mddNMR
The User manual

Version 2.7

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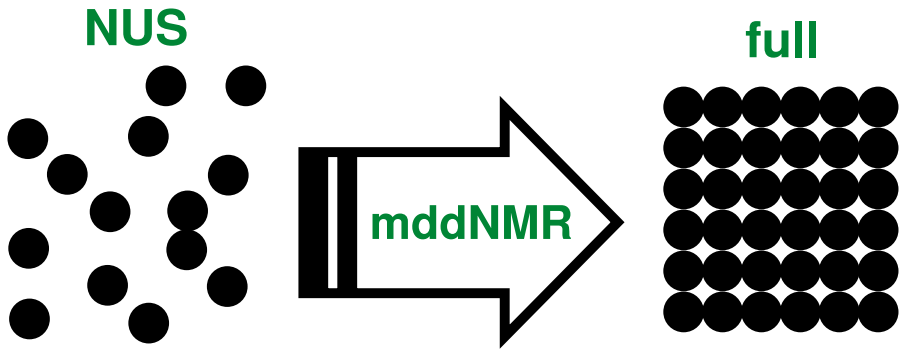

Figure 1: Reconstruction of NMR spectra from non-uniformly sampled signal using multidimensional decomposition (MDD) and Compressed Sensing (CS)

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Contents

1 Overview

MddNMR is a program for processing of non-uniformly sampled (NUS) multidimensional NMR spectra. The package contains also a routine to produce NUS schedule that can be used to setup N-dimensional NUS NMR experiments. Potentially any pulse sequence can be run in the NUS mode. In the NUS acquisition, only a fraction of full (conventional) data set is recorded. *MddNMR* uses multidimensional decomposition (MDD), compressed sensing (CS), and Low Rank (LR) reconstruction to replenishing missing data points in the full matrix followed by regular FT processing of the complete data.

2 Copyright

3 Citing the software

When presenting results obtained using the software, please cite at least one of the following papers:

1. Orekhov, V. Y. and V. A. Jaravine, Analysis of non-uniformly sampled spectra with Multidimensional Decomposition. Prog. Nucl. Magn. Reson. Spectrosc., 2011, 59, p. 271–292.
2. Kazimierczuk, K. and V. Y. Orekhov, Accelerated NMR Spectroscopy by Using Compressed Sensing, Angew. Chem.-Int. Edit., 2011, 50, p. 5556–5559.
3. Qu, X.; Mayzel, M.; Cai, J.-F.; Chen, Z.; Orekhov, V. Y, Accelerated NMR Spectroscopy with Low-Rank Reconstruction. Angew. Chemie - Int. Ed. 2015, 54 (3) p. 852–854
4. Mayzel, M., K. Kazimierczuk, and V.Y. Orekhov, The causality principle in the reconstruction of sparse NMR spectra. Chem Comm, 2014, 50, 8947–8950.

4 Downloads and updates

The software is available for download at http://mddnmr.spektrino.com/download or upon request from:

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Swedish NMR Center at Gothenburg University
Box 465, Gothenburg, SE 40530, Sweden
E-mail: orov@nmr.gu.se

All users of the program are encouraged to join news-group "mddnmr" at http://groups.google.com/group/mddnmr. The group is a forum for the MDD, CS, and software related discussions, as well as a billboard to inform the users about updates and bug fixes.

5 Installation

Note that to run the software you must have functioning *nmrPipe* package[1]. In order to run GUI *qMDD*, you also need python2 with additional libraries. For this, we suggest to install, for example, latest Anaconda python package from https://www.anaconda.com/products/individual, or which is free for academic use. Additionally, you can also use your system's standard python2 distribution and

[1] Delaglio, F., et al., 1995, J. Biomol. NMR, 6, 277-293

install missing packages using pip package manager. There are alternatives for installations on different platforms and python distributions. For these check attached file install_mddNMR.txt and posts in user group http://groups.google.com/group/mddnmr.

MddNMR software is distributed as a compressed Unix tar archives, e.g. *mddnmr2.0_29Jun2011.tgz*. Current version supports Linux (32 and 64 Bit) and Mac (Intel) OS X 10.4 and later. The corresponding binaries are automatically selected during installation. The step-by-step installation procedure is the following:

1. Uncompress and unfold the archive
2. Read content of *Copyright* file
3. Change directory to mddnmr2.xx and run command

   ```
   ./Install
   ```

   from the terminal
4. Add several lines into to your *.cshrc* file, as suggested by the terminal output produced by the script
5. [Optional] Download and install examples by unfolding corresponding tar archives in your preferable data location directory.

