av400 NMR spectrometer

Getting started + Troubleshooting

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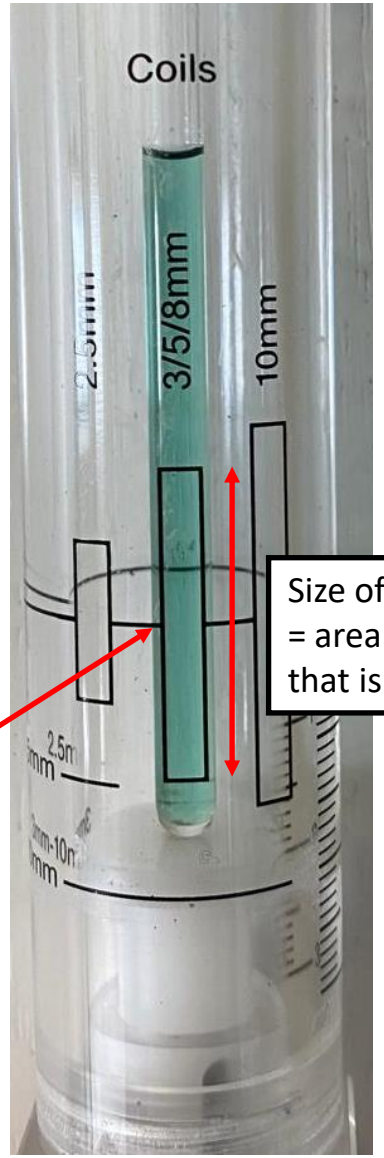
Sample centering



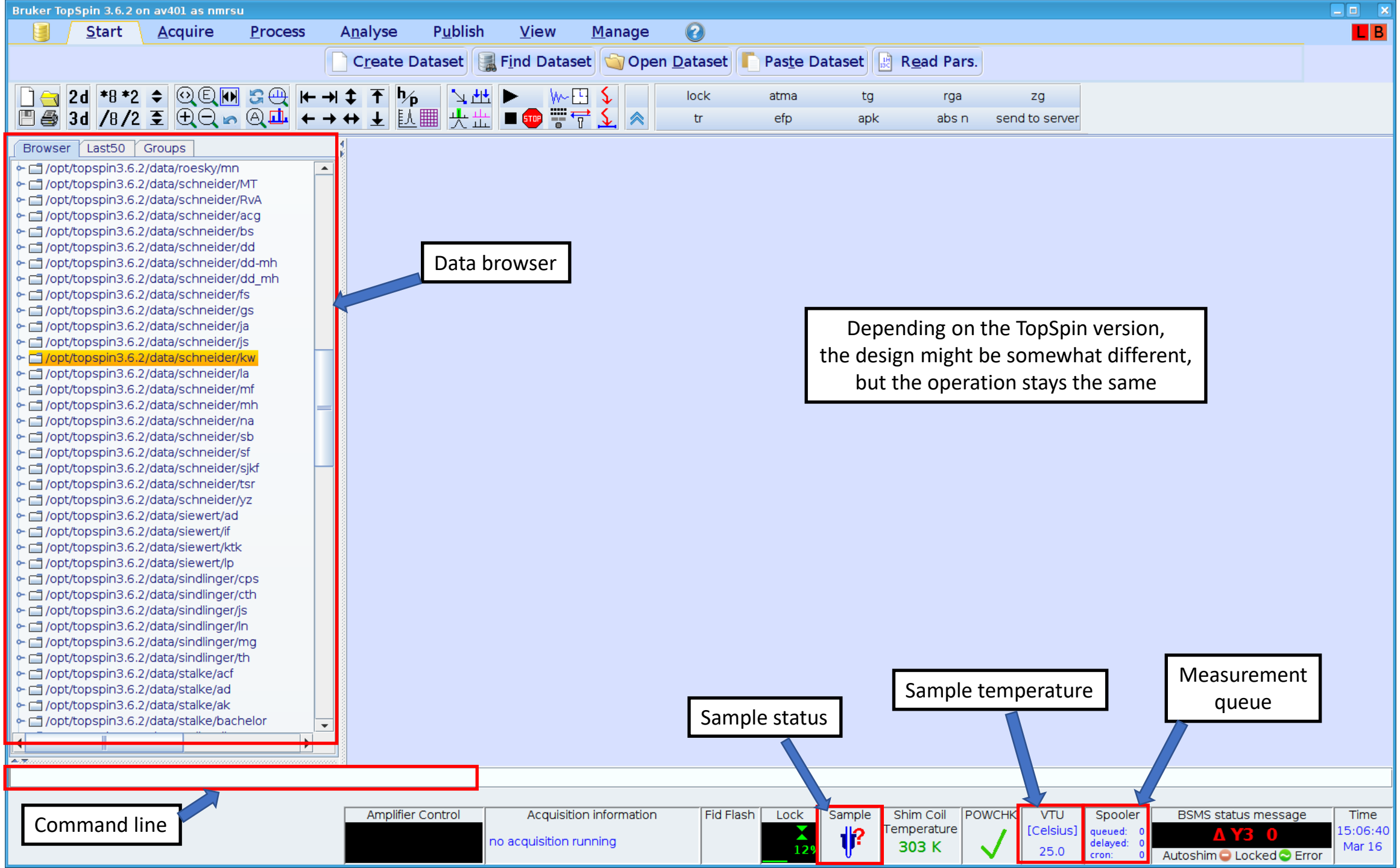
Place spinner on top of insertion tool and push sample through to the bottom

The rectangle marking the size of the rf coil should be “filled“ with sample

For samples with lower filling height, you can carefully pull the tube out of the spinner until the rectangle is “filled” with liquid. If the bottom of the tube is inside the rectangle then, add more solvent

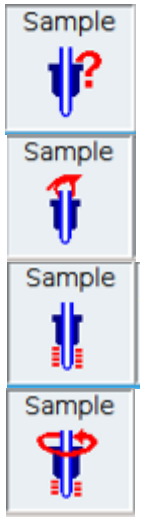
There should be no liquid-gas or liquid-solid interfaces within the rectangle, otherwise this might lead to lineshape distortions

Do not push the tube further into the spinner after taking it out of the insertion tool! The bottom of the tool marks the bottom of the probe, if the sample sticks too deep in the spinner, the tube might break while inserting it into the magnet!


Bruker TopSpin 3.6.2 on av401 as nmrsu
Start
Acquire
Process
Analyse
Publish
View
Manage
Create Dataset
Find Dataset
Open Dataset
Paste Dataset
Read Pars.
lock
atma
tg
rga
zg
tr
efp
apk
abs n
send to server
Browser
Last50
Groups
/opt/topspin3.6.2/data/roesky/mn
/opt/topspin3.6.2/data/schneider/MT
/opt/topspin3.6.2/data/schneider/RvA
/opt/topspin3.6.2/data/schneider/acg
/opt/topspin3.6.2/data/schneider/bs
/opt/topspin3.6.2/data/schneider/dd
/opt/topspin3.6.2/data/schneider/dd-mh
/opt/topspin3.6.2/data/schneider/dd_mh
/opt/topspin3.6.2/data/schneider/fs
/opt/topspin3.6.2/data/schneider/gs
/opt/topspin3.6.2/data/schneider/ja
/opt/topspin3.6.2/data/schneider/js
/opt/topspin3.6.2/data/schneider/kw
/opt/topspin3.6.2/data/schneider/la
/opt/topspin3.6.2/data/schneider/mf
/opt/topspin3.6.2/data/schneider/mh
/opt/topspin3.6.2/data/schneider/na
/opt/topspin3.6.2/data/schneider/sb
/opt/topspin3.6.2/data/schneider/sf
/opt/topspin3.6.2/data/schneider/sjkf
/opt/topspin3.6.2/data/schneider/tsr
/opt/topspin3.6.2/data/schneider/yz
/opt/topspin3.6.2/data/siewert/ad
/opt/topspin3.6.2/data/siewert/if
/opt/topspin3.6.2/data/siewert/ktk
/opt/topspin3.6.2/data/siewert/lp
/opt/topspin3.6.2/data/sindlinger/cps
/opt/topspin3.6.2/data/sindlinger/cth
/opt/topspin3.6.2/data/sindlinger/js
/opt/topspin3.6.2/data/sindlinger/ln
/opt/topspin3.6.2/data/sindlinger/mg
/opt/topspin3.6.2/data/sindlinger/th
/opt/topspin3.6.2/data/stalke/acf
/opt/topspin3.6.2/data/stalke/ad
/opt/topspin3.6.2/data/stalke/ak
/opt/topspin3.6.2/data/stalke/bachelor
Data browser
Depending on the TopSpin version, the design might be somewhat different, but the operation stays the same
Sample status
Sample temperature
Measurement queue
Command line
Amplifier Control
Acquisition information
no acquisition running
Fid Flash
Lock
129
Sample
Shim Coil Temperature
303 K
POWCHK
VTU
[Celsius]
25.0
Spooler
queued: 0
delayed: 0
cron: 0
BSMS status message
Autoshim Locked Error
Time
15:06:40
Mar 16

Sample insertion and ejection

-> first check whether there already is a sample inside
-> enter command **ej** in the command line to activate air stream
-> wait until previous sample is ejected (if necessary)
-> put down sample onto air stream and enter command **ij**
-> after ejection of your own sample, turn off the air stream with **ij**,
even if you don´t inject a new sample



No sample inside the magnet

Sample lift/air stream on

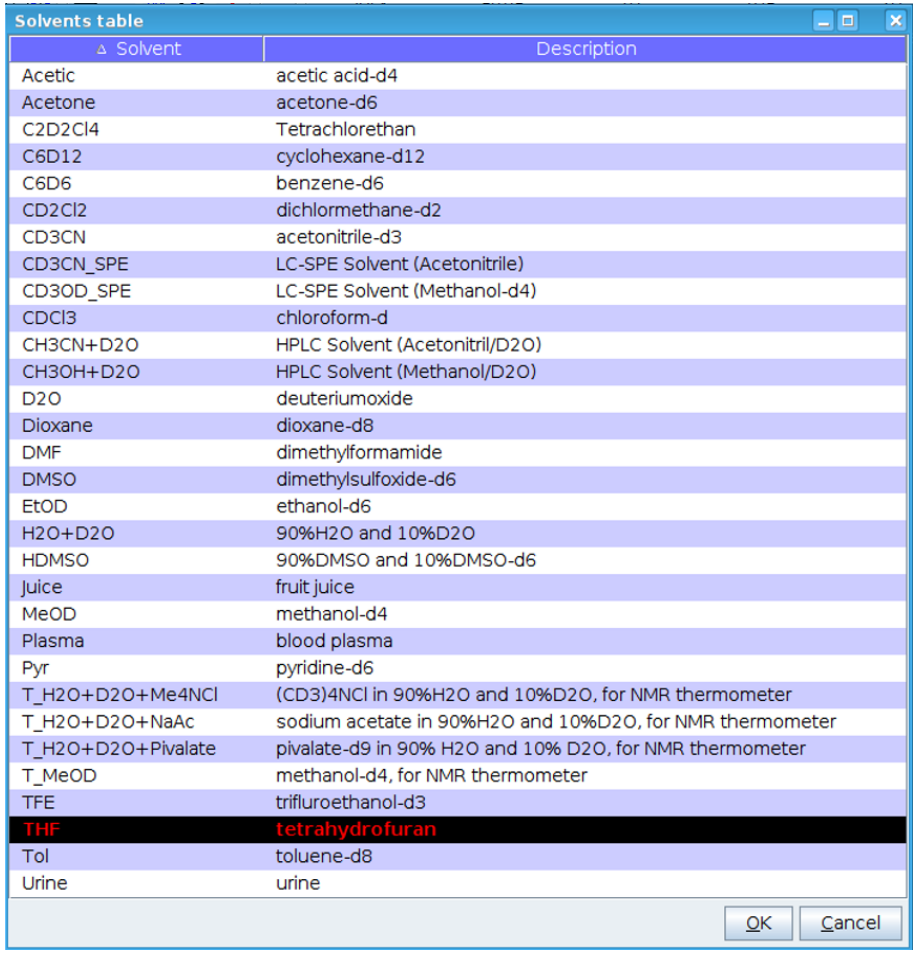
Sample inside the magnet (non-spinning)

Sample inside the magnet (spinning)

Young NMR tubes are heavier than conventional tubes and sometimes take longer to eject. If the sample eject does not work and you are sure that there still is a sample inside, you can increase the air flow carefully stepwise to max. 1200 lph. To achieve this, double-click on sample temperature and change the target gas flow. Please reset the air flow to the initial value afterwards (usually 400 to 600 lph). If you are uncertain about anything, do not hesitate to ask the technicians!

