Not So Short Introduction To TopSpin

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Disclaimer:

This presentation is intended as a basic introduction to TopSpin 3.2 for those who are not familiar with it and the content wishes to cover initial steps to handle the software, sample and spectrometer properly, be able to acquire simple 1/2D NMR spectra and some hints for processing.

If imprecise or unclear formulations encountered, talk to the authors or to more experienced spectroscopist or facility staff.

Everybody is encouraged to study literature and discuss with more experienced colleagues to clarify all below written and become autonomous NMR operator.

Before the TopSpin runs OR some useful UNIX commands (in **blue**) and results (in **red**)

[karelk@nmrcf ~]$ **whoami** - this command displays who's logged in

karelk

[karelk@nmrcf ~]$ **hostname -a** – displays full name of the computer you're working on

nmrcf.ncbr.muni.cz

[karelk@nmrcf ~]$ **quota** – displays space available for the current user

```
Disk quotas for user karelk (uid 16329):
     Filesystem  blocks   quota   limit   grace   files   quota   limit   grace
/dev/nmrspect1/home
                 25964  500000  600000            1146       0       0
```

[karelk@nmrcf ~]$ **quota -g** `lrpi` – displays space available for the nmr data

```
Disk quotas for group lrpi (gid 2005):
     Filesystem  blocks   quota   limit   grace   files   quota   limit   grace
/dev/nmrspect1/data1
               53431604  100000000 110000000           54667       0       0
```

[karelk@nmrcf ~]$ **pwd** – where are you working (print working directory)

/home/karelk

[karelk@nmrcf ~]$ **cd** `/d1/data/karelk/nmr/` - change directory to /d1 …

[karelk@nmrcf nmr]$ **pwd**

/d1/data/karelk/nmr

This is one of the key directories for your NMR experiments, generally it is as follows:

/<disk>/**data**/*<user>*/**nmr**/*<dataset>* - the items in <> and italic (can) change depending on spectrometer and user. Datasets are of any name. Think of a good ordering system at the beginning of your NMR experience …

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>

```
[karelk@nmrcf nmr]$ cd 130118_700b_Dataset/
```
- change directory to a Dataset

```
[karelk@nmrcf 130118_700b_Dataset]$ ls
```
– list directory contents

```
1  10005  2  3  4  5
[karelk@nmrcf 130118_700b_Dataset]$ grep PUL */acqu
```
– print lines matching a pattern, e.g. PUL – it is useful to see what kind of spectra were measured within the dataset, the output is however not sorted

```
10005/acqu:##$PULPROG= <c_con_iasq>
1/acqu:##$PULPROG= <zgpr>
2/acqu:##$PULPROG= <hsqcetf3gpsi2>
3/acqu:##$PULPROG= <c_caconcaco2_ct_nove>
4/acqu:##$PULPROG= <hccconhgp3d3>
5/acqu:##$PULPROG= <c_con_iasq>
[karelk@nmrcf 130118_700b_Dataset]$ grep PUL */acqu | sort -n
```
– here it is sorted by number

```
1/acqu:##$PULPROG= <zgpr>
2/acqu:##$PULPROG= <hsqcetf3gpsi2>
3/acqu:##$PULPROG= <c_caconcaco2_ct_nove>
4/acqu:##$PULPROG= <hccconhgp3d3>
5/acqu:##$PULPROG= <c_con_iasq>
10005/acqu:##$PULPROG= <c_con_iasq>
```

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>

```
[karelk@nmrcf 130118_700b_Dataset]$ cd 1
```
– change directory to Experiment number 1 (ExpNo 1)

```
[karelk@nmrcf 1]$ ls
acqu  acqus  audita.txt  fid  format.temp  pdata  pulseprogram  scon2
shimvalues  specpar  uxnmr.info  uxnmr.par
```
– Note that there are several files and directory pdata.

acqu – contains acquisition parameters prior measurement
acqus – contains info about the parameters as they were at the end of measurement
audita.txt – info about who/where/when ran the measurement
fid – raw one dimensional data
pulseprogram – compiled pulseprogram
pdata – directory containing processed data in different processing number directories (ProcNo, mostly 1)

```
[karelk@nmrcf 1]$ cd pdata
[karelk@nmrcf pdata]$ ls
1
[karelk@nmrcf pdata]$ cd 1/
[karelk@nmrcf 1]$ ls
```
– 1r and 1i are the real and imaginary part of your processed 1D spectrum

```
1i  1r  assocs  auditp.txt  outd  proc  procs  thumb.png  title

[karelk@nmrcf 1]$ pwd
/d1/data/karelk/nmr/130118_700b_Dataset/1/pdata/1
```

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>/<*ExpNo*>/**pdata**/<*ProcNo*>/ - where ExpNo and ProcNo may be integer 1..9999 (may be more but never tested)

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>

Let's move back to the Dataset directory and change directory to nD experiment, here ExpNo 2

```
[karelk@nmrcf 130118_700b_Dataset]$ pwd
/d1/data/karelk/nmr/130118_700b_Dataset
[karelk@nmrcf 130118_700b_Dataset]$ cd 2
[karelk@nmrcf 2]$ ls
acqu  acqu2  acqu2s  acqus  audita.txt  cpdprg3  format.temp  pdata
pulseprogram  scon2  ser  shimvalues  specpar  uxnmr.info  uxnmr.par
```

Note that there is similar content as of 1D experiment with two important differences – **fid** is replaced by **ser** and as there are two dimensions, there are **acqu2** and **acqu2s** files.

```
[karelk@nmrcf 2]$ cd pdata/1/
[karelk@nmrcf 1]$ ls
```

– in case of a 2D spectra, the processed data are in 2rr. Practically speaking one can ignore the imaginary files once the spectrum is properly processed and phased. Keeping just the 2rr file saves disk space.

```
2ii  2ir  2ri  2rr  assocs  auditp.txt  clevels  outd  proc  proc2  proc2s
procs  thumb.png  title
[karelk@nmrcf 1]$ pwd
/d1/data/karelk/nmr/130118_700b_Dataset/2/pdata/1
```

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>/<*ExpNo*>/**pdata**/<*ProcNo*>/

/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>

Let's move back to the Dataset directory and change directory to another nD experiment, this time ExpNo 4 and the pdata to see ProcNos different from 1

```
[karelk@nmrcf 130118_700b_Dataset]$ pwd
/d1/data/karelk/nmr/130118_700b_Dataset
[karelk@nmrcf 130118_700b_Dataset]$ cd 4/pdata
[karelk@nmrcf pdata]$ ls
1  13  23  998  999
```

This is typical content for a 3D dataset where in ExpNo 1 there is the processed 3D, as one has dimensions 1-2-3, ProcNo 13/23 are first planes of the spectral cube in 13/23 direction. 998 and 999 represent that any ProcNo is allowed.

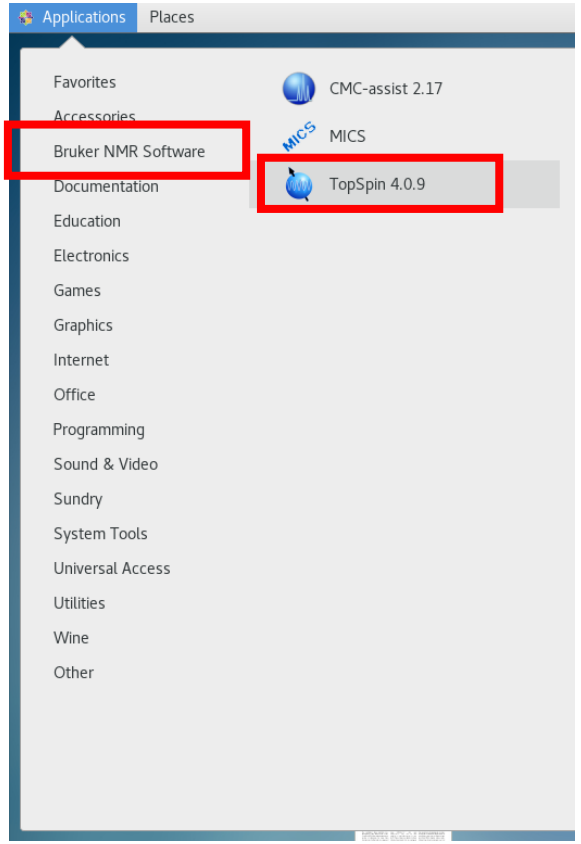
/<*disk*>/**data**/<*user*>/**nmr**/<*dataset*>/<*ExpNo*>/**pdata**/<*ProcNo*>/

NMR Acquisition

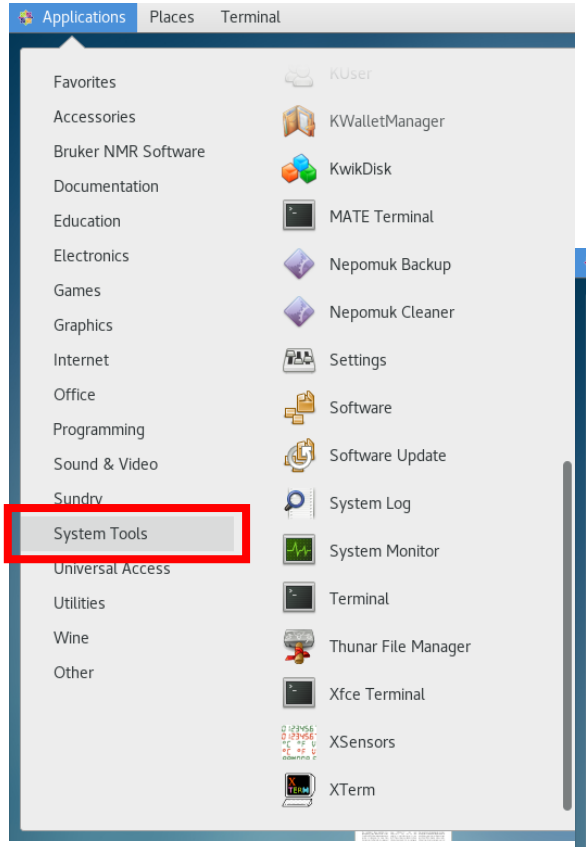
TopSpin

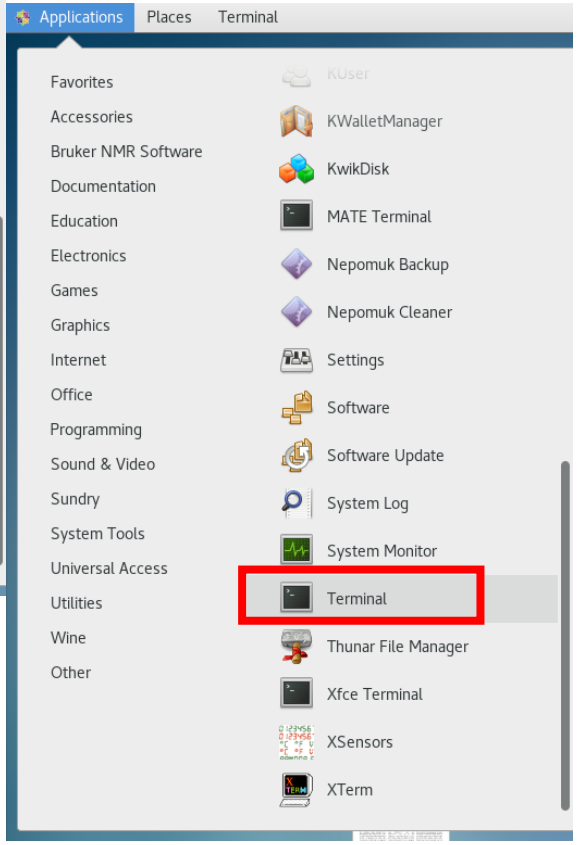
Starting TopSpin – A) Application menu or B) command line