6 General concept

Traditionally, multidimensional NMR experiments are collected on regular grid of equally spaced points in the time domain. The signal is processed by Discrete Fourier transform (DFT). NUS or sparse data are generally processed by other methods. Sparse recording of spectra can save a lot of measurement time, especially for high-resolution nD datasets with extensive phase cycling.

Processing of a regular NMR spectrum includes several steps:

(i) conversion of the FID and parameters into *nmrPipe* format

(ii) Fourier transform in the directly detected dimension

(iii) Fourier transform in all indirect dimensions

(iv) view of the result and, if needed, fine-tuning of the processing parameters.

If spectrum is recorded in the NUS mode, the indirect dimensions cannot be Fourier transformed right away and *mddNMR* software intervenes between steps (ii) and (iii). Steps i-iii are performed using *nmrPipe*. The role of the *mddNMR* is to replenish complete data matrix with reconstructed points (Fig. 2).

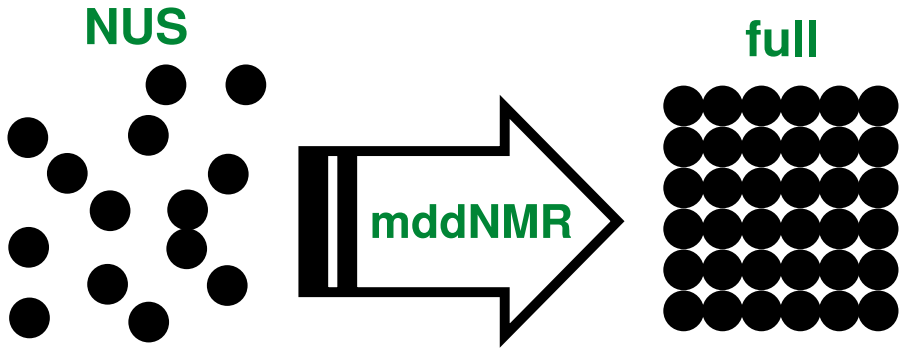


Figure 2: The software replenishes time domain data points in the indirect dimensions that are missing in the NUS set and produces the full data set amenable for regular Fourier transform

The software offers four general possibilities:

- *Direct Fourier transform*
  The complete data matrix is obtained by setting all missing points to zero. This spectrum has the lowest power among all possible spectra compatible with measured data
- *Multidimensional decomposition (MDD)*
  Spectrum constructed using MDD model
- *Compressed sensing (CS)*
  The sparsest spectrum, which is compatible with measured data
- *Low-rank method (LR)*
  The FID containing lowest number of components, which is compatible with measured data

In this manual, usage of the software is described by several commented examples. In addition, complete set of parameters and formats of essential files are given in Table 1 and in Appendices. Description of underlying mathematical algorithms and processing protocols can be found in our papers listed above and references cited therein.

7 *qMDD* graphical user interface

7.1 MDD calculation

The primary mode of *mddNMR* usage, which gives access to the full set of the software functionalities, is by C-shell scripts. There is, however, a graphical user interface (GUI) that simplifies work with the program, and is especially recommended for beginners. It is started with command

```
qMDD
```

for Python2 version, or

```
qMDD3
```

for Python3 version.

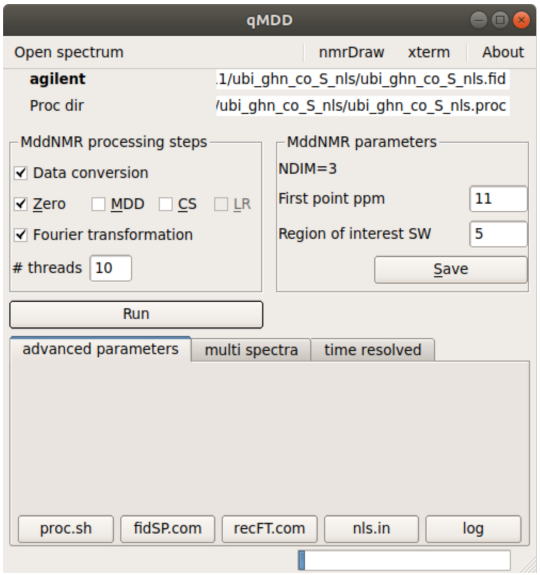
A window is opened, which invites to select a spectrum for processing. For example, you may select *ubi_ghn_co_S_nls.fid*, which is one of the example experiments in http://mddnmr.spektrino.com/download (data type is recognized by directory name, e.g. *.fid* for Variant/Agilent). Working directory named *ubi_ghn_co_S_nls.proc* is created, which is the place where you find all files discussed below. Answer 'yes' for the question (if any) about overwriting existing processing files. You are ready to process the spectrum by pressing button "RUN" (figure below, left).

