California State University, Los Angeles
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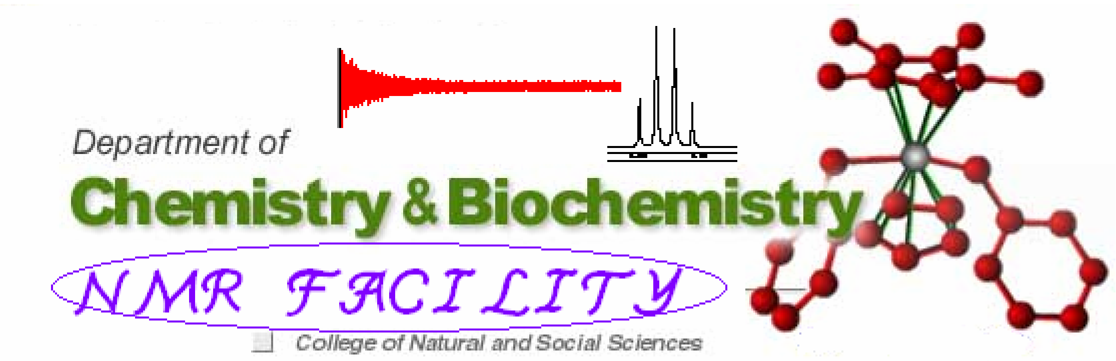

NTNMR/BRUKER SHORT MANUAL

Dissimilar

This manual is intended to be only very brief introduction for using the Bruker/Tegmac 300 spectrometer at California State University LA NMR Facility. For complete information about the NTNMR, software or running experiments please refer to the documentation that Tegmac provides. Most of the information in this manual was taken from different Tegmac's manuals and modified and edited to fit our need in this facility.

Richard Perrigan
& Ali Jabalameli

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- Data Manipulation
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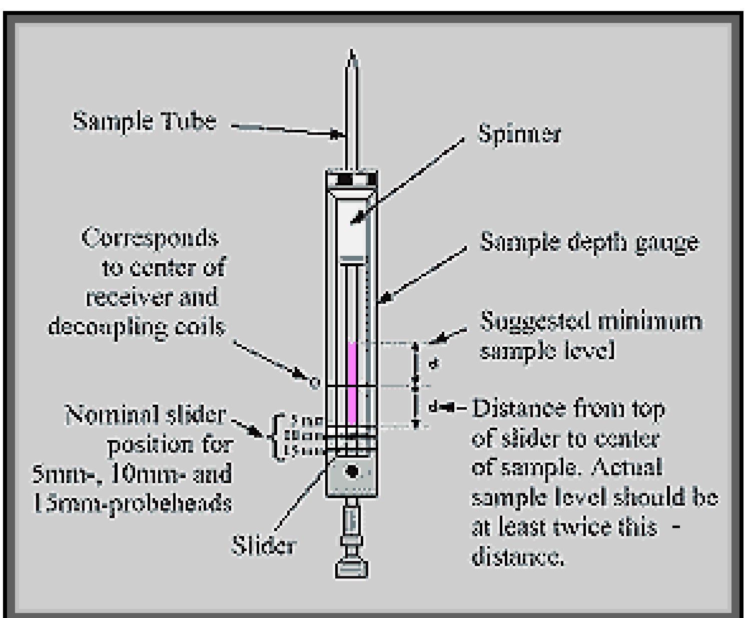
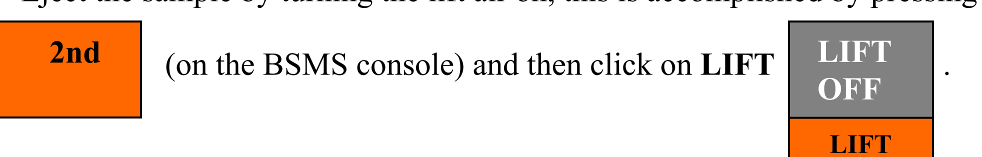
Basic ^{1}H NMR Experiment

1. Place your sample inside the spinner.

Important Note: Make sure your sample height in spinner is measured and adjusted prior to lowering your sample as it is shown below; otherwise, you may damage the probe.

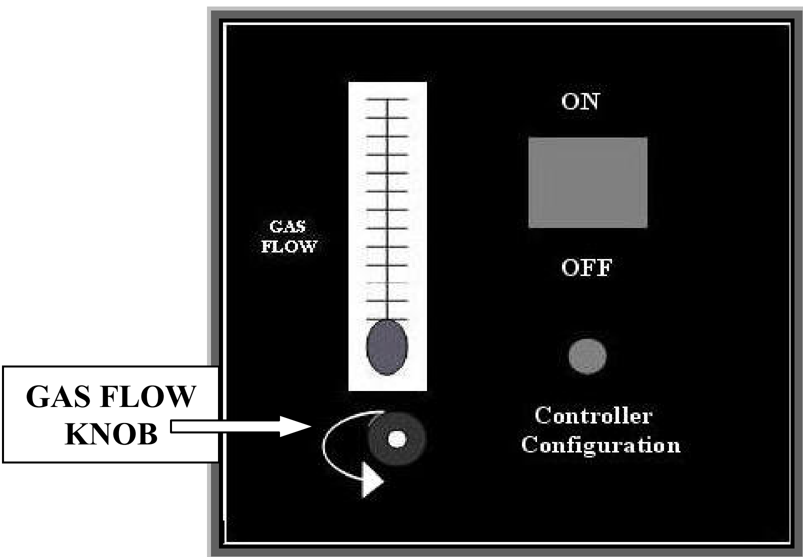


2. Eject the sample by turning the lift air on, this is accomplished by pressing **2nd**



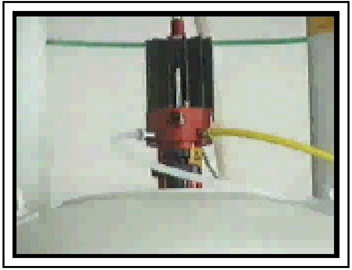
(on the BSMS console) and then click on **LIFT** .

3. Make sure the lift air is on; you should be able to hear and feel the air from the top of the magnet. If the air is not lifting up you should increase the **GAS FLOW** from the main console by turning the knob counterclockwise.



*(**GAS FLOW** Controller on the Main Console)*

4. Gently place the sample into the top of the magnet and make sure the sample is floating on the air cushion before you let the sample go.



