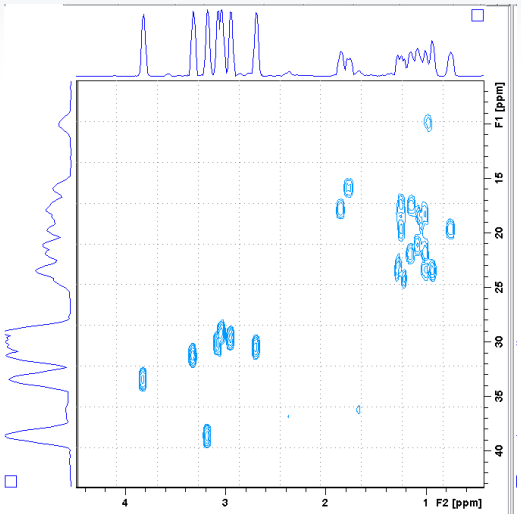
- On left is the 1st FID of an HMBC, note how signal builds from start, this uses the less common SSB=0,1 (these have the same effect)
- On right is the 1st FID of an HMQC. This looks more "normal" with max signal at beginning, and traditional decay. This uses SSB=2.



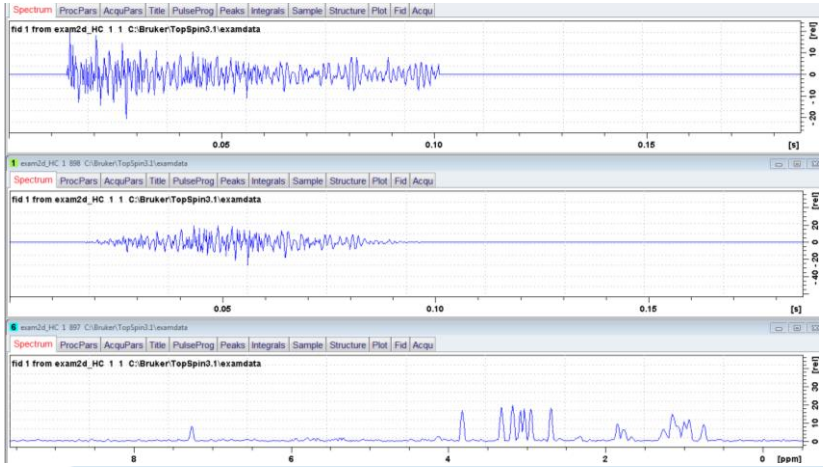
Window function – HMQC

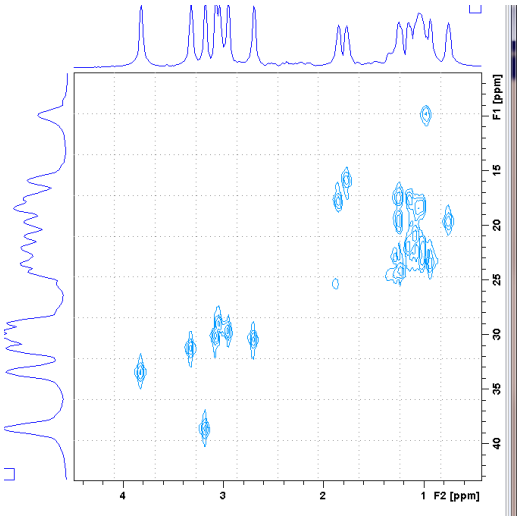
- HMQC with correct apodization





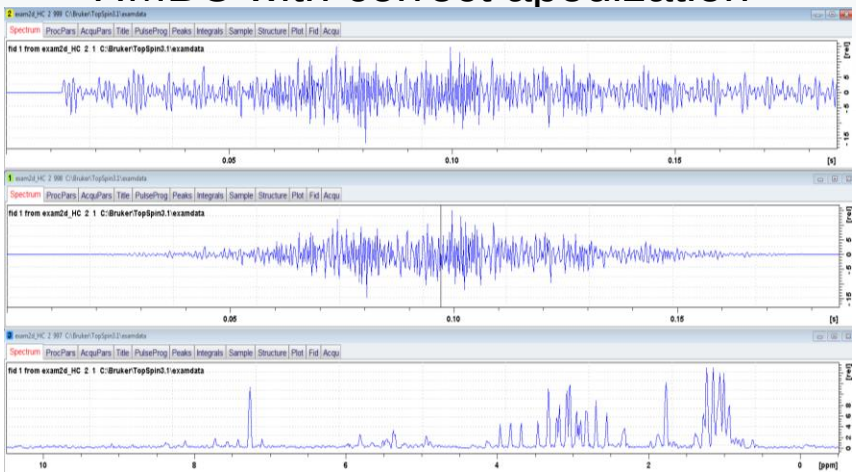
- HMQC with incorrect apodization

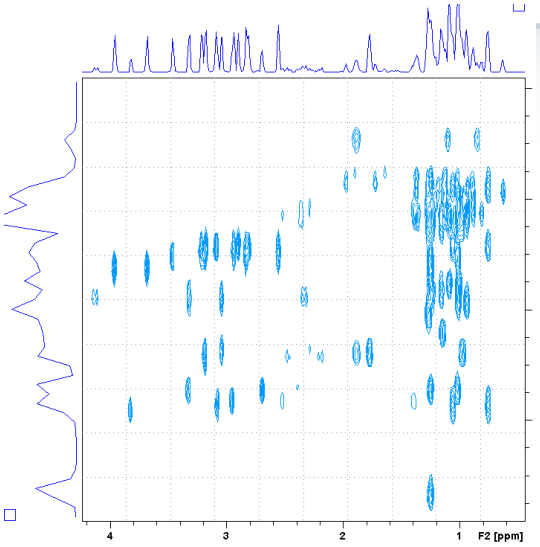




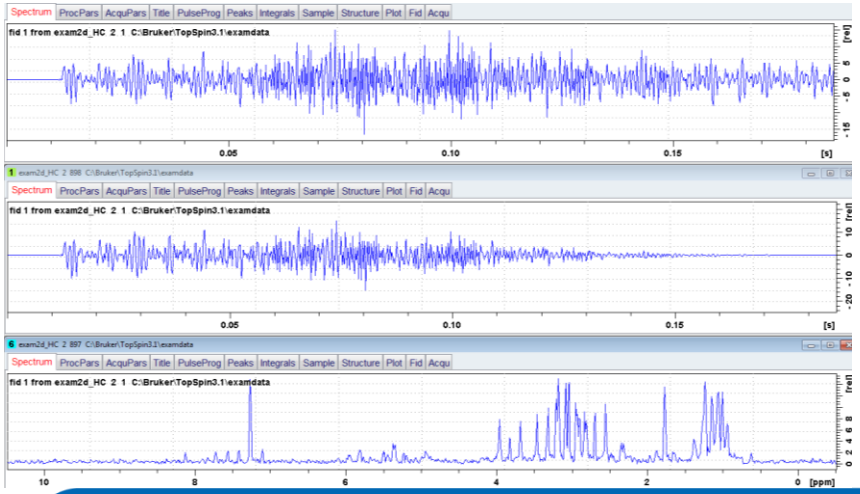
Window function – HMBC

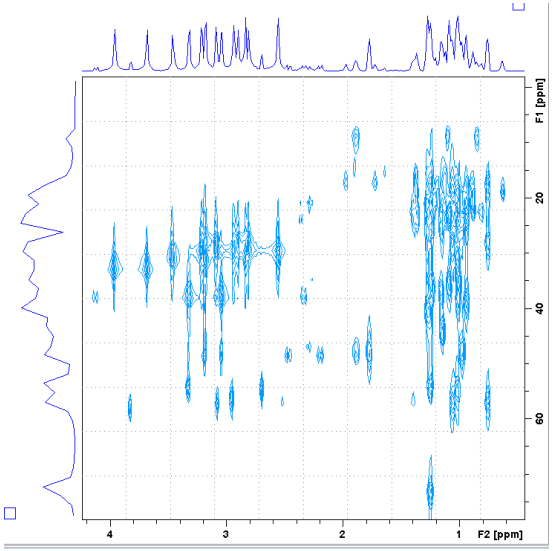