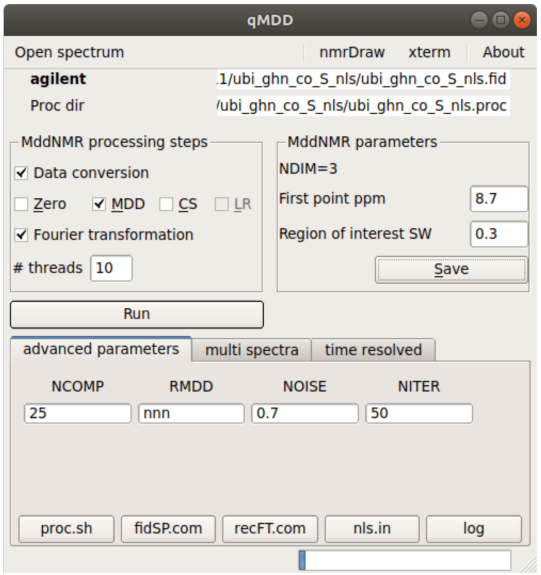
When button "RUN" is pressed, *qMDD* runs script *proc.sh*, which is found in the working directory (*ubi_ghn_co_S_nls.proc*). Active "Stop" button and sliding bar in the low-right corner of the window indicate calculations in progress. Details of the progress can be seen also in "log" window, which is opened with the corresponding button in the bottom right part of the program panel.

When the script is successfully finished, look at the resulting spectrum in *nmrDraw* by pressing button "nmrDraw" or starting *nmrDraw* in a terminal window. In *nmrDraw* file look at three projections of the 3D spectrum stored in *H1.C13.dat*, *H1.N15.dat* and *C13.N15.dat* or the 3D spectrum located in directory *ft*.

In the figure below (left), you may notice that the calculations were performed with "Zero" mode, which is essentially normal Fourier transform with all missing data points set to zero. This is the fastest and most robust method, albeit it provides the poorest results due to massive aliasing artefacts. Nevertheless, "Zero" mode (minimal power reconstruction) is useful since it allows fast look at the spectrum and adjustment of *nmrPipe* processing parameters, e.g. phases.

MddNMR software uses *nmrPipe* for spectral data conversion and traditional processing. There are two *nmrPipe* scripts that deal with these: *fidSP.com* and *recFT.com*. The former is responsible for conversion of the spectrum to the *nmrPipe* format and processing of the directly detected dimension. The latter script processes all indirect spectral dimensions after the missing data in the time domain interferogram is replenished by *mddNMR*. The scripts can be viewed and edited from "advanced parameters" by pressing the corresponding buttons.





Phases for all dimensions can be set in *fidSP.com* by modifying parameters for var2pipe (bru2pipe): -xP0, -xP1, -yP0, -yP1, etc. Set value for -xP0 to -48 (deg). Do not forget saving the script before closing the editor window. Rerun the calculation to see effect of the phase change.

Check the MDD box in order to try MDD algorithm for the same data. The GUI allows modification of several most important parameters. This is done in the "advanced parameters" display (fig. right). For example, you may set parameter CEXP to "yyn" in file in order to activate R-MDD mode for the indirect dimensions (13C, 15N) of the experiment.

Prior to pressing "RUN", you may define a small region of interest in the directly detected (amide proton) dimension by setting "First point ppm" and "Region of interest SW", as shown in the figure (right). This reduces the calculation time proportionally to the region size.

Check also parameter "# threads", which specify how many computational tasks can run simultaneously on your computer. **You need to press "Save" to activate the changes.** Meanings and recommended values for the parameters are indicated in contextual help, which appears when the mouse cursor is placed on the parameter field.

7.2 Adjusting conventional processing parameters

MddNMR software uses *nmrPipe* for spectral data conversion and traditional processing. There are two scripts that deal with these: *fidSP.com* and *recFT.com*. The former is responsible for conversion of the spectrum to the *nmrPipe* format and processing of the directly detected dimension. The latter processes all indirect spectral dimensions after the missing data in the time domain interferogram is replenished by *mddNMR*. The scripts can be viewed and edited from "advanced parameters" display by pressing the corresponding buttons.