The sample floating on air cushion on the top of the magnet

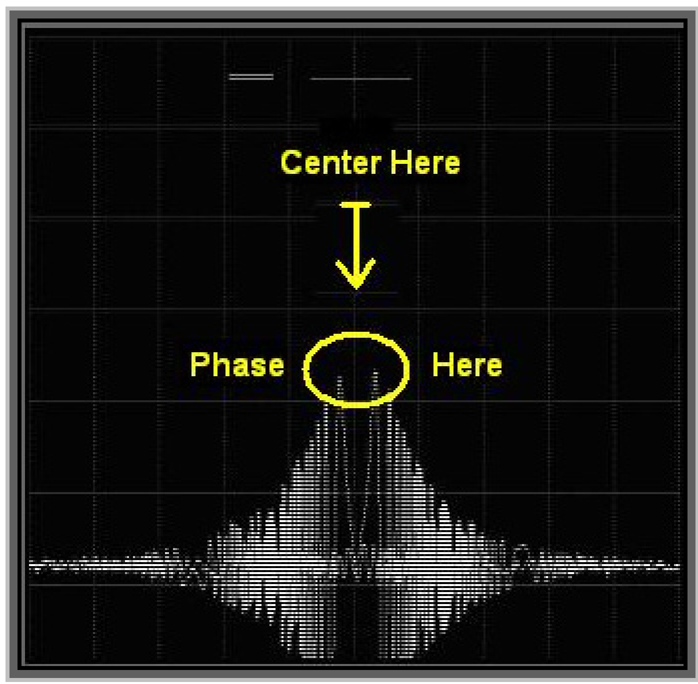
5. Press **LIFT OFF** LIFT OFF to lower your sample inside the magnet and decrease the **GAS FLOW** (turn the knob clockwise) to ensure that sample is down completely.
6. Once the sample is inside, start spinning it by pressing **SPIN** (on the BSMS console) SPIN and wait until green light stops flashing.
7. Start the WinTV2000 program WinTV2000 from the desktop for lock display.

8. Adjust the following knobs on the BSMS console in order to locate the deuterium lock signal for your solvent.

- **FIELD=about 8350**(varies for each solvent) **FIELD** **(Center the signal)**
- **SWEEP AMPL= about 25** **SWEEP AMPL** **(needs to be readjust for the solvent with more than one signal).**
- **LOCK POWER= about 40** **LOCK POWER** **(too much power will saturate the lock signal and will cause it to fluctuate).**
- **LOCK GAIN= about 120** **LOCK GAIN** **(too much gain will increases the signal noise).**

LOCK POWER and **LOCK GAIN** are used to adjust the height and amplitude of the lock signal.

9. Adjust the phasing using **LOCK PHASE** **LOCK PHASE** and make sure the signal is centered using **FIELD** . Click on **DUAL SWEEP** **DUAL SWEEP** display for better visualization: To achieve phasing and centering the lock signal, the height of two signals in dual sweep display ought to be equal while the middle of them should be middle of the lock display as it is shown below.



Lock Signal Display in Dual Sweep Mode

(Centered and Phased)

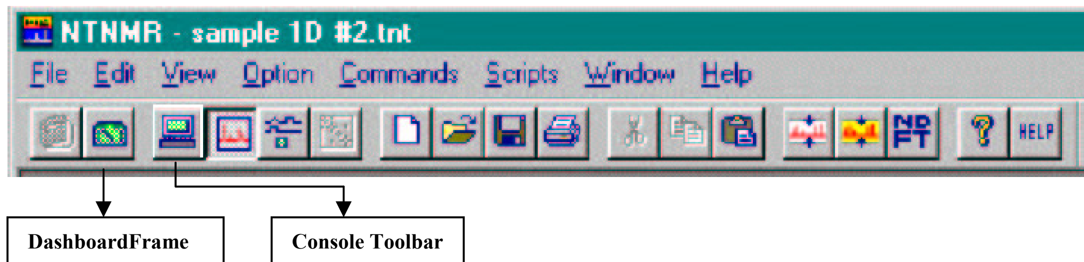
10. Once you have the signal phased and centered then you can press **AUTO LOCK** AUTO LOCK on BSMS. This will automatically lock on the signal by optimizing the Filed and Lock gain value. Wait until the lights (Filed and Lock gain) stop flashing then you can change it to manual lock by pressing LOCK . This will prevent auto lock to interfere during the shimming.
11. Reduce the gain LOCK GAIN so that you can see the lock level on the lock display. The lock level should be about 70 to 80 percent of lock display to maximize the sensitivity.
12. You may need to optimize the **Z1**, **Z2** and **Z3** shims in following order: First adjust **Z1**, and then **Z2** and **Z**1, and then **Z3**, **Z2** and last **Z1**. You may repeat this cycle for better optimization.

Note: you may have to keep reducing the LOCK GAIN in order to keep the lock display visible during the shimming as you improve the homogeneity.

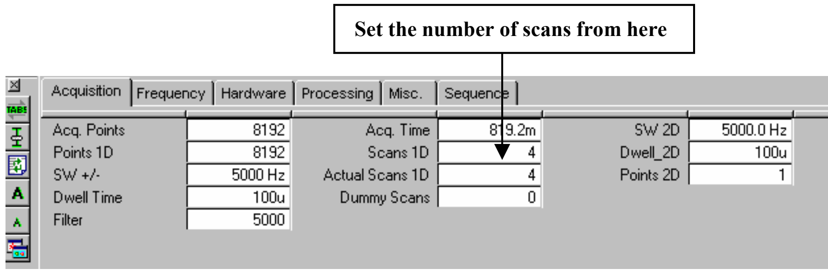
Order of shim adjustment

- **Z1**
- **Z2 Z1**
- **Z3 Z2 Z1**

13. Once done with shimming click on **STDBY** .
14. Start the NTNMR program NTNMR from the desktop.
15. From the NTNMR program under the file menu in the toolbar, open the Standard Proton file with related solvent (Example; H1 CDCl3). You may change/set the parameters by clicking on the **DashboardFrame** button in the upper left corner of **NTNMR**. For instance, make sure to change the number of scans to a higher number (multiple of 4) for a very dilute sample.

