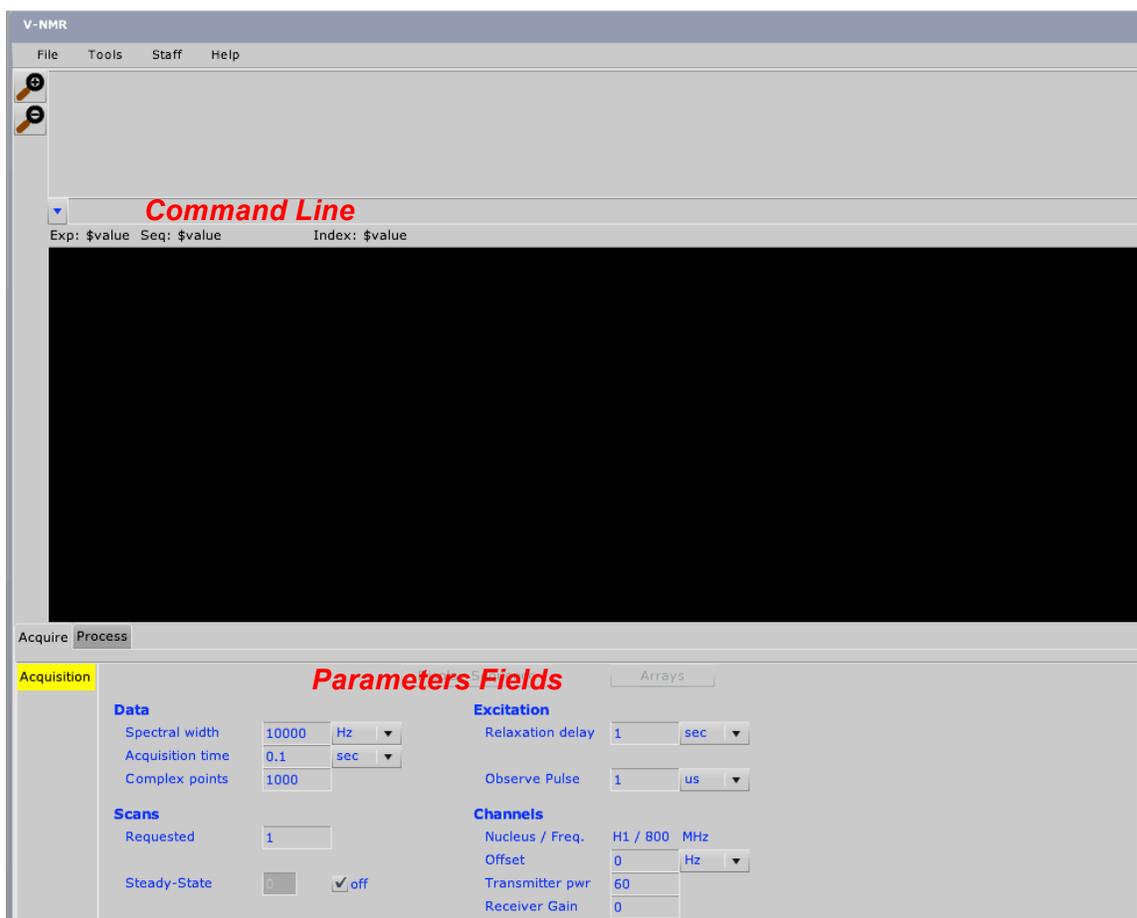


Your first NMR measurement

Introduction

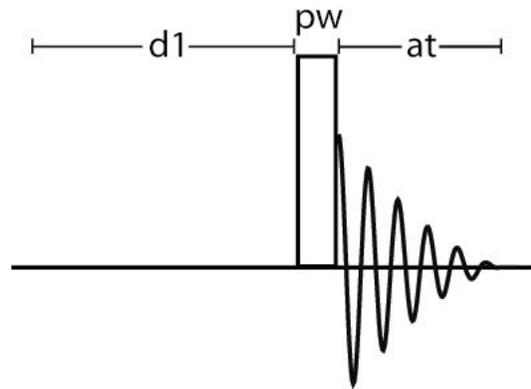
Select “10mM water in D2O” as NMR sample. The NMR spectrum of such sample consists of only two signals: the water signal and the peak of the reference (TSP). Follow the guide to log in (see <http://www.VirtualNMR.org/manual>), select 800 MHz as measuring frequency for 60 minutes and “Water_10mM_in_D2O” as NMR sample. The first time you log in the virtual spectrometer, a default setting of acquisition/processing parameters is loaded:



The acquisition parameters can be modified by directly typing the new values in the corresponding fields of the interface; alternatively, the command line can be used.