The procedure can be illustrated on *gNhsqc_S.fid* spectrum example. Load the spectrum using "Open spectrum" button and answer 'Yes' to the question (if any) to discard the existing processing scripts. Process the spectrum with "Zero" (FT). Inspection of the spectrum in *nmrDraw* shows that phase in the directly detected dimension requires adjustment by ca. 70 degrees. Press "fidSP.com" button in the "advanced parameters" display and set the phase correction as shown below (in the highlighted line). Save the script and rerun the calculations.

```
var2pipe -in fid -noaswap -aqORD 0              \
-xN          2048         -yN      192          \
-xT          1024         -yT      96           \
-xMODE       Complex      -yMODE   Rance-Kay    \
-xSW         13008.100    -ySW     2600.300     \
-xOBS        800.128      -yOBS    81.085       \
```

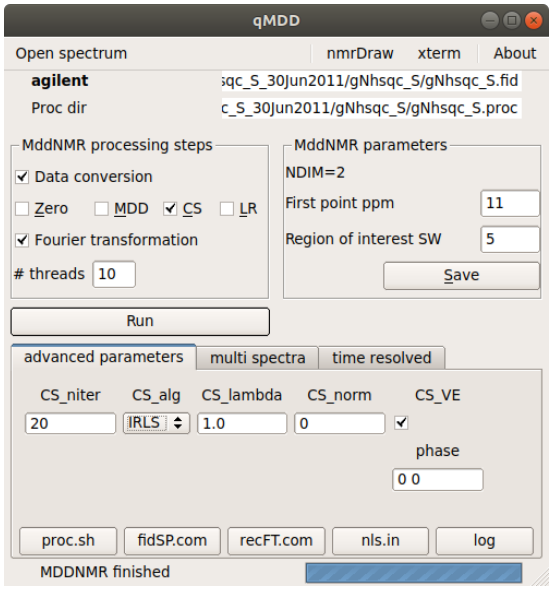
```
-xCAR       4.755       -yCAR   116.641         \
-xP0        156.1       -yP0    0.0             \
-xP1        0.0         -yP1    0.0             \
-xLAB       H1          -yLAB   N15             \
-ndim       2           -aq2D States            \

| nmrPipe -fn SOL                                          \
| nmrPipe -fn SP -off 0.450 -end 0.970 -pow 2 -c 0.500     \
| nmrPipe -fn ZF -auto                                     \
| nmrPipe -fn FT -auto                                     \
| nmrPipe -fn PS -hdr                                      \
| nmrPipe -fn PS -p0 70 -p1 0 -di                          \
| nmrPipe -fn EXT -x1 11ppm -xn 5ppm -sw -round 16         \
| pipe2xyz -z -out ft/data%03d.DAT -ov -nofs -verb
```

7.3 Compressed Sensing

CS processing is activated by selecting "CS" checkbox. You may try CS on *gNhsqc_S.fid* spectrum example. Note, that from version 2.5 mddNMR can be used to process 2D, 3D, and 4D spectra with CS. First, process the spectrum with "FT". Adjust the phase for the directly detect dimension as described in the previous section. Check the "CS" box and press "RUN" to calculate the spectrum. The result, which is stored in *test.dat* file, can be viewed in *nmrDraw*.

"Advanced parameters" view (see figure on the right) allows checking and editing the essential parameters for CS. For example, one can chose to use Iterative Reweighted Least Squares (IRLS) or Iterative Soft Thresholding (IST) algorithms (for 4D spectra only IST is allowed). Meanings and recommended values for the parameters are indicated in contextual help, which appears when the mouse cursor is placed on the parameter field.

Result of CS calculations is better, if Virtual Echo is used (see below).



7.4 CS with Virtual Echo

Compressed sensing is based on the assumption of sparsity (compressibility) of NMR spectra. Sparsity can be enhanced by a trick called Virtual Echo leading to better reconstruction quality (especially at low sampling levels)[2].

Two practical aspects of VE should be mentioned:

1. To use VE set CS_VE to "y" and phase parameter to appropriate value corresponding phase in the indirect dimensions (for half-dwell time, set phase to 0 (in a given dimension in *proc.sh*) and f180 to y. For instance, 2D spectrum requiring phase correction by 90 deg in F1 can be processed with VE if following lines are added into a script *proc.sh*:

```
setenv CS_VE y
setenv phase '90 0'
```

2. Calculation times and memory consumption are different for VE:

algorithm	Memory consumption (vs non-VE)	Calculation time (vs non-VE)
2D IRLS	ca. 2x higher	ca. 4x higher
3D IRLS	ca. 4x higher	ca. 4x higher
2D IST	ca. the same	ca. the same
3D IST	ca. the same	ca. 2x lower
4D IST (VE is obligatory!)	ca. the same	ca. 4x lower

7.5 Master script *proc.sh*

The role of GUI *qMDD* is to collect and check input from the user and to produce all necessary files for the calculations. The actual calculations are performed by C-shell script called *proc.sh* (or alike). The GUI

[2] For details see: Mayzel, M.; Kazimierczuk, K.; Orekhov, V. Y. The Causality Principle in the Reconstruction of Sparse NMR Spectra. Chem. Commun. 2014, 50 (64), 8947–8950.

