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Measuring Proton NMR Spectra on the old 500 MHz spectrometer

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This guide is adapted for use with the old 500 MHz spectrometer model Avance II that is in room 3 of the Philadelphia building at the Hebrew University of Jerusalem.

This guide is intended for use with the spectrometers of the Chemistry Institute of the Hebrew University.

Contents

1. Summary of instructions for measuring a proton NMR spectrum

(Use the full guide for full details. For troubleshooting see ch. 5)

For all the instructions in the table there is an icon that appears in the menu at the top of the program window. You can place the mouse cursor on it to be the pop-up help. Clicking on the icon is equivalent to entering the command.

From the opening screen please enter the username and password	Login:
Click on the icon to run the program	Topspin 3.2
Before the first spectrum and after changing the probe, define the probe. Usually the system selects the probe in blue, select **Define as current probe** from the menu then **Exit** then **Save and Close**	edhead
Before the first sample, read the shim file. Choose the file from the list that appears. Each probe has its own shim file **bbi** for BBI and **bbo** for BBO.	rsh bbo/bbi
Open a new file, a window will appear in which you can set the experiment **name**, its number (**expno**) and the **experiment** type. Ensure that you use the correct experiment in the directory: C:\Bruker\TopSpin3.2\exp\stan\nmr\par\user	edc
Set the experiment type - in case you didn't choose the correct type of file at the previous stage (**edc**) or there is a need to change the experimental parameters. After entering this command there will appear a long list of different NMR experimental parameters. Ensure that you choose a suitable set from the directory C:\Bruker\TopSpin3.2\exp\stan\nmr\par\user	rpar 1_Proton(BBO)
Insert the sample like this: Click on **LIFT** on the control panel to the right of the keyboard, wait till you hear a rush of air, put the sample in the spinner, check that it is the right height, put the sample in the upper opening of the magnet, click again on **LIFT**.	click on LIFT
Lock the spectrometer to the solvent deuterium frequency, choose the solvent from the list that appears	lock
Tune the frequency - you must do this when you start work or change solvent	atma exact
Adjust receiver gain	rga
Shimming is the magnetic field homogeneity correction stage. The topshim command corrects the field for the pure Z functions. Afterwards you need to correct the other functions.	shimming
Automatic shimming (for routine purposes after the 1st sample in the same solvent and ample height you might get satisfactory results quicker by manually adjust Z and Z^2).	topshim

For the 1st sample: Adjust the shimming (even if you used topshim on the 500) according to this order: Without spinning, correct X, XZ, Y and YZ, XZ^2, YZ^2, XY X^2-Y^2, repeating as necessary until the maximum lock level is achieved. Start the spin and rerun topshim.	shimming contd.
Initial scan with ds = 0 and ns = 1	zgfp
If the shimming is good please change ns as desired and ds to 2	
Final acquisition	zgfp
Phase correction – the correction is done in two stages – in the first the biggest signal is corrected by dragging the mouse on the number 0 and the second stage is dragging the mouse on the number 1. The phase is correct when the whole spectrum is straight.	.ph
Calibration of the spectrum to TMS or a residual solvent signal. Expand the region of the calibration signal, bring the line to the peak and click. A new window appears with a chemical shift. Enter the correct chemical shift and save.	.cal
Baseline correction: a menu appears – choose the second option.	.basl
Integration – click on the second left button on the menu bar in the window that appears and select the signals by dragging the mouse over them. Right clicking on a selected signal allows calibration.	.int
Printing – click on the printer icon on the menu bar of press Ctrl p. A new window with options appears, the second option is recommended allowing the appearance of the plot to be edited.	plot
Eject the sample	click on LIFT
Logout from the computer	Lougout:

2. General description of the equipment

Figure 1. The spectrometer magnet

Figure 2. The console of the spectrometer with the door closed on the left and open on the right

Figure 3. The computer that controls the spectrometer

Figure 4. The control panel for the sample and magnet

3. Routine experiments

For a routine proton experiment (http://chem.ch.huji.ac.il/nmr/techniques/1d/row1/h.html#BM1H) follow these instructions. For proton NMR with decoupling from other nuclei see chapter 4.

a. Logging on

Log on to the computer using your *username* and *password* (Fig. 5). The password will appear as black circles for security reasons. Click on OK to enter.

If the computer is already logged on then log off as described in chapter 3r.

Figure 5. User logon window

Run the Topspin 3.2 program by double clicking on the symbol  or start>All Programs>Bruker NMR Software>TOPSPIN 3.2>TopSpin 3.2

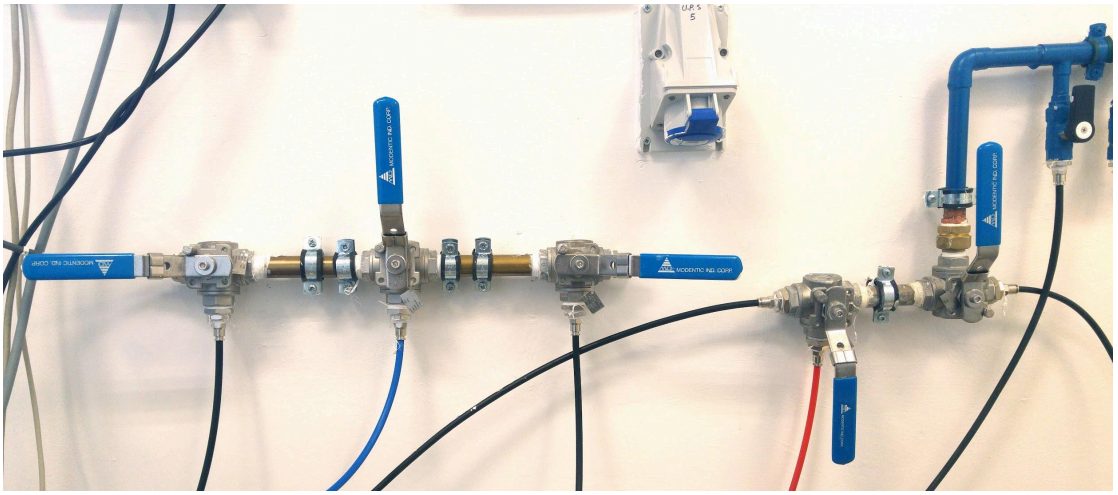
The spectrometer is used for a wide variety of experiments. Therefore, unless sure that the spectrometer is in the standard configuration, you should check the following. Otherwise go to section b.

You should check the that air bags and magnet legs are activated in order to prevent interference from vibration. The switches are on the floor next to the magnet and on one of the magnet legs (fig. 6).

Figure 6. Switches for raising the magnet (on the floor - right and on a magnet leg - left)

Ensure that the valves behind the console are in the correct positions (fig. 7).

Figure 7. Valves behind the console set for regular use.



Ensure that the signal cable (DA-15 connector) from the BVT 3000 unit (and not the HR-MAS box) is connected behind the BCU1 cooling unit (Fig. 8).

Figure 8. Signal cable connected behind the BCU1 cooling unit.

Check that the BCU1 unit is turned on to mode 1 on the top-front panel and that the status light is green or flashing green (Fig. 9). If it has been turned to mode 0 or turned off do not turn it to mode 1 until at least two hours have passed since turning off. If the unit keeps cutting off and/or the a red status-light is on or flashing, turn it to mode 0 and wait for at least two hours before turning on. You can still do ^{1}H-NMR at room temperature with the cooling off but the temperature stabilization will not be as good.

Figure 9. BCU1 top-front panel at the routine setting, mode 1, with status light green

Ensure that the cable connections are correct for ^{1}H-NMR. There should be a cable connected to the top output socket of the preamplifiers at the entrance and exit (fig. 10, 11).

Figure 10. Connections exiting the preamplifiers at the front for regular acquisition. The deuterium stop filter on the broadband channel (2nd from top) is optional and has no effect on regular ^{1}H acquisition.

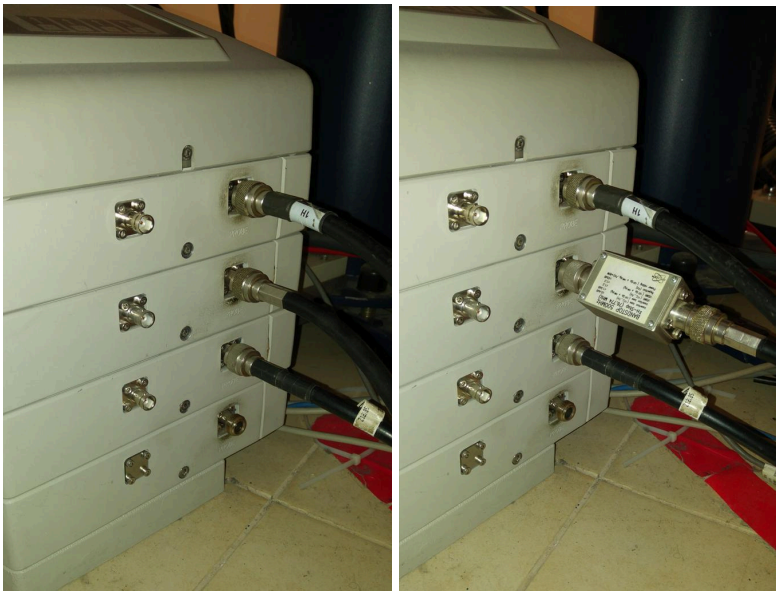
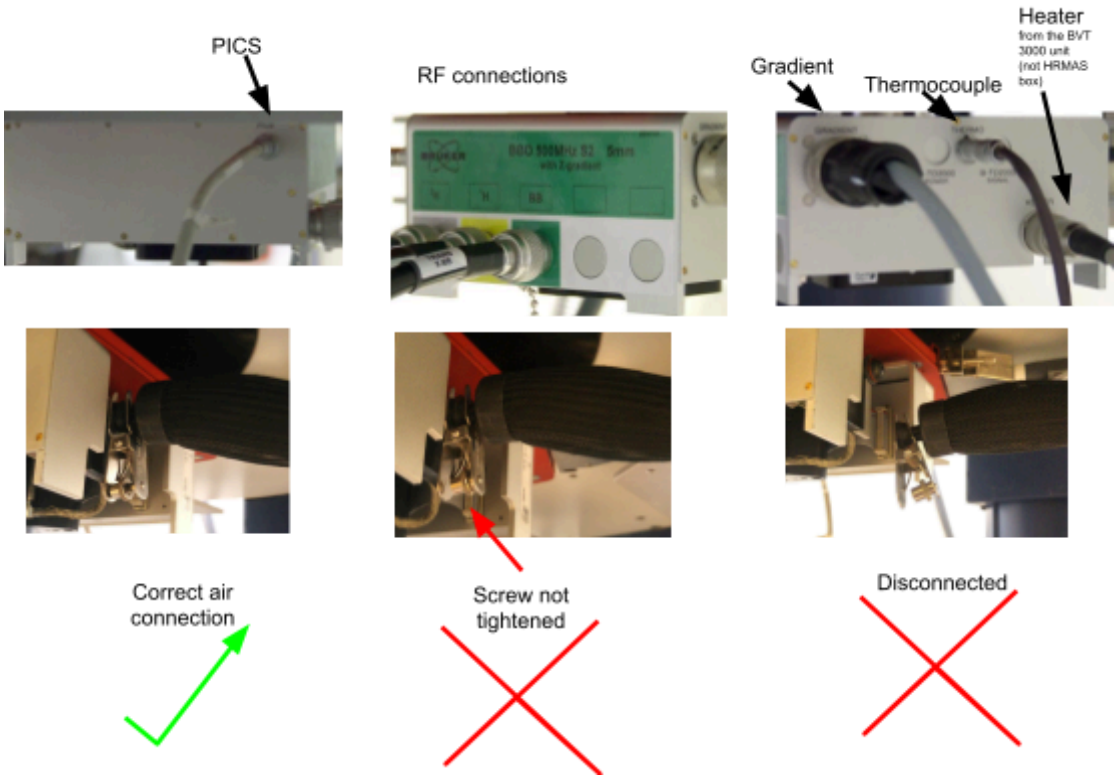


Figure 11. Connections entering the preamplifiers from the back for regular acquisition.

Ensure that the probe is connected correctly (fig. 12).

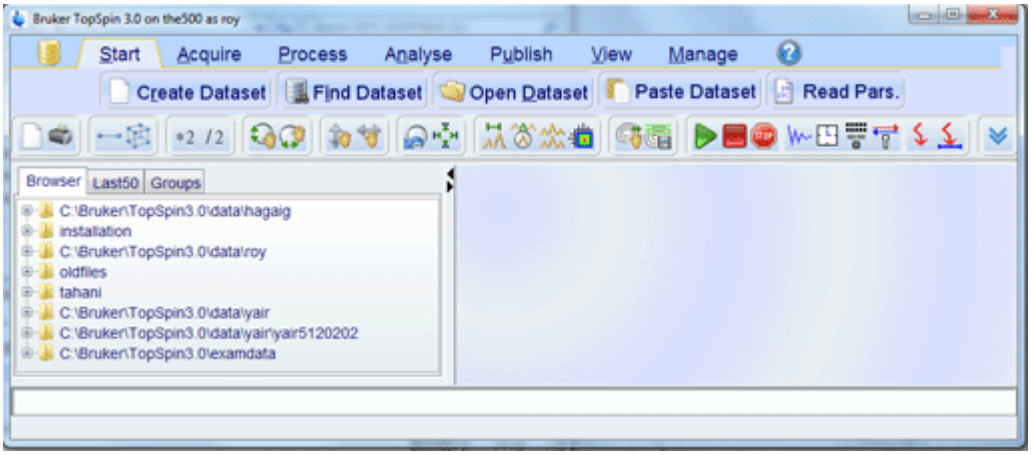
Figure 12. Probe connections for BBI and BBO probes for regular use



b. Creating a work file

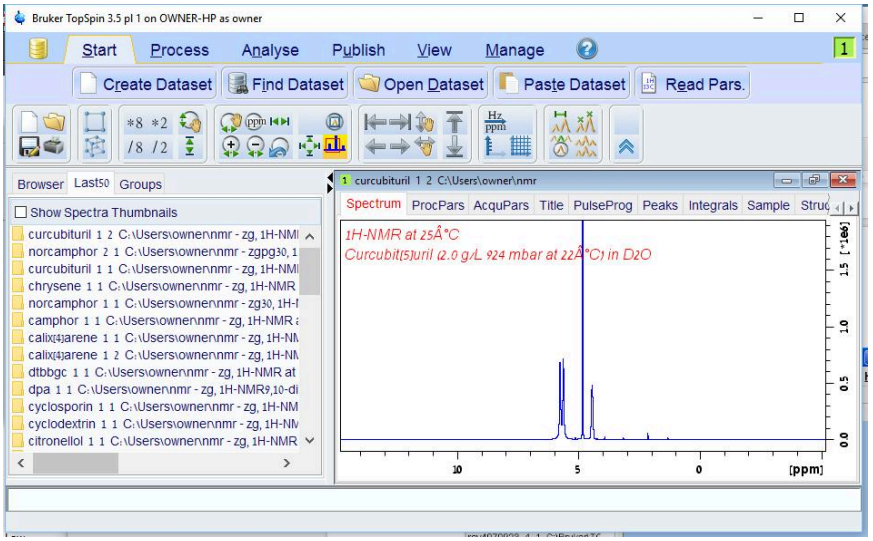
The program will appear as in fig. 13. Below the title are menus. The next rows is a toolbar for file management, editing, viewing options and acquisition commands. In the last row there is a toolbar for controlling spectral appearance. On the left hand side there is a list of files and on the right the spectrum will be displayed. The browser can be used to open existing files (fig. 13).