Acquisition of a NMR spectrum

The figure below gives a schematic representation of a simple pulse-acquire experiment.



The parameters $d1$, pw and at have a fixed meaning in V-NMR:

$d1$ denote the duration (in s) of the “so-called” preparation period; during $d1$ the magnetization relaxes along the direction of the external magnetic field. This field is called “Relaxation delay” in the interface.

pw (abbreviation for *pulse width*) represents the duration (in $\mu\text{s} = 10^{-6}$ s) of the irradiation period. This field is called “Observe Pulse” in the interface.

at (abbreviation for *acquisition time*) denotes the duration (in s) of the acquisition of the NMR signal (also called Free Induction Decay or FID).

The measurement consists of these steps:

- 1) wait for a period of duration $d1$
- 2) irradiate the sample for a period of duration pw
- 3) acquire the NMR signal for a period of duration at

The acquired signal depends on several additional parameters:

- tof (abbreviation for *transmitter offset*) allows a fine regulation (in Hz) of the irradiation frequency ($sfrq$). Called “Offset” in the interface.
- sw (abbreviation for *spectral width*) is the width of the region (in Hz) where NMR signals are measured.
- ss (abbreviation for *steady state*) is the number of executions of the pulse sequence without data acquisition, before starting the experiment.
- nt (abbreviation for *number of transients*) is the number of executions of the pulse sequence. The NMR signals results from the sum of all individual acquisitions. Called “Requested” in the interface.
- np (abbreviation for *number of points*): the digitized NMR signal consists of $np/2$ complex points. This value isn't shown directly in the interface but can be set by setting the “Complex points”.

The parameters np , sw and at are related by:

$$at = \frac{np}{2 \times sw}$$

- $gain$ controls the amplification (in dB) of the acquired NMR signal before digitalization. This parameters can take integer values between 0 (no amplification) and 60. This field is called “Receiver gain” in the interface.
- $tpwr$ (abbreviation for *transmitter power*) controls the amplification (in dB) of the intensity of the radiofrequency field. This parameters can take integer values between 0 (no amplification, low intensity) and 60 (maximum intensity).

The default setting of the acquisition parameters is:

$d1$ = 1.0 s
 pw = 1.0 μ s
 at = 0.1 s
 tof = 0
 sw = 10000 Hz
 ss = 0
 nt = 1
 $gain$ = 0
 $tpwr$ = 60

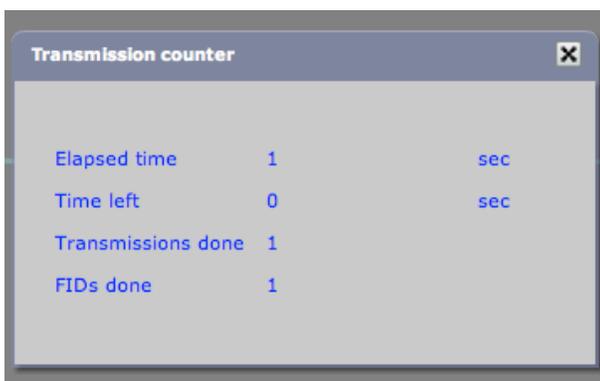
Acquire a 1D spectrum with these parameters setting.

To start a NMR experiment you can simply type “go” in the command line:

Go

<Enter>

In order to check the Acquisition Status, open the *Transmission Counter* window from the menu *Tools*.

