USER MANUAL FOR BASIC OPERATION AND NMR DATA ACQUISITION ON BRUKER AVIII(HD) SERIES SPECTROMETERS

Ver 4.5
revised 2019

Contact: Dr. Alec Krunić
Facility Director
COP Rm B72
ext. 64364

CONTENTS

INTRODUCTION

This user manual is intended to be a beginner's guide for the acquisition of basic 1D and 2D spectra on NMR spectrometers operating under Bruker TOPSPIN™ software. Although it contains step-by-step acquisition protocols, it is not meant to be used as a comprehensive guide for advanced experiments. A more detailed version of the same can be found in Bruker's software manuals available through the 'Help' menu in the main programme window (User Manual, Command Index, Parameter Index etc.).

Each user is expected to follow the rules and regulations of the MCP Magnetic Resonance Facility (MRF) outlined in this manual, in order to minimise operational interruptions.

Rules and regulations of the MCP MRF facility:

Do's

1. *In order to reserve instrument time, sign up on the sheet posted on the notice board across from 539 (MCP Main Office). Follow the posted instructions, also found on the Facility website (http://lab.pharm.uic.edu/nmr/).*
2. *Instrument logbook must be signed after every instrument session. Record any instrument-related issue in the logbook(s) and notify the Facility Director.*
3. *While being in the MCP MRF, keep all metallic, electronic and magnetic appliances away from the magnets.*
4. *Visitors and guests to the MCP MRF must be accompanied at all time and must be familiarised with basic magnet safety.*
5. *Clean up after every use.*
6. *Users are strongly encouraged to pay attention to notices posted outside or on the message board inside the facility.*

Don'ts

1. *Unauthorized access is strictly prohibited.*
2. *Spectrometer and computer settings must not be changed under any circumstance.*
3. *Do not alter the positon of the sample depth gauge slider.*
4. *No personal notes, comments or complaints should be written in the logbook.*
5. *Eating, littering and irresponsible usage of the facility will not be tolerated.*

AVAILABLE INSTRUMENTATION

Magnetic Resonance Facility, located in the B71 Suite, houses two Bruker Avance AV 400 series digital NMR spectrometers (9.4T/400 MHz magnets, 2004 vintage). Both systems were installed in late 2016/early 2017.

Selected features include:

- Sample changer (BACS 60) for 5 mm NMR tubes on the AVIII 400
- Networked MS Windows®-based computers with TOPSPIN software suite for acquisition and off-line processing of NMR data
- ATM (automatic tuning and matching) probes on both spectrometers
- Nitrogen evaporator for low temperature experiments on both spectrometers

Both instruments are equipped with direct-observe 5 mm Z-gradient room temperature probes, in which inner coil is sensitive for a range of X-nuclei and the outer coil is sensitive to proton.

Default probe on the AVIII HD spectrometer is a room temperature SmartProbe™ that can be used for detection of nuclei from ^{19}F to ^{109}Ag. ***Allowed*** temperature range on these probes is –150 ºC to +150 ºC, however the operating range depends on additional factors that may effectively reduce it.

AVIII 400 spectrometer is equipped with a PABBO (broadband observe probe) that can be tuned to a variety of nuclei from ^{31}P to ^{109}Ag. Spectrometer is configured with a BCU-05 cooling unit for variable temperature experiments down to +5ºC. Alternatively, it can be fitted with a nitrogen evaporator when lower temperatures are needed.

LABORATORY SAFETY AND SECURITY

NMR superconducting magnets contain large volumes of cryogenic liquid (helium and nitrogen). Except in rare instances the cryogens boil off over time at normal rates. In case there is loud noise followed with abrupt discharge of gases in the room, LEAVE THE ROOM IMMEDIATELY!!!

Magnetic Resonance Facility is equipped with oxygen sensors and low oxygen alarms located on the outside (orange strobe) and inside the B71 suite (orange strobe). In the event the alarm is triggered, LEAVE THE ROOM IMMEDIATELY (USE THE NEAREST EXIT)!!!

NOTIFY MCP MAIN OFFICE (SUITE 539) PERSONNEL AND WAIT FOR "ALL CLEAR" NOTIFICATION FROM THE DEPARTMENT BEFORE RE-ENTERING THE FACILITY.

Magnetic objects may pose a hazard or may be permanently damaged, **including pacemakers and medical implants[1], mechanical watches, cards with magnetic stripes, diskettes, coins and keys.**

Magnet safety tip: BEFORE APPROACHING A MAGENT, LEAVE PERSONAL ITEMS IN B73. Moving magnetic material affects the magnetic field and disturb the measurements in progress.

Safety note: Magnetic field has no known harmful medical effects.

NETWORK AND DATA SAFETY

MRF is on its own internal network with limited Internet access.

Processed data is removed on the regular basis from each workstation.

Acquisition data is backed up periodically and eventually transferred to removable media upon user's departure from the Department.

Users may transfer their data for off-line processing, either via CD/DVD media or network file share (see Data transfer on p13). **USB devices are not supported because of security risks they may pose**.

[1] Certain medical implants may be allowed with proper clearance from the University Health Service. Contact Facility Director for further details.

ACCOUNTS AND LOGGING IN/OUT

Before logging in on any spectrometer, you must have a valid USER ID and password. A valid account and password can be acquired by:

1) Submitting a memo from your supervisor to the Facility Director and
2) Filling out new user form (can be found on the Web at http://lab.pharm.uic.edu/nmr/) and attending instrument training.

Users are expected to maintain the required code of conduct in the facility at all times in order to run the MRF smoothly and efficiently. Report any problem or abuse of the instruments immediately to the Facility Director.

We also welcome suggestions and ideas from all users.

Login procedure for PC workstations running Microsoft Windows® 7:

- **SIGN IN** the spectrometer logbook before you start

- **<Ctrl><Alt><Del>** to login or click the login window
  - Type in your login ID (group ID) and password to begin
  - click on the **TOPSPIN 3.2** icon to start the NMR software

If you fail to login after several attempts, the account will be temporarily frozen for the security of your stored data.

Remember, you can either use the buttons, pull down menus, tabbed menus, or the command-line equivalents in Topspin.

- Once a data collection is finished follow the procedures for sample handling. Exit the TOPSPIN programme, wait for all windows to close and logout of the workstation

- Fill out the spectrometer logbook to complete the sign out process

SAMPLE HANDLING

- **Remove the port cover!**

- **Press the [Lift On/Off]** button on the BSMS keyboard/BSMS display or type **ej^** to lift the standard H2O/D2O sample, remove it from the magnet and place in the sample holder found near each workstation

- **Insert a clean NMR sample tube (**0.5-0.6 mL of a clear solution represents an optimum sample volume) into the spinner, and set the tube position as indicated with the **5/8 mm** coil length marking on the SDG - **DO NOT CHANGE THESE SETTINGS ON THE SDG**. SDGs differ between AVIII and AVIIIHD, use the appropriate one for each instrument. Wipe the sample tube clean **BEFORE** loading it in the spinner, then clean the spinner as well. The sample tube should fit snuggly inside the spinner

    - *Note*: **Keep both the rotor and the NMR tube free and clear of fingertip grease! Use Kimwipes provided, once cleaned do not touch the spinner with bare hands. Check your tubes for any outside dirt**

