PINMRF

Bruker AV-III / Avance DRX NMR Spectrometers running TopSpin
Training Guide for Basic 1D NMR Spectroscopy

INCLUDING:
AV-III-400-HD w/ 5mm BBFO SmartProbe – 369 WTHR
AV-III-500-HD w/ 5mm BBFO Cryoprobe Prodigy - B055 DRUG
AV-III-800 w/ 5mm QCI Cryoprobe - LB124 BRWN
Avance DRX500-1 w/ 5mm BBFO ATM Probe - 367 WTHR
Avance DRX500-2 w/ 5mm BBFO ATM Probe - G43 RHPH

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04-01-2021: Revised - JSH.
10-28-2019: Revised - JSH.
06-15-2017: Created - JSH.

Basic Spectrometer Operation Guidelines - 1D Spectra
Bruker AV-III / Avance DRX NMR Spectrometers
PINMRF

NOTE - Different spectrometer types and TopSpin versions

The instructions below cover both our Avance DRX- and AV-III-type spectrometers. There are some operational differences between the spectrometer types and between TopSpin 1.3 (Avance DRX) and TopSpin 3.2 (AV-III). When this occurs, Avance/TopSpin 1.3 instructions will be given in BLUE and AV-III/TopSpin 3.2 instructions will be given in GREEN.

Logging on to the computer and starting TopSpin

1. Enter your login ID in the Username field.
2. Enter your password in the Password field.
3. After the window manager starts, move the mouse pointer to the Linux shell window and type `topspin` to start the NMR program.
4. The NMR program will start and you will see the main window appear.

H1 experiment setup

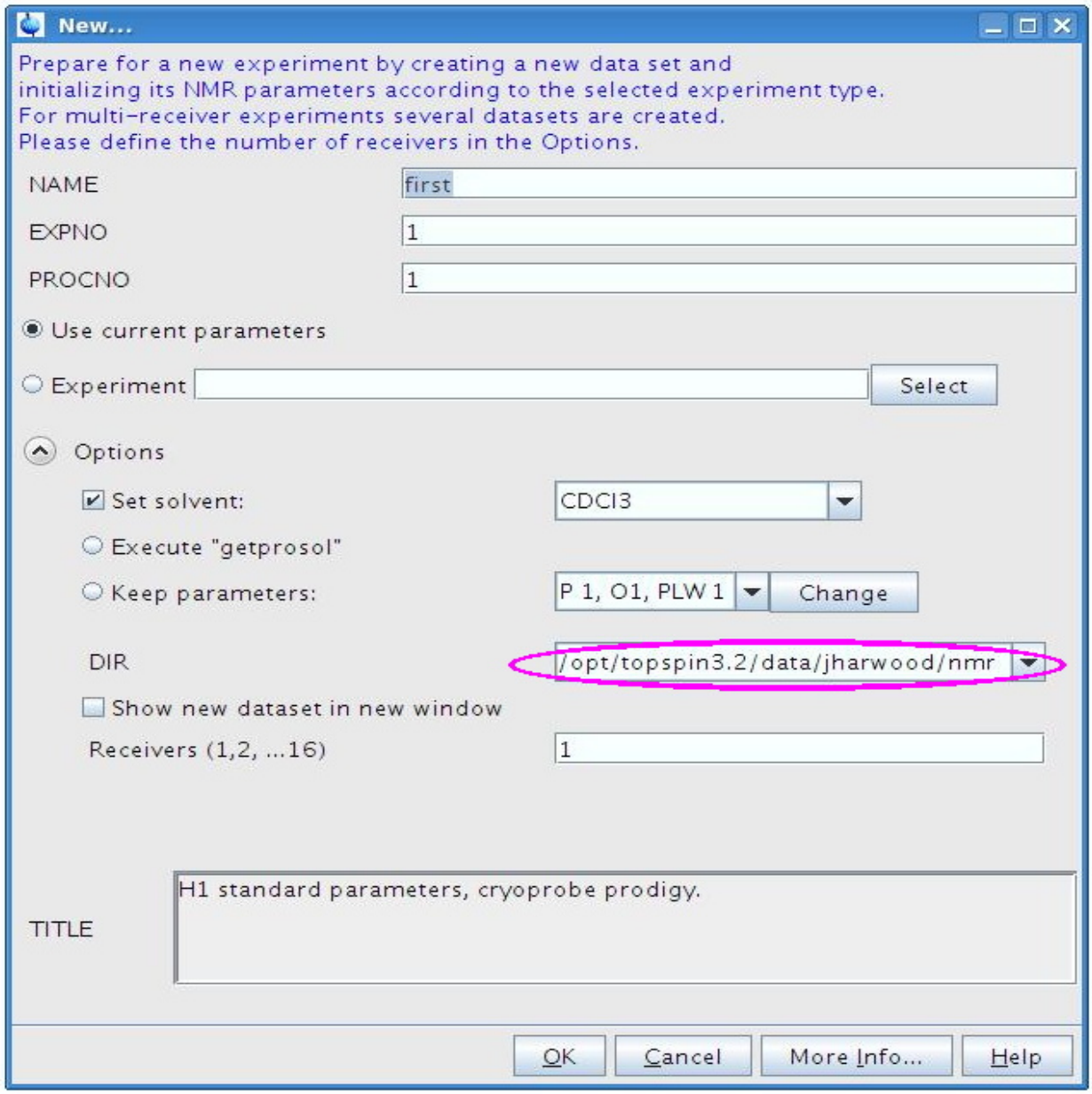
NOTE: if this is your very first usage of the spectrometer after getting your access set up, please follow the instructions in our document **First-time Use: Loading of the Initial Dataset** to assist you in getting all the fields in the edc window set up correctly.

