NMR spectrometer usage at the BioNMR facility ETH Zürich

Accounts

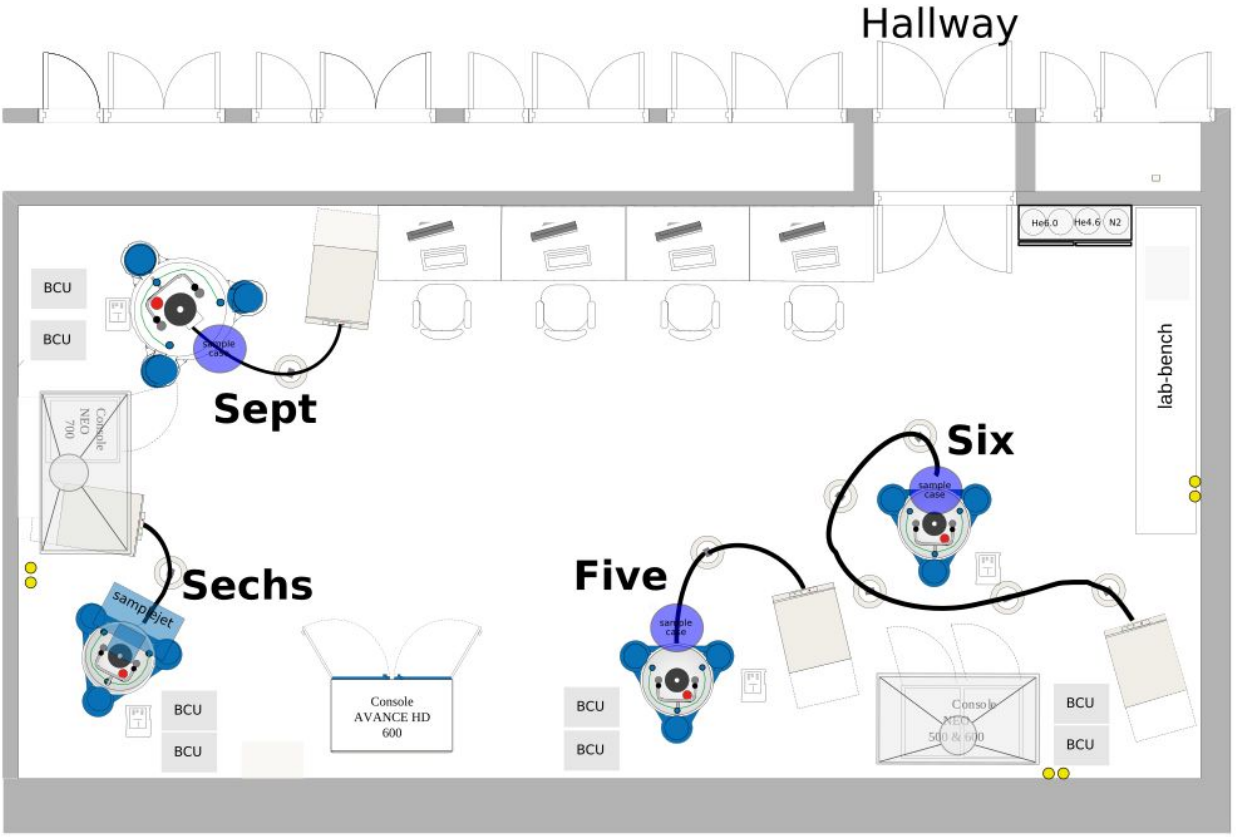
Logins on the NMR workstations are independent of ETH accounts on our institute & group computers. You will need to obtain an account from the NMR administrator before working independently at the spectrometer. There are also student accounts available for training, block course & semester projects. You may work on your supervisors account with his/her consent once you have received basic training.

Safety precautions: strong magnetic fields

- Keep magnetic materials (like iron tools or gas bottles) away from the magnets. Magnetic items may fly towards the magnet and hurt someone, or lead to a quench of the cryogenic magnet.
- Do not bring credit cards or electronic devices like cell phones close to the magnet. The memory may be erased by the strong magnetic fields.
- In case of pregnancy stay outside of 0.5 mT line.
- Do not bump into the magnets or other NMR equipment.