Sample lock

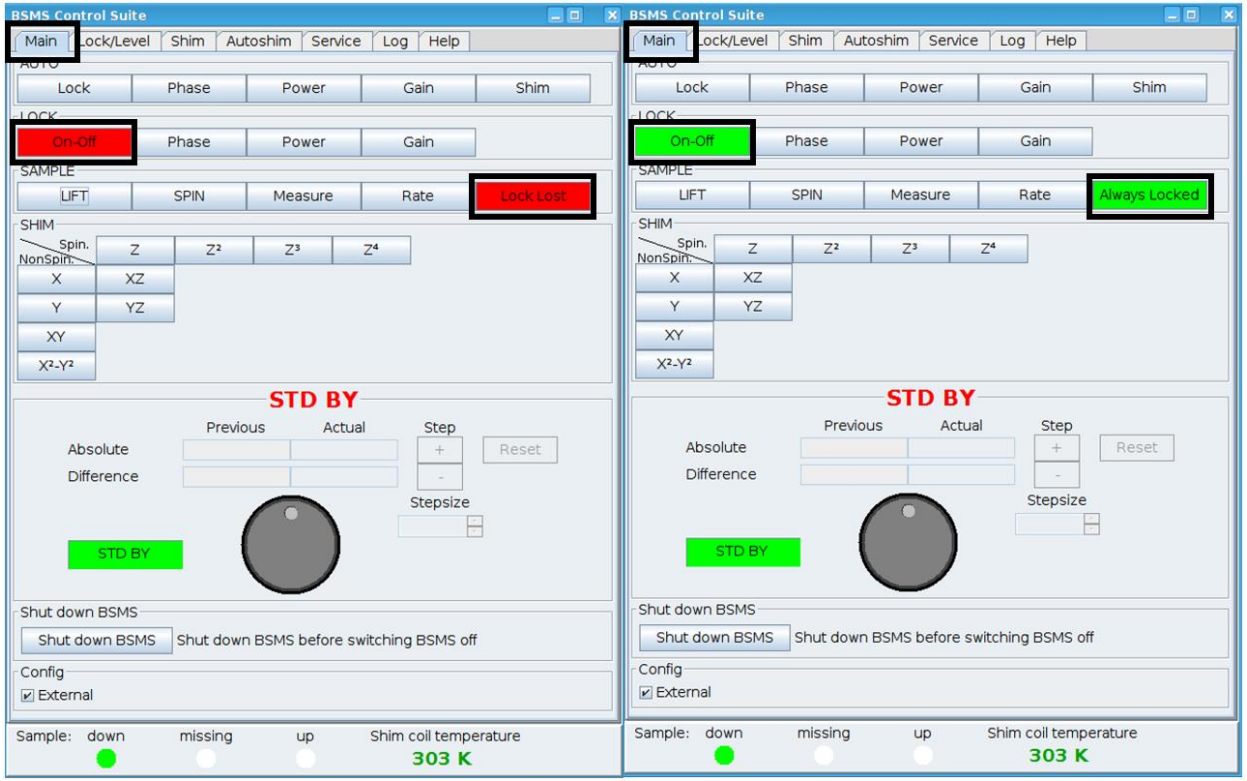
-> field frequency locking is performed on the deuterium signal of the solvent and is required for gradient shimming and to counteract the magnetic field drift for longer measurements
-> enter command **lock** into the command line, a table with all available solvents opens, select your solvent and press ok
-> alternatively, enter command **lock *solvent name*** and press enter (e.g. **lock thf** for locking on THF-d_8, for names of solvents as saved on the device enter **lock** or **edlock** to open solvent table, entry in command line is not case sensitive)
-> locking usually does not take longer than 1 min
-> repeat for every new sample with a different solvent than the previous one



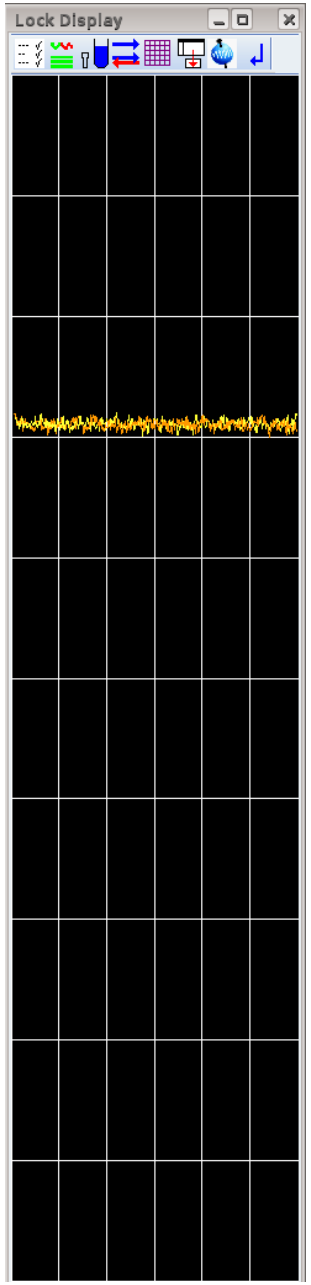
Sample lock

How do I know whether my sample is locked?

-> open BSMS window (doulbe-click on sample button)
-> check the color of the Lock On-Off button in the main menu
(red = lock off, green = lock on)



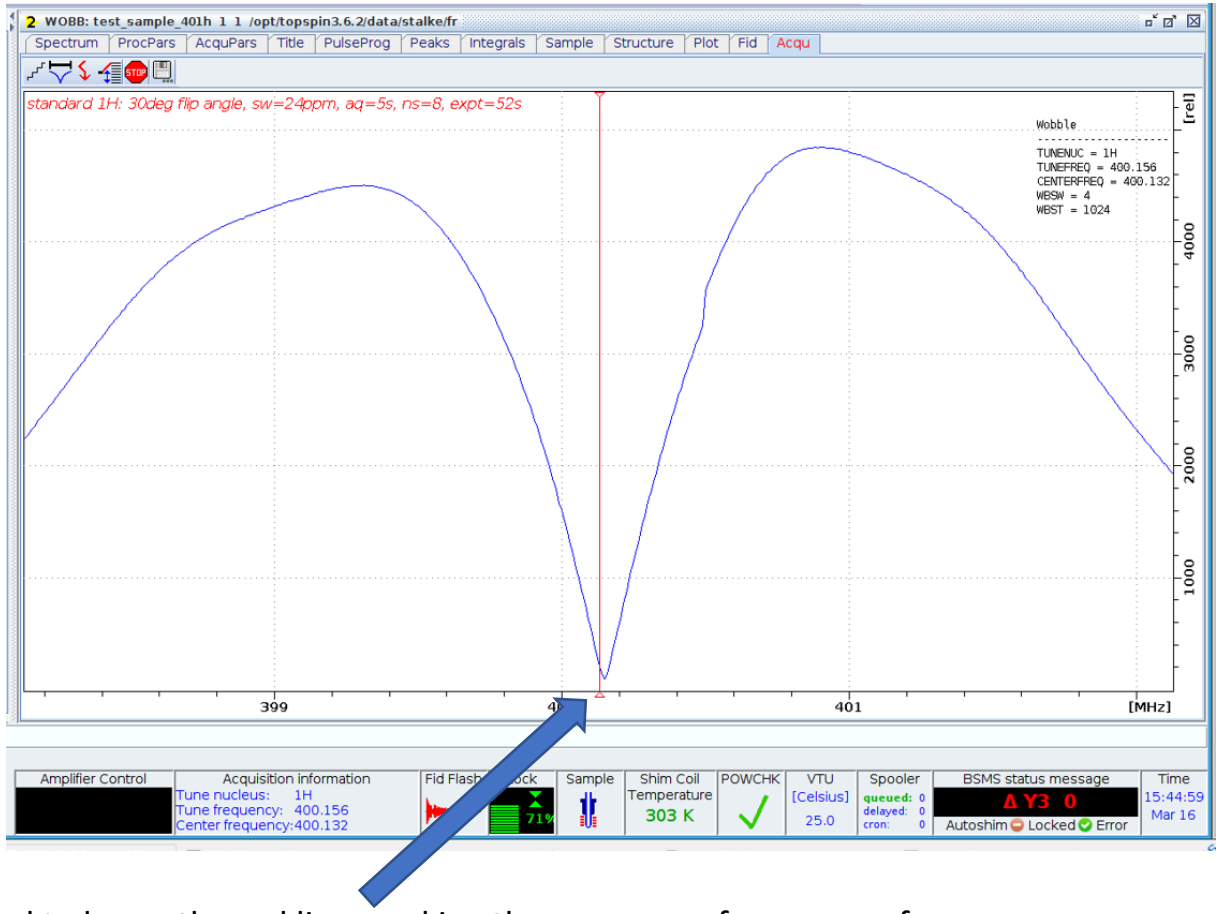
Lock display (usually on the right side of the screen)



If the lock display should be closed for some reasons, enter **lockdisp** into the command line

Tuning and matching the probe

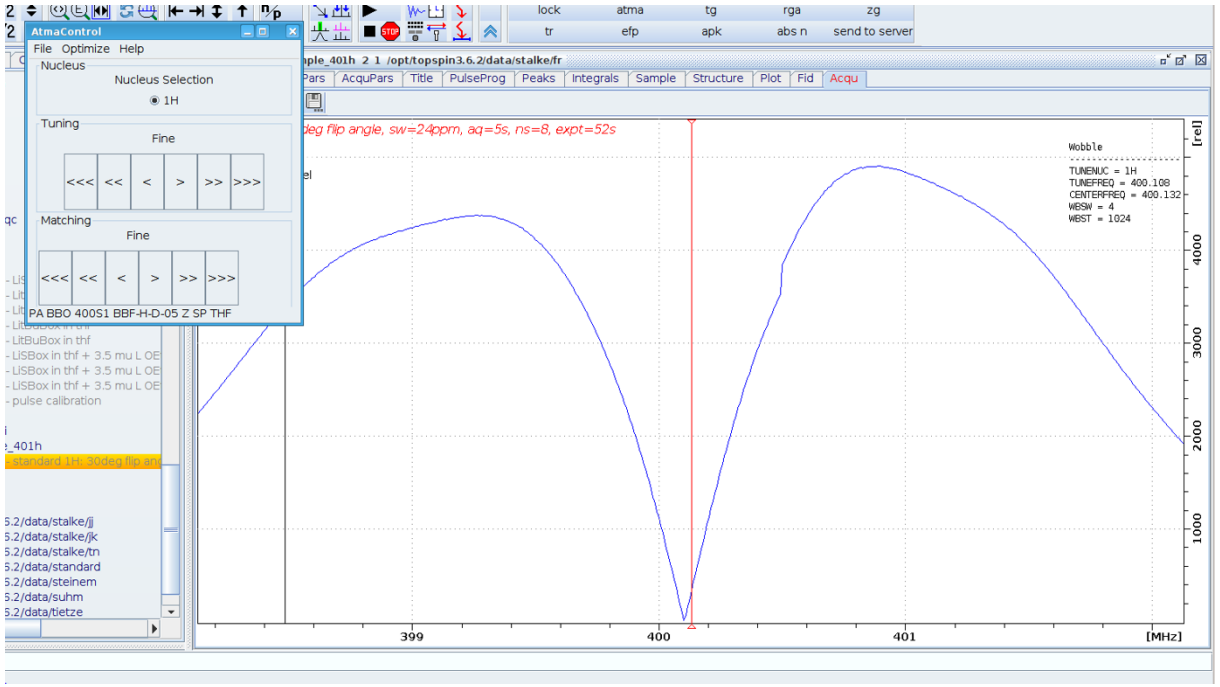
-> adjustment of the probe to the correct radiofrequency (e.g. 400 MHz for ^{1}H, 100 MHz for ^{13}C etc.)
-> open data set which you would like to use, then enter command **atma**
-> automatic tuning and matching will be performed for the nuclei contained in the open data set (one for non-decoupled 1D experiments, two for decoupled 1D and 2D experiments)
-> **atma** usually takes less than 1 min
-> repeat **atma** for each experiment containing a new nucleus



The dip is supposed to be on the red line marking the resonance frequency of the tuning nucleus and as deep as possible

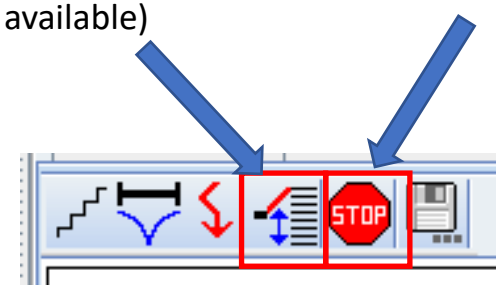
Tuning and matching the probe

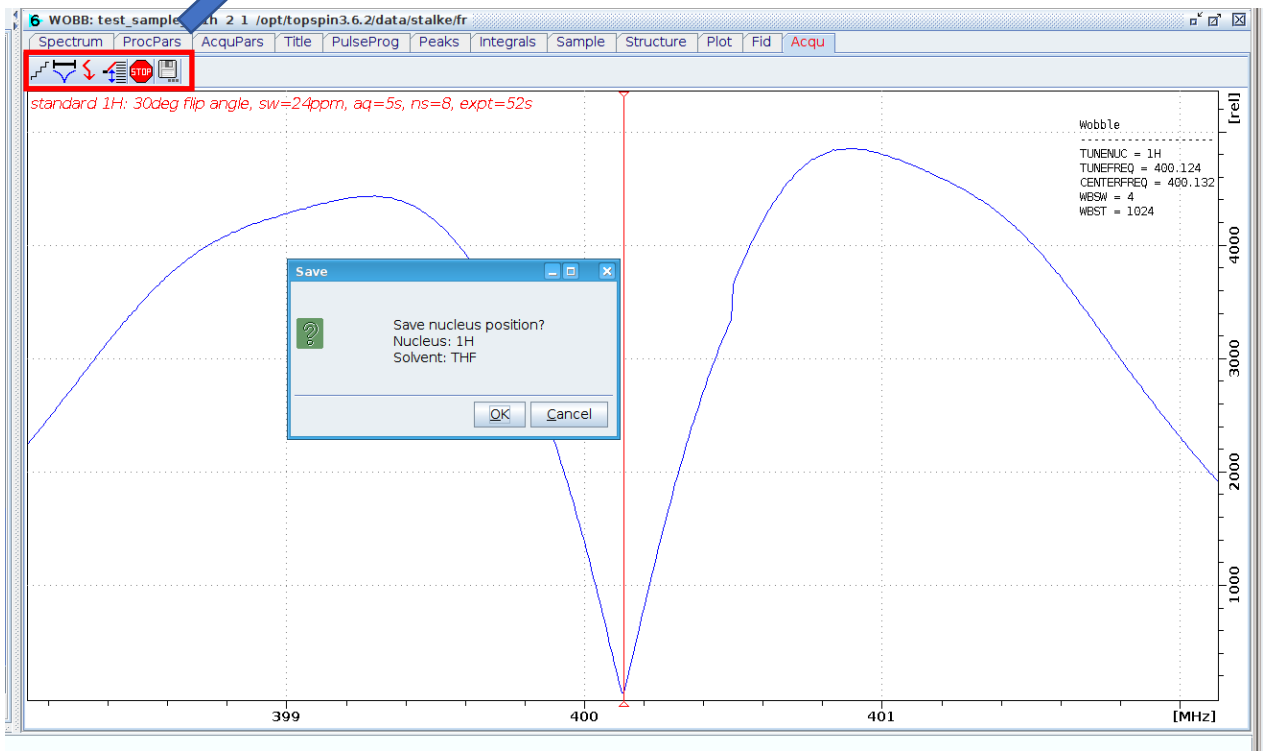
-> tuning and matching can also be performed manually using the command **atmm**
-> manually adjust dip by clicking on the respective buttond on the atma control panel that automatically opens when executing **atmm**
-> tuning moves the dip left and right, matching up and down
-> **atmm** is required especially for the first time measuring a certain nucleus



