Manual for Varian pure shift NMR pulse sequences developed by the NMR Methodology Group, University of Manchester

Rev. 1.0

1 Release notes

The aim of this manual is to help implementation and application of the Varian pure shift NMR experiments developed by the NMR Methodology Group at the University of Manchester. The package has been made available at the Pure Shift Workshop, Manchester 12 Sep 2017. Updated version may be provided in the future via our website.

The University of Manchester and the authors of this manual and software package cannot be held responsible for any damage or loss resulting from the use of these sequences.

This package was developed using a Varian VNMRS console and VnmrJ 4.0 software. Some of the pulse sequence statements are not compatible with older consoles (Inova/Mercury), and the use of VnmrJ 4.x or openVnmrJ is advised for data processing. Windows PC hosts are not supported. Please contact us if Inova support is required, but we regret that we cannot support old versions of VnmrJ for data acquisition.

2 Installation instructions

Download the Varian package from the Manchester NMR Methodology Group's website (http://nmr.chemistry.manchester.ac.uk/pureshift).

- Un-compress the archive
- Copy the contents of /psglib and /maclib to your pulse sequence (e.g. local user installation: /home/vnmr1/vnmrsys/psglib [vnmr1=linux user name]) and macro (e.g. /home/vnmr1/vnmrsys/maclib) directories.
  e.g. cp –p –r [path of downloaded package]/psglib /home/vnmr1/vnmrsys/psglib/.
- Copy the /wavelib/kp_WURST40 to /home/vnmr1/vnmrsys/wavelib/decoupling/
- Copy the /wavelib/kp2_wurst180 to /home/vnmr1/vnmrsys/wavelib/inversion/
- Copy the /wavelib/psyche to /home/vnmr1/vnmrsys/wavelib/inversion/
- Compile the new pulse sequences using seqgen

A set of example data is also provided, but is not needed to run the experiments.

3 Basic instructions for acquiring pure shift NMR spectra

- Set up a standard proton experiment and acquire the conventional proton spectrum
- Copy the data to a different experiment number, to use as a starting point for the pure shift NMR experiment
- Run the relevant **setup macro** (see Table 1) to convert the current (proton) experiment to the desired pure shift experiment .
- Set the `wexp` parameter, if desired, for data saving and/or queuing of experiments
- Run the experiment using **au** if `wexp` is used, otherwise using **go**.
- Save the raw data after acquisition, process the spectrum using the relevant processing macro (see Table 1), and save the processed data. Example saving macros are provided.

Table 1

1D interferogram experiments	**Pulse sequence filename**	**Setup macro (from ^{1}H)**	**Processing macro**	**Ref.**
BS (band-selective)	UoM_1d_if_PS.c	UoM_setup_1d_if_BS	UoM_proc_1d_if	1
Zangger-Sterk		UoM_setup_1d_if_ZS		2
PSYCHE (**P**ure **S**hift **Y**ielded by **CH**irp **E**xcitation)		UoM_setup_1d_if_PSYCHE		3
TSE-PSYCHE (**T**riple **S**pin **E**cho **P**ure **S**hift **Y**ielded by **CH**irp **E**xcitation)	UoM_1d_if_TSEPSYCHE.c	UoM_setup_1d_if_TSEPSYCHE		4
BIRD (**BI**linear **R**otation **D**ecoupling)	UoM_1d_if_BIRD.c	UoM_setup_1d_if_BIRD		5
real-time experiments				
1D BS	UoM_1d_rt_PS.c	UoM_setup_1d_rt_BS	UoM_proc_1d_rt	2c,6
1D Zangger-Sterk		UoM_setup_1d_rt_ZS		7
1D BIRD	UoM_2d_rt_PS_HSQC.c	UoM_setup_1d_rt_BIRD	UoM_proc_2d_rt	8
2D HSQC		UoM_setup_2d_rt_BIRD_HSQC		9