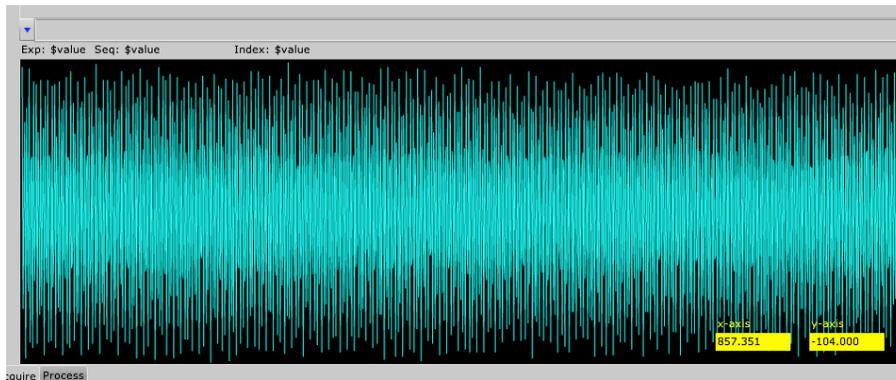


This indicates that the acquisition is completed.

You can display the acquired NMR signal by typing “df” in the command line.

df

<Enter>

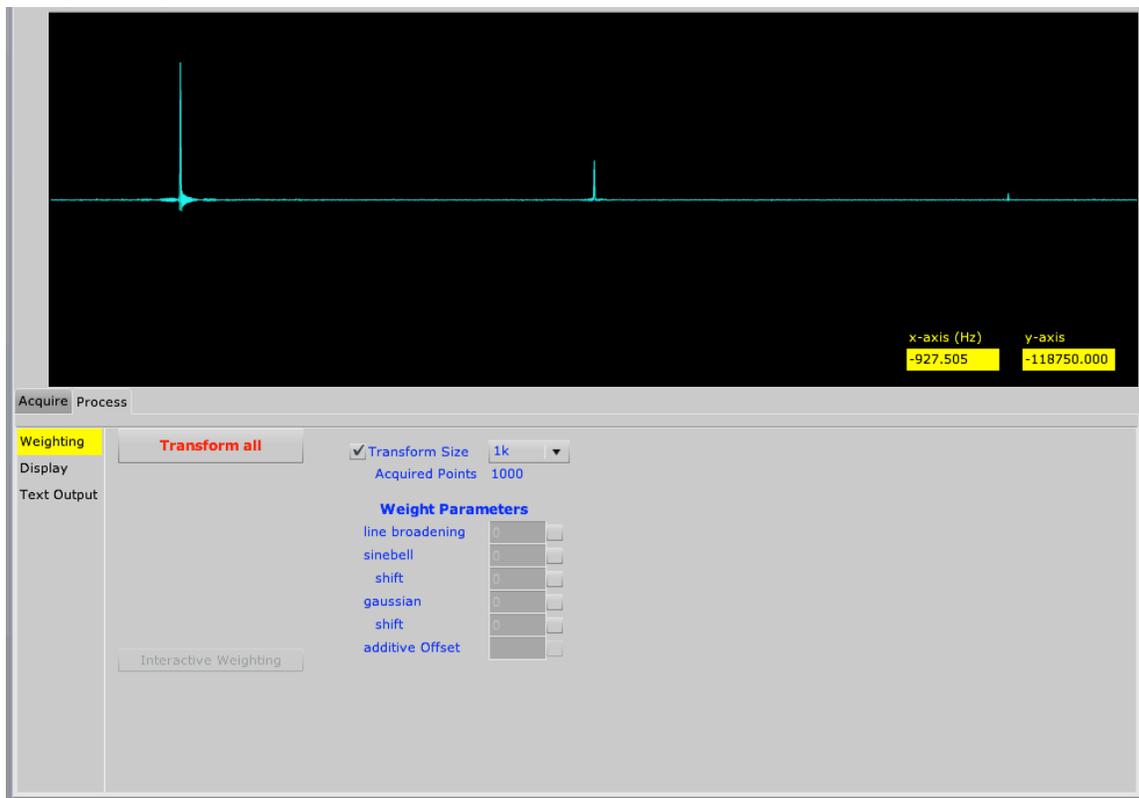


The NMR spectrum is obtained by a Fourier transformation of this FID. You can simply type “ft” in the command line:

ft

<Enter>

Alternatively you can press “Transform all” in the tab *Process*.