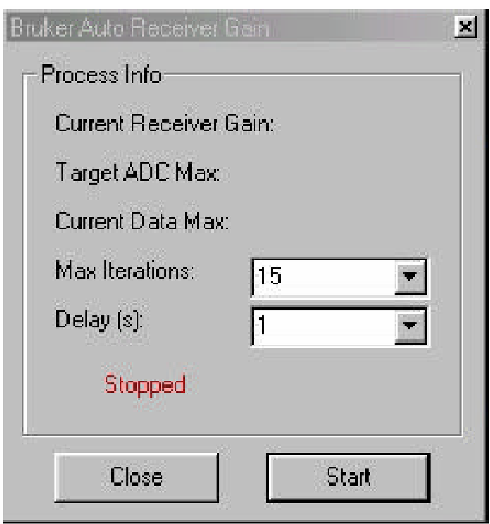


*(**NTNMR** Main Toolbar)*



*(**DashboardFrame** Window)*

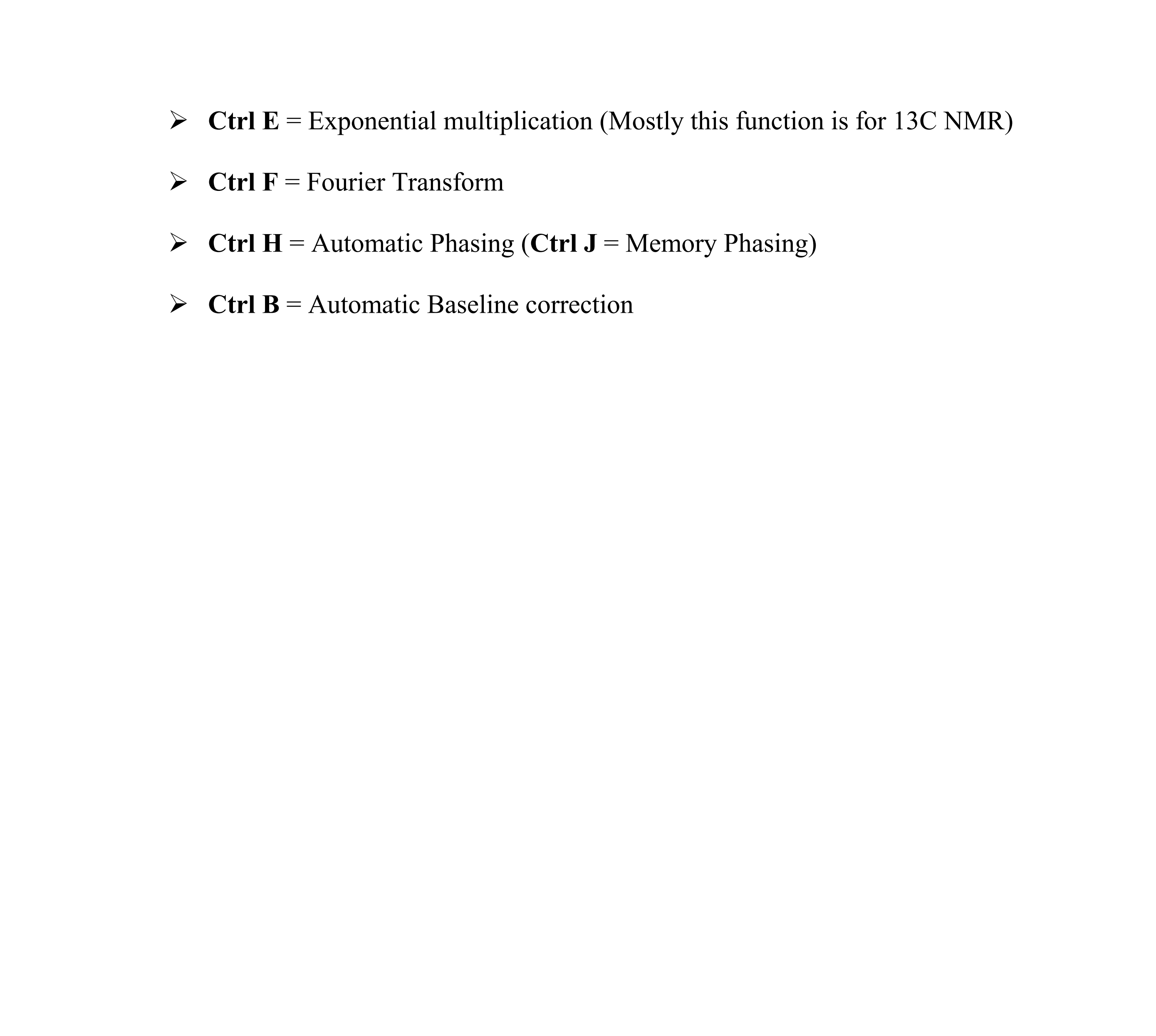
16. Start the **Bruker AG** (Bruker Auto Receiving Gain window) under **Scripts/hardware scripts** on toolbar. Adjust the receiving gain by clicking on **Start** button (**Working**), then wait until it stops (**Done**), and then click on **Close** button.



Bruker Auto Receiving Gain window

17. Now you may start the acquisition process clicking on the **ZG** (Zero and Go) icon on the NTNMR program toolbar.

18. Once the acquisition stops you should always **save** (in the main toolbar under **File/Save as**) the FID under your directory with new file name , and then process it by the following commands:



- **Ctrl E** = Exponential multiplication (Mostly this function is for 13C NMR)
- **Ctrl F** = Fourier Transform
- **Ctrl H** = Automatic Phasing (**Ctrl J** = Memory Phasing)
- **Ctrl B** = Automatic Baseline correction

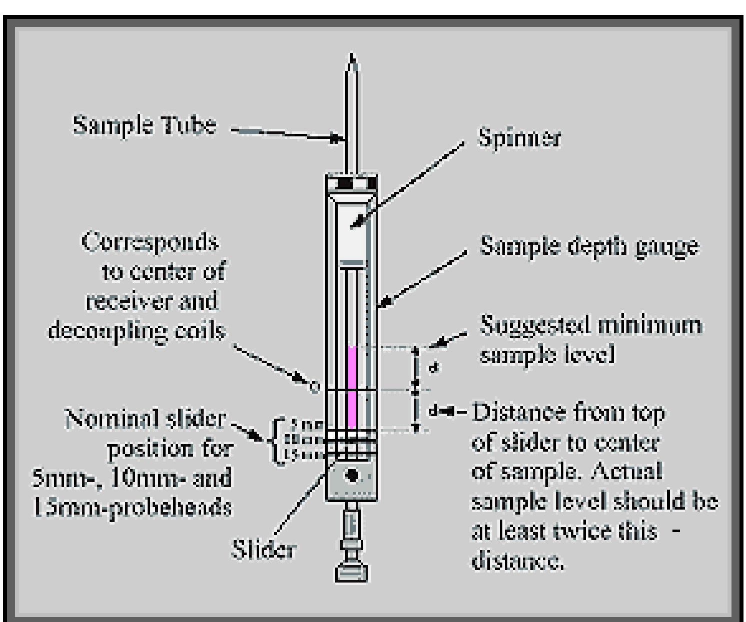
Basic ^{13}C NMR Experiment with Proton Decoupling ON

(Make sure that the probe inside the magnet is tuned for ^{13}C)

In order for the probed to be tuned for ^{13}C, the selective preamplifier for ^{13}C should be connected to the probe via the selective channel.