The 900 (lab HPK B7/9) and 750 MHz (lab HPM A54) spectrometers have a large stray field, almost up to the spectrometer computer consoles.

For the 500, 600, 600b and 700 MHz the 0.5 mT (=5 Gauss) line is the boundary of the red circles marked on the floor of the NMR labs. These spectrometers are located in lab HPK B1 (see floor plan).



Parts of an NMR spectrometer

1. Superconducting magnet and cryostat
   The coil of the superconducting magnet is cooled with liquid helium (4.2 K).
2. Console
   The console contains the electronics (amplifier, pulse generator, shim control, digitizer, etc.)
3. Preamplifier
   The weak signal from the probehead is amplified before it is transmitted to the console.
4. Probehead
   The probehead holds the sample and contains the sending and receiving coils for the individual nuclei (2H, 1H, 13C, 15N, 31P, 19F).
5. Spinner
   The NMR tube is inserted into the spinner and the spinner with the tube into the magnet.
6. Cryoplatform
   Cooling of the cryo probehead is provided by the cryoplatform.
7. BCU
   The sample temperature is controller with the BCU.
8. Workstation
   The spectrometer is controlled by the workstation computer with the program “topspin”.

NMR data storage

Data are stored locally on the spectrometer computers in **/opt/topspin/data/username/nmr.** The raw nmr data (ser files) are deleted automatically from the spectrometers computers after two weeks. Copy the data to a safe place immediately after completing measurements. If any file is accidentally deleted or changed you can retrieve the old versions from a rolling backup (up to 2 weeks). Data are also backed up at the central ETH backup for six months. Details on these backups and how to retrieve data are on our NMR Database pages.