There are a few local parameters in the experiments which may need to be changed for a particular sample. In BS/ZS the bandwidth of the ASR (active spin refocusing) shaped pulse is controlled by the parameter `bw_a`. The duration of the chunk is the inverse of sw_1, and is controlled by changing sw1. In interferogram experiments other than TSE, a quarter of the duration of the chunk needs to be long enough to accommodate the gradient pulse (`gt1`) and stabilisation delay (`gstab`). In real-time experiments the duration of the chunk is `kp_npoints/sw`. The total duration of the interferogram (or acquisition time in real-time experiments) is ni/sw1 (interferogram) and `kp_npoints/sw*kp_cycles` (real-time). The macro **_kp_npoints**, **_kp_cycles**, **_xxx** is run automatically whenever the value of the parameter kp_npoints, kp_cycles, xxx is changed, enabling parameters such as np to be kept correct. One may need to be careful with using long acquisition times when heteronuclear decoupling is applied in real-time experiments. In our experience, a real-time pure shift HSQC experiment typically causes slightly more sample heating than the parent HSQC experiment (because more ^{13}C pulses are used). Calibrations are needed to create the relevant shape pulses; proton and carbon 90° calibration values are taken from pw/tpwr and pwx/pwxlvl.

4 Summary of advanced options

All pulse sequences xxx have an associated **go_xxx** macro, which is executed when the experiment is started (no matter with what command). When the parameter kp_auto is set to 'y', the **go_xxx** macros will call the macro UoM_makePS9 with the relevant argument to create any pulse shapes that are needed on the fly. Local parameters are available to provide flexible control of the pulse shapes.

The *active spin refocusing* selective 180° pulse is defined by the parameters:

- shp_a shapefile name in shapelib (a for *active spin*)
- pw180_a length of the pulse [μs]
- pwr180_a power of the pulse [dB]

The user should select appropriate values for the parameters tof, bw_a, and offset (see below).

Fine calibrations and more advanced settings are supported via parameters kp_wave_a, kp_beta_a, kp_phincr_a, kp_stepsize_a.

The pulse needed is created when experiment started (or **UoM_makePS9** is called with the relevant argument; see **go_<pulse sequence filename>** macro of each pulse sequence), using the values of the following parameters:

- bw_a bandwidth (can be arrayed like offset; if the number of elements is less than that for the offset, then the value of the last element will be used automatically to make a diagonal array of bandwidth and offset) [default is 50 Hz for ZS]
- offset the frequency offset of the selective pulse (can be arrayed for simultaneous multi-frequency excitation, including Bloch-Siegert compensation) [default is 0 Hz]

- kp_wave_a the type of selective pulse - any valid name from the definitions in wavelib, for use by Pbox [default is rsnob for ZS/BS and psyche for PSYCHE]

- kp_beta_a flip-angle [typically 180°, except for PSYCHE experiments where smaller values between 2 and 8 are advised – n.b. for PSYCHE this is not actual flip angle, but is proportional to it); in Zangger-Sterk or band-selective experiments this parameter can be fine tuned either side of 180 to achieve a perfect 180° rotation.

- kp_phincr_a a small-angle phase shift of the selective pulse can be added via this parameter to correct the small phase difference between a hard 180° and a soft pulse, and needs to be calibrated whenever the values of tpwr and pwr180_a are changed; it is a property of the RF transmitter chain and does not depend on the sample. In interferogram experiments the result of using an inappropriate value is just a difference between the zero order phase correction of the conventional proton spectrum and the pure shift spectrum, but in real-time experiments miscalibration causes phase discontinuities between chunks and broadens the pure shift signals. [default: 0]

- kp_stepsize_a the duration of a single time-step in the shapefile, in units of µs; needs to be small enough with respect to pw180_a to achieve good digital resolution of the desired shape; typically between 0.25 and 10.0 (steps of 0.25); very selective pulses may require larger steps (otherwise memory is not sufficient for the shape file); normally does not need to be changed from the default value [0].

BIP pulses[10] on carbon are defined by the parameters:

- shp_XBIP shapefile name in shapelib for carbon

- pw_XBIP length of the carbon BIP pulse [µs]

- pwr_XBIP power of the carbon BIP pulse [dB]

- shp_HBIP set to an empty string '' to apply hard 180° pulse on proton

- pw_HBIP default value is 2.0*pw unless pulse sequence code is changed to use getval [µs]

- pwr_HBIP default is set to tpwr unless pulse sequence code is changed to use getval [dB]

The user should just set pwx and pwxlvl for a 90° pulse on carbon, and **UoM_makePS9** macro will call **UoM_bip125** to make the relevant shapefile and will also set the duration of the pulse and the power level.