Switch to 2nd nucleus (if available)

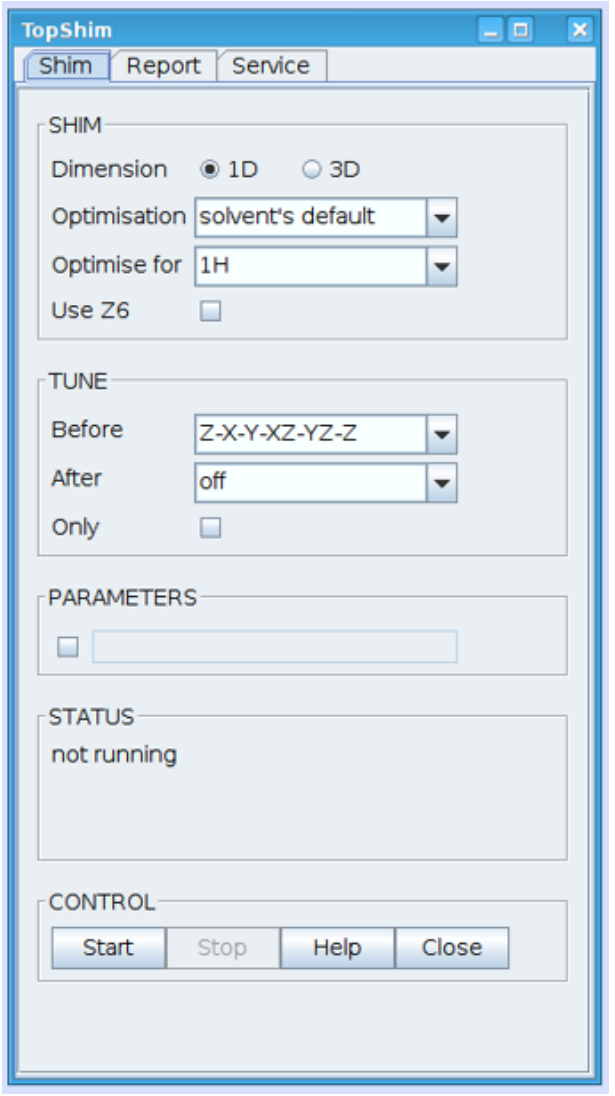
Exit atmm window





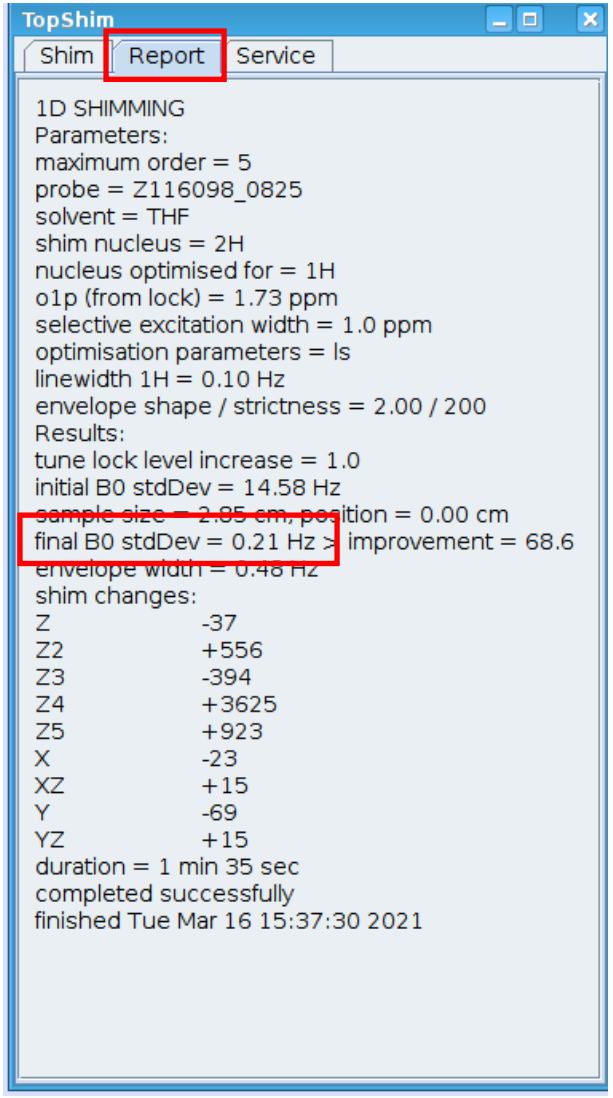
Gradient shimming

-> shimming is performed to increase the homogeneity of the magnetic field at the position of the sample
-> additional small magnetic fields into different directions are induced to correct residual inhomogeneities
-> fastest and easiest way to shim the sample is via the use of automatic gradient shimming (only works when the sample is locked!)
-> enter command **tg** into the command line and the TopShim window opens
-> press start button to start shimming process (usually takes approx. 2-5 min)
-> alternatively, the command **topshim** can be used (only gradient shimming will be performed without opening the TopShim window)



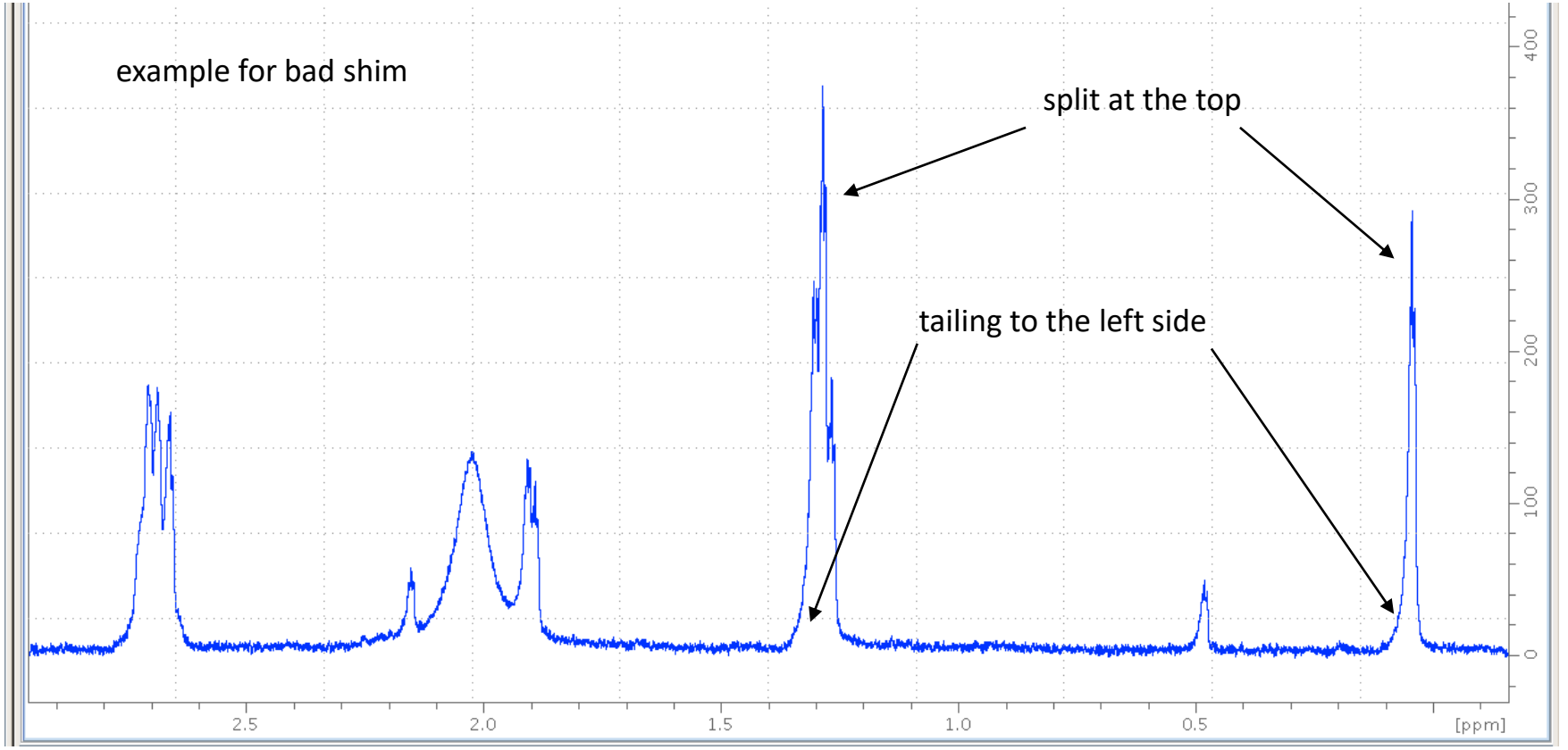
Gradient shimming

-> when shimming is successfully completed, the results can be viewed in the TopShim window under the tab „Report“
-> the final B0 stdDev indicates how well the automatic shim performed
-> the smaller the value, the more homogeneous is the magnetic field
-> for standard organic samples, the value should be lower than 0.4 Hz



Bad shim

-> when signal distortions are observed in the spectrum, this might indicate a bad shim
-> in case of a bad shim, all signals in the spectrum are equally affected by the same distortions
-> when signals are asymmetrically distorted, an inhomogeneous sample might be the reason



What to do when the shim is bad?

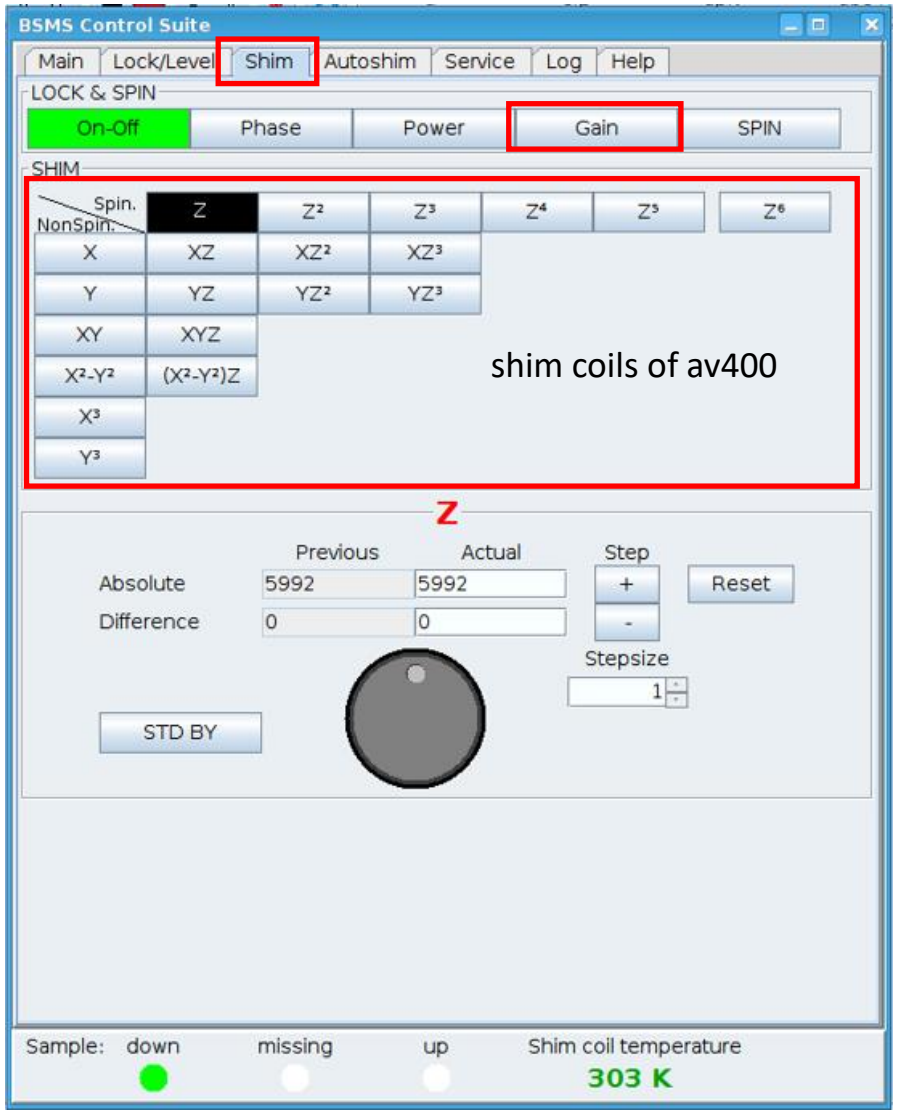
1) Adjusting the shim by hand

-> open BSMS window (double-click on sample button) and go to the shim menu
-> click on the respective shim coil and turn the mouse wheel to change its value while watching the lock signal
-> lock signal goes up: shim gets better, lock signal goes down: shim gets worse
-> when lock signal reaches top of lock display, click on Gain and turn the mouse wheel to adjust height of lock signal

-> usually, the adjustment of the coils Z, Z^2, Z^3, X, XZ, XZ^2, Y, YZ, YZ^2 is fully sufficient

-> proceed like this:
Z -> Z^2 -> Z^3
X -> XZ -> Z
Y -> YZ -> Z
X -> XZ^2 -> Z^2
Y -> YZ^2 -> Z^2