- HMBC with correct apodization





- HMBC with incorrect apodization

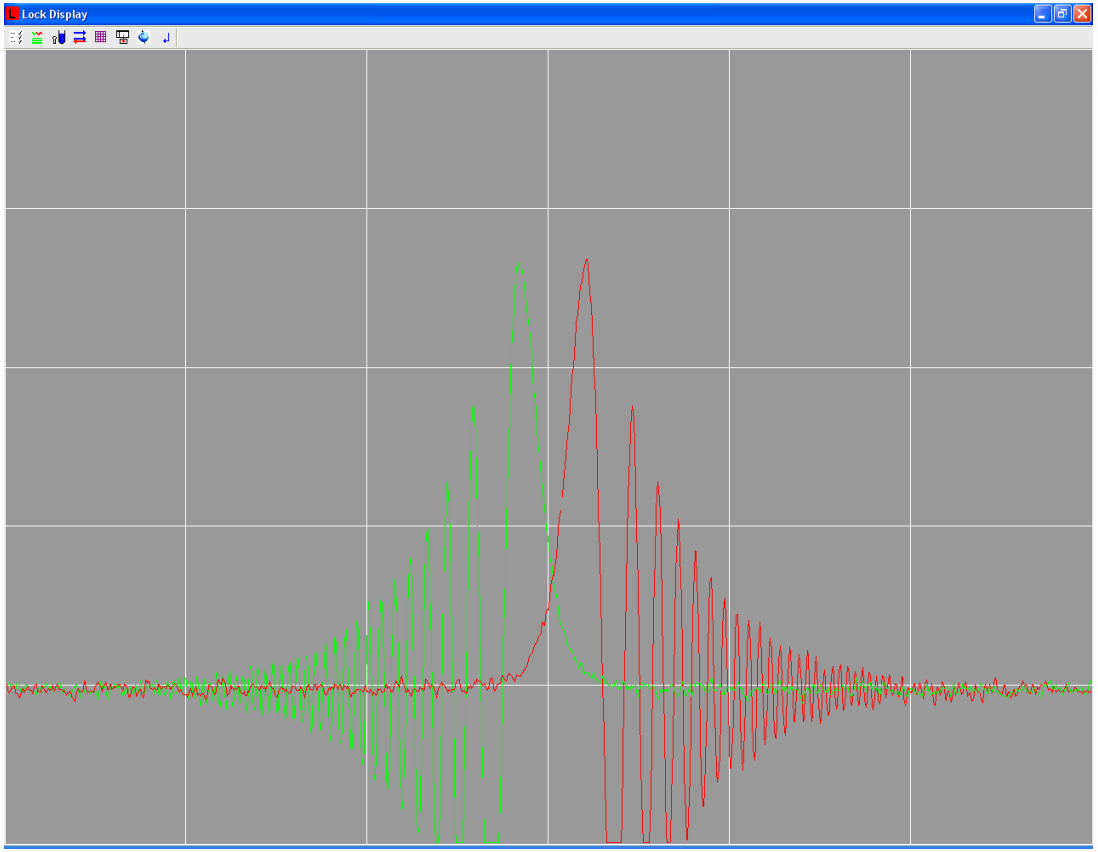




- **Lock phase**

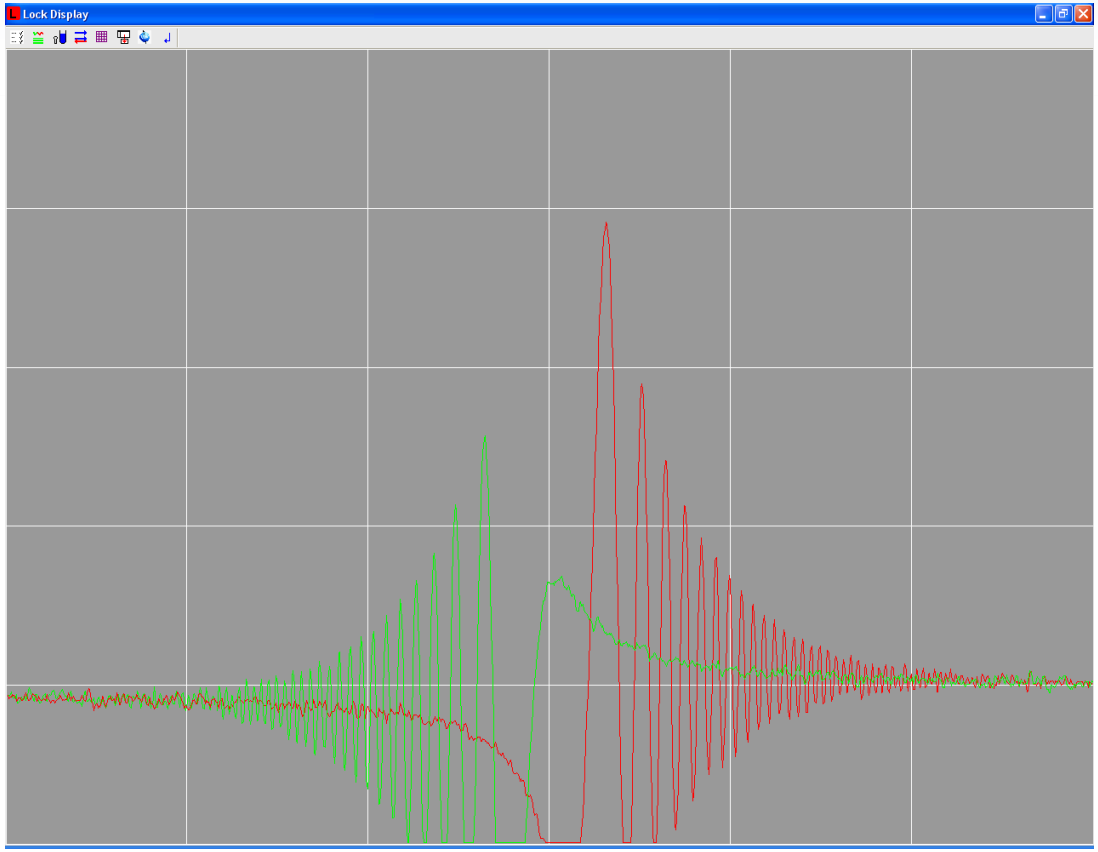
Lock phase

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



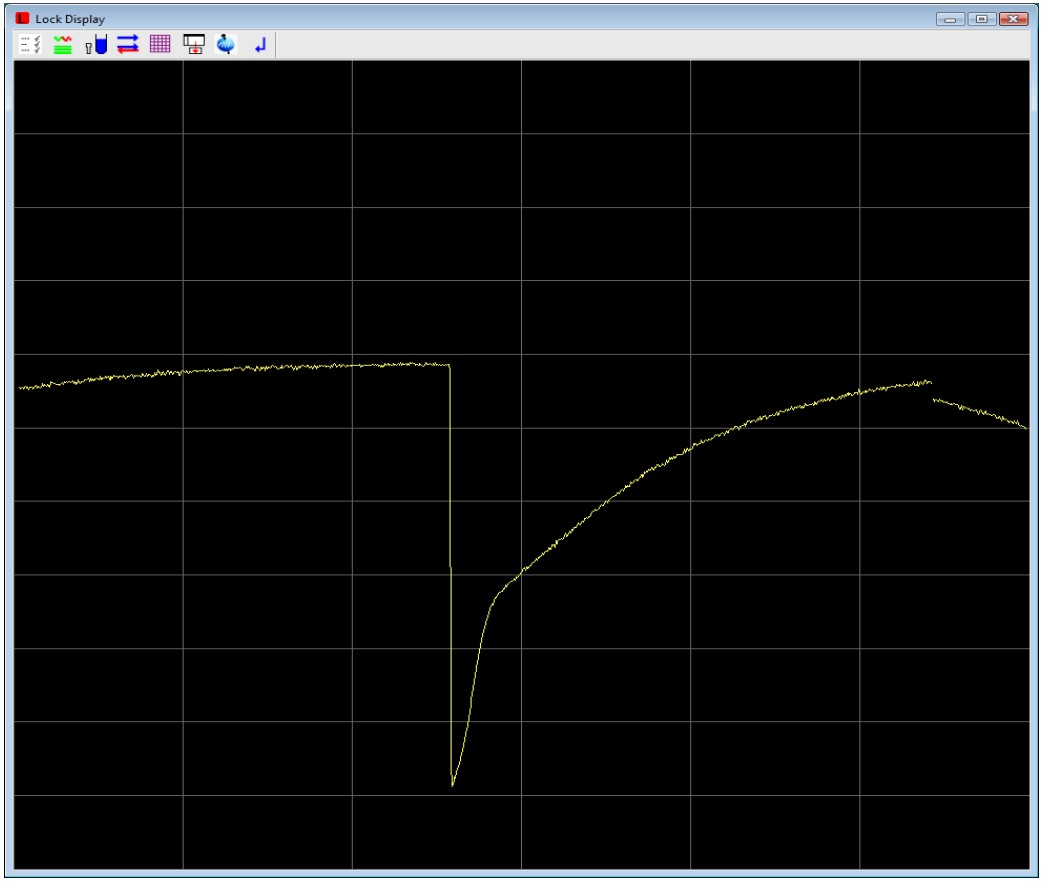
Wrong lock phase

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



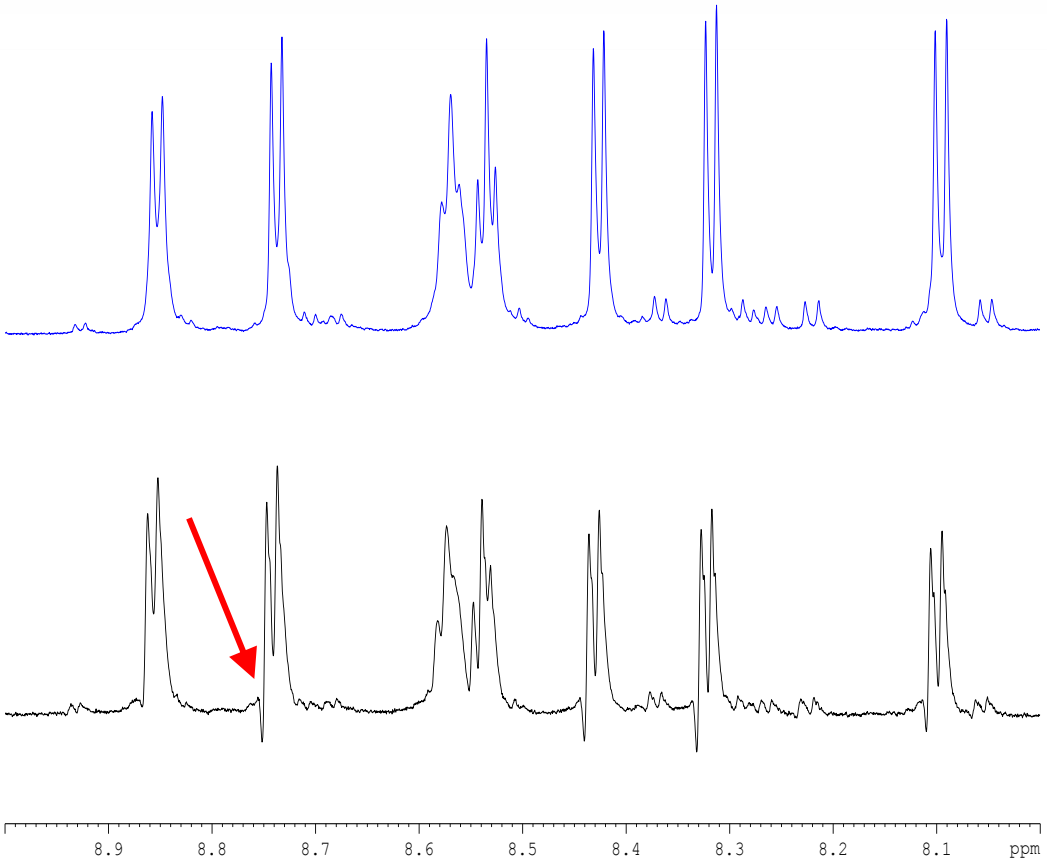
Wrong lock phase

- Lock phase off by 30°
- Lock has a knee during gradient experiment



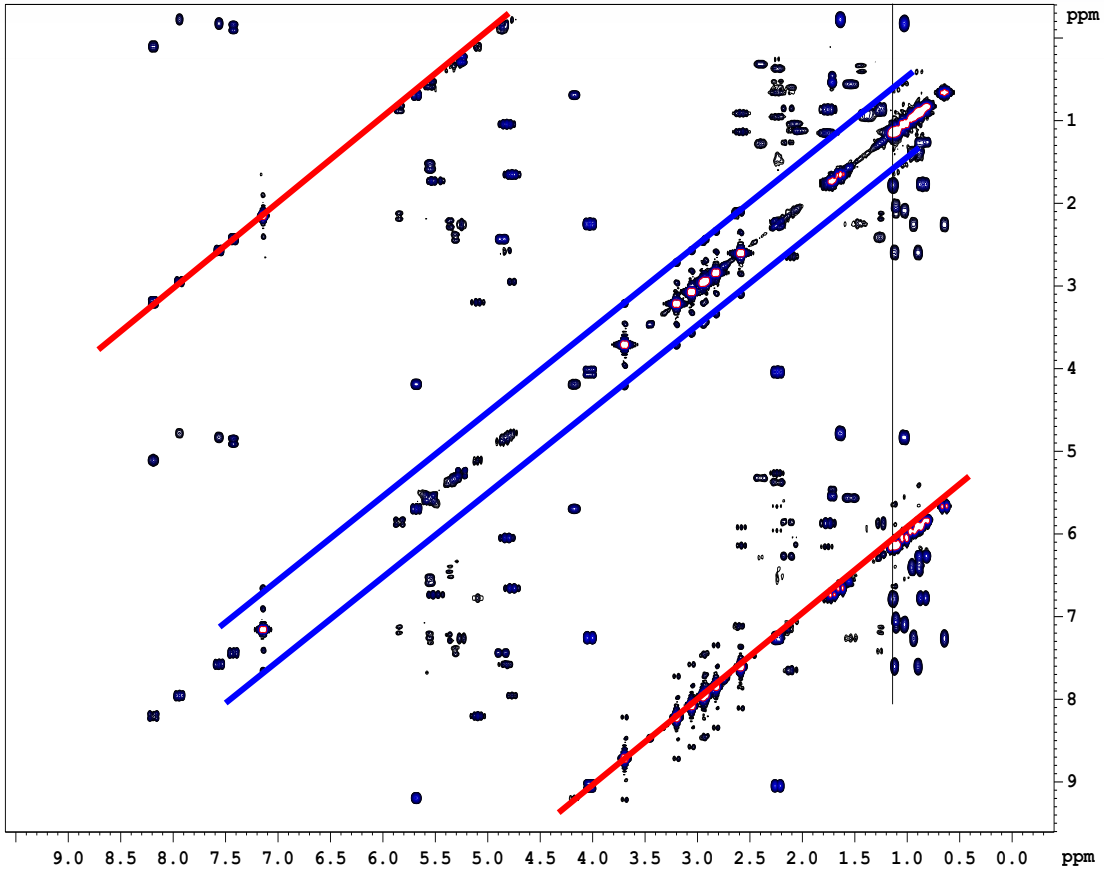
Wrong lock phase

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



Gradient-COSY

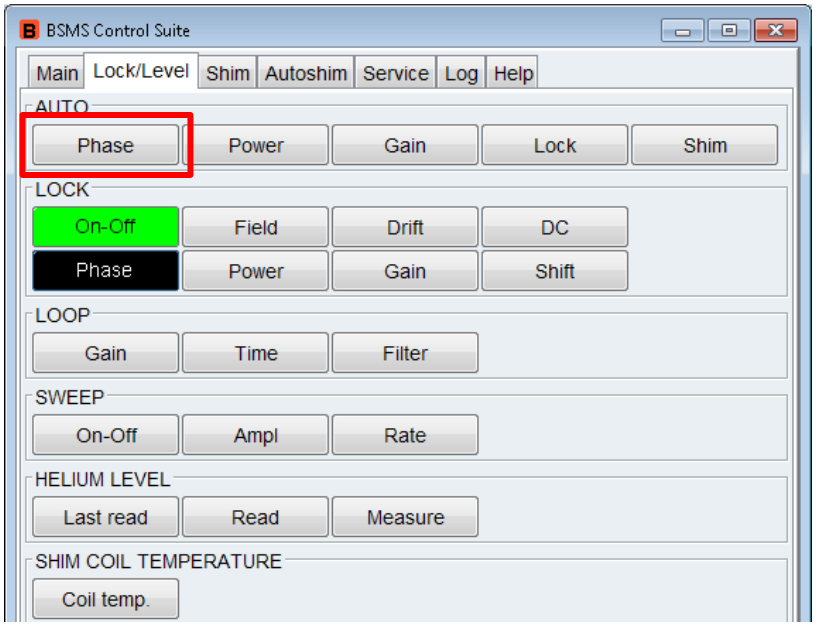
- COSYGPQF:
- Lockphase wrong by ca. 40°