Heteronuclear carbon decoupling in BIRD and HSQC:

- `dseq, dpwr, dmf, dres, dm, dof` as usual in VnmrJ

The user should select appropriate values for the parameters `bw_d, kp_wave_d, kp_beta_d, kp_pw_d, kp_scyc_d`, and `kp_stepsize_d`.

The decoupler waveform needed is created when the experiment is started (or **UoM_makePS9** is called with the relevant argument; see go_ macro for each pulse sequence), using the values of the following parameters (calibration values are provided via `pwx` and `pwxlvl`):

- `bw_d` bandwidth
- `kp_wave_d` the kind of decoupler pulse, using any valid name for Pbox from definitions in wavelib/decoupling [typically WURST40; kp_WURST40 uses higher Q factor which needs a bit more power but will reduce decoupling sidebands]
- `kp_beta_d` flip angle [typically 180]
- `kp_pw_d` duration of the decoupler pulse [typically between 1200 and 1600 μs]
- `kp_phincr_d` supports a small-angle phase shift in the waveform file [typically 0.0]
- `kp_scyc_d` the kind of decoupler supercycle, using one of the following options: 'd','m4','m8','m16','t5','t7','t9','t5,m4','t7,m4','t9,m4'.
- `kp_stepsize_d` the duration of a single point in the shapefile in μs; needs to be small enough with respect to `kp_pw_d` to achieve good digital resolution of the desired shape, but long enough to avoid out-of-memory runtime errors (the supercycle selected by `kp_scyc_d` also counts)

The broadband 180° pulses in TSE-PSYCHE are defined by the parameters

- `shp_bb` and `shp_bbR` shapefile names in shapelib (equivalent pulses with opposite sweep directions)
- `pw180_bb` duration of the pulse [μs]
- `pwr180_bb` power of the pulse [dB]

The user does not need to change the default values, but the following parameters are available:

- bw_bb bandwidth (should be the same as bw_a for the PSYCHE pulse)
- kp_wave_bb the kind of broadband inversion pulse, using any name valid for Pbox from definitions in wavelib [wurst180, or kp2_wurst180 which uses higher Q factor to ensure better inversion at the cost of higher RF power]
- pw180_bb the duration of the pulse [needs to be long enough to provide good dephasing of unwanted coherences, the longer the better but at the cost of more relaxation and diffusion/convection losses]
- kp_stepsize_bb the duration of a single point in the shapefile in μs; needs to be small enough with respect to pw180_bb to achieve good digital resolution of the desired shape; between 0.25 and 5.0 (steps of 0.25) typically 0.5 μs; very long shape files may need longer stepsize to avoid out of memory runtime error.

5 Complete list of macros

A comprehensive overview of the parameters is given in the previous section. In this section a list and description of the macros are provided.

_droppts1

Whenever the parameter droppts1 is changed this macro adjusts the value of np accordingly.

_droppts2

Whenever the parameter droppts2 is changed this macro adjusts the value of np accordingly.

go_UoM_1d_if_BIRD

If parameter kp_auto equals 'y', then pulse shapes for carbon broadband inversion (BIP) and heteronuclear decoupling are created when a 1D interferogram BIRD experiment is started.

go_UoM_1d_if_PS

If parameter kp_auto equals 'y', then pulse shapes for active spin refocusing (BS/ZS/PSYCHE) are created when a 1D interferogram pure shift experiment is started.

go_UoM_1d_if_TSEPSYCHE

If parameter `kp_auto` equals 'y', then pulse shapes for active spin refocusing using PSYCHE and broadband proton inversion pulses (ZQS elements in TSE-PSYCHE) are created when a 1D interferogram TSE PSYCHE experiment is started.

go_UoM_1d_rt_PS

If parameter `kp_auto` equals 'y', then pulse shapes for active spin refocusing (BS/ZS) are created when a 1D real-time pure shift experiment is started.

go_UoM_2d_rt_PS_gHSQC

If parameter `kp_auto` equals 'y', then pulse shapes for carbon broadband inversion (BIP) in BIRD and heteronuclear decoupling are created when either a 2D real-time pure shift HSQC or a 1D real-time BIRD experiment is started.

