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Measuring Proton NMR Spectra

by Roy Hoffman and Yair Ozery 20th September 2010

This guide is intended for routine use on the 400 MHz spectrometer

The sections that are adapted for use with the 200 MHz spectrometer are framed.

The sections that are adapted for use with the 500 MHz spectrometer are in a dotted frame.

This guide is intended for use with the spectrometers of the Chemistry Institute of the Hebrew University. See chapter 4 in order to adapt it for use in other laboratories.

Contents

1. Summary of instructions for measuring a proton NMR spectrum

(Use the full guide for full details)

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 2.1/1.3
Open a new file (not required) you can overwrite a previous file but then all the old data will be lost	edc
Read the parameters for a proton spectrum	rpar 1_Proton
Lock the spectrometer to the solvent deuterium frequency	lock
Tune the frequency - you must do this when you start work or change solvent, on the 400 you must use wobb and manual tuning	atma / wobb
	rga
Read the shim file. Each probe has its own shim file.	rsh bbo/bbi/mas/hrmas
Automatic shimming is available only on the 500	topshim
Adjust the shimming (even if you used topshim on the 500) according to this order: Press spin. On the 500 also click on 'on axis' and correct Z and Z^2. Cancel the spin and correct X, Xz, Y and YZ, restart the spin and readjust Z and Z^2. Sometimes it is necessary to repeat the process several times to get good shimming. Patience is recommended.	shimming
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	.int

calibration.	
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
Logout from the computer	Lougout:

2. General description of the equipment

Figure 1. The spectrometer magnets from left to right: the 200, 400 and 500 MHz magnets

Figure 2. The console of the 400 MHz spectrometer with the doors closed on the left and open on the right

Figure 3. The console of the 200 MHz spectrometer with the door closed on the left and open on the right

Figure 4. The console of the 500 MHz spectrometer with the door closed on the left and open on the right

Figure 5. The computer that controls the spectrometer

Figure 6. The control panel for the sample and magnet

3. Routine experiments

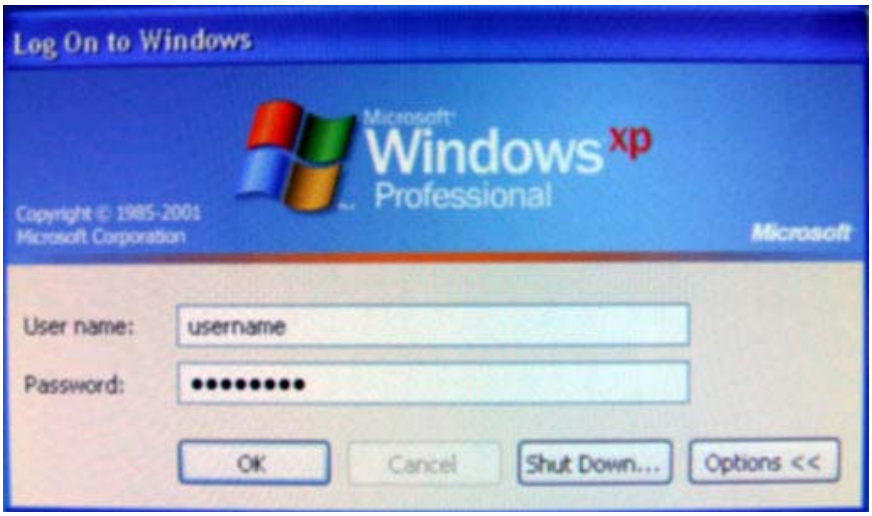
For a routine proton experiment (http://chem.ch.huji.ac.il/nmr/techniques/1d/row1/h.html#BM1H) follow these instruction. For proton NMR with fluorine decoupling see chapter 17 and for decoupling from other nuclei see chapter 18.

a. Logging on

Log on to the computer using your username and password. 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 7. User logon window

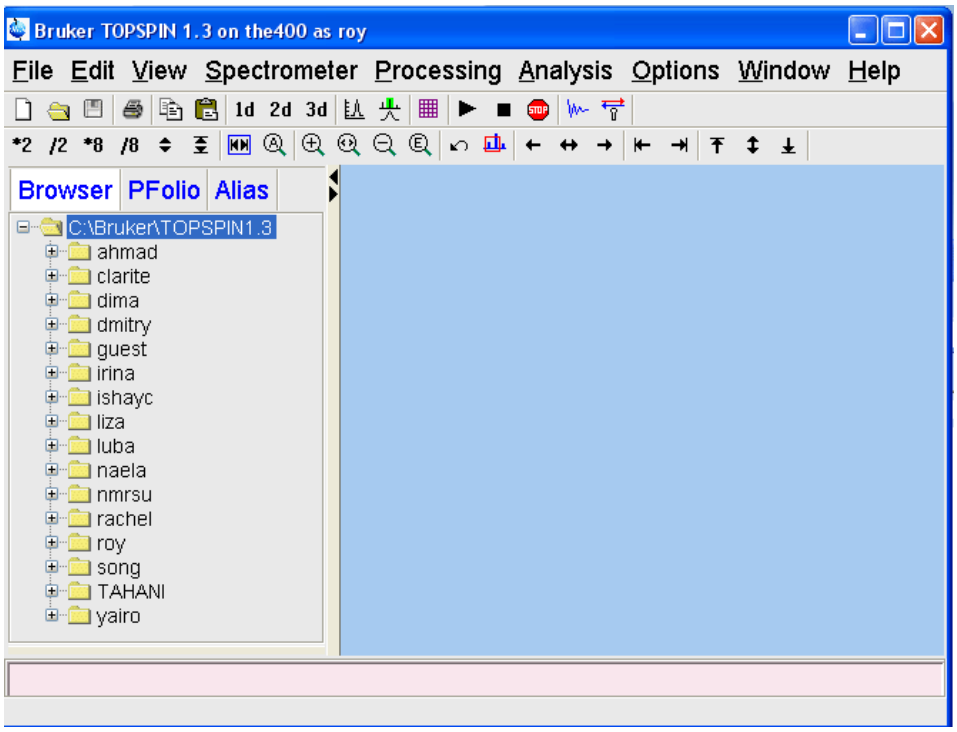


Run the Topspin 1.3 (Topspin 2.1 on the 500 MHz spectrometer) program by double clicking on the symbol or start>All Programs>Bruker>TOPSPIN>TOPSPIN1.3(2.1)>TOPSPIN1.3(2.1)

b. Creating a work file

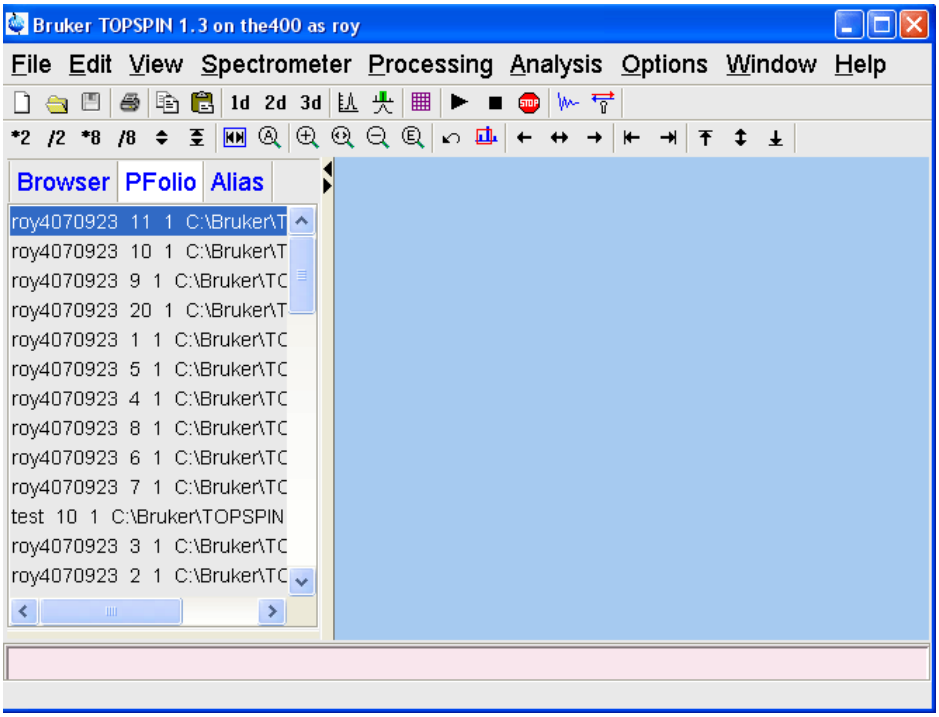
The program will appear as in fig. 8. Below the title are menus. The next rows is a toolbar for file management, editing, viewing options and acquisition commands. In the last row there is a toolbar for controlling spectral appearance. On the left hand side there is a list of files and on the right the spectrum will be displayed. The browser can be used to open existing files (fig. 8).

Figure 8. The file browser



Alternatively you can click on PFolio (Last50 in Topspin 2.0) to see recent files (fig. 9).

Figure 9. File selection from recent files

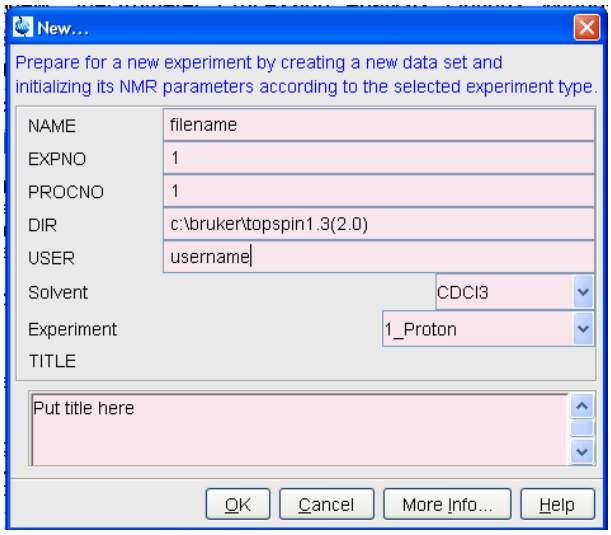


To create a new file, enter *edc* (fig. 10) and enter the parameters: experiment name in the field **NAME**, experiment number in **EXPNO** should be *1* (or a higher number if there are already experiments under that name), processing number in **PROCNO**

should be *1*, directory in **DIR** should be *c:\bruker\topspin1.3* or *c:\bruker\topspin* , username in **USER**, name of the solvent in solvent, *1_Proton* (for proton NMR) in **Experiment** 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 paramters* in the **Experiment** field.

On the 500 MHz spectrometer there is an extra field in the window **Experimental Dirs.** Choose the directory *C:/Bruker/TOPSPIN/exp/stan/nmr/par/user.*

Figure 10. Creating a new file with *edc*



c. Specifying the probe

The probe that is in the magnet must be specified by entering *edhead* (fig. 11) and choosing the relevant probe. Two probes are usually used on the 400 MHz spectrometer. (See below regarding the probes for the 200 and 500 MHz spectrometers.)

The connectors under the magnet of the probe 5 mm Multinuclear Z3918/086 [09] that is known as BBO look like this. It is used for carbon phosphorus and other nuclei but can be used for proton NMR although about a third of the sensitivity is lost compared with the BBI.

The connectors under the magnet of the probe 5 mm Multinuclear inverse Z-grad Z8202/0051 [11] that is known as BBI look like this. It is used for proton, fluorine, most 2D-NMR and diffusion.

If the probe is not one of these two then see chapter 5.

On the 200 MHz spectrometer there are three probes. For the routine purposes of measuring proton NMR, the 5 mm Multinuclear inverse Z03221/0022 [10] known as BBI is used.

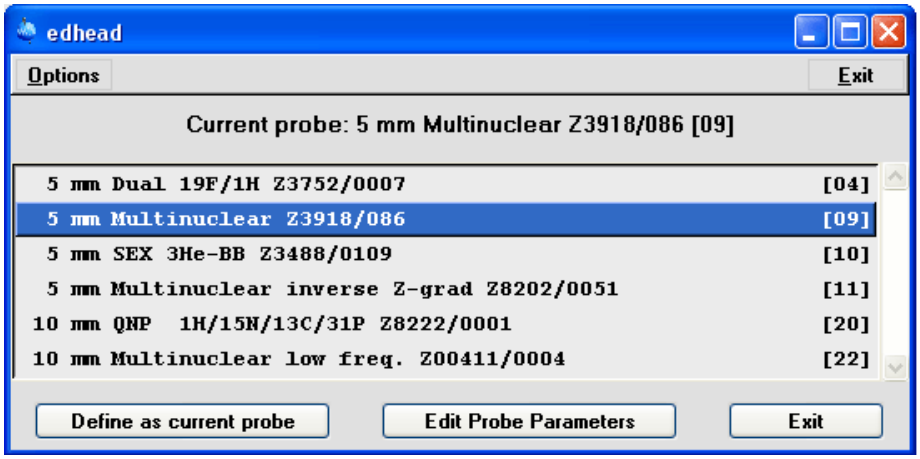
If the probe is not the BBI then see chapter 5.

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 5.