A) Application Menu

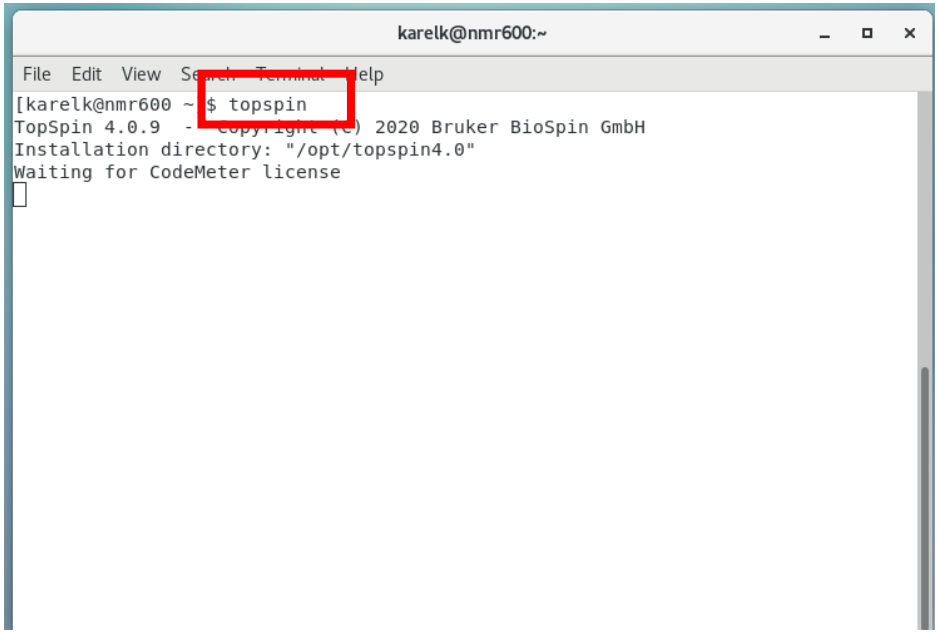


B) Terminal / command line



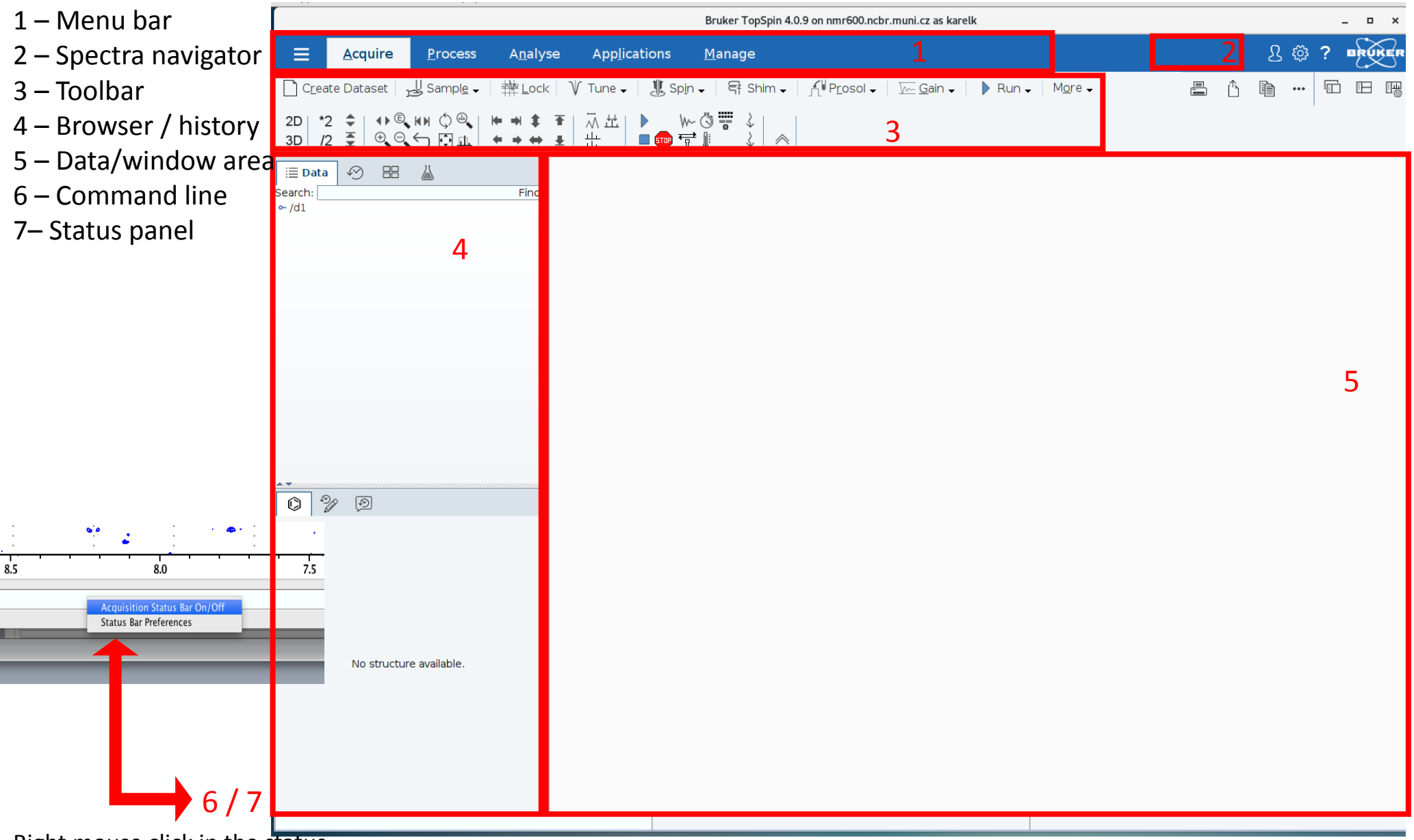


B) Terminal / command line



Topspin 4.X – layout description

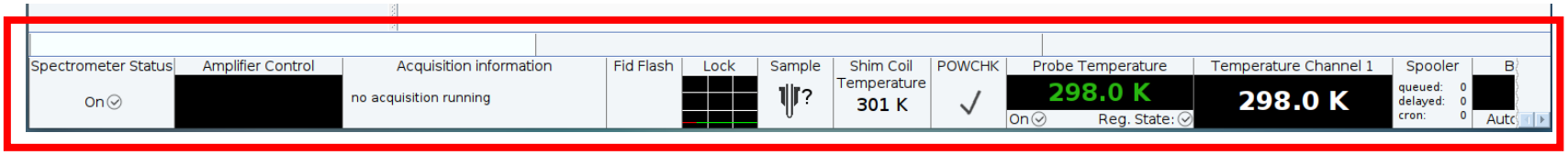
1 – Menu bar
2 – Spectra navigator
3 – Toolbar
4 – Browser / history
5 – Data/window area
6 – Command line
7– Status panel



Right mouse click in the status bar area toggles it on/off

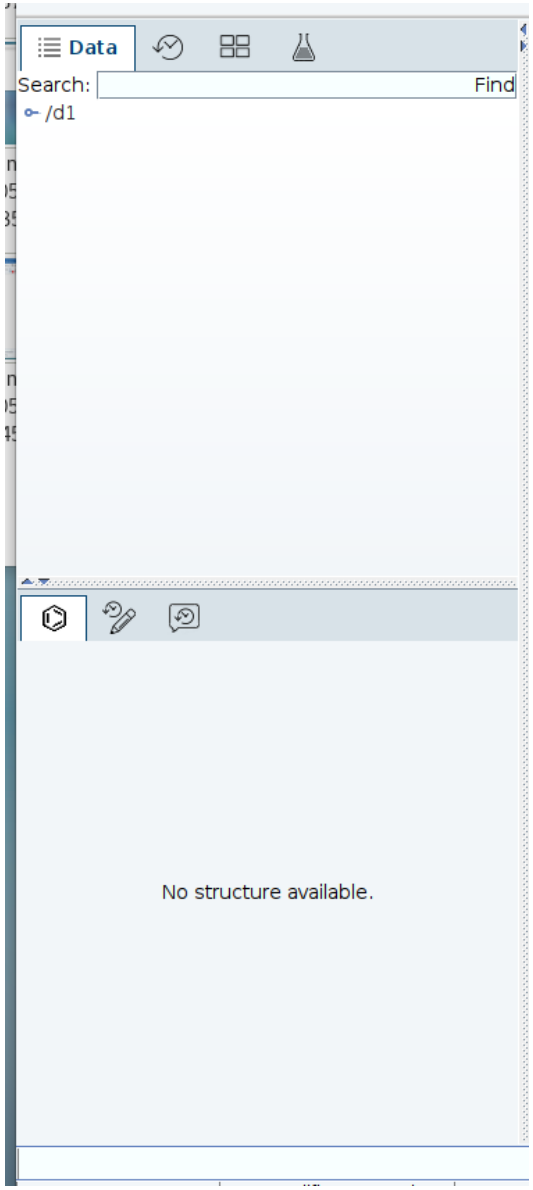
Topspin 4.X – layout description

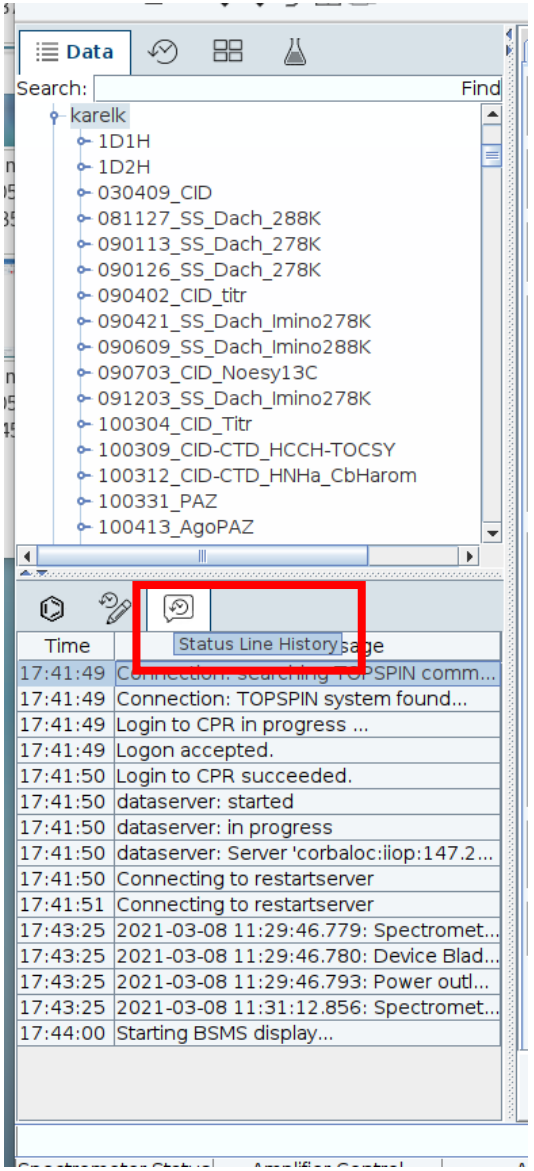
1 – Menu bar
2 – Spectra navigator
3 – Toolbar
4 – Browser / history
5 – Data/window area
6 – Command line
7– Status panel