On the Avance DRX-500s:

5. If you do not want to use the currently displayed dataset for your acquisition, type `edc` (edit current dataset) or `new` to create a new one. When the edc window appears, first type in the new dataset name in the NAME field and type in the desired experiment number in the EXPNO field (the PROCNO should be 1 and the DIR (/opt/topspin) and USER (your user login ID) fields should not need to be changed). Then, click on SAVE to close the window.

On the AV-III spectrometers:

5. If you do not want to use the currently displayed dataset for your acquisition, type `edc` (edit current dataset) or `new` to create a new one. When the edc window appears, first type in the



Above: Example TopSpin 3.2 edc popup Window. When creating a dataset, replace the user login id "jharwood" in the purple-circled text with your own user login id.

new dataset name in the NAME field and type in the desired experiment number in the EXPNO field. In the PROCNO field, enter 1. In the DIR field, you must enter the following text string exactly as shown: /opt/topspin3.2/data/user/nmr , where "user" is your user login ID. You can see an example of this shown in the figure above, the DIR entry is circled in purple. Then, click on OK to close the window.

NOTE 1: the EXPNO can be any number but we suggest 1 for the proton spectrum, 2 for carbon, 3 for DEPT, 4 for COSY, etc.

NOTE 2: for the NAME field, do not include spaces or special characters in the name - use only letters, numbers, and the - (dash) and _ (underscore) characters.

NOTE 3: in TopSpin we do not recommend using the edc command to read and move between existing datasets – use the re command or the browser window for this.

6. Type rpar h1.probe ("Probe" = bbo (Avance DRX500s & AV-III-400-HD); = cryo (AV-III-500-HD & -800)) to read the standard proton parameters. A window will appear listing the different sub-components of the parameter set; click the OK button to load all highlighted parameter types (or select specific types first).

7. To examine the acquisition parameters you can use either the eda (edit acquisition parameters) or the ased (acquisition setup editor) commands. Each brings up a window in the spectrum display area; after examining and changing anything (if necessary), click the mouse cursor at the text-entry area at the bottom of the window to allow further command entry. Alternatively you can click on the AcquPars tab above the spectrum to see the window.

Changing samples, locking and shimming

8. Move the mouse cursor to the text input area of the TopSpin window and type lockdisp to display the lock window. A sample of $CDCl_3$ resides in the magnet when the spectrometer is not in use, and this sample should be locked.

On the Avance DRX500s:

9. On the keypad, turn off LOCK (and SPIN, if spinning) function(s), then press the LIFT key to eject the $CDCl_3$ standard sample.

10. Place your sample (CLEAN IT!!) in the spinner and use the depth gauge to check the sample depth. Place the spinner on the air column at the top of the magnet, and press the LIFT key again to insert the sample. Wait for the sample to seat, then press the SPIN button to start spinning.

On the AV-III spectrometers:

9. Type bsmsdisp to open the BSMS control window. In the BSMS window, click on the SPIN button to turn off spinning (if it is on), then the LOCK button to turn off the lock, followed by the LIFT button to lift the sample.

10. Place your sample (CLEAN IT!!) in the spinner and use the depth gauge to check the sample depth. Place the spinner on the air column at the top of the magnet, and click the LIFT button again to insert the sample. Wait for the sample to seat, then click the SPIN button to start spinning (if desired). The buttons will change from red to green as the function is established.

NOTE: the command bsmsdisp is available on the DRX500 spectrometers also.

11. Make sure the main window text entry area is highlighted and type lock . Then select your solvent from the popup menu according to the solvent you are using. Wait for lock to be established, and for the lock button in the BSMS display or on the BSMS keypad to show green.

On the Avance DRX500s:

12. Shim your sample using Z1, Z2 and Z3 shims on the keypad. Adjust the LOCK GAIN and/or LOCK POWER as necessary. NOTE: the ON AXIS and FINE functions must be **ON** on the keypad.

13. Gradient shimming is available on the Avance DRX500 spectrometers. To open the gradient shimming control panel type gradshim . Use of this routine is presented in the supplemental instructions on pg. 10. After gradient shimming completes close all the gradient shimming windows and return to your previous spectrum display.

On the AV-III spectrometers:

12. Shim your sample using Z1, Z2 and Z3 shim buttons in the BSMS display main window. To do this, click the desired shim and then click the up/down arrows below (by the knob graphic) to adjust the shim value. Adjust the lock gain and/or power as necessary.