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

Figure 11. Window for specifying the probe



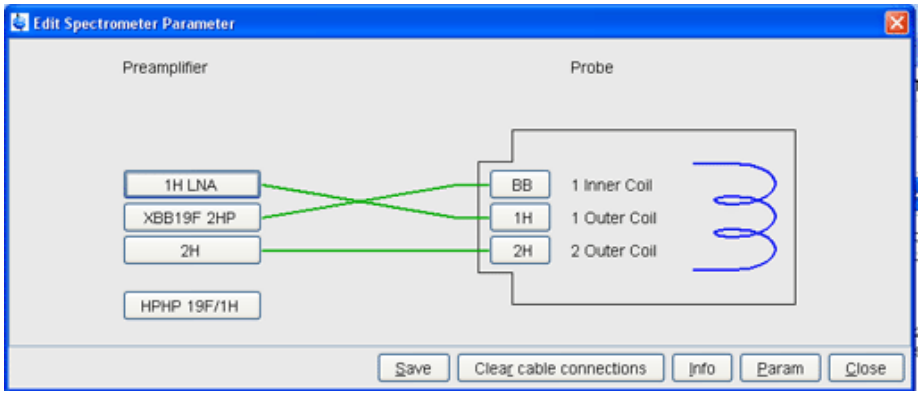
Read the shimming parameters by entering *rsh bbi* for the BBI probe or *rsh bbo* for the BBO probe.

If using the BBO probe on the 400 MHz spectrometer for proton NMR change the pulse width by entering *p1 9*. (The pulse width for the BBI is 5.6.)

If using the BBO probe on the 500 MHz spectrometer for proton NMR change the pulse width by entering *p1 11*. (The pulse width for the BBI is 8.5.)

After clicking on **Exit** another window will appear. The connections should appear as in fig. 12. Click on **Save**.

Figure 12. The connection window for the 500 MHz spectrometer (except of ^{19}F measurement and the CP-MAS probe)



If you need a quantitative spectrum see chapter 15 or if you want to optimize the sensitivity see chapter 12.

d. Inserting the sample

The NMR tube is inserted into a spinner. The spinner is then inserted into a sample gauge (fig. 13) 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 (fig. 14). If the sample depth is less than 4 cm then the center of the solution should be placed at the coil center (fig. 15).

Figure 13. Sample depth measurement

Figure 14. Sample position with enough sample depth

Figure 15. Placement of a short sample

One can use the control panel (fig. 16 and for the 500 MHz spectrometer fig. 17) or the *bsmsdisp* window (fig. 18) to insert the sample and for other actions mentioned later such as locking and shimming.

If using the control panel, air is passed through the magnet by pressing on **LIFT ON/OFF**. 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 16. The control panel for sample handling and magnet control as seen from above

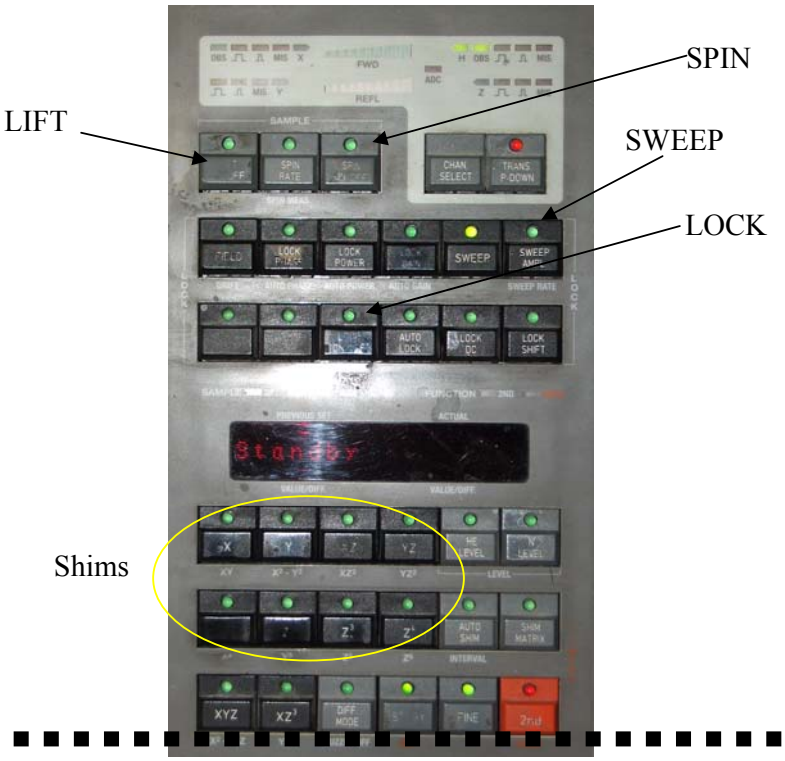
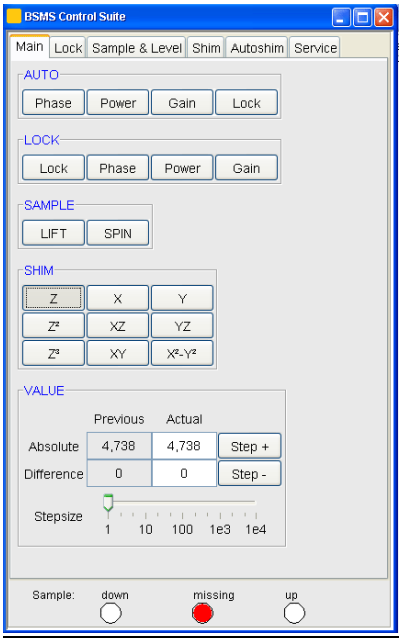


Figure 17. The control panel of the 500 MHz spectrometer for sample handling and magnet control as seen from above

If using *bsmsdisp*, enter *bsmsdisp* or, on the 500 MHz spectrometer, click on  in order to open the window (fig. 18). 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 air flow (see temperature control in chapter 7) eject and reinsert the sample and increase the air flow to what it was after insertion is confirmed.

Figure 18. The *bsmsdisp* window for sample and magnet control

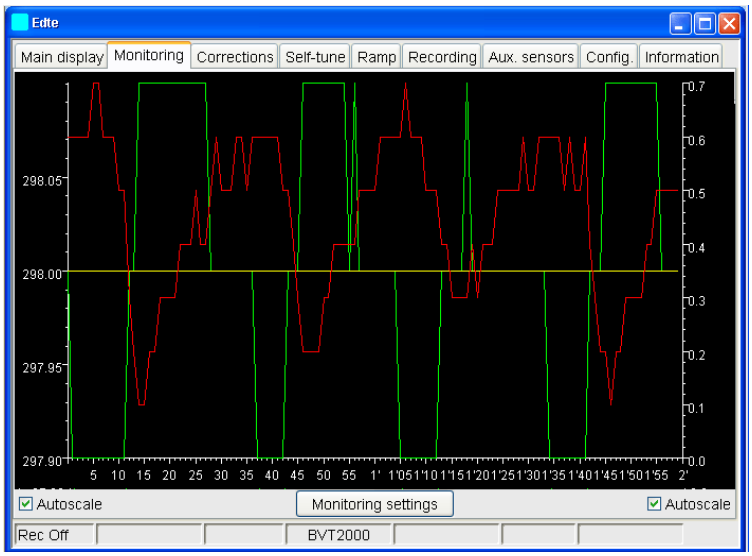


e. Check the temperature

Type *edte* and the temperature control window will appear. 298.0 K (24.85°C) is considered to be room temperature. The temperature can be monitored using the *Monitoring* tab (fig. 19).

On the 500 MHz spectrometer, the heater is shut off when leaving TOPSPIN and must be reactived by clicking on *Probe heater* under the *Main display* tab (see ch. 7 fig. 47). It may be that the temperature is displayed in Celsius. You can change it to Kelvin as described in ch. 7 figs. 48 and 49.

Figure 19. The *edte* window under the *Monitoring* tab for checking temperature stability



If the temperature is not correct or not stable to within 0.1 degrees then the parameters need to be adjusted – see ch. 7.

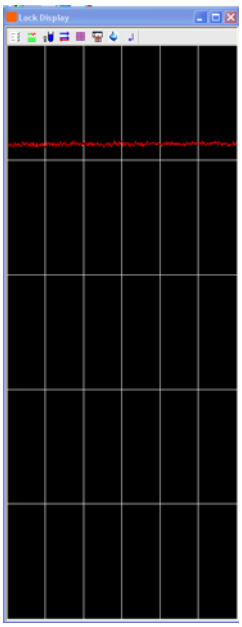
On the 200 MHz spectrometer the heater is usually left off for routine purposes. If you want to control the temperature see ch. 7.

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. 20).

Figure 20. 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 9.

If there is high dynamic range, improving the lock stability as descried in ch. 9a 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 for automatic tuning to finish. The computer will tell you when the process is complete.

On the other spectrometers enter *wobb* and a window will appear like in fig. 21. Likewise the preamplifier display next to the magnet will look like in fig. 22. If you do not see the minimum (dip) you can sweep the T (tuning) or click on  at the top of the wobb window and set a wider sweep width such as 20 MHz. It is recommended to return the range to 4 MHz after finding the minium.

Bring the bottom of the signal to the center (figs. 23 and 24) with the T (tuning) screw under the probe (fig. 25). Afterwards bring the signal down (figs. 26 and 27) with the M (matching) screw (fig.25). Continue tuning and matching until the probe is tuned (figs. 26 and 27).

Fig. 21. The wobb window showing the probe out of tune

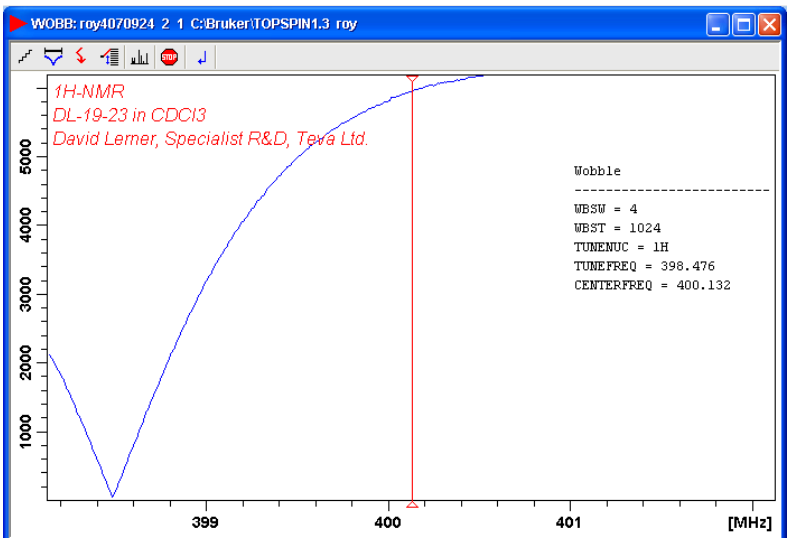


Fig. 22. The probe out of tune as seen on the display on top of the preamplifiers

Fig. 23. The wobb window showing that the probe needs matching

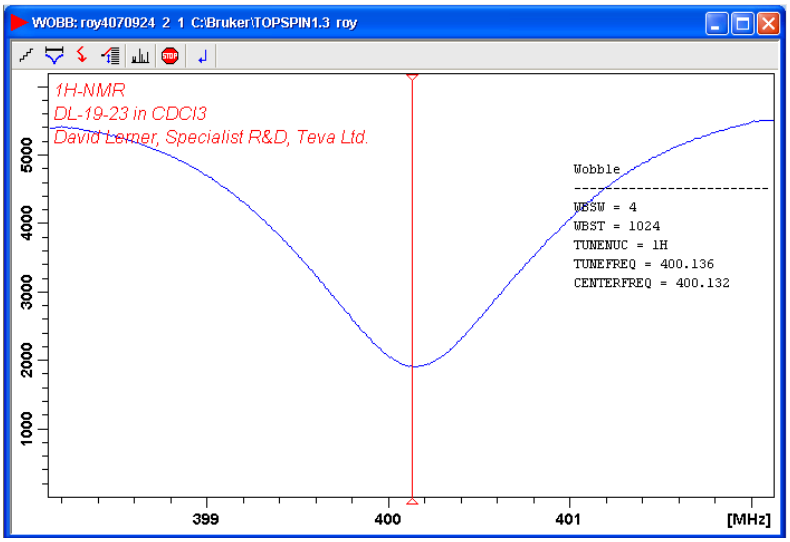


Fig. 24. The display on top of the preamplifiers showing that the probe needs matching

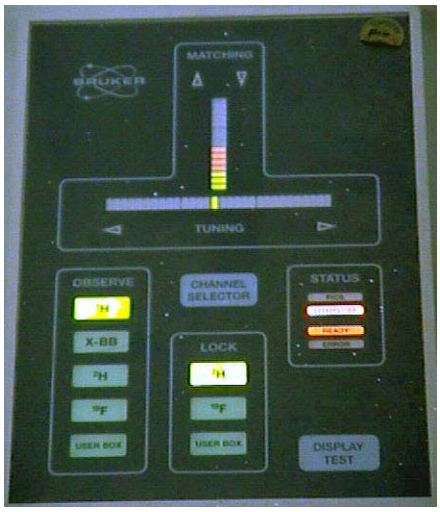


Fig. 25. The tuning and matching screws under the probe

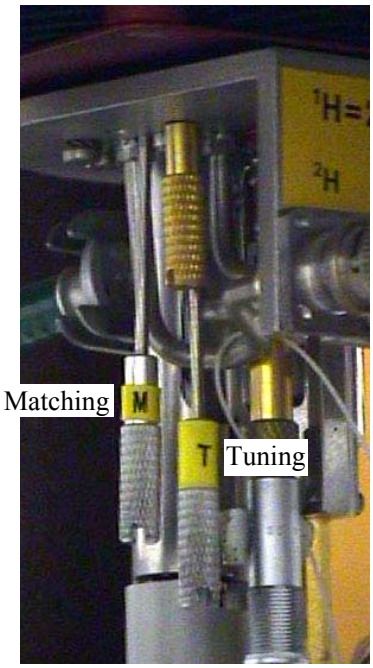


Fig. 26. The wobb window showing that the probe is tuned and matched

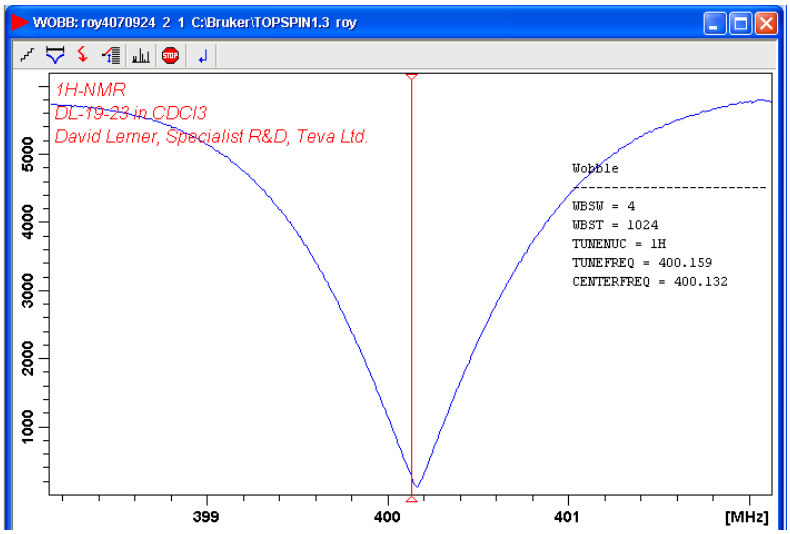


Fig. 27. The display on top of the preamplifiers showing that the probe is tuned and matched

h. Shimming

On the control panel (fig. 16) and the bsmsdisp window (fig. 18) 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*).