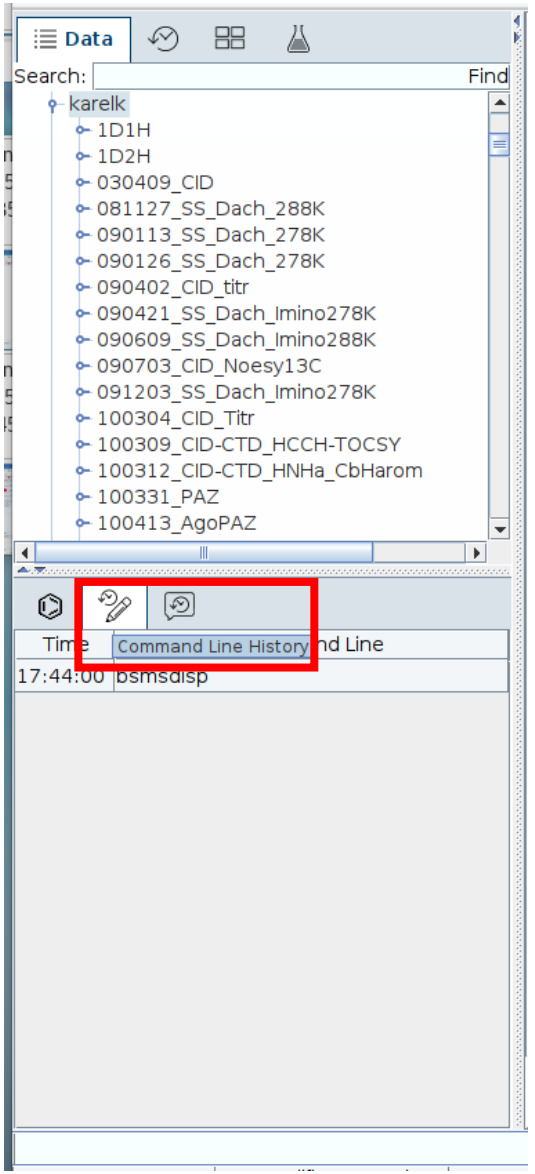


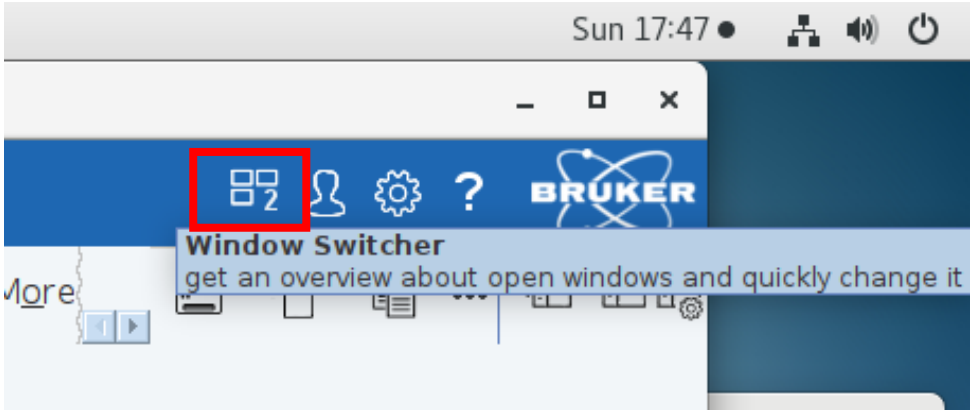
Topspin 4.X – layout description

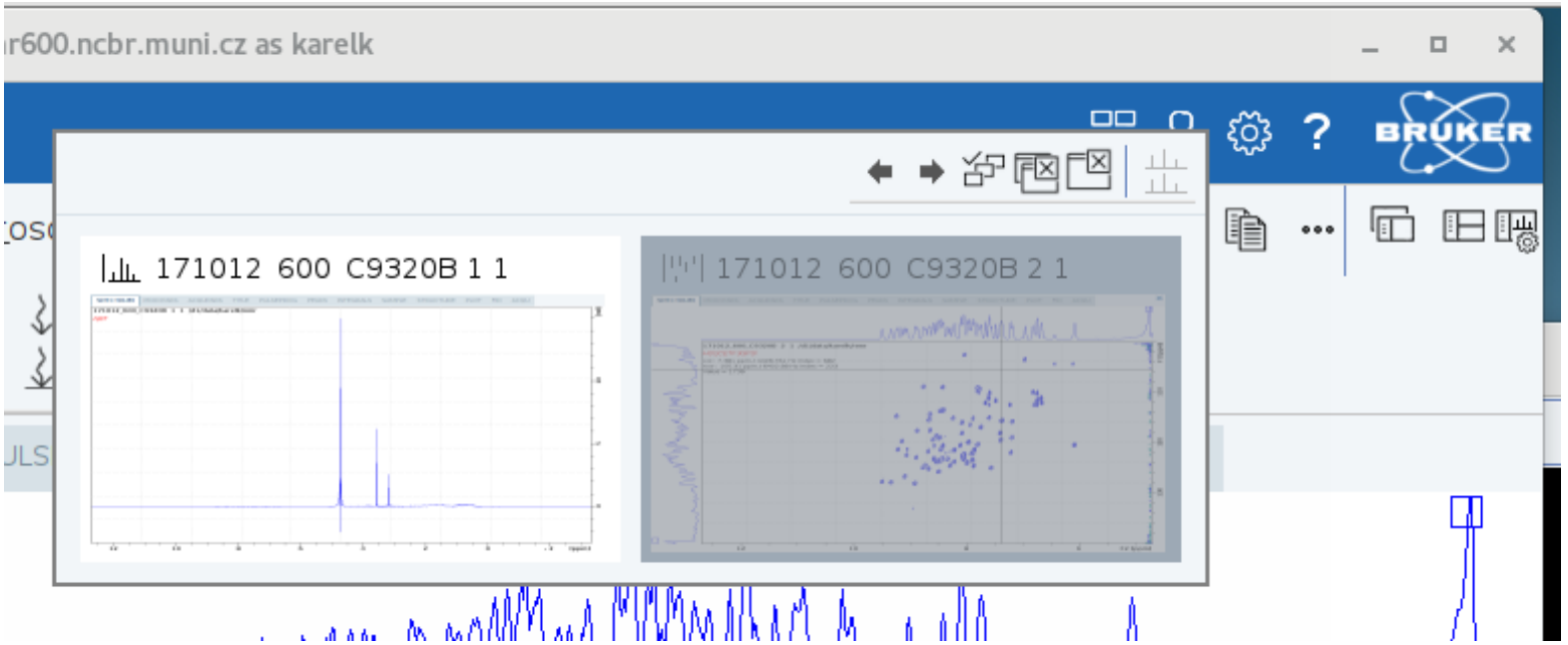
Browser / history

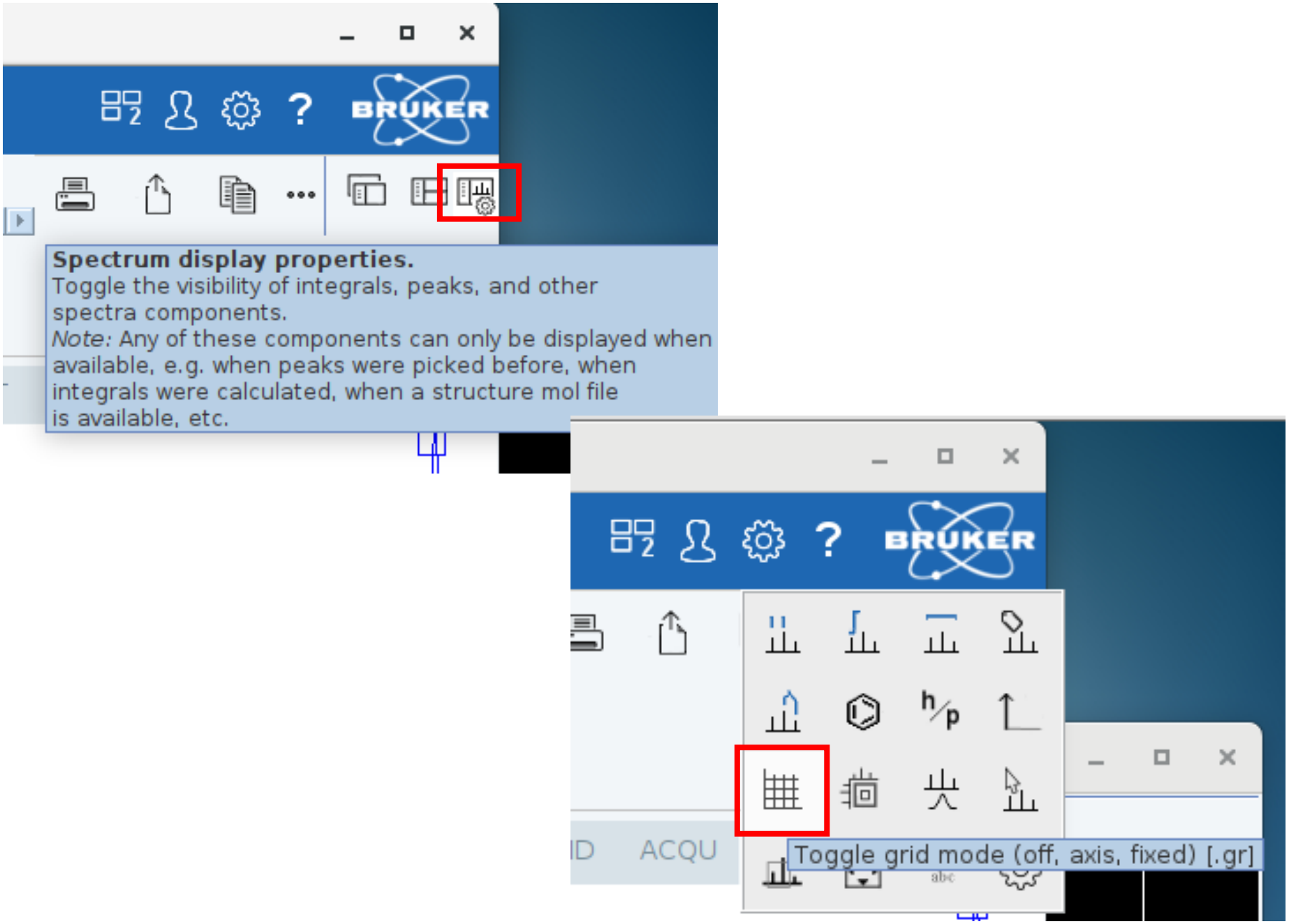







Sun 17:47
BRUKER
Window Switcher
get an overview about open windows and quickly change it


r600.ncbr.muni.cz as karelk
BRUKER
171012 600 C9320B 1 1
171012 600 C9320B 2 1


BRUKER
Spectrum display properties.
Toggle the visibility of integrals, peaks, and other spectra components.
Note: Any of these components can only be displayed when available, e.g. when peaks were picked before, when integrals were calculated, when a structure mol file is available, etc.
BRUKER
ACQU
Toggle grid mode (off, axis, fixed) [.gr]

Notes to TopSpin

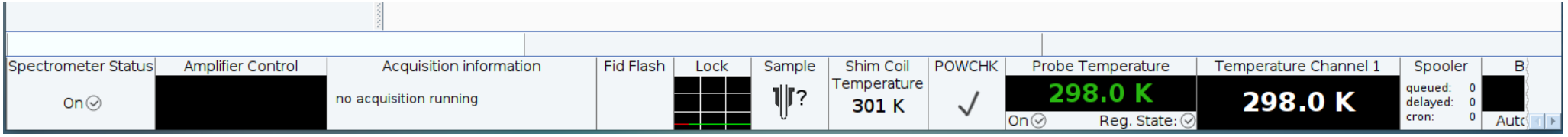
When you open Topspin for the first time under your own account, you need to set up a few things

-Set the path to data
-Create directory for your data
-Create a dataset in your data directory
-Open and customize the status panel
-Disable zg safety
-Other customizations available – colors, # of command lines, lock grid lines etc.

The preferences are stored in your home directory, e.g.
/home/fiala
In the directory .topspin1
May contain some junk, the essential file is
/home/fiala/.topspin1/prop/globals.prop

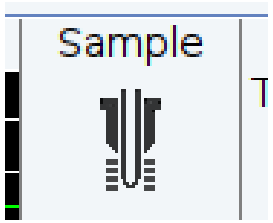
If you want to use someone else‘s setup, copy this direktory/file from his/her home directory to your home directory before starting TopSpin

- Prior any manipulation with the spectrometer or TopSpin, check the status panel first – namely, what is the acquisition status, temperature and what is the lock signal status.

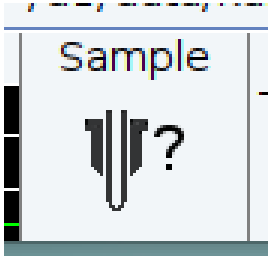


- In case there is a sample of your colleague still in the magnet, take it out from the magnet (*vide infra*) and take best care about the sample.

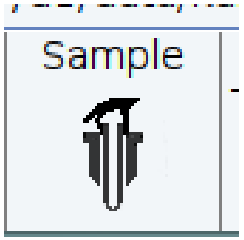
Sample status:



down

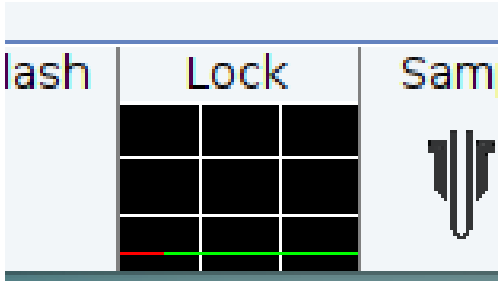


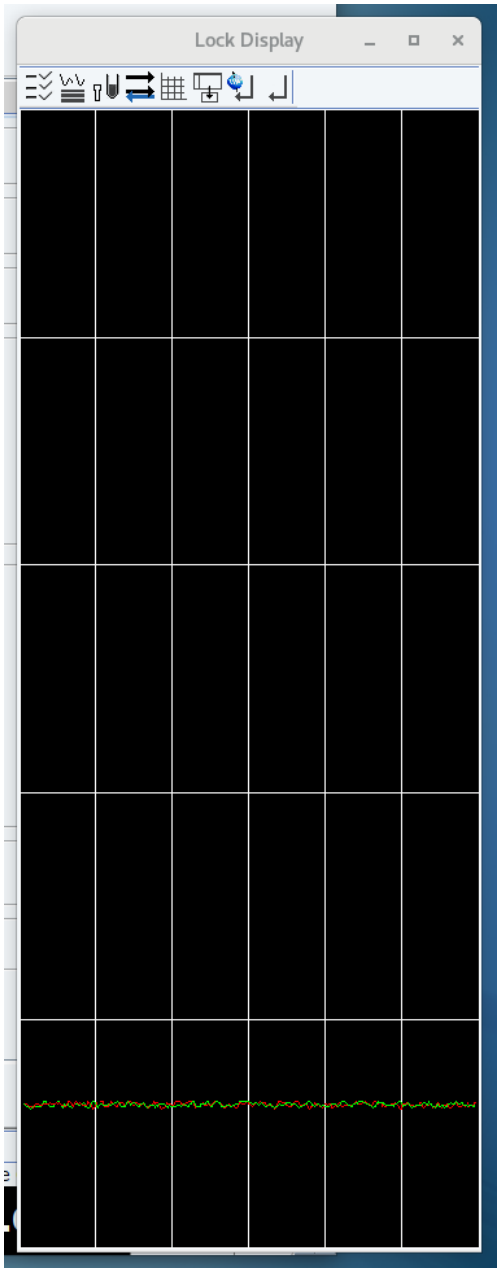
missing



up

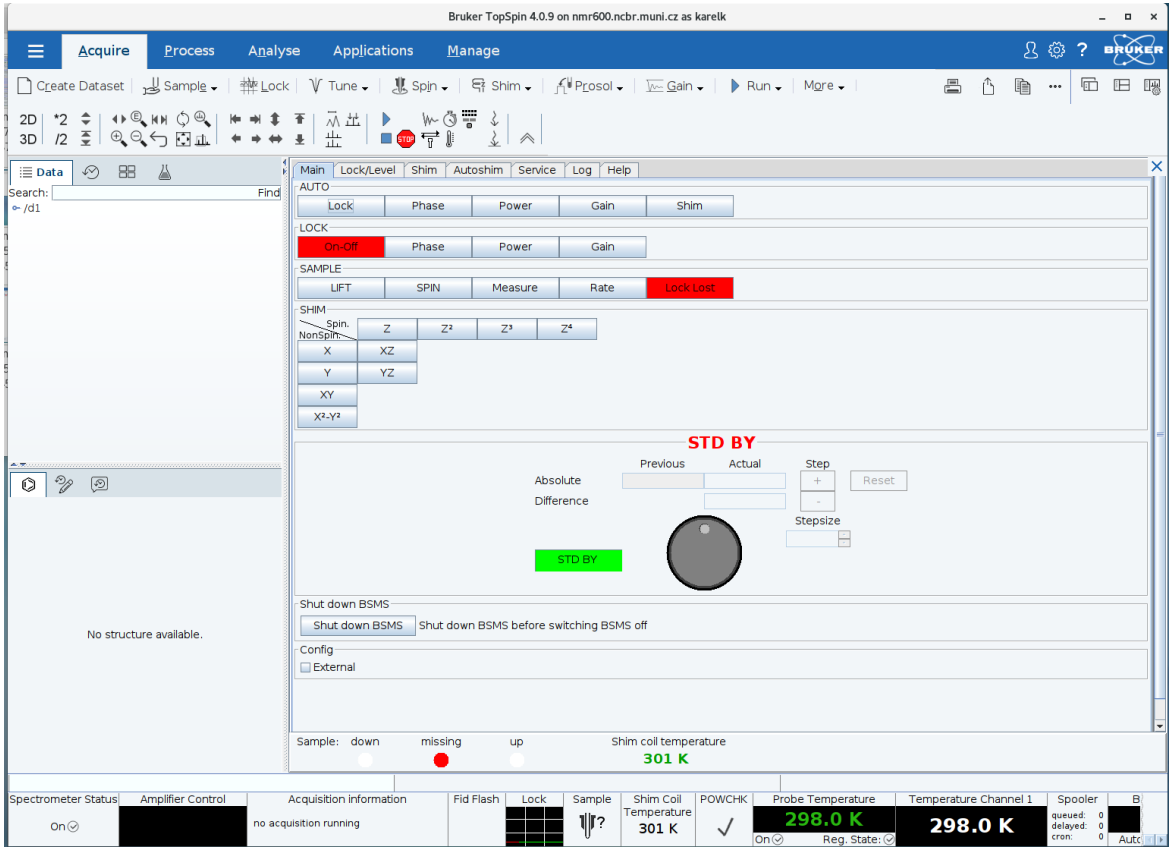
- To eject the sample from the magnet – assure yourself there is no acquisition running, the lock is off (no straight line is sweeping the lock window but dispersive sinusoidal curves can be seen in the lower area of the lock window – see the figure on the right) and there is no mechanical obstacle that could prevent sample ejection. Then either type **ej** on the cl or use the BSMS window to manipulate with the lift.