13. Automatic shimming is available on the AV spectrometers. To use this, type topshim to begin the shimming process. Wait for shimming to complete (check the messages in the spectrometer status area) before proceeding.

H1 data acquisition

14. Tune the probe, if necessary (probe tuning usually is not needed for survey H1 acquisitions). See the Probe Tuning Instructions (pg. 17) for details.

15. Type acqu to display the acquisition window (for FID display).

16. Type rga to set receiver gain - wait for message telling you it is finished.

17. Type zg to start acquisition. The command halt can be used to stop an acquisition before the requested number of scans (ns) is completed. NOTE: during a longer acquisition

the command tr can be used to transfer the FID to disk. The ef command will then transform that FID and display the result. Use the acqu command to see the data acquisition window.

C13 / other nuclei experiment setup, data acquisition

5a. Use the edc command to create a new EXPNO for the C13 / other nucleus acquisition.
6a. Type, for example, rpar c13.probe to read the standard carbon-13 parameters, then click the OK button to load all highlighted parameter types (or select specific types first). The available standard experiments are listed starting on page 13 of this handout.
7a. To examine the acquisition parameters you can use either the eda (edit acquisition parameters) or the ased (acquisition setup editor) command or click the AcquPars tab above the spectrum window.
14a. Tune the probe. See the Probe Tuning Instructions (pg. 16) for details.
15a. Type acqu to display the acquisition window (for FID display).
16a. Type rga to set receiver gain - wait for message telling you it is finished. NOTE: in general, do not use rga for C13 observation.
17a. Type zg to start acquisition. The command halt can be used to stop an acquisition before the requested number of scans (ns) is completed. NOTE: during a longer acquisition the command tr can be used to transfer the FID to disk. The ef command will then transform that FID and display the result. Use the acqu command to see the data acquisition window.

Data processing and plotting (please see screenshots on pages 7 and 8)

18. To examine the processing parameters you can use the edp (edit processing parameters) command or click the ProcPars tab. This brings up a window in the spectrum display area; after examining and changing anything (if necessary), click the mouse cursor at the text-entry area at the bottom of the window to allow further command entry. NOTE: the FID is always stored on disk so the ef command can be retyped at any time to generate a new spectrum after, for example, a processing parameter has been changed.
19. After acquisition is finished type ef to do the Fourier Transform. NOTE: after transformation the spectrometer will automatically switch to the spectrum display window.
20. **PHASING**: Using the left mouse button, click the phasing icon on the menu to the top of the spectrum display to enter the phasing routine, or type .ph . The biggest peak in the

spectrum is set by default as the reference point, denoted by a red line. Move the mouse pointer over the 0 icon just above the spectrum, hold down the left mouse button and move the mouse vertically up and down to adjust the 0-order phase correction. When the biggest peak is phased correctly, repeat the above process using the 1 icon, then iterate back and forth between 0 and 1 until the spectrum is phased correctly. Note the phasing window has an extra icon bar like the integral window does (figure, pg. 7).

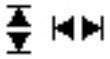
21. To save phasing and exit, click on the save/return icon above the spectrum, or type .sret .

22. **PHASING – alternate**: type apk to carry out automatic phasing on the spectrum. This will not work unless the signal-to-noise ratio of the spectrum is good.