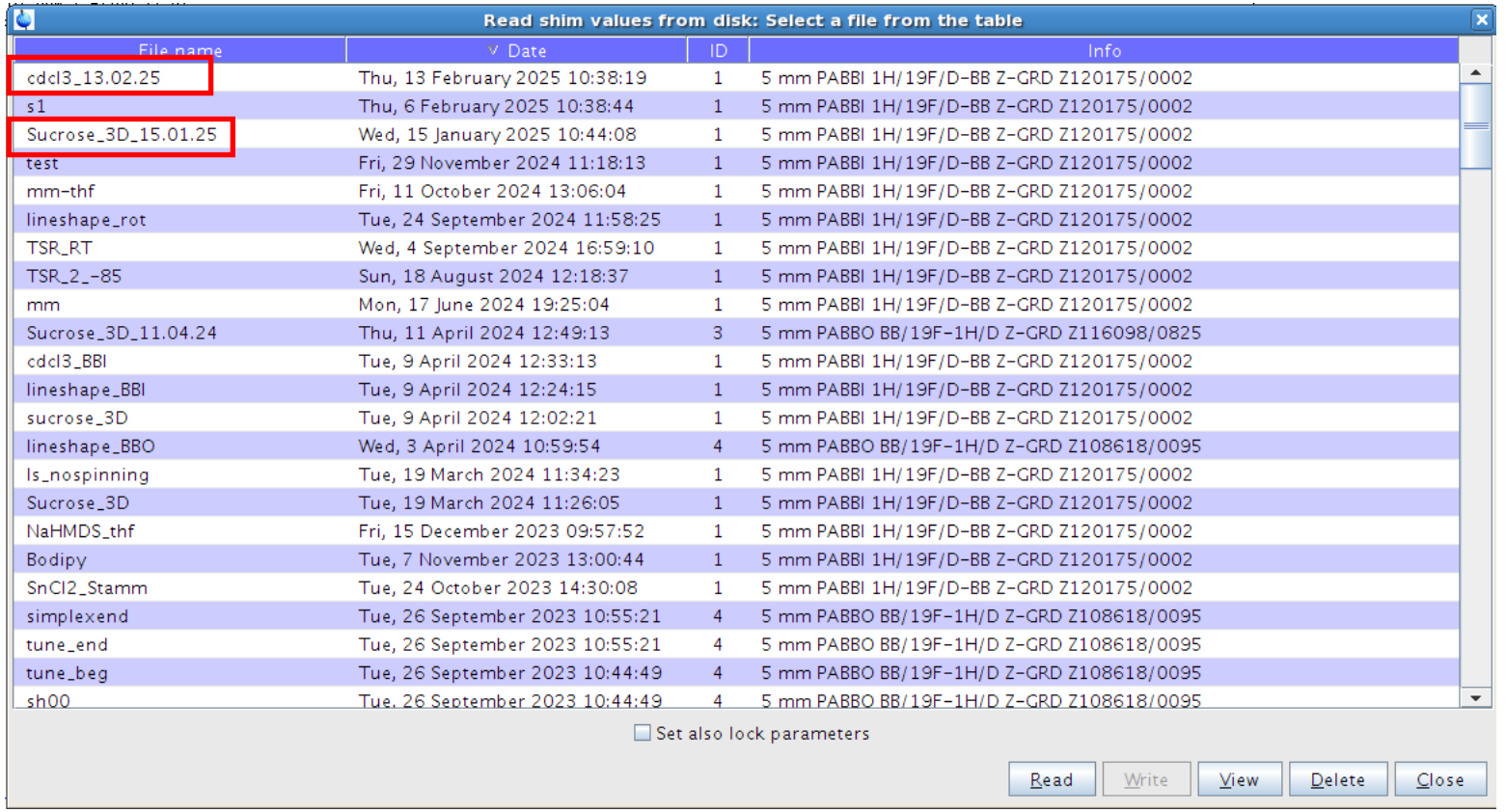
-> if you see a major change after optimizing e.g. Z^2, go back to Z and start the row anew



What to do when the shim is bad?

2) Read a shim file performed on a calibration sample

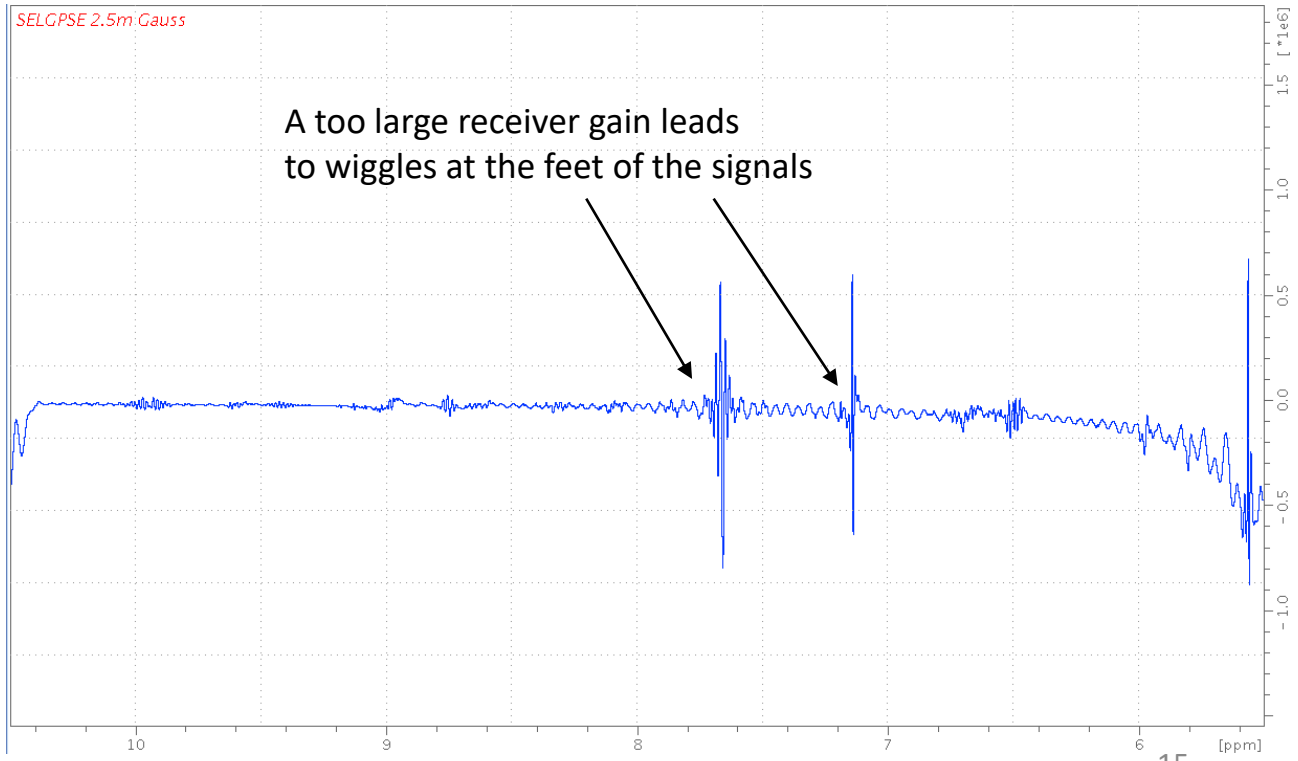
-> enter command **rsh** into the command line,
a table with saved shim files opens
-> select newest shim file called cdcl3 or Sucrose_3D and click on read
(make sure it was saved on the correct probe, usually, PABBI is inside av400)
-> execute TopShim again (either via **tg** or **topshim**)



Adjusting the receiver gain

-> signal emitted from the sample is enhanced by receiver gain
-> receiver gain is automatically determined by the spectrometer when entering the command **rga**
-> alternatively, a certain value can be chosen by entering **rg**
-> the receiver gain is small for concentrated samples and high for delute samples and varies depending on the channel (^{1}H, BB)
-> **rga** usually takes only a few seconds
-> repeat for each new measurement

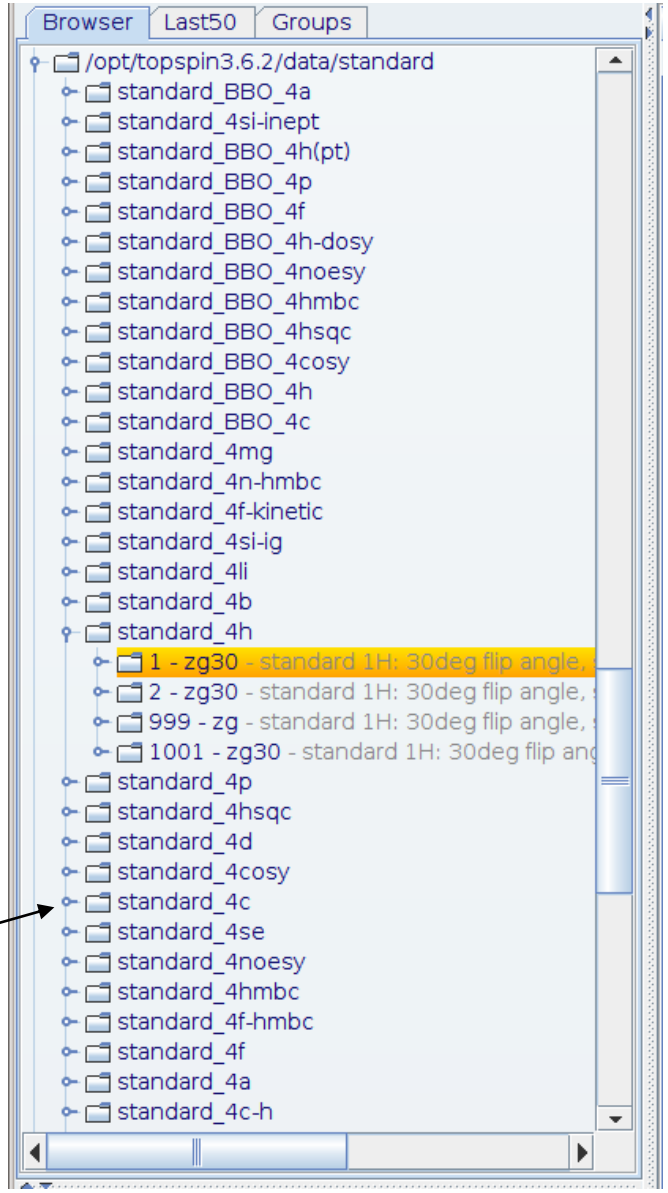
If you enter a command and the previous command is still running, the new one will be automatically added to the queue!



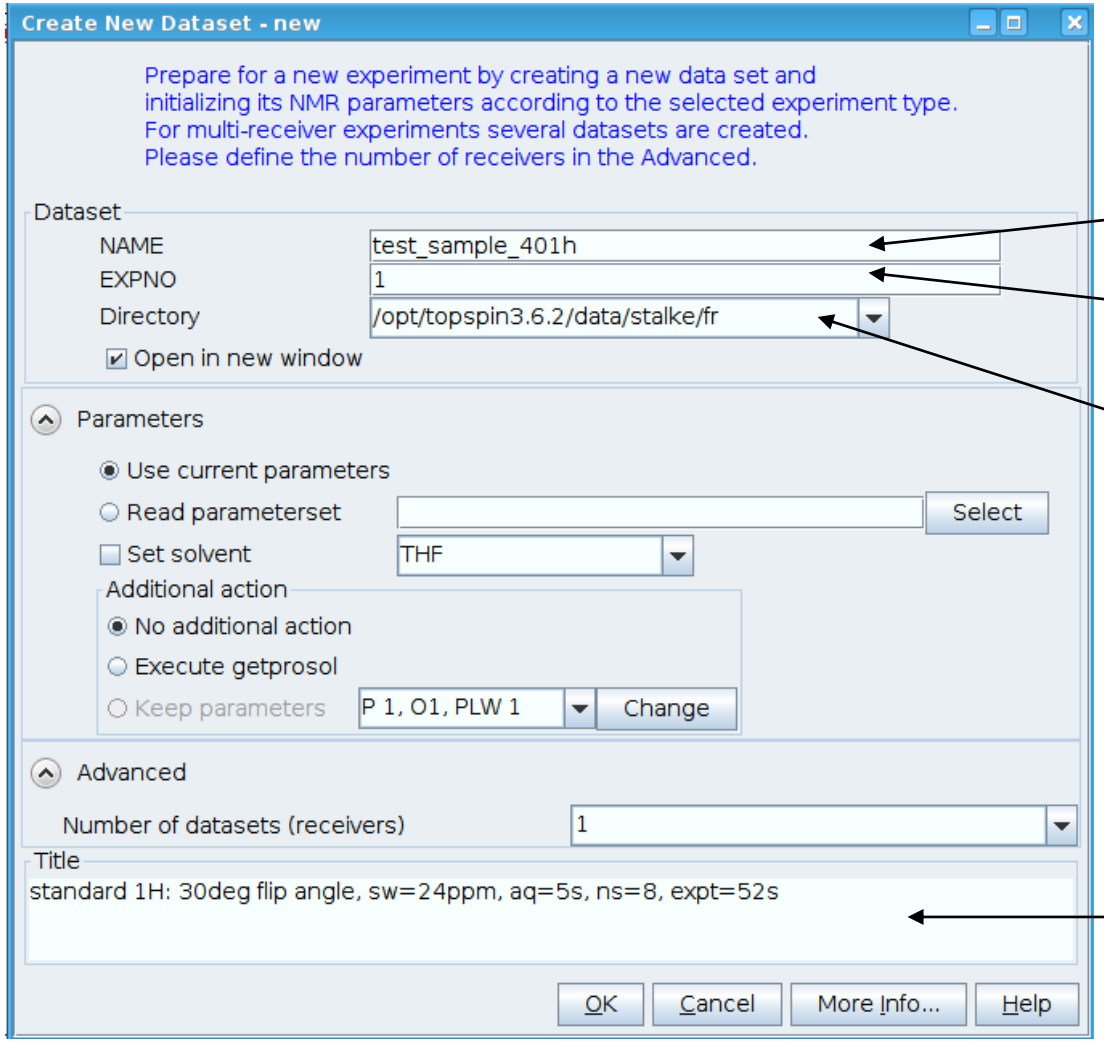
Creating a new dataset

-> easiest way to create a new dataset is via copying an already existing dataset
-> either copy one of your own experiments or take one from the standard file containing standard datasets with standard parameters
-> open the dataset you want to copy and enter command **new**
-> enter name of file, experiment number, directory of your research group and title (if you wish to) and press ok
-> **do not change the parameters of the standard datasets!**

The standard file contains a variation of different 1D and 2D experiments for several nuclei, e.g. standard_4c is a standard ^{13}C dataset