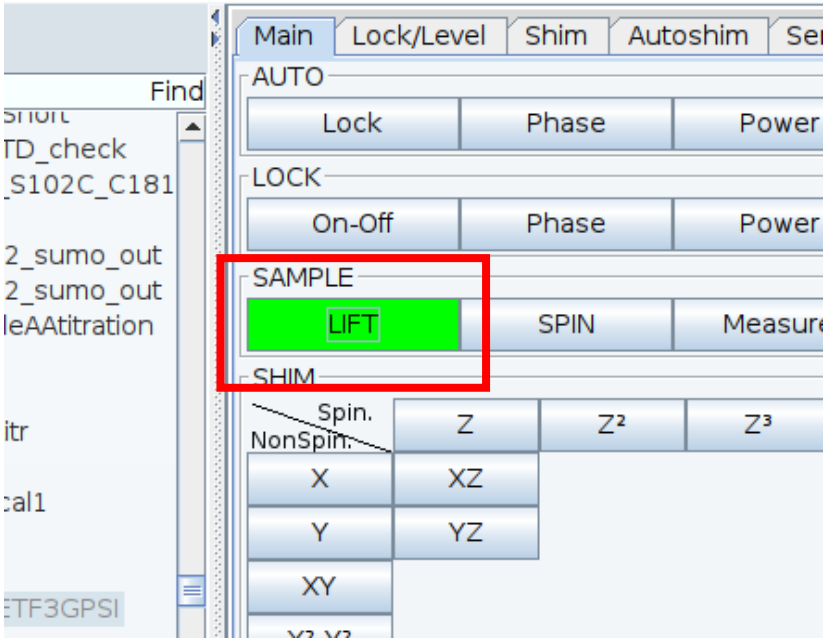




Ejecting the sample

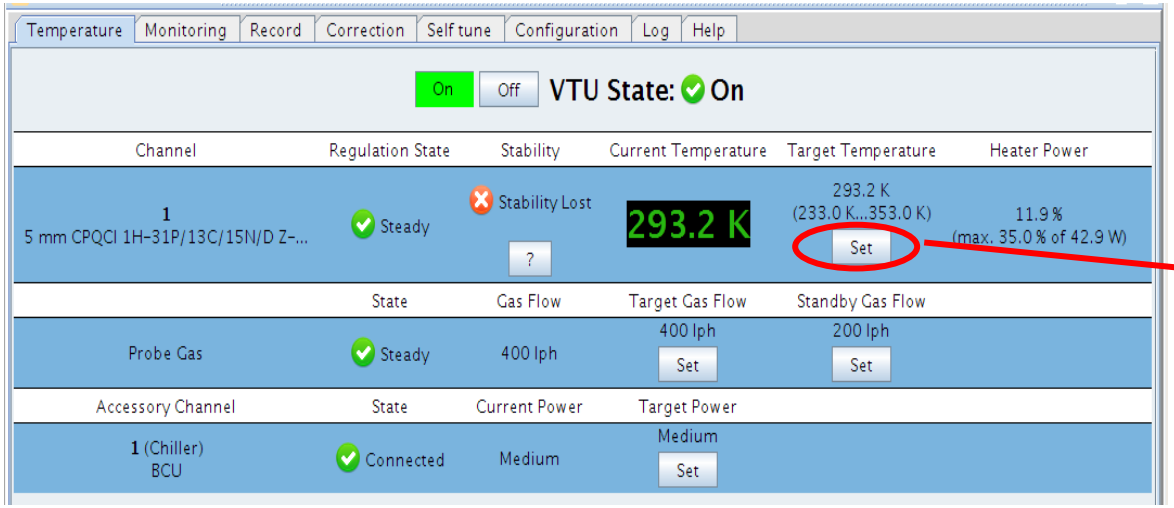
- In the Main panel of the BSMS window (Bruker Smart Matching /Shimming system), click Sample - Lift for turning on the airflow (button turns green)
- Sample mail will be set in action and brings the sample down
- Spectrometers that have no sample mail will announce the sample ejection by increased airflow and sample „dancing“ on the top of the magnet. In this case, after removing the sample from the magnet, do not forget to switch off the airflow by clicking the Lift button once again (turns grey)

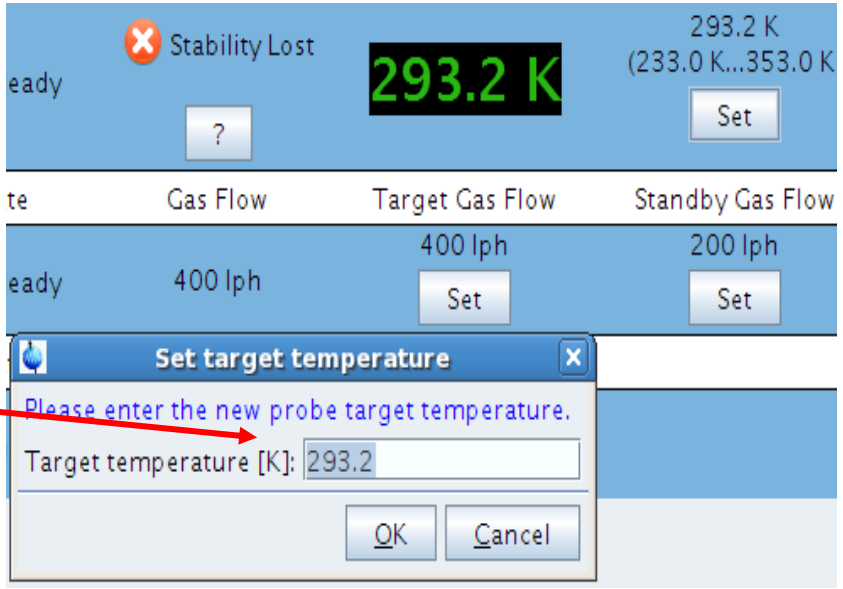


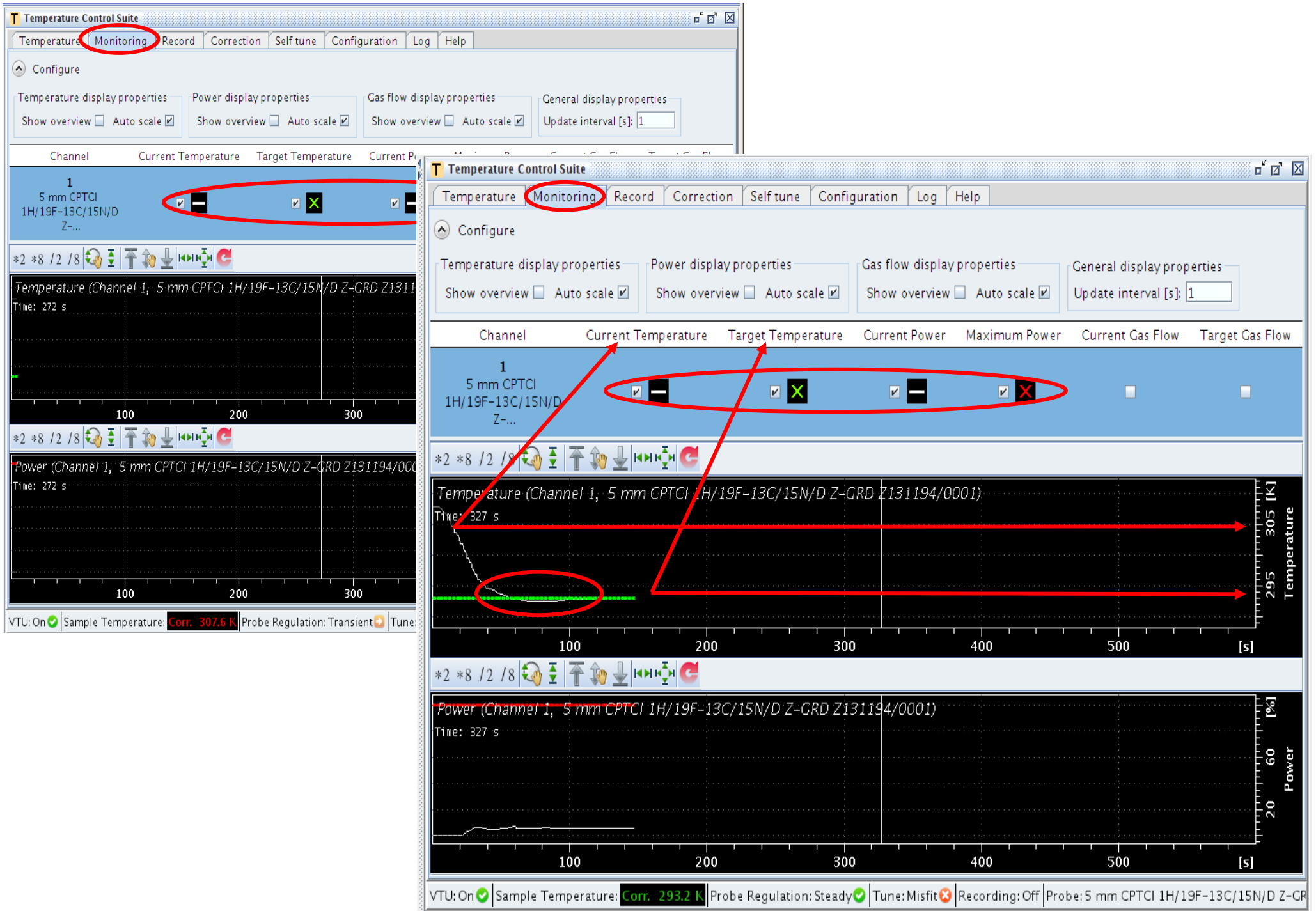


Temperature control

- Once there is no sample in the magnet, set the temperature you want for your sample/measurement – this is done by opening the Temperature control window. This can be done by ***i)*** double click the Sample Temperature window in the status panel, ***ii)*** type **edte** in the command line (cl). This will open new window called **T**. Set the temperature and in the monitoring tab check first four check-boxes and control the progress of temperature. As soon as the temperature is stabilized, you may insert your sample into the magnet.





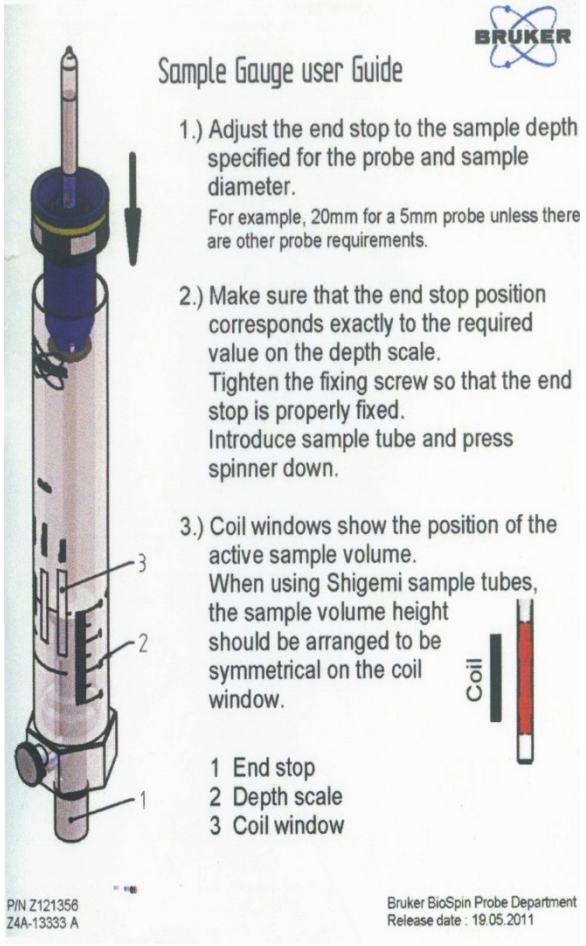

Temperature Control Suite
Temperature
Monitoring
Record
Correction
Self tune
Configuration
Log
Help
Configure
Temperature display properties
Show overview
Auto scale
Power display properties
Show overview
Auto scale
Gas flow display properties
Show overview
Auto scale
General display properties
Update interval [s]: 1
Channel
Current Temperature
Target Temperature
Current Power
Maximum Power
Current Gas Flow
Target Gas Flow
1
5 mm CPTCI
1H/19F-13C/15N/D
Z-...
Temperature (Channel 1, 5 mm CPTCI 1H/19F-13C/15N/D Z-GRD Z131194/0001)
Time: 327 s
Temperature [K]
305
295
100
200
300
400
500
[s]
Power (Channel 1, 5 mm CPTCI 1H/19F-13C/15N/D Z-GRD Z131194/0001)
Time: 327 s
Power [%]
60
20
VTU: On
Sample Temperature: Corr. 293.2 K
Probe Regulation: Steady
Tune: Misfit
Recording: Off
Probe: 5 mm CPTCI 1H/19F-13C/15N/D Z-GR

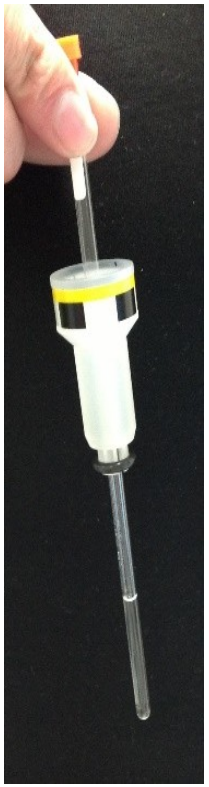
Sample/tube handling

- Inserting the tube into the spinner
  - Do not push the tube straight down the spinner, the tube may break down
  - Screw the tube slowly into the spinner and at the same time gently push the spinner upward the tube

Adjusting/centering the tube in the spinner

- Put the tube with the spinner into a depth gauge and screw the tube until the length of the sample is symmetrical around the middle line crossing the coil-representing boxes