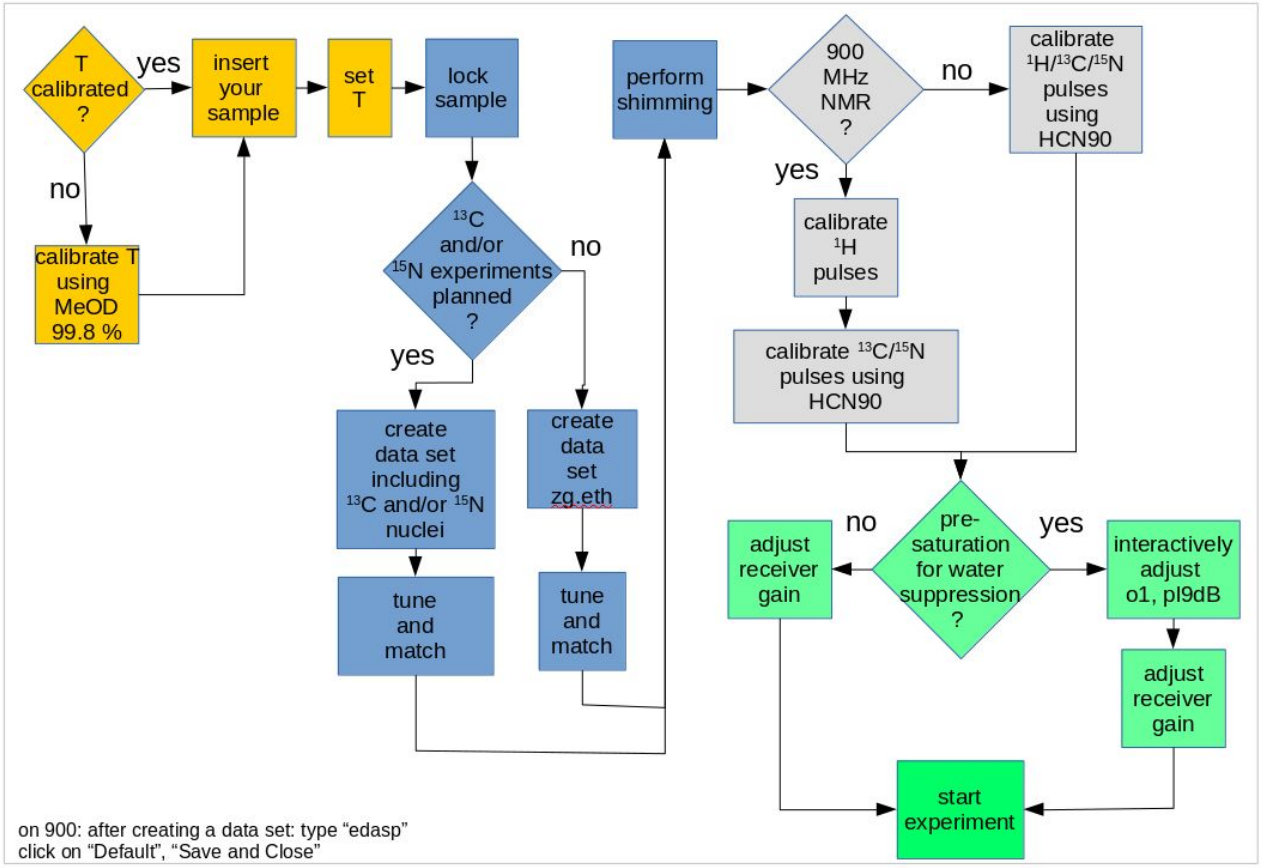
Start topspin software

1. Log in to the computer which is controlling the spectrometer Each workstation screen is labeled with the spectrometer name. Be careful not to confuse the six and the six2!

2. Start topspin by clicking on the topspin icon. Topspin is the software to control the spectrometer, process and analyze the data. The current topspin version is topspin

3.2..

Initial steps for performing NMR measurements



The sample

There must be a sample in the spectrometer at all times. If there is no lock signal when you start or no sample appears when you eject, contact an NMR manager. This could be a problem with a sample being stuck in the bore. Putting in a second sample can cause a major problem and may even require warming up the cryoprobe or loss of your sample.

Samples must be in standard or shigemi NMR tubes (3 mm or 5 mm) and correctly positioned in the spinner to prevent damage to the probehead and sample. Samples must be cleaned before inserting into the spinner using a chemwipe. No sticky or loose parafilm may be present. If the sample is loosely held by the spinner try a different one. Do not use the heavy white ceramic spinners.

At each spectrometer workstation desk there is a standard sample in a fused 5 mm standard NMR tube with a blue “D2O/H2O” label containing 10% D_2O in H_2O. The sample should be put in the spectrometer & locked after each user completes his/her session.

Insert sample

Magnets without SampleCase (nine, seven) and SampleJet in manual mode (sechs)

1. Eject sample: **`ej`** (H_2O/D_2O sample should be ejected if you just started your session)
2. Remove the previous sample from the magnet.
3. Insert your sample tube into a spinner, and adjust the insertion depth by centering the liquid around the active volume of the probe using the plexiglas device. The sample insertion depth should maximally be 21 mm from the center.
3. Insert sample on top of magnet, and type **`ij`**

Magnets with SampleCase (six, five, sept)

1. Put your sample with spinner into a free holder in the SampleCase carousel.
2. Type **`sx holder_nr`** to insert the sample from holder_nr into the magnet.
3. Holder 23 and 24 contain the standard samples for temperature calibration and sucrose to be inserted in the magnet in case there is no sample from the user.
4. To eject the sample type **`ej.`**