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 of the 500 MHz spectrometer (fig. 16) 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**, $\mathbf{X^2}$-$\mathbf{Y^2}$)

press the function then $\mathbf{Z^0}$. For mixed functions press both function components for example for XZ press **X** then $\mathbf{Z^1}$. To shim the 500 MHz spectrometer 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.

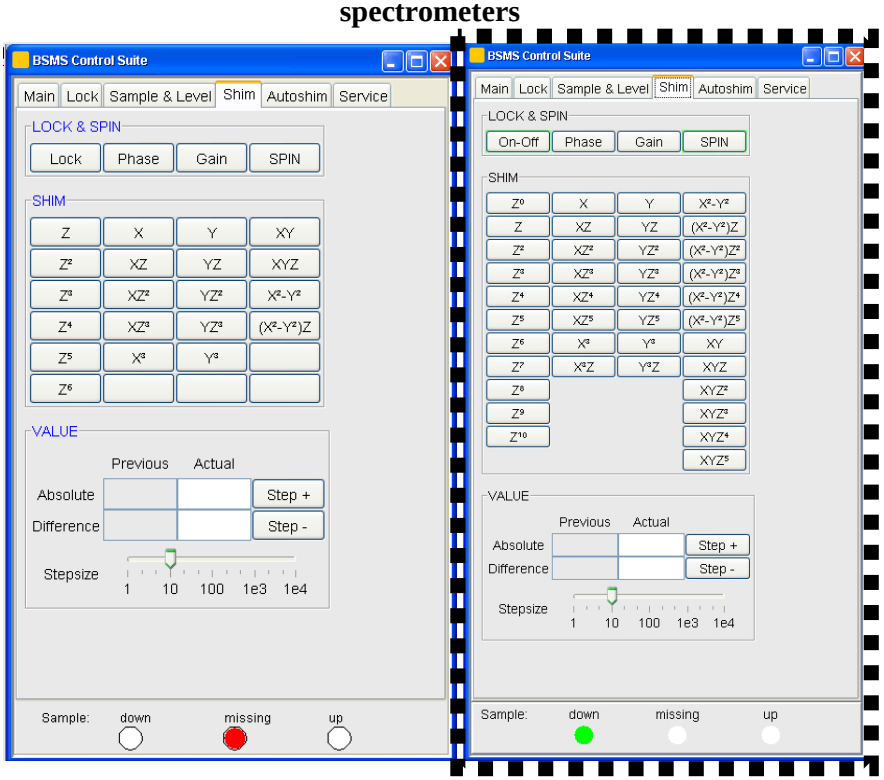
On the control panel the adjustment is carried out by turning the wheel. In the *bsmsdisp* window (fig. 18) under the **Main** tab in the **SHIM** frame there are buttons for the main shim functions. Choose the shim function that you want and change it by clicking on **Step +** and **Step –** in the **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.

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 $\mathbf{X^2}$-$\mathbf{Y^2}$. Spin the sample and 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 $\mathbf{Z^2}$.

If using *bsmsdisp* then you must go to the **Shim** tab (fig, 28) to change $\mathbf{XZ^2}$ and $\mathbf{YZ^2}$.

Figure 28. The *bsmsdisp* window with the *Shim* tab for adjusting XZ^2 and YZ^2; on the right is that for the 500 MHz spectrometer and of the left for the other spectrometers



Afterwards, acquire and phase the spectrum as explained below (see acquisition and phasing ch. 3i to 3k) and look at the signals (fig. 29). It is best to look at a singlet such as the solvent of TMS and correct as necessary.

Figure 29. 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.)

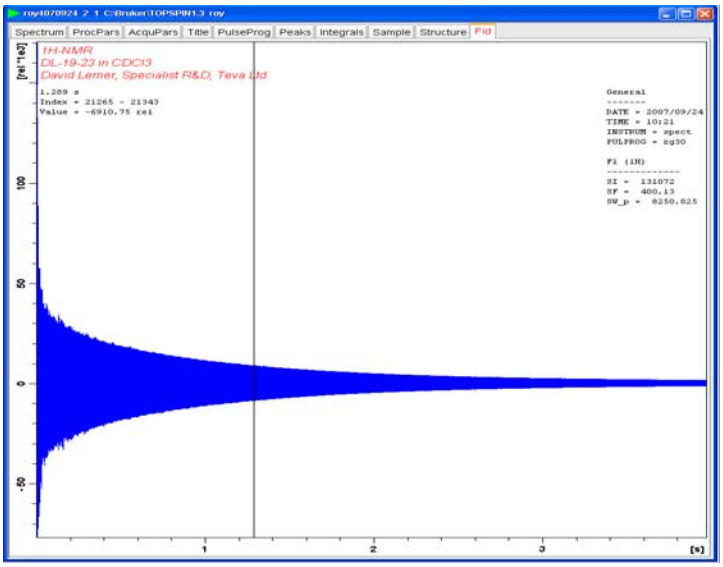
If you just copied a previous proton file (*e.g.*, by using *Use current parameters* in **Experiment** under the command *edc*) and did not read new parameters (by specifying *1_Proton* in **Experiment** under the command *edc* or by entering *rpar 1_Proton all*) then enter *ds 0* then *ns 1*.

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. 61), 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 adjustemenr, see ch. 10.

Fig. 30 shows the acquisition window.

Figure 30. 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. 31) that converts the acquired signal into the spectrum (fig. 32).

Figure 31. The Fourier transform

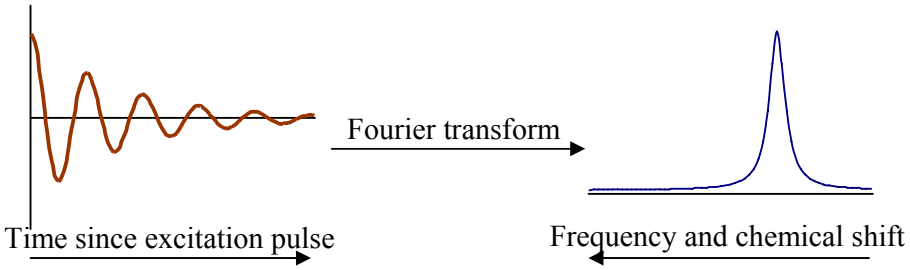
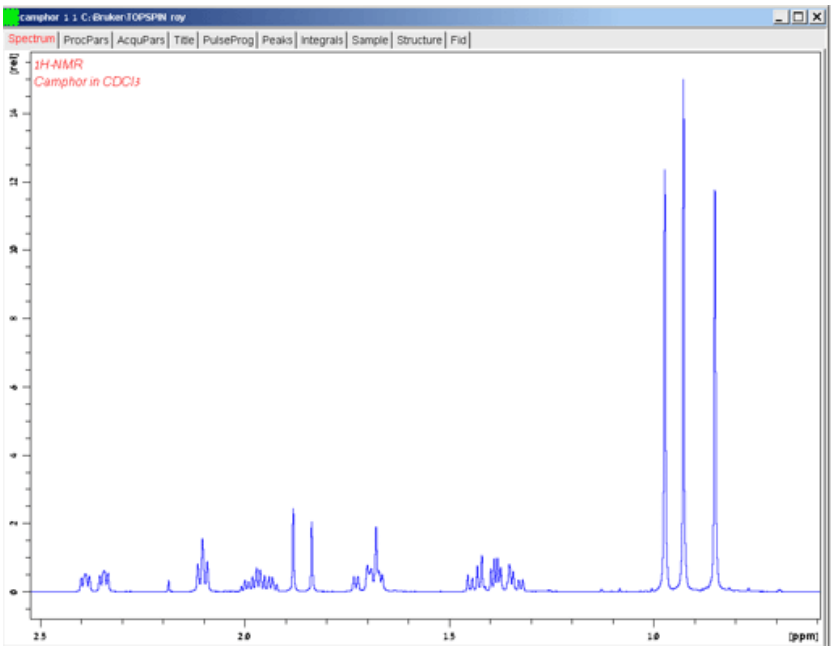
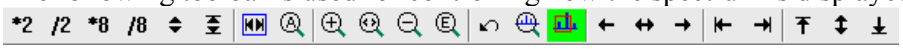


Figure 32. A spectrum



j. Control of the one-dimensional spectrum display

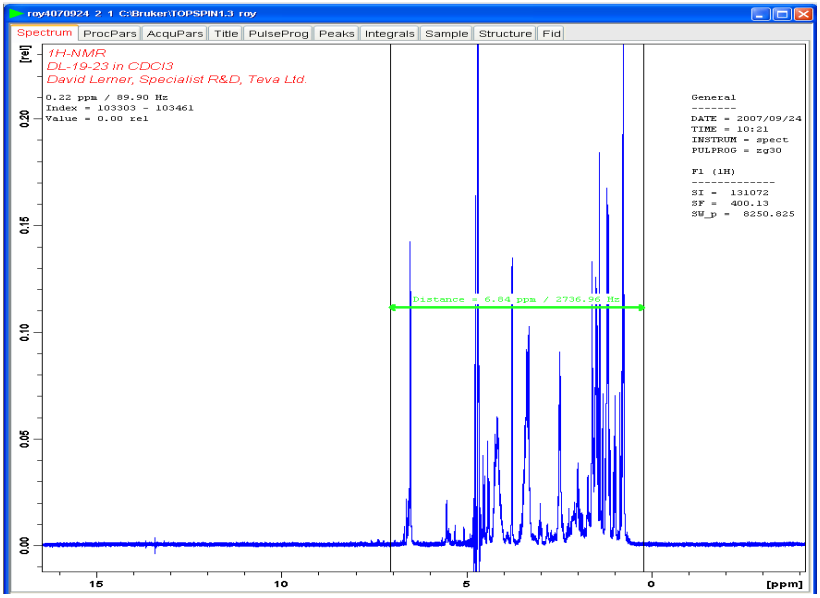
The following toolbar is used for controlling how the spectrum is displayed.



From left to right: double the height, half the height, increase the height 8 times, reduce the height 8 times, adjust the height interactively, display the full height, display the full spectral width, display the who spectrum, contract the width, adjust the width interactively, expand the width, define the region numerically, return to the previous expansion, expand, keep the same region when reading another spectrum, move half a screen left, adjust the horizontal position interactively, move half a screen right, display the left end of the spectrum, display the right end of the spectrum, raise the baseline to the center, adjust the vertical position interactively, lower the baseline to the bottom.

The spectrum may be expanded by dragging the mouse while left-clicked. Releasing the mouse key expands the spectrum (fig. 33).

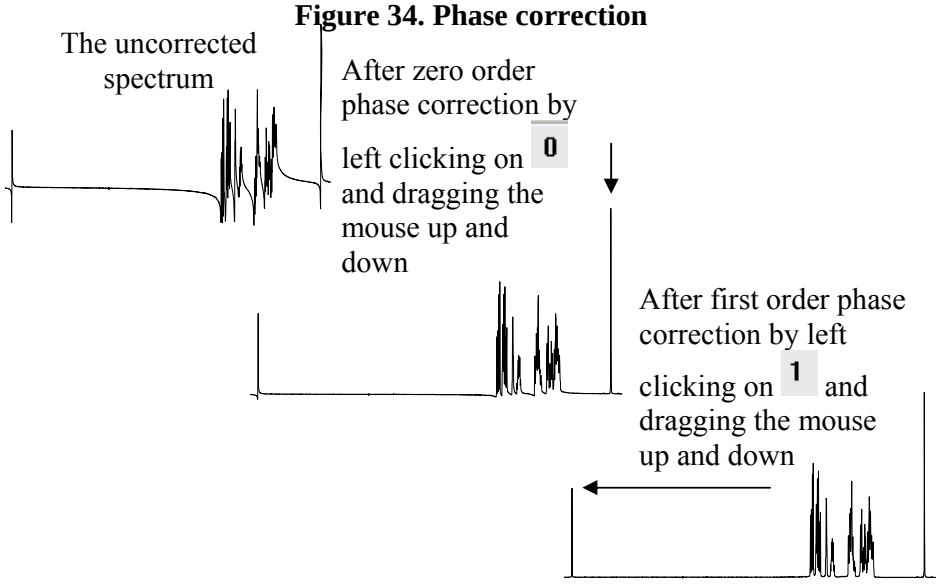
Figure 33. Selecting a region by dragging the mouse



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 0 and dragging the mouse up and down. First order phase correction (far from the red vertical line) is done by left clicking 1 and dragging the mouse up and down. When the phase correction is complete click on to save (or on to cancel) the correction (fig. 34).