Creating a new dataset



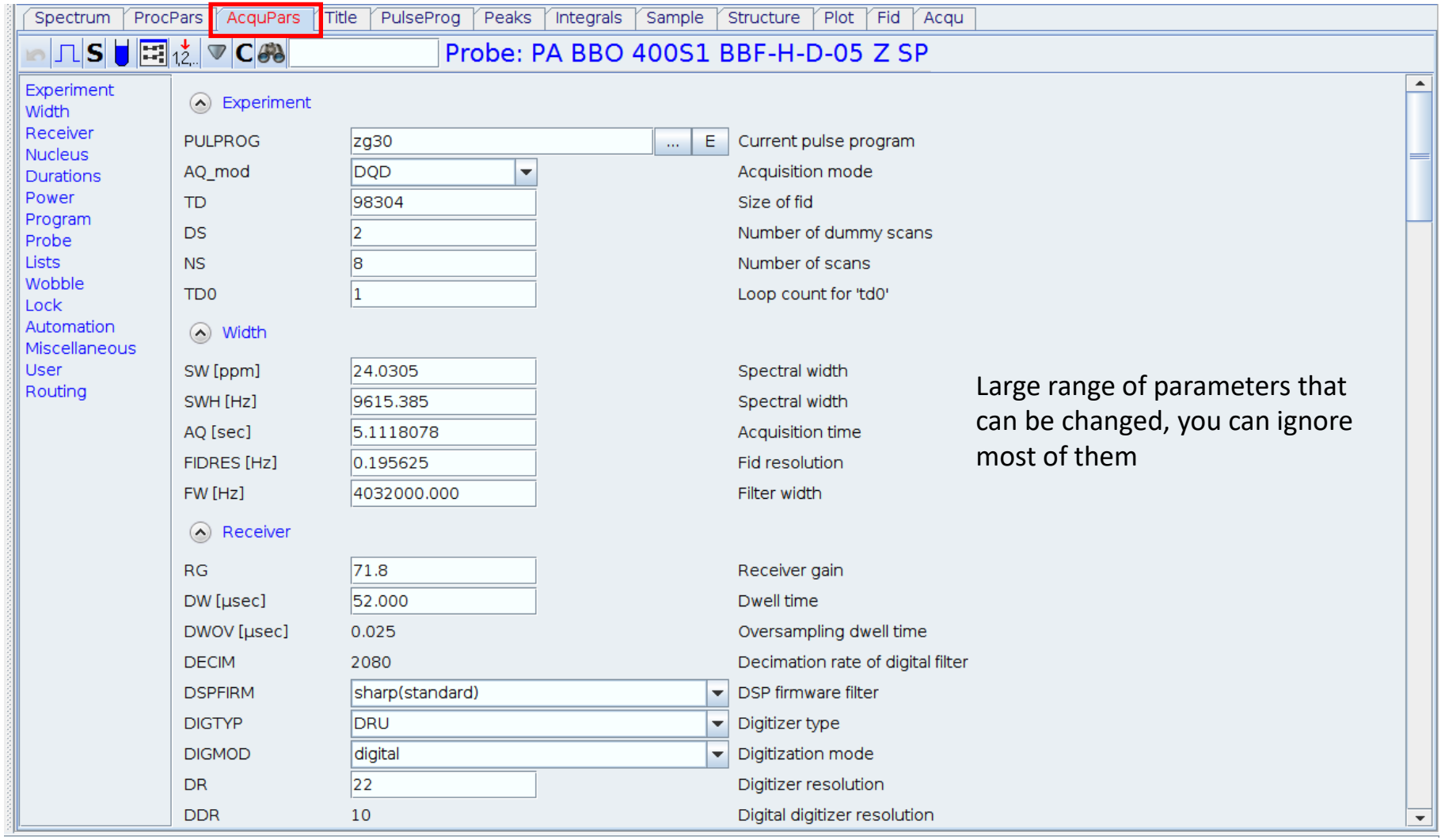
name of file (e.g. number of sample, acronym_4h,… ,
do not use spaces or special characters like ä, ö, ü)

experiment number (not always continued automatically)

directory into which the new dataset is copied
(don´t forget to change if you copy a standard dataset!)

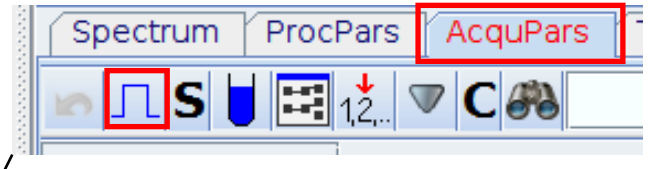
enter title of your experiment e.g. with experimental details
here (can be changed afterwards)

Optimizing acquisition parameters (1D)



Large range of parameters that can be changed, you can ignore most of them

Optimizing acquisition parameters (1D)



Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Plot | Fid | Acqu

Probe: PA BBO 400S1 BBF-H-D-05 Z SP

General
Channel f1

General

PULPROG	zg30 ... E		Pulse program for acquisition
TD	98304		Time domain size
SWH [Hz, ppm]	9615.38	24.0305	Sweep width
AQ [sec]	5.1118078		Acquisition time
RG	71.8		Receiver gain
DW [µsec]	52.000		Dwell time
DE [µsec]	6.50		Pre-scan-delay
D1 [sec]	0.100000001		Relaxation delay; 1-5 * T1
DS	2		Number of dummy scans
NS	8		1 * n, total number of scans: NS * TD0
TD0	1		Number of averages in 1D

Channel f1

SFO1 [MHz]	400.1320007		Frequency of ch. 1
O1 [Hz, ppm]	2000.65	5.000	Frequency of ch. 1
NUC1	1H Edit...		Nucleus for channel 1
p0 [µsec]	3.37		p0=p1/3
P1 [µsec]	10.100		F1 channel - 90 degree high power pulse
PLW1 [W, dB]	27.733	-14.43	F1 channel - power level for pulse (default)

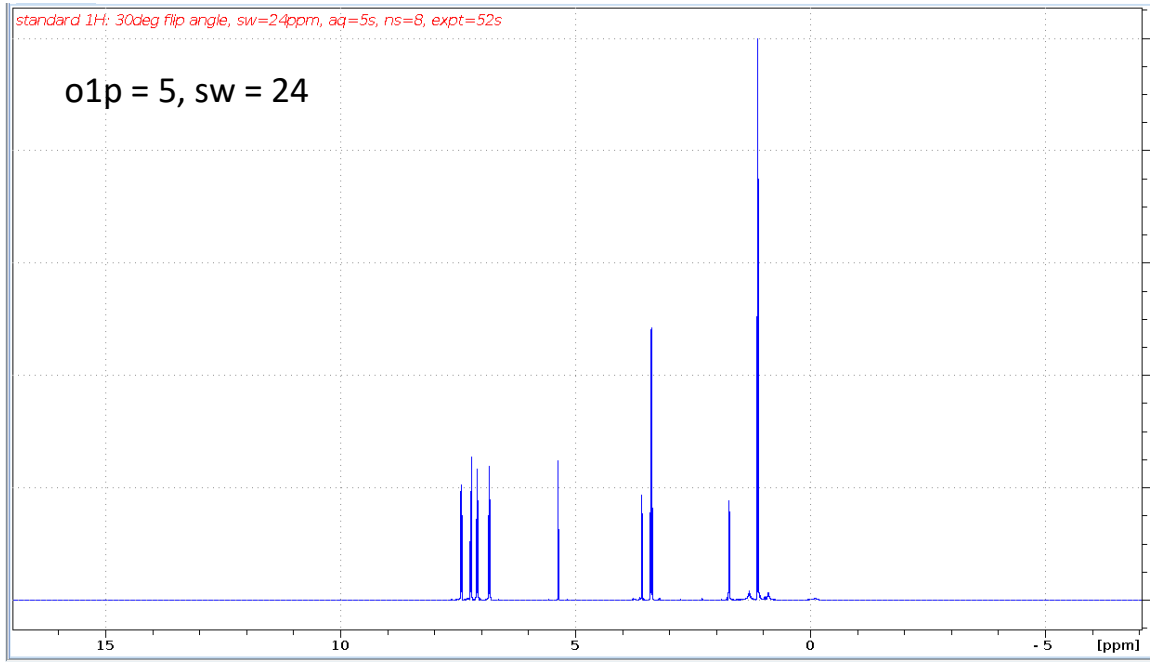
most important parameters

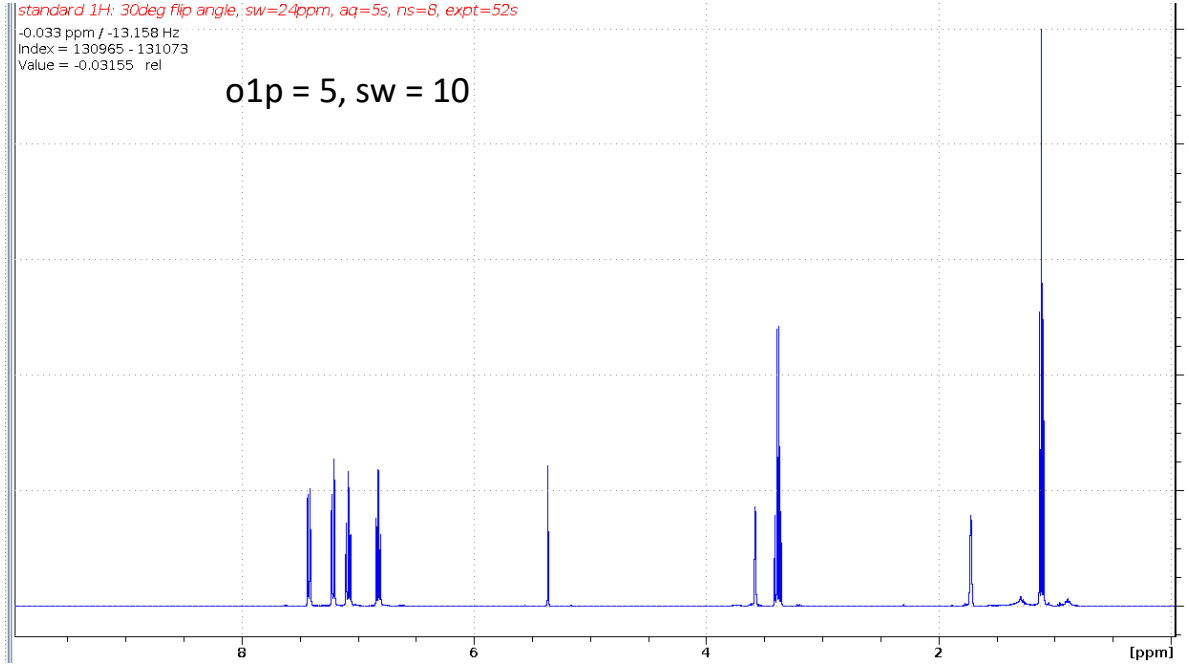
Parameters can be changed either in this screen or by entering the respective parameter into the command line, e.g. **o1p** for the transmitter frequency

Do not change the parameters in the standard datasets! Make a copy first!

Optimizing acquisition parameters (1D)

-> transmitter frequency (**o1p**, center of the spectrum) and spectral width (**sw**, width of the spectrum)
-> total ppm-region that is recorded goes from o1p – ½ sw to o1p + ½ sw
-> can be changed either in the acquisition parameters or by typing **o1p** and **sw** into the command line

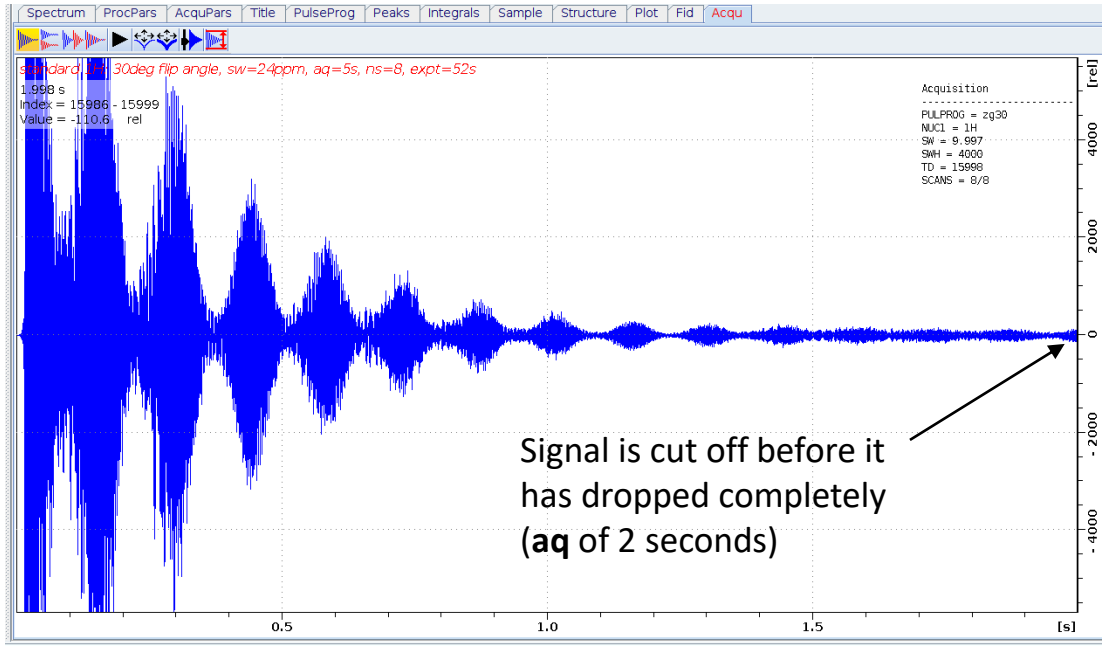


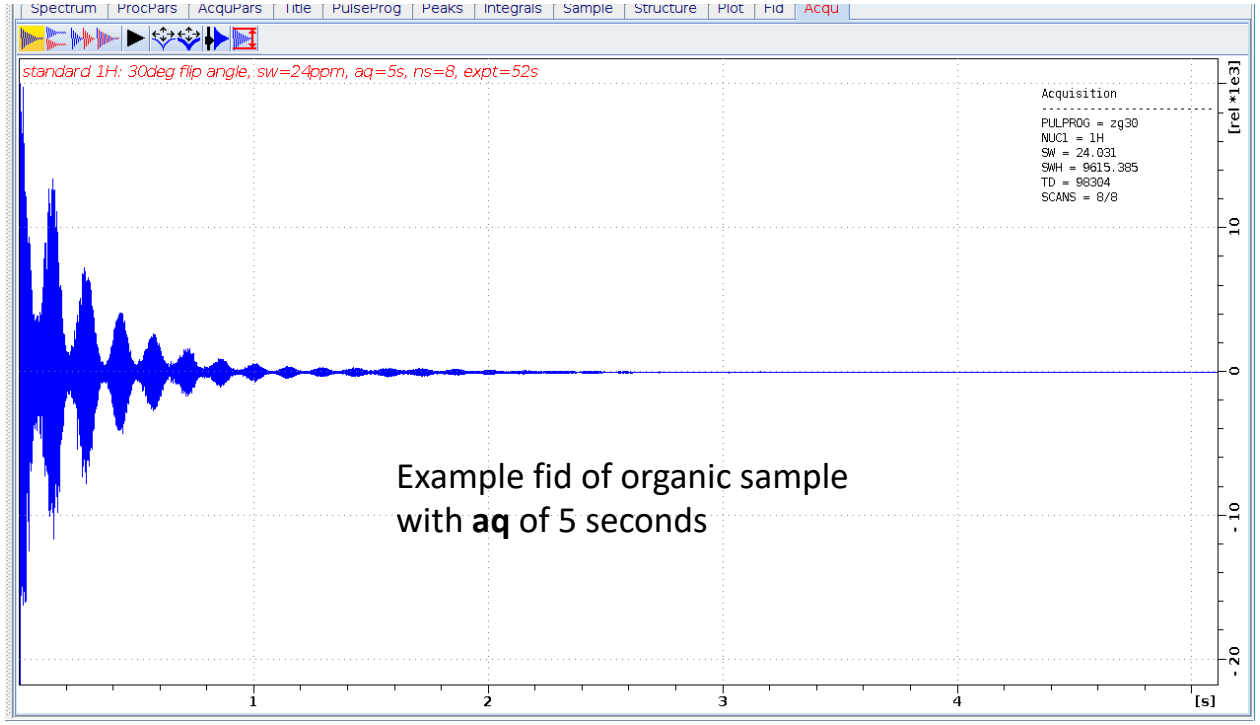


Optimizing acquisition parameters (1D)