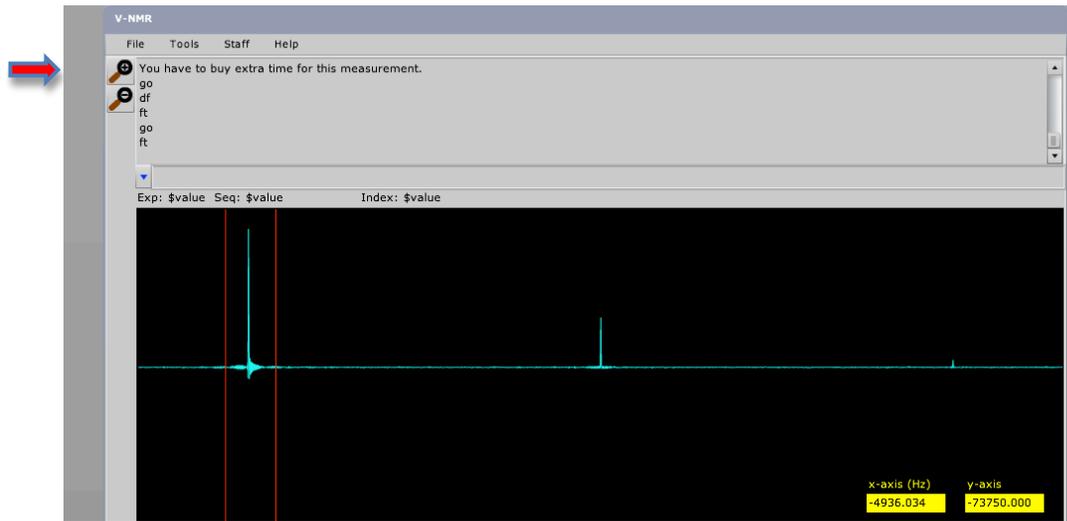


The peak at the left hand side and in the center of the spectrum are the water signal and the signal of TSP, respectively. The small signal at the right hand side of the spectrum is an artifact, as mirror image of the water signal.

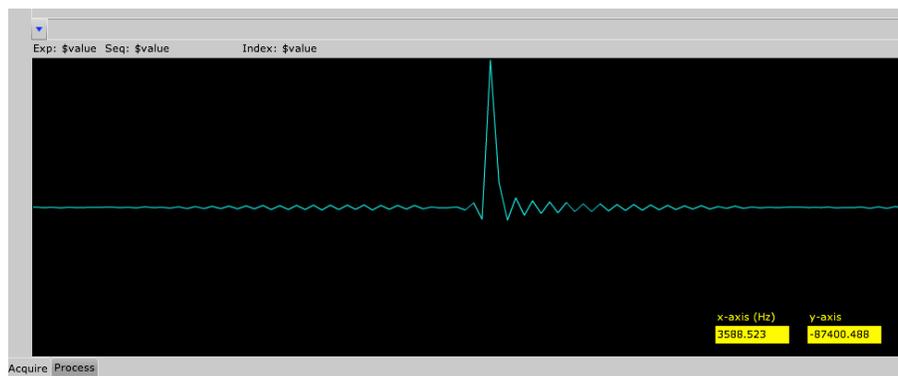
Horizontal and vertical scale

Horizontal scale

Use the left mouse button to define the left (click once) and right (click fast twice) margin of the spectral region you want to enlarge. These margins are displayed as two vertical red lines.



Now click on the magnifying lens (at the left hand side of the V-NMR interface).



Click on the symbol of the magnifying lens (minus sign) to reset the horizontal margins and display the full spectrum.