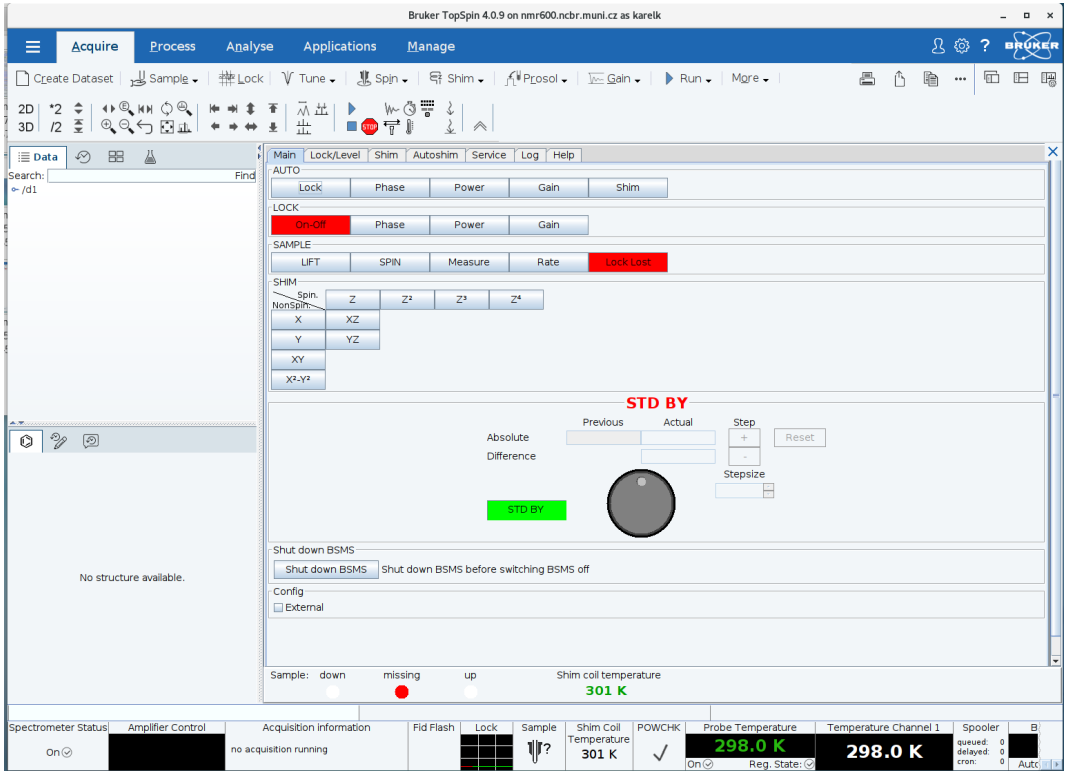


Notes to temperature control

- Temperature is maintained by flowing cooled/heated gas around the sample.
- Sample temperature may differ from the set temperature (sensor not in the sample, calibration of the sensor itself) – calibration needed
- Calibrations based on the change of chemical shift with temperature
- Methanol (neat, 4%, 0.2%), 25^{o}C and down
- Ethylenglycol, 30^{o}C and up
- NMR thermometer – calibration substance added to buffer, ^{2}H spectra measured during the experiment run, special locking parameters must include the calibration data

Inserting the sample

- After the desired temperature is reached, in the Main panel of the BSMS window (Bruker Smart Magnet System), click (skip this in case of sample mail) Sample - Lift for turning on the airflow (button turns green)
- Place the sample either into the sample mail or on the top of the magnet bore when „maximum“ airflow is reached
- Turn off the airflow by clicking the Lift button again (goes gray again)
- For the magnets equipped with sample mail, place the spinner with the tube into the sample rack and close it
- Wait until the sample is positioned in the probe



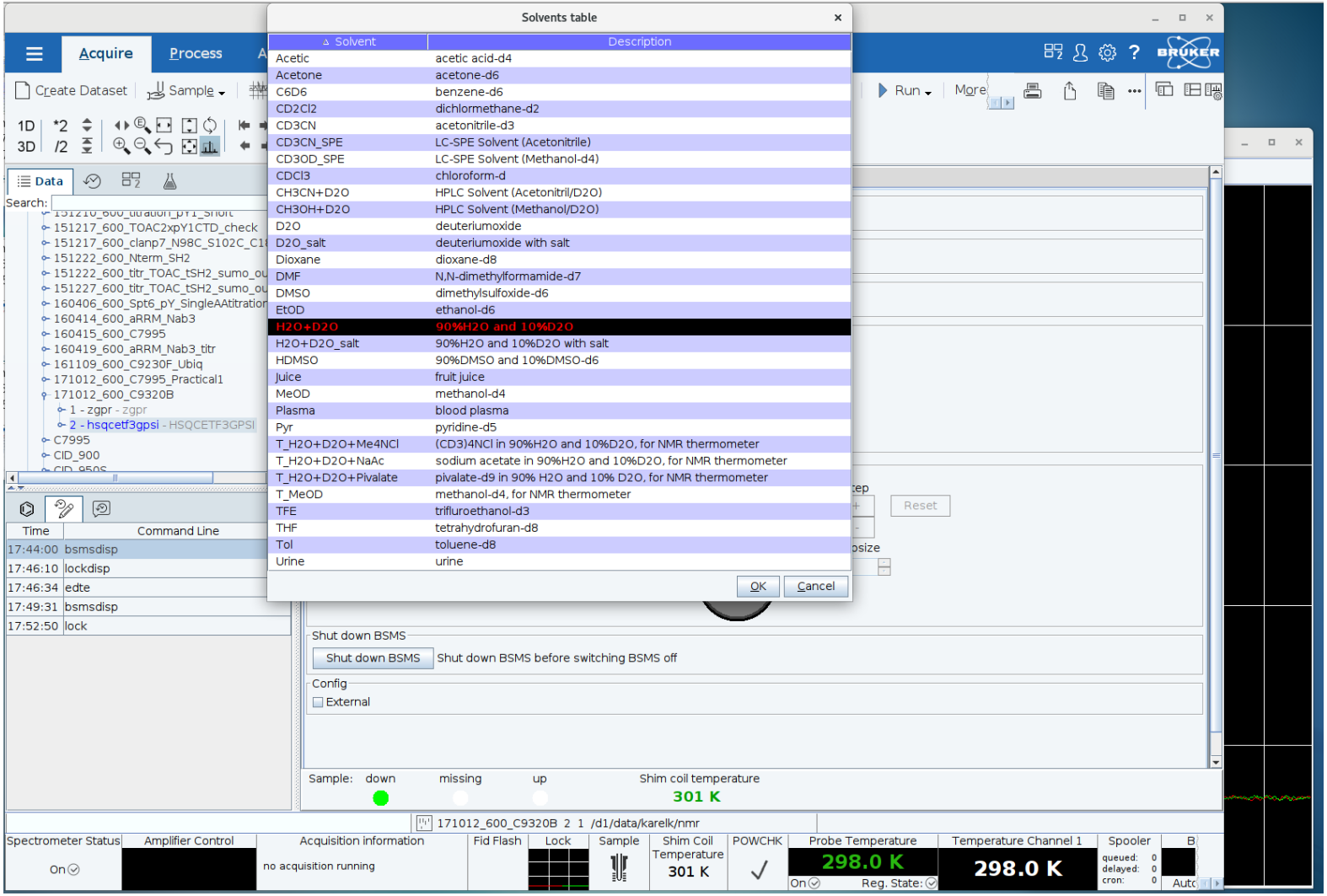
Locking the magnetic field

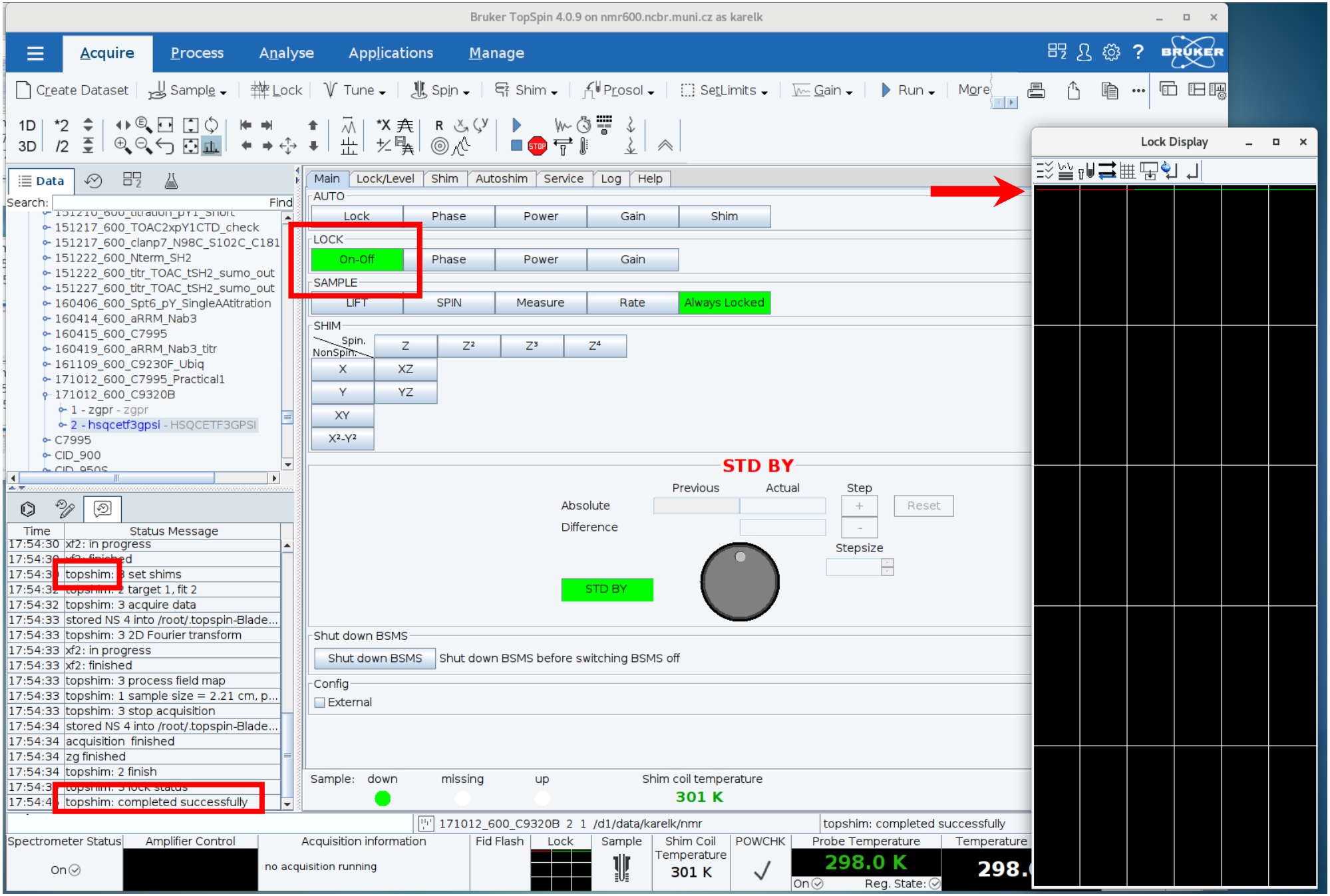
- Locking on the reference nuclei (deuterium)
- Once the sample is in the magnet (check Sample status icon on status bar, status LED of the sample mail etc.), usually lock signal appears as red/green signal in dispersion mode. In case you do not see that line – it may indicate that previous user was using different deuterated solvent or there is no ^{2}H in your sample. Undocumented **lopo** command reads in the lock parameters without engaging the lock.
- Type **lock** on the cl

Note: you may not be able to lock if the field homogeneity is poor. It is a good idea to read in suitable shims before you try to lock (command **rsh**). If you do not have suitable shims, look for a recent sucrose (for water samples) or lineshape (organic solvents) files.

Locking the magnetic field

- Table with solvents will pop up
- Select the right solvent from the table and click the OK-button or double click the solvent of choice



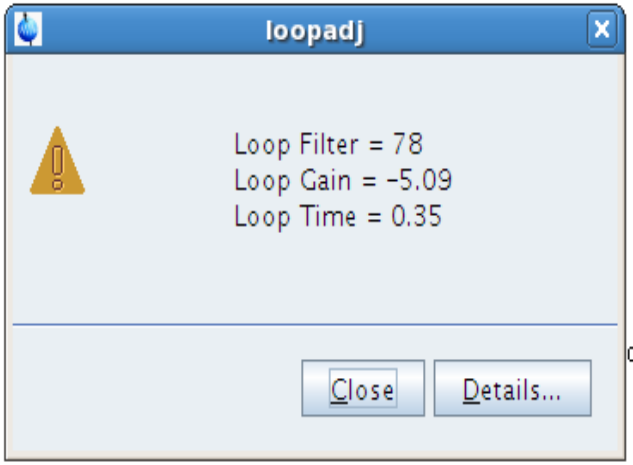

Bruker TopSpin 4.0.9 on nmr600.ncbr.muni.cz as karelk
Acquire
Process
Analyse
Applications
Manage
Create Dataset
Sample
Lock
Tune
Spin
Shim
Prosol
SetLimits
Gain
Run
More
Data
Search:
Find
151217_600_TOAC2xpY1CTD_check
151217_600_clanp7_N98C_S102C_C181
151222_600_Nterm_SH2
151222_600_titr_TOAC_tSH2_sumo_out
151227_600_titr_TOAC_tSH2_sumo_out
160406_600_Spt6_pY_SingleAAtitration
160414_600_aRRM_Nab3
160415_600_C7995
160419_600_aRRM_Nab3_titr
161109_600_C9230F_Ubiq
171012_600_C7995_Practical1
171012_600_C9320B
1 - zgpr - zgpr
2 - hsqcetf3gpsi - HSQCETF3GPSI
C7995
CID_900
Time
Status Message
17:54:30 xf2: in progress
17:54:33 topshim: set shims
17:54:32 topshim: 2 target 1, fit 2
17:54:32 topshim: 3 acquire data
17:54:33 stored NS 4 into /root/.topspin-Blade...
17:54:33 topshim: 3 2D Fourier transform
17:54:33 xf2: in progress
17:54:33 xf2: finished
17:54:33 topshim: 3 process field map
17:54:33 topshim: 1 sample size = 2.21 cm, p...
17:54:33 topshim: 3 stop acquisition
17:54:34 stored NS 4 into /root/.topspin-Blade...
17:54:34 acquisition finished
17:54:34 zg finished
17:54:34 topshim: 2 finish
topshim: completed successfully
Main
Lock/Level
Shim
Autoshim
Service
Log
Help
AUTO
Lock
Phase
Power
Gain
Shim
LOCK
On-Off
Phase
Power
Gain
SAMPLE
LIFT
SPIN
Measure
Rate
Always Locked
SHIM
Spin.
NonSpin.
Z
Z²
Z³
Z⁴
X
XZ
Y
YZ
XY
X²-Y²
STD BY
Previous
Actual
Step
Absolute
Difference
Reset
Stepsize
STD BY
Shut down BSMS
Shut down BSMS
Shut down BSMS before switching BSMS off
Config
External
Sample:
down
missing
up
Shim coil temperature
301 K
171012_600_C9320B 2 1 /d1/data/karelk/nmr
topshim: completed successfully
Spectrometer Status
On
Amplifier Control
Acquisition information
no acquisition running
Fid Flash
Lock
Sample
Shim Coil Temperature
301 K
POWCHK
Probe Temperature
298.0 K
On
Reg. State:
Temperature
298.
Lock Display

Homogeneity of the field

Adjusting homogeneity of the magnetic field – as the more homogeneous magnetic field results in a narrower lock signal which results in a higher d.c. voltage, one aims for an optimum lock signal by adjusting various shim currents

Shimming options

- Manual (in the BSMS display via the Shim tab)
- Automatic
  - Gradient shimming – Topshim (additional parameters, 3D only works with water samples)
  - Simplex (slow, works based on lock level, shims to optimize must be specified in a file)
  - Hardware autoshim (Autoshim tab in BSMS), activate before starting long experiments .
- As there may be needed to adjust the lock phase, lock gain and other parameters, type **loopadj** on the cl (optimizes lock phase, lock gain, loop phase, ...) which will take care about all of it. In case there is sufficient lock signal, menu with three lines will appear to confirm everything went smoothly and was set up. Should there be low lock signal, error message will appear.