-> acquisition time **aq** is the time in seconds in which the fid is recorded
-> is the **aq** too long, much noise will be recorded, leading to lower signal intensity
-> is the **aq** too short, the fid will be cut off, leading to signal distortions
-> for standard organic samples, an **aq** of 5 seconds for ^{1}H experiments is a good compromise

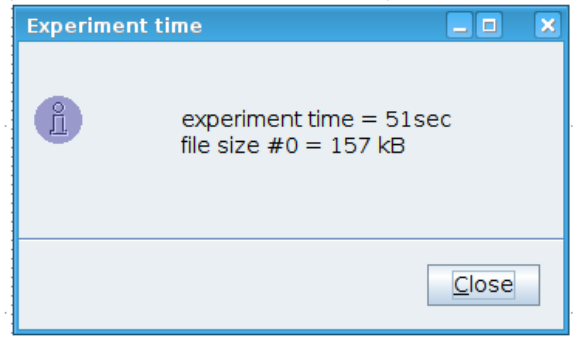
sw and **aq** are connected!
When the **sw** is halved, the **aq** doubles, so always check the **aq** after changing the **sw**!

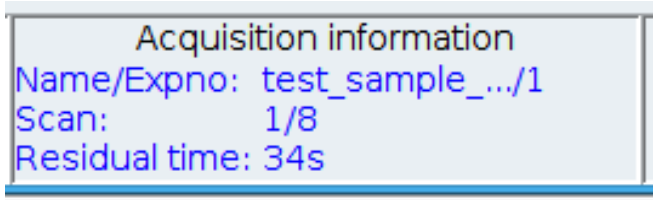


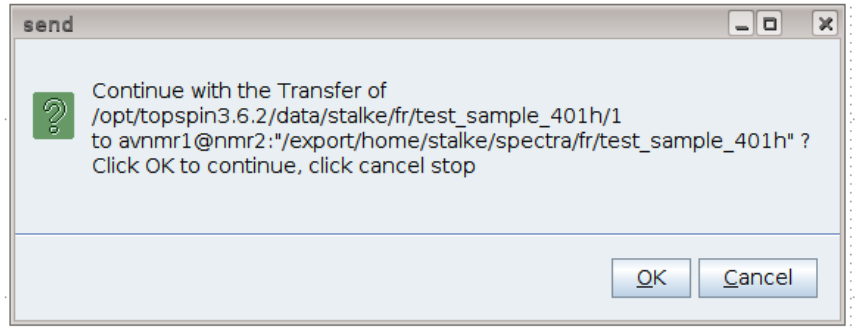


Starting & processing the measurement (1D)

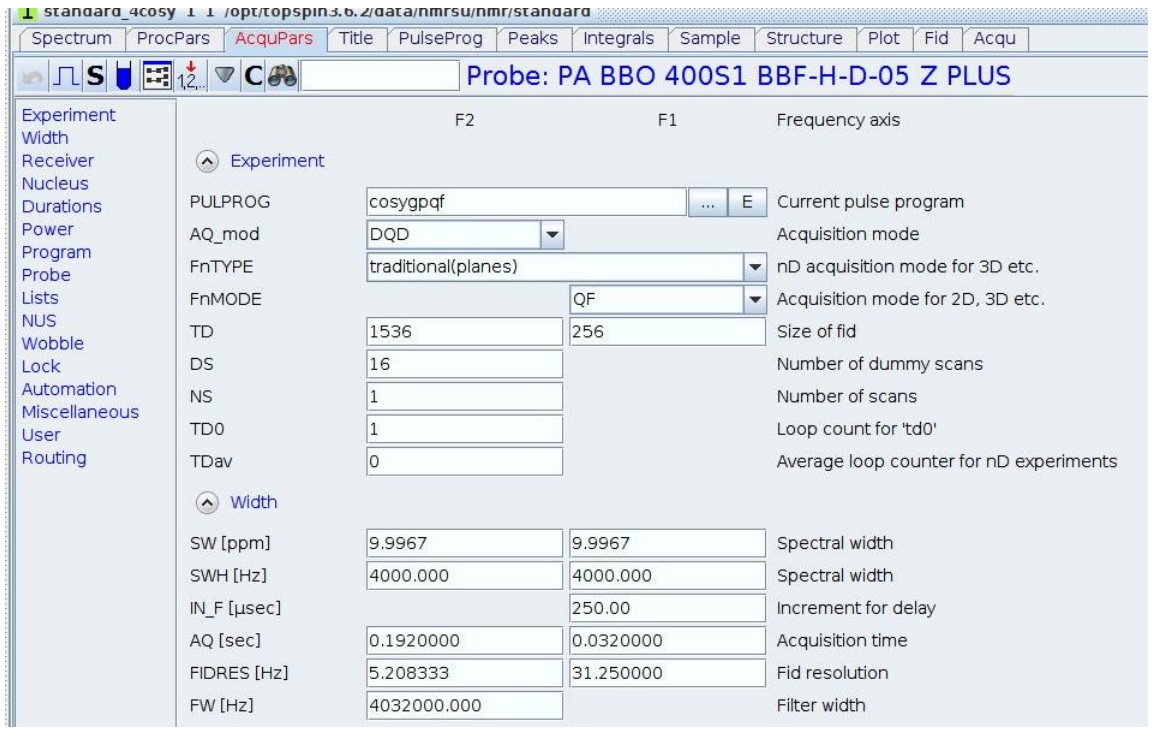
-> use command **expt** to see how long the experiment will take (dependent on **ns**, **aq**, …)
-> command **zg** starts the aquisition (valid for all 1D & 2D experiments except for DOSY)
-> acquisition information shows information about the currently running measurement
-> 1D fourier transformation is executed using command **efp**
-> 1D automatic phase correction (**apk**) and baseline correction (**abs n**, here the space is important) can be used
-> fourier transformation can also be performed while the measurement is still running
-> if this error occurs then, first use command **tr** to store already recorded scans to the disk before executing **efp** again
-> when you want to stop the acquisiton, use either **stop** (measurement stops immediately, fid is not saved) or **halt** (stops after currently running scan, fid is saved)
-> send your data to your group server via command **send**



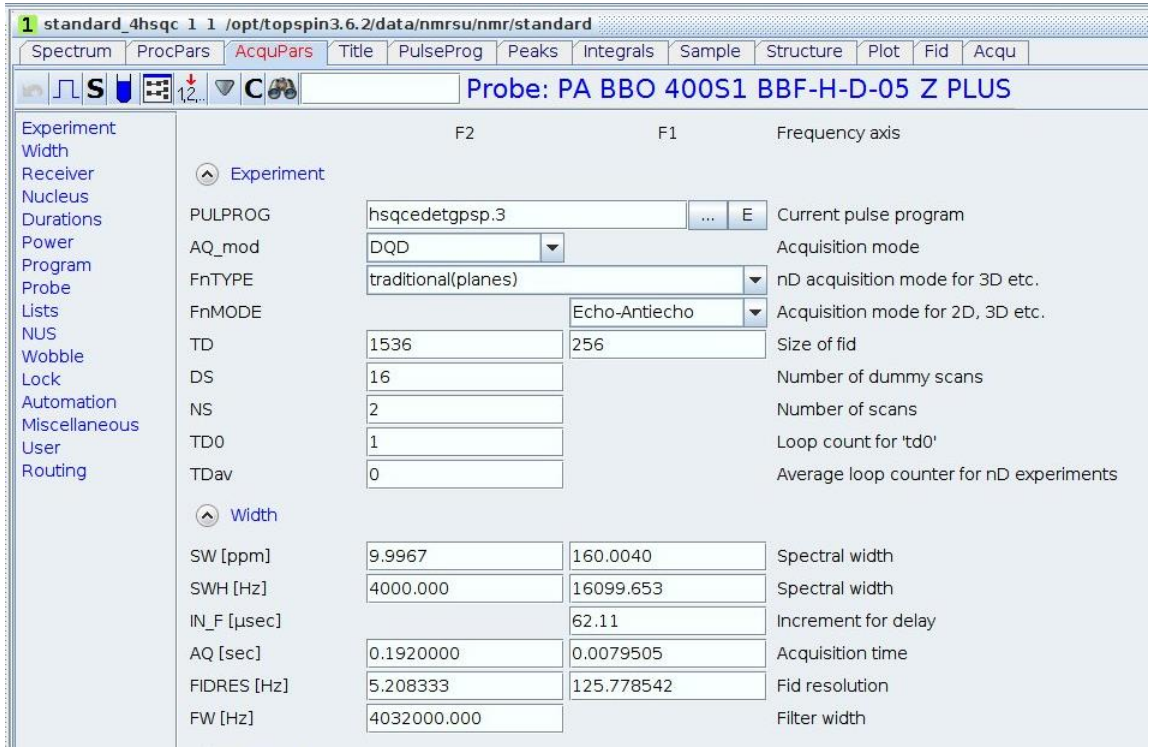




Optimizing acquisition parameters (2D)



Homonuclear (e.g. COSY)

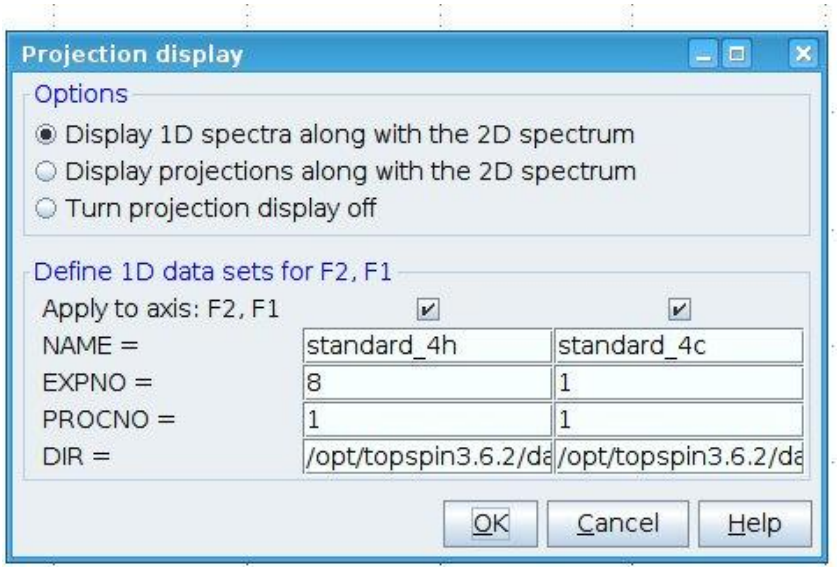


Heteronuclear (e.g. HSQC)

-> parameter optimization for 2D experiments very similar to 1D experiments, only there are often two values (F2 and F1)
-> F2 direct dimension (horizontal), F1 indirect dimension (vertical)

Processing the measurement (2D)

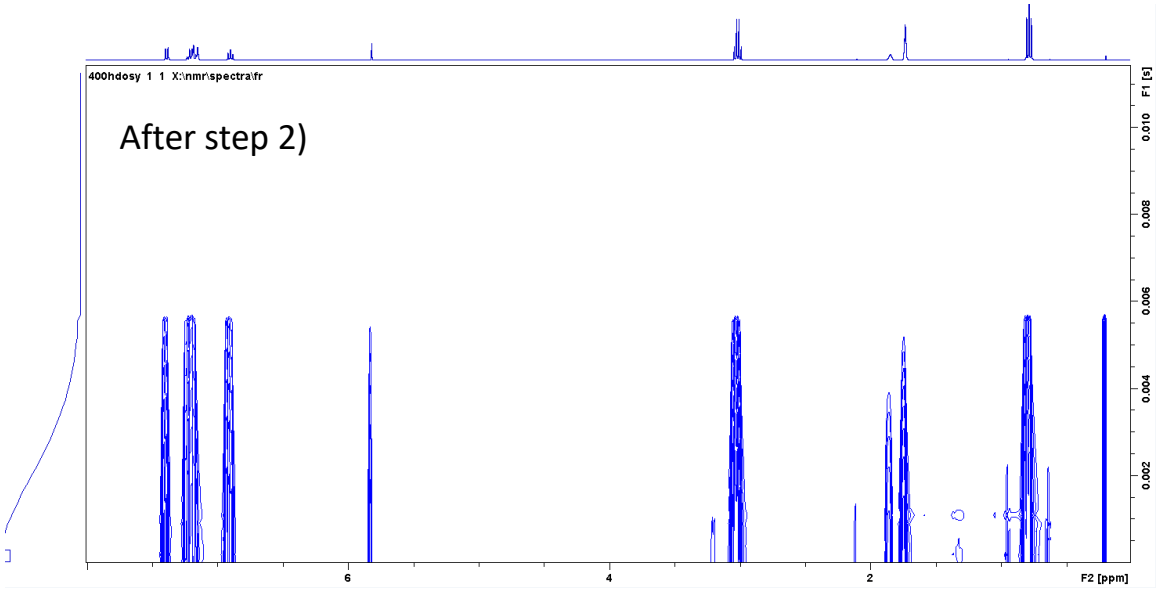
-> 2D fourier transformation works via command **xfb**
-> 2D baseline correction is executed either via **absb** (no space here) or separately
correcting the respective dimensions via **abs1** and **abs2**
-> using command **projd**, you can project already measured 1D spectra as traces to the 2D spectrum