1. Place your sample inside the spinner.

Important Note: Make sure your sample height in spinner is measured and adjusted prior to lowering your sample as it is shown below; otherwise, you may damage the probe.



2. Eject the sample by turning the lift air on, this is accomplished by pressing **2nd**

2nd (on the BSMS console) and then click on **LIFT**

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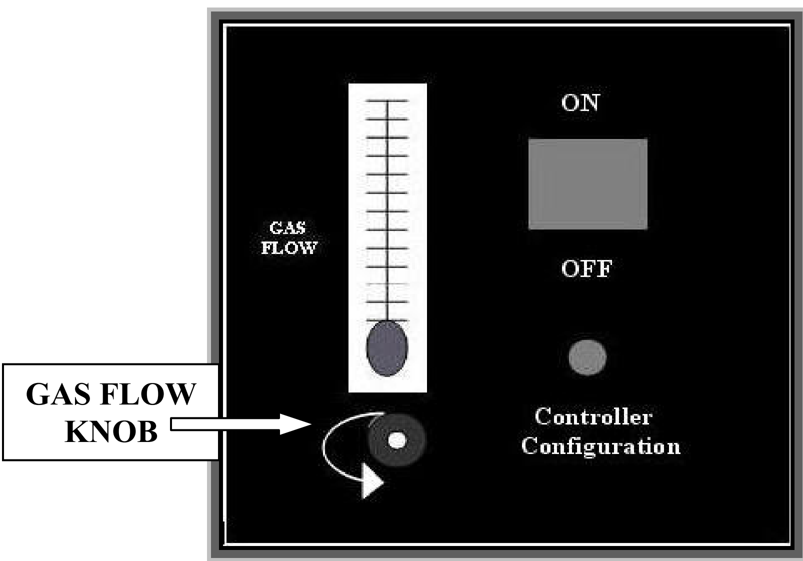
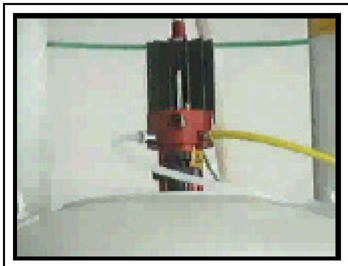
3. Make sure the lift air is on; you should be able to hear and feel the air from the top of the magnet. If the air is not lifting up you should increase the **GAS FLOW** from the main console by turning the knob counterclockwise.



*(**GAS FLOW** Controller on the Main Console)*

4. Gently place the sample into the top of the magnet and make sure the sample is floating on the air cushion before you let the sample go.



The sample floating on air cushion on the top of the magnet

5. Press **LIFT OFF** LIFT OFF to lower your sample inside the magnet and decrease the **GAS FLOW** (turn the knob clockwise) to ensure that sample is down completely.
6. Start the WinTV2000 program WinTV2000 from the desktop for lock display.
7. Adjust the following knobs on the BSMS console in order to locate the deuterium lock signal for your solvent.

- **FIELD=about 8350**(varies for each solvent) **FIELD** **(Center the signal).**

- **SWEEP AMPL= about 25** **SWEEP AMPL** **(needs to be readjusted for the solvent with more than one signal).**

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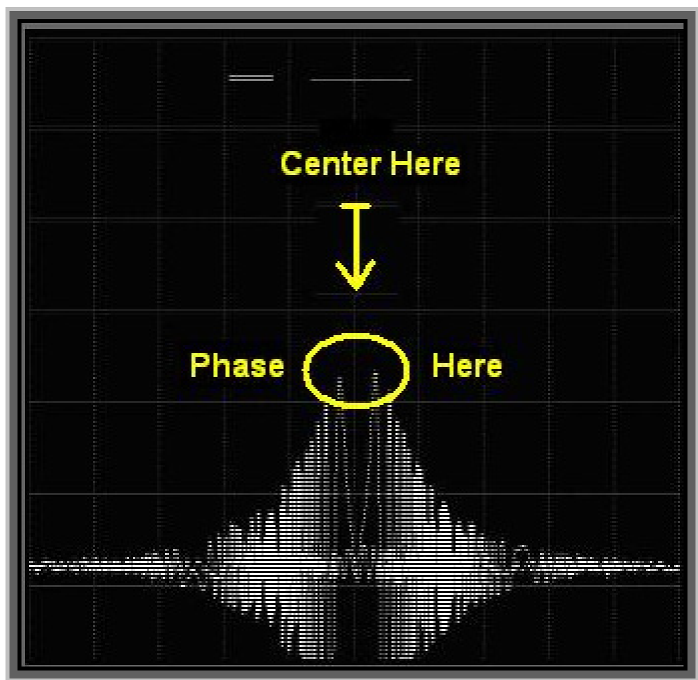


Lock Signal Display in Dual Sweep Mode

(Centered and Phased)

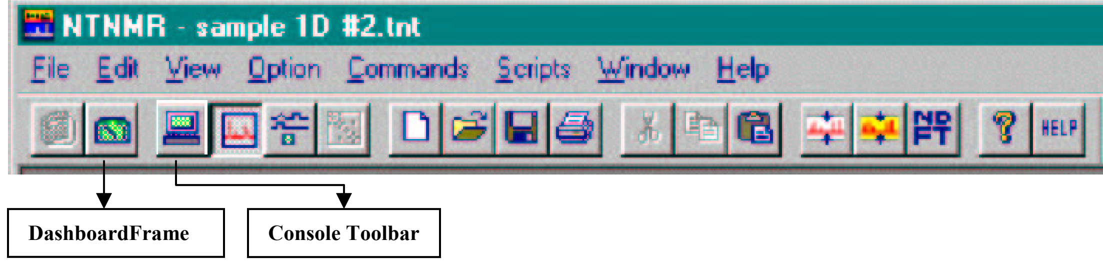
9. Once you have the signal phased and centered then you can press **AUTO LOCK** AUTO LOCK on BSMS. Wait until the lights (Filed and Lock gain) stop flashing then you can change it to manual lock by pressing LOCK .This will prevent auto lock to interfere during the shimming.
10. Reduce the gain LOCK GAIN so that you can see the lock level on the lock display. The lock level should be about 70 to 80 percent of lock display to maximize the sensitivity.

11. You may need to optimize the **Z1**, Z2, and **Z3** shims in following order: First adjust **Z1** and then **Z2** and **Z1** and then **Z3**, **Z2** and last **Z1**. You may repeat this cycle for better optimization.