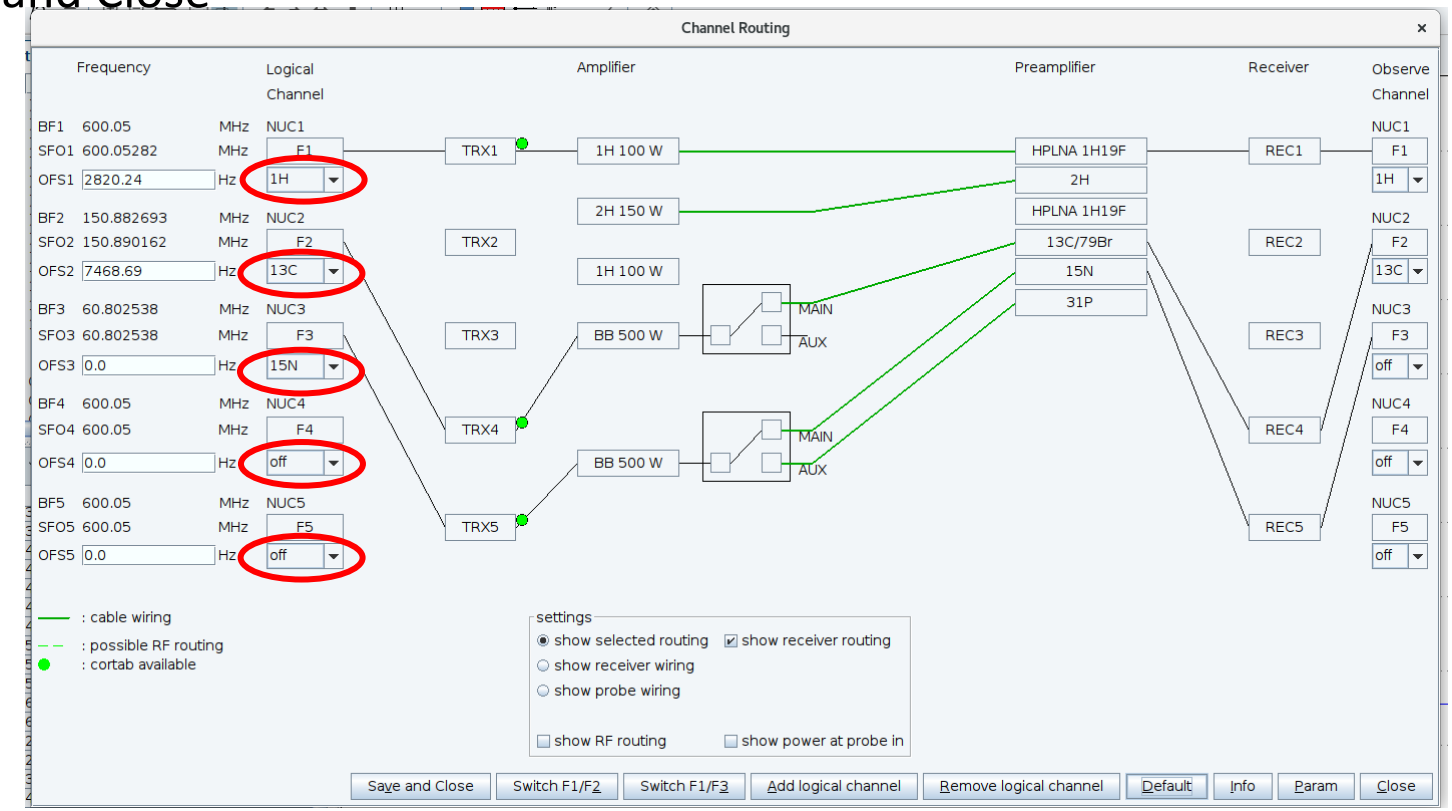


Selecting the nuclei

- Prior starting any set up, pulse checks or measurements, make sure the channel routing is correct and all the channels that are going to be measured are active.
- To do so, type **edasp** on the cl
- set the desired nuclei from the pop-up menus: typically channel 1 – ^{1}H, channel 2 - ^{13}C, channel 3 – ^{15}N
- Click on Default to set proper routing

for the channels and Save and Close

the settings.

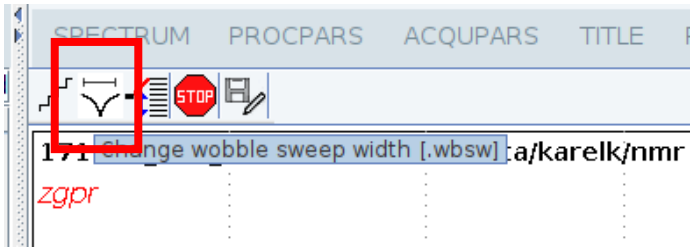


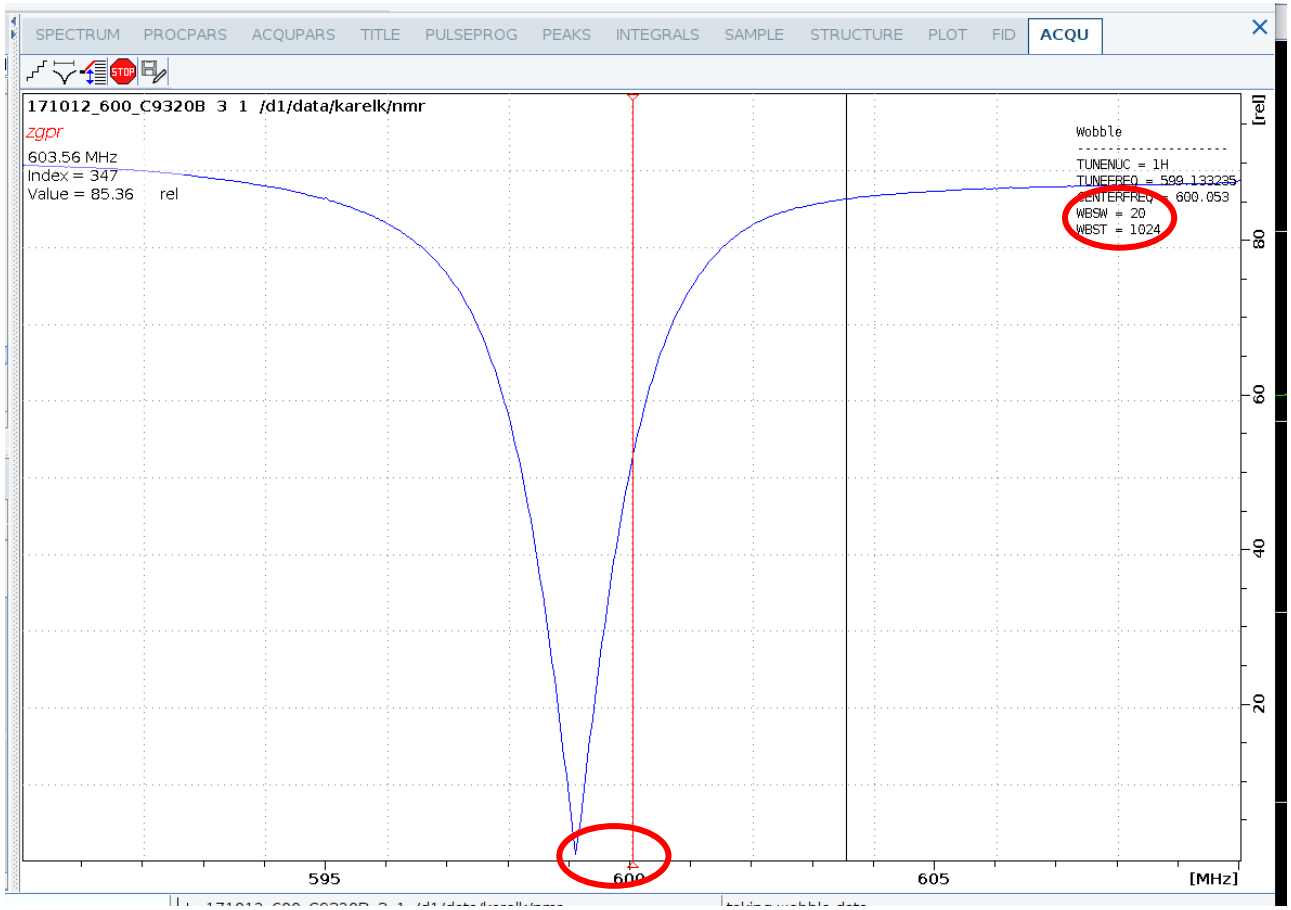
Tunning and matching the probe

- For obtaining optimal signal-to-noise ratio, one needs to tune the probe with the sample inserted. It is done by adjusting two mutually interactive capacitors. One tunes the circuit to the desired resonance frequency (**tuning**) and the other matches the impedance (**matching**). Tuning/Matching can be achieved either manually directly on the probehead that is NOT equipped with ATM unit or automatically from a PC in case the probehead IS equipped with the ATM unit. Automatic Tuning/Matching Automatically (atma)
- Start with **atma** (AutoTuneMatchAuto)on the cl – typically, this will tune the probehead automatically without any human intervention
- Once atma is done, it is recommended to check the tuning manually
- Type **atmm (**Automatic Tuning/Matching Manual**)** on the cl – window with a black wobbling curve that needs to be ftuned to the minimum indicated by vertical red line is opened
- The precision of the displayed curve is driven by wobble sweep width. Set it prior wobbling by command **wbsw** on the cl to 2 MHz or click wbsw icon and set it there.
- N.B. Sometimes the atmm gets stuck when trying to change the sweep width. Using of following command and its option **atmm manwbsw** has proven to improve the wobble behavior. Again, create own macro or use **wkk**.

Tunning and matching the probe

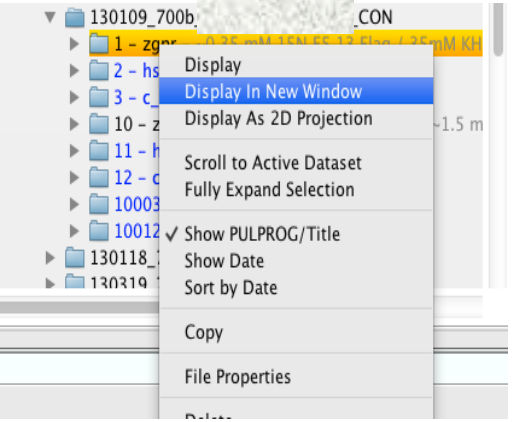
- While tuning the probehead „manually “ with atmm, keep an eye on the lock-level signal. It may drop significantly during the tuning so once finished, run topshim once again. Typically one is tuning the channels starting at lowest frequency (typically, ^{15}N followed by ^{13}C and then ^{1}H) but in case of (cryo)-probeheads with a new design (lock signal is dropping while tuning/matching) are supposed to be tuned from highest frequencies down (i.e. ^{1}H->^{13}C->^{15}N). In any case, check the tuning curve of a given nuclei iteratively.
- Tune/match each channel starting from the nuclei with the lowest frequency (on the 850/950MHz spectrometers in reversed order)
- Adjust the displayed sweep width (parameter WBSW (wobble sweep width) in the upper right corner, *vide infra*)
- Get the minimum of the curve to required position by clicking the arrows that represent expected step of the tune/match
- Start with matching (get the minimum to the bottom), then tune (center the minimum on the reference line). As tune/match are interconnected, the procedure becomes iterative but optimum has to be always reached.
- For samples with high salt [c(NaCl)>=250~300mM], use either shaped NMR-tube or 3mm tube. Both tubes require special rotors and extremely gentle handling!