    - IMPORTANT: Use ONLY high-quality NMR tubes in the 400 MHz

Wilmad	Norell	Kontes	New Era
535-PP	**CONSULT FACILITY MANAGER BEFORE PURCHASING**		
528-PP			
527-PP			
507-PP			
WG-1000			

- **Place the sample in the sample port**, then press the **[Lift On/Off]** button to lower the sample in the magnet (click on Lift button on the BSMS display or type **ij^** in the command line. Watch the sample, to ensure that it goes down (sometimes they get stuck at the top)

- **Insert the sample only if you hear the hissing sound of the sample lift gas**

- **Sample spinning is no longer necessary [SPIN] button should always be off**

- **If a sample tube is broken inside the instrument or the standard sample is missing, report it to the Facility Director immediately**

- **When you complete the data collection run, load the standard sample back and return the port cover to its place**

DATA ACQUISITION AND PROCESSING

ACQUISITION AND PROCESSING OF 1D ^{1}H, ^{13}C, ^{31}P AND ^{19}F SPECTRA:

NMR experiments are created from the TOPSPIN main window. TOPSPIN software features a data browser on the left hand-side, tabbed document interface in the centre, and additional options for processing and analysis at the top in the form of expanding pull-down menus and horizontally arranged buttons – **NOTE:** TOPSPIN will display your most recent experiment on startup, if not, open any of your datasets using browser on the left side of the screen.

TOPSPIN also supports a multi-window mode, where you can keep more than one spectrum opened at a time. To open a new window enter **newwin^** in the command line.

NOTE: *All buttons in the user panels are interactive. Point the mouse cursor to any button, and its description will show up at the bottom of the screen.*

The following instructions represent a stepwise protocol for acquiring 1D NMR spectra for the most common nuclei, once the sample has been loaded.
Character "^" denotes the Enter key.

(a) **edc**^ (or **new**^) - edit current data set. In TOPSPIN, your data will be stored in the user data folder defined when you open up the programme for the first time. This is usually the same as your login name.
In case the computer cannot find or read your data set, contact the Facility Director. Choose a new file name and set experiment/process values to 1. At this point you may also select an experiment from a list of available experiments and continue the basic setup from this window. This will allow you to set the appropriate solvent, load the proper probe-related acquisition parameters (execute 'getprosol' option), create a new experiment window, and enter additional information about your sample ('Title box') at this point. You can always revisit this parameters later on, and change them again if necessary. *When using this option skip the remaining steps and go to (e)*.

(b) **rpar**^ - reads parameter file. A parameter file contains a complete set of instrument parameters required for running an experiment.
NOTE: User defined and Bruker default experiments are kept separately in TOPSPIN. They are accessible from a pull down menu in the top-right corner of the rpar window.

(c) Select '+PROTON', representing the parameter file for running a ^{1}H experiment. A new window opens up. Click on the 'OK' button to load all the listed parameters.
List of available experiment on both spectrometer is included in Appendix A. Details about the particular pulse programme code used in data generation can be found under the 'Pulseprog' tab.

(d) **eda^** or click on the Acquisition (AcquPar) tab to edit acquisition parameters. Certain parameters need to be updated for each experiment loaded from the rpar list. You will have to scroll down and find a parameter named 'SOLVENT' and choose the correct solvent from the pull down menu. You will also have to find the 'GETPROSOL' button at the top of the window (looks like an NMR tube) and click on it.
If you already completed it under (a), skip this step.
If you want to see the abbreviated list of parameters click on the button that looks like the Greek letter "Π" (or type **ased^**), to go back to the full list click on the button "A". When done updating experimental parameters, click on the 'Spectrum' tab.

(e) Check spectrometer tuning: Type **wobb^** and observe the tuning curve that appears (see **Appendix C**). If no adjustments are needed, stop the acquisition (stop button or type **stop^**) and return to the 'Spectrum' tab.

(f) **lockdisp^** - Displays the lock signal. A new detached window will open up showing the lock signal strength and noise level of the deuterated solvent used. Observed signal can be used as an effective guide for shimming the instrument.

(g) **rsh^** - recall shim files. Shimming parameters are typically saved as shim files for several NMR solvents and are frequently updated. Less common solvent can be added at a user's request, as well.

(h) Select the latest shim file for your solvent (e.g. if your solvent is $CDCl_3$ LATEST_$CDCl_3$ is the name of the corresponding shim file). One cannot assume that the shims from the previous user are fine. Do **NOT** check the box "set also lock parameters".

(i) **lock^** - auto locks on the solvent selected from the list of available solvents. Your selection should match the entry in the AcquPars tab.
NOTE: Check Lock Field, Lock Phase, Lock Gain values on the BSMS keyboard before proceeding (or on emulated BSMS display).

(j) **Shimming procedure**: Start by opening up BSMS display with **bsmsdisp^** command or use the BSMS keyboard. If you are familiar with manual shimming you can shim the instrument for better line shape and resolution. The correct order is to shim Z1→Z2→Z1→X and Y, then you can shim X^2-Y^2, XY, XZ and YZ followed by X and Y/Z1 and Z2, again, using "FINE" for the rate of adjustment. [*On certain keyboards you need to press "ON AXIS" button to access on-axis shims and "X+Z0" combination for off-axis shims*] Monitor the lock signal in the lock display window. If the lock signal reaches the top, reduce the 'LOCK GAIN' setting. A good lock level is anywhere between the second and third row from the top of the grid.
Topspin 2.0 and later versions feature an improved protocol for gradient-based shimming, named TOPSHIM. For most samples use **topshim tuneb^** command from the command line (samples in D_2O require a different option). See **Appendix D** for details.
NOTE: Topshim procedure above adjusts only the low-to-mid order Z and X/Y shims.

(k) **rga^** - Adjusts receiver gain in order to ensure the generated FID is not saturating the instrument's receiver unit.
(l) **rg^** - Note the value. It gives you an idea about concentration of your sample.
(m) **ns^** - number of scans. Allows you to select the number of scans for your acquisition. It has to be a number in the form of 2^n, where n is any number ≥ 0.
(n) **expt^** -Calculates the experiment duration. You can adjust the number of scans to allow the experiment to run in the time alotted.
(o) **zg^** - Starts the experiment. You may want to observe the signal accumulation in the 'Acqu' tab. Complete FID is stored in the 'Fid' tab. Once you have acquired a ^{1}H NMR spectrum, you can follow the same set of instructions for starting a ^{13}C or ^{31}P or ^{19}F experiment. You will need to create a different experiment number with the **edc^** command. Otherwise, you will overwrite on an existing FID and lose your data permanently.
(p) **setti^** or click on the 'Title' tab – to add the title information. You can keep a record of any experiment and its particulars for future referencing.
(q) **lb 0.3^** - Sets line broadening to 0.3 Hz (3.0 Hz for carbon)
(r) **ft^** - Fourier transforms the FID
(s) **Phasing** – Click INTERACTIVE PHASE CORRECTION button on the screen (looks like a twisted peak) or type **.ph^**. New window appears with (PH)0 and (PH)1 phasing buttons among others. Click on the (PH)0 (zero order phase correction) with the left mouse button, hold and move the mouse up or down to make the baseline of the spectrum around the vertical red line (pivot point) as flat as possible. You can also expand sections of the spectrum and increase the peak height to make it easier to phase the spectrum. Next, use the (PH)1 for phasing the peaks farther away from the pivot point. Click on 'SAVE & RETURN' button if you are satisfied with the phasing result. If you want to start over again press the 'RETURN' to exit.
(t) **efp^** - This composite processing command should be used to optimise signal-to-noise ratio in the spectrum, and is usually observed as a reduction in the spectral noise. Considers user-defined lb parameter in its calculation, and also re-applies the phase correction values (if available).
(u) **sref^** - Sets reference on the TMS signal, if present. If the TMS signal is absent or you wish to calibrate on the solvent signal instead, use manual referencing. Click on the SPECTRAL CALIBRATION button (NMR peak with "0" underneath it) or type **.cal^**, and in the new window that opens find the signal of interest then click with the left mouse button to open a small window where you can enter new chemical shift information. 'SAVE and RETURN' to store the information and to close the window. **NOTE**: **Do this before peak picking procedure**

Further Processing of NMR spectra in TOPSPIN: Spectrum Calibration, Peak picking, Integration, Plotting and Display options

For historic reasons, Bruker software requires a three-button mouse or one with a mouse wheel, where the wheel replaces the middle button. In TOPSPIN, only Left (L) button and the wheel are used – Left for selection/action and wheel for the Y-scaling of the spectral data only. Right mouse button calls additional options available in each tab.