Figure 13. The file browser



Alternatively you can click on Last50 to see recent files (fig. 14).

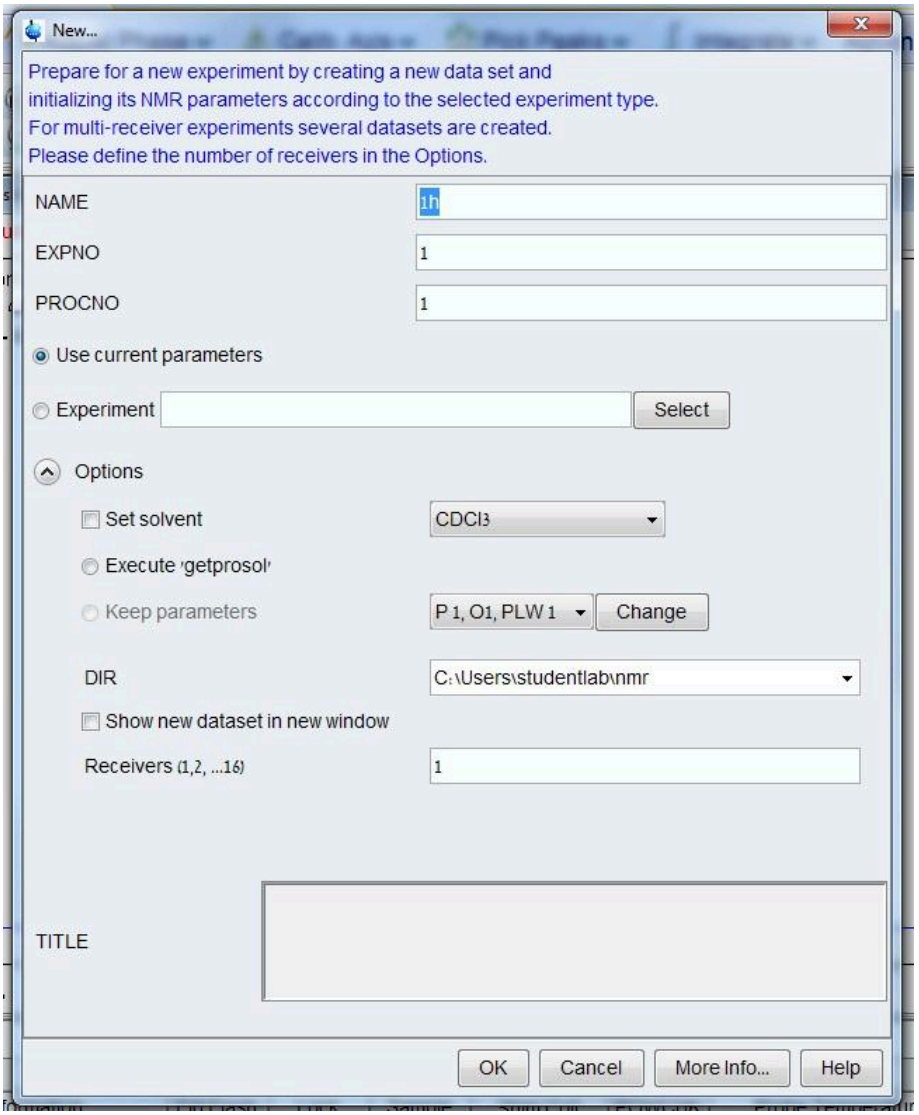
Figure 14. File selection from recent files



To create a new file, enter *edc* (fig. 15) and enter the parameters: experiment name in the field **NAME**, experiment number in **EXPNO** should be *1* (or a higher number if there are already experiments under that name), processing number in **PROCNO** should be *1*, directory in **DIR** should be C:\theOld500Nmr*username*, name of the solvent in solvent, **Experimental Dirs.** Choose the directory *C:/Bruker/TOPSPIN3.2/exp/stan/nmr/par/user*, *1_Proton* (for proton NMR) in **Experiment** or click on **Use current parameters**, and the title in **TITLE** (The title can be changed later by clicking on the **TITLE** tab on the spectrum window). Click

on **OK** or press enter to create the file. You can copy parameters from an existing file using *edc* by choosing *Use current parameters* in the **Experiment** field.

Figure 15. Creating a new file with *edc*



c. Specifying the probe

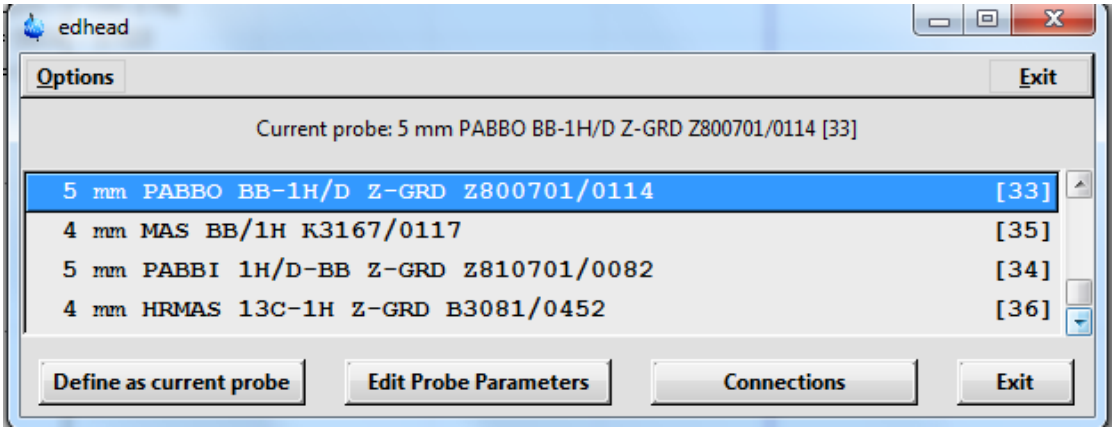
After login or changing the probe, the probe that is in the magnet must be specified by entering *edhead* (fig. 16) and choosing the relevant probe. If the probe is not one of these two then see chapter 5.

On the 500 MHz spectrometer there are four probes. For routine purposes such as proton NMR the 5 mm PABBO BB-1H/D Z-GRD Z800701/0114 [33] probe known as BBO that looks like this  or the 5 mm PABBI 1H/D-BB Z-GRD Z810701/0082 [34] probe known as BBI that looks like this  .

If the probe is not one of these two then see chapter 6.

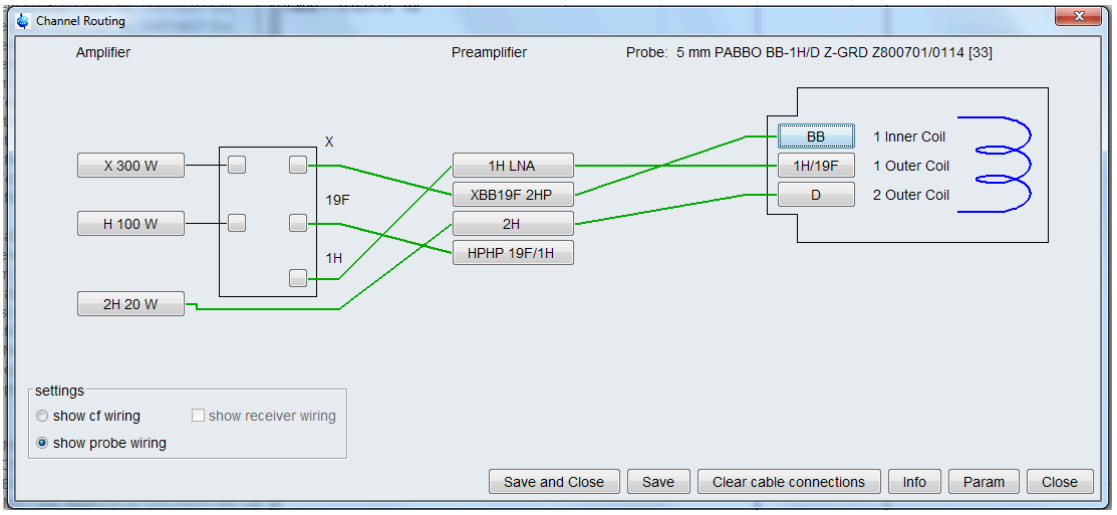
After choosing the probe, click on **Exit** or press **Return**.

Figure 16. Window for specifying the probe



After clicking on **Exit** another window will appear. The connections should appear as in fig. 17. Click on **Save and Close**.

Figure 17. The connection window (except of ^{19}F measurement and the CP-MAS probe)

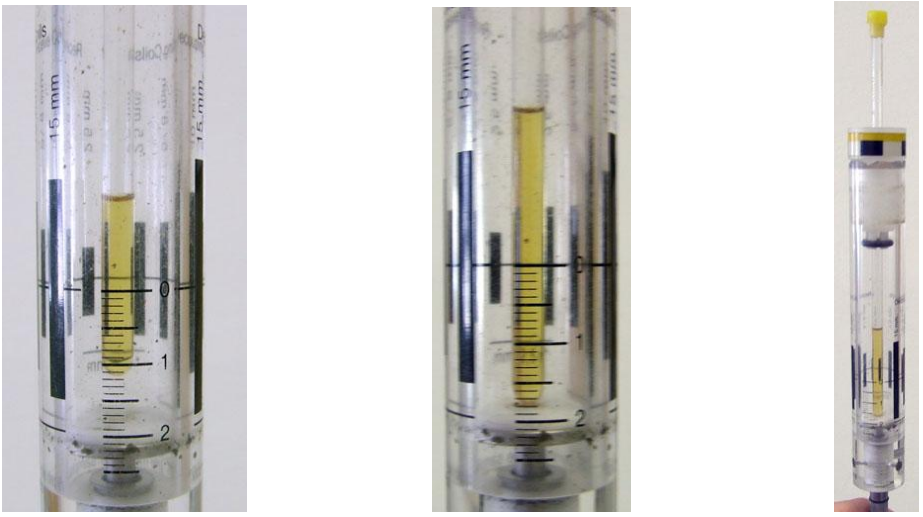


Read the shimming parameters by entering *rsh bbi* for the BBI probe or *rsh bbo* for the BBO probe.

d. Inserting the sample

The NMR tube is inserted into a spinner. The spinner is then inserted into a sample gauge (fig. 18) and is then checked to see that the sample is in the region of the black lines. The solvent height should be at least 4 cm from the bottom of the tube. The bottom of the tube should be 2 cm below the coil center. If the sample depth is less than 4 cm then the center of the solution should be placed at the coil center.

Figure 18. Right - measuring the sample depth, center - sample position for normal liquid depth, left - position for a short sample



The command *ej* causes air to flow through the magnet. It is important to hear the rush of air to ensure that the sample may be inserted safely (if no air is heard then do not insert the tube). If there is a sample in the magnet it will be ejected. Before inserting a sample ensure that there is not already a sample inside. Remove the previous sample and put the new sample in place. Enter *ij* and the sample sinks into the magnet.

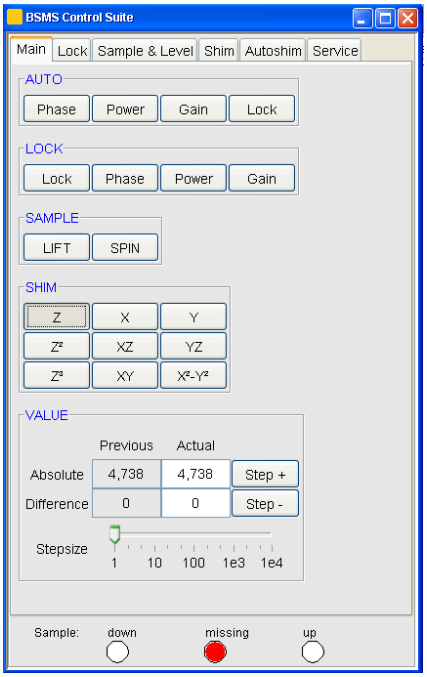
If you choose to use the control panel (fig. 19) you can start the airflow by pressing on **LIFT ON/OFF.** Again, it is important to hear the rush of air to ensure that the sample may be inserted safely (if no air is heard then do not insert the tube). If there is a sample in the magnet it will be ejected. Before inserting a sample ensure that there is not already a sample inside. Remove the previous sample and put the new sample in place. press on **LIFT ON/OFF** again and the sample sinks into the magnet.

Figure 19. The control panel for sample handling and magnet control as seen from above

If using *bsmsdisp*, enter *bsmsdisp* or, click on  in order to open the window (fig. 20). Under the **Main** tab that opens automatically most of the required actions are displayed. Air is passed through the magnet by clicking on the **LIFT** button in the **SAMPLE** frame. It is important to hear the rush of air to ensure that the sample may be inserted safely (if no air is heard then do not insert the tube). If there is a sample in the magnet it will be ejected. Before inserting a sample ensure that there is not already a sample inside. Remove the previous sample and put the new sample in place. Click on **LIFT** again and the sample sinks into the magnet.

Do not spin the sample until the control panel or *bsmsdisp* shows that the sample is inserted successfully (a green light appears). If the sample does not insert successfully then reduce the airflow (see temperature control in chapter 7) eject and reinsert the sample and increase the airflow to what it was after insertion is confirmed.