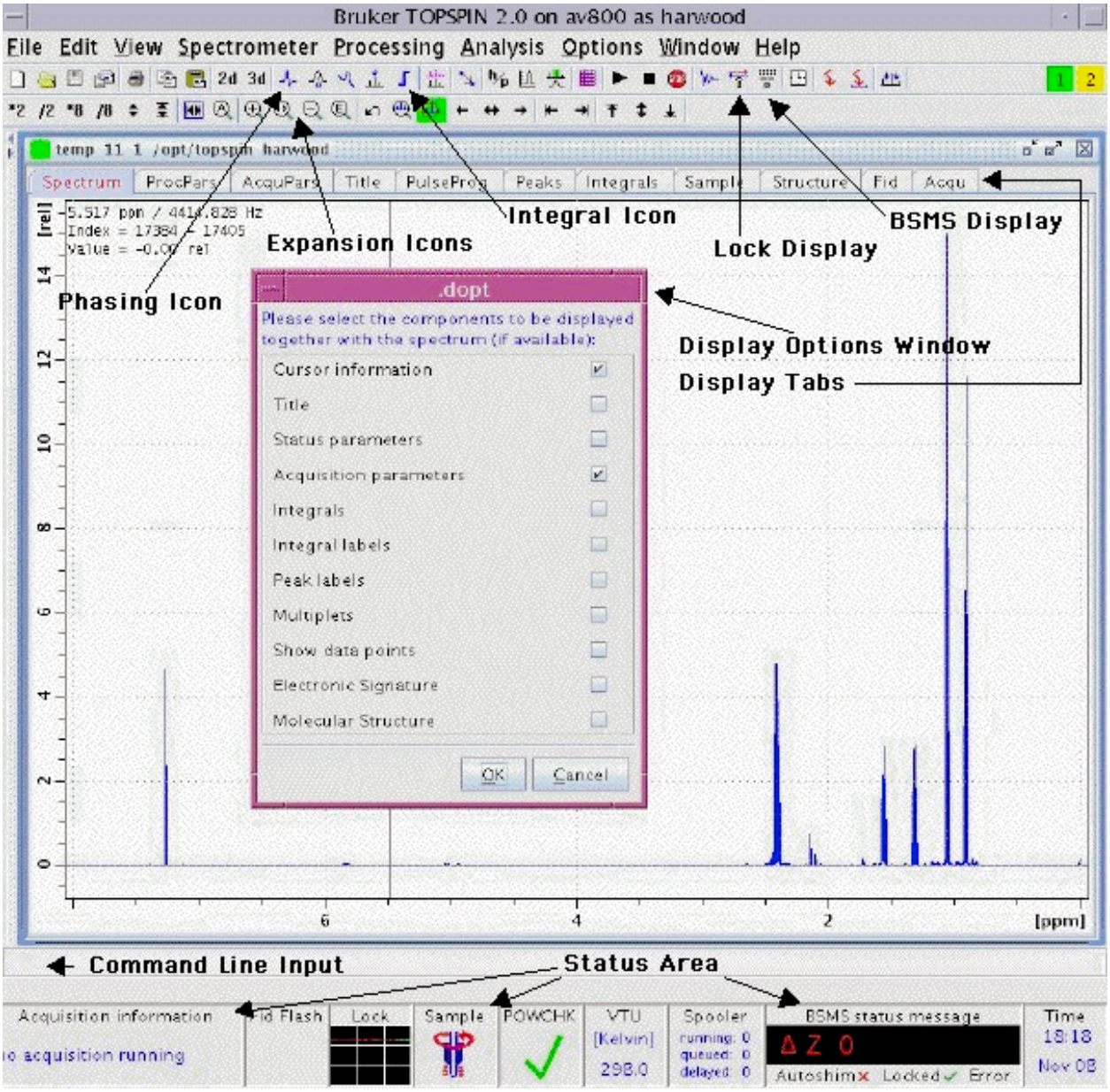
23. Use the abs or abs13c command to flatten the spectrum baseline.

24. **SPECTRUM MANIPULATION**: To expand the spectrum and to move and/or change the expanded region, use the arrow buttons above the spectrum display. These buttons have labels such as ↑ , → , etc. The mouse can be moved over the spectrum to give the chemical shift readout. The left mouse button will freeze the cursor in the desired location, and moving the mouse again will set an expansion region, which will become active when the left mouse button is released.

Other manipulations:

- *2, /2, *8, /8, adjust mouse wheel: adjust vertical scale up or down;
- < >, ><, ®, etc: expand or contract spectrum;
-  : click this icon to re-display the entire spectrum;
-  : click these icons to display the entire spectrum on the vertical or horizontal axes;
- type .zx to enter specific spectrum display limits (e.g. before #24, below);
- hold down right mouse button in spectrum window (or type .dopt)to bring up a menu of other display options - go to the "DISPLAY PROPERTIES" menu and turn on/off integrals, peak labels, etc., as desired - see the screenshot on the following page.

25. **CHEMICAL SHIFT REFERENCE**: Check the chemical shift of a known peak. If it is incorrect, left-click the calibrate icon above the spectrum display (or type .cal), then move the cursor to a known peak and click the left mouse button to set the chemical shift for this peak.



Above: Example TopSpin (version 2.0 shown) Window with Annotations

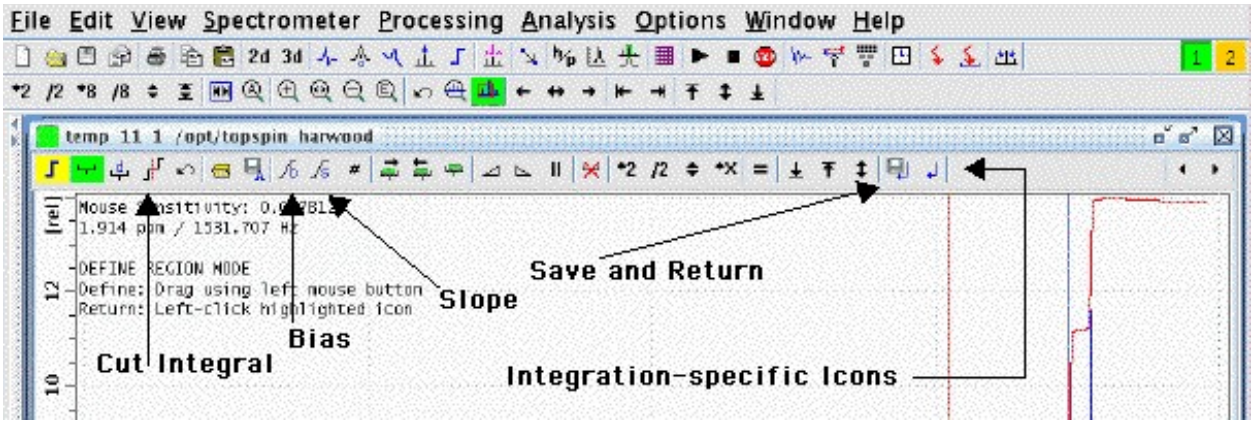
26. **DEFINE PLOT REGION**: To define a region for plotting, first display the desired region on the screen. Right-click on the spectrum to bring up the display options menu, then choose the "SAVE DISPLAY REGION TO PARAMETERS F1 F2…" option.