In general, whenever you open up either a processing or an analysis option, a new window will open, with a yellow button in the upper left-hand corner representing the selected option. Active tools inside this window will be highlighted in orange.

(a) General guidelines: Adjust your spectra such that you have a clear view of the peaks to be integrated and the peaks are well spaced out. You can use the navigating buttons in the main window to move from one peak to another in case they do all not fit in the same window.

(b) **Calibration** – calibrate the chemical shifts based on TMS (if present) or deturated solvent. Chemical shift reference chart is posted on the message board in B71. Type **.cal**^ or click on the button with a small '0' underneath a peak, find the appropriate signal for calibration and L-click on it. Enter the corrected value in the new window.

(c) **Peak picking** – Click on the PEAK PICKING button or type **.pp**^ (**pp**^ for advanced options). Peak picking screen that opens up will allow you to do semi-automatic peak picking on a defined range (= rectangular selection box), or manual selection. When done, click on the 'SAVE AND RETURN' button.

(d) **Integration** - Click on the INTEGRATE button or enter **.int**^. This will open up a new interactive integration window where you can either define integral ranges using the computer mouse L button or via dialog.
To calibrate the integrals, first deselect the range tool and point the cursor to the integral of interest and then R-click on. Integral value below will become highlighted in yellow. Another R-click to access the 'CALIBRATE' option, enter a number in the new window.
If you are satisfied with the integration press the 'SAVE' button. Otherwise, you can press 'RETURN' and try to integrate again. If you want to deselect a single integral double L click on the peak and select 'DELETE FROM CURRENT'.

(e) **prnt**^ - prints the default spectrum window. In TOPSPIN one can simply select the desired print area of a spectrum with the mouse cursor. **Plot**^ command calls up the plot editor which can be also found in the Plot tab. [Ctrl P] calls a general printing menu with more options. **Exportfile**^ opens up a new window to enter file name and data format in which you want to save the data.

(f) **Stacking multiple spectra** – click on the 'MULTIPLE DISPLAY' button or enter **.md**^. In the new window, there is an area underneath the data browser where you can drag-and-drop any number of spectra. Works for both 1D and 2D spectra, and supports printing.

PROCEDURE FOR SETTING UP STANDARD 1D ^{13}C, ^{31}P AND ^{19}F EXPERIMENTS

(a) **Create new experiment with edc^/new^ option-** change EXPNO to a different number from the proton experiment (usually 1) if running it after proton. Select the desired experiment (+carbon or +phosphorus or +fluorine_**with decoupling**) from the list of parameter files. Spectra are recorded with proton decoupling. If you need to acquire a non-decoupled data, please see the Facility Director.

(b) Check for tuning on the probe's BB channel by typing **wobb^**, observe the tuning curve (**see Appendix C**), then type **stop^** when done.

(c) Type **atma f1^** (or **atma^**) to tune the ATM probe to nucleus of your choice. Observe tuning curve on both BB(X-) and ^{1}H channels.

NOTE: ATM probe does not automatically switch back to carbon frequency. Unless you tune it back as a courtesy to other NMR users, the next user will have to check it/adjust it prior to acquiring data.

Always check the tuning before starting any acquisition setup.

(d) The rest of setup is exactly the same as for 1D proton spectrum, except that you can skip the rga step. Rg value is pegged at 203 on the AVIII (and newer) consoles.
For a ^{19}F experiment you should change the following parameters in the processing tab ('ProcPar') before going through the next step:
ME_mod = LPbc, *NCOEFF = 32*, *TDoff = 16*
There is an option to reprocess already processed data, as well. See the Facility Director for instructions on how to do this.

(e) Following **ft^**, you may use **apk^** (auto phase correction) then **efp^** (set **lb^** to 3 first)

(f) **abs^** (to correct the baseline if necessary).

(g) Use peak picking routine **pp^**.

(h) Picked peaks will appear in the Peaks tab afterwards and the peak list can be printed from there.

LOGGING OUT:

Before you logout, replace your sample with standard sample provided (1:1/H_2O-D_2O). Create a new experiment folder, e.g. lock, and load proton experiment. Change solvent to D_2O or D_2O+H_2O and lock on the solvent.
Proceed with programme exit and log out.

DATA TRANSFER:

Recorded NMR data is saved in each user's data folders found in the following directory: D:\data*username*\nmr

Flash memory devices for data transfer are not permitted for security reasons.

MRF workstations are linked to a network fileshare (NMRFILESHARE, W:\). The share can be accessed through offline processing workstation (currently Dell Optiplex) in Room B73. For login information contact the Facility Director.

To obtain data for offline processing or backup, go to your respective data folder(s) on each NMR workstation, select data you want to transfer and copy/paste it into NMRFILESHARE/data/*username* folder. Data can be also transferred onto CD/DVD(RW) media directly from the NMR workstations.

OFF-LINE DATA PROCESSING:

(a) Start TOPSPIN 3.x/4.x on the NMRDATASTATION in B73
(b) Locate your NMR data by clicking on the "File" menu, then "Open" option. Your data is either on the W:\ or on the drive lettter assigned to your portable memory device. Append the dataset you wish to open and click "Apply". Data in the form of NMR spectrum or FID will appear in the window.
Alternatively, drag-and-drop your data file into the main programme window.
(c) You can also type **new^** in the main TOPSPIN window, and change the DIR, NAME, EXPNO and PROCNO fields accordingly, to link to your existing data.
(d) Network printing from all workstation is enabled to the network HP4100 printer located in B73.

ACQUISITION AND PROCESSING OF 2D SPECTRA

Before starting an acquisition it is important to achieve optimal spectrometer conditions. Tube and solvent quality are equally important, refer to the section on Sample Handling of the manual for details.

Standard set of 2D experiments available on both 400 MHz spectrometers are listed in Appendix A.

All the 2D acquisition parameter files come with a preset spectral widths, covering most common region of proton chemical shifts (0-16 ppm for ^{1}H, 0-160/220 ppm for ^{13}C). Hence, user should adjust the spectral width according to proton/carbon reference spectra of the sample of interest. Alternatively, spectral widths can be narrowed to maximise resolution in t1 dimension.

Experiment setup protocol for a 2D experiment is similar to that of a 1D experiment, although there are many additional parameters than need to be checked. Processing strategies and parameter are largely different, **xfb^** command automatically processes and phases most common experiments except for the phase sensitive ones.