Figure 20. The *bsmsdisp* window for sample and magnet control



e. Check the temperature

Stabilizing the temperature improves the quality and resolution of the spectrum. Type *edte* and the temperature control window will appear (Fig. 21). 25°C is considered to be room temperature and according to our calibration setting the temperature to 24.2°C gives a true value of 25.0°C for the BBI and BBO probes. The temperature can be monitored using the *Monitoring* tab (fig. 22).

Check that the heater is on and if not turn it on by pressing next to Probe Heater in the Main display tag (fig. 21). If the sample temperature is below the target temperature and it does not immediately start heating you can open the *Self-tune* and click on *Apply PID changes* or wait a few minutes for it to start on its own.

Figure 21. The edte window

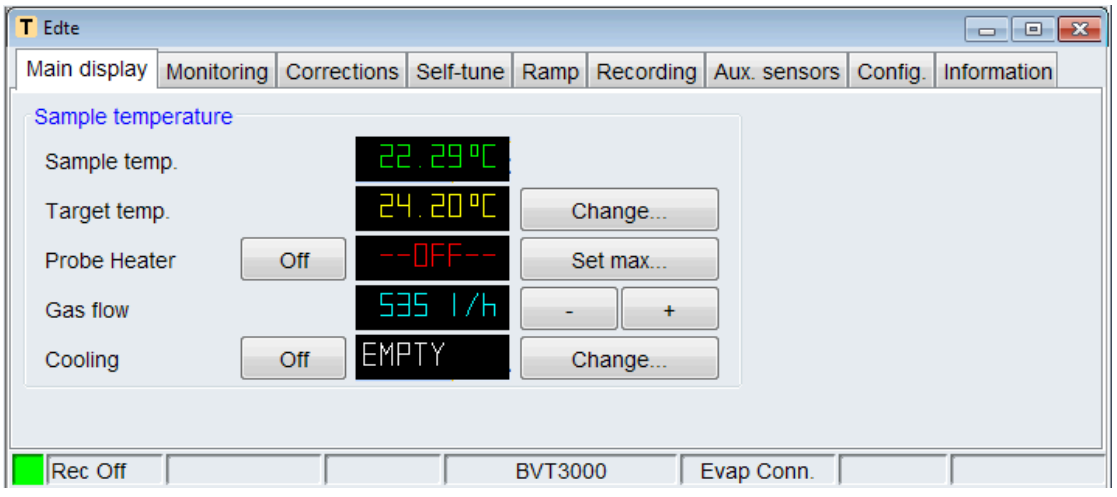
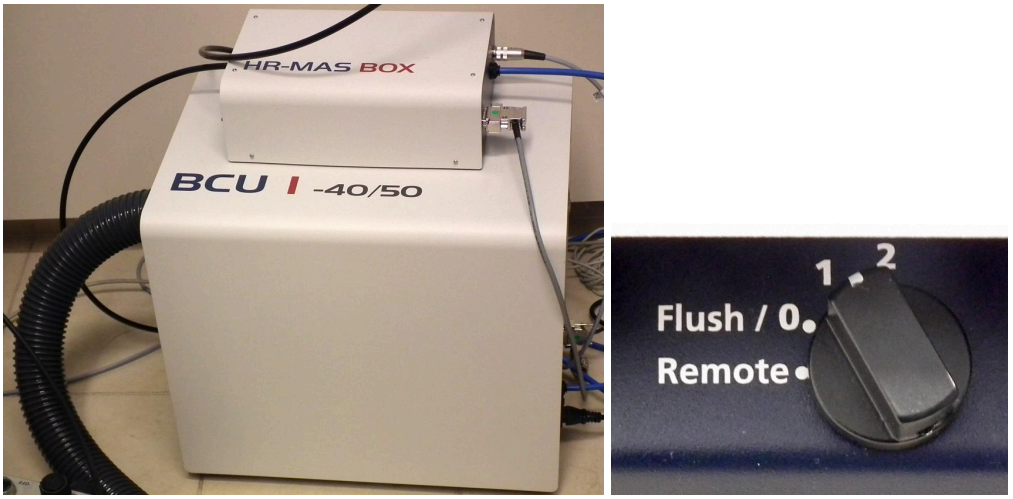


Figure 22. The *edte* window under the *Monitoring* tab for checking temperature stability

The BCU 1 unit should be activated and set to mode 1 (fig. 23).

Figure 23. The BCU 1 temperature stabilisation unit and the mode selection switch



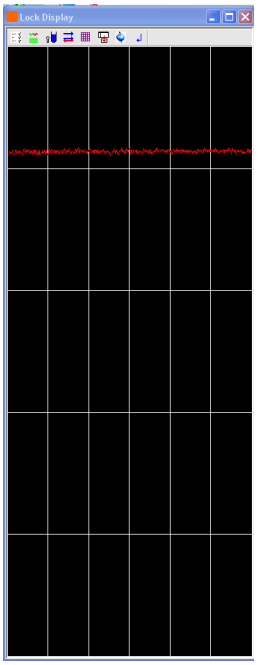
If the temperature is not correct or not stable to within 0.1 degrees then the parameters need to be adjusted – see ch. 9.

f. Field-frequency lock

Because the magnet field strength varies slightly affecting the frequency of resonance. the resonance frequency of deuterium is locked by making slight adjustments to the magnetic field. This is one of the reasons that deuterium substituted solvents are used.

In order to see the lock status click on  or enter *lockdisp*. A new window will appear showing the lock signal (fig. 24).

Figure 24. The lock window (*lockdisp*) showing that the sample is locked



If the lock sweep signal appears in two colors clock on  so that it only appears in one color. (The use of two colors can be misleading during shimming.) One can lock by entering *lock* and then the solvent name (for example *lock cdcl3*). The sample then usually locks automatically.

If the solvent has more than one type of deuterium of similar intensity such as THF-d_8 or DMF-d_7 or there is insufficient deuterium in the sample or the sample is not homogeneous or isotropic enough then lock manually or do not lock as described in chapter 11.

If there is high dynamic range, improving the lock stability as described in ch. 11a may improve the line-shape near the baseline of tall peaks.

g. Tuning the probe

Each time the solvent or probe is changed and at the start of your work you should tune the probe.

On the 500 MHz spectrometer enter *atma* (or for better results *atma exact*) and wait a minute or two (right after changing a probe it will take four minutes) for automatic tuning to finish. The computer will tell you when the process is complete.

If the process is slow or does not work, use semi-automatic tuning (ch. 12).

h. Shimming

Note that for routine samples, starting from the second one in the same solvent, you can save time by adjusting the Z and Z^2 shims since this is faster than doing *topshim* and the procedure described below.

On the control panel (fig. 19) and the bsmsdisp window (fig. 20) there are buttons for the different shim functions for correcting magnetic field homogeneity.

(While shimming you can also do *rga* – see ch. 3i.)

If you have not read the shim file already the do it now by entering *rsh* probename (*rsh bbi* or *rsh bbo*).

Shimming with the control panel

Check that the **DIFF MODE** button is not on.

The shims **Z**, $\mathbf{Z^2}$, **X**, and **Y** are adjusted with **FINE** on and the remainder of the shims with **FINE** off.

The pure **Z** axis shims (**Z**, $\mathbf{Z^2}$, $\mathbf{Z^3}$) are adjusted while the sample is spinning (the **SPIN** button is on) and the other shims are adjusted without spinning (**SPIN** button off).

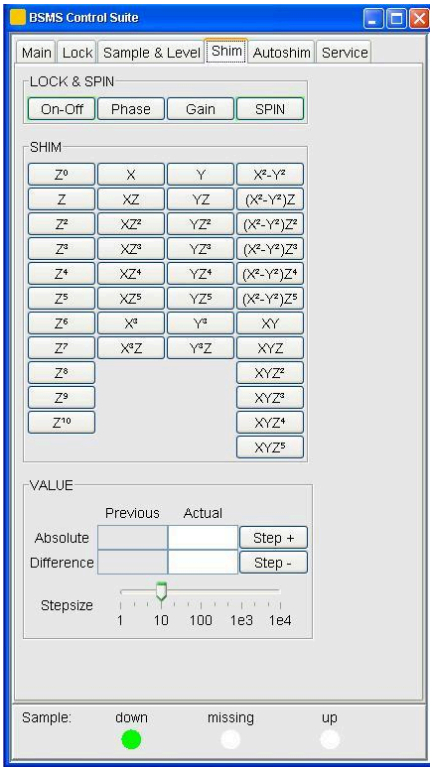
On the control panel (fig. 19) you need to press two buttons to select each shim function. For a pure Z function ($\mathbf{Z^1}$, $\mathbf{Z^2}$, $\mathbf{Z^3}$) you must press **ONAXIS** and the required functions. For functions without Z (**X**, **Y**, **XY**, $\mathbf{X^2\text{-}Y^2}$) press the function then $\mathbf{Z^0}$. For mixed functions press both function components for example for XZ press **X** then $\mathbf{Z^1}$.

Shimming with the *bsmsdisp* window

In the *bsmsdisp* window (fig. 20) under the **Main** tab in the **SHIM** frame there are buttons for the main shim functions. Choose the shim function that you want and change it by clicking on **Step +** and **Step –** in the **VALUE** frame. You can also enter the value numerically under the word **Actual**. The higher the signal in the *lockdisp* window, the better the shimming.

You must go to the **Shim** tab (fig, 25) to change $\mathbf{XZ^2}$ and $\mathbf{YZ^2}$.

Figure 25. The *bsmsdisp* window with the *Shim* tab for adjusting XZ^2 and YZ^2



Enter *topshim* without sample spinning and wait about three minutes for the process to finish. For the first sample you should also correct the non-spinning shims as explained below and if there is a large change repeat *topshim*. You may be able to further improve the shimming slightly by manually adjusting **Z** and $\mathbf{Z^2}$ with spinning. Sometimes, particularly for non-homogenous samples or samples in non-standard tubes, *topshim* will fail and you should shim manually as explained below.

For manual shimming, start with the functions **Z** and $\mathbf{Z^2}$ (with spinning).

For the first sample that you do, adjust (without spinning) also functions **X** and **Y** then **XZ** and **YZ** (if there is a large change return to **X** and **Y**), then $\mathbf{XZ^2}$ and $\mathbf{YZ^2}$ (if there is a large change return to **X**, **Y**, **XZ** and **YZ**) and then **XY** and $\mathbf{X^2\text{-}Y^2}$. Spin the sample and rerun *topshim*. If shimming manually, readjust **Z** and $\mathbf{Z^2}$ and if the spectrum (see acquisition and phasing ch. 3i to 3k) looks alright then you may adjust $\mathbf{Z^3}$ and return to adjust **Z** and $\mathbf{Z^2}$.

Afterwards, acquire and phase the spectrum as explained below (see acquisition and phasing ch. 3i to 3k) and look at the signals (fig. 26). It is best to look at a singlet such as the solvent of TMS and correct as necessary.

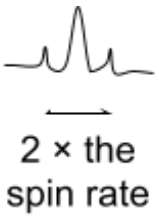
Figure 26. Signal distortion due to bad shimming

If the signals look like this increase $\mathbf{Z^2}$ 

If the signals look like this reduce $\mathbf{Z^2}$ 

If the signals look like this correct $\mathbf{Z^3}$ 

If the signals look like this correct **Z** and perhaps $\mathbf{Z^3}$ 

If the signals look like this correct **X**, **Y**, *etc.* 

i. Initial acquisition

Adjust the sensitivity of the ADC by entering *rga*. (If you already did it while shimming there is no need to do it again.)

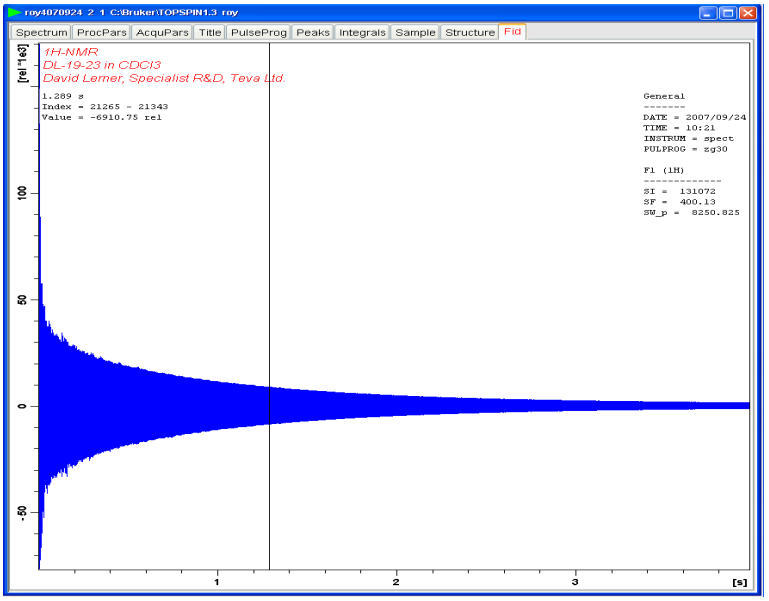
If you just copied a previous proton file (*e.g.*, by using *Use current parameters* radio-button under the command *edc*) and did not read new parameters (by specifying *1_Proton* in **Experiment** under the command *edc* or by entering *rpar 1_Proton all*) then enter *ds 0* then *ns 1*. If using the BBO probe use the *1_ProtonBBO* parameter file.

Enter *zgfp* to run the spectrum.

Usually the spectral region and the acquisition is appropriate. Sometimes the fid may be truncated and ringing will appear in the spectrum (fig. 50), that the signals may be broad wasting time on acquiring noise or there may be signals outside the range. Whenever any of these conditions are suspected the spectral range and acquisition time need adjustment, see ch. 13.

Fig. 27 shows the acquisition window.

Figure 27. The acquisition window with an fid



After the acquisition a Fourier transform (http://chem.ch.huji.ac.il/nmr/techniques/1d/1d.html) is carried out (fig. 28) that converts the acquired signal into the spectrum (fig. 29).