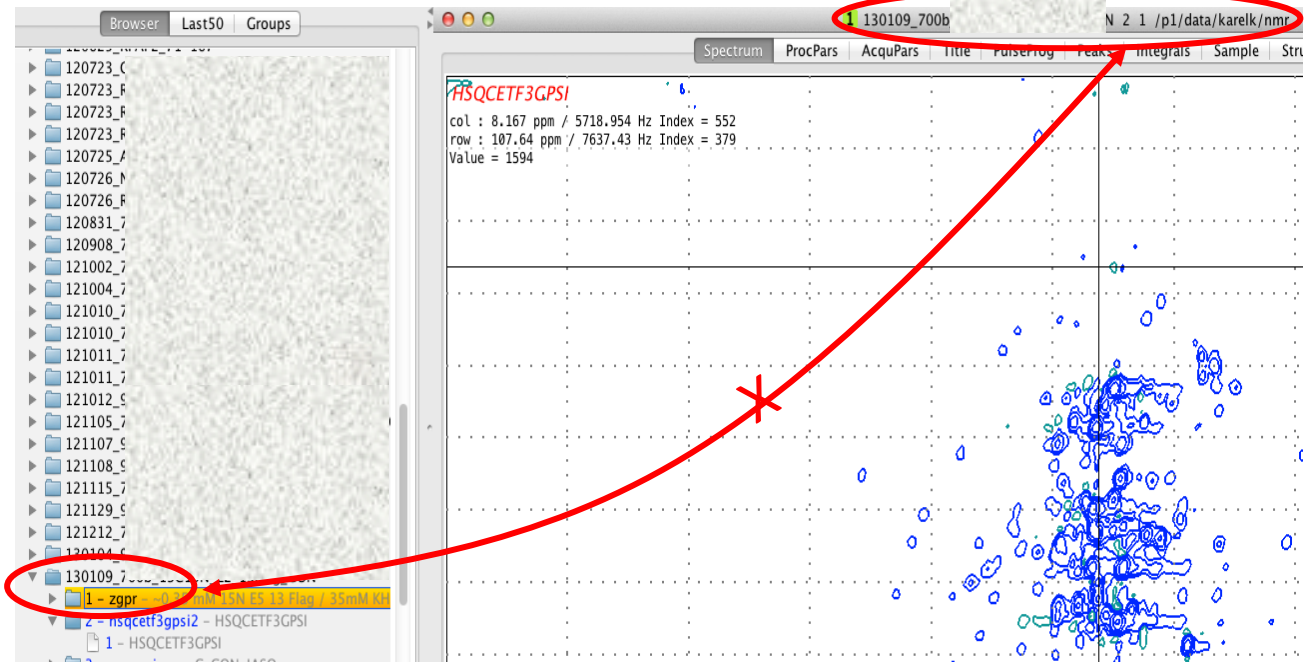

SPECTRUM
PROCPARS
ACQUPARS
TITLE
STOP
change wobble sweep width [.wbsw]
a/karelk/nmr
zgpr


SPECTRUM
PROCPARS
ACQUPARS
TITLE
PULSEPROG
PEAKS
INTEGRALS
SAMPLE
STRUCTURE
PLOT
FID
ACQU
171012_600_C9320B 3 1 /d1/data/karelk/nmr
zgpr
603.56 MHz
Index = 347
Value = 85.36 rel
Wobble
TUNENUC = 1H
TUNEFREQ = 599.133235
CENTERFREQ = 600.053
WBSW = 20
WBST = 1024
[rel]
80
60
40
20
595
600
605
[MHz]

Creating a dataset

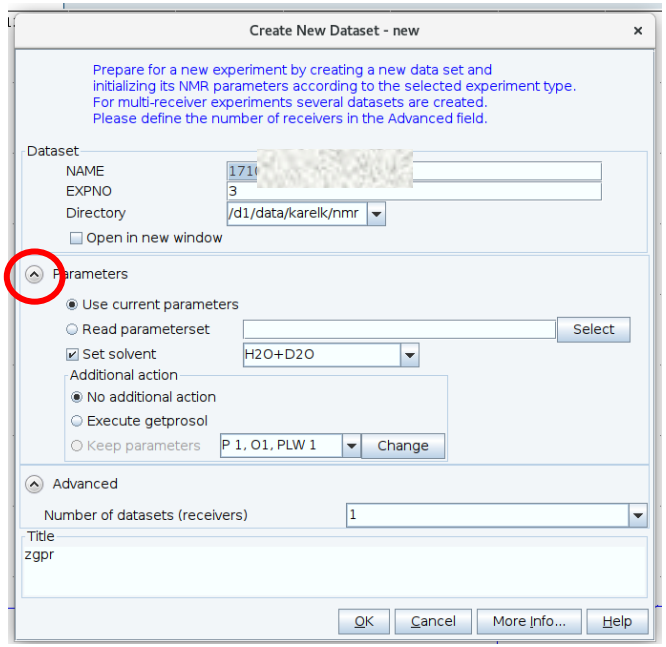
- Steps involving edasp and atma/atmm require an existing dataset with parameters. Users typically keep in their data-tree directorie Setup/Calibration or something similar. In those dataset one usually doesn‘t change more than power-levels, (de)activates nuclei but not much more ...
- If one wants to create a dataset, there are several ways to do it
  - Copy an existing dataset you or someone else used previously
    - Select a dataset that worked previously, ideally by clicking right button in the browser and open in a new window. Often, different set is selected in browser and different in the window. Always check which spectrum you are working with!!!
  - Read in a standard parameter set (rpar)

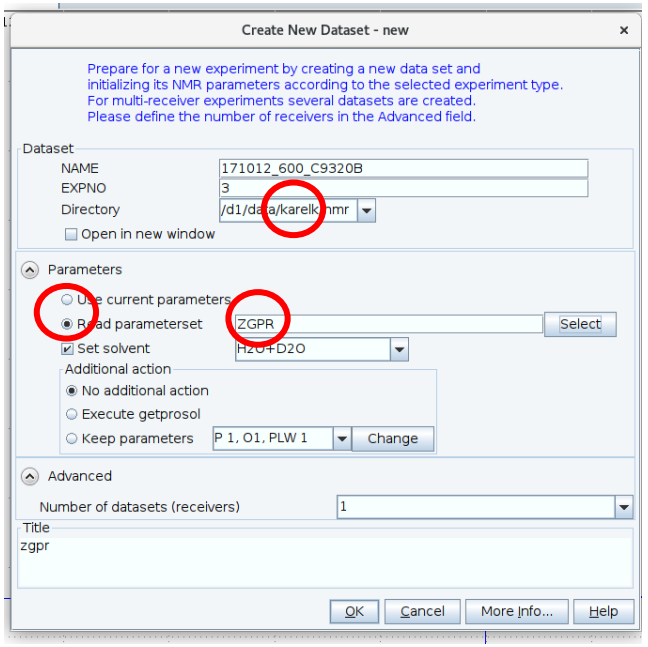




Creating a dataset

- Once being in the right dataset, type **edc** on cl and set the new destination of the experiment, experimental number, title, user ...
- In case dataset should be copied to the next ExpNo, use command **iexpno** which will copy the dataset to next ExpNo





- If creating a new dataset, type **rpar** on cl to read standard dataset parameters of a required pulse sequence
- Type **getprosol** on cl to set actual pulse calibration. Should calibration of any nuclei vary from the standard ones (typically ^{1}H), type **getprosol 1H 13.55 13W** which means that 90° ^{1}H pulse is 13.55µs@13W taken as reference.
- Modify the rest of the parameters in AcquPars in Data area (TD – number of points collected during FID, DS – number of dummy scans, NS – number of scans, SW – spectral width, O1P – carrier frequency, ...)

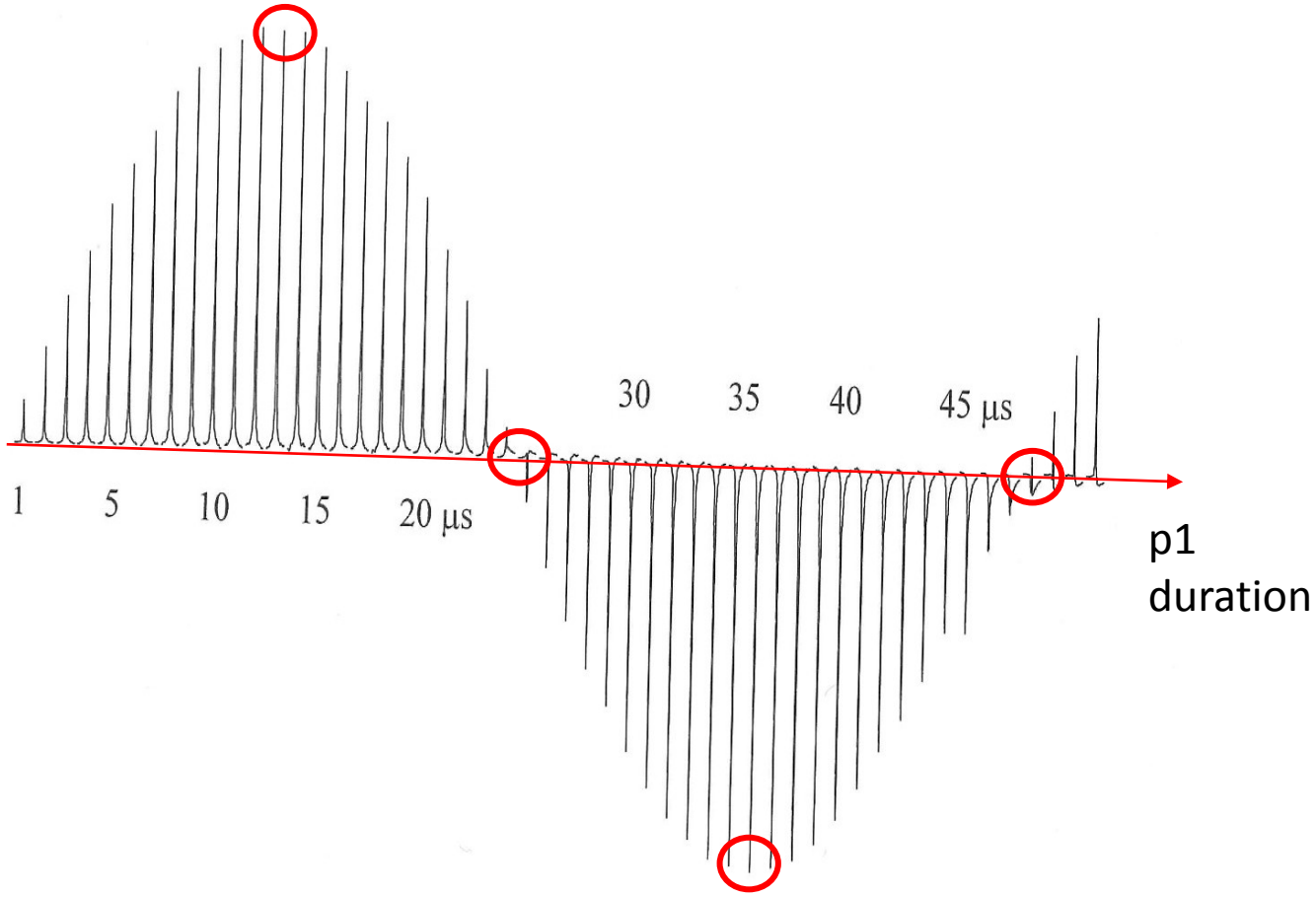
Calibration of 90° ^{1}H pulse

- Automatic calibration
  - Type **pulsecal** on cl – info-panel will pop-up with the length and power of 90° pulse. The parameters will be also automatically updated within the dataset
  - Pulsecal gives results reasonable enough to use them for nD experiments or to use them as a starting point for manual calibration

- Manual calibration
  - Type **p1** on cl, set the pulse length 1 µs and type **zgqfp**. This will run a macro that is composed of **zg** (zero go - start an experiment), **qsin** (multiply resulting FID with QSINE window function) and **fp** (perform Fourier transformation with phase correction). Alternatively zgefp does the same but instead of qsine exponential window function is used for FID apodization.
  - Water-line signal will appear at about ~4.7 ppm. Phase the line to pure absorptive line either manually through Process menu and manual phase adjusting. Phasing window can also invoked by typing **.ph**. If there are few signals in the spectrum automatic phase correction – **apk** command will work well. Check also symmetry of the signal and/or any abnormalities of the line-shape. Also measure the LWHH to get an estimate about the quality of the shimming.
  - Proton hard pulse (90°or $\pi/2$) is strongly dependent on the tube diameter, salt concentration, temperature and solvent used. The pulse-length can be as short as 8µs but can reach ~17µs for high salt samples. As the 90°pulse is usually determined by measuring the 360° since the pulse gives a minimum signal, type **p1** on cl and set it to four times the expected length of 90° ^{1}H hard pulse (i.e. 32 to 68µs). From the knowledge of sine-function one can easily guess whether the signal is longer 360°or shorter.
  - Once minimum in 360° is achieved, take one fourth the length of the pulse that gave a zero signal to obtain length for the 90° pulse.
  - Samples in organic solvents or D_2O can be calibrated on 180° pulse (first pass through zero).

Calibration of 90^{o} ^{1}H pulse

Another option how to calibrate pulse in to use command **paropt**, which will pop-up a window where one sets up initial value [of the pulse], increment, and number of increments. Result of such paropt will provide similar output (initial value: 1μs, increment:1μs, number of increments: 50).