Step-by-step preparations for 2D data acquisition:

(a) Check the RF probe temperature displayed in the "Acquisition Bar" in the main programme window (298K by default). Load the sample in the magnet.
(b) Tune the probe to ensure maximum sensitivity for detection. Tuning/matching is automated with the ATM module on both spectrometers. If you are setting up a 2D heteronuclear experiment, you will also need to tune the other nucleus (usually ^{13}C). This can be done either by setting up a ^{13}C observe experiment and repeating the procedure above, or by waiting until you set up your 2D experiment. In that case, "**wobb^**" command will display the curve of the X-nucleus first; in order to observe proton you will need to type "**wobb f2^**".
(c) Lock and shim on the sample (manual/topshim), run **rga**
(d) Collect a survey 1D proton spectrum with ns=8, ds=0 o1p=6 ppm, sw=20 ppm
(e) Calibrate proton high-power 90 degree pulse (^{1}H PW90)
(f) Reacquire 1D proton spectrum using the optimised PW90 value
(g) After phase correction and **efp^** identify the spectral region containing peaks of interest.
(h) Click on the **sw-sfo1** button on the screen. Values for **sw** (spectral window), **o1p** (transmitter frequency offset in ppm) and **rg** are used for the 2D setup.
(i) Reacquire 1D proton spectrum, to be used as external projection.
(j) Collect a survey carbon, if possible.
(k) Start a new experiment with **new^**/**edc^** and select a 2D experiment of your choice

(l) **eda^** and set solvent parameters including 'getprosol'. Verify that TD parameter for the F2 dimension is set to 2048/1024, in particular for heteronuclear experiments utilising ^{13}C decoupling. Next to it is the value for F1 dimension. Select values of 128/256/512 for *low/medium/high* spectral resolution. NOTE: experiment time will proportionally increase when you increase this value.

(m) Adjust sw and o1p/o2p values

(n) **sw** <values from optimized 1D experiments>^

(o) **o1p** <value from optimized 1D experiment>^

(p) **o2p** <value from optimized 1D experiment>^

(q) Enter the **rg** value determined from the **PROTON** experiment. DO NOT RUN the **rga** command.

(r) **expt^** to calculate the experiment time

(s) **zg^** (in Topspin 3.x this does not open acquisition window ('Acqu tab')!), wait for 1st increment to complete (1/TD counter in the acquisition bar)

(t) **rser1^** (**rser2^** for TOCSY)

(u) **sinm^/ft^** or **qsin^/ft^**

(v) Observe the FID, it should resemble a proton spectrum in most cases. Phase the 1D spectrum and click the 'SAVE AS 2D' button then 'RETURN'.
If you acquired enough increments (signal-to-noise is good), proceed with the processing the 2D spectrum as described on the next page.

(w) Click on the '2D' button to return to the current 2D dataset.

Processing of magnitude mode experiments (e.g.: COSY, HMBC)

(a) **xfb^**
(b) **syma^** - to symmetrize a spectrum (optional, for COSY only), **xfb^** should you want to return to the original display mode.
(c) Add projections using the 'Toggle projection display' button (multicoloured squares with lines surrounding them). Projections are not enabled by default. Using the R mouse button near either horizontal or vertical projection will display a menu to define new external/internal projection and its position on the display (centre/bottom options).
(d) Change pseudo colour mode to contour display mode or the other way around. Add gridlines if you find them helpful in data analysis later.
(e) Select plotting region. This can be done in two ways:
(1) Move the mouse over the starting point of your choice in a spectrum (main window) and click the left mouse button. Hold the L button and drag the mouse to the lower/upper left edge of the region you want to expand. Release the button to define the expansion box
(2) Use the exact zoom button (E) to manually define the boundaries. Should you need to revert back to the initial layout, click on the (A) button. You may use zoom in/out buttons but they are not as precise.
(f) Adjust vertical scaling to desired level and print or open up the Topspin Plot editor tab for further editing.
(g) **prnt^** to print the spectrum from the screen.

Processing of phase sensitive experiments (e.g.: NOESY, HSQC). For detailed description see **Appendix B**.

(a) **rser 1^** (**rser 2^** for TOCSY)
(b) **sinm^** or **qsin^**
(c) **ft^**
(d) phase 1D spectrum. Then click on the 'SAVE AS 2D AND RETURN' button.
(e) Select the "2D" button (in the button menu) to return to your 2D spectrum.
(f) **edp^** ProcPar tab - change processing parameters if required.
(g) **xfb^**
(h) click on the "PHASE" button.
(i) Select (at least three) peaks for phasing, follow the procedure outlined in the **Appendix B**.
(j) Phase "Columns" then "Rows", wait for the phase information to update.
(k) **syma^** - to symmetrize the spectrum (optional for TOCSY, NOESY/ROESY).
(l) Define plot region and include external projections and gridlines, if you wish.
(m) Print, re-define plot region and print again.

UNATTENDED RUN OF A SERIES OF NMR EXPERIMENTS

This procedure enables you to run several experiments consecutively as opposed to one by one. It is an extremely useful option for overnight/weekend runs.

(a) Create a 1D/2D experiment as previously described. Prepare for running a NMR experiment similar to 1D proton spectrum (Lock and Shim etc.)
(b) Create another new experiment and increase the experiment number (EXPNO parameter) by one. DO NOT change the dataset name. Use **re (expt. no.)**^ to switch between experiments.
(c) Adjust acquisition parameters as necessary (sw, o1p, o2p, ns, rg etc.)
(d) Repeat steps **(b)-(c)** for each new data set.
(e) Calculate the total runtime for all experiments in the sequence. Use the multiexpt command: type **re 1**^, then **multiexpt**^, enter the number of queued experiments.
(f) type **multizg**^
(g) Enter the total number of datasets you plan to run and press enter. This starts the run.
(h) Place the "experiment in progress" sign on the table and leave a note with your contact information and instructions for the next user, if any.

TOPSPIN PLOT EDITOR

TOPSPIN has a built-in plot editor (formerly Bruker's external plot editor for creating high quality printouts of NMR data) included as a separate tab (Plot) in the main programme window. Current version 3.x allows export of NMR data in graphical (JPEG, TIFF, BMP) and Adobe PDF formats. Microsoft (WMF, EMF) and PostScript (EPS) file formats are no longer supported.

By clicking on the Plot tab, your current NMR spectrum will be reprocessed and displayed in a slightly different manner then in the TOPSPIN main window. In addition to chemical shift, integral and title information, this plot includes experiment parameters and Bruker logo on the right-hand side of the spectrum. All elements appear on the screen as objects that can be freely moved around, re-sized or deleted. Clicking on the each of these elements will open up available options menu on the left-hand side of the spectrum.

General options found on the on the left-hand side of the main window:

Layout: select the desired spectrum layout, the defaults are usually called 1H, X, 2D etc. Click on the button to access additional options.
Print: select the desired paper size, the default is A4 (approximately US letter size, although printer will complain about not being able to proceed). You may need to re-scale your spectrum depending on your selection.
View: options to zoom in or select parts of the displayed spectrum.

There are additional menus located on the screen for inserting new elements, text or graphics.

Spectral data can be exported from the main programme window through the **Export** command in the **File** menu or by typing **exportfile^** in the command line. Enter file name and the file extension from one of the supported file formats in the pop-up window.