27. If you want to expand the spectrum vertically for plotting, type `cy` and enter the desired value; the default is 12 - 15 cm.

28. **TITLE:** To enter a title for the plot, type `setti` or click on the Title tab above the spectrum. Enter desired text. It is now not necessary to explicitly save the title file.

29. **PEAK PICKING**: To show peak picking type pps . If you want to change the peak picking threshold, type mi and enter a new value in the window that appears. Use the DISPLAY PROPERTIES menu to turn off peak labels on the display.

30. **INTEGRATION (see below)**: Click on the integral icon above the spectrum window or type .int . Move the mouse pointer to the spectrum and drag using the left mouse button to define the overall integral region – upon releasing the button the integral trace will be displayed. Adjust the slope and bias of the trace using the /b and /s icons above the spectrum in a similar fashion to phasing. NOTE: the bias affects the whole integral trace equally while the slope is frequency dependent with the downfield end of the trace as a reference point. If baseline correction abs has been used there is usually no need to adjust the integral slope and bias.



Above: Example TopSpin 2.0 Window during Integration with Annotations. Window during phasing is similar in that it has an extra row of icons just above the spectrum.

31. Now click the cut integral icon and using the left mouse button cut the integral trace as desired, then left-click again on the icon when finished. To delete the unwanted parts of the integral trace, move the mouse pointer above the trace, right-click, then select "DELETE CURRENT INTEGRAL." Repeat this for all unwanted integral regions. The same procedure is used to set an integral value, except the "CALIBRATE CURRENT INTEGRAL" choice is used.

32. When the integration is complete, adjust the vertical scale of the integrals using the *2 or /2 buttons immediately above the spectrum, then click on the save/return icon above the spectrum, or type .sret .

33. **PLOTTING**:

a) To start the plot efitor using the existing dataset and display limits type `plot0` . This will open a new window with the XWinPlot editor displaying your plot. The appearance of the spectrum can be altered prior to plotting by right-clicking on the spectrum in the XWinPlot window and selecting the 1D/2D-edit choice. The window that appears can be used to manipulate the scaling and expansion of the spectrum. To print the spectrum left-click on the File pulldown, followed by Print → Print (click-box). Please see the hardcopy manual "Plotting" for further details on the plotting routine.

b) There is a rudimentary "internal" WYSIWYG printing routine accessible using the command `prnt` (p r n t). Items that are displayed in the spectrum window will be on the print output. This will bring up a window with various printing-related options - choose as appropriate.

c) The command `print` (p r **i** n t) will bring up a menu allowing you to choose XWinPlot (with or without editing) or direct printing. If you chose XWinPlot with editing, check that the correct LAYOUT= is selected, as follows: for H1, +/1D_H.xwp; for C13, +/1D_X.xwp.

Finishing up with TopSpin and logging off the computer

34. Using the BSMS display or keypad (Avance DRX500s), remove your sample and replace it with the $CDCl_3$ standard following steps 9 & 10 above.

35. If you have been running spectra other than proton, use the `re` command or the data browser (step 5, note 3) to load a proton dataset.