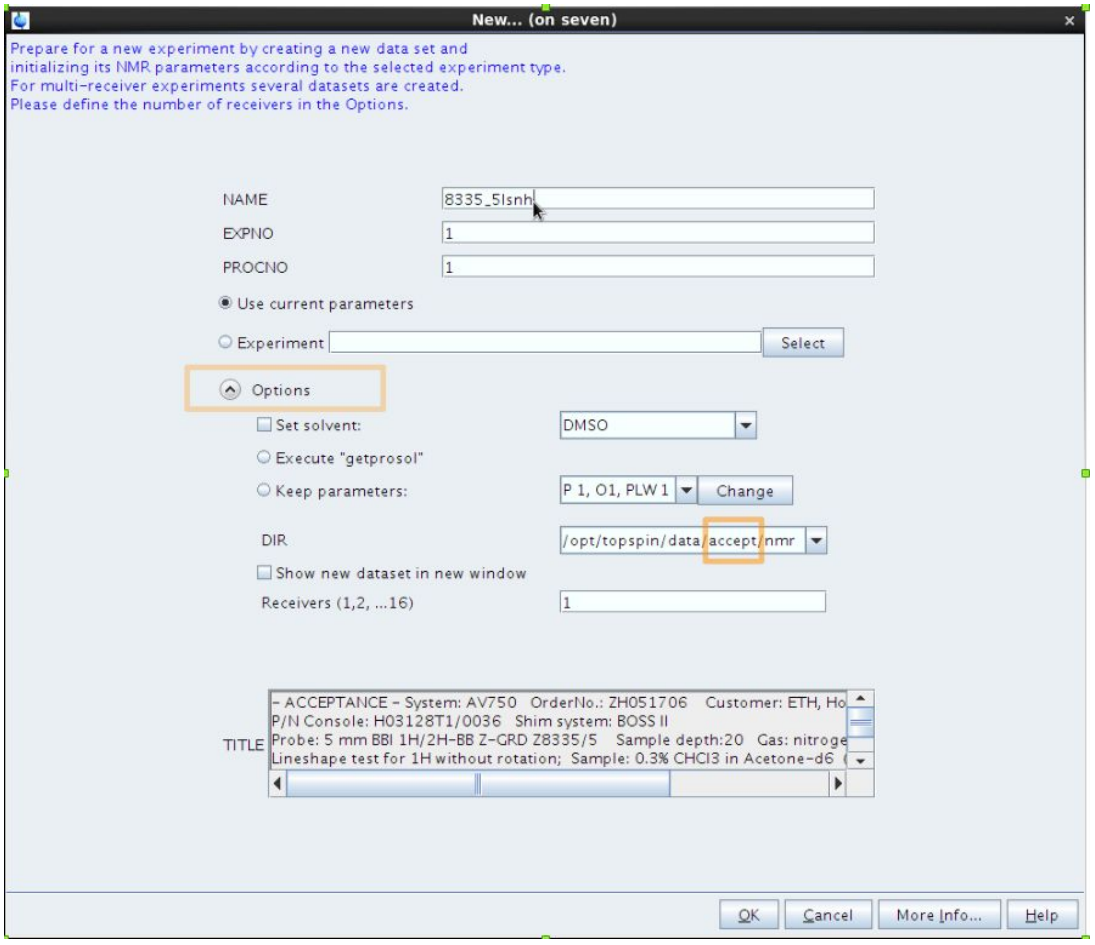
SampleJet (sechs)

Please ask Simon or Alvar if you like to use the SampleJet. The SampleJet robot needs special caps for the tubes.

Create new experiment

Create a data directory for your experiments.
This is done by copying a directory from one of your earlier experiments or from another user or by creating a brand new experiment by entering edc and loading a parameter set using **`rpar`**.
To copy an experiment directory you first have to load that data set into topspin: navigate to the directory on the left panel. Right click on the directory and select “display”… The experiments you want to copy is now the currently active dataset in topspin.

In order to copy the currently active dataset directory type **`edc`**.
The following window will appear:



To create a copy of the current dataset in your folder you need to change the username (in the example above “accept”) to your username. In case the DIR window is not visible, click on “Options”. You may also want to change the name of the directory so that you can easily identify the type of sample, experiment and date later. A good naming convention is for example DATE_SAMPLE_EXPTYPE, for instance 20180103_Ubiquitin_bbassignment where DATE is in YYYYMMDD format.

Set Temperature

Type **`edte`** or double-click on the temperature display icon at the bottom of the topspin window.

Allowed temperature range: 0 °C to 80 °C.

Allow the temperature to equilibrate during 3–5 minutes (3 min for 3 mm tubes, 5 min for 5 mm tubes)
The temperature should be calibrated before each experimental session. See section “Temperature calibration”.