APPENDIX A

LIST OF COMMONLY AVAILABLE NMR EXPERIMENTS

The following parameter sets have standard parameters already entered, their names starting with a plus sign prefix to distinguish them from the Bruker's parameter sets.

PROTON
CARBON
DEPTQ(v2)
UDEFT
PHOSPHORUS
FLUORINE **on AVIIIHD only**

gHSQC edited
gDQFCOSY
gTOCSY
gCOSY
gHMBC
gHSQC
gH2BC
gNOESY
gROESY

gHSQC-TOCSY
gLR-HSQMBC

g1,1-ADEQUATE
sel1D_TOCSY/NOESY/ROESY

Additional experiments are available upon requests. AVIII (HD) consoles are capable of executing the most current pulse sequences published in the literature (for Bruker spectrometers).

Relative order of sensitivity of 2D experiments:

COSY>TOCSY>DQF-COSY>HSQC, HMQC>HMBC, NOESY, ROESY

PULSE PROGRAMME ABBREVIATIONS USED BY BRUKER

Certain common abbreviations are listed below. Pulse programme name can be checked by entering **pulprog^** in the command line or going into acquisition control screen the 'Pulseprog' tab. More details can be found in the pulse programme file itself (**edcpul^**):

qf – magnitude acqu mode **ph** –phase sensitive acqu mode **lp** – lowpass filter
gp/gs – gradient experiment **pr** – presaturation **et** – Echo/Antiecho **lr** – long range
df/mf – quantum filtered **l9** – WATERGATE **nd** – not decoupled **sel** - selective
mlev, dipsi, dip – TOCSY **es** – excitation sculpting **si** – sensitivity enhanced
sp – using shaped pulses

APPENDIX B

PHASE CORRECTION PROCEDURE FOR 2D PHASE-SENSITIVE EXPERIMENTS

Before you begin: Generally in a 2D spectrum, F2 dimension (rows) is phase-corrected first, and then the F1 dimension (columns). To phase-correct the spectrum in F2, at least three rows each containing crosspeaks should be selected. The crosspeak of the first row should be from the far left of the spectrum, the cross peak of the second row should be close to the middle, and the one of the third row should be from the far right of the spectrum.

Enter the phase correction menu by clicking on the phase correction button or by entering **.ph^** in the command line. New window will open with a cross-hair selection cursor. Move the cursors to the upper right-hand corner and select a crosspeak using R(ight) mouse button and choose 'Add' option from the menu that appears. Selected peak will appear within in a circle with row/column positions marked with horizontal/vertical lines. Moving diagonally across the spectrum, select a couple of more peaks in the same way. When done, click on the R button to start phasing the rows. Yet another (1D phasing) window will open, with 1D slices of the crosspeaks appearing top to bottom in order you selected them. Click on the topmost spectrum and adjust the zero order phase correction with the '0' button. Check the bottom spectrum and adjust the first ('1' button) order phase correction, if necessary. When done, click on the 'SAVE AND RETURN' button.

Although the abovementioned procedure works in most cases, you can improve the quality of phasing by going through the each 1D slice with the +/- buttons to make sure that your crosspeak section has included the peak maximum/largest peak area.

NOTE: To phase correct the spectrum in F1 dimension (columns), repeat the above procedure by selecting three (or more) columns rather than rows.

APPENDIX C

PROBE TUNING AND MATCHING.

In order to check for the probe tuning you need to type **wobb**^, then wait for the wobble curve to appear in the window. Vertical red line marks the spectrometer frequency. Actual tuning profile is displayed in blue, with a "dip" at the actual resonant frequency. From this observe window you can call up autotune and match control panel by typing **stop**^ then **atmm**^ or **atma**^, in case you need to adjust tune and match. From the same window, you can check the tuning for other channel (X, if present) by typing **wobb f2**^ (f2 is the probe second channel).
NOTE: wobb curve for an X-nucleus may appear slightly different from the ^{1}H curve.

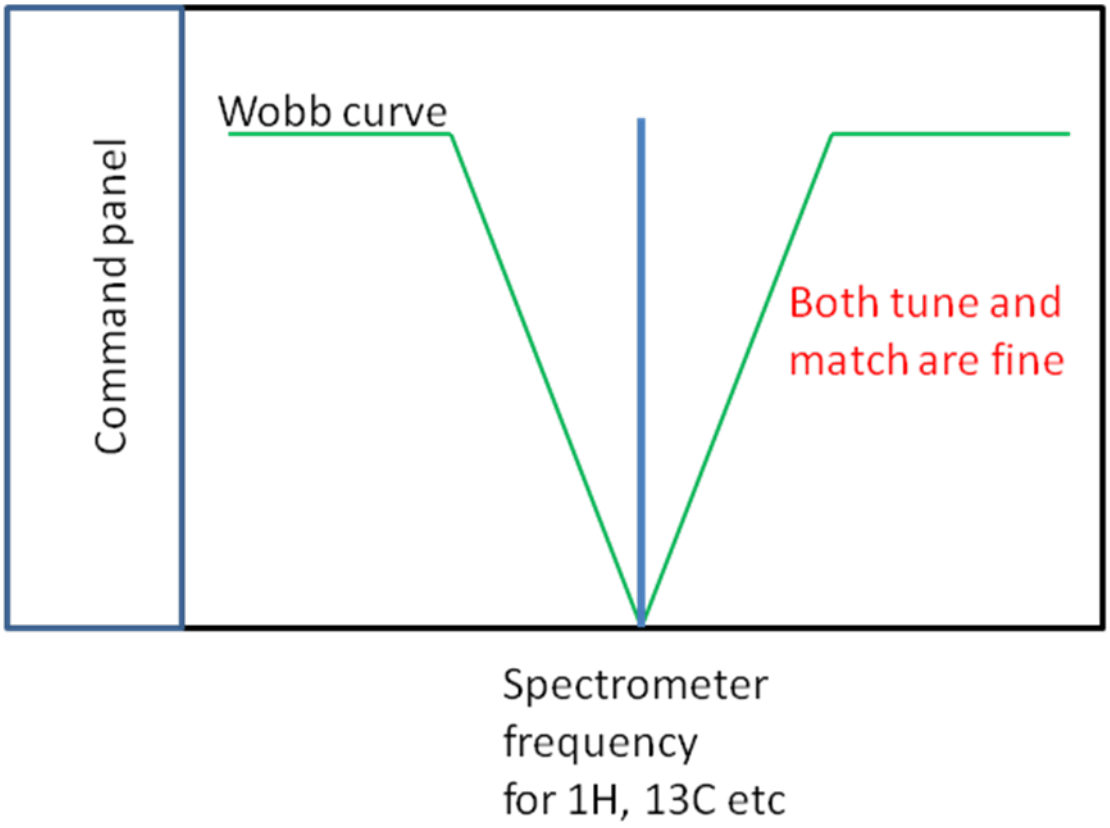
Stop the acquisition with **stop^** command, and exit the window by typing **return^** or clicking on the 'RETURN' button.

Autotuning and matching routine (atma) approach: From the wobb window type **stop^** then **atma^** (or atma f1^). Routine will autoadjust both tuning and matching on both channels (if present). It will stop and close the acquisition windows when it is done. This procedures is recommended when switching X-nuclei back and forth (carbon to fluorine to carbon, for an example).