Figure 28. The Fourier transform

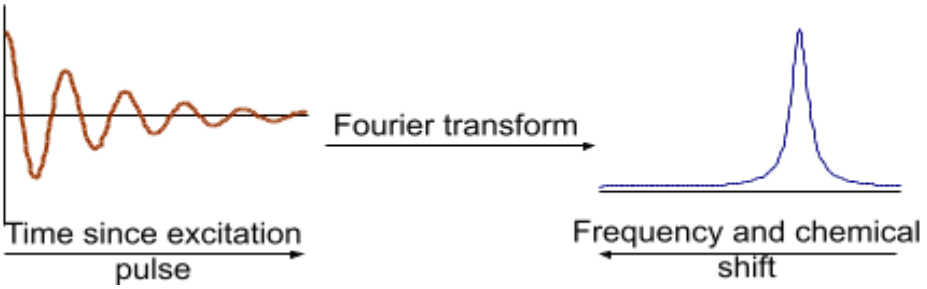
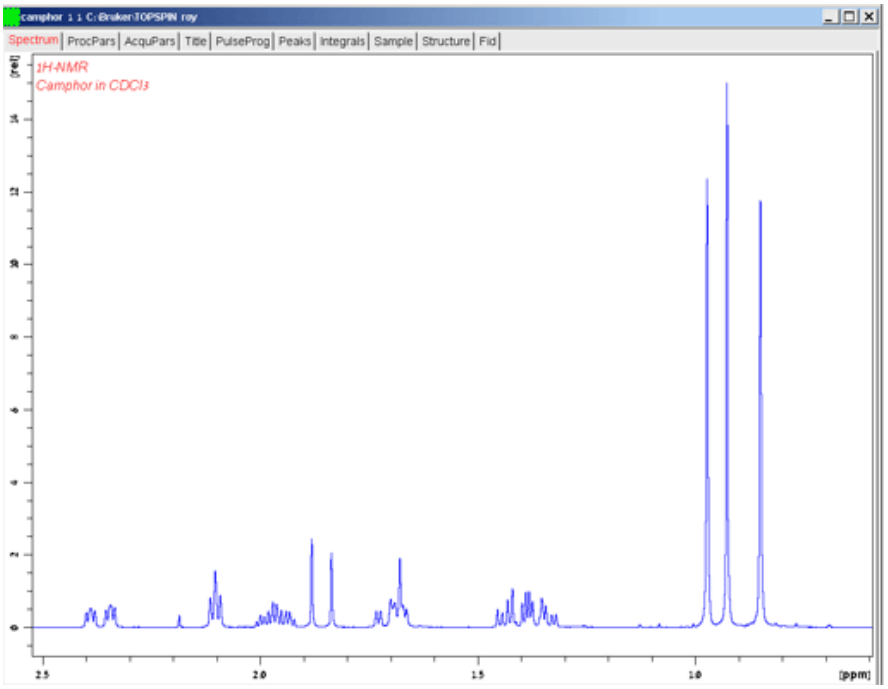


Figure 29. A spectrum



j. Control of the one-dimensional spectrum display

The following toolbar is used for controlling how the spectrum is displayed.

From left to right: create new file, print screen contents, go to last 2D dataset, go to last 3D dataset, double the height, half the height, adjust the height interactively, adjust width interactively, move up and down, move sideways, return to last expansion, display the whole spectrum, measure horizontal separation, open a full-spectrum window, open a multiple-spectrum window, start acquisition. halt and save acquisition, stop acquisition without saving, show acquisition window, calculate time required for the experiment, open the BSMS window, open the lock window, set the center of the acquisition window, set the center and width of the acquisition window, show the double-row toolbar.

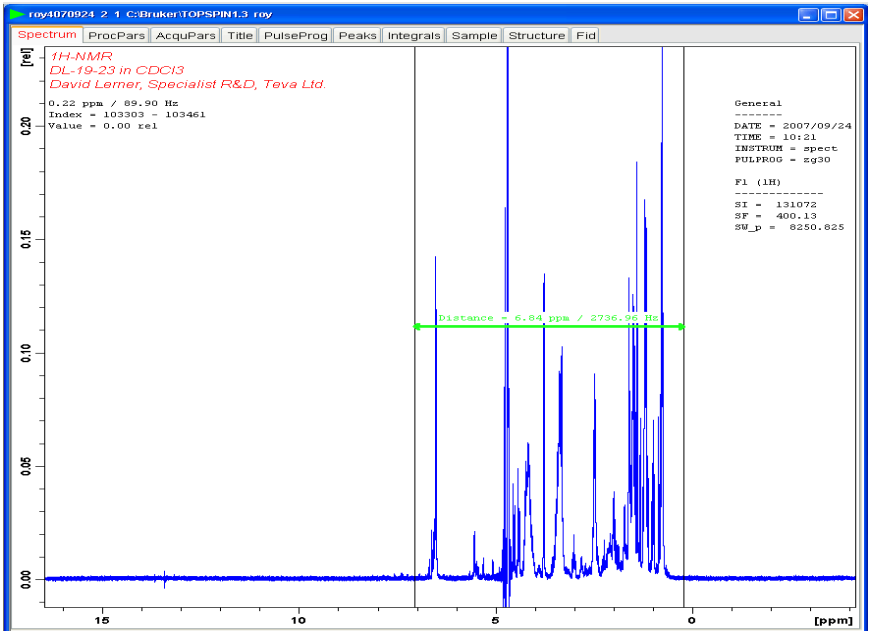
If the double-row toolbar is opened, it will look like this:

Upper row: create new file, open an existing dataset, go to last 2D dataset, increase the height 8 times, adjust the height interactively, adjust the width interactively, define the region numerically, display the whole spectrum width, toggle interactive zoom mode, display the left end of the spectrum, display the right end of the spectrum, move up and down, raise the baseline to the center, switch between Hz and ppm scales, measure horizontal separation, set up frequency list for pulse programs, start acquisition. show acquisition window, calculate time required for the experiment, set the center of the acquisition window.

Lower row: Save data elsewhere or in a different format, print screen contents, go to last 3D dataset, reduce the height 8 times, half the height, display the full height, increase expansion, decrease expansion, return to the previous expansion, display the whole spectrum, retain expansion when changing datasets, move half a screen left, move half a screen right, contract the width, expand the width, adjust the horizontal position interactively, lower the baseline to the bottom, show vertical scale, show grid, halt and save acquisition, stop acquisition without saving, open the BSMS window, open the lock window, open MAS window, set the center and width of the acquisition window, show the single-row toolbar .

The spectrum may be expanded by dragging the mouse while left-clicked. Releasing the mouse key expands the spectrum (fig. 30).

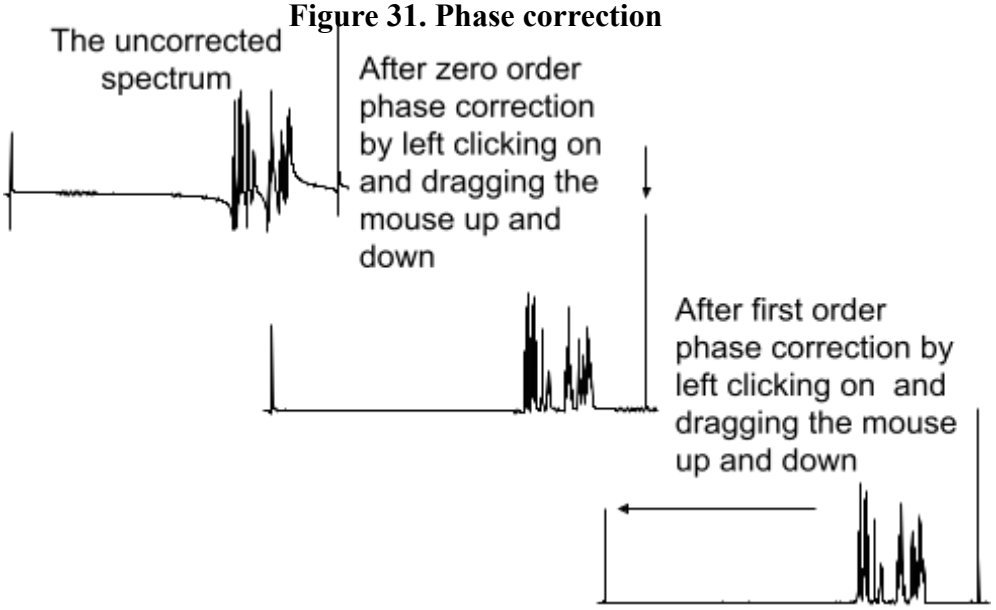
Figure 30. Selecting a region by dragging the mouse



k. Phase correction

Enter *.ph* or click on  . The phasing window will open. The zero order phase correction (at the point where there is a red vertical line) is done by left clicking on  and dragging the mouse up and down. First order phase correction (far from the red vertical line) is done by left clicking and dragging the mouse up and down. When the phase correction is complete click on  to save (or on  to cancel) the correction (fig. 31).

Figure 31. Phase correction



l. Final acquisition

If you need a quantitative spectrum see chapter 7 or if you want to optimize the sensitivity see chapter 8.

ns is the number of scans. If the sensitivity is good then 16 scans is enough. The less sensitivity the more the scans needed: 32, 64, 128, *etc.* (see ch. 14).

ds is the number of dummy scans to allow the system to equilibrate. Set *ds* to 2.

Enter: *ds 2*

ns 16

zgfp

Correct the phase.

Check that the spectrum is alright.

At the processing stage one can improve the sensitivity or the resolution of the spectrum but not both at the same time using a window function (apodization) – see ch. 13. If there is still not enough sensitivity, then other parameters may be adjusted – see ch. 14 or use the BBI probe instead of the BBO probe.

m. Baseline correction

Enter *bas* or use the menu **Procession > Baseline Correction…[bas]**. A menu window for baseline correction will open. Choose the option (second from the top) **Auto correct baseline using polynomial**. You can change the baseline polynomial order in the field **Degree of polynomial ABSG (0..5)=**. The default is 5 but 0, 1 or 2 usually work better. Click on **OK** to save (or **Cancel** to abort).

n. Chemical shift calibration

Enter *.cal* or click on  . Bring the cursor (vertical red line) to the calibration peak and left click. Enter the chemical shift and click on **OK** to save (or **Cancel** to abort).

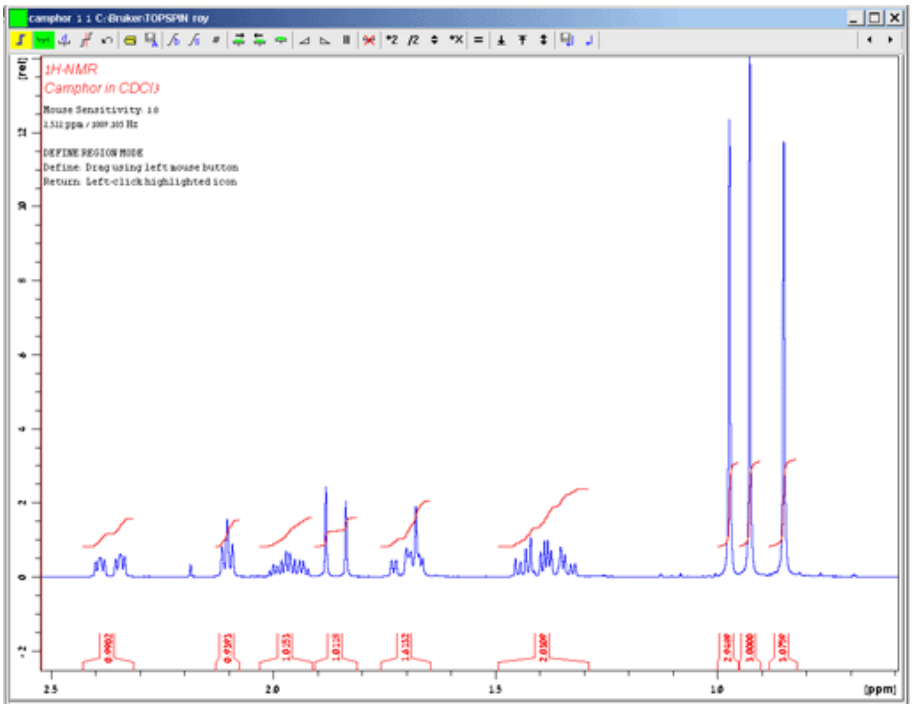
The most common chemical shift references at room temperature are: TMS 0, $CHCl_3$ 7.262, DMSO-d_5 2.503, HOD 4.81 and CD_2HOD 3.312. Other chemical shifts are given in table 2 ch. 15. See http://chem.ch.huji.ac.il/nmr/whatisnmr/chemshift.html.

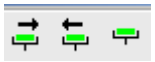
Be careful not to confuse the reference signal with other overlapping signals. The solvent and TMS usually have especially sharp signals. Be especially careful not to confuse the signal of silicone oil at 0.05 ppm with that of TMS.

o. Integration

Enter *.int* or click on  or Process-> Ingerate. The integration window (fig. 32) will open.

Figure 32. The integration window



You can add an integral by clicking on  and left dragging the cursor over the regions of the spectrum that you want to integrate. To select an existing integral use the  buttons. The right button selects and deselects all the integrals and the other buttons select the integrals one by one.  deletes the selected integral(s).  splits and reconnects the selected integral. The integral window should look something like in fig. 32 after manual integration.

Calibrate the integral intensity by right clicking on an integral of known intensity (of a known number of protons). A menu will appear; select **Calibrate current integral** and enter the intensity in the **New value** field.

The integrals in a regular proton spectrum are accurate to approximately ±10%. It is possible to improve the accuracy to ±1% by acquiring a quantitative spectrum – see ch. 7.

p. Peak picking

For the purposes of routine printing the peak picking is carried out automatically. See ch. 16 to peak pick manually.

q. Printing

To print press *ctrl-p*, click on  or use the **Plot** tab.

1. Press *ctrl-p* or click on  to print what appears in the spectrum window. Click on **OK** to print (or **Cancel** to abort). Usually it prints without parameters and the print is difficult to read.

2. For more options, click on the **Plot** tab. This opens a window where you can edit the appearance of the spectrum but this takes more time. You can do many things in the editor but these are the most important. Click on the spectrum away from the title and use the regular toolbar above to adjust the spectrum. Click on an empty region or press Esc or the return symbol on the sidebar to leave this mode. Click on  on the toolbar above to print, choosing the default or other printer.

r. Saving printouts to a file and sending them by email and fax

Instead of printing on paper, one can prepare a printout file for sending by emailor to save it on the computer. If this is a single plot then choose the printer Adobe PDF (if you are using your own computer, check if Adobe PDF writer is installed), Microsoft XPS Document Writer or Microsoft Document Image Writer from the printer menu. If you want to insert a number of printouts into one document, chose the Adobe PDF printer and choose a filename. If you want to save more than page, create a file for each page and from within the Adobe PDF window, that appears after each such 'printing', choose from the menu File -> Create PDF -> From Multiple Files…' click on Browse, choose a filename, click on OK and repeat for each file. Click on Save and choose a filename. You can send the file by email although it is not recommended to send more than 30 pages at once.