_kp_cycles

Whenever the parameter `kp_cycles` is changed this macro adjusts the value of np; the standard _np macro then adjusts the value of at.

_kp_npoints

Whenever the parameter `kp_npoints` is changed this macro adjusts the value of np; the standard _np macro then adjusts the value of at.

UoM_bip125(<shapename>,<ref.power>,<ref.pw>)

This macro creates BIP pulses based on the calibration data supplied.

UoM_makePS9(<mode>)

This is used to create the pulse shapes needed by pure shift experiments in this package. It can be called from the command line, but is mainly intended to be used by the go_ macros of the pulse sequences. The argument <mode> can be the following:

1: interferogram BS/ZS

2: real-time BS/ZS

6: real-time HSQC or any BIRD

7: PSYCHE

UoM_nowt

Removes use of weighting functions in 1D/2D/3D experiments.

UoM_proc_1d_if

Constructs pure shift FID(s) from interferogram 1D experiment data.

UoM_proc_1d_rt

Removes the droppts collected in real-time 1D experiments. The result is a pure shift FID that can be processed as normal.

UoM_proc_2d_rt

Removes the droppts collected in real-time 2D experiments. The result is the a pure shift FID that can be processed as normal.

UoM_save_1d_if

Example data saving macro, which saves raw data, processes 1D interferogram data, and saves pure shift data.

UoM_save_1d_rt

Example data saving macro, which saves raw data, processes 1D real-time data, and saves pure shift data.

UoM_save_1d_rt_BIRD

Example data saving macro, which saves raw data, processes 1D real-time BIRD data acquired using an HSQC experiment with t_1 = ni = 0, and saves pure shift data.

UoM_save_2d_rt

Example data saving macro, which saves raw data, processes 2D real-time data, and saves pure shift data.

UoM_setup_1d_if_BIRD

Changes a proton parameter set to a 1D interferogram BIRD experiment.

UoM_setup_1d_if_BS

Changes a proton parameter set to a 1D interferogram band-selective experiment.

UoM_setup_1d_if_PSYCHE

Changes a proton parameter set to a 1D interferogram PSYCHE experiment.

UoM_setup_1d_if_TSEPSYCHE

Changes a proton parameter set to a 1D interferogram TSE-PSYCHE experiment.

UoM_setup_1d_if_ZS

Changes a proton parameter set to a 1D interferogram Zangger-Sterk experiment.

UoM_setup_1d_rt_BIRD

Changes a proton parameter set to a 1D real-time BIRD experiment using the real-time pure shift HSQC sequence with t_1 = ni = 0.

UoM_setup_1d_rt_BS

Changes a proton parameter set to a 1D real-time band-selective experiment.

UoM_setup_1d_rt_ZS

Changes a proton parameter set to a 1D real-time Zangger-Sterk experiment.

UoM_setup_2d_rt_BIRD_HSQC

Changes a 1D proton (or 2D HSQC) parameter set to a 2D real-time BIRD HSQC experiment.

UoM_unpureshift

This macro can be used to recall the raw data after processing pure shift experiment results.

6 List of example data files

A set of example data was acquired using a 50 mM quinine sample in dmso-d_6. The experiments in /data will be referred to using the number at the beginning of each fid directory.