Locking

Lock the sample by typing **`lock`** and selecting the lock solvent.
The purpose of the lock is to keep the magnetic field constant by monitoring a reference signal, which is the deuterium signal of the lock substance, and adjusting the magnetic field to compensate for external (like moving metal objects) or internal (drift) perturbations.
Type **`lock`** to open a window to select the lock solvent. Select the solvent for your sample (usually 10 % D_2O / 90 % H_2O for biological samples). The spectrometer now reads the parameters for the corresponding lock solvent, lock power, gain, frequency and searches for the lock signal within a given range.
Wait until "lock finished" appears.

Tuning and matching

Start tuning and matching by typing **`atmm`**.
A new window will open and show the wobble-curve of the channel with the lowest frequency. E. g. if you loaded the parameter set zgpr.eth it has three channels defined

channel 1: H1

channel 2: C13

channel 3: N15

The tuning and matching curve of N15 is displayed first. The Larmor frequency of N15 is shown by a vertical red line. You can adjust TUNE and MATCH using the < and > buttons for small steps, << and >> for medium steps, and <<< and >>> for large steps. When done Save, Clicking *switch nucleus* will change to C13 which has the next higher frequency. Repeat procedure. Click switch nucleus again & finally adjust H1 and save.

You can also do this entire procedure automatically by entering the command **`atma`**. It says "atma finished" once atma is completed.

Shimming

Shim the sample by typing **`topshim gui.`** Start shimming by clicking on "Start". The message "topsim complete" indicates that shimming is finished. The quality of the shim can be assessed from the "report" tab. The parameters envelope width and B_0 inhomogeneity are supposed to be below 0.5 and 0.2 Hz, respectively.
Shimming is required to create a homogeneous magnetic field. An in-homogeneous magnetic field result in broad and/or asymmetric lineshapes.

^{1}H pulse calibration

Calibrate the pulse length for the proton 90 deg pulse using the water resonance at 4.7 ppm.

1. Change to your data directory.

2. **`rpar zg.eth`** : load the data set called zg.eth.
3. **`pl1db -13`** (-13 dB, the exact value depends on the spectrometer) : Set the power level for the high power proton pulse.
4. **`p1 1 us`** : Set the pulse width to a small value (1 µs) and acquire a spectrum to set the phases of the water signal correctly.
5. **`ns 1`** : number of scans
6. **`ds 0`** : dummy scans
7. **`rg 1`** : receiver gain set low to avoid receiver overflow because of the intense water signal
8. **`zg`** : zero go
9. **`ft`** : fourier transform
10. **`apk`** : automated phase correction. Observe the intense water signal at around 4.7 ppm and make sure it is a symmetric gaussian curve.
11. Set the carrier at the center of the water signal at about 4.7 ppm using the cursor by clicking on the  icon. Move the cursor to the center of the peak and click the left mouse button, select O1. The o1 values is now stored for the current parameter set.

For the actual calibration, the 360 deg pulse duration is determined and divided by four to give the 90 deg pulse.

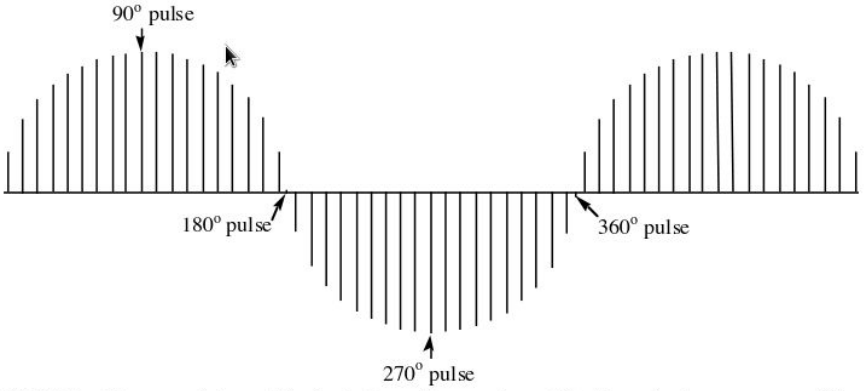


Figure 1. Representation of typical view of arrayed *pw* data for a single resonance. The null points are at 180° and 360°. Notice that determining the 90° pulse-width directly is more difficult than finding the null points.