supports basic and most frequently used features of the software, while more advanced processing may require editing of the master script. The script can be viewed and modified in "advanced parameters" view by pressing button "proc.sh" or in any text editor. As soon as the script is ready, it can be run in a terminal window or by pressing "Run" button. For example, *proc.sh* script for MDD/CS processing of *gNhsqc_S.fid* spectrum described in the previous section is:

```
% #!/bin/tcsh
setenv FST_PNT_PPM      11
setenv ROISW            5
setenv NUS_POINTS       96
setenv NDIM             2
setenv MDDTHREADS       4
setenv METHOD           MDD

% # CS algorithm related parameters
setenv CS_alg           IRLS
setenv CS_norm          0
setenv CS_lambda        1.0
setenv CS_niter         10

% # MDD algorithm related parameters
setenv SRSIZE           0.1
setenv NCOMP            25
setenv NITER            50
setenv MDD_NOISE        0.7
setenv CEXP             nn
setenv lambda           0.001

mddnmr4pipeN.sh 1 2 3 4 5
```

The master script sets all parameters that have to be altered from defaults (description of the parameters is in section 'Processing parameters'). This is done by setting C-shell environment variables using command `setenv`. The actual calculations are started by command `mddnmr4pipeN.sh` at the end of the script. As arguments, the command takes list of tasks to be performed (1–5), which are defined by unique numbers (described in section Advanced processing).

8 Advanced processing

Several examples presented in this manual illustrate most of the software features. The commands are typically arranged into short C-shell (Unix) scripts. The master script, which is called *proc.sh* in this manual and in all examples, first sets several parameters (most of the parameters have good defaults values, and are not set explicitly). The parameters are set as Unix environment variables with command `setenv` (see examples in Section 8.4). They can be changed by modifying the script. Finally the processing is performed by `mddnmr4pipeN.sh` command. As line parameters `mddnmr4pipeN.sh` takes a list of steps, which are integer numbers. Typically steps 1 - 5 are executed sequentially because output from a previous step serves as an input for the next one. The steps are:

0 – print full list of parameters recognized by the program.

1 – conversion of ser/fid to *nmrPipe* format; processing of the direct dimension and extraction of region of interest (ROI) (see *nmrPipe* script defined by parameter fidSP

2 – preparing input for MDD/CS calculations.

3 – MDD/CS calculations over all sub-regions of the ROI.

33 – estimate memory required for the reconstruction 4

4 – full spectrum matrix is produced.

5 – the full time domain reconstruction obtained in step 4 is processed using an *nmrPipe* script (see parameter REC2FT)

42 – MDD mode only: MDD shapes obtained on step 3 are processed by *nmrPipe* and written into Unified Spectral Format (USF3) (see parameters Proc3D_* and Proc4D_*).

Several steps can be executed in one line, e.g.

```
mddnmr4pipeN.sh 1 2 3 4 5
```

or can be done by consecutive calls of `mddnmr4pipeN.sh`, for example:

```
mddnmr4pipeN.sh 1 2 3
mddnmr4pipeN.sh 4 5
```

In the first case, the program passes the data from one step to another in memory. In the latter example, intermediate results are stored in files in working directory MDD. This can be useful to skip lengthy calculations on initial steps when changes are required for the later downstream steps only. For example, lengthy MDD calculations on step 3 may be performed only once for all possible *nmrPipe* processing of the reconstructed spectrum obtained at steps 4 and 5.

8.1 Input files

There is at least one file, which needs to be prepared for the processing. Name of this file is conveyed to *mddNMR* by parameter fidSP. The file *fidSP* is an *nmrPipe* script that performs conversion from spectrometer to *nmrPipe* data formats using programs bruk2pipe or var2pipe, Fourier transform of the directly detected dimension and storing of region of interest (ROI) to disk. In this manual and in all examples, the script is called *fidSP.com*. The script is automatically generated by *qMDD* GUI or can be produced by command `nus2pipe` from *mddNMR* software or can be prepared by editing the data conversion script produced by programs *bruker/varian* from *nmrPipe* package. For example, for experiment 57 from the examples, correct *fidSP.com* file is produced by:

```
nus2pipe -f 57 -t Bruker
```