You can send files to other computers (ch. 17) and install the processing software there (ch. 18).

s. Exiting the program when finished work

When finishing work remove the sample (see ch. 3d) and close the window or enter *exit*. A message will appear **Close TOPSPIN This will terminate all possibly active commands. Exit anyway?** Click on **OK** or return. Leave your account: **Start > Log off**. A message **Are you sure you want to log off?** will appear. Click on **Logout**.

If no one is going to use the spectrometer in the next hour set the BCU 1 to mode Flush 0.

4. Proton acquisition decoupled from other nuclei

It is possible to decouple other nuclei such as phosphorus from a proton spectrum. It is preferable to use a BBI probe but a BBO probe can be used. However, on this spectrometer, only on the BBO probe can decoupling from rhodium be carried out.

Run a regular proton spectrum as described in ch. 3. If the chemical shift range of the coupled nucleus is unknown then run a spectrum of the coupled nucleus (see the guide

"Measuring NMR spectra of carbon and other non-proton nuclei") or if there is insufficient sensitivity run a heteronuclear correlation (see the guide "Measuring 2D NMR spectra"). If using the 1D spectrum of the coupled nucleus click on  in that spectrum, place the cursor at the center of the signals and note down the frequency.

Put the 2H stop filter in the BB channel (fig. 33).

Figure 33. Connections at the preamplifier exit for broadband decoupling

Table 3. Parameters for decoupling other nuclei from proton

Decoupled nucleus	Parameter name	pl12 BBI	pl12 BBO
Phosphorus, ^{31}P	1e_Protonpdec	-7.7	-1.07
Rhodium, ^{103}Rh	1f_Protonrhdec		-4

Open a file with the correct parameters according to table 3. If you have not tuned the BB channel then do it now according to the instructions in the guide "Measuring NMR spectra of carbon and other non-proton nuclei" ch. 1b. If you measured the frequency of the center of the signals of the decoupled nucleus from the 1D spectrum then set *sfo2* to this value. Otherwise set *sfo2* to $sf \times \Xi_X(1 + 10^6\delta_X)$ where Ξ_X for the coupled nucleus is as found in "Measuring NMR spectra of carbon and other non-proton nuclei" table 3 and δ_X is the chemical shift in ppm of the center of the signals of the coupled nucleus. For example if phosphorus is the coupled nucleus and the chemical shift of the center of the spectrum is 20 ppm:

$$sfo2 = sf \times \Xi_X(1 + 10^6\delta_X)$$

$$= 400.13 \times 0.40480742(1 + 10^6 \times 20)$$

$$= 161.9788325$$

If using a BBO probe (except for rhodium decoupling) you must change the value of *pl12* according to table 3.

Run and process the spectrum like a regular proton spectrum

You cannot decouple fluorine from proton on the old 500 MHz spectrometer.

5. Troubleshooting and problems with the instrument

Do not shut down the instrument under normal circumstances

a. The spectrum does not appear at the expected shift or does not look good

Check that the sample is clear, in position and has the correct depth. If the sample is not visibly faulty, check another sample or a standard sample. If the standard sample does not give a good spectrum, read the standard parameters and try again.

b. Lock does not work

Check the lock phase. It sometimes gets set wrong in autolock.

If the lock still does not work, especially after a reset, delete the file exp/stan/nmr/lists/bsms/.BSMS_init_values_lost

c. Tuning does not work

If the tuning command atma or atmm gives a 'null' error, leave and restart Topshim.

Even without errors, some samples are difficult to tune - see ch. 12.

d. BSMS panel does not react to pressing keys

If the BSMS panel does not work, open the bsmsdisp window (enter *bsmsdisp* or, click on  , fig. 20) then close it. If BSMS panel still does not work, its functions can be controlled from the bsmsdisp window.

e. Topspin window freezes

You can right-click on the frame and close the program but this will stop the acquisition.

You can keep the acquisition running and restart the graphic user interface (GUI) by entering the Windows Task Manager (Ctr-Alt-Del and select Start Task Manager). Go to the Processes tab, select the process java.exe *32 and click on End Process. The Topsin GUI will restart without stopping the acquisition and you can now close the Windows Task Manager.

f. Power failure

If there is a power failure for five minutes or another emergency situation that requires a system shutdown:

Turn off:

- The computer from the Start button
- The console - the red switch at the top left
- The MAS unit - switch on the right
- The temperature unit - switch on the left

Do not start turn back on until at least 10 seconds passed since turning off.

Switch on:

- The computer - button on the right half-way up
- The console - the green switch at the top left, do not turn on the DRU unit at this stage
- The MAS unit - switch on the right
- The temperature unit - switch on the left

If the BSMS control panel shows an error, press the STD BY button repeatedly to clear the errors.

Log on to the computer with your username and password (fig 5). The password will appear as black circles for security reasons. Click on OK to enter.

Wait for the network to connect (a few seconds).

Open the console door and turn on the AQS/R unit - switch at the top

Wait 90 s.

Start the Topspin 3.2 program by double clicking on the symbol  or start>All Programs>Bruker NMR Software>TOPSPIN 3.2>TopSpin 3.2

Enter *edhead* - See ch. 3c. Ignore any errors by clicking OK.

If the BSMS control panel shows an error, press the STD BY button repeatedly to clear the errors.

Enter *ii* and wait for the command to finish.

Enter *rsh* and wait for the command to finish.

If upon trying to lock for the first time an inconsistent frequency error occurs, enter cf then the nmr superuser password then enter then click: Next>, Next>, Next>, Next>, Save and Close, Finish>, No.

If the lock does not work, see ch. 11 b.

6. Less common probes

In addition to the commonly used probes there are four extra probes for special applications.

On the 500 MHz spectrometer there is a CP-MAS probe that looks like this  and an HR-MAS probe that looks like this  . See instruction for solid-state. Do not put a regular NMR tube into these probes.

7. Accurate quantitative acquisition

It is possible to achieve integration to an accuracy of 1% by quantitative acquisition. First measure the longitudinal relaxation, T_1 (see ch. 19).

Change *ds* and *ns* according to requirements and enter *pulprog zg*. Enter *aq* and note down the value. Change (usually increase) *d1* to $7T_1 - aq$ (or $7.21d7 - aq$) but not less than 0.03 s. For example, if *d7* is 4 s then T_1 is 5.76 s. If *aq* is 3.972 s then

$$d1 = (10.1 \times 4 - 3.972)\ \text{s} = (7 \times 5.76 - 3.972)\ \text{s} \approx 36.4\ \text{s}$$

(Theoretically five T_1 is sufficient but due to inaccuracies in relaxation-time measurement and our experience, it is recommended to use seven T_1.)

Enter *rga* and wait for it to complete. This may take a minute or more.

Reacquire the spectrum which may take longer than usual. Accuracies of 1% can be achieved by integration.

8. Parameter adjustment for optimizing sensitivity

If the sensitivity is too low despite a large number of scans (*ns*) it is possible to increase the sensitivity a little. Measure the pulse width (see ch. 19) and the relaxation time (T_1) (see ch. 18) of the signals of a similar compound under similar conditions. Enter *pulprog zg*. Change *p1* so that the pulse width is 68°.

9. Temperature control and stabilization

Use the ceramic spinner for temperatures over 60°C (333 K) and don't use the ceramic spinner when cooling.

In the *edte* window under the *Main display* tab (fig. 47) you can change the temperature by clicking on **Change…** and entering the new temperature in the **Sample temp.** field. The actual measured temperature appears in **Target temp.** You can use any temperature up to 453 K as long as the solvent does not boil.

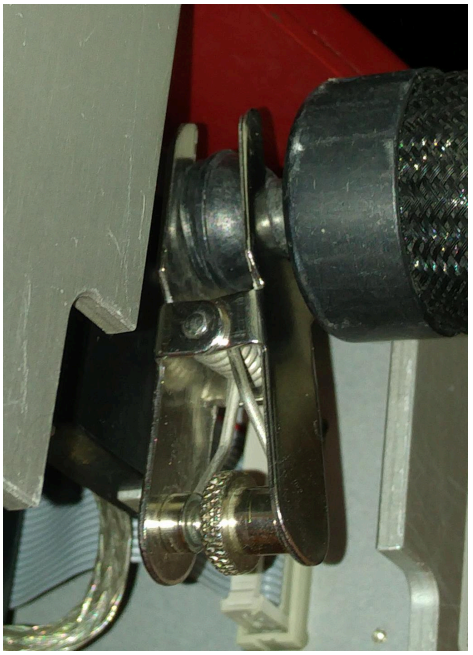
At -5°C and above, air is passed to the probe from under the magnet via a black pipe shown in fig. 34.

Figure 34. Air connection (black tube) for room temperature and above

In order to cool below -5°C, use the cooling unit. Do not use it without special permission.

To cool, fill the Dewar with liquid nitrogen, insert the transfer tube with its O-ring and close it tightly. Turn off the **HEATER** and release the air hose clip (fig. 35).

Figure 35. The air hose clip



The cooling connection is opened by pushing the plastic sheath (fig. 36) and connecting it to the probe (fig. 37). On finishing work with cooling disconnect the cooling pipe with heating from a hair-dryer, connect the air pipe and start the air-flow and heating.

Figure 36. Connection of the cooling pipe on the 500 MHz spectrometer

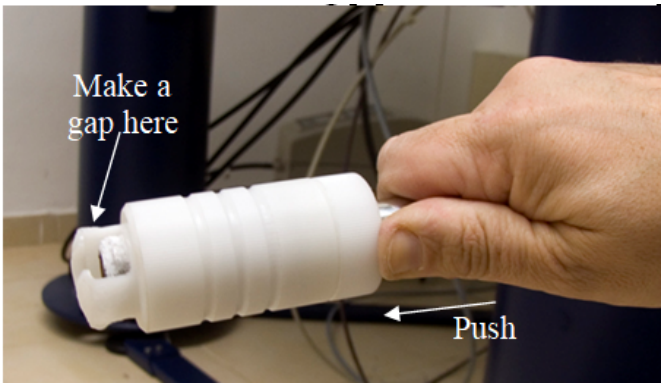
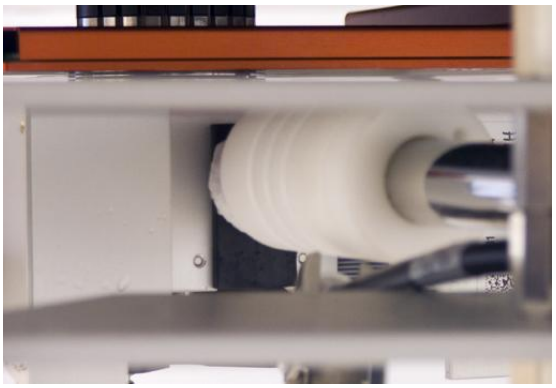


Figure 37. The cooling pipe connected to the probe of the 500 MHz spectrometer

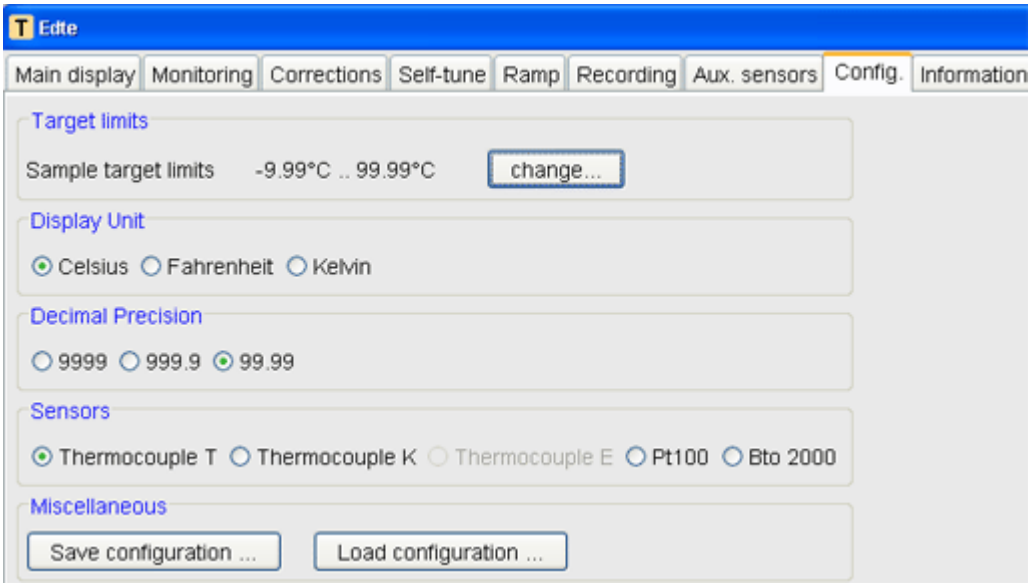


All the control over the temperature unit is performed from the **edte** window (fig. 38). Check that the Probe heater is on and the Gas flow is 535 l/h for heating or zero when Cooling is on.

Figure 38. The *Main display* tab of the *edte* window for temperature control

On the 500 MHz spectrometer it is possible to display the temperature in Celsius in the range -9.99°C to 99.99°C that allows an extra decimal place of precision. Under the **Config.** tab, change the **Display unit** to **Celsius** and the **Decimal Precision** to **99.99**. In the frame **Target limits** click on **change…** and select a minimum of ***-9.99*** and a maximum of *99.99*. The resulting window should look like in fig. 39.

Fig. 39 The *Config.* tab of the *edte* window in Celsius mode



If you want to work outside this temperature range or to display the temperature in Kelvin, change the **Display unit** to **Kelvin** and the **Decimal Precision** to **999.9**. In the frame **Target limits** click on **change…** and select a minimum of ***123.15*** and a maximum of *423.15*.

If the temperature does not stabilize, set these parameters manually. They are correct for room temperature. For other temperatures see fig. 40.

Proportional Band: *74.5*

Integral Time: *232*

Derivative Time: *38*

After making these changes click on **Apply PID changes**. There is no need to change these parameters for small changes in temperature. When cooling it is best to use a higher nitrogen flow rate than that in fig. 40 and then to reduce it. If the temperature does not stabilize to ±0.1 K, use **self-tune** (fig. 41) and wait several minutes while the unit calibrates itself. If the temperature still does not stabilize then there is a leak or insufficient gas flow.