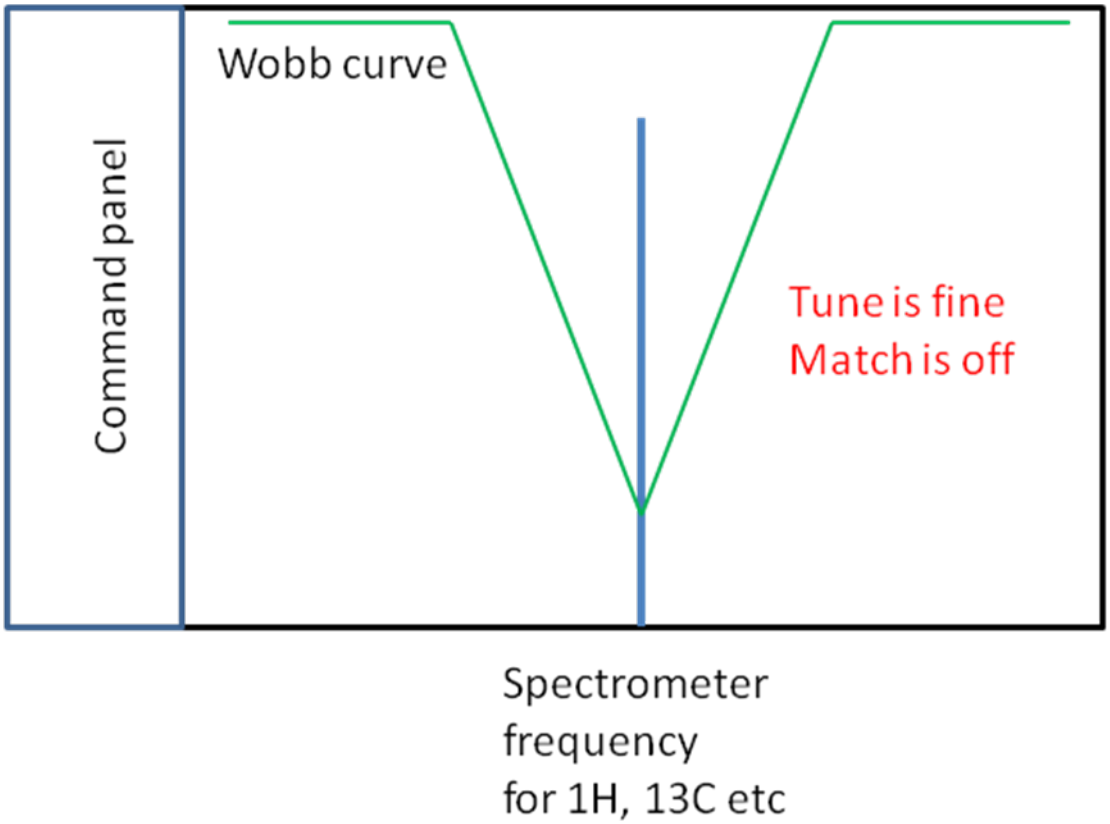
In case you are unable to observe a wobb curve, contact the MRF Director.

Following pages contain examples (1-4) of wobb curves in respect to tune and match settings.

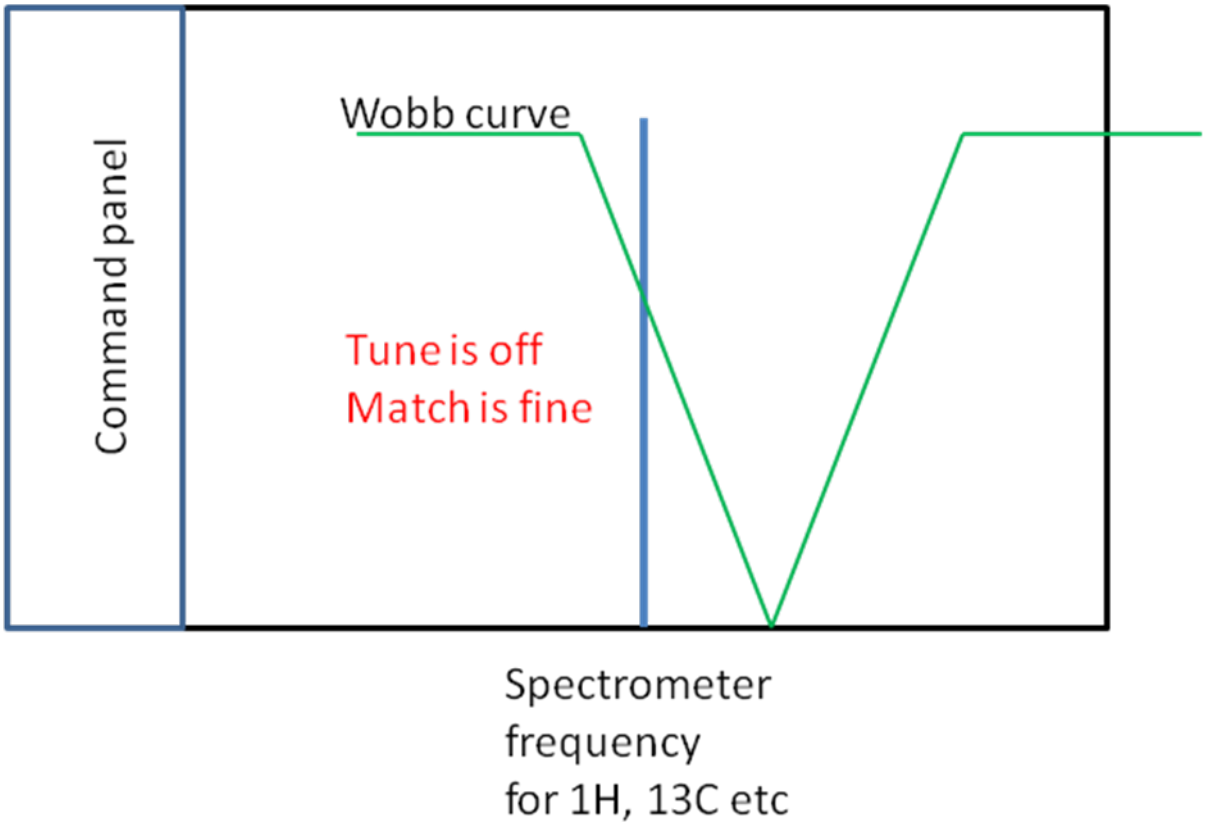
1)


Command panel
Wobb curve
Both tune and
match are fine
Spectrometer
frequency
for 1H, 13C etc