Figure 34. Phase correction



l. Final acquisition

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. 12).

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. 11. If there is still not enough sensitivity then other parameters may be adjusted – see ch. 12.

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**. 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).

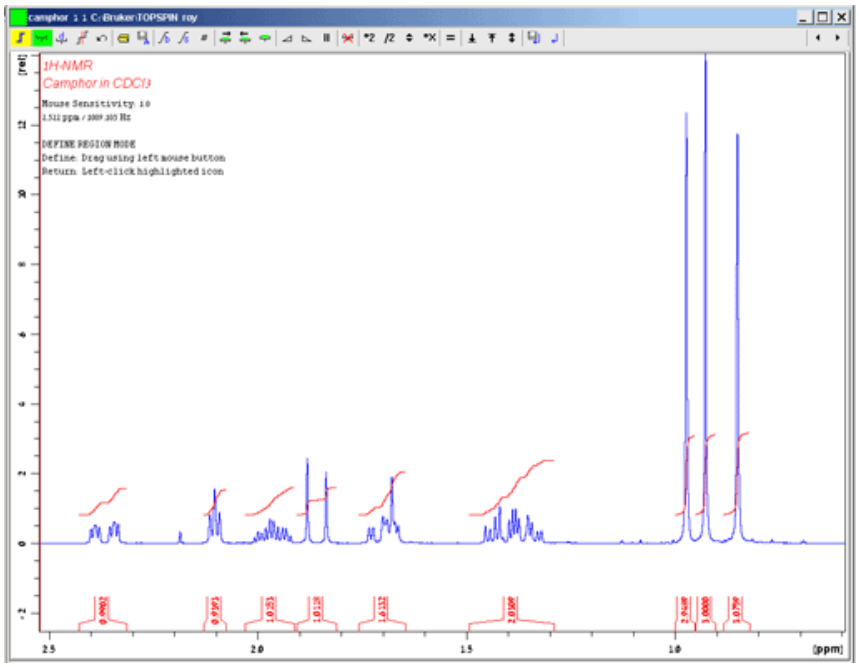
The most common chemical shift references at room temperature are: TMS 0, $CHCl_3$ 7.261, DMSO-d_5 2.504, HOD 4.81 and CD_2HOD 3.312. Other chemical shifts are given in table 2 ch. 14. See http://chem.ch.huji.ac.il/nmr/whatisnmr/chemshift.html.

Be careful not to confuse the reference signal with other overlapping signals. The solvent and TMS usually have especially sharp signals.

o. Integration

Enter *.int* or click on  . The integration window (fig. 35) will open.

Figure 35. 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. 35 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. 15.

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 menu **File > Print…[Ctrl P]**.

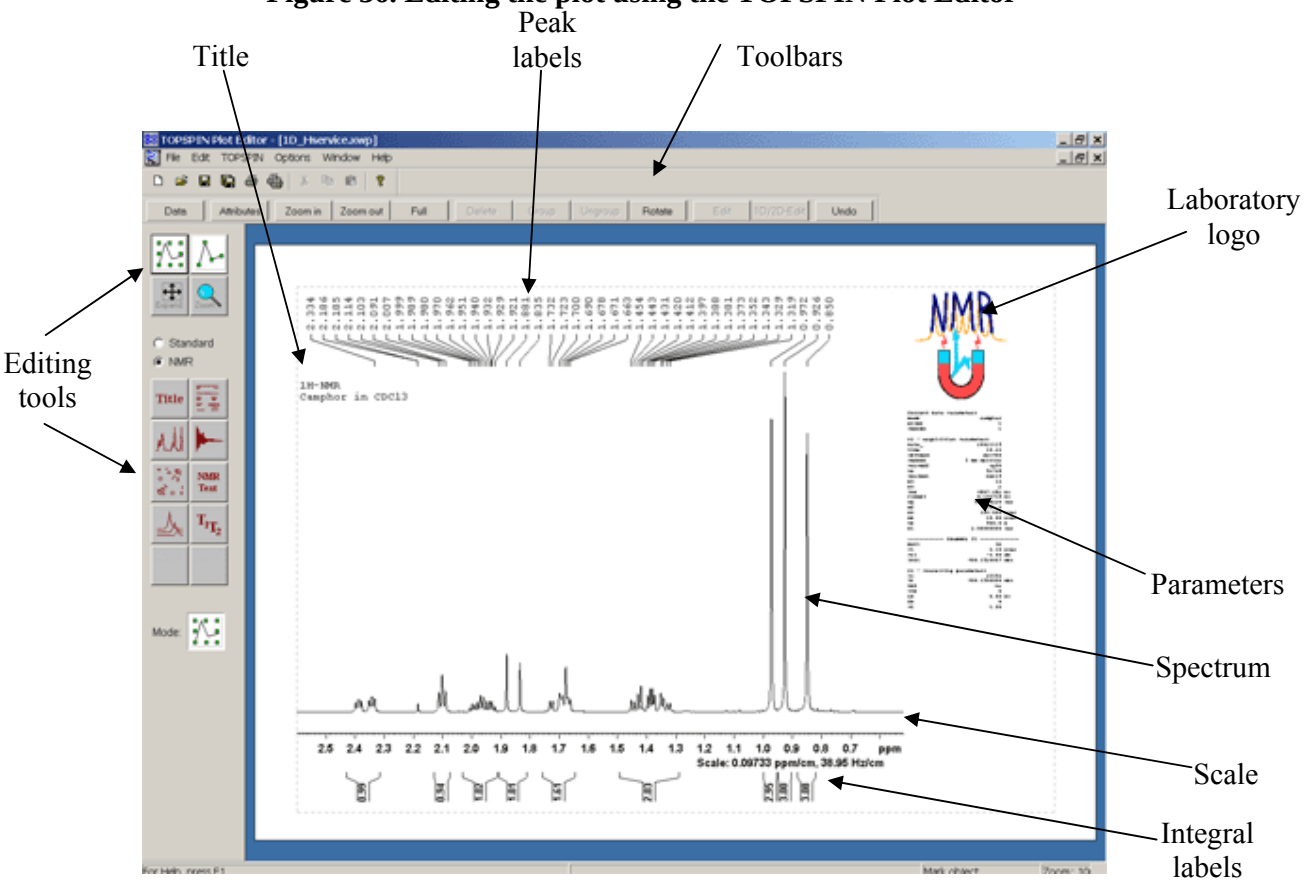
There are three print options:

1. **Print active window (prnt)** prints 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. **Print with layout – start plot editor (plot)** opens a plot editor (fig. 36) and you can change the plot appearance. Choose the *+/1D_H.xwp* **LAYOUT**. Click on **OK** to

open the editor (or **Cancel** to abort). This is the preferred option even though it takes more time.

Figure 36. Editing the plot using the TOPSPIN Plot Editor



The are many things that can be adjusted, the most important are as follows. Click on the spectrum region far from the title. Small green squares appear. Click on **1D/2D-Edit** in the upper toolbar. An extra window appears. Use it to move and expand the spectrum as necessary. Click on **Close** on that window when finished. Click on the printer symbol on the upper toolbar to print. Choose the default printer (for our 400 and 500 MHz spectrometers this is **Xerox Phaser 3117**).

(For the 200 MHz spectrometer the default printer is **HP LaserJet 5L**.)

You can return to **1D/2D-Edit** several times to print different regions of the spectrum. When finished close the window and answer **No** to the question **Save changes to 1D_H.xwp**.

3. **Plot with layout – plot directly (autoplot)**
Save changes to 1D_H.xwp. Choose the *+/1D_H.xwp* **LAYOUT**. Click on **OK** to open the editor (or **Cancel** to abort). This prints the region in the spectrum window at the height in that window.

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 email or fax or 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. If you have a fax installed on the computer (there is a fax in the 400 and 500 MHz spectrometers) you can send the 'printout' by fax. Open the file and print to fax. A fax window will appear. (The first time that you use the fax, a window will appear for you to enter your personal details.) Click on **Next>** then enter the name and phone number as dialed from the university (there is no international line). Click on **Next>**, **Next>** and **Finish** to send it.

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**.

4. Use of the guide in other laboratories

In order to prepare the parameters for acquisition, read the parameters for **PROTON** (*rpar PROTON all*) change the parameters below and save under the name **1_Proton** (*wpar 1_Proton all*). Afterwards go into the new parameter directory and set all the files in it to read only.

PL1 to the minimum permitted -6, -3 or 0

P1 length of the 90° pulse in μs

CY 12.5

NS 1

DS 0

SI 64k

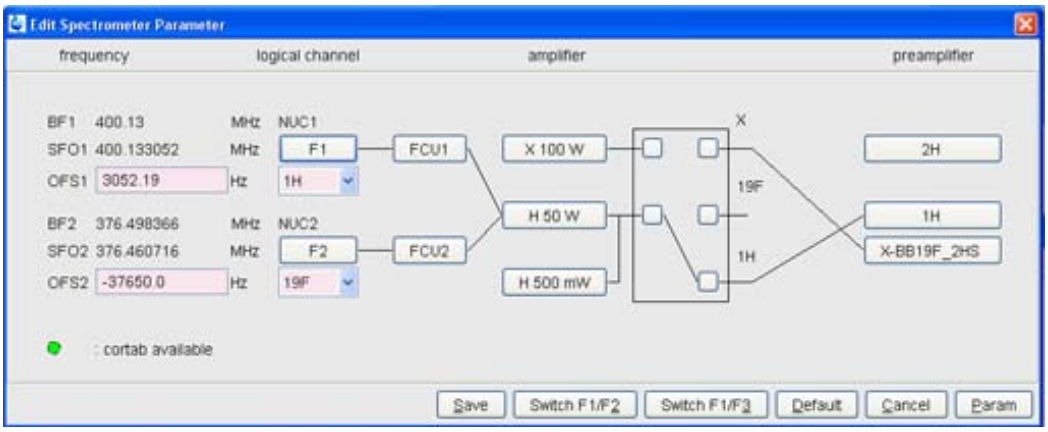
Prepare another file from this one, modify it as below and save it as **1c_Protonfdec**.

PL12 the fluorine pulse attenuation such that the 90° pulse is 100 μs

PL13 the fluorine pulse attenuation such that the 90° pulse is 100 μs

Enter *edasp* and change the parameters according to fig. 37.

Figure 37. The *edasp* setup for proton with fluorine decoupling



Copy the file 1D_H.xwp to 1D_Hold.xwp and cancel the readonly property from 1D_H.xwp. Go into the plot editor and change the file as you wish as explained in the book "Topspin plotting" from Bruker. Reinstate the readonly property on 1D_H.xwp.

Prepare the following macros:

zgft =

zg

ft

zgfp =

zg

fp

zgef =

zg

ef

zgefp =

zg

efp

5. Less common probes

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

The **5 mm Dual 19F/1H Z3752/0007 [04]** probe is used for measuring fluorine with proton decoupling and proton with fluorine decoupling. With this probe change *p1* to *6.9*.

The **5 mm SEX 3He-BB Z3488/0109 [10]** probe that was once the BBI probe of the 300 MHz spectrometer is now used only for measuring ^{3}He.

The **10 mm QNP 1H/15N/13C/31P Z8222/0001 [20]** probe is used for measuring ^{15}N, ^{13}C and ^{31}P in 10 mm tubes for samples that are too insoluble to measure in 5 mm tubes.

The **10 mm Multinuclear low freq. Z00411/0004 [22]** probe is used for measuring low frequency nuclei such as ^{57}Fe without lock.

The **5 mm Dual 13C/1H Z3225/0347** probe is used for carbon spectra but can be used for proton NMR with half the sensitivity of te BBI probe. If using the dual probe on the 200 MHz spectrometer change the proton pulse *p1* to *10.4*. See ch. 3c.

The **10 mm Multinuclear Z01400/992** probe is used only for other nuclei such as phosphorus. It is not recommended to use the BBO probe of the 200 MHz spectrometer for proton but if using it set *p1* to *20*. See ch. 3c.

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

6. Measuring the pulse width

Acquire a regular spectrum and correct the phase.

Enter *pulprog zg*.

Set *p1* to close to the 360° pulse: *20* for the BBI and *34* for the BBO.

On the 200 MHz spectrometer the 360° pulse for the BBI is close to *20*, dual *39* and BBO *90*.

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 $(p_{360°} - 0.8) \times \varphi / 360 + 0.8$

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

On the 500 MHz spectrometer the value of *p1* for a φ° pulse is

($p_{360°}$ - 0.16) × φ / 360 + 0.16

For a regular pulse of 90°: *p1* = $p_{360°}$/ 4 + 0.12

For a fluorine decoupled proton spectrum you can calibrate the fluorine decoupling pulses. Open a file for measuring fluorine as described in the guide "Measuring NMR spectra of carbon and other non-proton nuclei" ch. 1 and calibrate the decoupler pulse as described there in ch. 6.

7. Temperature control and stabilization

On the temperature unit that is in the console on the right hand side at the top, check that there is an airflow of about 270 L/h and that the **HEATER** is on (fig. 38).

Figure 38. The temperature unit on the 400 MHz spectrometer

On the 500 MHz spectrometer all the controls are in the software so there is no need to physically touch the temperature unit.

