### BRUKER AV-500 OPERA TION MANUAL

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**BRUKER AV-500 OPERATION MANUAL**

Open TopSpin by double-clicking on the program icon:

+ Place your sample in one of the spinners, use the sample gauge to center it, and place it in one of the empty slots in the SampleCase system, noting the sample number. Minimum sample height is 40 mm. Failure to use the depth gauge correctly will result in bad shims and may even break your sample and/or the instrument. The depth gauge should be set to 2.

+ Type `sx ##` (where ## is the location of your sample). This will place your sample inside the magnet. You will see this icon at the bottom of the screen when there is a sample in the magnet and the lights will turn yellow.

+ Type `new` or select **[Start] - [Create Dataset]**. This creates a directory where your files will be saved (automatically). Select **[OK]** when finished filling out the dialog box below:

   ![New dialog box](image)

   For both EXPNO and PROCNO, start with “1”; increment EXPNO as you add more experiments on this sample, i.e., 1 is 1H, 2 is 13C, 3 is COSY...

   Click on **[Select]** for the list of parameter sets (see next page) or type it in if you know it.

   Where your data is saved (you shouldn’t need to change...
Check the “Show Recommended” box for the shortened list of experimental parameters, then

**Common 1D experiments**

**PROTON** (default 16 scans)

To decouple F19, use 1H_dec19F_BBO or 1H_dec19F_TBO in the /user directory.

**C13CPD** (default 1024 scans, 54 min)

**C13DEPT90** (CH carbons only, default 256 scans, 13 min)

**C13DEPT135p** (CH, CH\(_3\) up; CH\(_2\) down, default 256 scans, 13 min)

**P31CPD** (default 16 scans)

F19 needs probe specific parameters in the /user directory:

- **F19_dec1H_BBO** (128 scans) and **F19_TBO** (default 16 scans)

Alternatively, you can read in a set of parameters using the **Read Pars.** button.

**Caution:** reading in a set of parameters on top of a currently open dataset will overwrite that dataset.

**1D Acquisition**

+ Select the [Acquire] tab. The TopSpin acquisition menu is as follows.

Since we are using the autosampler, **SKIP** .

**Do not spin.** The shims on the AV-500 are quite good without spinning.

+ Type **lock** or select **Lock** , choose your solvent from the list, then select [OK].

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Select by clicking on it. This performs the automatic tune and match (atma) for your individual sample. You will need to tune for every new nucleus run on this sample.

To load the standard shims, type rsh in the command line, and choose the correct shim file, then [Read]. There will be shim files for both available probes on the instrument, BBO and TBO. See instrument logbook for current shim file name.

Skip and use instead, which will execute automatic shimming (topshim) with convection compensation for non-spinning samples. Type topshim gui to see results or you can also shim from this interface. See below for manual lock/shim.

Select to load in probe specific parameters, e.g., pulsewidths and power levels (getprosol). Execute this command for every experiment run on your sample or you will get no signal.

Select to automatically set receiver gain (rga).

Changing parameters

In the [AcquPars] tab, you can change experimental parameters, or type in the code and enter the new value in the dialog box. After typing in the new value, make sure to hit the “enter” key on the keyboard.

Some key parameters are:

ns = number of scans
d1 = relaxation delay
sw = sweepwidth
o1/o1p = offset (center) of sweepwidth
aq = acquisition time
in Hz/PPM
Start acquisition

+ Select 🎥 to start the acquisition ( zg ).

When the acquisition is finished, your data will be saved automatically in the filename and expno you specified in the [Create Dataset] window.

Processing 1D data (see processing manual for more details)

+ Type efp (exponential multiplication, fourier transform and phase) to process the spectrum and apk to phase it.

During the acquisition, you can type tr in the command line to transfer current data so you can process it (similar to the bs command in vnmr).

Other useful buttons and commands

- start acquisition ( zg )
- halt acquisition ( halt ) and retain data. halt 16 will halt after 16 scans have been acquired.
- stop acquisition ( stop ), but lose all data
- or open BSMS window for manual locking and shimming
- use displayed region to set desired sweepwidth ( sw ) and offset ( o1 )
- use displayed region to set desired offset ( o1 ), i.e., center of spectrum
- or expt calculates the experiment time.

iexpno will increment to the next experiment number so that you can run an identical experiment without having to repeat the tune and prosol commands. If you want to run a different experiment, use the 📜 Read Pars. button to read in a new parameter set. iexpno 2 will increment to expno 2.

tr will transfer data during an acquisition so that you can look at it by typing efp.

   tr 8 will transfer data after 8 scans.

re 2 will move to expno 2.
2D Acquisition

+ Collect a 1D spectrum as above, in expno 1. Note: If you don’t want to use the default C13 sweepwidth for HSQC data (default = 160 ppm), you will also need a C13DEPT135; for HMBC (default = 222 ppm), you will need a C13CPD.
+ Start a new dataset by typing new or select [Start]- [Create Dataset]
+ Change EXPNO to 2 (if your 1D was in 1) or enter a new filename.