Below is an example of the fidSP file for experiment 57.

```
bruk2pipe -in ./ser -bad 0.0 -aswap -DMX -decim 2000                  \
                    -dspfvs 20 -grpdly 67.9862518310547               \
-xN          2048         -yN          1            -zN          3712        \
-xT          1024         -yT          0            -zT          856         \
-xMODE       DQD          -yMODE       Complex      -zMODE       Complex     \
-xSW         10000.000    -ySW         2500.000     -zSW         2500.000    \
-xOBS        600.130      -yOBS        150.903      -zOBS        60.811      \
-xCAR        4.702        -yCAR        175.327      -zCAR        115.840     \
-xP0         -46.9        -yP0         0.0          -zP0         0.0         \
-xP1         22.4         -yP1         0.0          -zP1         0.0         \
-xLAB        1H           -yLAB        13C          -zLAB        15N         \
-ndim 3 -aq2D States                                                  \
| nmrPipe -fn POLY -time                                  \
| nmrPipe -fn SP -off 0.450 -end 0.970 -pow 2 -c 0.500    \
| nmrPipe -fn ZF -auto                                    \
| nmrPipe -fn FT -auto | nmrPipe -fn PS -hdr               \
| nmrPipe -fn PS -p0 0 -p1 0 -di                          \
| nmrPipe -fn EXT -sw                                     \
% #| nmrPipe -fn POLY -auto                                  \
| pipe2xyz -z -out ft/data%03d.DAT -ov -nofs -verb
```

Script *fidSP.com* may be modified, for instance, for adjusting phase in the indirect dimension or chemical shift references. On step 1, script *fidSP.com* is used as a template for generating several scripts (*FTx.sh**) , which are actually used in the processing.

Name of another important *nmrPipe* script is set by parameter REC2FT. This is an *nmrPipe* script that performs regular processing of the full data matrix in all indirect dimensions. User may need to change some parameters, e.g. phase corrections, weighting functions, linear prediction, etc. To do this, make and/or edit a local copy of the script *recFT.com* in the processing directory. The templates for *recFT.com* files can be found in the ${MDD_NMR}/com directory.

8.2 Real dimensions

The processing of series of 2D and 3D spectra, e.g. relaxation, arranged as pseudo-3D and 4D NMR data, respectively, is possible in both CS and MDD modes and is performed using "real" dimensions. CS performs a separate processing on each of the 2D or 3D spectra in a series (thus forming pseudo-3D or pseudo-4D input and output). MDD treats all data together, which provides superior reconstruction, if peaks do not change their positions. To process pseudo-3D or pseudo-4D data, the dtype parameter has to be set to define which dimensions are 'real', i.e. enumerate spectra in a stack. For serial 2D data, the parameter should be set to rcr, for serial 3D data to rccr. The example serial data can be found at http://mddnmr.spektrino.com/downloadE/Real-dim_pseudo-3D_Nhsqc_T2.zip.

Please note the following :

1. Only 1st indirect dimension can be real
2. *qMDD* does yet not support script setup for real dimensions
3. Sampling schedule should include all indirect dimensions, also the real one.

Below are the example pre- (fidSP) and post-processing (recFT) scripts for pseudo-3D (series of 2D) data:

fidSP:

```
bruk2pipe -in ./ser -bad 0.0 -noswap -DMX -decim 1584 -dspfvs 21 -grpdly 76 \
-xN     1536            -yN     1           -zN         400                 \
-xT     768             -yT     1           -zT         200                 \
-xMODE  Complex         -yMODE  QF          -zMODE      Echo-AntiEcho       \
-xSW    12626.263       -ySW    4409.222    -zSW        2553.626            \
-xOBS   899.890         -yOBS   899.890     -zOBS       91.185              \
-xCAR   4.698           -yCAR   4.698       -zCAR       117.500             \
-xP0    -122.1          -yP0    0.0         -zP0        90.0                \
-xP1    0.0             -yP1    0.0         -zP1        0.0                 \
-xLAB   1H              -yLAB   T2          -zLAB       N15                 \
-ndim 3 -aq2D States    \

| nmrPipe -fn POLY -time                                 \
| nmrPipe -fn SP -off 0.48 -end 0.95 -pow 2 -c 0.5       \
| nmrPipe -fn ZF -auto | nmrPipe -fn FT -auto            \
| nmrPipe -fn PS -hdr                                    \
| nmrPipe -fn PS -p0 0 -p1 0 -di                         \
| nmrPipe -fn POLY -auto -xn 5.0ppm -ord 1               \
| nmrPipe -fn EXT -sw                                    \
| pipe2xyz -z -out ft/data%03d.DAT -ov -nofs -verb
```

recFT:

```
#!/bin/tcsh -f
cat $1
| nmrPipe -fn TP -auto                                  \
| nmrPipe -fn ZTP -auto                                 \
| nmrPipe -fn SP -off 0.48 -end 0.95 -pow 2 -c 0.5      \
| nmrPipe -fn ZF -auto | nmrPipe -fn FT                  \
| nmrPipe -fn PS -hdr                                   \
| nmrPipe -fn PS -p0 0.0 -p1 -0.0 -di                   \
| nmrPipe -fn TP -auto                                  \
| pipe2xyz -out $proc_out -x -verb -ov
echo $proc_out ready
exit
```