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

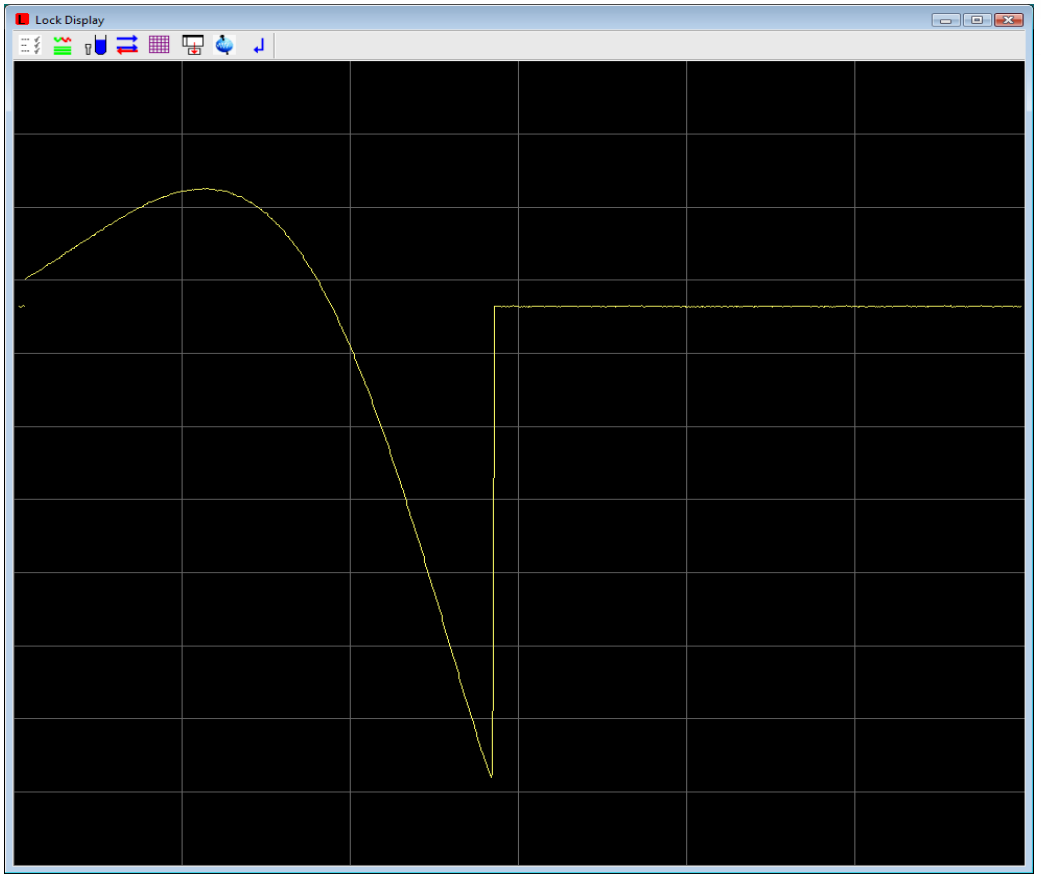
- Software:
  - loopadj (AU)
  - Tune during Topshim ([**topshim tunea**])
  - Autophase on BSMS display



Lock power

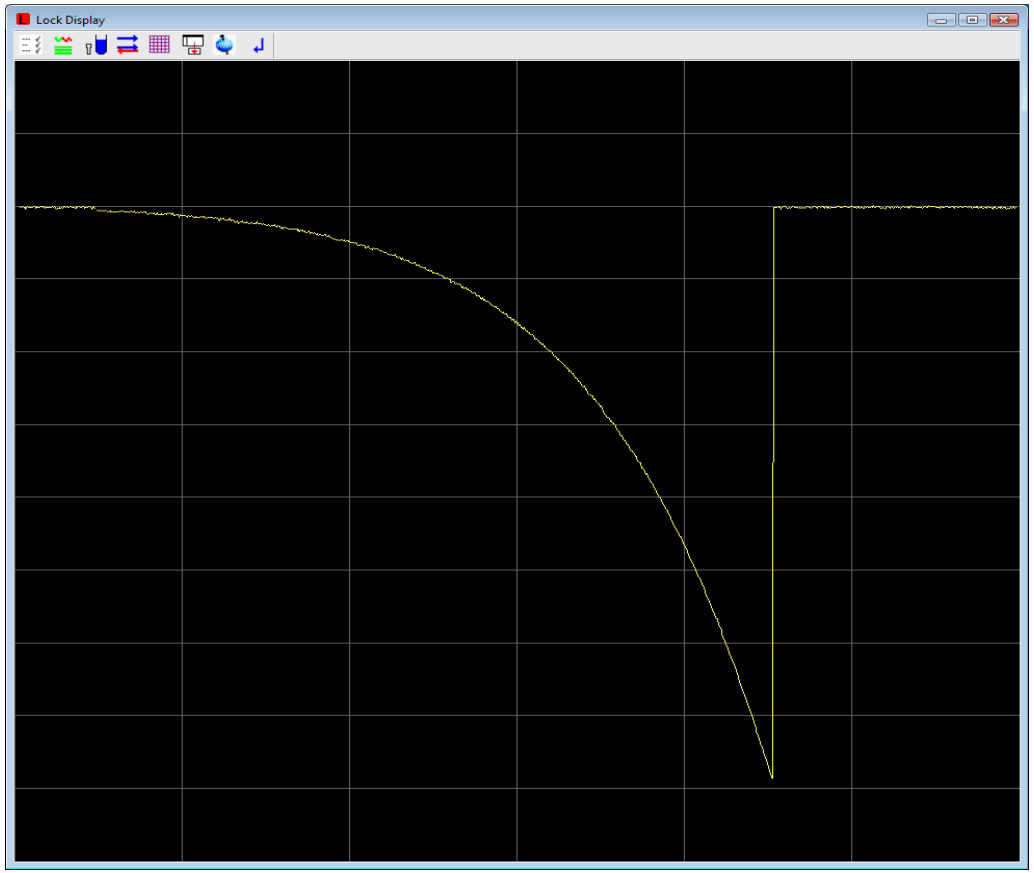
- «overshoot» after gradient

➢ Lock power too high



Lock power

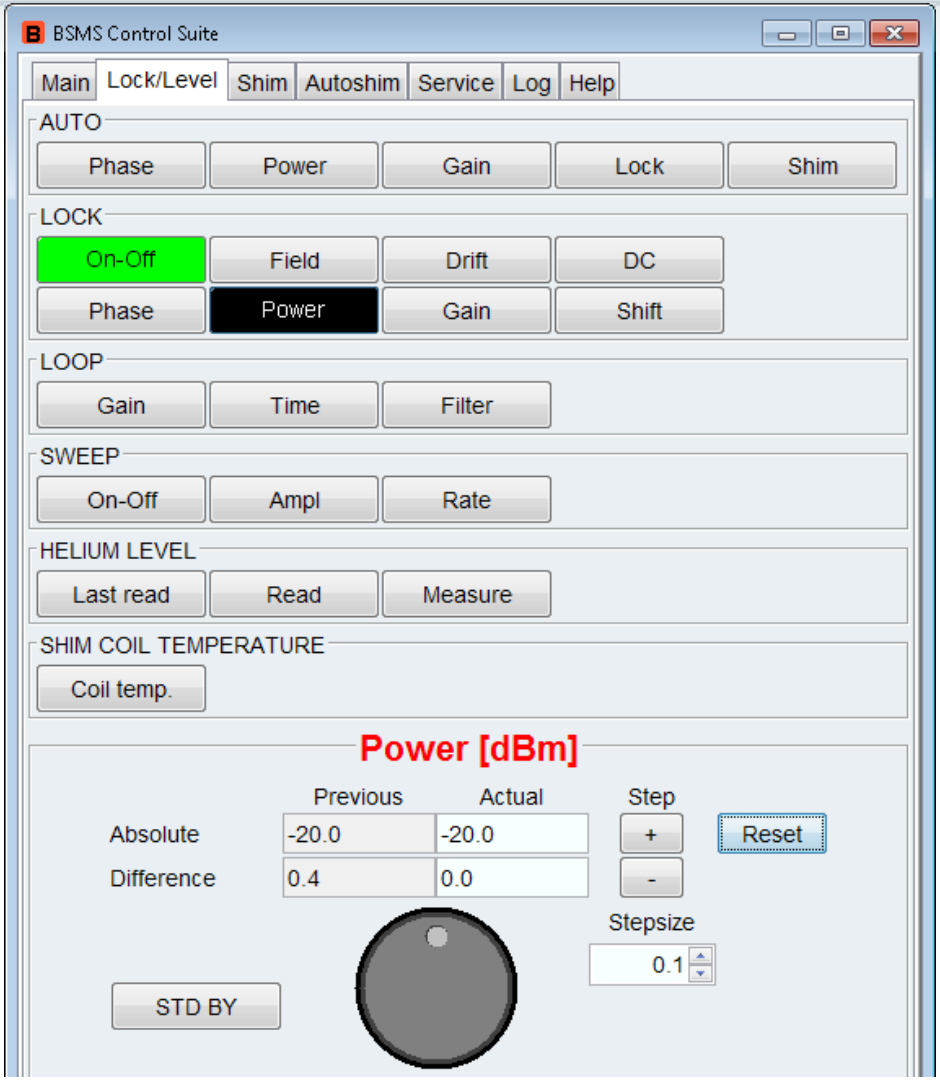
- Lock power set correctly



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.



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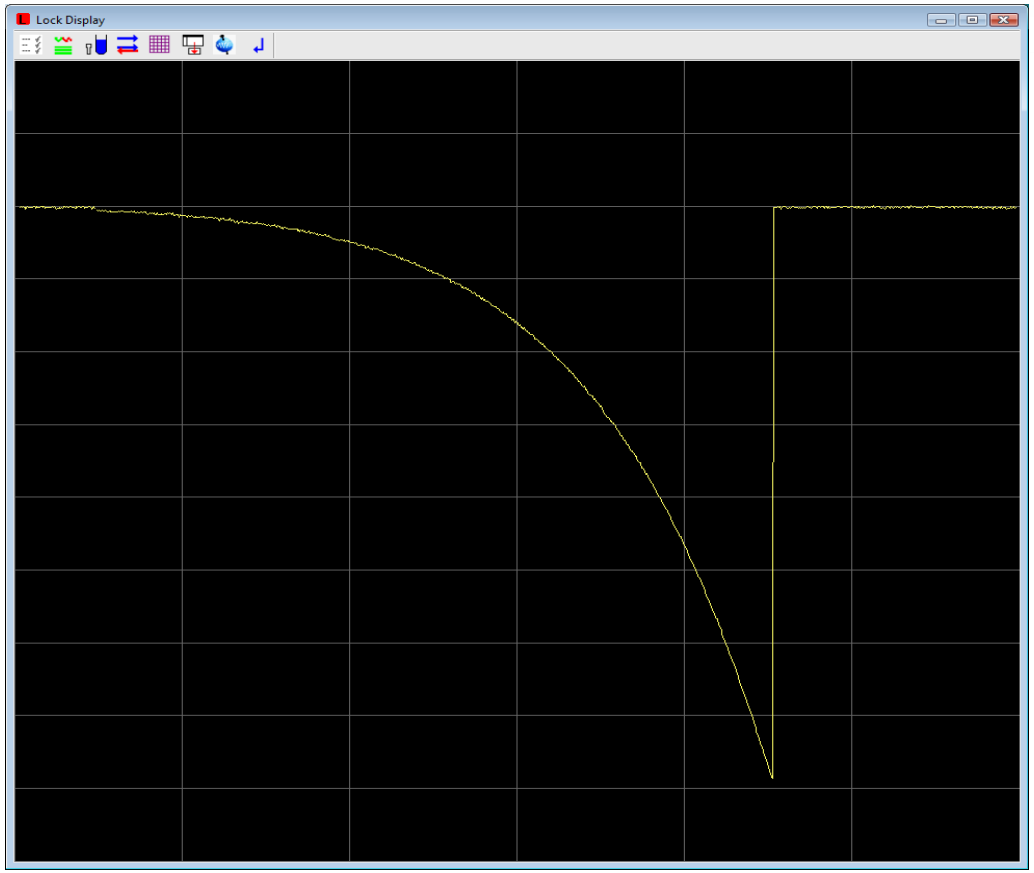