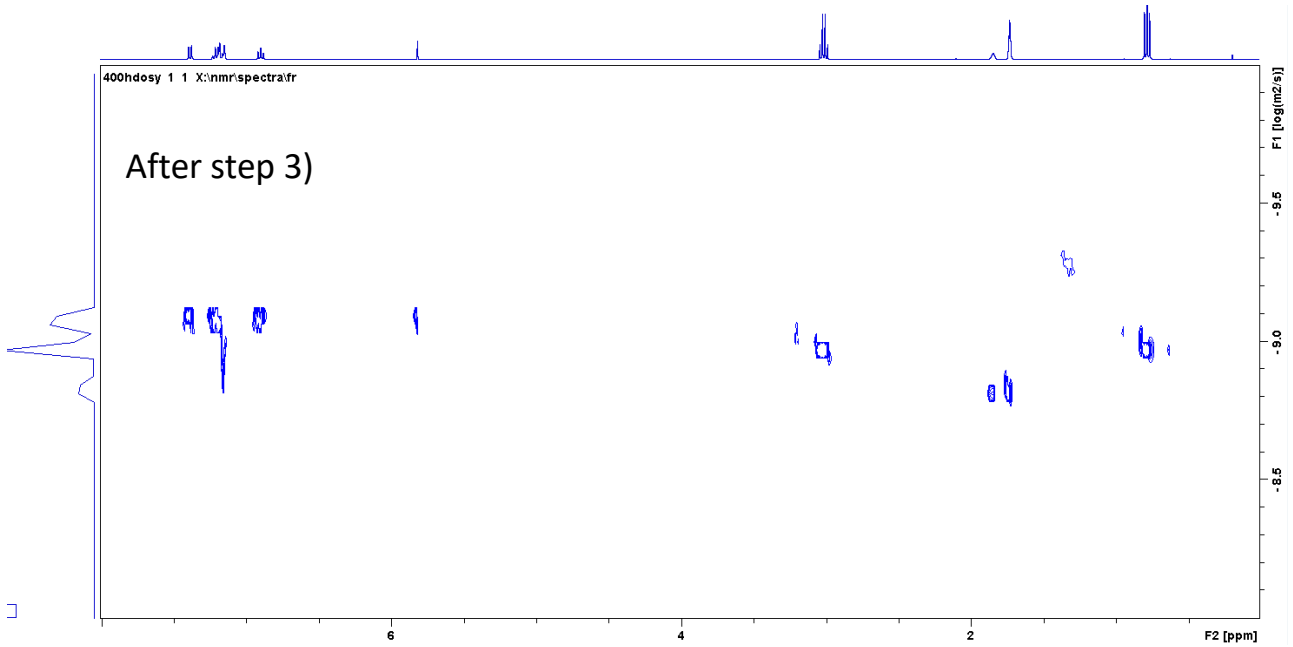


Measuring and processing DOSY

-> unlike all other experiments, DOSY is started via command **xau dosy**
-> a window opens, asking about gradient setup, just press enter until the measurement starts

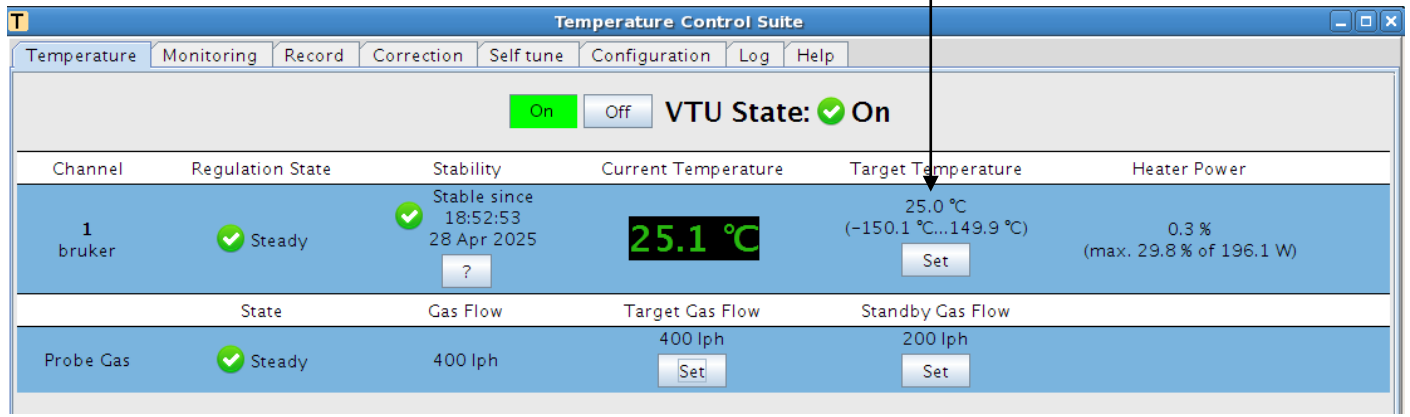
-> processing DOSY requires multiple steps:
1) process a 1D FID via **efp** and do a phase correction using **.ph** (press  to transfer the correction to all FIDs)
2) enter command **to2d** to return to 2D view, then process the pseudo-2D experiment with **xf2** and **abs2** (baseline correction)
3) to create the diffusion dimension, enter command **setdiffparm** and afterwards **dosy2d** to obtain the typical DOSY spectrum





Variable temperature (VT) measurements

-> please make sure your chosen solvent is suitable for the temperature range you plan to measure
-> temperature is chosen and monitored via temperature control suite (double-click on probe temperature)
-> for temperatures **higher than 25 °C**, click on „set target temperature“ and enter desired value
-> the spectrometer will adjust the heater power by itself to reach the new target temperature
-> is the current temperature lower (higher) than the target temperature, the value is shown in blue (red)
-> the color of the value will change to green when the target temperature is reached and stable



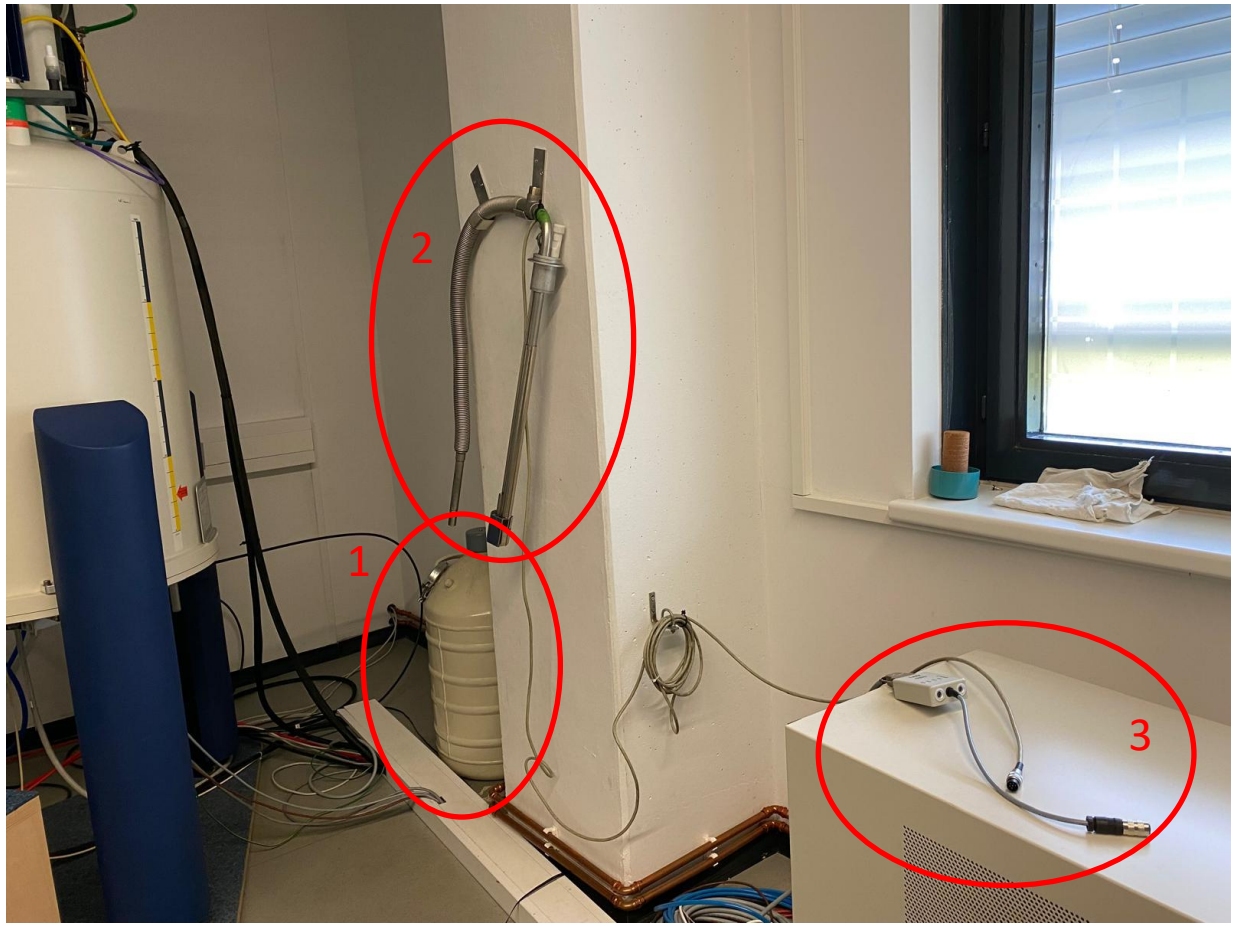
Plan more time for your VT measurements than for measurements at room temperature! Your booked time should include enough time to reach your desired temperature and turn the spectrometer back to 25 °C afterwards!

Variable temperature (VT) measurements

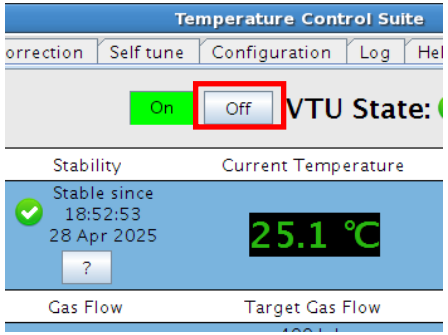
-> for temperatures **beneath 25 °C**, an external cooling with (liquid) nitrogen is necessary (N2 chiller)
-> cooling equipment ready to use next to av400

1) Dewar with liquid nitrogen (bring your own in case it´s empty)
2) Nitrogen transfer line (connects dewar with probe)
3) Connection cable between console and chiller (for regulation)

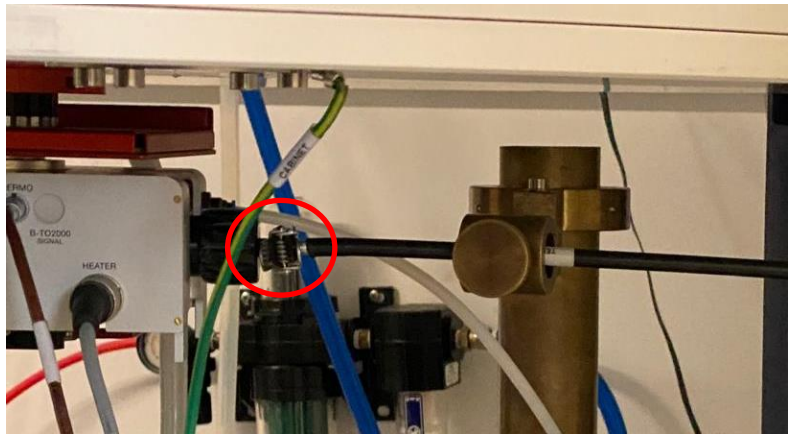
If it´s your first time using the low temperature setup, let an experienced person show you how to install and use it. Don´t use the setup on your own until you´re 100% sure how to use it. If you are unsure about anything, do not hesitate to ask someone with experience!