Vertical scale

Before changing the vertical scale select the option *Absolute* for the parameter *Amplitude Scaling* in the tab *Process-Display*. Alternatively you can type on the command-line:

ai **<Enter>**

Now you can zoom in or out on the spectrum by scrolling with the mouse. (*Note that when you set the vertical scale to Absolute for the first time, the signal may be displayed as an almost flat line, i.e. maximum zoomed out.*)

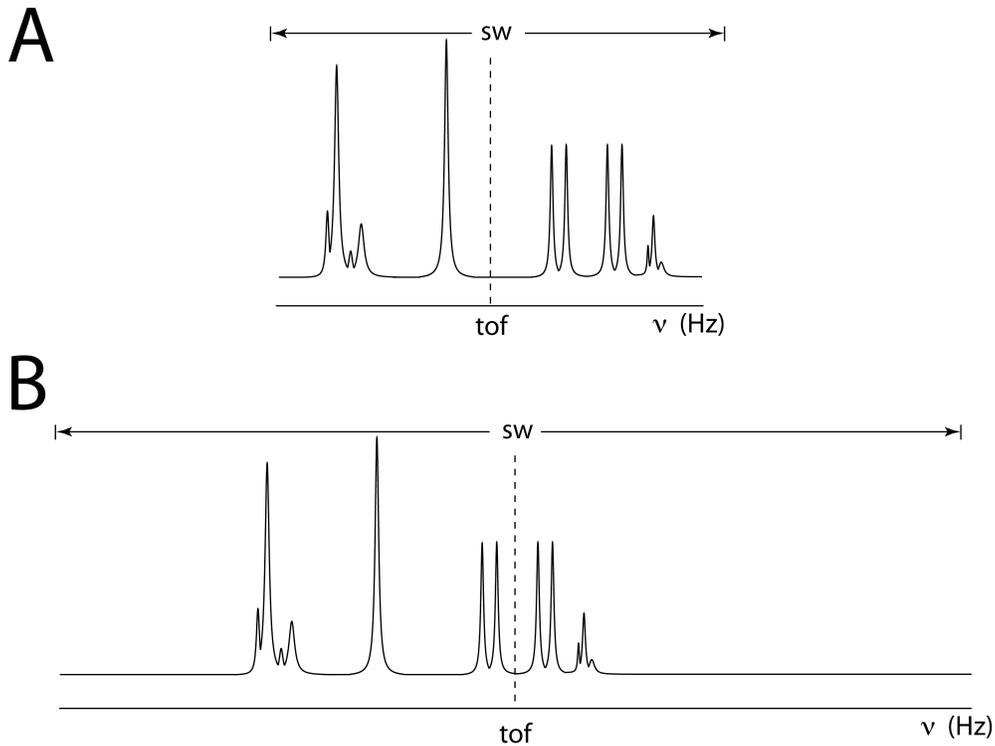
Alternatively, you can use the parameter *vs* (abbreviation of *vertical scale*) to change the vertical scale (*vs*=[*value*]).

By selecting the option *Normalized* for the parameter *Amplitude Scaling* in the tab *Process-Display*, the vertical scale is adjusted so that the highest signal fits exactly in the spectral area of the interface. The same result is obtained by typing

nm **<Enter>**

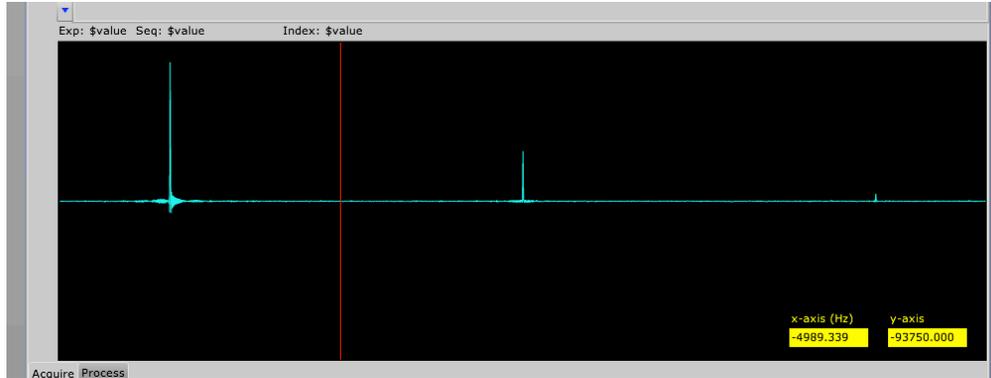
Adjust transmitter frequency and spectral width

For an efficient irradiation the transmitter frequency (parameter *tof*) should be set approximately in the middle of the spectrum. The width of the spectral region can be adjusted to comprise all signals (see example below).