8.3 Deconvolution

The MDD and CS processing with IRLS algorithm allows deconvolving assumed modulation in the indirect dimensions, e.g. J-modulation or exponential decay. To perform the deconvolution, the parameter deconv has to be set to specify the dimension and deconvlolution function. For example, to perform virtual decoupling of doublets in the first indirect dimension with J-coupling of 35 Hz use:

```
setenv deconv               'ynn cos:J=35'
```

Moreover, CS allows to switch off the deconvloution for a spectrum part located below a defined ppm value. This allows to separately process the "singlet region" in the first indirect dimension, which is useful in the case of HNCA and similar spectra (as described in: https://arxiv.org/abs/2009.08813). The upper border of the "singlet region" is set by adding extra number to the CS_lambda parameter. For example, to deconvolute J-modulation of 35 Hz in the first indirect dimension of a 3D spectrum, but not for peaks below 45 ppm in that dimension, one has to set the processing parameters in the following way:

```
setenv deconv               'ynn cos:J=35'
setenv CS_lambda             '1.0 45'
```

Importantly, the spectral width (SW) **in Hz** also has to be set, either in `nls.in` or in `proc.sh` :

```
setenv SW                    '6031.36 2918.86 9615.385'
```

The singlet region extraction requires passing FT processing mode (`-alt` and `-neg`) used in the indirect dimension as FT_FLAGS parameter:

```
setenv FT_FLAGS '3 0 0' # FT -neg -alt
```

or

```
setenv FT_FLAGS '2 0 0' # FT -alt
```

or

```
setenv FT_FLAGS '1 0 0' # FT -neg
```

8.4 Processing parameters

The parameters are typically set in the master C-shell script using command `setenv`. Full list of parameters with their current values can be viewed by command

```
mddnmr4pipeN.sh 0
```

Since most of the parameters have good default values, typically only few parameters need to set explicitly. For an illustration, let us look at the commented master script for processing scripts for experiment 57, which is one of the examples provided with the software. The experiment is a 3D HNCO spectrum recorded on Bruker spectrometer using NUS acquisition mode in TopSpin 3.0.

```
# input/output files
setenv FID ../57                    # location of directory with the experiment
setenv fidSP fidSP.com              # script for conversion to nmrPipe
setenv in_file nls.in               # parameters of the NUS schedule
setenv selection_file nls.hdr_3     # nus schedule

# processing algorithm
setenv METHOD MDD                   # FT/MDD/CS/LR

setenv REC2FT recFT.com             # pipe procession of the indirect dimensions
setenv proc_out ft/test%03d.dat     # nmrPipe template for the final spectrum

# Definition of a small region of interest (ROI) in the direct dimension
setenv FST_PNT_PPM 8    # first point in ppm
setenv ROISW 0.5        # ROI size in ppm

#MDD related parameters
setenv MDDTHREADS 2     # maximal number of parallel processes
setenv NCOMP            # number of components per sub-region 25
setenv NITER 100        # number of iterations
setenv SRSIZE 0.1       # approximate size of sub-region in ppm
setenv MDD_NOISE 0.7    # factor for adding residuals to the MDD reconstruction
setenv lambda 0.01      # MDD lambda
setenv CT_SP nnn        # parameter CT_SP in file nls.in is overridden
setenv CEXP nnn         # parameter CEXP in file nls.in is overridden

# start actual calculations
mddnmr4pipeN.sh 1 2 3 4 5
```

In the script above, only first four parameters (highlighted) are needed to be set explicitly. The remaining parameters are there mostly for display. The MDD solver has one parameter that mostly affects quality of the solution, namely, number of components (parameter NCOMP) per sub-region. Guidelines on correct setting for the parameter can be found in our papers. In most cases, however, a default value of ca 30 for a sub-region strip of 0.1-0.2 ppm (parameter SRSIZE) in the directly detected ^{1}H dimension is a good guess. In short, the number of components must be 20-50% larger then number of expected cross-peaks for 2D's and triple resonance backbone experiments, or number of diagonal peaks for 3-4D NOESY/TOCSY experiments. Note that number of components refers to a sub-region. NCOMP value must be sufficient for a sub-region with maximal expected number of peaks.