Common 2D experiments

COSYGPSW (gradient COSY using a narrow sweepwidth; can do single scan, default 1 x128, 5 min)
NOESYGP (phase-sensitive gradient NOESY; use multiples of 2 scans, default 16x256, 3 h)
NOESYPHSW (phase-sensitive NOESY with narrow sweepwidth; use multiples of 2 scans, default 4x256, 44 min)
HSQCEDETGP (gradient HSQC, phase sensitive: CH₂ opposite phase than CH and CH₃; can do single scan; default 2x256, 14min)
HSQCEDETGPSISP_ADIA (gradient HSQC, phase sensitive: CH₂ opposite phase than CH and CH₃; sensitivity enhanced with adiabatic pulses; can do single scan; default 4x256, 39min)
HMQCGP (gradient HMQC; can do single scan, default 4x128, 14min)
HMBCGP (gradient HMBC with decoupling during acquisition; multiples of 2, default 4x128, 15 min)
HMBCGPND (gradient HMBC without decoupling; see single-bond CH peaks as doublets; multiples of 2, default 8x128, 32 min)

Alternatively, you can read in a set of parameters using the read pars. button. Caution: reading in a set of parameters on top of a currently open dataset will overwrite that dataset.

+ In the [Acquire] window, select Tune if this is a heteronuclear experiment. (If this is a COSY, and you just ran a proton, the probe should still be tuned.)

+ Select Proso to load the pulse width and power levels into the parameter set

+ Then setLimits. This will open up a new dialog box. Do not click [OK] yet.
To open the 1D proton spectrum, click and hold the left mouse button to drag the 1D proton dataset into the data window. Expand the spectrum to display all peaks, leaving ca. 0.5 ppm of baseline on either side of the spectrum.

Then click [OK]. You will get the following message:

Click on [Close].

Select [AcquPars], go to A, and check that F1 and F2 dimensions (esp. for paramagnetic COSY) are equal if you are collecting a symmetrical homonuclear dataset (ie, COSY, NOESY).

To change parameters, see below. Otherwise,

Select Gain and then Go.

Changing parameters (2D)

Under the [AcquPars] tab, click on A to display all the acquisition parameters.

Alternatively, type the parameter in the command line, e.g., sw, o1p, or td [note td in F1 dimension is the number of increments (ni) in VNMR], and enter the desired value in the dialog box:
For NOESY:

For a NOESY spectrum, it is important to select the appropriate mixing time: d8

<table>
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<tr>
<th>D8 [sec]</th>
<th>0.450</th>
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NOTE: The mixing time depends on the size of the molecule. The range for biomolecules is typically from 0.05 to 0.2 sec, medium size molecules from 0.1 to 0.5 sec and small molecules 0.5 to 0.9 sec.

For TOCSY:

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<th>D9 [sec]</th>
<th>0.08000000</th>
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NOTE: A mixing time of 0.06 to 0.08 sec is typical for the TOCSY experiment.

For HSQC:

The parameter set HSQCEDTGP has a fixed F1 sweep width of 160 ppm, which is big enough to cover the protonated resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the button for a second time. In this case a 1-D carbon experiment on the same sample has to have been collected previously. Drag in the carbon experiment and expand the spectrum to display all peaks, leaving ca. 2 ppm of baseline on either side of the spectrum.

For HMBC:

The parameter set HMBCGP has a fixed F1 sweep width of 222 ppm, which is big enough to cover all carbon resonances for a broad range of samples. If desired, changes to the F1 sweep width can be done by using the button for a second time. In this case a 1-D carbon experiment on the same sample has to have been collected previously. Drag in the carbon experiment and expand the spectrum to display all peaks, leaving ca. 2 ppm of baseline on either side of the spectrum.

Processing 2D data (see processing manual for more details)

+ Type xfb to process the spectrum.
Logging out

When you are finished with your experiments, go to the button and Terminate TopSpin. This will exit the program and log you out of the workstation. To retrieve your sample, advance the carousel manually so that you can remove your sample safely.

Manual lock and shim

+ Open the BSMS window with either of these buttons:  or

+ Check the External box so that you can move the window around. This Main window has the key shims to adjust. The Shim tab will have a lot more.
+ Type `lockdisp` or double-click and the lock display will open. The figure on the left shows an unlocked signal; the signal is locked on the right.

+ Go to the [Lock/Level] tab in the BSMS window and select Lock [On-Off] (green is on; red is off); select [Field] to adjust Z0 (field offset) so that the 2 peaks are centered in the screen, and [Phase] to adjust the peaks to the same height, by stepping +/- or by using the wheel. Adjust [Power] or [Gain] to put signal on screen if it goes offscale. Turn lock back on when you are finished and it should look like the figure on the right, above.

+ Select [Main] to manually shim. Select desired shim (Z, Z2, Z3, etc) and change steps (+/-) to increase the position of the lock.
To shim the XY shims automatically

The Topshim routine does not adjust the non-spinning shims automatically. This is a useful procedure to do if your peaks are slightly broader than expected and fine splitting is not observed. Broadening can result from poor quality tubes (or good quality tubes that have been treated poorly).

This probably will not help much for C13 spectra or paramagnetic proton spectra, or spectra with naturally broad lines (large MW polymers) and it will add time to the shimming routine.

+ Enter `topshim gui` on the command line. The TopShim graphical user interface dialog box will open.

![TopShim GUI](image)

+ In the Tune section, select either the Before or After buttons (or both), and use the dropdown menu to select the Z-X-Y-XZ-YZ-Z option. This will shim these shims using the lock level either Before or After (or both) the regular TopShim routine.

+ Type `convcomp` in the PARAMETERS box. (Note: this compensates for possible convection currents in your sample due to temperature gradients; this is what we set up the TopShim button to do.)

+ Select [Start] and wait for it to finish.