Order of shim adjustment

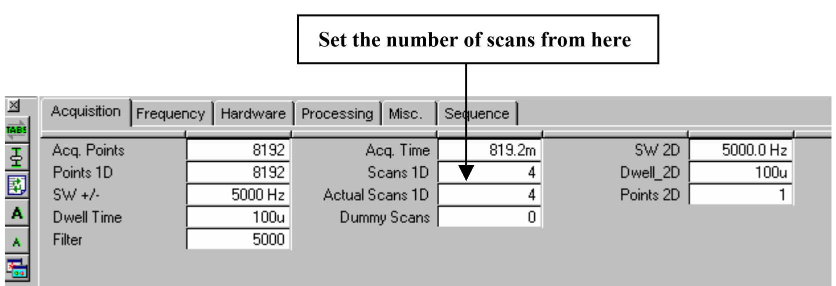
- **Z1**
- **Z2 Z1**
- **Z3 Z2 Z1**

12. Once done with shimming click on **STDBY** .
13. Start the NTNMR program NTNMR from the desktop.
14. In NTNMR program, open the ^{13}C NMR file with related solvent (Example; C13 CDCl3). This will load the Standard parameters for the routine ^{13}C Decoupled Experiment. You may change/set the parameters by clicking on the **DashboardFrame** button in upper left corner of **NTNMR** main toolbar. For instance, make sure to change the number of scans to a higher number (multiple of 4) for a very dilute sample.

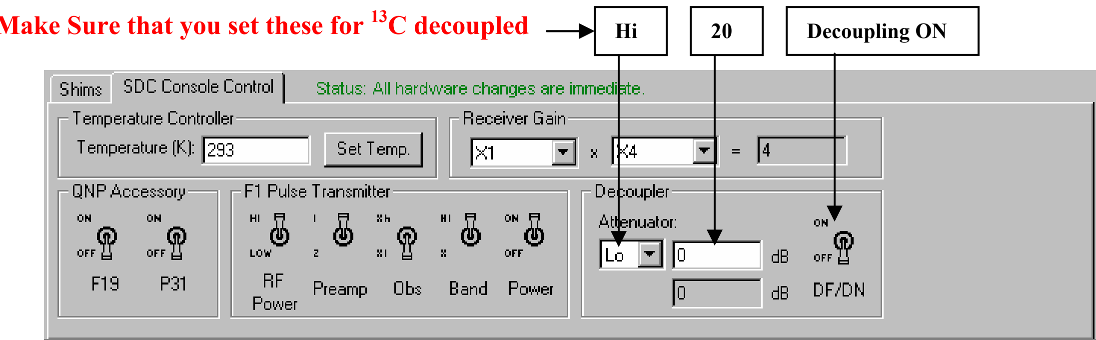


*(**NTNMR** Main Toolbar)*

15. You may turn the Decoupling off or on by changing its status from the **SDC** window. You can activate the **SDC Console Control** window by clicking on the **Console Toolbar** [icon] on the **NTNMR** main toolbar.

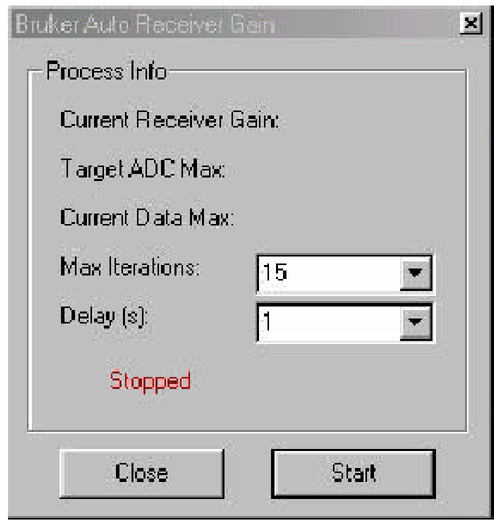


*(**DashboardFrame** Window)*



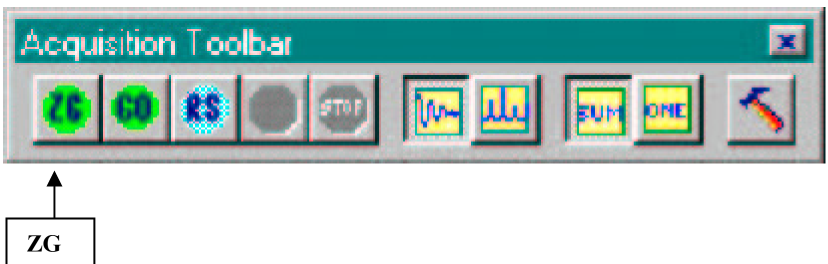
*(**SDC Console Control** Window)*

16. Start the **Bruker AG** under **Scripts/hardware scripts** on toolbar. Adjust the receiving gain by clicking on **Start** button (**Working**), then wait until it stops (**Done**), and then click on **Close** button.



Bruker Auto Receiving Gain window

17. Now you may start the acquisition process clicking on the **ZG** (Zero and Go) icon on the NTNMR program toolbar.



18. once the acquisition stops you should always **save** (in the main toolbar under **File/Save as**) the FID under your directory with new file name and then process it by the following commands:

- **Ctrl E** = Exponential multiplication (Mostly this function is for 13C NMR)

  Note: For this window function, the value (LB) needs to be set form the DashboardFrame window and usually is between 3 to 5 HZ for ^{13}C.

- **Ctrl F** = Fourier Transform
- **Ctrl H** = Automatic Phasing (**Ctrl J** = Memory Phasing)
- **Ctrl B** = Automatic Baseline correction

Data Manipulations

1. **Setting The Reference Pick**

2. **Manual Phase Adjustment**

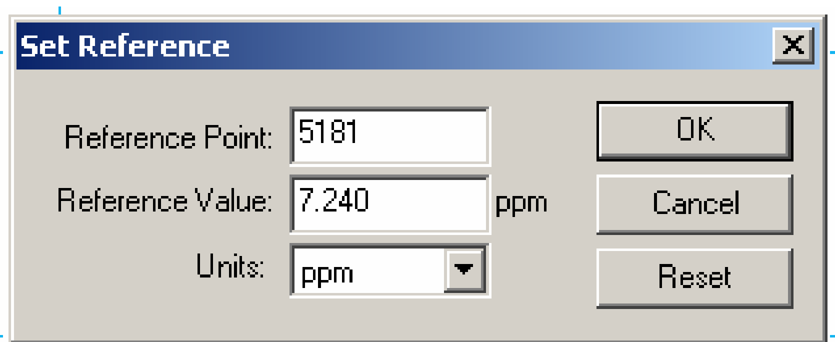
3. **Peak Pick procedure**

4. **Integrals**

5. **1D Printing**

1. Setting The Reference Pick

Position the cursor on the desirable pick for referencing (TMS or solvent pick). Now, invoke the **Set Reference Dialog box** (below) by clicking on **View/Reference/Set Reference** from Toolbar.