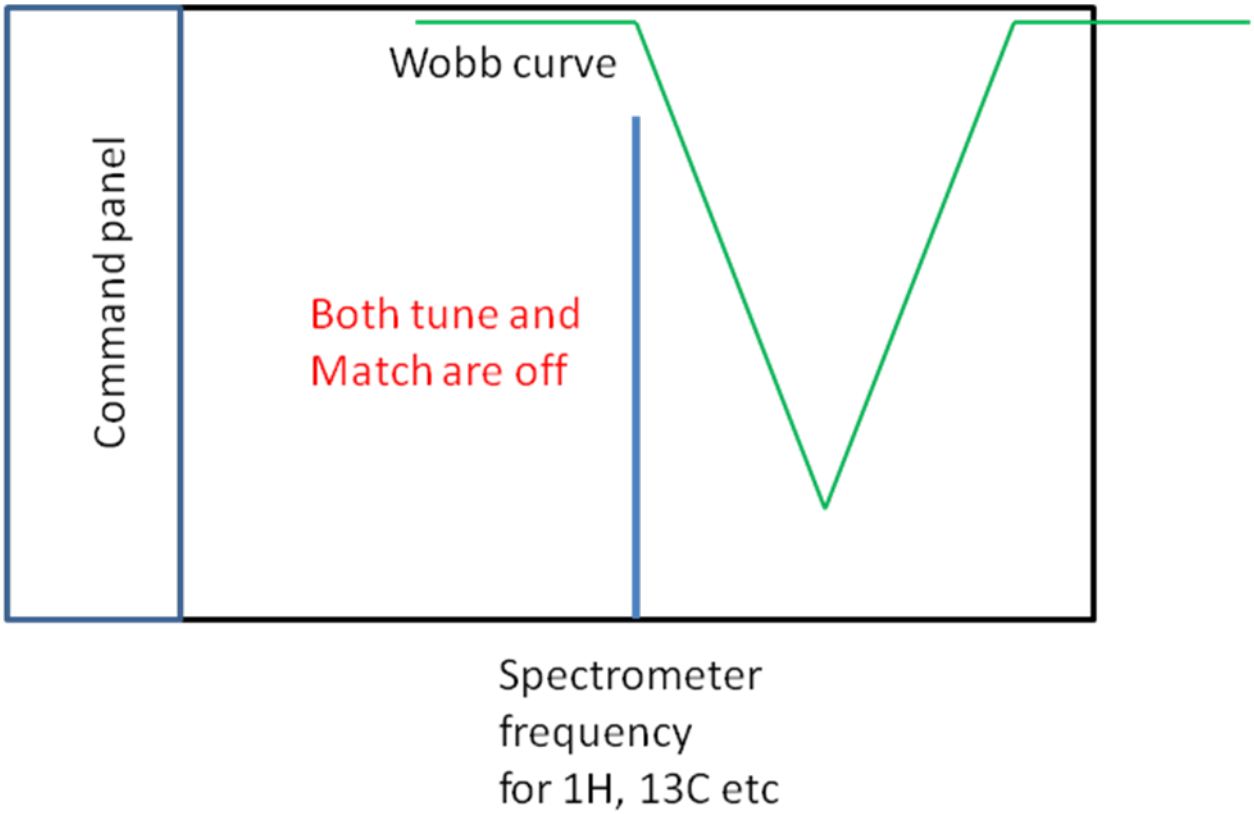
2)


Command panel
Wobb curve
Tune is fine
Match is off
Spectrometer
frequency
for 1H, 13C etc

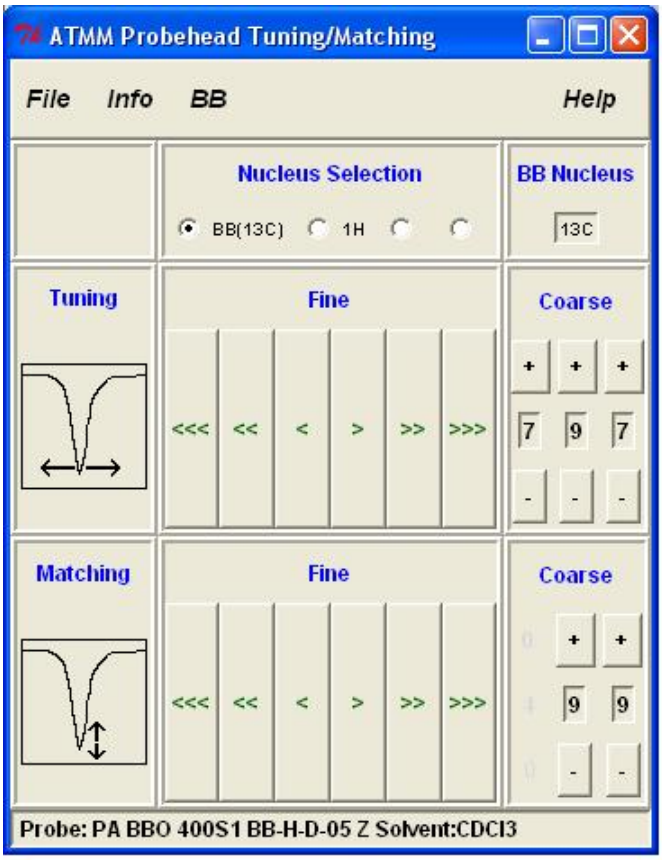
3)


Command panel
Wobb curve
Tune is off
Match is fine
Spectrometer
frequency
for 1H, 13C etc

4)


Command panel
Wobb curve
Both tune and
Match are off
Spectrometer
frequency
for 1H, 13C etc