Installing the N2 chiller

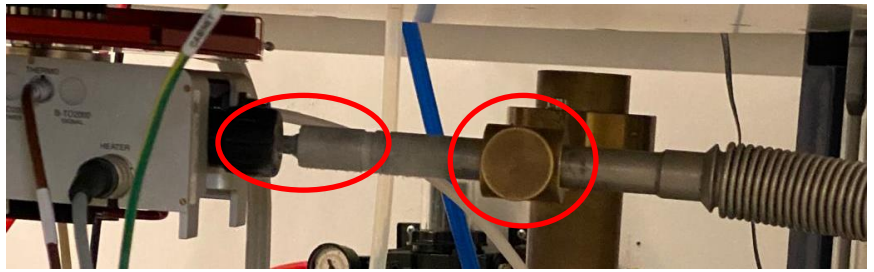


1) Open temperature control suite and press „off“



3) Remove the hose and the respective clamp regulating the air flow at the back of the probe

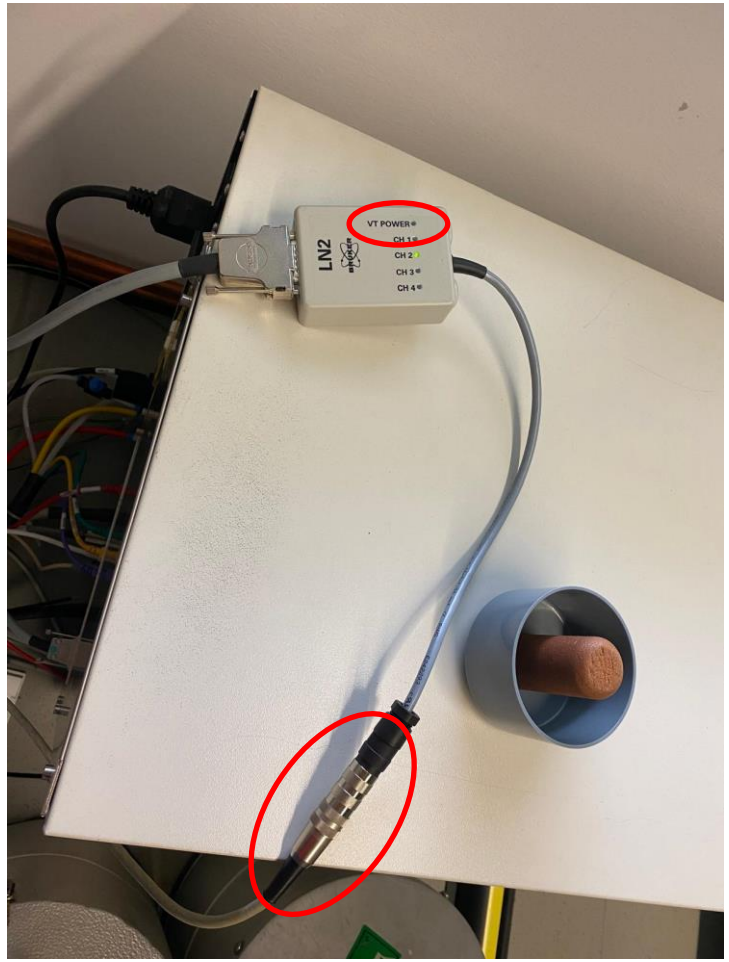
2) Insert the transfer line into the dewar and secure it with the clamp (don´t forget the gasket)



4) Put the hose of the transfer line through the bronze mount and secure it with the screw. It should sit tightly at the gas inlet to prevent leakage

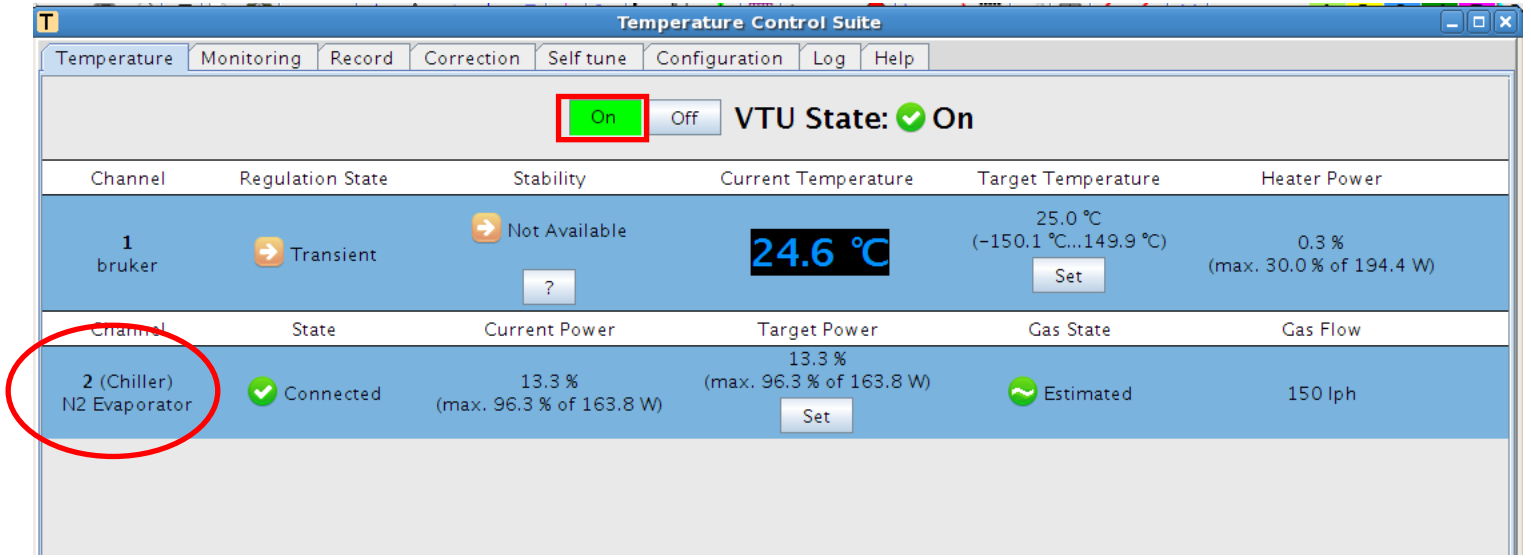
Please don´t remove any equipment parts from the room and put them back to where you found them after use!

Installing the N2 chiller



5) Connect the cable of the transfer line with the port lying on the av400 console (no brute force!). The light behind „VT power“ should blink after a few seconds

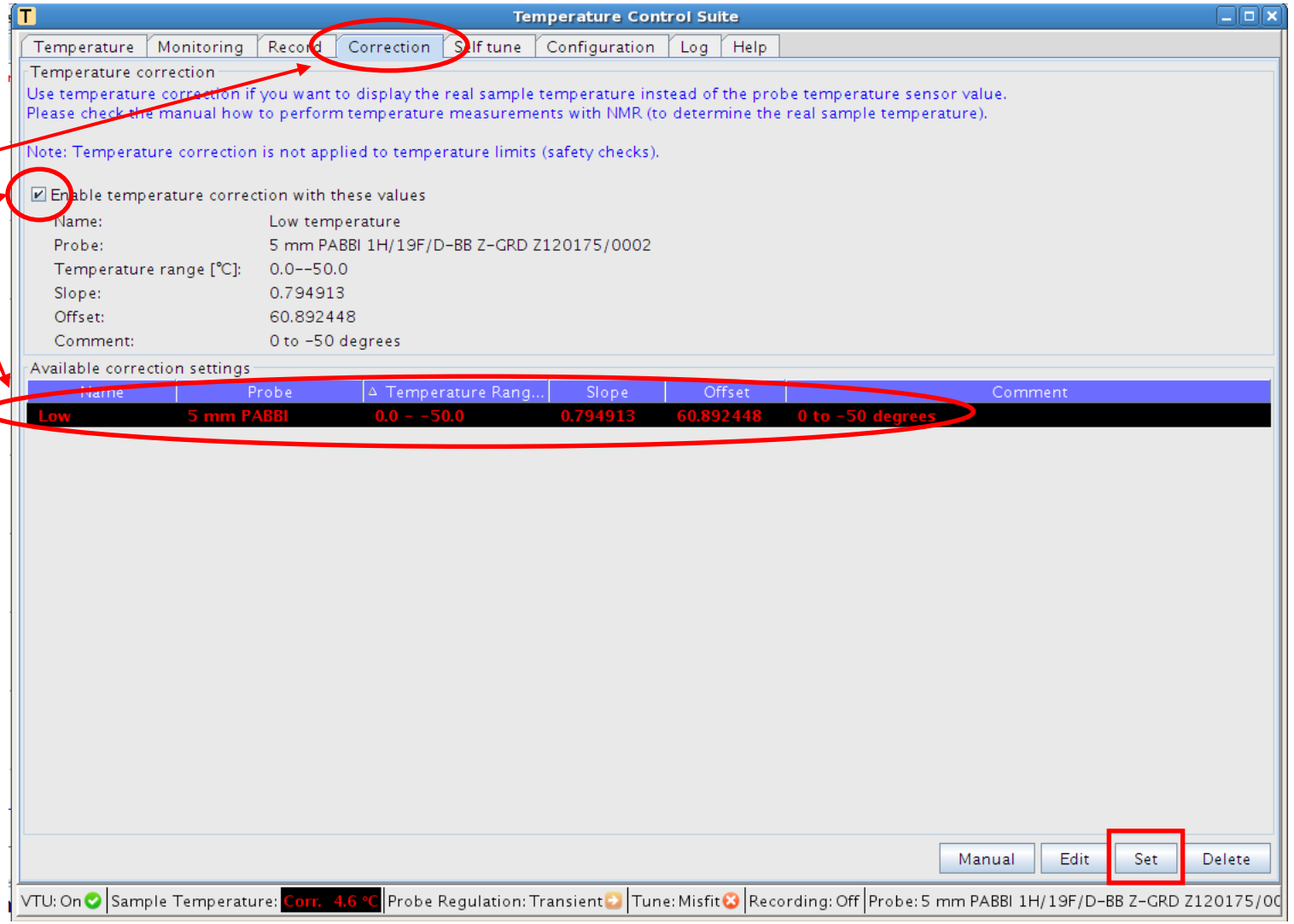
6) Click on „on“ again. After successfull installation, the N2 chiller should be shown in the temperature window



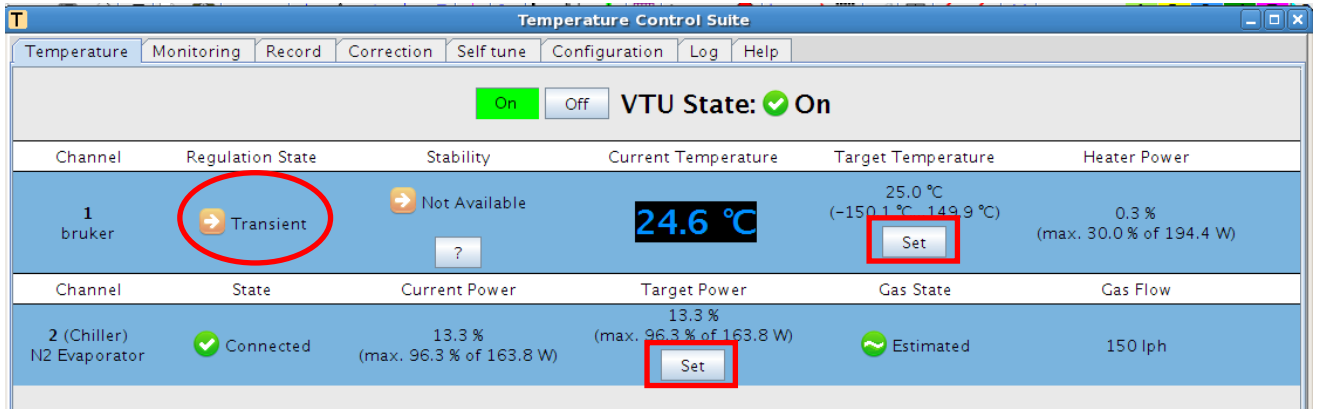
Low temperature measurements

-> low temperature measurements require a temperature correction
-> after installing N2 chiller, open temperature window and go to correction window
-> place tick and select available correction plot
-> click on „set“
-> the temperature window will now show a corrected (actual value) and a measured temperature value (as measured by the device)
-> when correction is active, just enter the desired temperature as target temperature
-> temperature correction works reasonably well between 0°C and -75°C

-> after going back to room temperature, just remove the tick again



Low temperature measurements



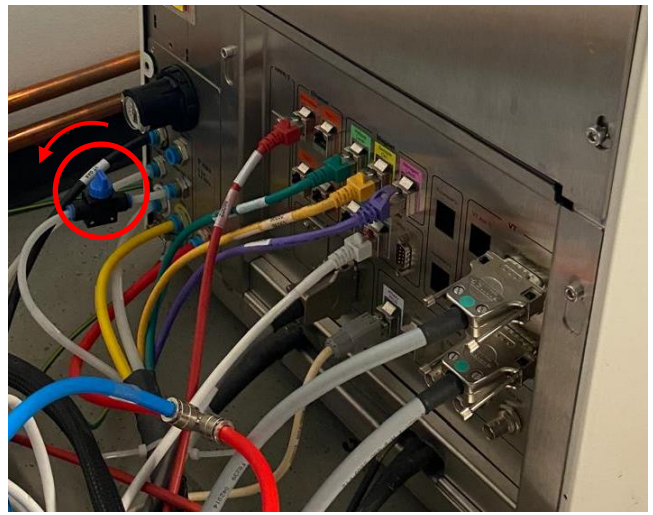
-> enter desired temperature and appropriate evaporation power
-> wait until target temperature is reached and stable (this may take some time, especially when cooling down from room temperature as all the equipment is still warm then)
-> regulation state should be transient

Use 3 % for 0 °C
Use 5 % for -25 °C
Use 7 % for -50 °C
Use 10 % for -75 °C

What to do if the regulation state says „insufficient chilling“?

-> check whether the transfer line hose sits tightly on the gas inlet and tighten it further if necessary
-> putting a towel around the hose helps to prevent ice formation on the magnet
-> slightly increase the evaporation power

For longer measurements (overnight) at temperatures lower than 0 °C or higher than 80 °C, switch on the shim gas to prevent ice formation in the shim system by turning the blue knob at the back of the console. Use this preferably after 3 pm as the shim gas of av400 is pretty loud



abs1/abs2	Baseline correction of F1/F2 in 2D spectrum
absb	Baseline correction of both dimensions in 2D spectrum
abs n	Baseline correction in 1D spectrum
apk	Phase correction in 1D spectrum
aq	Show/change aqcuisitioin time
atma	Automatic tuning and matching of the probe to the frequency of the nucleus to be measured
atmm	Manual tuning and matching of the probe to the frequency of the nucleus to be measured
dosy2d	Creates typical pseudo-2D DOSY spectrum
edlock	Show table of available solvents and their locking properties
efp	Performs 1D fourier transformation
ej	Ejects sample/turns on air stream
expt	Shows estimated duration of experiment
halt	Stops running experiment after currently running scan and saves fid
ij	Injects sample/turns off air stream
lock/lock *solvent name*	Select solvent for locking/starts locking on the stated solvent
lockdisp	Opens lock window

new	Creates new data set by copying open data set
ns	Show/change number of scans
o1p	Show/change transmitter frequency (center of spectrum)
projd	Project already existing 1D data sets as traces of 2D data set
rg/rga	Show/change receiver gain/automatically adjust receiver gain
rsh	Read existing shim file from disk
send	Send your data to your group server after measurement is complete
setdiffparm	Creates diffusion dimension during DOSY processing
stop	Stops acquisition immediately without saving fid
sw	Show/change spectral width
tg	Opens TopShim window
topshim	Performs gradient shimming on sample
tr	Save already recorded scans to disk while experiment is still running
xau dosy	Starts DOSY experiment
xfb	Performs 2D fourier transformation (both dimensions)
zg	Starts any 1D and 2D experiment (except for DOSY)