(A) Optimal setting of the parameters *tof* and *sw* **(B)** Example of a sub-optimal setting

Let's optimize *tof* and *sw* for the water sample, using the spectrum we have just recorded. Position the mouse between the water and the TSP signals and click with the left mouse button. The cursor is indicated by a vertical red line:



Type:

movetof

<Enter>

to set the transmitter at frequency of the cursor. We can now measure a new spectrum.

Type:

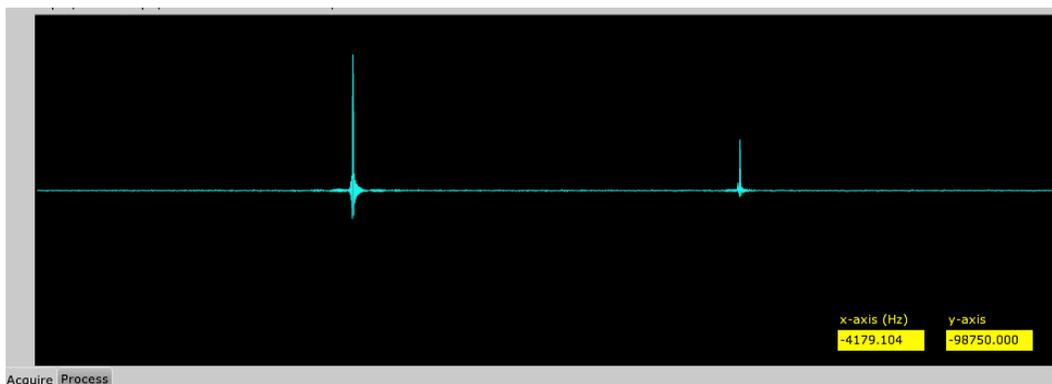
go

<Enter>

After a few seconds, at the end of the measurement, type:

ft

<Enter>



To adjust the spectral width it is more convenient to report the spectrum in frequency units, rather than ppm. Type:

axis=h

<Enter>

Use the cursor to determine the width of the empty regions at the sides of the spectrum (2500 Hz at the left hand side of the water signal and 2500 Hz at the right hand side of the reference signal, i.e. 5000 Hz in this case). Type:

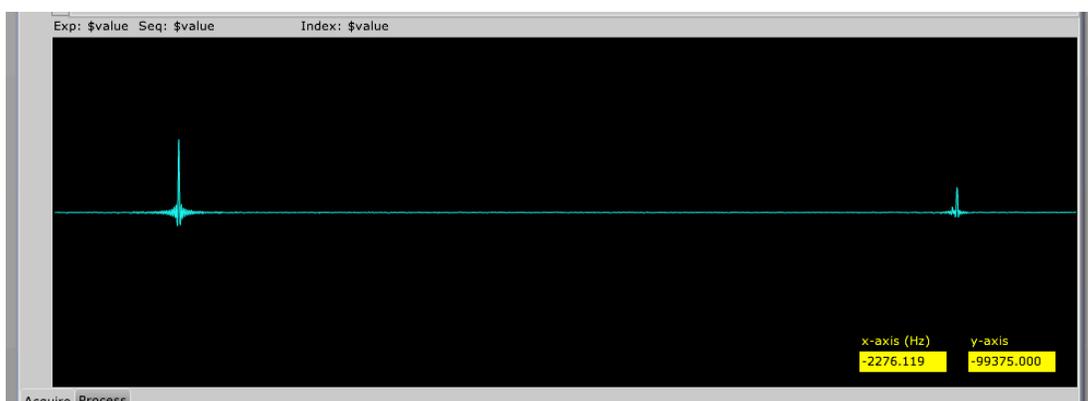
sw=sw-5000 **<Enter>**

Measure the spectrum with the new setting of *sw*. Type:

go **<Enter>**

wait for the measurement to finish, ...then type:

ft **<Enter>**



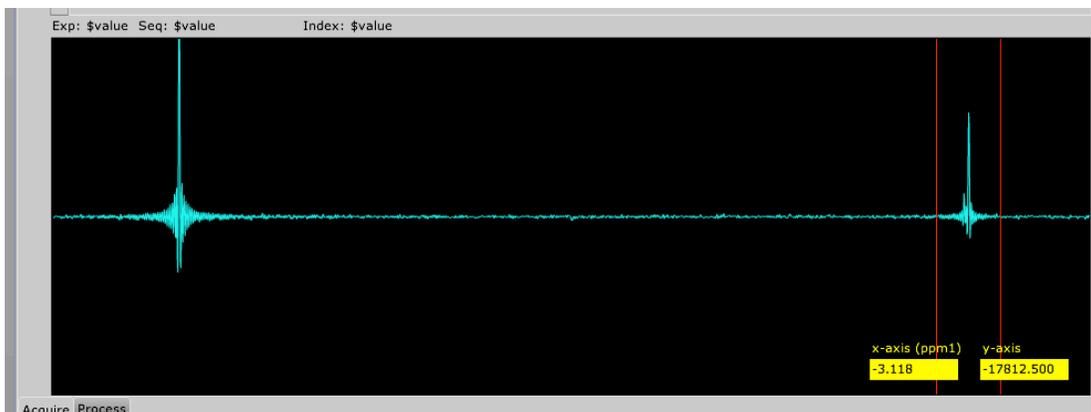
Both *tof* and *sw* are now optimized.

Spectrum calibration

Calibration of the spectrum is performed by setting the chemical shift of the proton signal of TSP equal to zero. First set the “ppm” as unit for x-axis. Type:

axis=p **<Enter>**

Now zoom in on the region of the TSP signal (at the right hand side of the spectrum):



Set the cursor on the peak of TSP:



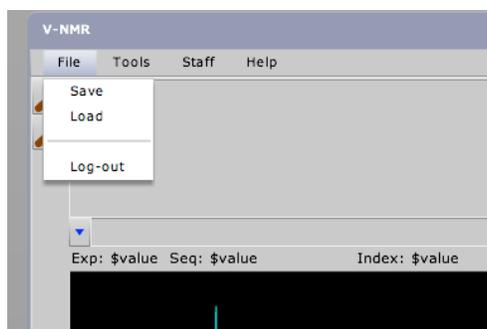
In order to set the chemical shift at zero ppm at the cursor position type:

`r1 (0p)`

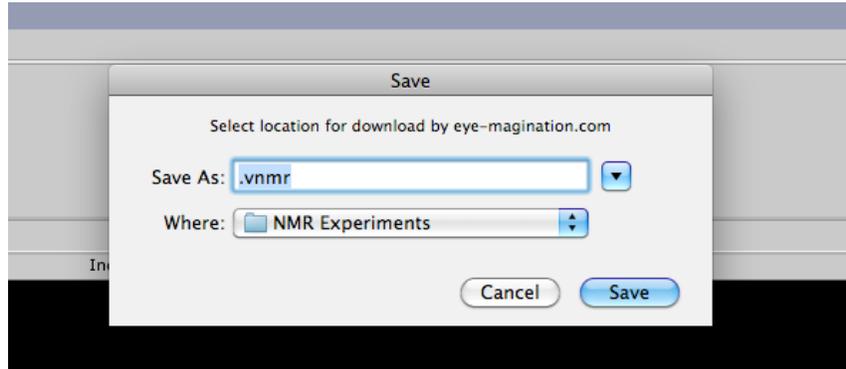
`<Enter>`

Save data

It is possible to save *locally* (i.e. on your own computer) the acquired FID and the acquisition parameters. Select *Save* with the left mouse button from the *File* menu.



Type a filename for the acquired data.