Figure 40. Parameters for temperature control

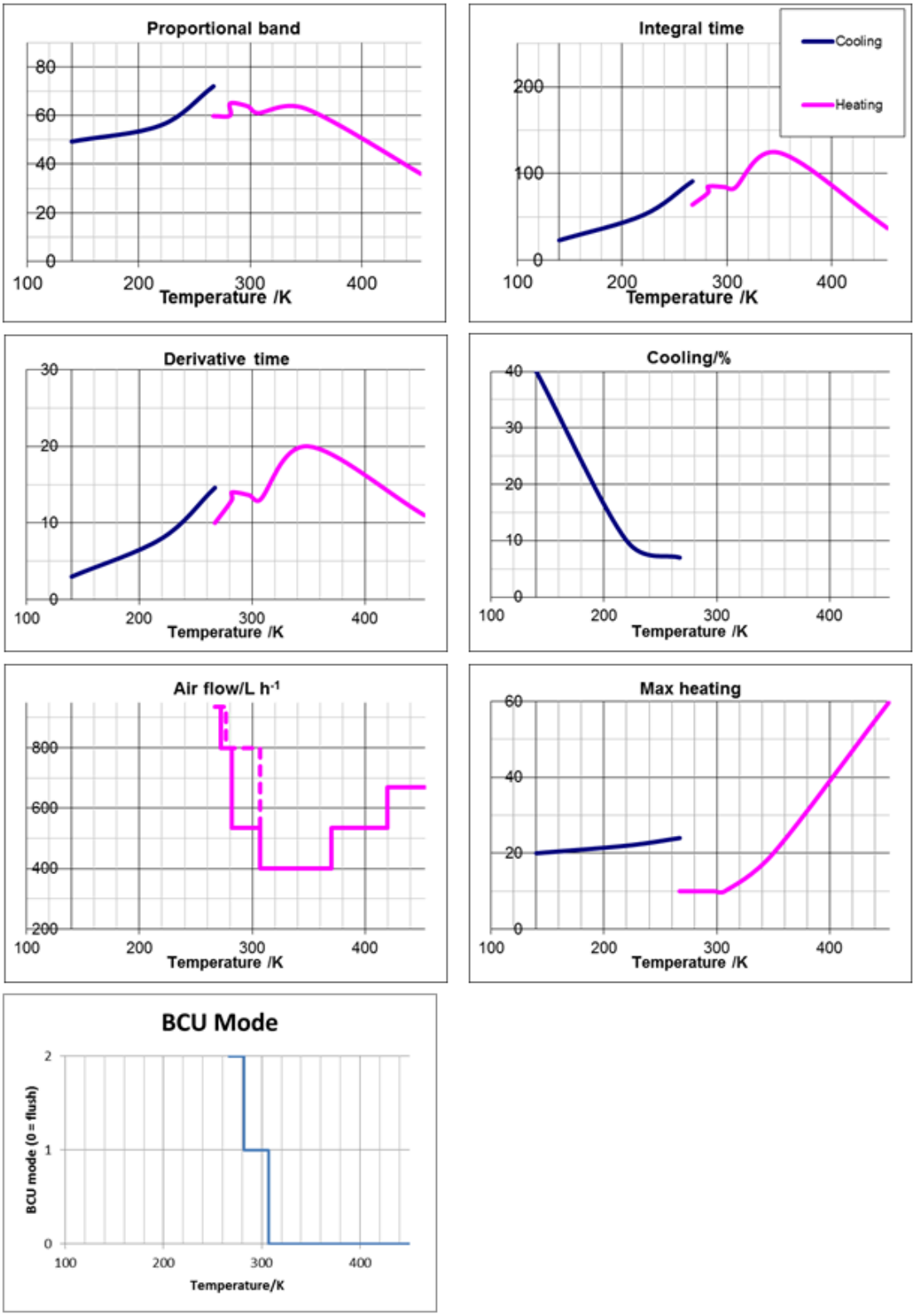
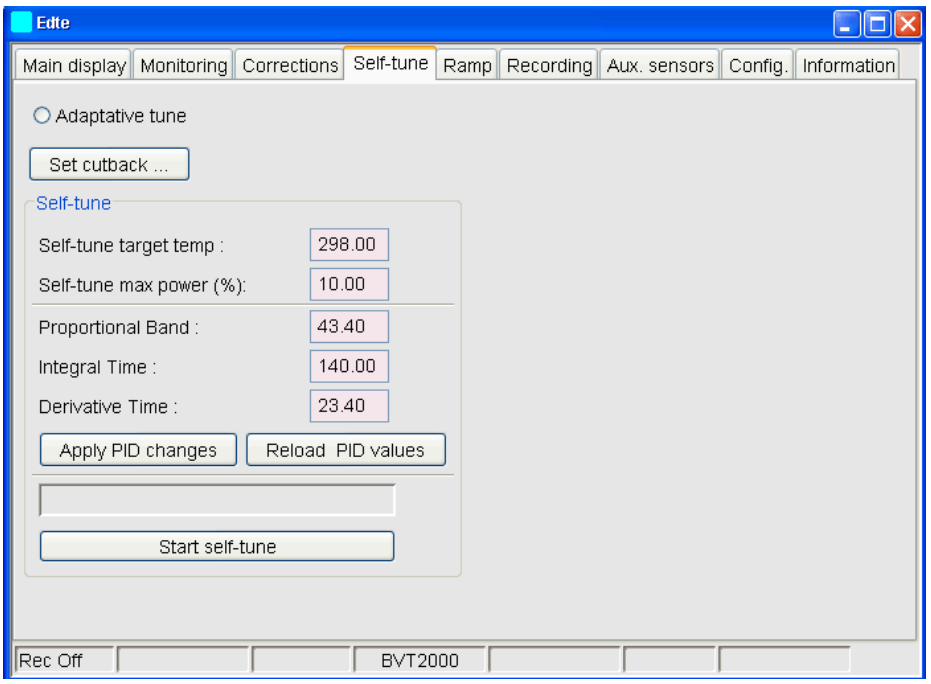


Figure 41. The *Self-tune* tab of the *edte* window for calibrating the temperature unit



When the temperature is close to room temperature it is accurate to the nearest degree. If you want to be more accurate you can calibrate the temperature as described in ch. 10.

10. Temperature calibration

When the temperature is close to room temperature it is accurate to about one degree. The accuracy becomes worse the further the temperature is from room temperature. If you want better accuracy you can calibrate the temperature using methanol or CD_3OD for room temperature and below, D_2O near room temperature or glycol for above room temperature. You can also do the calibration after the acquisition.
When using CD_3OD or D_2O the acquisition is as usual.

When using regular methanol or glycol you need to off-tune as follows. Enter *atmm* wait for *wobb* to start then click six times on <<<. To return the tuning afterwards enter *atma*. On leaving *atmm* it will ask you if you want to save. Click on **Cancel**. Cancel the lock – click on **LOCK** on the control panel or **Lock** in the **LOCK** frame of the *bsmsdisp* window. Wait a few seconds until the **SWEEP** light comes on then cancel the sweep by clocking on **SWEEP** on the control panel or **On-Off** in the **SWEEP** frame of the *bsmsdisp* window. Shim without lock as described in ch. 11.

Acquire the NMR spectrum by entering *zgfp*. Correct the phase as described in ch. 3k. Measure the chemical shift difference ($\Delta\delta$) between the two main peaks (fig. 42). The measure of temperature stability is that the result of repeated acquisitions is the same to within 0.001 ppm. Repeat the measurement until such stability is

obtained. This usually takes 10 to 15 minutes. If the temperature does not stabilize, check the temperature controller (ch. 9).

Calculate the temperature using the relevant equation like the one for methanol in fig. 42 or use the program (fig. 43):
http://chem.ch.huji.ac.il/nmr/software/thermometer.html.

$$T(CD_3OD)/K = 419.0746 - 52.513\Delta\delta - 16.7467(\Delta\delta)^2,\ 287\ K < T < 330\ K$$

Otherwise $T(CD_3OD)/K = 461.37 - 115.86\Delta\delta + 16.664(\Delta\delta)^2 - 6.2701(\Delta\delta)^3$

$$T(D_2O/TMS) = 297.14 - 90.86(\Delta\delta - 4.8),\ 287\ K < T < 310\ K$$

$$T(MeOH)/K = 409 - 36.54\Delta\delta - 21.85(\Delta\delta)^2,\ 180\ K < T < 330\ K$$

$$T(Glycol)/K = 465.77 - 100.91\Delta\delta - 0.39(\Delta\delta)^2,\ 275\ K < T < 450\ K$$

Figure 42. Temperature calibration spectrum

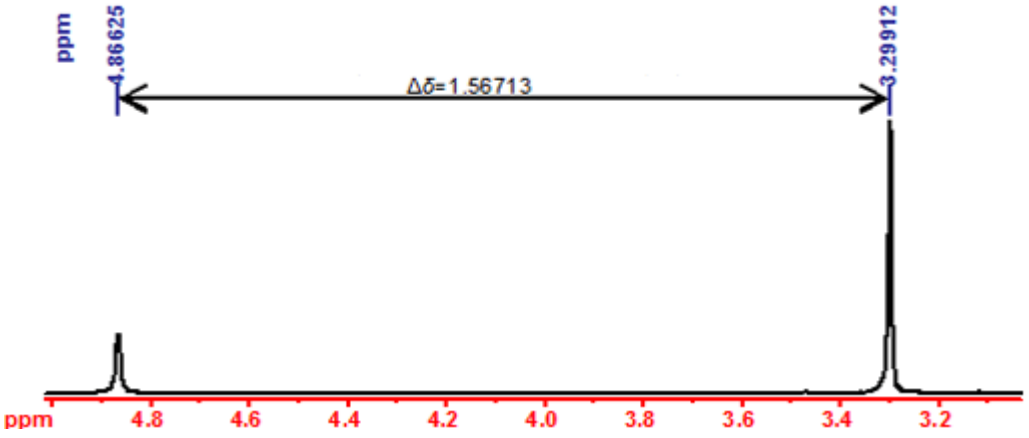
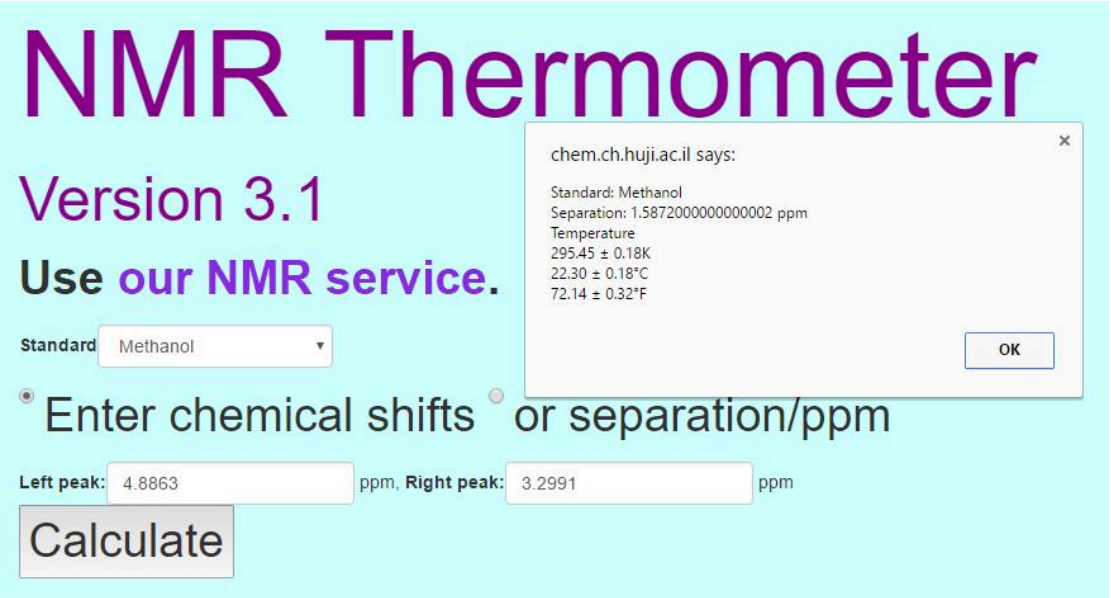


Figure 43. Program for calculating the temperature



11. Difficulties in locking and acquiring without lock

It is possible that the lock will fail when there are multiple solvents, the signal is weak or there are multiple signals in the deuterium spectrum such as with THF-d_8 and DMF-d_7 when you will need to release the lock by pressing on **LOCK ON/OFF** on the control panel and relock manually.

a. Improving lock stability

High dynamic range spectra are especially sensitive to lock stability. The lock stability may be improved by adjusting the lock parameters. If the lock goes up and down in a wavy manner, reduce the **LOCK POWER** by 6 dB. If the lock is stable, note the lock level, increase the **LOCK POWER** by 6 dB, note down the **LOCK GAIN** and reduce it until the lock returns to it s previous level. If the reduction in **LOCK GAIN** is significantly less than 6 dB then return the **LOCK POWER** to what it was. Continue until the optimum **LOCK POWER** is found. Enter *loopadj2* and wait for it to finish. This makes the lock more stable.

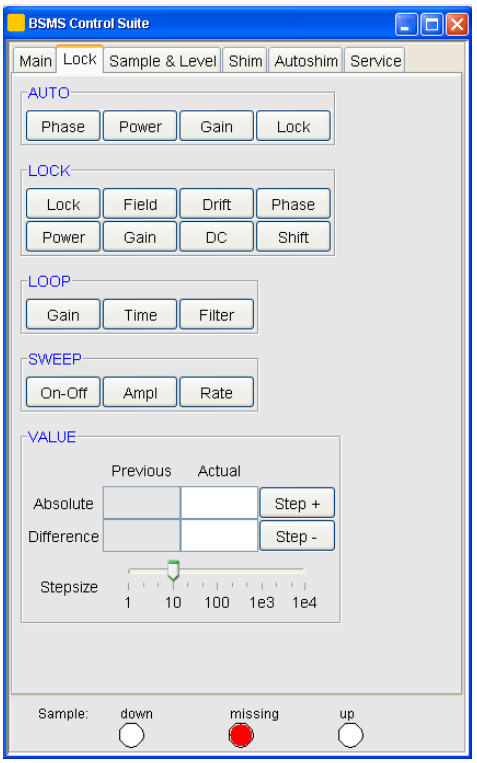
b. Manual locking

Finding the field: on the control panel (fig. 19, 20) click on the **FIELD** button. The current value of the **FIELD** appears in the small window. Using the wheel, search for the signal. When you see a lock signal that is reminiscent of a butterfly, bring it to the center.

If you use *bsmsdisp* (fig. 20) open the **Lock** tab (fig. 44). There you can change the value of **Field**.