36. Type `standard` to set up the spectrometer for the standard $CDCl_3$ sample.

37. Touch up the shims of the $CDCl_3$ standard sample following step 12 above.

38. Close the lock window using the button in the upper right corner or the icon on the upper left.

39. Type `exit` to leave the NMR program.

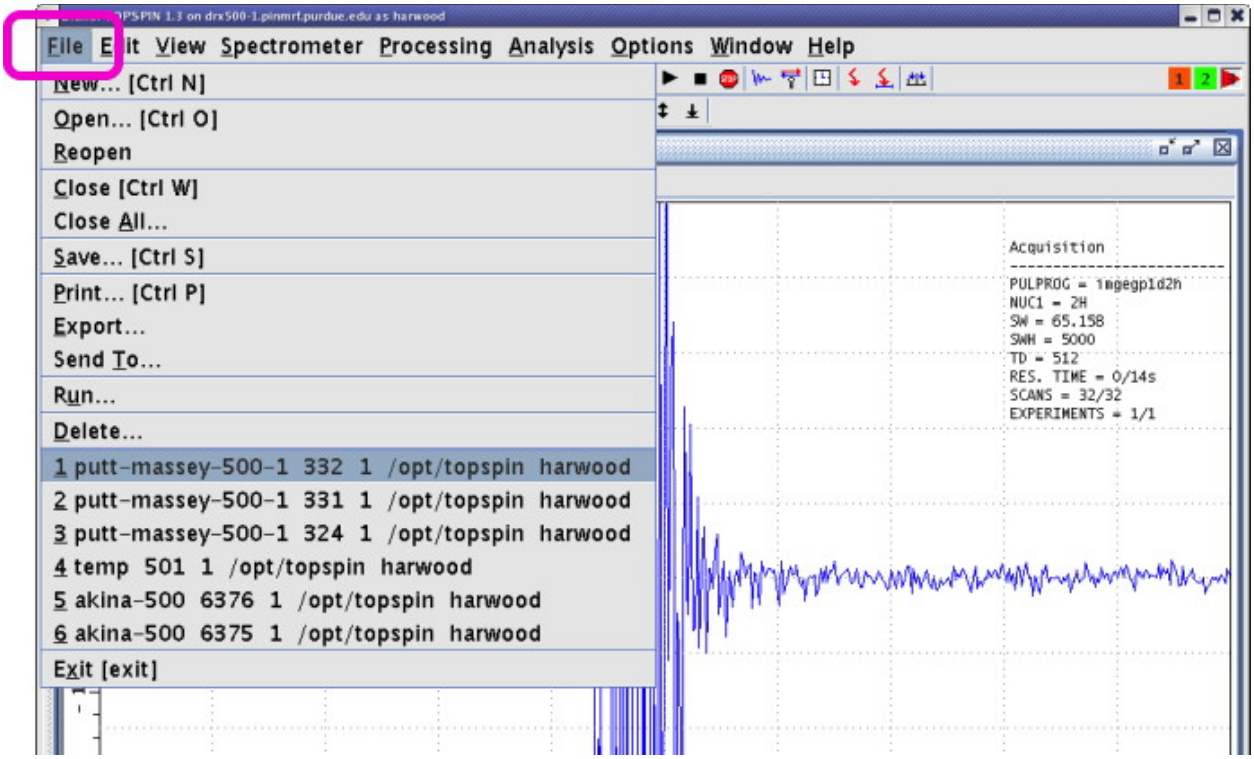
40. Move the mouse pointer to the background of the Linux desktop and hold down the right mouse button. A desktop menu will appear. Keep the right mouse button depressed and move the cursor to the Logout choice, then release the right mouse button. A logout screen will appear, click Logout to confirm your logout from Linux.

Supplemental Instructions

S1. **USING THE SAMPLE TEMPERATURE CONTROLLER:** On all of our TopSpin spectrometers the temperature controller is always operating, and the default sample temperature should be set to 20 - 26˚C (293 – 300K). To set the sample temperature, within the range of -40˚C to +80˚C type `edte` to open the temperature-control window (NOTE: not all of our TopSpin spectrometers will achieve this temperature range without additional hardware being set up. Some of our TopSpin spectrometers are capable of a wider sample-temperature range. Please consult with PINMRF staff prior to attempting any variable-temperature work). To change the temperature set point, click the SET or CHANGE button next to the "Target temp." readout. In the pop-up window, enter the new set point (**in degrees Kelvin!**) in the "Sample target temp" field, then click APPLY and OK to load the new set point. Next, set the Probe Heater power limit by clicking on the SET MAX.. button adjacent to the "Heater" readout (this is not necessary on the AV-III-400-HD & AV-III-500-HD). In the pop-up window, set the heater power limit value either by typing it in the field or adjusting the slider, then click on APPLY and OK to load the new heater limit. Ensure that the Probe Heater is enabled – the button adjacent to the power limit readout should read "on." If it shows "Off" click it to turn it on. To close the edte window, click the X icon in the upper right-hand corner of the window. Be sure to set the temperature back to 20 - 26˚C (293 – 300K), and wait for the sample temperature to equilibrate at this point, before logging off the spectrometer.

S2. **USING THE GRADSHIM ROUTINE (Avance DRX-500s):** Type `gradshim` to open the control panel. See picture on next page. In the "Shimming Method" (upper) part of the panel highlight the button for the 1D2H choice. Next, check that your userid is entered in the USER field in the "Data Set" panel. In the "Iteration Control File" panel check that the FILENAME is deflt1d2h . Then, click on the "Start Gradient Shimming" button to start the process. The gradshim routine will then switch from the currently displayed data to a new window showing the acquisition of the gradshim data. At some point a new window will pop up showing the shimming results. The shimming process will repeat and the results window will be updated.

After the process completes you can close all of the gradshim popup windows by clicking the "Exit" button in the upper left corner of the gradshim control panel (you may have to click on the border of this window to make it active). Then, make sure you return to your original spectrum display window before proceeding (NOTE: the gradshim data acquisition window will still be shown in TopSpin even after terminating the gradshim process). One way to do this is to click on the "File" button in the upper left of the TopSpin window, then select your dataset from the pop-up list. The most recent dataset (i.e., the one which was loaded when gradshim was started) is dataset #1 in the list. See the figure below.



Above: "File" Pulldown Menu in TopSpin 1.3.