Set Reference Dialog Controls

• **Reference Point:** the data point in the spectrum to which the new frequency or PPM value is to be applied.

• **Reference Value:** the new value to be assigned to the **Reference Point** entered

• **Units:** the units for the Reference Value

• **OK** will reference the current data set at the Reference Point to the Reference Value. The current cursor position and its value for the currently selected units are loaded by default. These values can be changed directly by typing in the associated text fields. The units can be selected from the Units pop-up.

• **Cancel** will remove the dialog with no change in reference

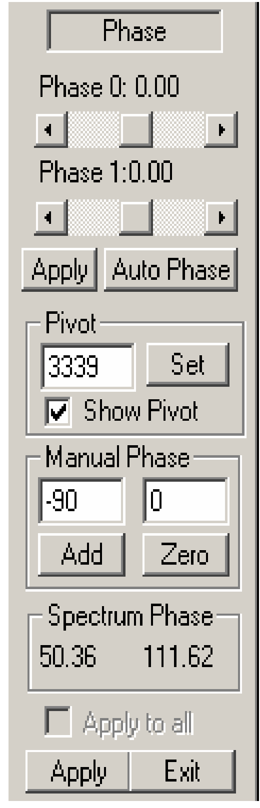
• **Reset** will re-position zero Hertz in the center of the frequency axis

Setting the Spectrum Reference

When the Set Reference dialog is open, the current cursor position is displayed in the **Reference Point** window. A new **Reference Points** can be entered directly. Entering a new **Reference Value** will assign the value entered to the point in the spectrum indicated by the **Reference Point**.

2. Manual Phase Adjustment

The Phase Adjustment allows you to perform manual zero and first order phase correction on the spectrum. Selecting **Option|Phase Adjustment** causes the Control Swap Area to change to the Phase Area and the phase window to appear as shown below. Phase Adjustment mode is only available for frequency domain data.

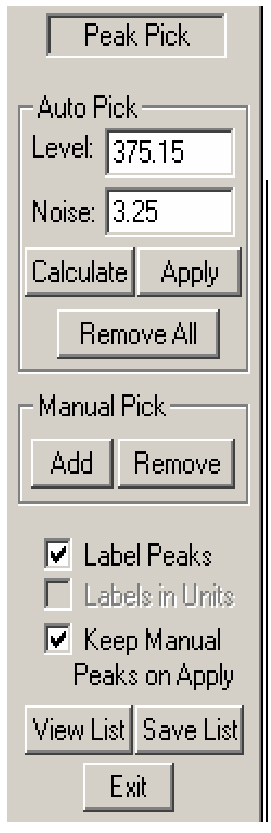


Phase Adjustment Summary

- **Position your mouse curser (Black vertical line) to the one side of your spectrum (tallest pick).**
- **Click on Set button to Set the Pivot value, this will turn the black curser line to a yellow line.**
- **Adjust Phase 0 Value using Slider until the tallest peak is phased.**
- **Adjust Phase 1 Value using Slider until the rest of the spectrum is phased. *If the slider is maximized, click on Apply button (below the slider) to reset the slider.***
- **Click on Apply button to apply the changes to the spectrum the click on Exit.**

3. Peak Pick procedure

The **Peak Pick** routine is entered by selecting the **Peak Pick** item under the **Option** menu. Choosing the Peak Pick option causes the Control/Swap Area to change from its current selection to Peak Pick, as shown in the figure. Note that in the NTNMR Data Window, Peak Pick are displayed only by label, i.e. a number assigned to each peak found ordered from left to right in the display. When printing, the user has the option to display either the peak pick labels or the chemical shift values directly on each peak.



Auto Pick:

- **Click on Calculate button** to calculate the Level and Noise Parameters for the current display area. If an area has been highlighted by a click-and-drag operation, the Level and Noise parameters are calculated for the highlighted region.
- **Apply** Executes an automatic peak pick using the current Level and Noise parameters.
- **Remove All** Removes all Peak Picks.

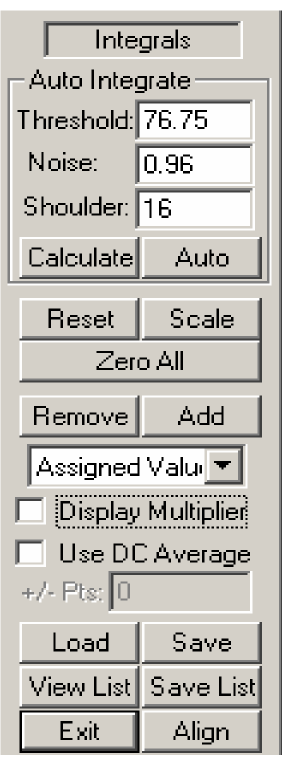
Manual Pick:

- **Click on Add** to add a peak pick at the current cursor position. The manual picks appear in blue. If a region of the spectrum is highlighted, the Add button will execute an Auto Peak Pick for the highlighted region only using current Level and Noise parameters.
- **Click on Remove** to remove the peak pick at the current cursor position regardless of whether it was generated automatically or manually. If a region of the spectrum is highlighted, all picks that fall inside the highlight will be removed.
- **View List** Opens the Peak List window. (See below)
- **Save List** Saves the current peak list to a text file.
- **Exit** Exits the Peak Pick Mode.

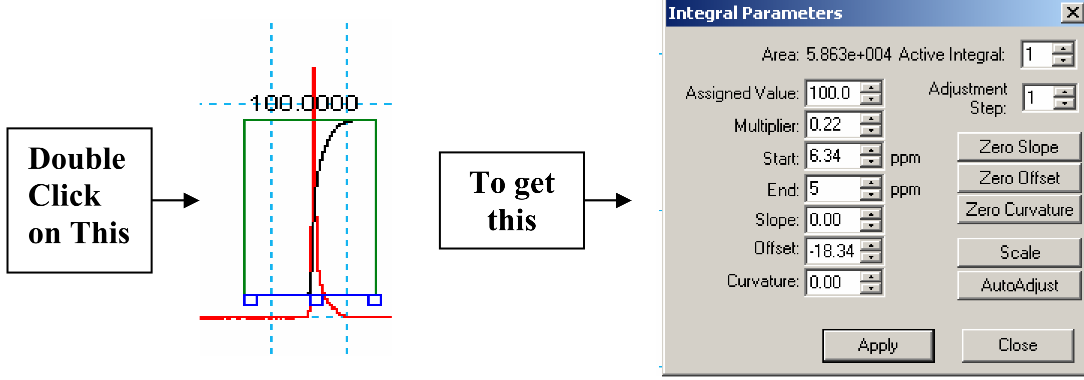
The Peak Pick area provides two edit boxes: Level and Noise. The Level threshold sets a limit on the height of peaks selected. The Noise threshold tests for the difference between potential peaks when moving from point to point in the peak search. NTNMR will calculate default values for Level and Noise if no values are saved with the data. To set the value for Level or Noise, enter a new value in the appropriate text box. The Level can also be adjusted by positioning the mouse pointer at the top of the level area on the display by dragging up or down.