Lock power optimized

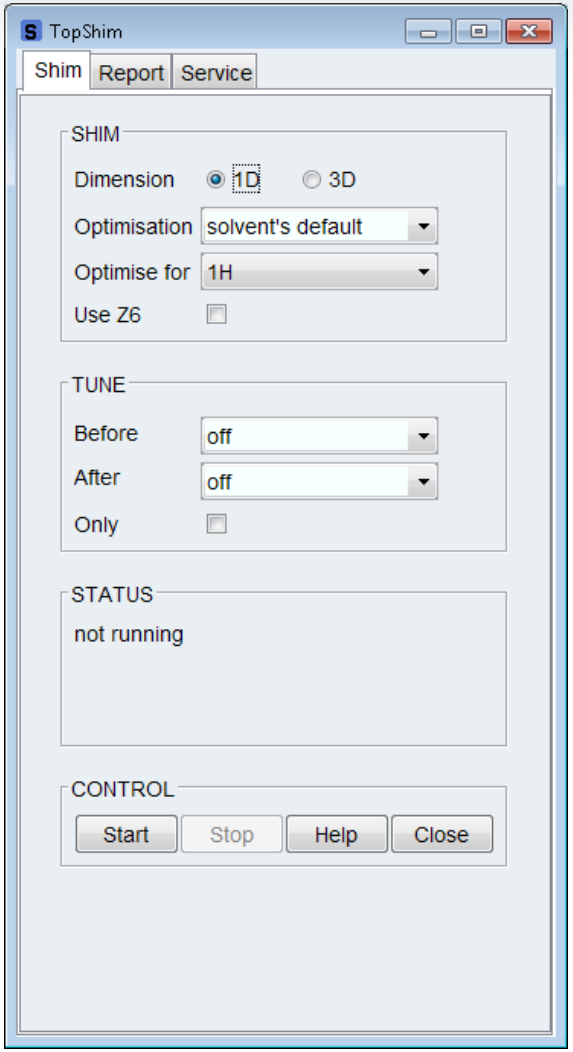
- **TopShim**

TopShim GUI

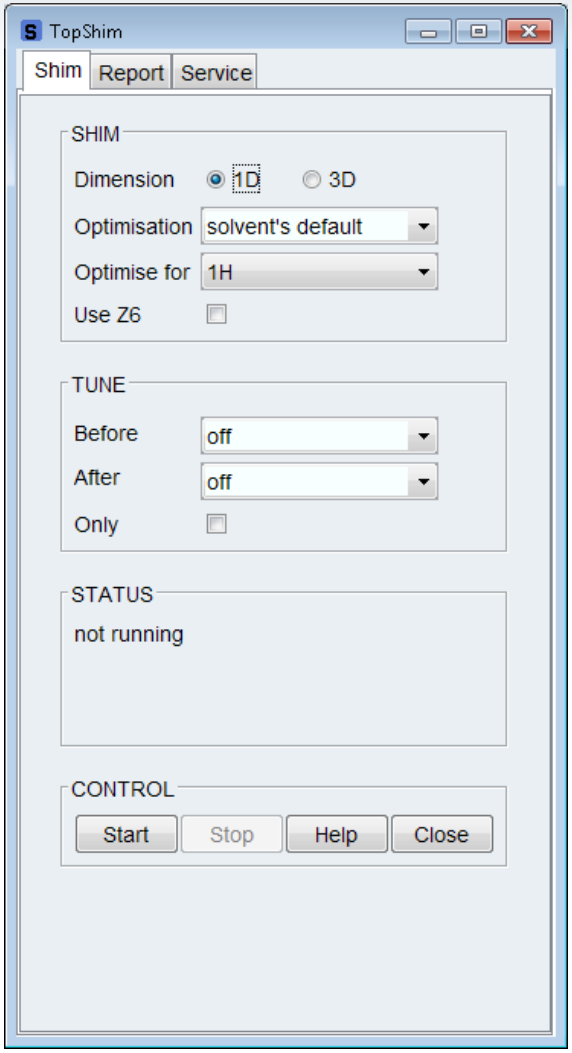
- Can be opened with command

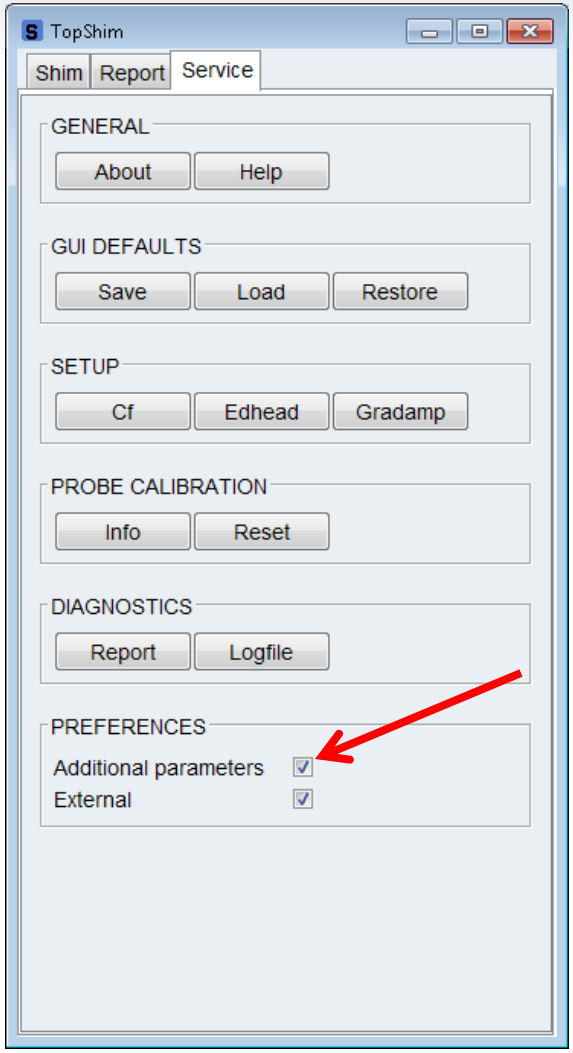
  [**topshim gui**]

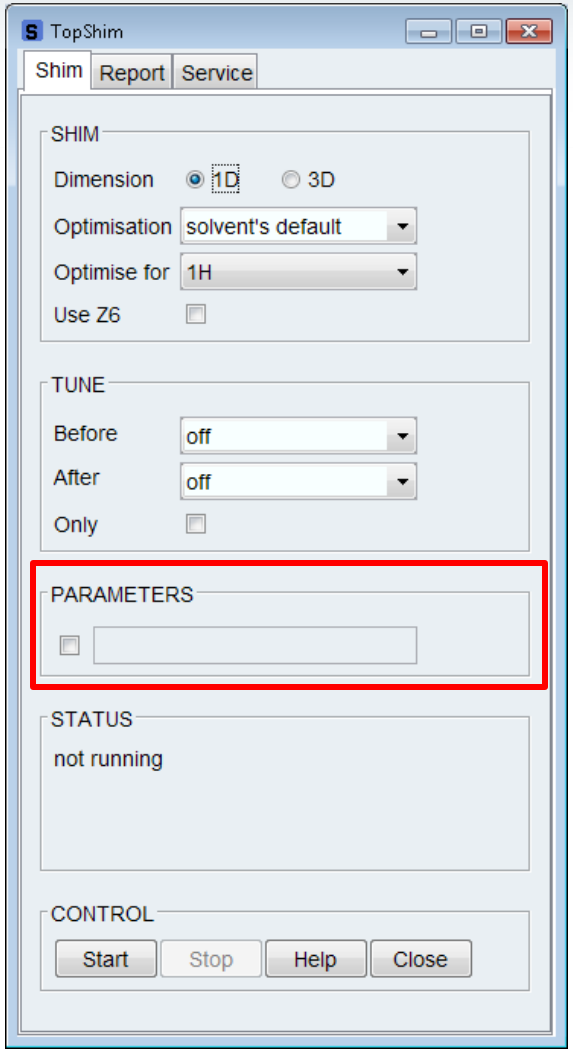
- Allows several options



TopShim GUI – Additional Parameters







Useful Arguments

- **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_6)

- **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_3CN+D_2O)

- **durmax=** maximum duration per 1D field map acquisition (expressed in seconds)
  default = 7

Useful Arguments

- **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)

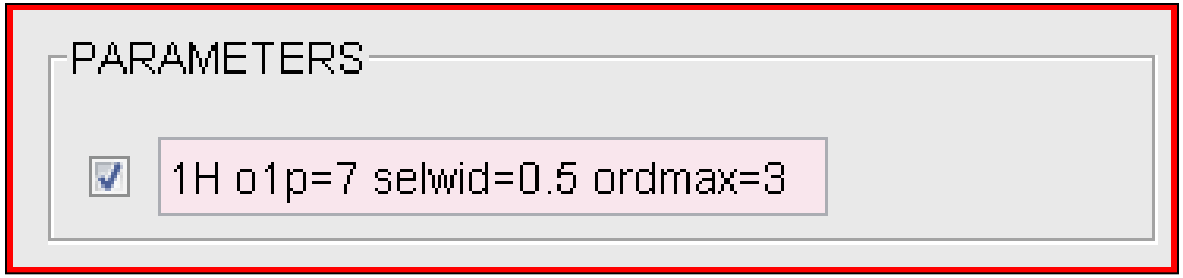
- **plot** Saves data after completion in <TopSpin_home>/data/topshimData

… read about more in the Topshim manual!

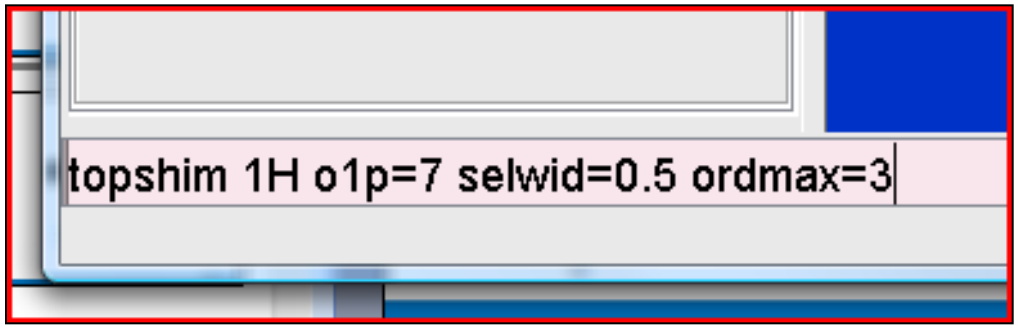
Type [***help topshim***] from the Topspin command line.