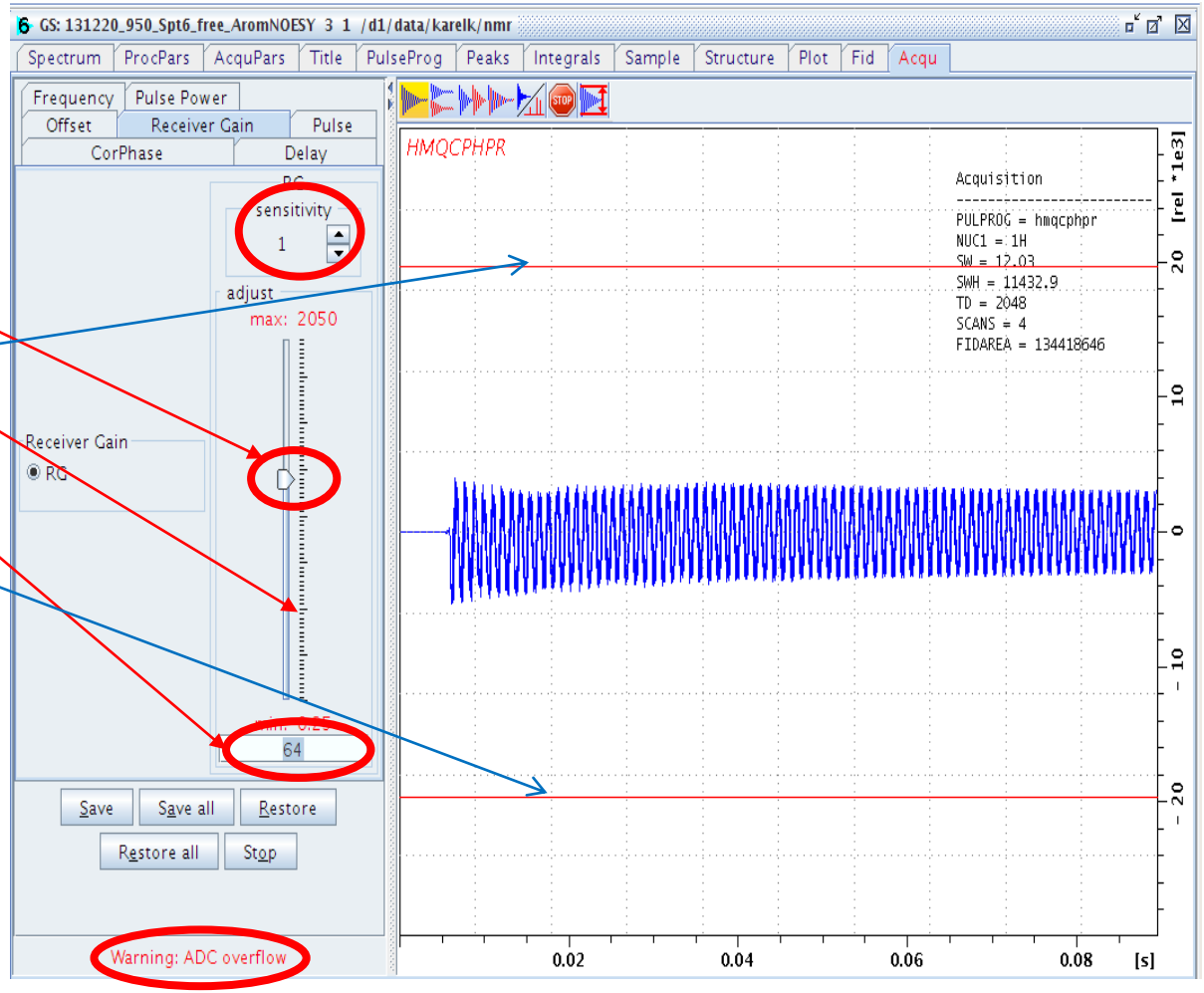
Optimizing acquisition parameters

- Before starting any acquisition, it is wise to check whether all parameters are at their optimum or can be further improved. For the sake of optimization there is command **gs** (go scan) that will run an “infinite” loop enabling real-time manipulation with pulse-lengths, delays, receiver gain etc. Immediate impact on the FID is observed.
- Start with optimizing receiver gain – either by typing **rga** prior running gs or manually till ADC overflow disappears*
- The parameters can be adjusted by:
  - dragging the slider (not recommended)
  - directly typing desired value
  - clicking on the slider-scale

  (clicking will be affected by the sensitivity)

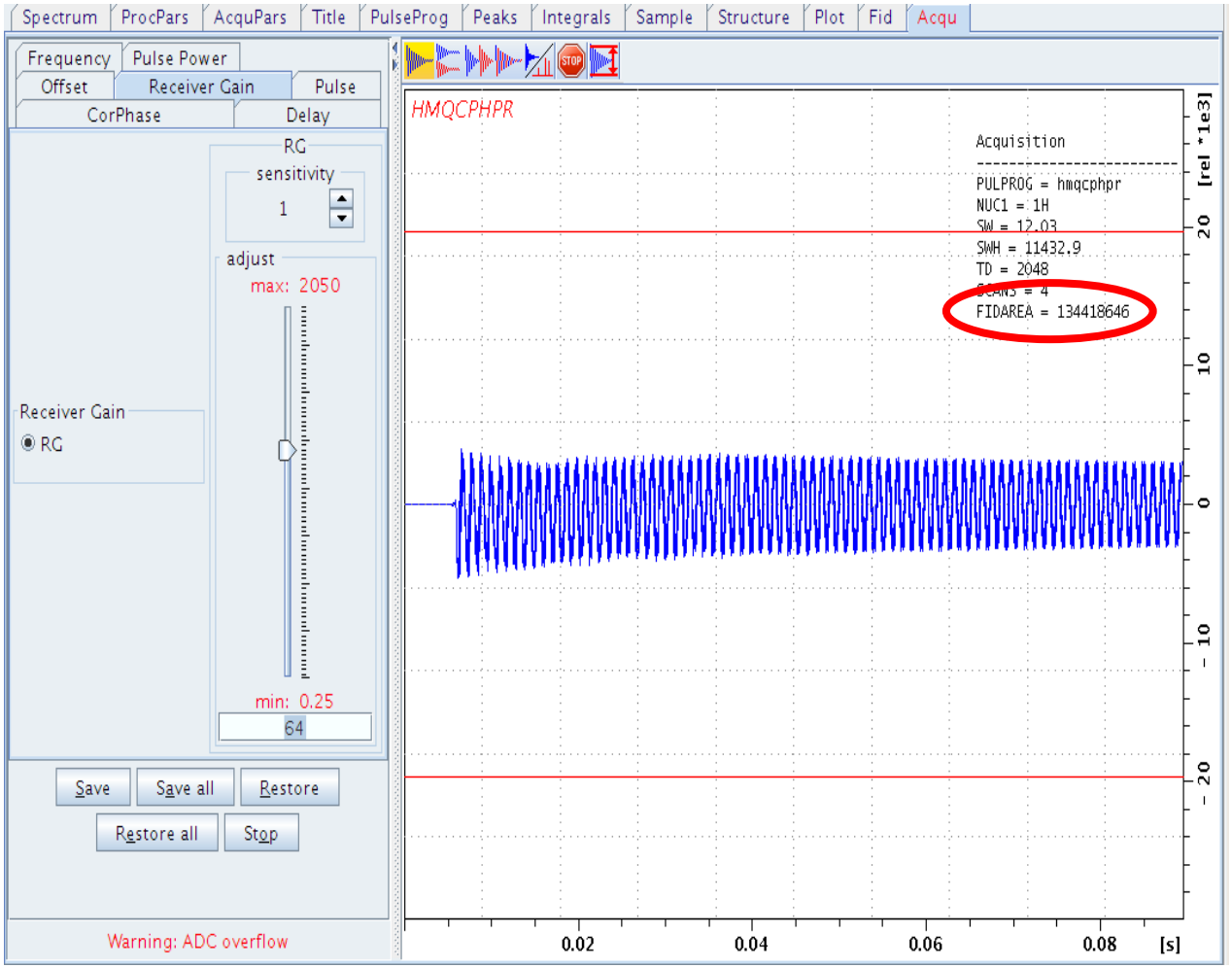
ADC range lines

*note: cryoprobes tend to give false ADC overflow warnings



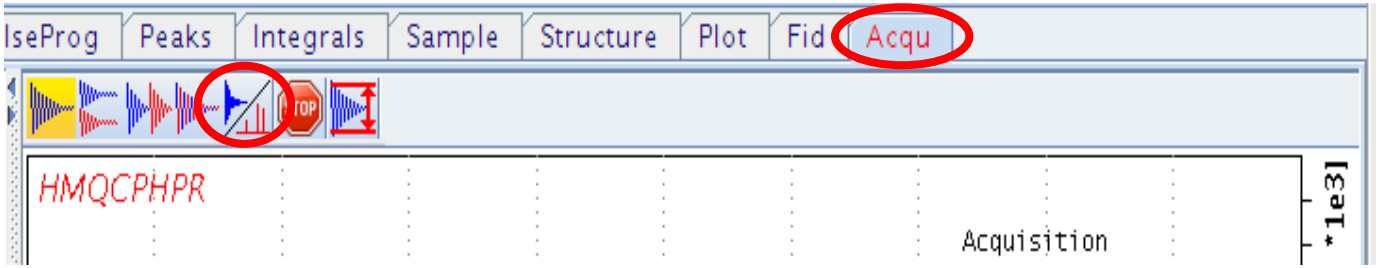
Optimizing acquisition parameters

- Other parameters that could be set are the exact position of receiver (Offset-tab), phase correction (particularly in case of WATERGATE water suppression; CorPhase) and duration of calculated shaped pulses (Pulse-tab).
- Always check the Warning message and FIDAREA which one wants to reach as low as possible as the signal in biomolecular samples is dominated by water which is supposed to be efficiently suppressed.



Start of the experiment

- Duration of the experiment
  - Type **expt** (experimental time) – gives the experimental time, required space on disc for the current dataset
  - Type **multiexpt** – allows to estimate duration of multiple experiments that are in row (e.g. ExpNos 1, 2, 3, 4 ...). Returns the time and date, when the experiments are finished.
- Running the experiment
  - Type **zgqfp** – starts and processes 1D experiment (composite macro of zg+qsin+fp) – in case of low concentrated samples, one set number of scans as high as 128 or even 1024. As such experiment takes already a significant amount of time, it is wise to check whether something is being acquired. Either check the on-line Fourier transform in the acquisition window or type **tr** to transfer the so far acquired data from spectrometer to computer and process them with **efp** or **qsin**+**fp**.



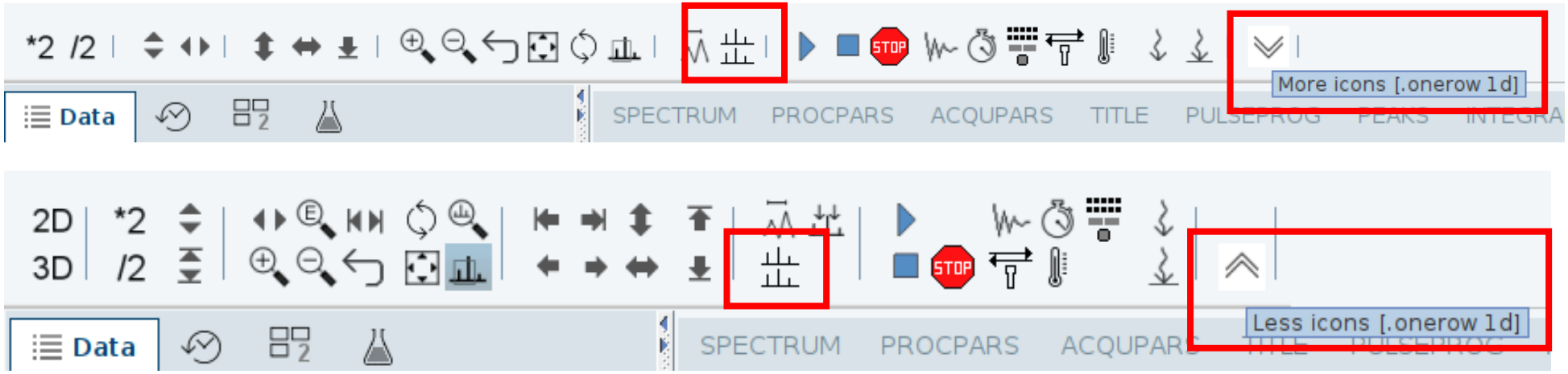
  - Type **zg** – starts the experiment without processing it
  - Type **stop** – immediately stops the experiment
  - Type **halt** – stops the experiment after the current scan

Start of the experiment

- In case of an nD experiment follow the same steps as in case of 1D, i.e. first set all parameters properly and check with gs-command that everything is correct and is not hurting sample/probe. Before typing zg, check the duration with expt or multiexpt to see when the experiment is going to finish.
- When the experiment is started with the zg-command, wait till the first FID is acquired. In the mean-time check that the temperature and lock are stable and not affected by the running experiment.
- Once first FID measured and stored, type **rser 1**, which will transfer the first FID to ~TEMP directory ExpNo 1, ProcNo 1. Process the FID with qsin+fp and phase it. This will give you a rough information about signal/noise ratio of your experiment – if there is no signal in the firs row, there will be not much signal at the end of the experiment (exceptions are, e.g. HNCACB or DQF COSY, but these are not experiments for beginners:-).

Start of the experiment

- Should it be possible to compare the running experiment with previous dataset, store the current ProcNo to ProcNo 2 by typing **wrp 2 y** – **wr**ite **P**rocNo to **2** and if there is already ProcNo 2, **y**es, overwrite it. Then go to the previous/reference experiment, type again rser 1; qsin; fp, phase the spectrum. Type **.md** or click the multiple spectra icon and then type **rep 2** – **re**ad **P**rocNo **2**. Of course one can do all the overlay with mouse only but the clicking may be more time consuming as the directory gets filled with experiments and ~TEMP remains at the top (scrolling up, double-clicks …, keyboard is keyboard:-).



Quick reference:

1) bsmsdisp – open control panel
2) ej – eject sample
3) edte – open temperature control panel
4) ij – inject sample
5) lockdisp – display lock window
6) lock – select solvent for locking the magnet
7) topshim – automatic shimming
8) loopadj – adjust lock parameters
9) edasp – set up spectrometer routing
10) wbsw – set up wobble sweep width
11) atma – automatic tuning/matching
12) atmm – manual -----”-----
13) edc – copy dataset
14) iexpno – copy dataset to next ExpNo
15) rpar – read parameter set
16) getprosol – set up pulses according to prosol
17) pulsecal - calibrate ^{1}H 90° pulse
18) paropt – optimize parameter (e.g., p1 length)
19) rga – automatically set up receiver gain
20) gs – go scan – optimize acquisition parameters
21) expt – estimate duration of the experiment
22) multiexpt - ----”---- for multiple expts in row
23) zg – start acquisition
24) zgqfp – acquire 1D and apply qsin apodization
25) qsin – multiply FID with qsin window function
26) xfb – process 2D
27) xfb n – process 2D and remove 2ri, 2ir, 2ii
28) tr – transfer data 1D from spectrometer to PC
29) edmac *name* – create or edit macro *name*
30) wrp 2 y – write processed data to ProcNo 2 and if exists, overwrite
31) rser 1 – extract first FID of an nD experiment
32) .ph – start phase menu
33) .md – spectra overlay window
34) apk – automatic phase correction
35) show – show active processes
36) curplot – set up printer
37) print – print spectrum
38) acqu – switch to acquisition window
39) ii – if spectrometer doesn’t communicate
40) ii restart – if ii doesn’t help
41) stop – stop immediately acquisition, rough
42) halt – stop it smoothly, recommended
43) pulse – calculate pulse length based on 90°hard pulse parameters
44) calcpowlev – similar to pulse