4. Integrals

The **Integrals** routine is entered by selecting the **Integrals** item under the **Option** menu. Choosing the **Integrals** option causes the Control/Swap Area to change from its current selection to **Integrals**, and a window will appear as shown below.



- **Auto Integration:**
  - **Click on Calculate** to calculate the Threshold, Noise and Shoulder values for an auto integration. Values are calculated for the current data region displayed, or if a region is highlighted for the highlighted region.
  - **Click on Auto to** execute an auto integrate command and draws the results on the data display window. Only the data region displayed is integrated. If a region of the spectrum is highlighted, only that region is integrated.
- **Manual Integration:**
  - **Adding Integrals**. Add an integral to the spectrum by clicking and dragging to a select region of the spectrum and then either clicking on the **'Add'** button or right clicking and selecting **'Add Integral'**.
  - **Removing Integrals.** Remove a current integral by clicking and dragging to select the region of the Spectrum that contains the integral and either click on the '**Remove**' button or right-click and select **'Del Integral'**.
- **Assigned Value**
  - Allows assignment of a value to a particular integral in the spectrum. When a value is assigned to a particular integral all other integrals are given new assigned values. This is used to normalize all integrals in a spectrum for assigning and adjusting the integral value see below.
- **Adjusting Integrals.**
  - Integrals can be adjusted by mouse or by parameter. Double clicking on any integral will open the 'Integral Parameters' dialog, which contains adjustments for all parameters for each individual integral in the spectrum (as shown below).



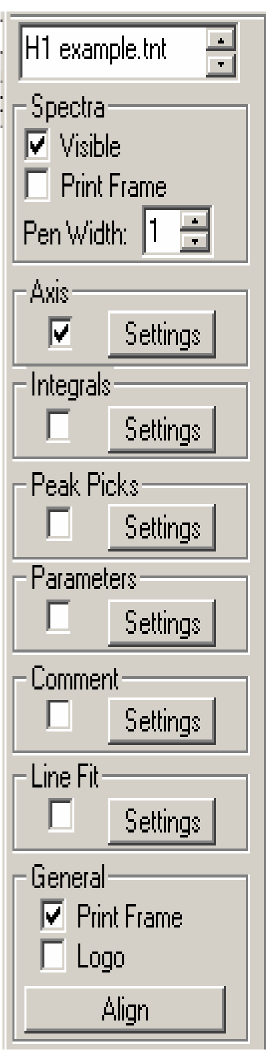
5. 1D Printing

1D Print Commands

All printing functions are accessed from the File menu in the Print section Four 1D printing commands are available: Print Setup, Print Layout, add to Print Layout, and Print (Ctrl + P). Print templates are saved using the 1D Print Layout mode. Once a default print template has been saved, NTNMR will apply the default template to all print outs that are executed by selecting 'Print' from the file menu or by clicking the 'Print' button on the main toolbar. The default print template will also be applied upon entering 1D Print Layout mode. **Print Layout** Activates the NTNMR Print Layout mode in which you can manipulate and control various print parameters for your printouts(see below).

Customizing 1D Printouts

Select 'Print Layout' from the File menu. This will add current data set to the print Layout mode.



1D Print Layout Toolbar

- **Data Set Selection Box:** The text box at the top of the window shows the filenames for the data sets that have been added to the 1D Print Layout.
- **Spectra / Visible:** Determines whether or not the selected spectrum will be printed.
- **Spectra / Print Frame:** Determines whether or not NTNMR will draw a frame around the active spectrum.
- **Spectra / Pen Width:** Allows selection of the pen width to be used to draw the active data set.
- **Axis Checkbox:** Toggle on/off the axis for the selected data set.
- **Integrals Checkbox:** Toggle on/off the integrals for the selected data set.
- **Peak Picks Checkbox:** Toggle on/off the peak picks (if any) for the selected data set.
- **Parameters Checkbox:** Toggle on/off the parameters display for the selected data set.
- **Comment Checkbox:** Toggle on/off the comment display for the selected dataset.
- **Line Fits Checkbox:** Toggle on/off the line fits (if any) display for the selected data set.
- **General / Print Frame:** When selected, a frame will be printed around the entire plot *(this is a global setting for the entire plot)*.
- **General / Logo:** When selected, a logo will be added to the plot *(this is a global setting for the entire plot)*.
- **Align:** Snaps all print preview elements to the invisible grid.

Frequenly Ask Questions:

Q1. How should I prepare my NMR sample?

Q2. Can I run my sample neat (or without deuterated solvent)? or What if I can not use the deuterated solvent due to the special circumstances?

Q3. Why I don't see anything coming up the magnet, when I press the lift button?

Q4. Why doesn't my sample spin?

Q5. Why do I see my lock signal after I put my sample into the magnet?

Q6. Why does the lock signal display, fluctuate (moving up and down) after I locked?

Q7. Why does the lock display fluctuate too much (too sensitive) when I try to adjust the shims?

Q8. Why are all my signals in the spectrum split into two (or more) peaks?

Q9. How do I improve the resolution for my spectrum? The peaks look awful!

Q10. How can I adjust phasing for the spectrum if the first order phasing is maximized?

Q11. Why don't I see any peaks in my spectrum?

Q12. Why are the peaks in my spectrum are shifted to one side? Why are some of the peaks in my spectrum missing?

Q13. Why are the chemical shifts for the peaks in my spectrum incorrect?

Q14. Why can't I change the scale (vertical) of my spectrum?

Q15. I ran "Bruker AG" Script and the computer froze. How can I unfreeze the computer?

Q16. Why the integrals do not show the values that I assign to them?

Q1. How should I prepare my NMR sample?

Dissolve your compound in a suitable deuterated solvent. You may have to filter the solution because there should not be any undissolved particles. Place the solution in an NMR tube with a cap. The sample height in the tube should be around 5.0 cm. Wipe and clean the outside of the sample

Click to go back to the menu

Q2. Can I run my sample neat (or without deuterated solvent)? or What if I can not use the deuterated solvent due to the special circumstances?