Sometimes two 'butterflies' appear because the solvent (such as CD_3OD) has two different types of deuterium. Then it is preferable to choose the larger signal and if they are of the same intensity, the right-most signal.

Figure 44. The *Lock* tab of the *bsmsdisp* window for advanced lock functions



Click on **lock** in the **LOCK** frame and the 'butterfly' should disappear and become a flat line.

Click on **LOCK GAIN** on the control panel or **Gain** in the **LOCK** frame of the *bsmsdisp* window to bring the lock level up or down to near, but not completely to, the top of the lock window.

c. Normal values for the lock parameters

The lock parameter values are adjusted by clicking on the relevant button and changing the value.

FIELD in the **LOCK** frame is the position of the magnetic field and changes between solvents. For example for $CDCl_3$ the field is about 300.

SWEEP AMP on the control panel or **Ampl** in the **LOCK** frame of the *bsmsdisp* window is used for controlling the width of the signal. It is usually 2.0 but may be increased to 10 in order to search for a signal or reduced to 0.1 to separate very similar deuterium signals such as in DMF-d_7 and nitrobenzene-d_5.

SWEEP RATE on the control panel or **Rate** in the **SWEEP** frame of the *bsmsdisp* window is usually 0.15.

LOCK PHASE on the control panel or **Phase** in the **LOCK** frame of the *bsmsdisp* window corrects the symmetry of the signal. If the two 'wings' of the 'butterfly' then the phase must be corrected until their heights match.

LOCK POWER on the control panel or **Power** in the **LOCK** frame of the *bsmsdisp* window depends on the solvent. The more deuterium in the solvent molecules the lower the value. There are solvents where the signal after lock are more likely to be

unstable and wobbling like a wave. This phenomenon is called saturation. In such a case, reduce the value of the lock power until the signal stabilizes then reduce further by five units. (Solvents that are more likely to give saturated signals are methanol-d_4, acetone-d_6 and acetonitrile-d_3.)

LOCK GAIN on the control panel or **Gain** in the **LOCK** frame of the *bsmsdisp* window controls the height of the lock signal and can be varied according to need. Usually it is increased so that the signal is in the upper part of the window.

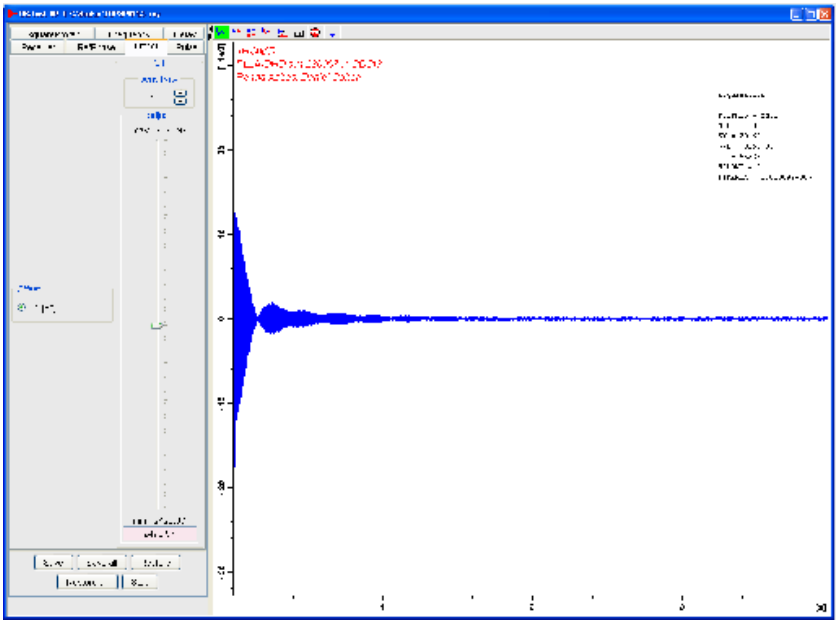
d. Acquisition without lock

If the sample contains less than 2% of deuterated solvent or the sample is ansotropic (common in liquid crystals) it is impossible to lock the field. For a regular (isotropic) sample, as long as it does not adversely affect the measurement, you can add 10% deuterated solvent containing lots of deuterium such as acetone-d_6, benzene-d_6 or even D_2O.

If there is no possibility of locking the field then the acquisition is slightly different from normal. It is preferable to take a similar but deuterated sample and do all the preparations for acquisition: locking, tuning and shimming. If this is not possible then enter the *lock* command and select a deuterated solvent most similar to the sample and after the lock fails, cancel the lock. Click on **LOCK** on the control panel or **Lock** in the **LOCK** frame of the *bsmsdisp* window. Wait a few seconds till the **SWEEP** light comes on. Cancel the **SWEEP** on the control panel or **On-Off** in the **SWEEP** frame in the *bsmsdisp* window. Enter *locnuc off*.

Tune the probe and do *rga*. Use *gs* to do the shimming. This is done interactively by observing the fid and spectrum. First acquire an initial spectrum and correct the phase then enter *gs* (fig. 45).

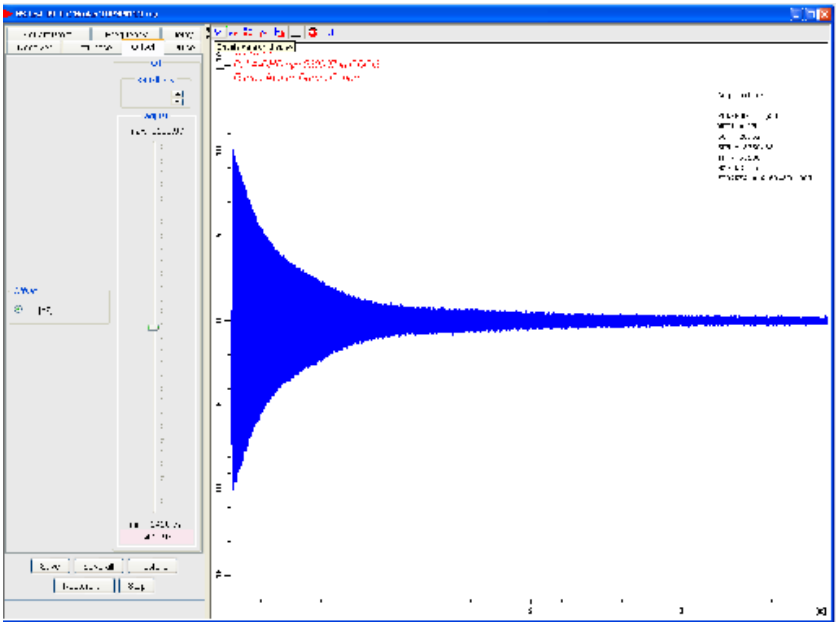
Figure 45. fid with poor homogeneity that requires shimming



You can correct the **Z** shims with spinning and the shims containing **X** and **Y** components without spinning in order to improve the fid so that its decay is slow

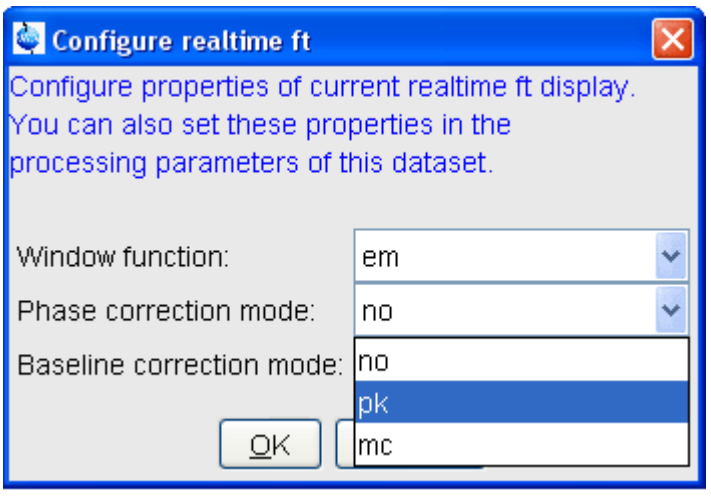
and monoexponential (fig.46). If the spectrum contains a strong multiplet then ringing will appear in the fid rather than a monoexponential.

Figure 46. fid in a homogeneous field with good shimming



In order to correct shim $\mathbf{Z^2}$ it is easier to use the real-time spectrum. Click on  and an unphased spectrum will appear. Click on  and the window in fig. 47 will appear. Change the **Window function:** to *none* and the **Phase correction mode:** to *pk*. The spectrum will appear like in fig. 48.

Figure 47. Parameter window for real-time spectrum display



Correct the shape of the signal according to fig. 26 which in the case of fig. 48 means correcting $\mathbf{Z^2}$ such that the spectrum appears like in fig. 49. Return to the fid display by clicking on  and continue shimming until both the fid and spectrum look alright. Enter *stop* to leave the *gs* mode. The final acquisition and all the subsequent steps are identical to those with lock. To return to acquisition

with lock you must read new parameters and enter *ii* or *locnuc 2h*. Afterwards click on **SWEEP** on the control panel or **On-Off** in the **SWEEP** frame in the *bsmsdisp* window.

Figure 48. Real-time spectrum with poor homogeneity that requires shimming

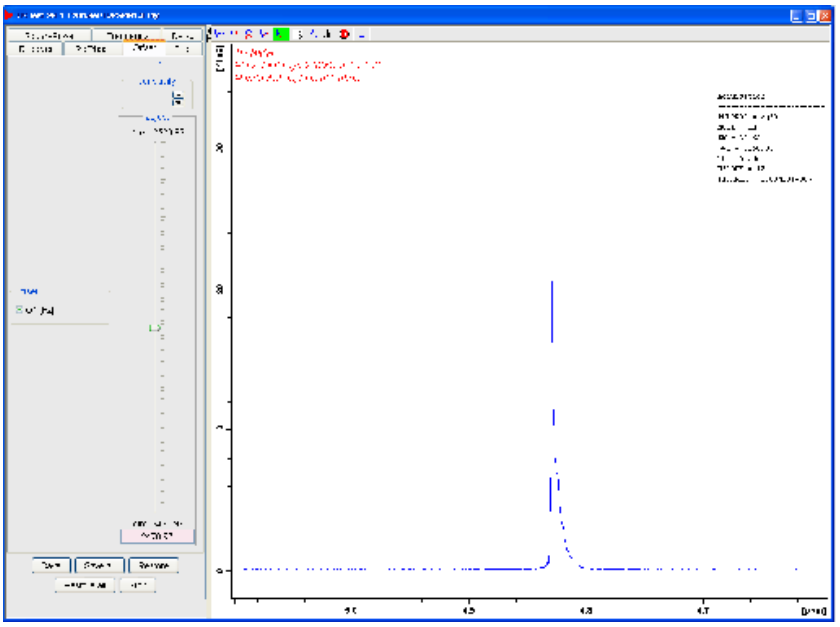
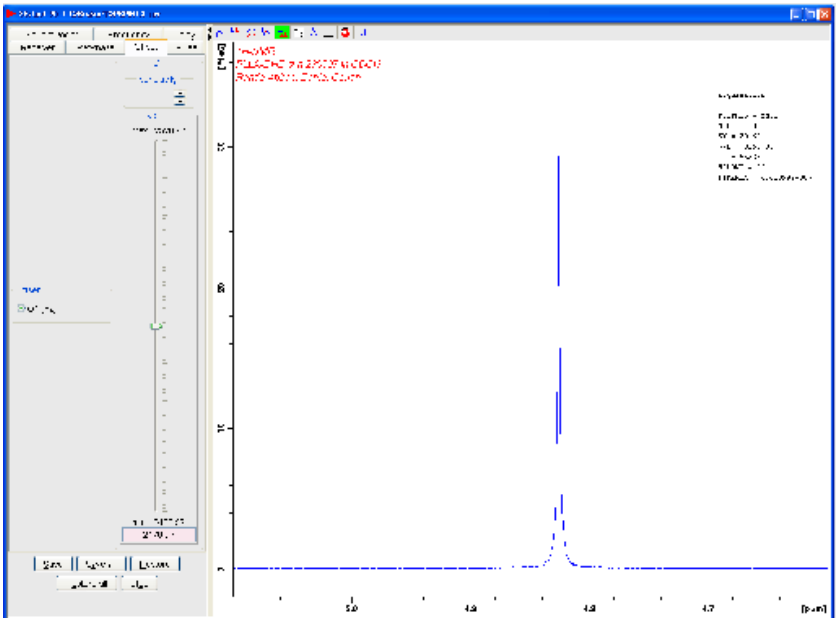


Figure 49. Real-time spectrum in a homogeneous field with good shimming



12. Semi-automatic tuning

Sometimes (especially for ionic samples) the automatic tuning (atma) does not work or works slowly and semi-automatic tuning is needed. enter *atmm* and a window with arrows for **tune** and **match** will appear. Move the **tune** sideways so that the minimum

moves to the center and the **match** so that the minimum moves to the bottom and repeat until there is no further improvement. For ionic samples the minimum will be very wide and maybe nearly flat.

13. Apodization (window function) for increasing sensitivity or resolution

To increase sensitivity set *lb* to the number of Hz that you want to broaden the signal. Choose a value less than or equal to the line-width without a window function. Up to the line-width, the larger the *lb* the greater the sensitivity but the worse the resolution. Enter *efp* to obtain the result. See http://chem.ch.huji.ac.il/nmr/techniques/1d/1d.html.

To improve the resolution set *lb* to a negative value but not of greater magnitude that the line-width without a window function. Set *gb* to a positive number up to 0.5. Up to these limits the greater the magnitude of *lb* and the greater *gb* the better the resolution but the worse the sensitivity. Enter *gfp* to obtain the result.

Commands that include the window functions, Fourier transform and acquisition appear in table 1. The regular command for acquiring and processing a proton spectrum is *zgfp* but it is best to be familiar with the other commands in table 1 in order to use window functions.