Command Line Arguments

- Command line arguments
  - Multiple arguments can be used simultaneously



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



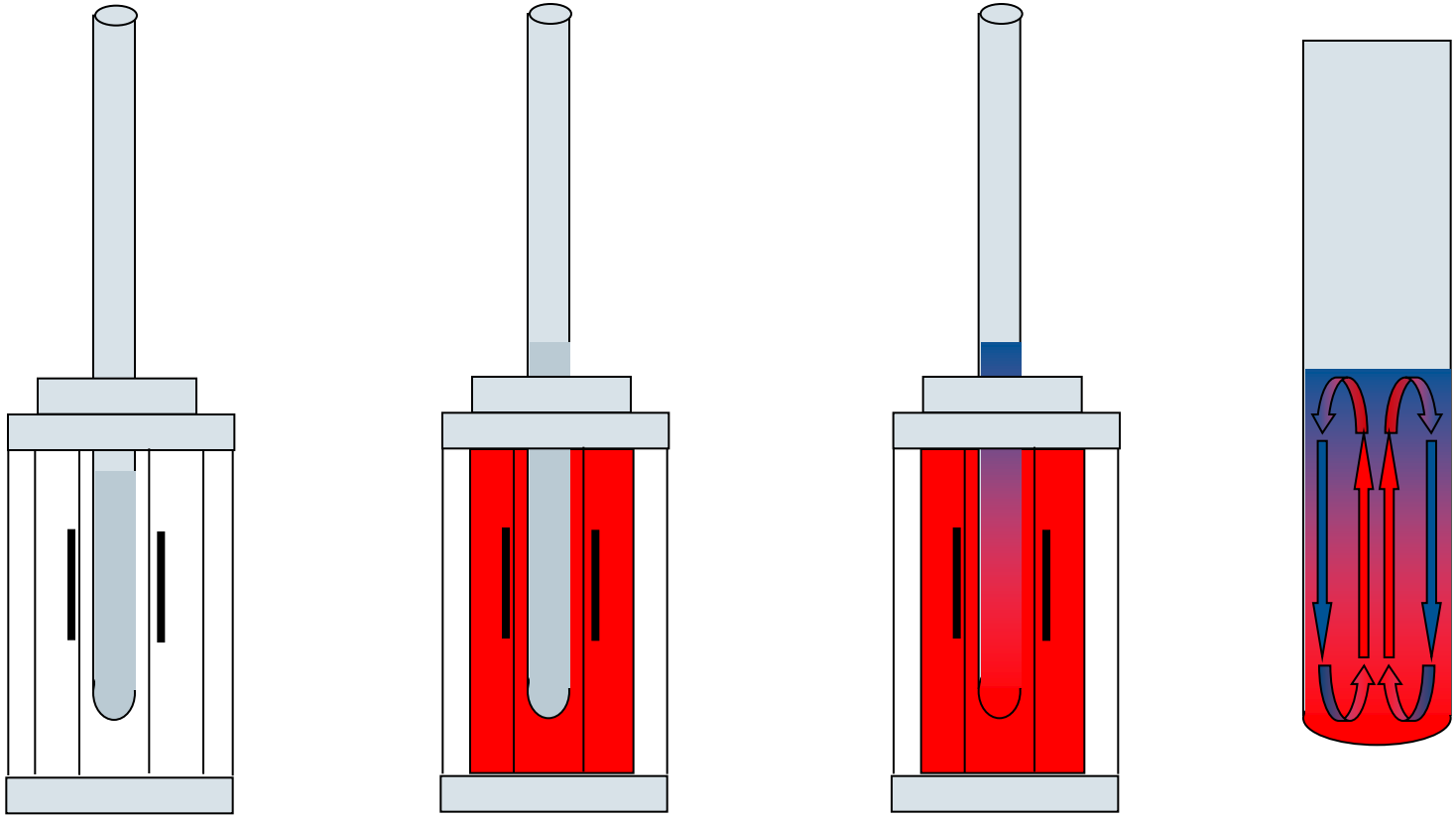
  - Can be used in macros as well

Topshim Tips

- Check ^{2}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

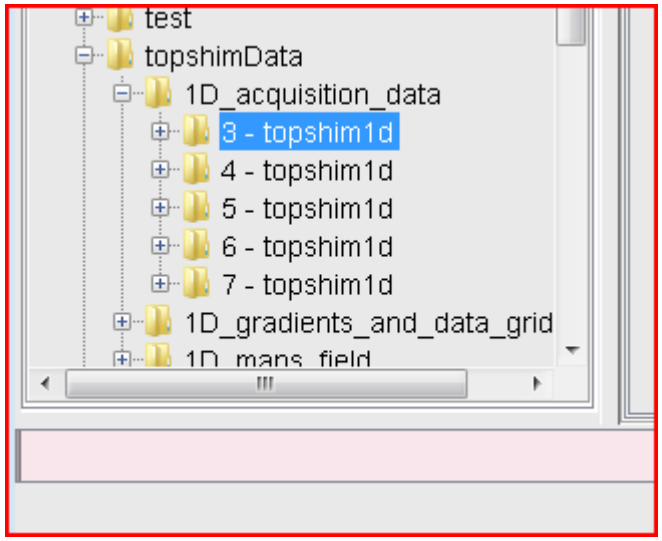


Convection Compensation

- In order to eliminate / reduce convection:
  - Sample filling height =< 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

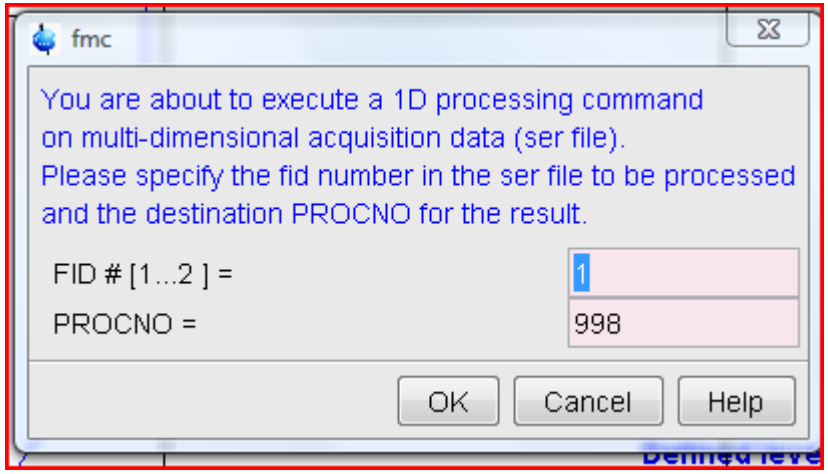
- Troubleshooting Tips
  - Topshim deletes data immediately after execution. The command [***topshim plot***] can be used to save topshim data.



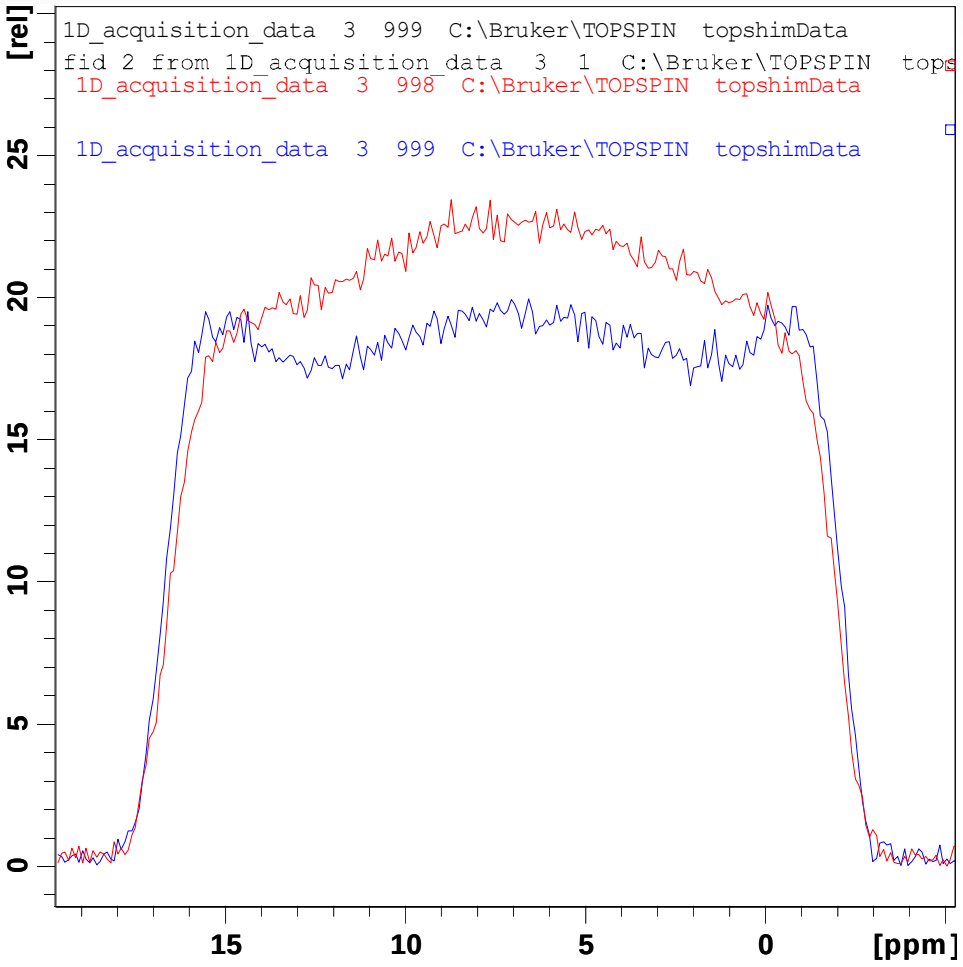
  - Data located in ***<topspin_home>***\data\topshimData

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



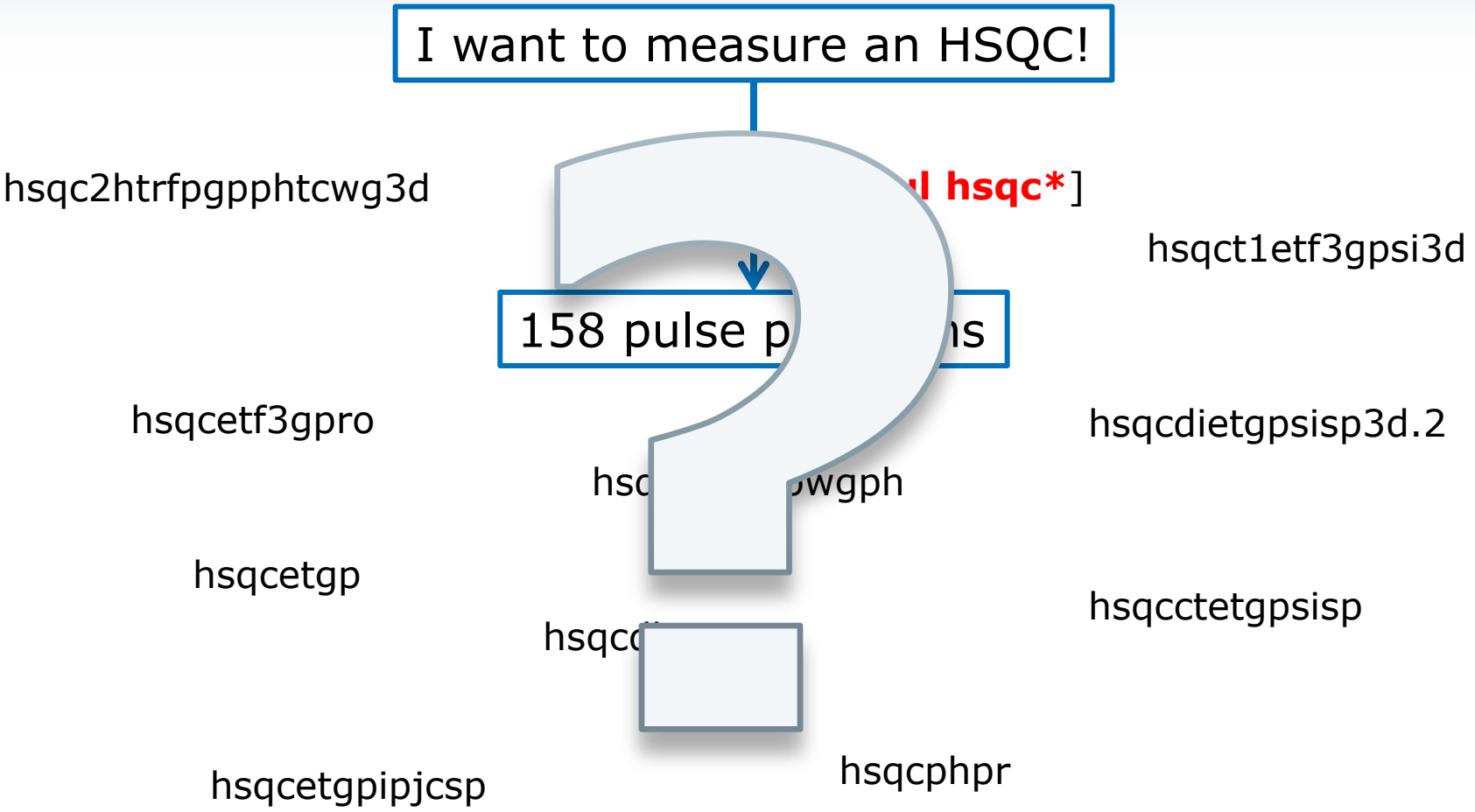
- Profiles should have similar intensity. If long echo time spectrum is <0.5 then there may be homogeneity issues.
- For homogeneity issues try