1) Conventional proton experiment
2) 1D interferogram band-selective pure shift experiment (δ_{tof} = 5.8 ppm); raw 2D data
3) 1D interferogram band-selective pure shift experiment (δ_{tof} = 5.8 ppm); processed 1D data
4) 1D interferogram band-selective pure shift experiment (δ_{tof} = 7.9 ppm); raw 2D data
5) 1D interferogram band-selective pure shift experiment (δ_{tof} = 7.9 ppm); processed 1D data
6) 1D interferogram band-selective pure shift experiment (δ_{tof} = 5.2 ppm); raw 2D data
7) 1D interferogram band-selective pure shift experiment (δ_{tof} = 5.2 ppm); processed 1D data
8) 1D interferogram multiple frequency band-selective pure shift experiment (δ_{tof} = 5.0 ppm and selected regions centred at 5.2 ppm, 5.8 ppm, and 7.9 ppm); raw 2D data
9) 1D interferogram multiple frequency band-selective pure shift experiment (δ_{tof} = 5.0 ppm and selected regions centred at 5.2 ppm, 5.8 ppm, and 7.9 ppm); processed 1D data
10) standard 1D interferogram Zangger-Sterk pure shift experiment; raw 2D data
11) standard 1D interferogram Zangger-Sterk pure shift experiment; processed 1D data
12) multiple frequency selective 1D interferogram Zangger-Sterk pure shift experiment; raw 2D data
13) multiple frequency selective 1D interferogram Zangger-Sterk pure shift experiment; processed 1D data
14) standard 1D interferogram PSYCHE experiment (15+15ms saltire pulses); raw 2D data
15) standard 1D interferogram PSYCHE experiment (15+15ms saltire pulses); processed 1D data

16) 1D real-time band-selective pure shift experiment (δ_{tof} = 5.8 ppm); raw 2D data

17) 1D real-time band-selective pure shift experiment (δ_{tof} = 5.8 ppm); processed 1D data

18) 1D real-time band-selective pure shift experiment (δ_{tof} = 7.9 ppm); raw 2D data

19) 1D real-time band-selective pure shift experiment (δ_{tof} = 7.9 ppm); processed 1D data

20) 1D real-time band-selective pure shift experiment (δ_{tof} = 5.2 ppm); raw 2D data

21) 1D real-time band-selective pure shift experiment (δ_{tof} = 5.2 ppm); processed 1D data

22) 1D real-time multiple frequency band-selective pure shift experiment (δ_{tof} = 5.0 ppm and selected regions centred at 5.2 ppm, 5.8 ppm, and 7.9 ppm); raw 2D data

23) 1D real-time multiple frequency band-selective pure shift experiment (δ_{tof} = 5.0 ppm and selected regions centred at 5.2 ppm, 5.8 ppm, and 7.9 ppm); processed 1D data

24) standard 1D real-time Zangger-Sterk pure shift experiment; raw 2D data

25) standard 1D real-time Zangger-Sterk pure shift experiment; processed 1D data

26) multiple frequency selective 1D real-time Zangger-Sterk pure shift experiment; raw 2D data

27) multiple frequency selective 1D real-time Zangger-Sterk pure shift experiment; processed 1D data

28) 2D real-time BIRD HSQC experiment (with multiplicity editing option activated); raw data

29) 2D real-time BIRD HSQC experiment (with multiplicity editing option activated); processed data

30) parent, conventional HSQC experiment (with multiplicity editing option activated)

31) standard 1D real-time BIRD pure shift experiment (using HSQC with t_1 = ni = 0); raw data

32) standard 1D real-time BIRD pure shift experiment (using HSQC with t_1 = ni = 0); processed 1D data

33) 1D interferogram BIRD pure shift experiment (using a J-filter instead of HSQC with t_1 = ni = 0); raw 2D data

34) 1D interferogram BIRD pure shift experiment (using a J-filter instead of HSQC with t_1 = ni = 0); processed 1D data

35) standard 1D interferogram PSYCHE experiment (15+15ms saltire pulses); raw 2D data [same as 14]

36) standard 1D interferogram PSYCHE experiment (15+15ms saltire pulses); processed 1D data [same as 15]

37) Better suppression of artefact signals, compared to the standard experiment, at the cost of more T_2 and diffusion/convection losses. 1D interferogram PSYCHE experiment (50+50ms saltire pulses); raw 2D data

38) Better suppression of artefact signals, compared to the standard experiment, at the cost of more T_2 and diffusion/convection losses. 1D interferogram PSYCHE experiment (50+50ms saltire pulses); processed 1D data

39) standard 1D interferogram TSE-PSYCHE experiment; raw 2D data

40) standard 1D interferogram TSE-PSYCHE experiment; processed 1D data

41) conventional proton experiment

7 References

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