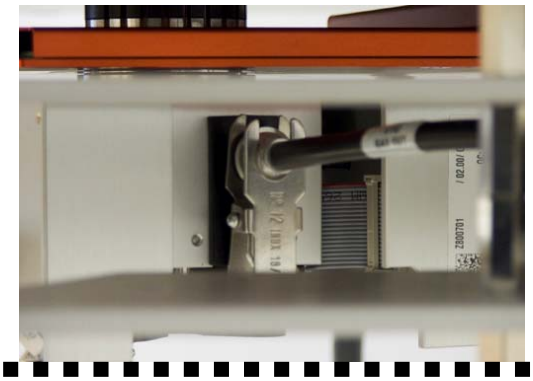
Use a ceramic spinner for temperatures over 310 K but do not use a ceramic spinner for cooling. The ceramic spinner is fragile and expensive. Do not drop it.

In the *edte* window under the *Main display* tab (fig. 46) 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 room temperature and above, air is passed to the probe from under the magnet via a black pipe shown in fig. 39 and for the 500 MHz spectrometer shown in fig. 40.

Figure 39. Air connection (black tube) for room temperature and above

Figure 40. Air connection for the 500 MHz spectrometer (black tube) for room temperature and above



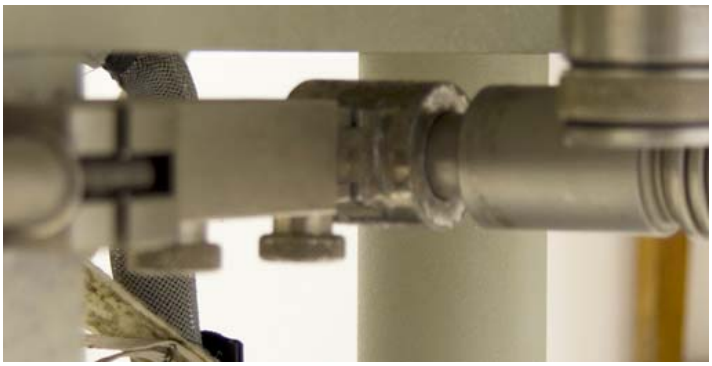
In order to cool or to, stabilize a temperature close to that of the room better than normally required, use the cooling unit. Do not use it without special permission.

To cool, fill the Dewar with liquid nitrogen, insert the transfer tube with its O-ring and close it tightly. Turn of the **HEATER** and release the air hose clip (fig. 41).

Figure 41. Opening the air hose clip

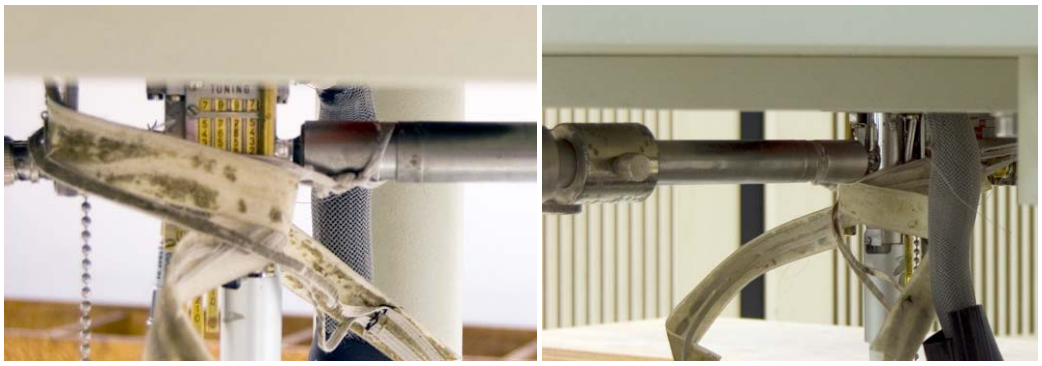
Position the cooling pipe against the probe opening using the clamp attached to the magnet leg (fig. 42) and check that it is in exactly the right place. The air opening is very fragile so the pipe has to be positioned accurately.

Figure 42. Clamp for the cooling pipe under the magnet



Connect the pipe to the probe and attach the straps (fig. 43).

Figure 43. Connection of the cooling pipe to the probe



Press the **LN_2** button on the temperature unit then turn off the air flow. Adjust the nitrogen flow using the buttons either side of the **LN_2** button according to fig. 47.

When you finish working with cooling stop the cooling (pres the **LN_2** button) then turn the air flow on.

Before touching the cooling pipe heat the joint between the pipe and the probe with a hairdryer until it is totally thawed. The connection is very fragile and trying to disconnect it without heating may break it. After the joint has thawed disconnect it gently and connect the air pipe.

On the 500 MHz spectrometer the cooling connect is different and is opened by pussig the plastic sheath (fig. 44) and connecting it to the probe (fig. 45). On finishing work with cooling disconnect the cooling pipe with heating from a hairdryer, connect the air pipe and start the air flow and heating.

Figure 44. Connection of the cooling pipe on the 500 MHz spectrometer

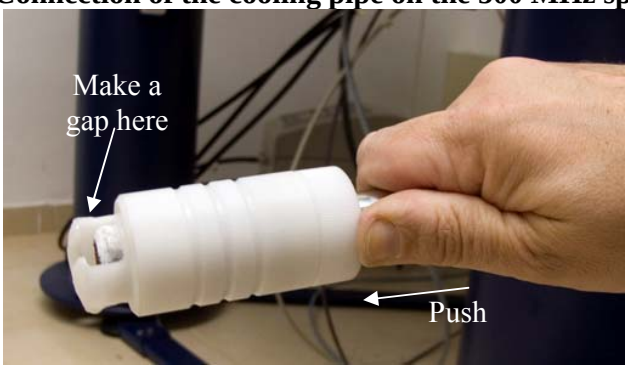


Figure 45. The cooling pipe connected to the probe of the 500 MHz spectrometer

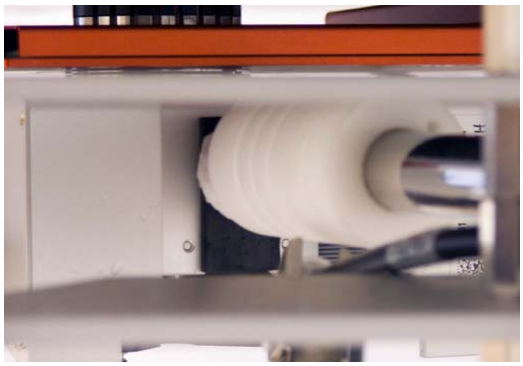


Figure 46. The *Main display* tab of the *edte* window for temperature control

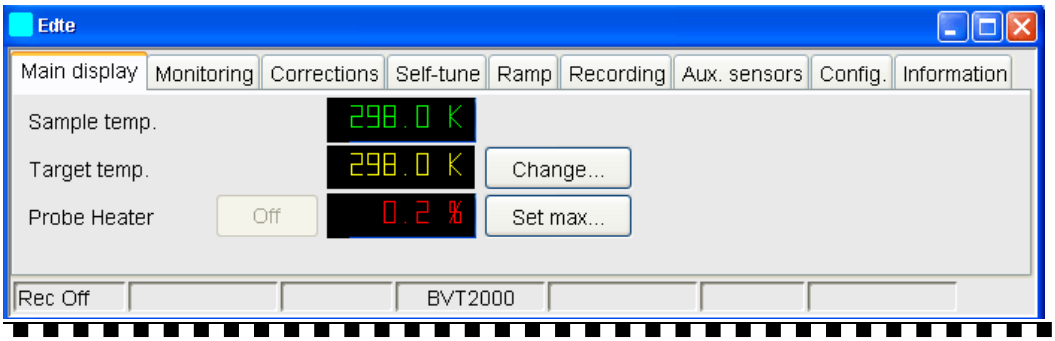
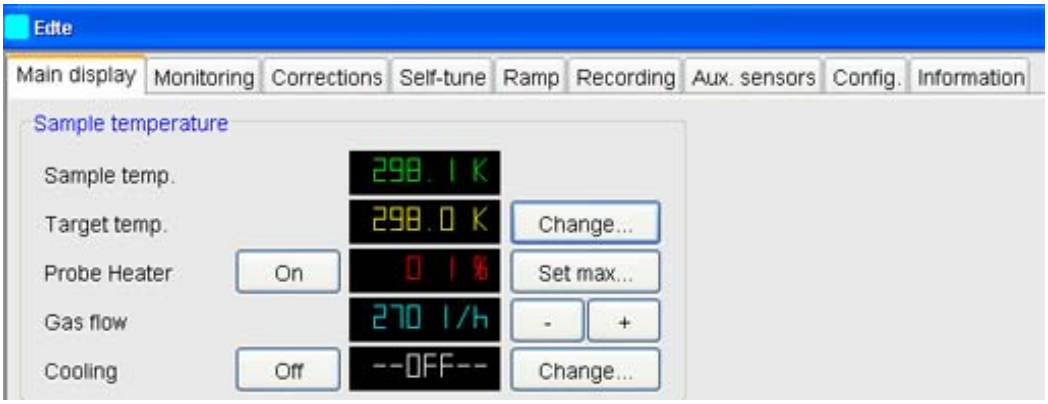


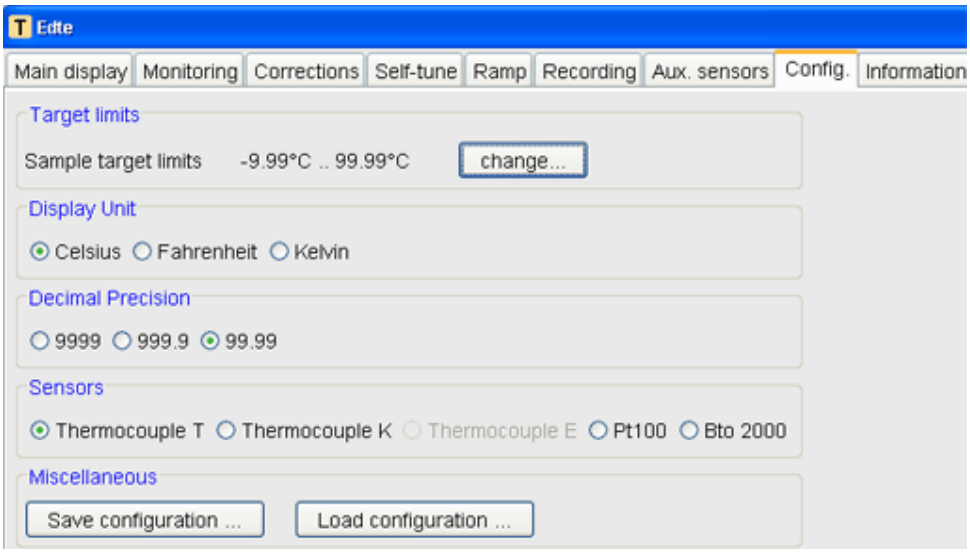
Figure 47. The *Main display* tab of the *edte* window for temperature control on the 500 MHz spectrometer



On the 500 MHz spectrometer the temperature unit is entirely controlled from the *edte* window (fig. 47). Check that the **Probe Heater** is **On** and that the **Gas flow** is *270 l/h*. To cool, fill the Dewar, connect it to the probe and set **Cooling** to **On** and **Gas flow** to *0* L/h.

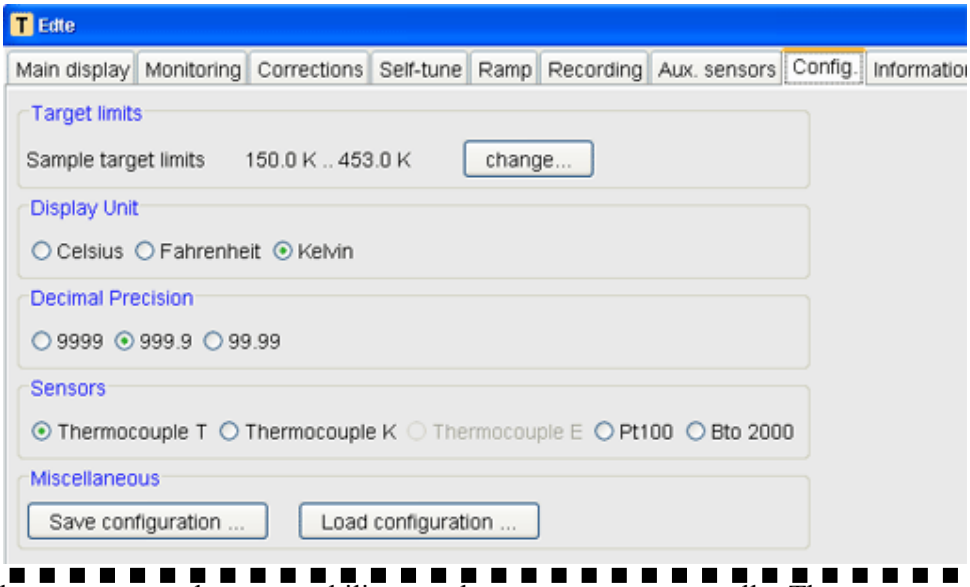
On the 500 MHz spectrometer it is possible to display the temperature in Celsius in the range -9.99°C to 99.99°C that allows an extra decimal place of precision. Under the **Config.** tab, change the **Display unit** to **Celsius** and the **Decimal Precision** to **99.99**. In the frame **Target limits** click on **change…** and select a minimum of ***-9.99*** and a maximum of *99.99*. The resulting window should look like in fig. 48.

Fig. 48 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 *453.15*. The resulting window should look like in fig. 49.

Fig. 49 The *Config.* tab of the *edte* window in Kelvin mode



If the temperature does not stabilize, set these parameters manually. They are correct for room temperature. For other temperatures see fig. 50.