S3. **OPENING A LINUX TERMINAL WINDOW:** Left-click the RedHat icon in the lower left corner of the screen, go to the "System Tools" menu, then highlight the Terminal choice and release the mouse button.

TopSpin

Commonly Used Keyboard Commands

NOTE THAT ALMOST ALL COMMANDS ARE AVAILABLE FROM THE PULL-DOWN MENUS AT THE TOP OF THE SPECTRUM DISPLAY

Parameter Setup
edc, new - create new datasets
eda - edit acquisition parameters - full menu
ased, edasp - edit acquisition parameters - pulse program driven or nucleus-related
gpro - update current experiment with current probe's pulse calibration parameters
edte - set up temperature controller parameters
edp - edit processing parameters
wrpa - copy current dataset to a new one - specify new name and experiment number
re - read existing dataset - specify its name and experiment number
rpar - read parameter set

Data Acquisition
lockdisp - display lock window
lock - start autolocking routine - select solvent from popup window
solvent - set solvent parameter
rsh - read shim set
wsh - save shim set - enter a unique name in the menu
bsmsdisp - open the BSMS panel display
topshim - start automatic shimming routine (AV-III spectrometers)
gradshim – open automated gradient shimming control panel (Avance DRX-500s)
ii - initialize interfaces
acqu - display acquisition window - shows FID on screen
atma - start automatic probe tuning routine
rga - set receiver gain automatically (do not use for C13 observation)
ns - number of scans
zg - zero current data and start acquisition
go - start data acquisition
tr - transfer FID to disk for processing
halt - halt data acquisition after next scan
stop - stop data acquisition immediately
standard - set up spectrometer for $CDCl_3$ standard sample

Custom Acquisition AU Programs for Advanced 1D Spectra
deptjsh – turn current C13 dataset into DEPT experiment and acquire
homodecjsh – turn current H1 dataset into a homodecoupling experiment and acquire
noediffjsh – turn current H1 dataset into a 1D nOe-difference experiment and acquire
presatjsh – turn current H1 dataset into a presaturation experiment and acquire

Data Processing and Plotting
.dopt - open display options menu
setti - enter title for plot
ft - Fourier transformation
ef - exponential multiplication and Fourier transformation
efp - carry out "ef" plus apply existing phase corrections to spectrum
apk - automatic phase correction of 1D spectrum
abs - apply automatic baseline straightening to spectrum
abs13c - apply automatic baseline straightening to a C13 spectrum
lb - controls amount of exponential multiplication

.ph - enter phasing routine
.int - enter integration routine
.sret - save results and return from phasing, integration or peak-picking routine
.ret - return from phasing, integration or peak-picking routine without saving
sref - set chemical shift scale reference using TMS or default parameters
.cal - enter spectrum calibration routine
nzp - number of data points to zero at start of FID
zp - zero nzp points at start of FID
.basl - enter baseline correction routine
.zx - enter numeric limts for display/plot region
pscal - define plot vertical scaling method
cy - plot vertical scaling
mi - threshold for peak picking
pps - peak picking with output on screen
.pp - peak picking with menu choices
print - open plotting menu to define plotting choices
plot0 - start XWinPlot standalone program for plotting
prnt - start internal plot routine

Data Handling
dir - show a listing of your experiment names
dirf - show a listing of all 1D FID files (includes experiment names and numbers)
dirser - show a listing of all 2D SER files (includes experiment names and numbers)
dirs - show a listing of all 1D spectra (includes experiment names and numbers)
dir2d - show a listing of all 2D spectra (includes experiment names and numbers)
dels - delete 1D processed spectra (FID's are not deleted)
del2d - delete 2D processed spectra (SER files are not deleted)

Notes

Bruker Avance DRX Spectrometer Parameter Sets - 10-28-2019

Parameter Sets

DRX-500-1 & 2 (BBFO probe)
h1.bbo
c13.bbo
c13dept.bbo
f19.bbo
p31.bbo
p31nd.bbo (no decoupling)
c13hsqc.bbo
cosy.bbo
hmqc.bbo
hmbc.bbo
noesy.bbo
tocsy.bbo
h2.bbo
al27.bbo
b11.bbo
li7.bbo
n15.bbo
n15hsqc.bbo
si29.bbo
si29ineptrd.bbo
sn119.bbo

Parameter sets for other nuclei can be set up upon request.