AUTOTUNE AND MATCH (ATM) UTILITY FOR ATM ENABLED PROBES

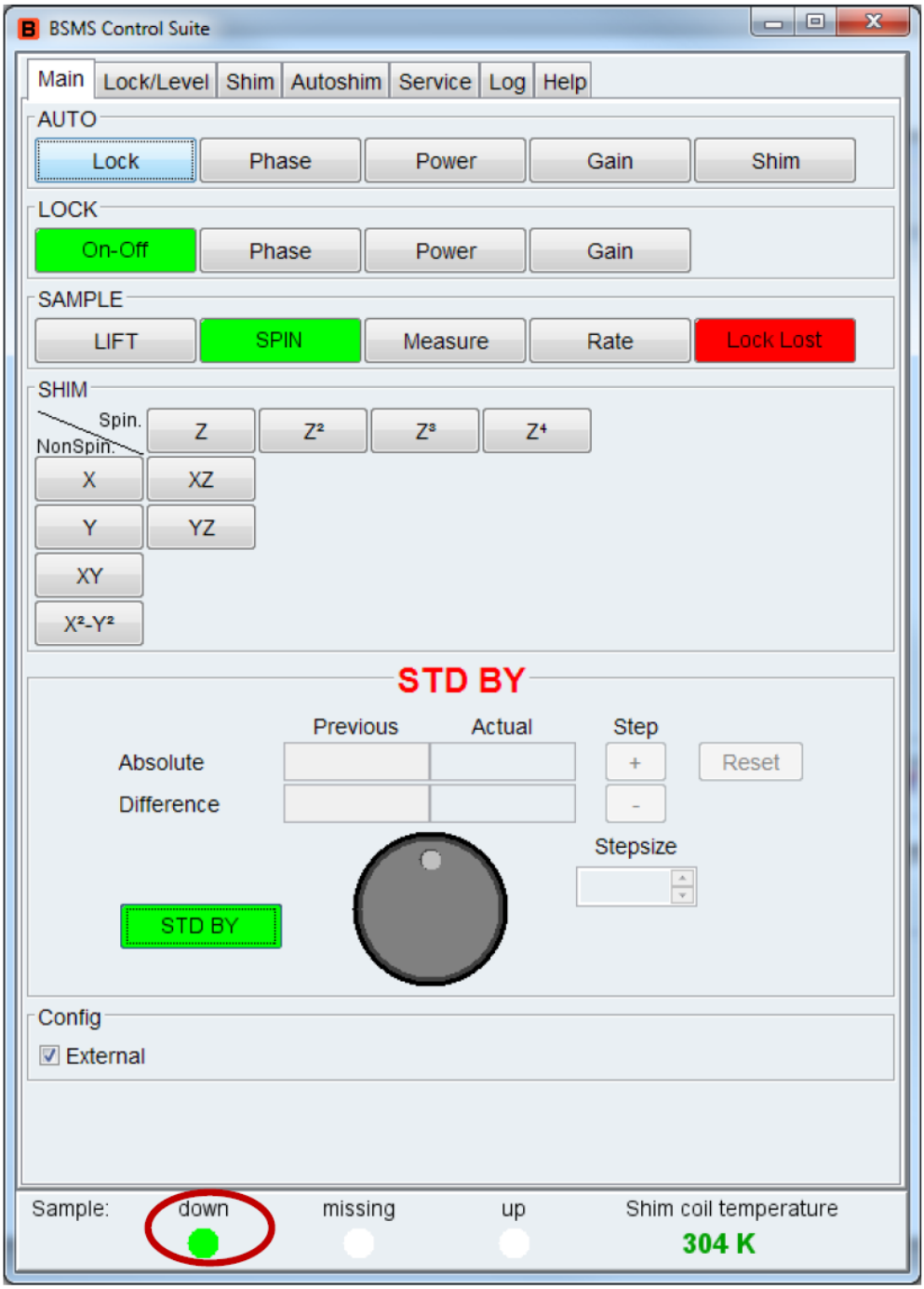


ATM control panel is split into two sections, one for adjusting the tuning and the other for matching of an RF probe. Each section has single/double/triple arrows representing the rate at which the motor in the probe box turns tuning/matching rods. **DO NOT USE TRIPLE ARROWS**, as you can seriously damage the probe!!!
Click on the arrow button, wait for response, then click again, starting with tuning then proceed to matching. Your goal is to match this dip with the vertical line. When the wobb curve lines up with the vertical line (Figure 1 on p22), click on "File" then "Exit" (answer "No" to the question about saving the current position).

In Topspin 3.x, the ATM panel may look slightly different then pictured above.

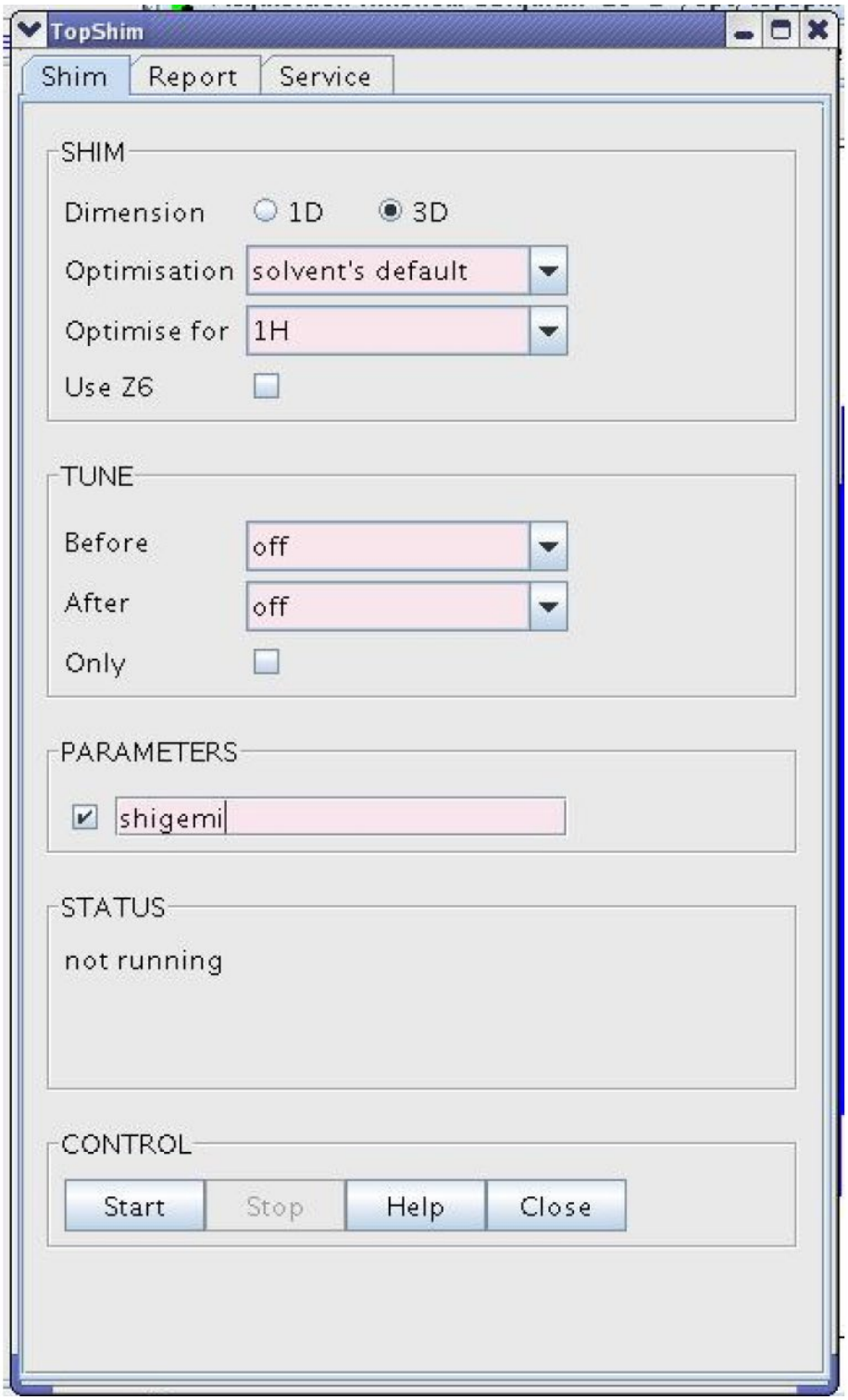
APPENDIX D

BSMS DISPLAY/CONTROL SUITE IN TOSPIN 3.x



Buttons on the display have the same functions as buttons on a BSMS keyboard (found on the AVIII spectrometer)

APPENDIX E
TOPSHIM UTILITY IN TOPSPIN ;)



Topshim interface in Topspin 3.x, accessible with **topshim gui^** command

TOPSHIM CONTROL PANEL

SHIM section (TOP) – leave the default settings. Dimension button should be set to 1D (3D is to be used with water sample).

TUNE section (MIDDLE) – select shims that you wish to optimise (tune) before and after the topshim procedure. Unless the Z6 option is set in the section above, use Z-X-Y setting from the drop-down menu. If you check the box, the procedure will run with shim tuning only, without the gradient shimming component (topshim).

PARAMETERS section (MIDDLE) – use it to enter special parameters described below. Type in the parameter box, if available, and click on the check box next to it to apply one of the following options:

Durmax= Maximum duration per 1D field map acquisition (in seconds). Default = 7 (try 15, 30 or even 120)

Rga Forces receiver gain optimisation before shimming. Or from the command line type **topshim rga^**. Used when S/N is low.

Tune Shim on the lock signal before and/or after gradient shimming, for example tuneb switch shims X, Y, Z, XZ, YZ before running gradient shimming. Another example would be **topshim tuneaz^** – shims Z *after* running gradient shimming

Shigemi Used to eliminate unreliable data at axial Shigemi tube walls when using 1D shimming

Zrange Sets the range in cm in the Z-axis direction used for shimming **– topshim zrange=-0.8,0.8^** (short sample)

Plot Saves data after completion of topshim routine in <Topspin_home>/data/topshimdata

Help **helptopshim^** (always useful)