Proportional Band: *60*

Integral Time: *72*

Derivative Time: *18*

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. 50 and then to reduce it. If the temperature

does not stabilize to ±0.1 K, use **self-tune** (fig. 51) 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 50. Parameters for temperature control on the 400 MHz spectrometer

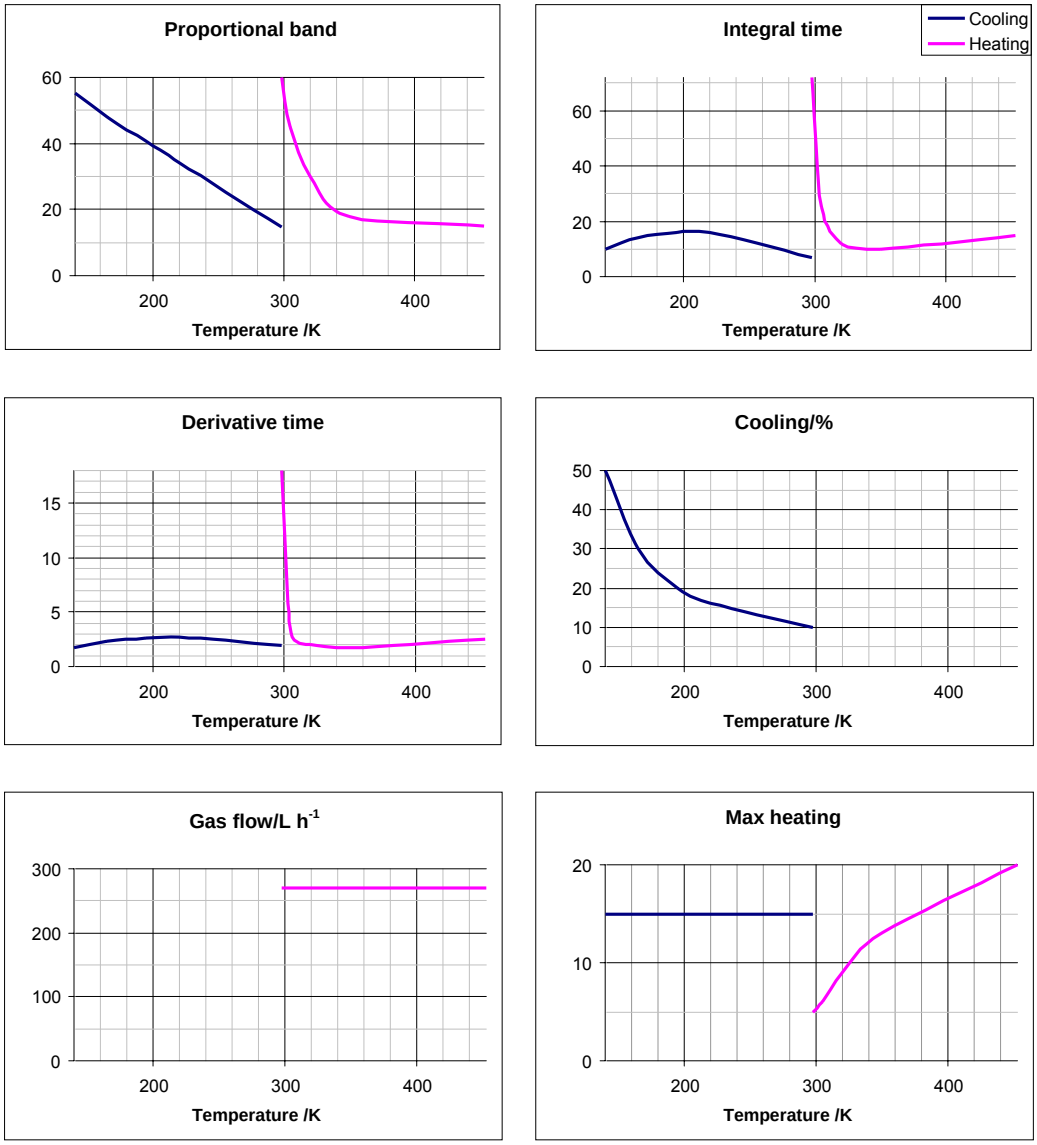
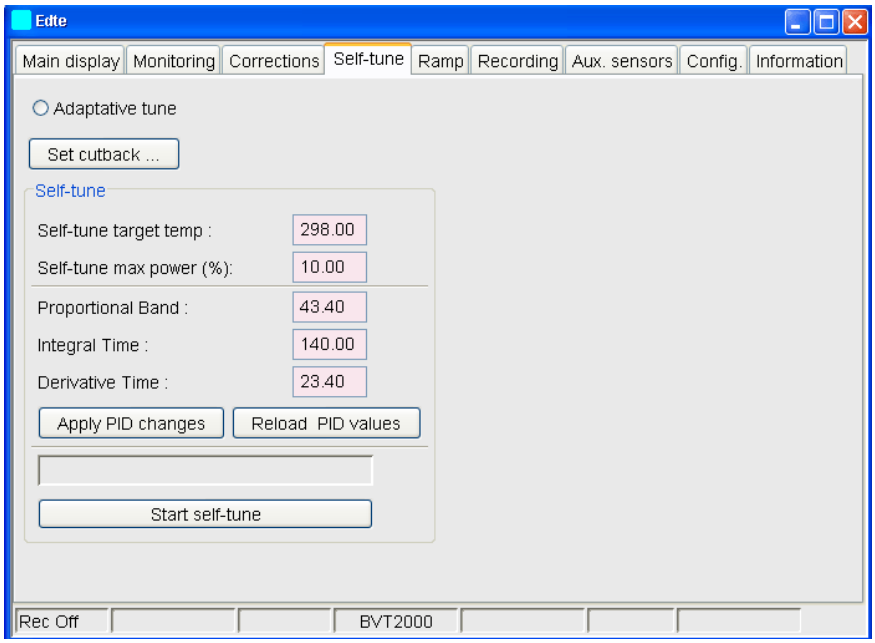


Figure 51. The *Self-tune* tab of the *edte* window for calibrating the temperature unit



On the 500 MHz spectrometer the parameters for room temperature are:

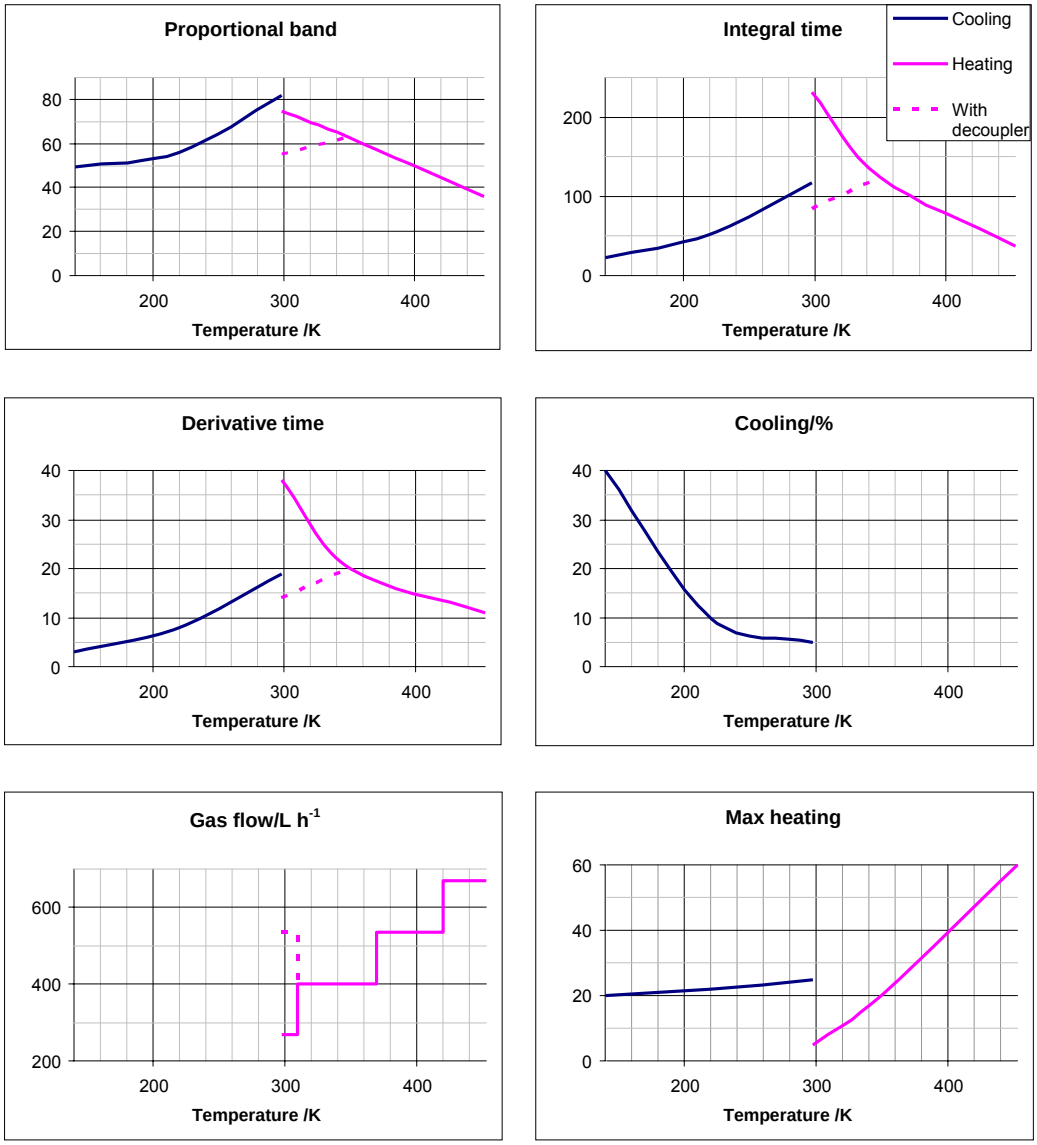
Proportional Band: *74.5*

Integral Time: *232*

Derivative Time: *38*

When cooling or heating use the values in fig. 52 (just like fig. 50 for the 400 MHz spectrometer.

Figure 52. Parameters for temperature control on the 500 MHz spectrometer



On the 200 MHz spectrometer the heater and the temperature controller are not used for routine purposes.

If despite this you want to control the temperature. Turn the heater on in the temperature controller (fig. 53) that is in middle of the console. The unit is accurate to one degree.

Figure 53. The temperature controller of the 200 MHz spectrometer

The software control is similar to that of the 400 MHz spectrometer with the following differences. Unter the *Main display* tab of *edte* there is control of the air flow. 650 L/h is recommended. The manual paramters for *Self-tune* are:

הפיקוד עליו בתוכנה דומה לזה של ספקטרומטר ה-400 מגהרץ עם השינויים האלה. בלשונית Main display של edte ניתן לפקח על מהירות זרימת האוויר. מומלץ 650 ליטר לשעה. המומלצים הם: הפרמטרים הידניים ל-self-tune

Proportional Band: *2*

Integral Time: *7*

Derivative Time: *1*

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. 8.

8. 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 for room temperature and below or glycol for above room temperature. You can also do the calibration after the acquisition.

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.

Read the methanol file by entering *re meoh*.

If the file does not exist an error will appear: **Data set does not exist** and you will need to create it using *edc*. In the menu that appears (fig. 10) set the **NAME** to *meoh*, **EXPNO** to *1*, **PROCNO** to *1* and **Experiment** to *1_Proton*. Click on **SAVE** and the window will disappear. Enter *p1 60* then *rg 64*. The file is then ready.

Off-tune the probe by three turns of the **TUNING** screw (**T**, fig. 24). It is a good idea to remember the direction that you turned the screw so that you can correct it later.

On the 500 MHz spectrometer 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**.

Acquire the NMR spectrum of methanol or glycol by entering *zgfp*. Correct the phase as described in ch. 3k. Measure the chemical shift difference ($\Delta\delta$) between the two main peaks (fig. 54). The measure of temperature stability is that the result of repeated acquisitions is the same to within 0.001 ppm. So 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. 7).