Shim Files

DRX-500-1 & 2 (BBFO probe)
shims.bbo

Bruker AV-III Spectrometer Parameter Sets - 10-28-2019

Parameter Sets

AV-III-800 (QCI cryoprobe)
h1.cryo
presat.cryo
c13.cryo
c13dept.cryo
p31.cryo*
p31nd.cryo*
c13hsqc.cryo
cosy.cryo
hmqc.cryo
hmbc.cryo
noesy.cryo
noesypr.cryo
noesypr1d.cryo
tocsy.cryo
tocsypr.cryo
3d-hnca.cryo
3d-hnco.cryo
h2.cryo
n15.cryo
n15ineptrd.cryo
n15hsqc.cryo
n15hsqc3919.cryo

*PINMRF staff assistance required

Shim Files

AV-III-800 (QCI cryoprobe)
shims.cryo
shigemi.cryo

AV-III-500-HD (BBFO cryoprobe Prodigy)
h1.cryo
presat.bbo
c13.cryo
c13dept.cryo
f19.bbo
p31.cryo
p31nd.cryo (no decoupling)
c13hsqc.cryo
cosy.cryo
hmqc.cryo
hmbc.cryo
noesy.cryo
tocsy.cryo
h2.cryo
b11.cryo
n15.cryo
n15ineptrd.cryo
n15hsqc.cryo
n15hsqc3919.cryo
si29.cryo

AV-III-500-HD (BBFO cryoprobe Prodigy)
shims.cryo

AV-III-400-HD (BBFO SmartProbe)
h1.bbo
c13.bbo
c13dept.bbo
f19.bbo
p31.bbo
p31nd.bbo (no decoupling)
c13hsqc.bbo
cosy.bbo
hmqc.bbo
hmbc.bbo
noesy.bbo
tocsy.bbo
h2.bbo
li7.bbo
b11.bbo
n14.bbo
n15.bbo
n15ineptrd.bbo
n15hsqc.bbo
si29.bbo
si29nd.bbo
si29ineptrd.bbo
as75.bbo
se77.bbo
sn119.bbo
sn119nd.bbo
te125.bbo
te125nd.bbo
pt195.bbo

AV-III-400-HD (BBFO SmartProbe)
shims.bbo

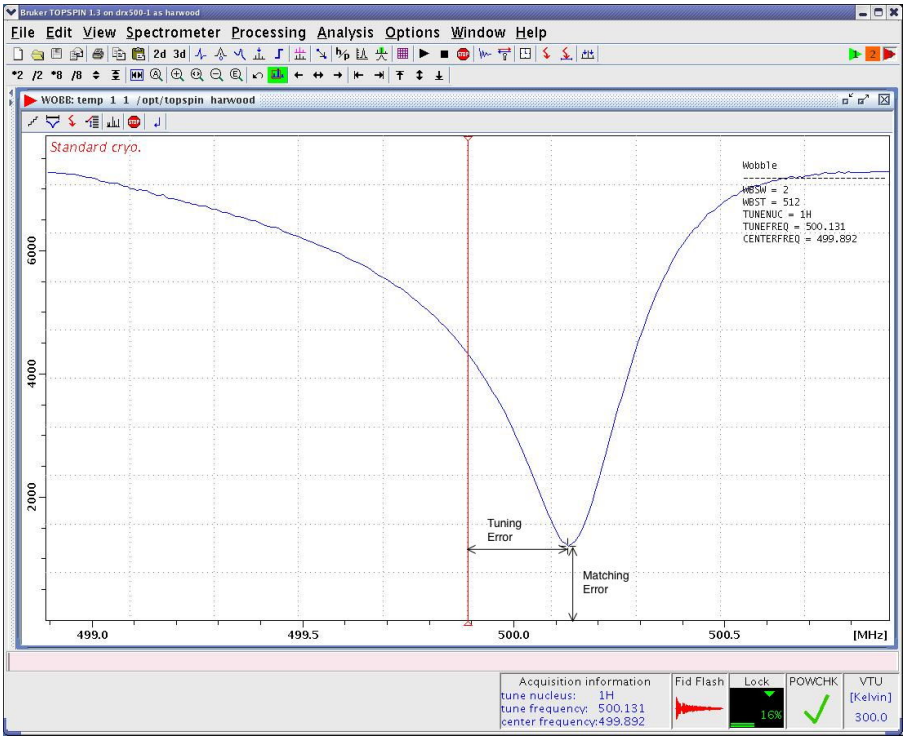
PINMRF TopSpin Spectrometers – Probe Tuning Instructions

1. After the desired experiment is set up, type `atma` to start the automated probe tuning routine. If you are planning on running several experiments on the same sample, for probe tuning be sure to first set up the experiment which uses all the different nuclei which you will be measuring.

2. A `wobb` window will appear as shown below. You will observe the computer optimize the probe tuning for all relevant nuclei. When probe tuning is optimized the dip will be centered on the red line and will extend as close as possible to the baseline.

3. When all tuning is completed `atma` will terminate itself.

4. NOTE: on all of our Bruker TopSpin spectrometers, **all experiments** except for H1 survey acquisitions will require tuning of **all relevant nuclei**.



5. After running experiments using any nuclei other than H1 or C13 you must create a C13 dataset and tune the probe in this new dataset. Do this prior to finishing up as in step 34, page 9.

PINMRF TopSpin Spectrometers – Approximate Sensitivity

Console/Probe	H1 sensitivity	C13 sensitivity
AV-III-800, QCI cryoprobe	6200:1	1000:1
AV-III-500-HD, BBFO cryoprobe Prodigy	1350:1	750:1
AV-III-400-HD, BBFO SmartProbe	450:1	200:1
DRX500-1 or -2, BBFO ATM probe	450:1	200:1