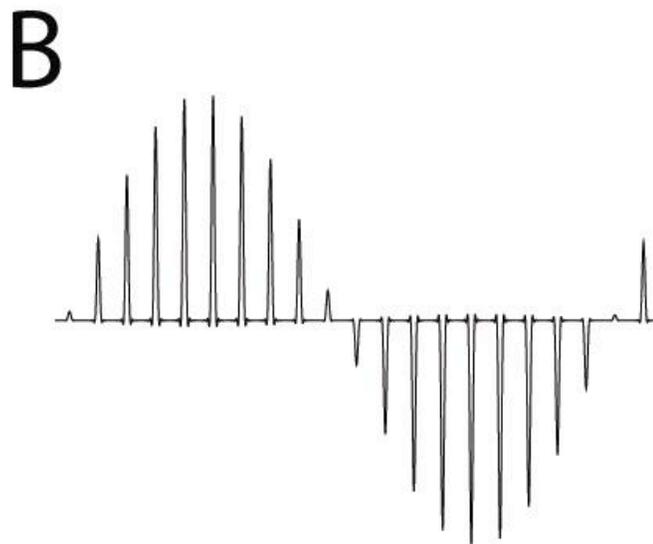
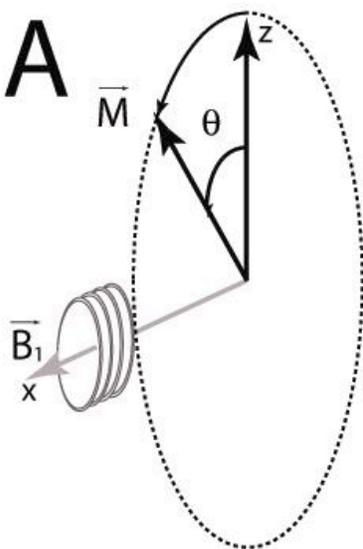
The data can be retrieved at any moment for further analysis (no extra costs involved).

90° pulse calibration

The intensity of the NMR signal depends on the transverse magnetization generated by a RF pulse:

$$S \propto M_{x,y} \propto \sin(\vartheta) = \sin(\gamma B_1 \times pw)$$

where pw denotes the pulse duration and B_1 indicates the strength of the oscillating field B_1 . By acquiring a series of spectra at increasing pw duration, the signal intensity should follow a sinusoidal profile.



(A) Rotation of the magnetization vector around the B_1 axis.

(B) Determination of the duration of the 90° pulse as one fourth of the 360° pulse.

For a correct calibration of the 90° pulse it is important that the signal we follow during the experiment is exactly on resonance. In the present case we will follow the water peak. Set therefore the cursor on the water signal and type:

movetof <Enter>

By using the command *array* we can measure a series of NMR spectra where one or more parameters are systematically changed. In the present case we want to acquire 11 spectra where *pw* will be regularly increased in steps of 4 μ s. Type:

array <Enter>

The interface will inquire us about the parameter to be changed, the number of steps, the starting value and the increment:

parameter to be arrayed:

Type:

pw <Enter>

Number of steps

Type:

11 <Enter>

Starting value

Type:

2 <Enter>

Array increment

Type:

4 <Enter>

You can list the arrayed values of *pw* by selecting *Text Output* in the tab *Process* and typing:

da <Enter>

In order to start the series of measurements type:

go <Enter>

From the *Transmission Counter* we can follow the acquisition of all 11 NMR signals. At the end of the measurement set a proper EM apodization and zero filling:

fn = 4096 <Enter>

Then type:

wft

<Enter>

and

ds (1)

<Enter>

to display the first spectrum of the series. Enlarge the region around the water signal and adjust the vertical scale if necessary. Then type:

dssh

<Enter>

The water peaks of all NMR spectra of the series are displayed in succession. As expected, the signal intensity follows a sinusoidal profile. The 90° pulse is calculated as one fourth of the 360° pulse, which is easy to determine as it corresponds to a no-signal situation.

The spectrum corresponding to a 360° rotation can be recognized from the followings:

- a. the signal should be absent, or very weak
- b. the spectrum measured with a shorter pw should show a negative signal
- c. the spectrum measured with a longer pw should show a positive signal

We can perform, if necessary, a new array measurement in proximity of the approximate 360° pulse, using a smaller step for a more accurate pulse calibration.