Calculate the temperature using the relevant equation like the one for methanol in fig. 54 or use the program (fig. 55): **Start > NMRThermometer > NMRThermometer**.

$$T(\text{MeOH})/\text{K} = 409 - 36.54\Delta\delta - 21.85(\Delta\delta)^2$$

$$T(\text{Glycol})/\text{K} = 465.77 - 100.91\Delta\delta - 0.39(\Delta\delta)^2$$

Figure 54. Temperature calibration spectrum

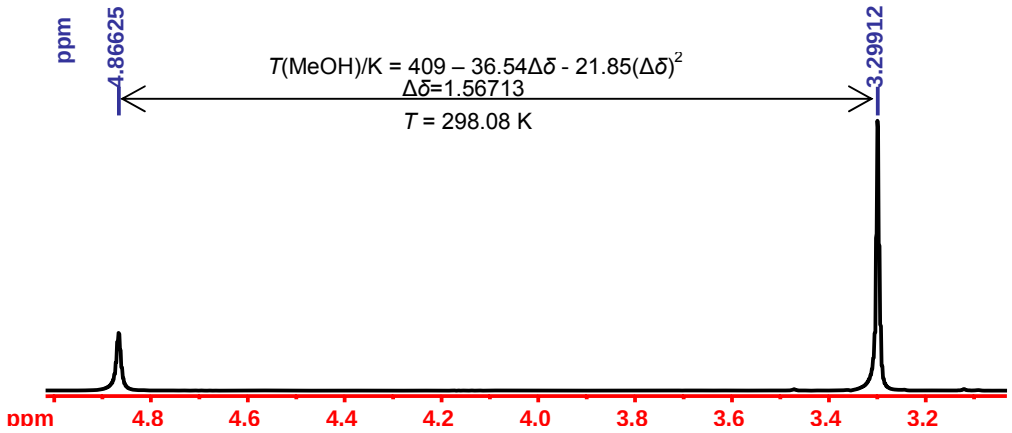
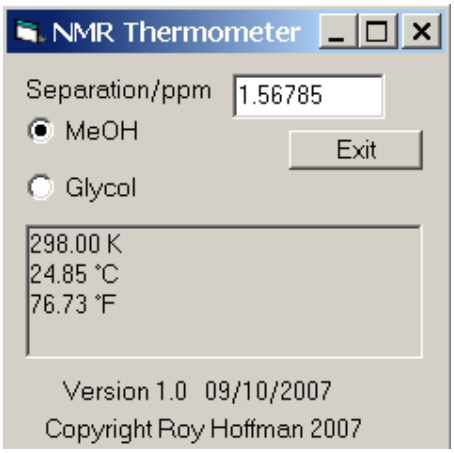


Figure 55. Program for calculating the temperature



9. 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

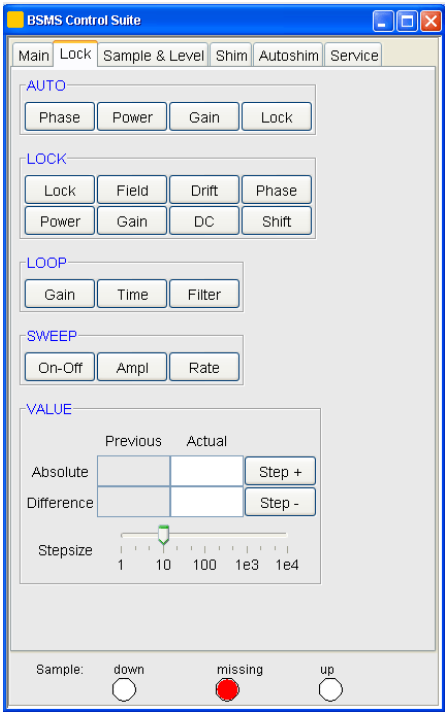
High dynamic range spectra are especially sensitive to lock stability. The lock stability may be improved by adjusting the lock parameters. If the lock goes up and down in a wavy manner, reduce the **LOCK POWER** by 6 dB. If the lock is stable, note the lock level, increase the **LOCK POWER** by 6 dB, 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. 16, 7) 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. 18) open the **Lock** tab (fig. 56). 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 56. 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 $CDCl_3$ on the 400 MHz spectrometer the field is about 2750 and on the 500 MHz spectrometer it is about 1000.

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. This is usually about 280 on the 400MHz spectrometer and 50 on the 500 MHz spectrometer. 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.

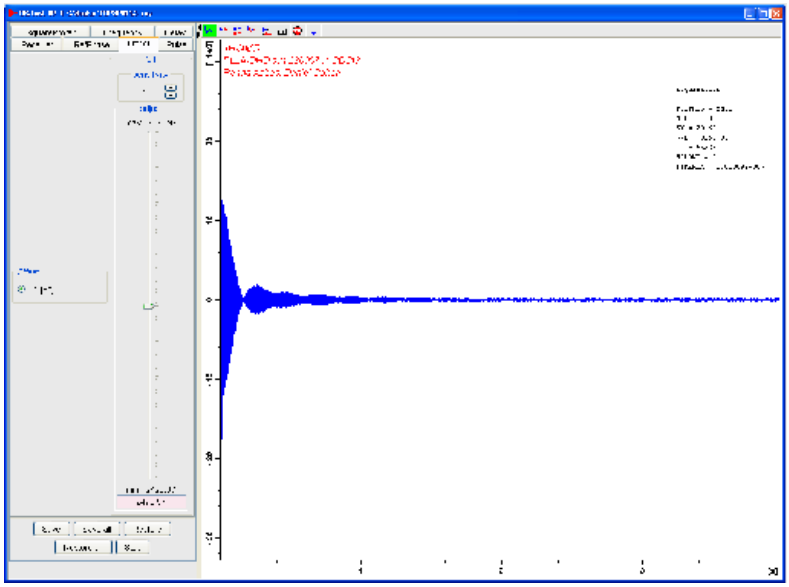
d. Acquisition without lock

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 this is not possible but at least 2% of solvent can be added without disturbing the experiment and there is a BB channel on the probe, then tune the BB channel to deuterium using the sliders as explained in the guide "Measuring NMR spectra of carbon and other non-proton nuclei" and then connect the deuterium cable (2H) to the BB channel of the probe. (When finishing work please return the cables to their normal configuration: 2H to 2H and BB to BB. Tune the BB channel away from deuterium. This way, the next experiment can be run with normal locking.)

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*.

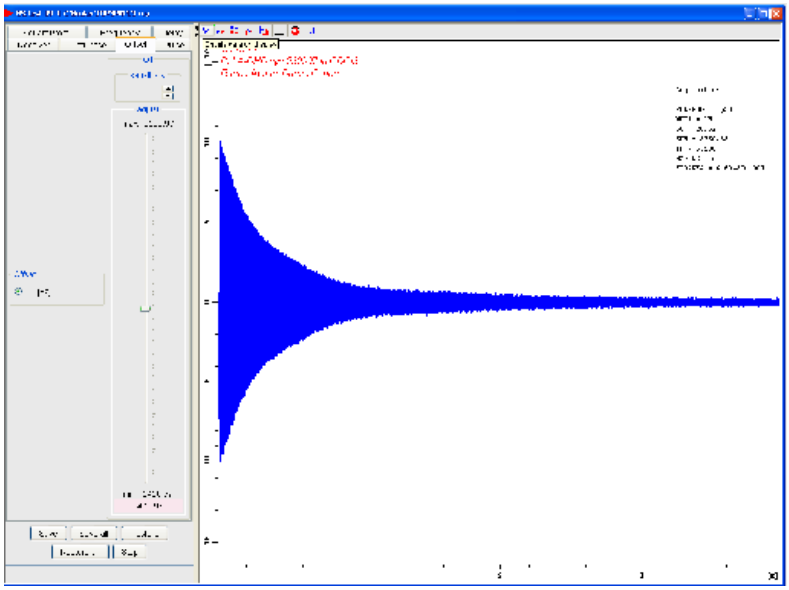
Tune the probe and do *rga*. Use *gs* to do the shimming. This is done interactively by observing the fid and spectrum. First acquire an initial spectrum and correct the phase then enter *gs* (fig. 57).

Figure 57. 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.58). If the spectrum contains a strong multiplet then ringing will appear in the fid rather than a monoexponential.

Figure 58. 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. 59 will appear. Change the **Window function:** to *none* and the **Phase correction mode:** to *pk*. The spectrum will appear like in fig. 60.

Figure 59. Parameter window for real-time spectrum display

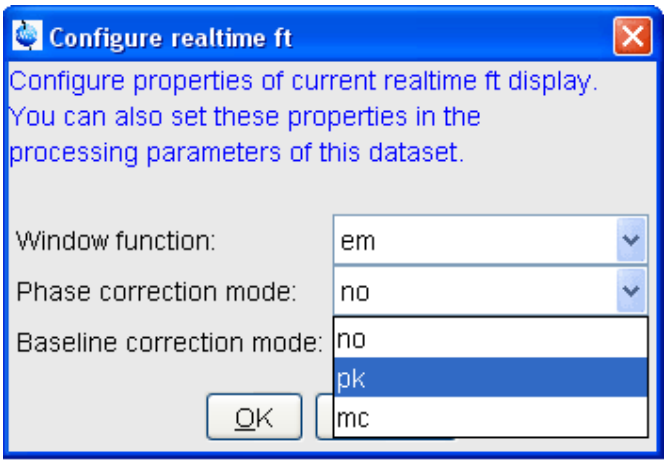


Figure 60. Real-time spectrum with poor homogeneity that requires shimming

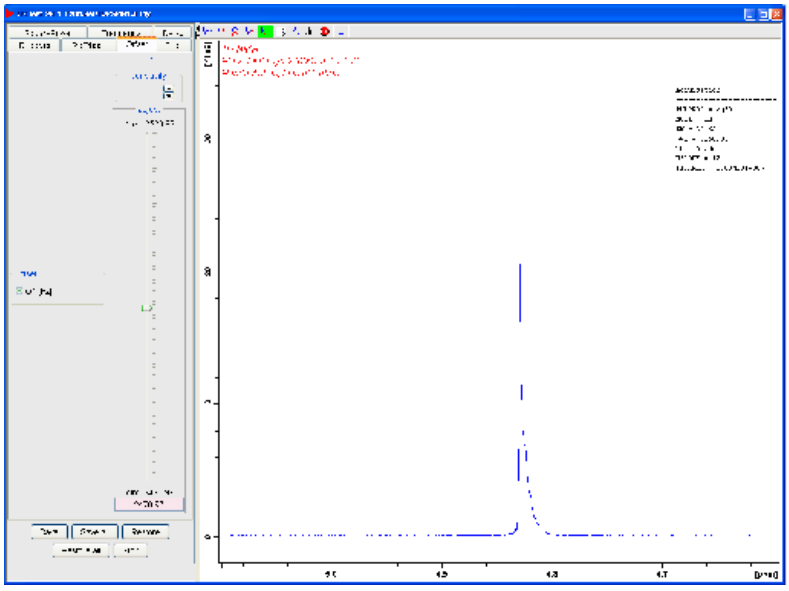
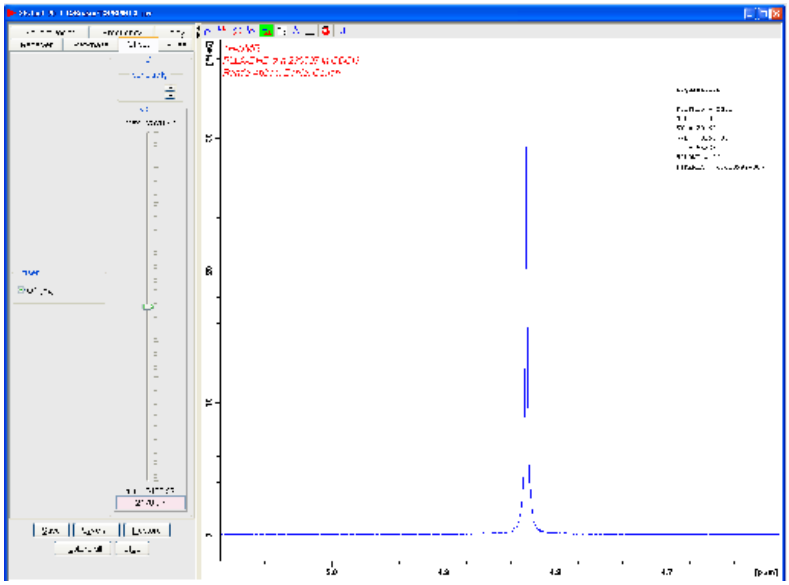


Figure 61. Real-time spectrum in a homogeneous field with good shimming



Correct the shape of the signal according to fig. 29 which in the case of fig. 60 means correcting $\mathbf{Z^2}$ such that the spectrum appears like in fig. 61. 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.

10. 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. 59). 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:

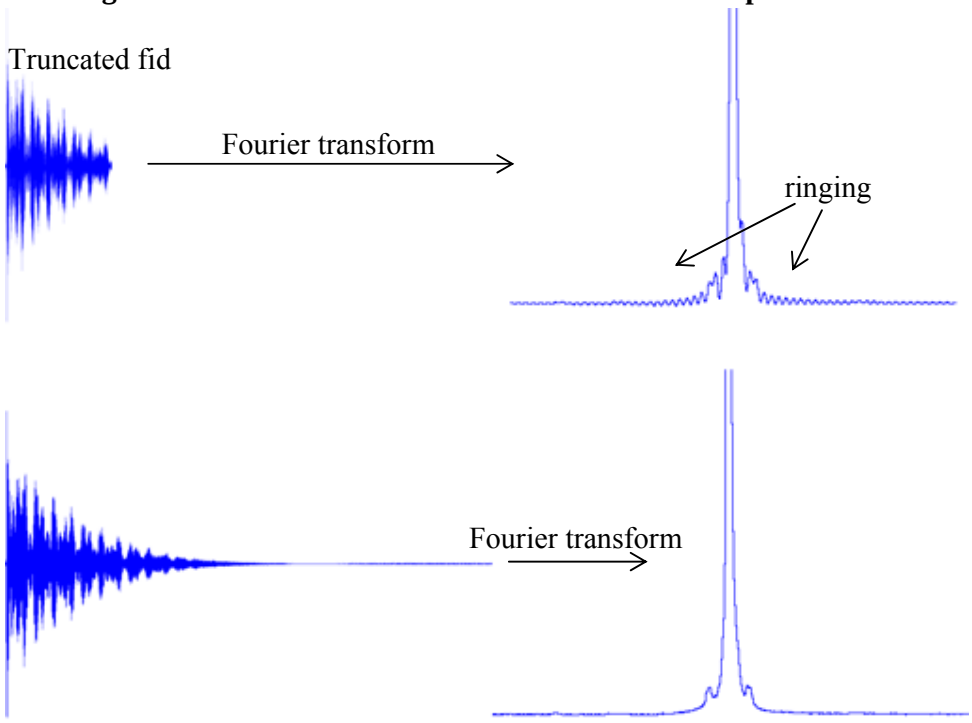
$$o1p = (\delta_{max} + \delta_{min}) / 2$$

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. 62) then you should increase *td* and *si*.

Figure 62. 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*.