Table 1. Fourier transform commands

Command	Meaning
zg	Aquisition
em	Sensitivity enhancment
ft	Fourier transform
pk	Phase correct
zgft	zg;ft
zgfp	zg;ft;pk
zgef	zg;em;ft
zgefp	zg;em;ft;pk

14. Optimizing the spectral width and acquisition time

The default spectral width is between -4 and 16 ppm. In rare cases signals may appear beyond this range. Signals may occur at up to 22 ppm in aldehydes, carboxylic enols and around macrocycles. There may be signals at low chemical shifts down to -20 ppm in metal hydrides in organometals and protons within or over macrocycles. Paramagnetic signals may appear tens or hundreds of ppm from the normal region. On the other hand it is possible to reduce the range if all the signals appear in a small region and there is a need to cure fid truncation (fig. 50). If it is suspected that there are signals outside the default region then it needs to be expanded. Change o1p to the chemical shift at the center of the region such that:

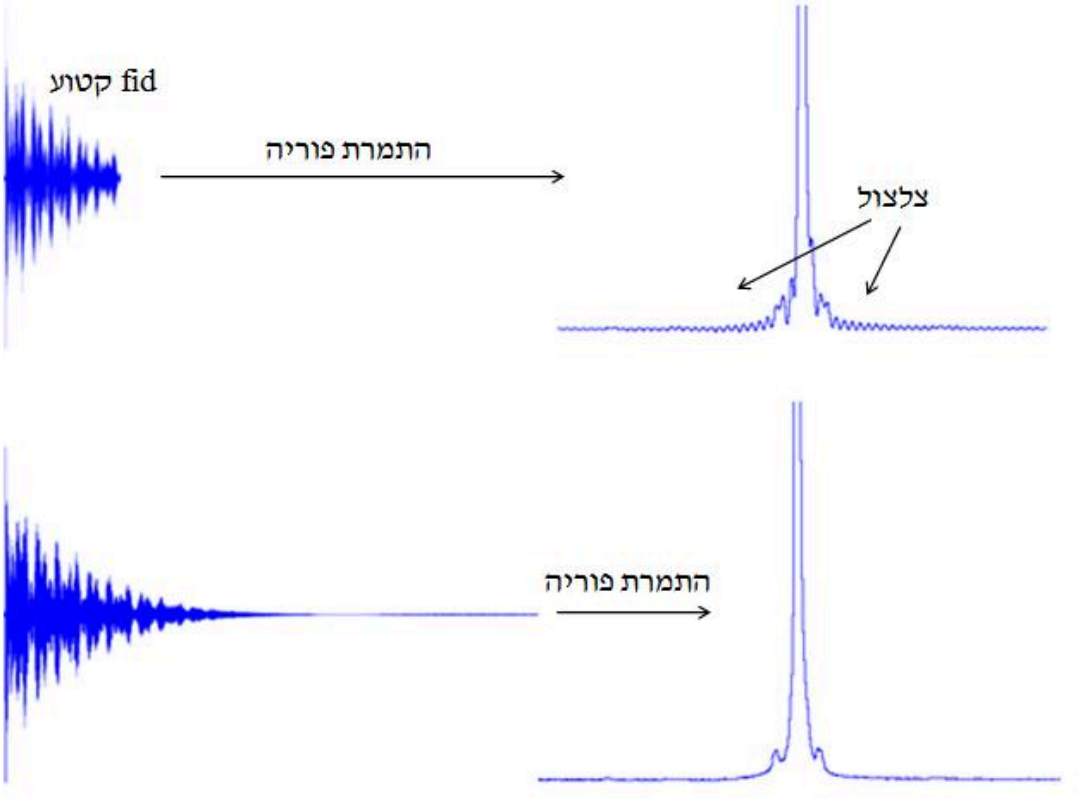
$$o1p = (\delta_{max} + \delta_{min}) / 2$$

and *sw* to the spectral width such that:

$$sw = \delta_{max} - \delta_{min}$$

Alternatively you can display the required region in the spectrum window and click on  . Once the spectral region has been selected it is possible to adjust the acquisition time by changing *td* and *si*. Carry out one scan and look at the fid and the spectrum. If the fid decays into the noise in the first half of the acquisition you can reduce *td* and *si*. If the fid is truncated or there is ringing in the spectrum (fig. 50) then you should increase *td* and *si*.

Figure 50. The effect of truncation of the fid on the spectrum



The value of *td* must be a power of two and *si* should be double *td*. You can use the letter '*k*' instead of '× 1024'. Therefore if *td* is *64k* that means that it is equal to *65536* and *si* should be set to *128k* or *131072*. Look at the fid in the acquisition window or under the **fid** tab of the spectrum window. It the fid does not decay into the noise at the end of the acquisition then double *td* and *si*. On the other hand if the *fid* decays into the noise before halfway through the acquisition then halve the *td* and *si*. Do not increase the *td* to more than *1024k* or reduce it below *256*.

15. Chemical shifts of solvents for calibration purposes

See http://chem.ch.huji.ac.il/nmr/whatisnmr/chemshift.html.

Table 2. Proton chemical shifts of deuterated solvents relative to internal TMS at 298 (25.15°C).

Solvent name	Solvent formula	Chemical shift
Acetic acid-d_4	CD_3COOD	1.899, *10.60

Acetone-d_6	$(CD_3)_2CO$	2.052
Acetonitrile-d_3	CD_3CN	1.940
Benzene-d_6	C_6D_6	7.154
Chloroform-d	$CDCl_3$	7.262
Deuterium chloride (1M) in D_2O	DCl	*5.17
Deuterium oxide	D_2O	*4.81
Dichloromethane-d_2	CD_2Cl_2	5.306
DMF-d_7	$(CD_3)_2NCH$	2.744, 2.915, 8.025
DMSO-d_6	$(CD_3)_2SO$	2.504
Formic acid-d_2	DCOOD	8.308, *10.24
Methanol-d_4	CD_3OD	3.312, *4.867
Nitrobenzene-d_5	$C_6D_5NO_2$	7.41, 7.67, 8.12
Sodium deuteroxide (1M) in D_2O	NaOD	*4.97
THF-d_8	C_4D_8O	1.721, 3.574
Toluene-d_8	C_7D_8	2.08, 6.98, 7.00, 7.12
Trifluoroacetic acid-d	CF_3COOD	*10.88

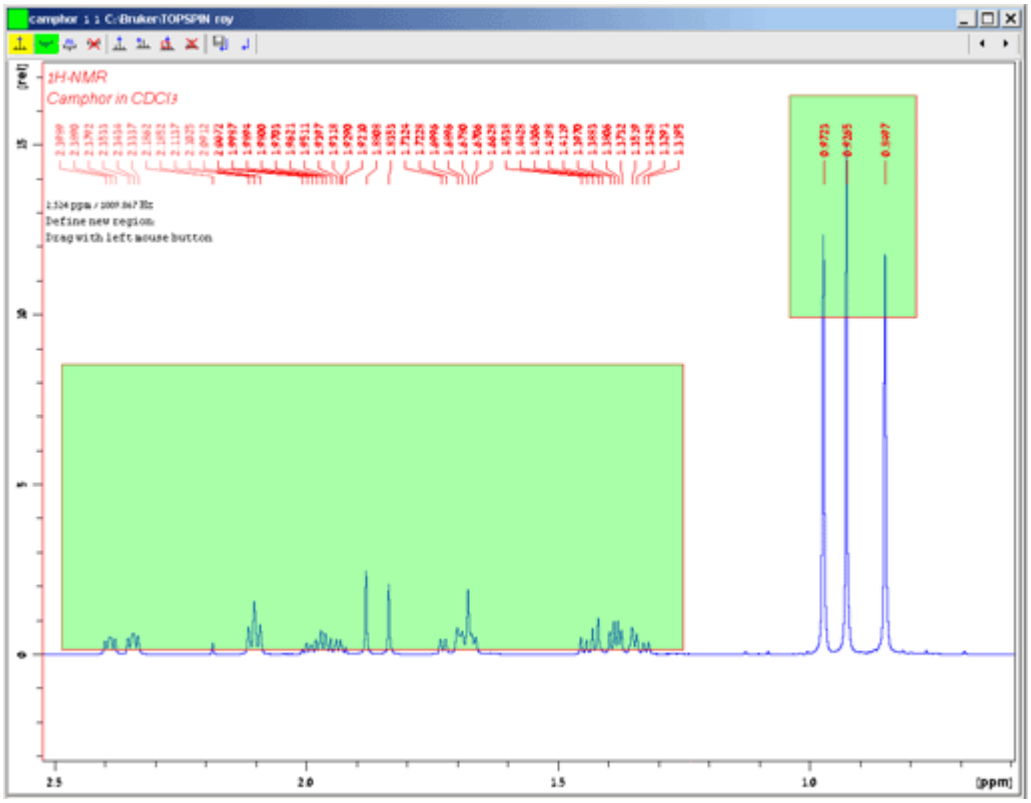
*The chemical shift of this signal is very temperature dependent and therefore not accurate for the purposes of chemical shift calibration.

16. Manual peak picking

The command *pp* does an automatic peak pick. Once can change the parameters of the automatic peak pick: MI, MAXI and PC. The automatic peak pick chooses the peaks between the heights MI and MAXI where the height relative to the local minima is greater than PC times the noise. The noise level is determined automatically. One can increase PC to reduce the number of peaks or reduce PC to increase the number of peaks.

One can change the peak picking manually as follows. Enter *.pp* or click on  . The peak picking window will open. Click on  and drag the mouse over the spectrum (fig. 51). If you want to restrict the number of signals stop the selected region above the baseline.  cancels the selected peaks.  allows individual peak picking.  cancels one peak. When finished click on  to save (or on  to cancel). Figure 51 shows the peak picking window.

Figure 51. Manual peak picking in selected regions



17. Transferring files from the spectrometers to other computers

Request an account on the backup server of the laboratory that is called nmrnas,myqnapcloud.com. You can access it from within the University or with special permission from outside.

The files on the 500 MHz spectrometer are in the directory DataVol1/theOld500Nmr/nmrData/*username*. If you need today's files then use a disk-on-key to transfer them directly from the instrument where they are in the directory C:\theOld500Nmr*username*.

18. Use of TOPSPIN on other computers

You can use for TOPSPIN from any computer with Windows 7, 8, ot 10 or Linux RHEL3 or higher or MacOS operating systems. Your computer must have at least a 1 GHz CPU, 512 MByte of RAM, a 64 MByte graphics card, a screen resolution of 1280 × 1024 and a three-button mouse.

The software is free to University staff and students (and also for schools and government workers). Go to the Bruker site **bruker.com** and under **Service -> Support&Upgrades -> Software Downloads -> Nuclear Magnetic Resonance** log on or create an account. Click on **Free Academia license**. Click on **Dowload for Windows**. Click on the latest TopSpin version (4.0.2 at the time of writing) and click **Save**. Run the package with Administrator (root in Linux) privileges. After a few seconds or a minute or so, the computer may ask you to confirm Administrator privileges.

Follow instructions. For TopSpin 4.0.2 on Windows 10 (other versions may vary):

Click **Next>**

Next>

Next>

Next>

Next>

Create a password for NMR superuser and enter it twice

and follow the instructions.

Click **Next>**

Next>

Next>

Next>

Finish

Run Topspin. It will ask for a license code.

Use the back button in your browser to return to the license page. Click **Request personal license ticket now**. You will then be given a code that you can copy into the TopSpin software.

A message requesting configuration will appear.

Click on **Expinstall**

Next>

Select the types of acquisition files that you wish to be able to process

Next>

Close

Next>

Next>

Next>

Finish

You will be asked to create an backup.

Click on **Automatic backup**

Automatic backup

OK

OK

Save

Close

You can then copy your spectrometer configuration into **C:\Bruker\TopSpin4.0.2\conf\instr** in order to make processing more convenient.

Once the installation is complete you can transfer your files to your computer (see ch. 17) and use TOPSPIN from your computer.

19. Measuring the longitudinal relaxation time – T_1

These measurement techniques for longitudinal relaxations (T_1) are accurate enough for accurate integration and optimizing sensitivity. These methods do not provide very accurate measurements of T_1. For more accurate measurements see the quide "Measuring relaxation" and http://chem.ch.huji.ac.il/nmr/techniques/other/t1t2/t1t2.html#t1.

a. The inversion-recovery method

The inversion-recovery method is suitable for relaxations times up to approximately 10 s if there is enough sensitivity to see the spectrum in one pulse.

Calibrate the pulse width and set *p1* to 90°, see ch. 6.

Enter *ds 0* and *ns 1*.

Acquire a regular spectrum and phase correct.

Enter *pulprog t1ir1d*.

Put the initial guess for T_1ln2 (0.69 T_1) into *d7*. If you do not have a guess use 1 s.

Acquire a spectrum by entering *zgfp*.

If the signal of interest is negative increase *d7* and if it is positive decrease *d7*.

Wait at least 7 times T_1 (10.1 *d7*) between acquisitions.

(Theoretically five T_1 is sufficient but due to inaccuracies in relaxation-time measurement and our experience, it is recommended to use seven T_1.)

Repeat the experiment until you find a value of *d7* that gives near zero intensity for the signal of interest. Usually the signal of interest is that with the longest relaxation but is not a solvent signal.

Calculate the relaxation time: T_1 = d7/ln2 = 1.44d7

b. The DESPOT method

When T_1 is longer than 10 s or the sensitivity is low it is preferable to use DESPOT. Set *pulprog* to *zg*. Note down the values of *aq* and *d1*. Change *ds* to int[$7T_{1max}$/(*aq* + *d1*)]+1. Acquire the spectrum with a pulse width of 90° (*p1* = $p_{360°}$ / 4 + 0.6 and on the 500, *p1* = $p_{360°}$ / 4 + 0.12). Open another file with the same parameters and change the pulse width to 45° (*p1* = $p_{360°}$ / 8 + 0.7 and on the 500, *p1* = $p_{360°}$ / 8 + 0.14). Run both spectra.

From the second spectrum click on  to enter multi spectrum display. If other spectra appear, select them in the left window and cancel them by clicking on  .

Add the first spectrum by opening it in the usual manner and select it in the left window. Match the heights of the signal of interest by dragging with the mouse up and down on  . Note down the value that appear at the upper left next to **Scale:**. Calculate T_1 as follows.

$$T_1 = (aq + d1)/\ln[1/(1\text{-}\mathbf{scale}/\sqrt{2})]$$

Click on  to return to the normal spectrum window.

.20. Measuring the pulse width

Acquire a regular spectrum and correct the phase.

Enter *pulprog zg*.

Set *p1* to close to the 360° pulse: On the 500 MHz spectrometer the 360° pulse for the BBI is close to *34* and the BBO *44*.

Acquire a provisional spectrum by entering *zgfp*.

If the spectrum is positive reduce *p1* and if it is negative increase *p1* until you find a value of *p1* that yields a spectrum close to zero intensity.

The value of *p1* for a φ° pulse is $(p_{360^\circ} - \alpha) \times \varphi / 360 + \alpha$

For the BBI and BBO probes α is near zero so: $p1 = p_{360^\circ}/ 4$

For a regular pulse of 90°: $p1 = p_{360^\circ}/ 4 + 0.6$