  [***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



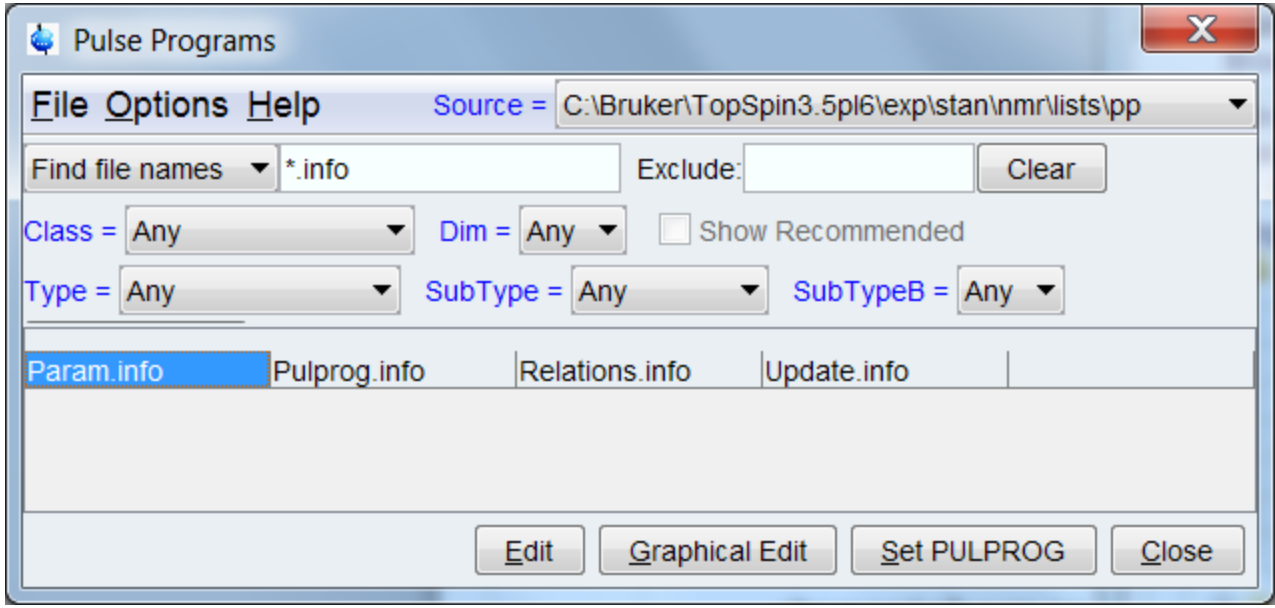
.info files

- .info files can be found in the pp folder

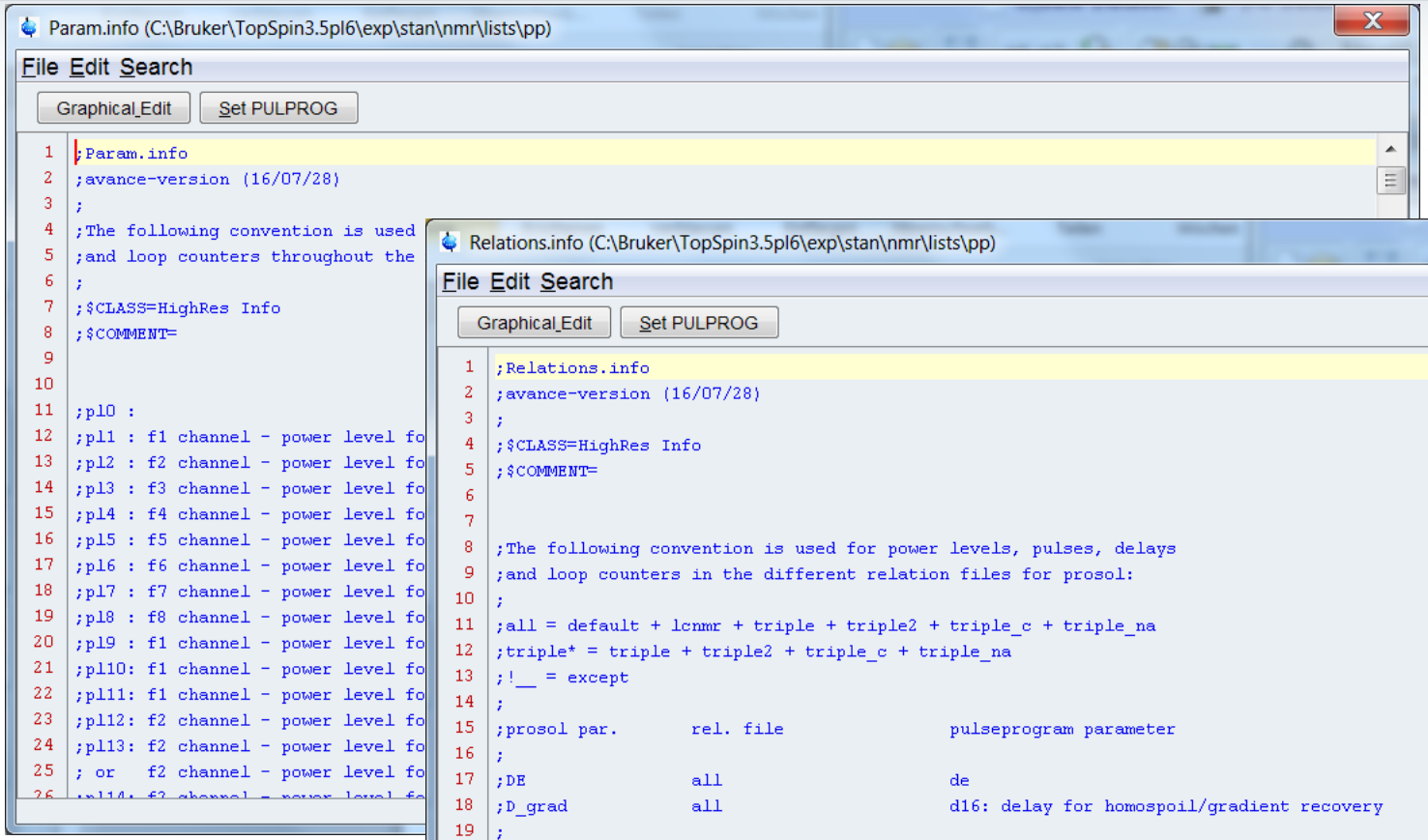
  <topspinhome>\exp\stan\nmr\lists\pp

- [**edpul *.info**]

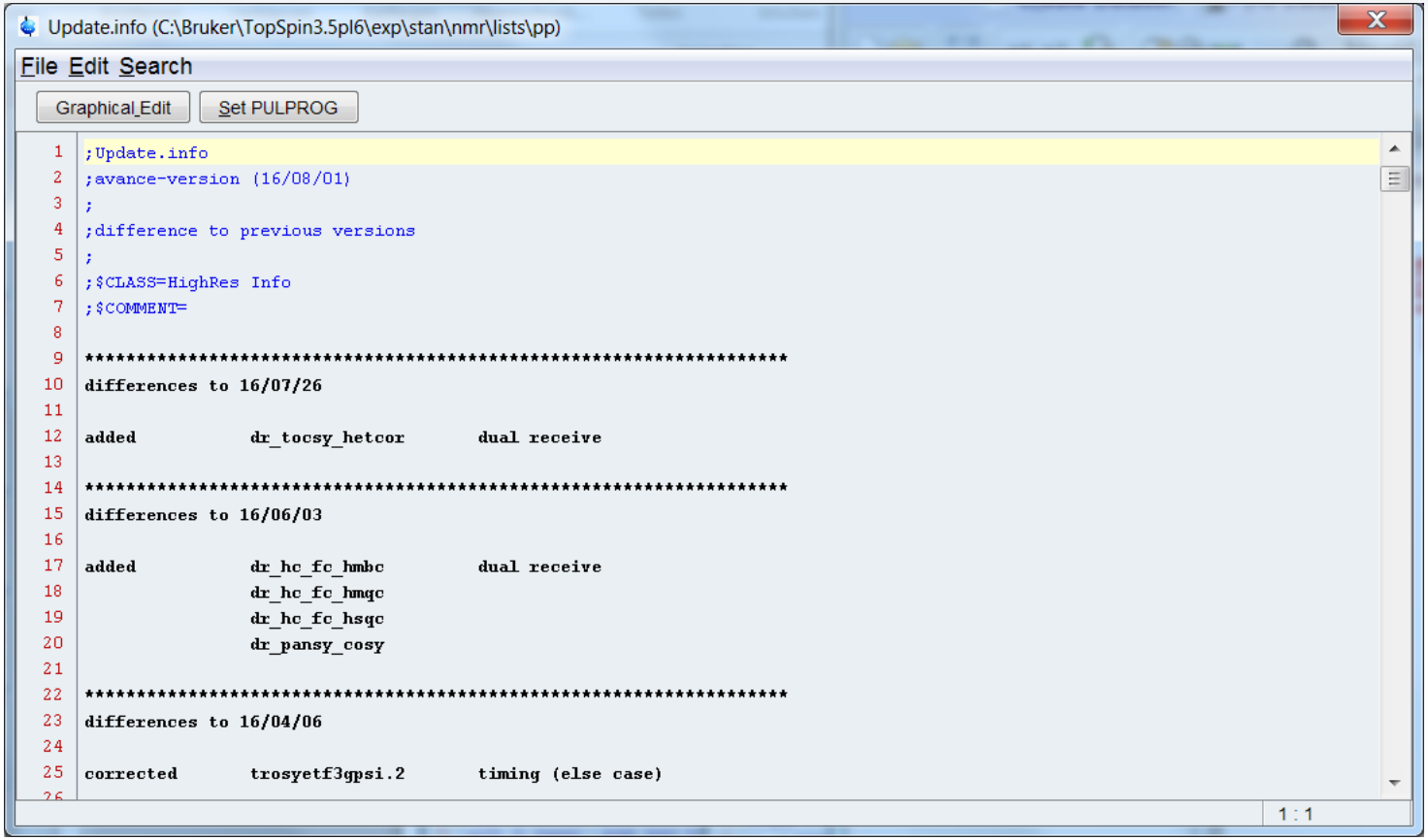
- Available file:
  - Param.info
  - Pulprog.info
  - Relations.info
  - Update.info