Determining the 360 deg pulse has two advantages: once the p1 is close to 360 the water magnetization is returned to the positive z axis and therefore only a short equilibration delay is needed. Furthermore, it is visually more straightforward to identify a zero transition, like in the figure above, than a maximum in a sine curve.
Start with p1 = 4 * p90. p90 is an estimate to the 90 deg pulse width. The value from the logbook is a good starting point.
Acquire spectrum like described in 8, 9, 10.
If the p1 value is too high, this will result into a positive water signal and vice versa. For a perfect 360 deg pulse, the spectrum will look almost like a flat line with some wiggles at the position of the water signal. Adjust p1 accordingly and repeat steps 8, 9, 10.

^{1}H, ^{13}C and ^{15}N pulse calibration using HCN90

Type **HCN90**. HCN90 will automatically determine the 90 deg pulses for ^{1}H, ^{13}C and ^{15}N. It takes a few minutes for HCN90 to finish. Note that your sample needs to contain reasonable concentrations (> 50 µM) of ^{13}C and/or ^{15}N labeled molecules (protein or RNA) to work. Unless you are using the 900 MHz, you can use the automatically determined ^{1}H pulse from HCN90. HCN90 does not give good values for ^{1}H on the 900 MHz.

Write down the pulses and power levels in the logbook.

Type **xxx90** to read calibrated parameters into current data-set.

Type **geteth** to calculate and set decoupling power, shaped pulse power, and recommended maximum TD values for the current spectral widths. If you change spectral widths, you must repeat geteth to obtain maximum TD values. Type DOTALL in the command line to open documentation on the DOTALL parameter sets, which includes and more detailed explanation of geteth.

Temperature calibration

The sample temperature is regulated with a stream of cooled or heated air which constantly passes the sample in the probehead. The temperature which is displayed in topspin show the temperature of the airstream which can deviate from the actual sample temperature. The actual temperature within the NMR tube is calculated from the temperature dependent chemical shifts of the methanol signals.

1. Insert fused 5 mm NMR tube with yellow label “MeOD 99.8% 2H sample”.
2. Lock on deuterated methanol by typing **lock** and selecting the solvent MeOD (methanol-d4).
3. Observe the lock signal. Open the large lock-signal window by double-clicking on the lock icon at the bottom panel of the topspin window or by typing **lockdisp** in the topspin command line.
4. Open the BSMS panel by clicking on its icon  or by typing **bsmsdisp**.
5. Adjust the height of the lock signal by changing the lock gain (in the range between 100 to 115) and power (depending on the solvent: for 10% D_2O around -18 dB). Ideally, the lock signal (green and red moving lines) is at the same height as the top horizontal grid line.
6. Automatically adjust lock parameters by typing **loopadj** in topspin command line.
7. Perform tuning and matching (**atmm**).
8. Read the parameter set zg.eth (**rpar zg.eth**).
9. Make sure the following parameters are set properly for the zg.eth experiment:
   ns 1 (number of scans)
   ds 0 (dummy scans)

rg 1 (receiver gain)
lb 8 (line broadening)

10. Perform shimming (**topshim gui**) and click on "Start".
11. Start the experiment by typing **zg**.
12. Process the data using the following commands:
    em (exponential multiply)
    ft (fourier transform)
    apk (automated phase correction)
13. Calculate the sample temperature from the processed methanol spectrum by typing **calctemp** (select D, which is the default)
    Calctemp computes the actual sample temperature using the chemical shift difference between the methanol methyl and OH proton resonances.
14. Compare the temperature calculated by calctemp with the set temperature. If the calculated and the set temperature are different then adjust the set temperature and repeat the temperature measurement (steps 10 to 13).