11. 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

12. 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. 6) and the relaxation time (T_1) (see ch. 13) of the signals of a similar compound under similar conditions. Enter *pulprog zg*. Change *p1* so that the pulse width I 68° such that *p1* $=0.189 \times p_{360^\circ} + 0.65$ and set *d1* to $T_1 - aq$ or $1.44d7 - aq$.

On the 500 MHz spectrometer this is $p1 = 0.189 \times p_{360^\circ} + 0.13$

For example if *d7* = 4 s then T_1 = 5.76 s. If *aq* = 3.972 s then
d1 = (1.44 × 4 – 3.972) s = (5.76 – 3.972) s ≈ 1.79 s
If p_{360° = 20 then *p1* = 0.189 × 20 + 0.65 = 4.43

13. 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 $T_1 \ln 2$ (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 lesat 5 times T_1 (7.2 *d7*) between acquisitions.

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: $T_1 = d7/\ln 2 = 1.44d7$

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[$5T_{1max}$/(*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.

14. 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	$(CD_3)_2CO$	2.053
Acetonitrile-d_3	CD_3CN	1.940
Benzene-d_6	C_6D_6	7.16
Chloroform-*d*	$CDCl_3$	7.261
Deuterium chloride (1M) in D_2O	DCl	*5.17
Deuterium oxide	D_2O	*4.81
Dichloromethane-d_2	CD_2Cl_2	5.279
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	8.309, *10.241
Methanol-d_4	CD_3OD	3.312, *4.877
Nitrobenzene-d_5	$C_6D_5NO_2$	7.503, 7.671, 8.120
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, 6.97, 7.01, 7.10
Trifluoroacetic acid-*d*	CF_3COOD	*10.98

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

15. 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. 13).

Change *ds* and *ns* according to requirements and enter *pulprog zg*. Enter *aq* and note down the value. Change (usually increase) *d1* to $5T_1 - aq$ (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

$$d1 = (7.21 \times 4 - 3.972)\ \text{s} = (5 \times 5.76 - 3.972)\ \text{s} \approx 24.8\ \text{s}$$

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

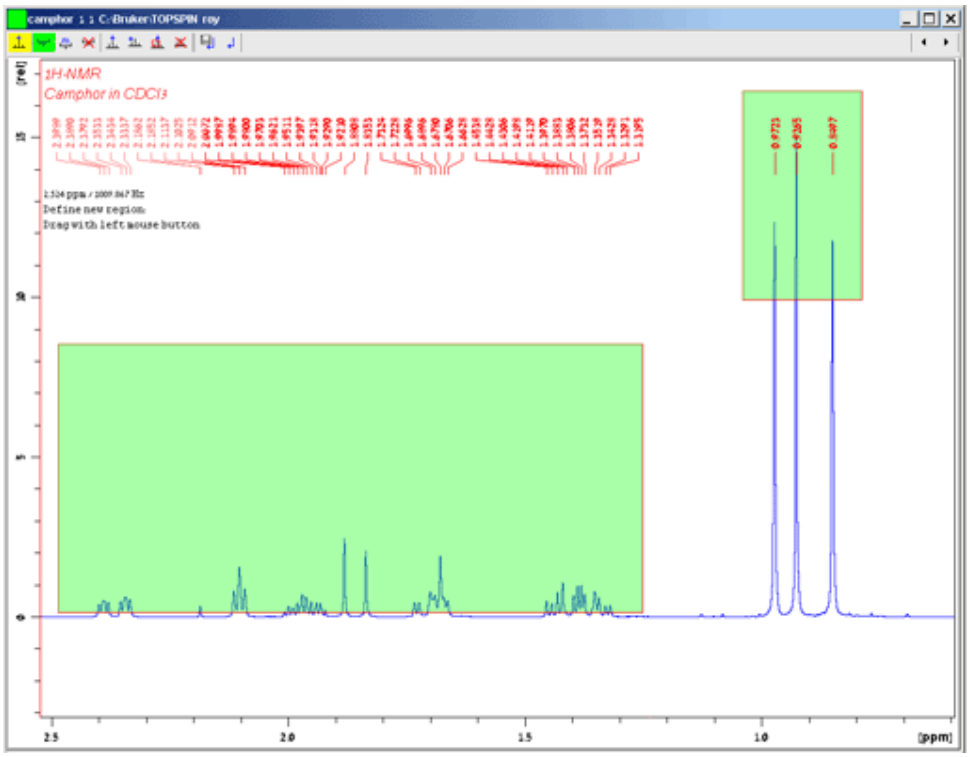
Reacquire the spectrum which may take longer than usual.

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. 60). 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 63 shows the peak picking window.

Figure 63. Manual peak picking in selected regions



17. Fluorine decoupled proton acquisition

A fluorine decoupled proton spectrum is acquired just like a regular proton spectrum but with the following differences.

You cannot acquire a fluorine decoupled proton spectrum on the 200 MHz spectrometer.

You cannot acquire a fluorine decoupled proton spectrum on the 500 MHz spectrometer.

Use only the 5 mm Dual 19F/1H Z3752/0007 [04] probe (see ch. 5), Put the 2H stop filter in the X channel (fig. 64) and read the shims by entering *rsh dualf*.

Figure 64. The 2H stop filter connected to the X channel of the preamplifiers

Set *p1* to *6.9* and run a regular proton spectrum (see ch. 3). Afterwards, run a fluorine spectrum (see the guide "Measuring NMR spectra of carbon and other non-proton nuclei") with the parameter *p1* set to *13*. In the fluorine spectrum click on  , and put the cursor at the center of the signals and note the frequency.

Tuning the proton and fluorine is carried out using adjustment screws (fig. 65).

Figure 65. Adjustment screws for tuning the dual proton fluorine probe

Create a file with the parameters *1c_Protonfdec* in the **Experiment** field (see ch. 3b). Change the parameter *sfo2* to the frequency that you noted down. Run and process the spectrum like a regular proton spectrum.

18. 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.

Only on the 500 MHz spectrometer and 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. 64).

Table 3. Parameters for decoupling other nuclei from proton

Decoupled nucleus	*Parameter name*	*pl12 BBI 400*	*pl12 BBO 400*	*pl12 BBI 500*	*pl12 BBO 500*
Phosphorus, ^{31}P	1e_Protonpdec	10.1	19.6	11.0	17.7
Rhodium, ^{103}Rh	1f_Protonrhdec				13.8

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.

On the 500 MHz spectrometer when decoupling at a temperature close to room temperature, increase the gas flow to 535 L/h and change the parameters (**Proportional band**, **Integral time** and **Derivative time**) according to the dashed lines in fig. 49. Even so, the temperature may not stabilize and you will need to raise the temperature by as much as five degrees or attach the cooling unit.

Run and process the spectrum like a regular proton spectrum.

19. Transferring files from the spectrometers to other computers

Request an account on the backup server of the laboratory that is called nmrdisk.ch.huji.ac.il.

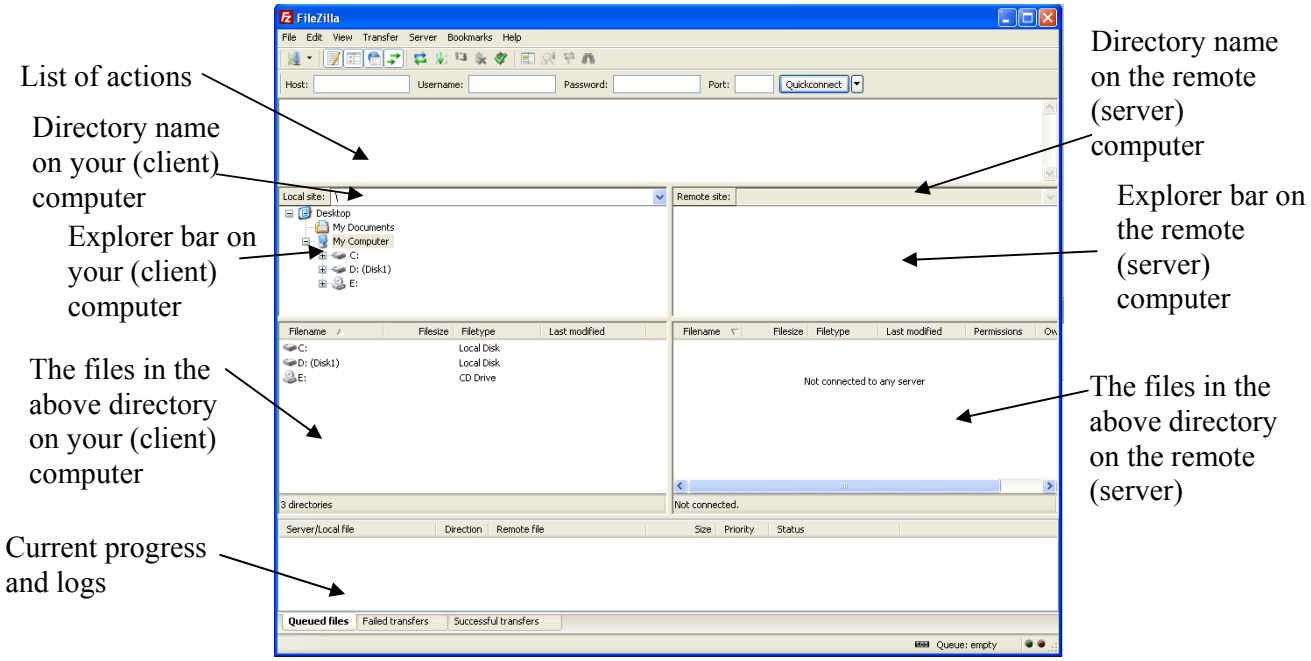
The files on the 200 and 400 MHz spectrometers are in the directory c:\Bruker\TOPSPIN1.3\data*username*\nmr and on the 500 MHz spectrometer in the directory c:\Bruker\TOPSPIN\data*username*\nmr. The files are backed up every night to /Public/*spectrometername*/Bruker… (where *spectrometername* is *the200*, *the400* and *the500*) on the server nmrdisk.ch.huji.ac.il.

On your personal computer you can install TOPSPIN as described in chapter 20. The default directory for NMR files on your computer is c:\Bruker\TOPSPIN\data*username*\nmr but you can use any directory in the form *homedirectory*\data*username*\nmr and transfer your NMR files to there.

In order to transfer files to your computer you need to use an ftp client: either the command line based client that comes with Windows or for greater convenience a GUI ftp client such as FileZilla (download from http://filezilla-project.org/). To install FileZilla from their website click on **Download FileZilla Client**, choose your operating system and follow instructions.

In order to run the program click on  . A window will open like in fig. 66.

Figure 66. The FileZilla Client program



The first time the program is used, enter *nmrdisk.ch.huji.ac.il* in the **Host:** field, your username is the **Username:** field, your password in the **Passowrd:** field and *21* in the **Port:** field. On subsequent occasions, click the down arrow next to the **Quickconnect** button and select the nmrdisk entry..

You can open directories on your computer (top left window) and on the server (top right window) and drag files and directories from one to the other (fig. 66).

Likewise the software is already installed on the spectrometers and you can transfer files to the server so that you can process them elsewhere on the same day. If you want to transfer files before they are automatically transferred every night then you can transfer them to your personal directory on the server: /*username*.

20. Use of TOPSPIN on other computers

There are floating licenses that you can use for TOPSPIN from any computer with Windows XP Pro SP2 or Linux RHEL3 or RHEL4 operating systems that is connected to the University network. If you want to use the software outside the University then you need to request permission to connect to the University network. For details see http://ca.huji.ac.il/services/internet/connect/home.shtml. 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, DVD drive and a three-button mouse.

Check that you have the SSH Secure Shell program installed on your computer (see ch. 18). Install it if it is not there but do not run it yet.

Install the TOPSPIN2.1 program from the DVD. Log in as an administrator (*root* on Linux). Insert the TOPSPIN2.1 DVD. After a few seconds an install shield will appear. Click on **Next>** and a **Adobe Acrobat Professional – [rellet.pdf]** window will appear. Close the window by clicking on the **X** at the top right then click on **Next>**. If you have a previous version of TOPSPIN installed, it will ask you to uninstall the previous version. If there is no previous version skip to "continue here…"

Click on:

Yes

Next>

Next>

Next>

OK

Next>

OK

Next>

OK

Next>

OK

Next>

OK

OK

OK

Continue here if the was no previous version of TOPSPIN to uninstall.

Click on:

Next>

Yes

Next>

A **Password Input** window will appear. Choose and enter a password in both places on the window and click on **Next**. Wait several minutes (about 10 minutes with a 3.4 GHz single processor and 16× DVD). At the end a message will appear **TOPSPIN Setup Installation Complete**. Click on **Finish**.

If this is an upgrade from a working copy TOPSPIN 2.0 then skip to the next paragraph. Otherwise, copy (see ch. 18) the license file *license.dat* from the directory /shares/internal on nmrdisk.ch.huji.ac.il to the directory c:\flexlm\Bruker\licenses on your Windows computer (or the directory /user/local/flexlm/Bruker/licenses on Linux). After the installation run TOPSPIN (in Linux you must log on as a user other than root). A **LICENSE** window will appear. Accept the condition by clicking on **I Accept**.

A **Configuration check** window will appear. Click on **Expinstall** and enter the password that you chose earlier.

Click on:

OK

Next>

Next>

Choose the basic frequency (such as *400.13* or *500.2*). Specify the printer/plotter. Specify the page size as **A4/Letter**. Click on **Next>** then **Finish** then **Close**. After a few seconds a message will appear at the bottom **expinstall: Done** and then you can use the program.

Once the installation is complete you can transfer your files to your computer (see ch. 18) and use TOPSPIN from your computer. The number of licenses are limited so please leave the program when you finish using it.