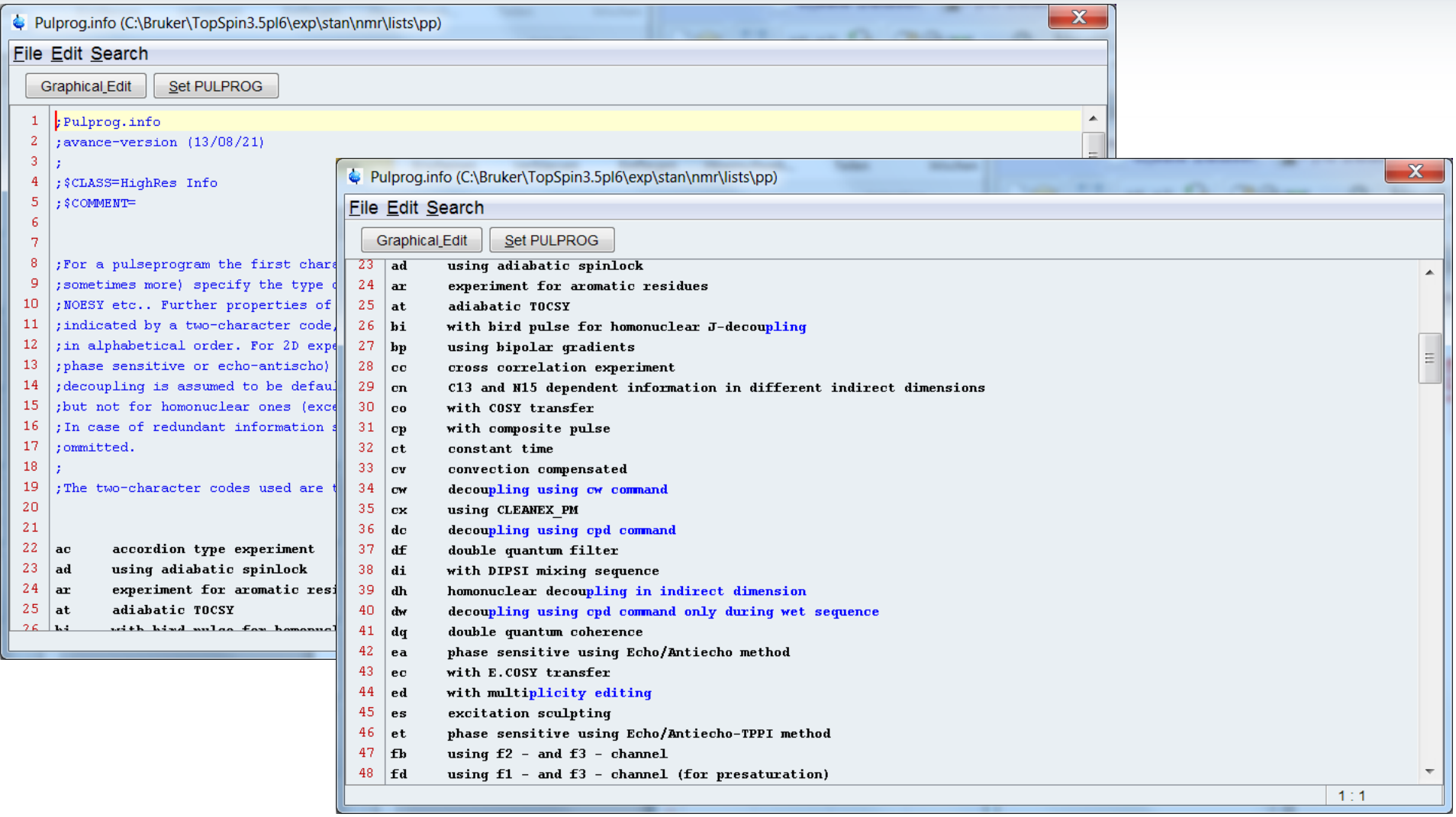
Param.info and Relations.info



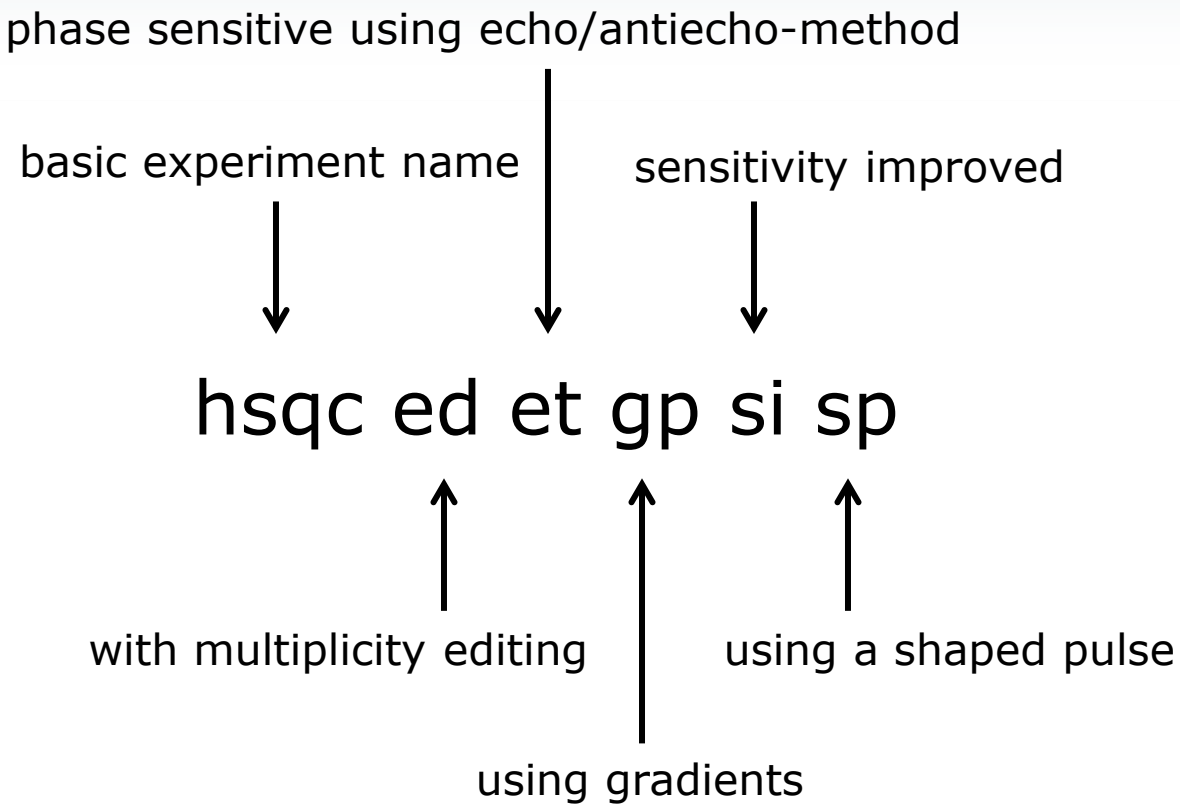
Update.info


Update.info (C:\Bruker\TopSpin3.5pl6\exp\stan\nmr\lists\pp)
File Edit Search
Graphical_Edit
Set PULPROG
;Update.info
;avance-version (16/08/01)
;
;difference to previous versions
;
;$CLASS=HighRes Info
;$COMMENT=
****
differences to 16/07/26
added dr_tocsy_hetcor dual receive
****
differences to 16/06/03
added dr_hc_fc_hmbc dual receive
dr_hc_fc_hmqc
dr_hc_fc_hsqc
dr_pansy_cosy
****
differences to 16/04/06
corrected trosyetf3gpsi.2 timing (else case)
1 : 1

Pulprog.info

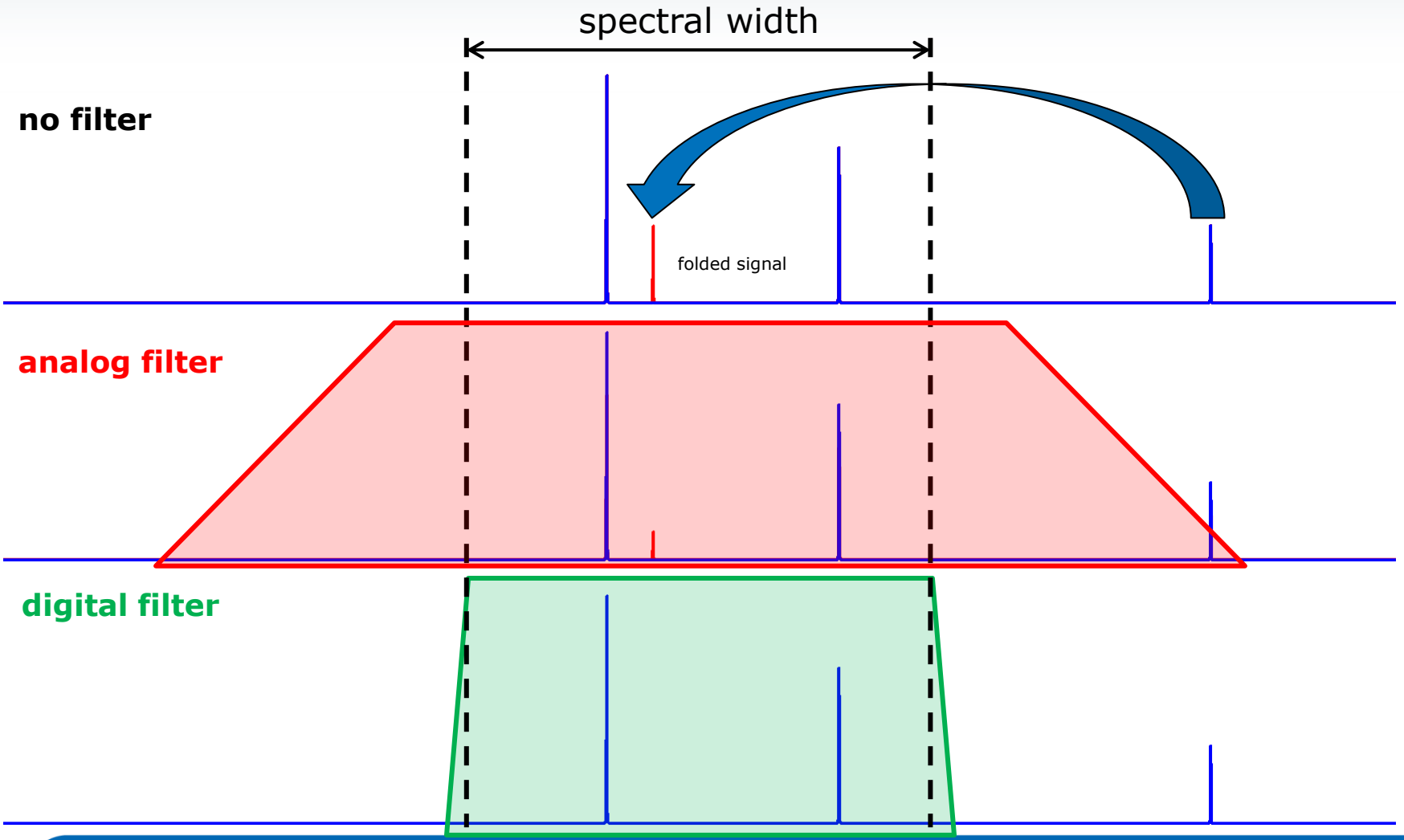


HSQC


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

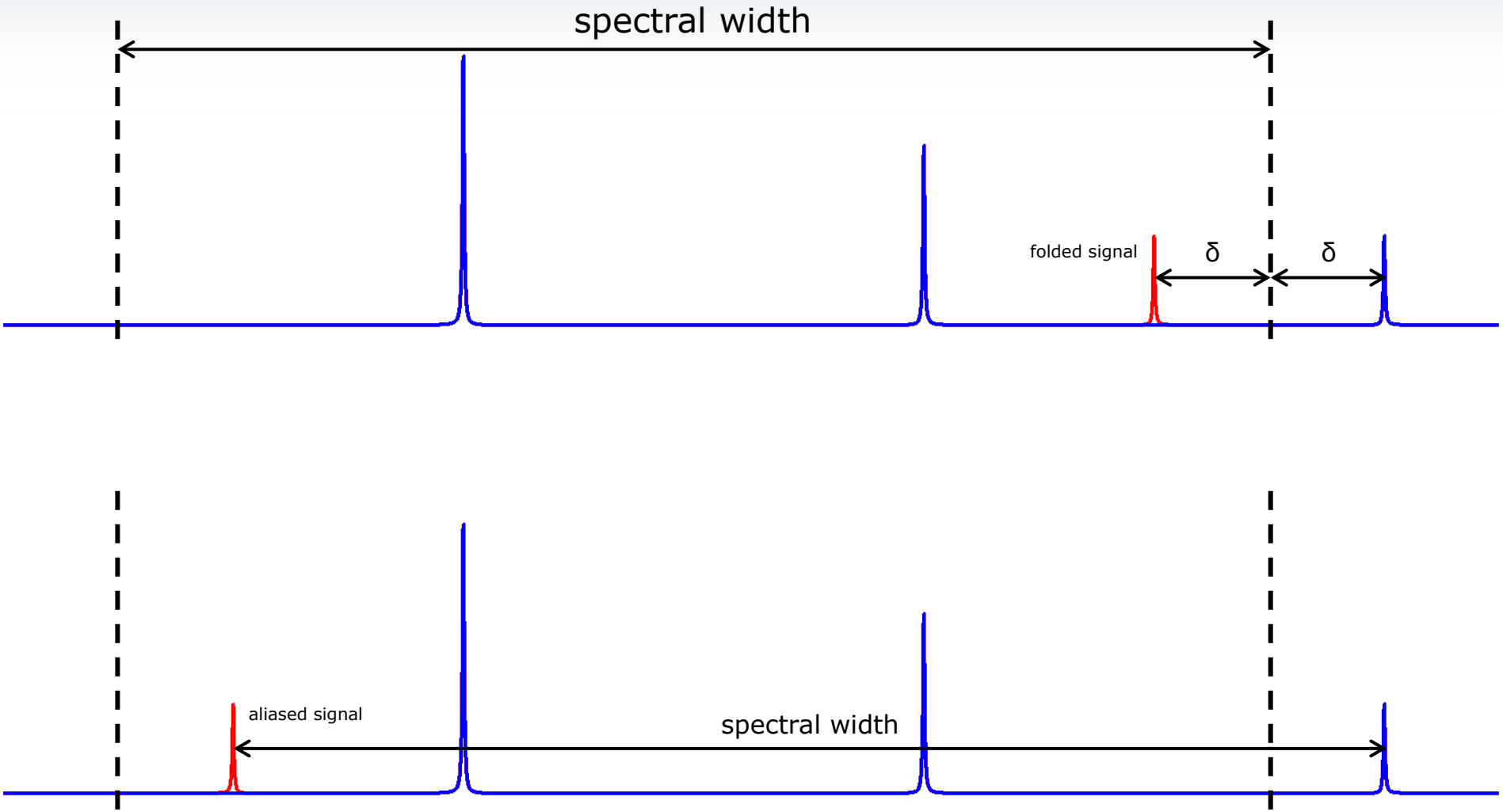
Folding is not a problem for 1D spectra in modern spectrometers due to very efficient filters.