You still can run your sample however, you need to make sure that the lock is in standby mode and also you need to shim the magnet using the FID instead of using the deuterated lock signal. Additionally, you may need to use the external reference for assigning the chemical shifts.

Click to go back to the menu

Q3. Why I don't see anything coming up the magnet, when I press the lift button?

1-There is no air

2- Air flow is too low.

3- There is no sample in the probe to be ejected by pressing the lift button.

4- In the worst scenario; the sample is broken inside the magnet.

Click to go back to the menu

Q4. Why doesn't my sample spin?

1-There is no Air.

2- Air flow is too low.

3- Air spin rate on BSMS console is set to zero. You can adjust that from BSMS keyboard.

4- The black and white tape on the spinner turbine is worn or the spinner turbine is damaged. Try to use another one.

5- Sample height is not adjusted properly. Use the depth gauge to measure it.

6- The sample is too heavy. If you are using the heavy wall NMR tubes or the NMR tubes with heavy air values, you may have a spinning problem. You may be able to fix this problem by increasing the gas flow, or increasing the spinner rate form the BSMS keyboard.

7- If you are doing a low temperature experiment, most possibly there is some ice formation due to moisture condensation. You should raise the temperature and flush the system with dry air or preferably with nitrogen gas.

Click to go back to the menu

Q5. Why do not I see my lock signal after I put my sample into the magnet?

1- Deuterated solvent was not used.

2- The sample has not settled down into the probe properly because of (a) too much airflow, and (b) the sample height either was not adjusted prior to putting the sample into the magnet, or the sample height changed and moved due to the sample being lose inside the spinner. If the spin indicator on the BSMS console is showing that the sample is spinning, then that is not the problem. You can turn the spinner on and off to verify this matter.

3- Make sure the field sweep (on the BSMS Console) is on.

4- Make sure the field value is set correctly (about 8400). If you still do not see the signal, increase the lock power, lock gain, and sweep amplitude (turn clockwise) and then adjust the field until you see the signal.

5- Someone has done some radical shimming and has ruined the minimal homogeneity required for observing the lock signal. You should try to reload the shims by calling their values from the shim files.

6- Worst scenario; there is no magnetic field because the magnet has quenched ***OUCH!***

Click to go back to the menu

Q6. Why the lock signal display fluctuate (moving up and down) after I locked?

The lock power is too high for your solvent. Typically this happens for the strong deuterium solvents (like acetone-d_6 and methanol-d_4), when their spins are saturated with too much irradiation power. To solve the problem turn down the lock power until the flunctuation stops. In order to maintain the level of the lock display, you should compensate the power reduction by increasing the gain power, otherwise; you may lose the lock.

Click to go back to the menu

Q7. Why does the lock display fluctuate too much (too sensitive) when I try to adjust the shims?

1- Click on the **Fine** button on the BSMS console to toggle to fine adjustment from coarse adjustment.

2- The lock power is too high for your solvent. Typically this happens for the strong deuterium solvents (like acetone-d_6 and methanol-d_4), when their spins are saturated with too much irradiation power. To solve the problem, turn down the lock power until the fluctuation stops. In order to maintain the level of the lock display, you should compensate the power reduction by increasing the gain power; otherwise, you may lose the lock.

Click to go back to the menu

Q8. Why are all my signals in the spectrum split into two (or more) peaks?

Bad shimming could cause that. Try to adjust the shimming

Click to go back to the menu

Q9. How do I improve the resolution for my spectrum? The peaks look awful!

1- Make sure you are using the lock, or alternatively, if you can not use the lock, the lock sweep is off.

2- Bad shimming could cause that. Try to improve the shimming

2- Bad sample. Sample preparation is important. Make sure that you have enough sample solution; the sample height in the tube should be around 5.0 cm. Sometimes metallic impurities in the sample could cause that. Make sure you sample look clear; any undissolved solid floating in your sample may have an undesirable effect your spectrum.

Click to go back to the menu

Q10. How can I adjust phasing for the spectrum if the first order phasing is maximized?

1- Click on apply button by the first order phase slider to reset it.

Click to go back to the menu

Q11. Why don't I see any peaks in my spectrum?

1- The vertical scale is too low . Try to increase it.

2- The **SW** is out of range and needs to be readjusted for your sample. You can increase the **SW** to a large number (5000, you can do that from the **Dashboardframe** button on the main menu) and do one (or a few) scans and then process the data. Once your determine what region of the spectrum contains your peaks, you can reduce the **SW** in this manner; first, select (zoom) on the desirable region the run the " **Set New SW**' script under **Scripts/ Acquisition Scripts** in the toolbar menu.

3- If you are doing any low temperature experiment, you might have frozen your sample. This problem usually led to the loss of the lock signal as well. You can eject your sample and see if it is frozen. You may need to use different solvent for your low temperature experiment.

5- For some reason, the gas flow has changed and has elevated your sample away where it is suppose to be. Try to lower the gas flow.

Click to go back to the menu

Q12. Why are the peaks in my spectrum shifted to one side? Why are some of the peaks in my spectrum are missing?

1- The **SW** is out of range and need to be readjusted for your sample. You can increase the **SW** to a large number (5000, you can do that from the **Dashboardframe** button on the main menu) and do one, (or a few) scans, and then process the data. Once you determine what region of the spectrum contains your peaks, you can reduce the **SW** in this manner ; first, select (zoom) on the desirable region the run the " **Set New SW**' script under **Scripts/ Acquisition Scripts** in the toolbar menu.

Click to go back to the menu

Q13. Why are the chemical shifts for the peaks in my spectrum are incorrect?

You need to set the reference peak (TMS or your solvent peak).

Click to go back to the menu

Q14. Why can't I change the scale (vertical) of my spectrum?

In NTNMR there are two icons in main toolbar, one for "Auto Scale" and other one for "Fit to window". Click on the "Fit to window" icon to enable the keyboard option to change the scale.

Click to go back to the menu

Q15. I ran "Bruker AG" Script and the computer froze. How can I unfreeze the computer?

Try "Control-Alt-Delete" and then under Task manager, close the Bruker AG program by clicking on end of task.

Click to go back to the menu

Q16. Why the integrals do not show the values that I assign to them?

In the integrals toolbar window, there is a small pull down window that allows you to set the option to Area Labels, Number Labels, or Assign Values. Make sure you set it to Assign Values. If you are not able to display these values in the final print out, do the following. Click on the Integrals Setting in the Print Layout toolbar to start the pop out window. In there, make sure that "Show Value" is checked.

Click to go back to the menu