Basic experiments

1. zg-wg3919.eth : one dimensional proton experiment using the 3-9-19 watergate for water suppression
   important parameters: o1, p1, pl1 (pl1db), ns, rg
2. 15Nhsqc.eth: 2D 1H15N HSQC
   important parameters: o1, p1, pl1, p3, pl3, ns, rg, 1 td (number of increments)

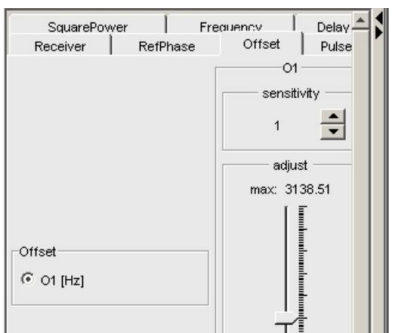
Interactive optimization of acquisition parameters

In the previous sections we have described how to determined all parameters required to start the experiment, like the pulse lengths and o1.

However, for samples in aqueous buffer the parameters for water suppression using the presaturation method need to be adjusted interactively. Once the water suppression is optimized, the receiver gain (rg) can be set to the highest possible value.

Interactive optimization of parameters is done by adjusting the parameters during acquisition. Clicking above or below the slider button moves the slider a small increment in that direction. The command **gs** open a dialog box and starts interactive acquisition. Note that in gs mode the acquired data is not stored on the disc.

Open the experiment whose parameters you need to optimize and type **gs**.



gs left panel

Modify the parameter using the slider.

• Save - save the parameter that was changed last.

• Save all - save all changes.

• Restore - restore the parameter that was changed last.

• Restore all - restore all changes.

• Stop - stop the acquisition and leave the gs dialog box.

Optimize receiver gain (rg)

The receiver gain determines the amplification of the raw NMR signal in the receiver. A low receiver gain results in noisy spectra. A too high rg results in receiver overflow by the high intensity water signal. The goal is to set rg to at least 64.

1. Start gs mode by typing **`gs`**.
2. Click on the receiver tab on the left panel. A slider appears to interactively adjust the receiver gain.
3. Move the slider up or down to increase or decrease the receiver gain. Changes in the rg can be immediately observed in the intensity of the FID (free induction decay) on the right panel.
4. *Too high rg* values result in a intense FID which will be chopped off at the top and bottom of the FID window and the message: “Warning ADC overflow” will appear. Low values of rg result in a weak FID signal and noisy spectra.
5. Increase the rg to just below ACD overflow.
6. Click on “Save” and “Stop” on the left panel. The new rg value is now stored for the current data set.

Low rg values are usually caused by poor water suppression. Reasons for poor water suppression are (1) Misadjusted presat power level (pl9) (2) Misadjusted proton carrier frequency (3) poor shims or a combination of the three.

Optimize water suppression (presaturation)

One of several possibilities for water suppression is the presaturation method. The parameters to be optimized are the power level for the presaturation (pl9 in W or pl9db in dB) and the offset frequency for the proton channel (o1). The goal is is to keep the intensity of the water signal low. Typical values for pl9dB are around 48 dB. How to obtain the o1 value see section “1H pulse calibration” (point 11).

1. Start gs mode by typing **gs**.
2. Adjust the rg according to the previous section.
3. Click on “Square Power” on the left panel.
4. Select pl9dB using the radio button.
5. Change the sensitivity for the slider to 0.1 by clicking on the sensitivity arrow down button.
6. Adjust pl9dB using the slider in 0.1 dB steps. Note that it takes 2 or 3 scans (about 3 seconds) for the change to become visible in the FID.
   In case of poor water suppression most of the FID intensity result from water. A decrease in the water signal intensity can therefore be observed by an overall intensity decrease of the FID.
   Decrease pl9dB until the FID intensity does not decrease any further. In case decreasing pl9dB does not result in decreased FID intensity try to increase pl9dB.
7. Click on “Save” to store the optimized pl9dB value for the current data set.
8. Click on the “Frequency” tab on the left panel.
9. Select o1 using the radio button.
10. Change the sensitivity for the slider to 0.1 by clicking on the sensitivity arrow down button.
11. Adjust o1 in 0.1 increments (increase or decrease) and observe the change in the shape of the FID. If the o1 value is off the center of the water signal the FID appears “wavelike”. Try to adjust o1 to obtain an FID which is symmetric around the time axis.
12. Click on “Save” and “Stop” on the left panel.