BUT

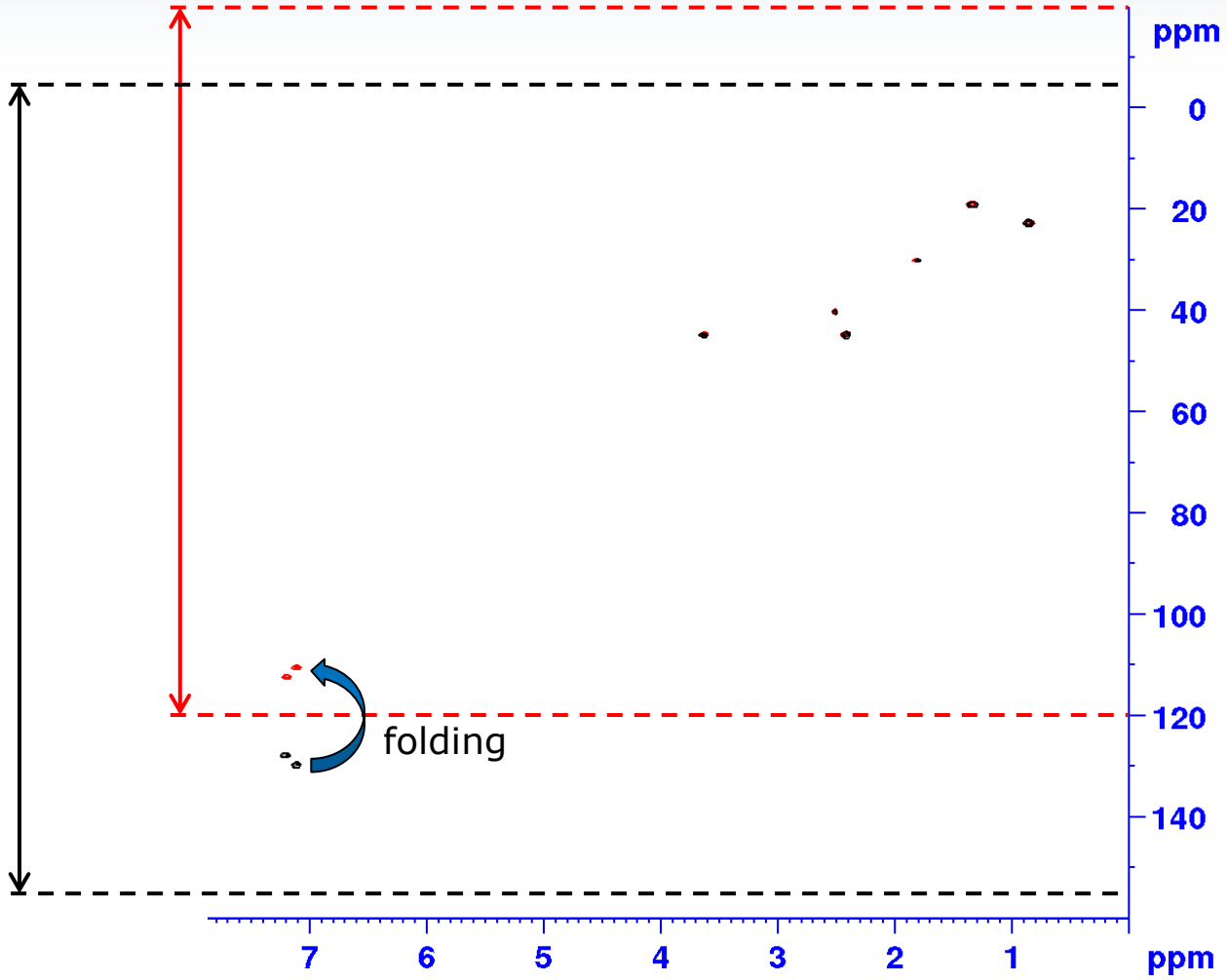
There are no filters in the second dimension!

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

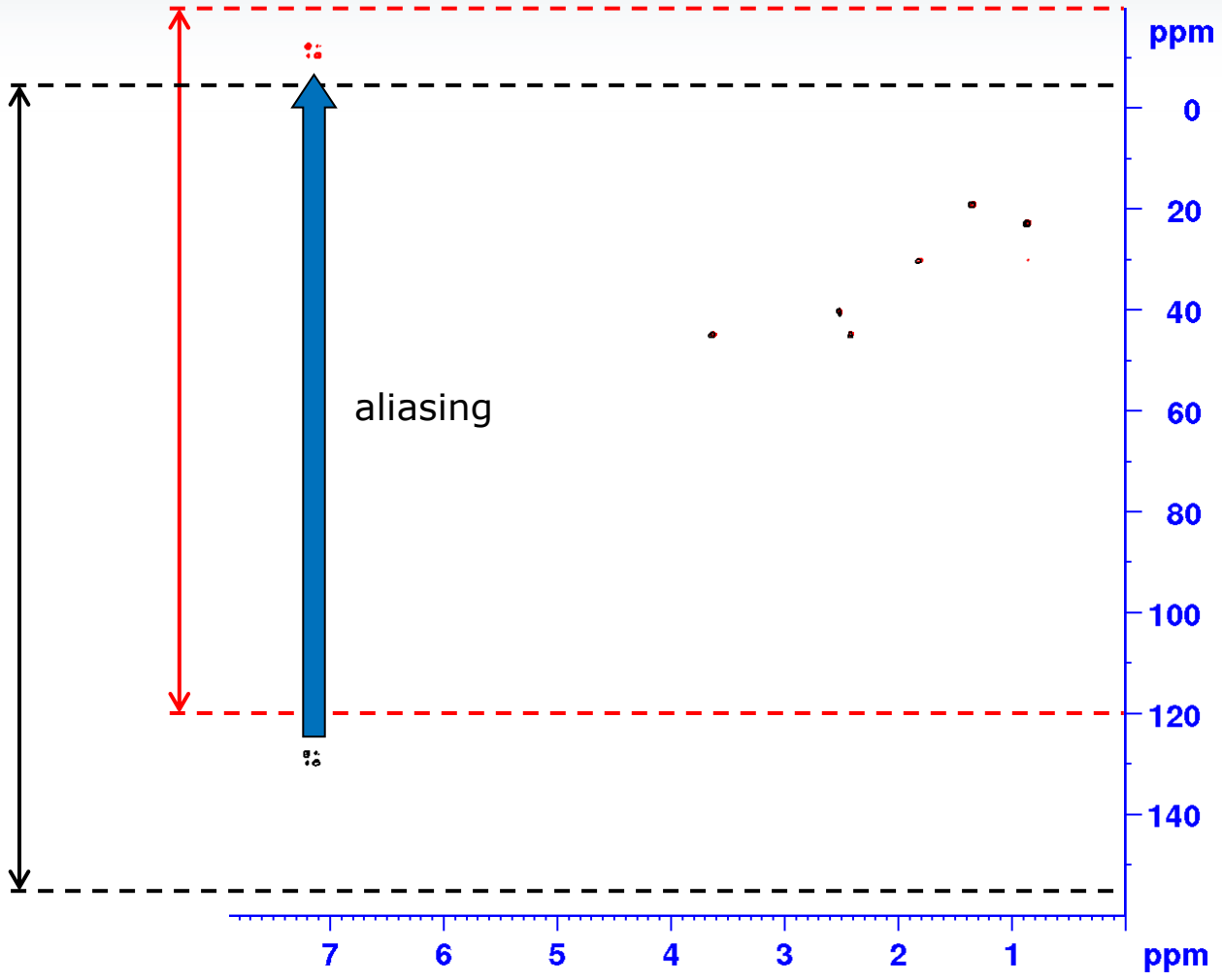
Folding and aliasing



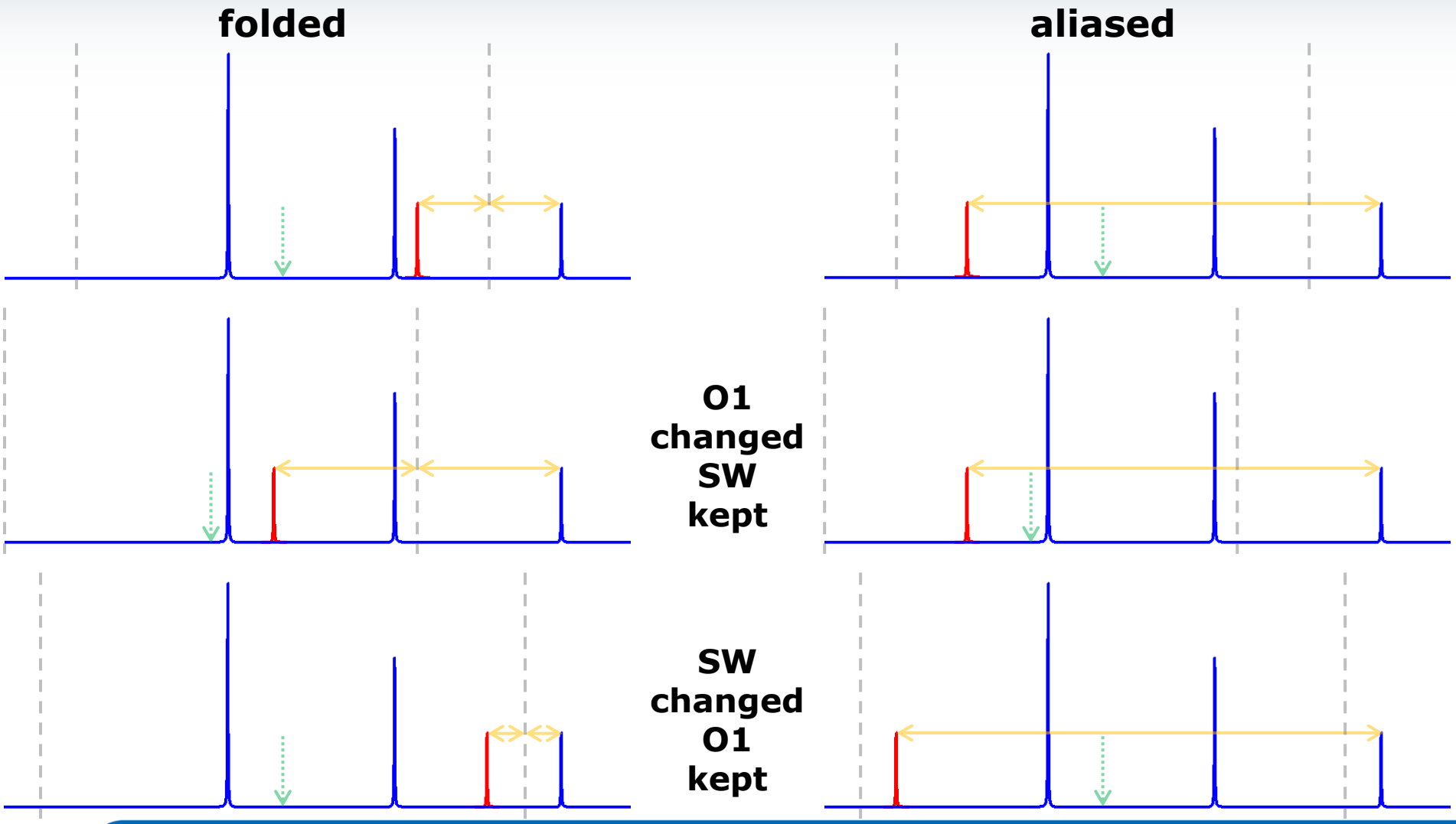
Folding and aliasing

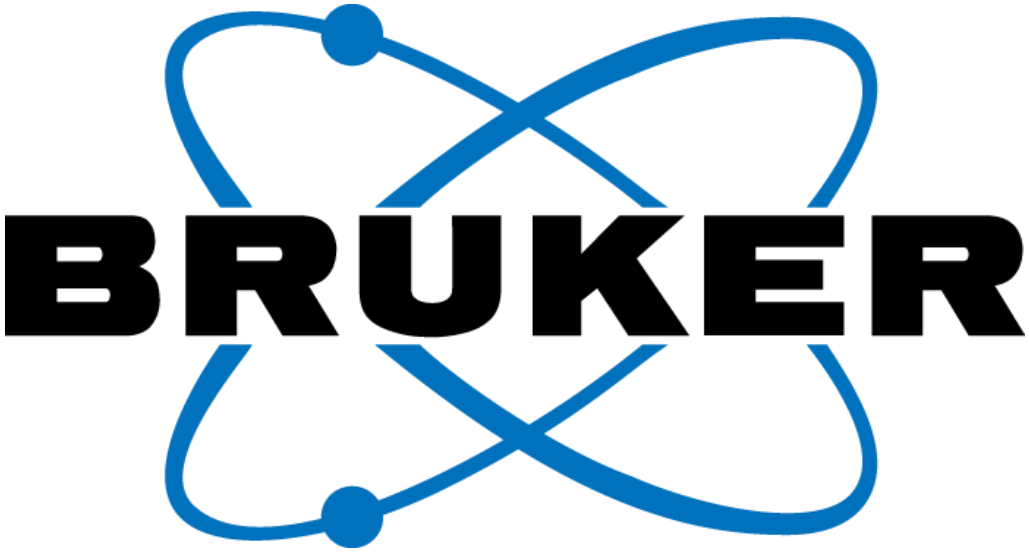


Folding and aliasing



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





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