Table 1: Parameters recognized by *mddNMR* software

Parameter name	**Default value**	**Meaning and references for examples**
CEXP	nn...	MDD only parameter. y or n for indirect dimensions, toggles RMDD mode for the corresponding dimension, i.e. assumption of exponent or complex exponent functional form for the signal. If set, override value in nls.in file.
CS_alg	IRLS	CS algorithm: IRLS – iterative reweighted least squares, or IST – iterative soft thresholding

CS_lambda	1.0	CS regularization (default is ok for all studied cases). For IRLS, to make spectrum less sparse decrease lambda (applicable, if noise seems to be 'peaky', i.e. turned into sparse representation). For IST, CS_lambda has to be =<2 (values <1 make spectrum less sparse). If deconv parameter is active, then optional second number in a CS_lambda array sets the upper border of the 'singlet region', e.g. '1.0 45'. A non-zero third number enables the division mode for deconvolution, but is not recommended.
CS_niter	10	Number of iterarations for CS (default is ok for IRLS). Change to 100-10000 for IST.
CS_norm	0	Norm for CS IRLS algorithm: 0 - 1
CS_VE	y	Virtual echo on/off. If turned on (y), please set phase parameter to a proper value
CS_ZF	2	Frequency domain "over-digitization" in CS algorithm (best results for 2)
CT_SP		
DATAMAP_FILE		
deconv		Works for MDD and CS-IRLS 2D&3D Defines whether the deconvolution is used and in which dimensions. For example 'ynn cos:J=35' means, that first indirect dimension is deconvoluted from cosine of 35 Hz. Requires proper setting of SW parameter (in Hz). If singlet region extraction is used, the upper border of the region should be set in a CS_lambda parameter and FT processing flags should be set in FT_FLAGS parameter.
DIM_MERGE		If defined, dimensions to merge. Used, e.g. to process 4D spectrum with 3D MDD
dtype		Defines which dimensions are "real" (r) and which are complex (c). For example, for the serial 2D HSQC relaxation experiment dtype should be set to rcr. Only first indirect dimension can be real. Direct dimension (last) is real by default
f180		Defines the 1/2-dwell time (180 deg linear phase) mode. If set, overrides value in nls.in file
FID		Directory with experimental data
fidSP		name of the step 1 script
FIX_FREQ		Reserved
FIX_FREQ_FILE		Reserved
FST_PNT_PPM	10	Start (highfield) of the region of interest (ppm) in the directly detected dimension
ft4	./XYZA/ft4.xyza	Reserved
FT4DX	FTx.sh	Reserved
FTX_2D	./XYZA/ft4sp.xyza.2D	Reserved
FTXTREC	/XYZA/ft4trec.xyza	Reserved
FT_FLAGS	'0 0 0'	Required when deconvolution with singlet region extraction is used. Flags defining the processing mode used. 3: `-alt -neg`, 2: `-alt`, 1: `-neg`, 0: no flags
in_file		Name and location of NUS parameter file.

Jsp		Modulation of the sampling schedule produced by the nussampler program
lambda	0.005	Tikhonov regularization for MDD: 0.001-0.1
MAP_FACTOR	1	Reserved
MDD_DIR	./MDD	Reserved
MDD_FILE	./MDD/region	Reserved
MDD_NMR	..../mddnmr2.0	Location of the software directory
MDD_NMR_COM	..../mddnmr2.0/com	
MDD_NOISE	0.7	A factor that scales residuals of the MDD and IST calculations as they are added to the reconstructed spectrum: 0 - 1
MDD_STDERR	stderr	If set to a file name, all error terminal messages are redirected to the file
MDD_STDOUT	stdout	If set to a file name, all terminal messages are redirected to the file
MDD_WORK_DIR	.	Processing working directory
MDDRUNS	./regions	Reserved
MDDTHREADS	2	Maximal number of threads, i.e. number of processes that can be run on your computer at the same time. The parameter relates to number of processors and cores on the computer
METHOD	FT	Processing method: FT, MDD, CS, LR
NCOMP	30	Number of MDD components per subregion
NDIM		If set, override value in nls.in file
NI		If set, override value in nls.in file
NIMAX		If set, override value in nls.in file
NIMIN		If set, override value in nls.in file
NITER	300	Number of iteration for MDD calculations
NUS_POINTS		Usually, number of the NUS points in the experiment, but can be less to use only part of the data
NUS_TABLE_ORDER		reshuffles columns in the nuslist
OVLP	3	Overlap [points] between sub-regions in points
phase		If set, override value in nls.in file. Important to set it properly for CS_VE='y'
PHASE_ORDER		If set, reshuffle FID's inside each hypercomplex point, e.g. setting '1 3 2 4' results in swapping of the 2nd and 3rd FID's. The parameter is analogous to -aqORD flag in nmrPipe, which does not work for NUS processing. See also programs fid_shuffle and ser_shuffle .
Proc3D_X	shapeProc3D_%c.sh	Reserved
Proc3D_Y	shapeProc3D_Y.sh	Script for processing MDD shapes for Y dimension
Proc3D_Z	shapeProc3D_Z.sh	Script for processing MDD shapes for Z dimension
Proc4D_A	shapeProc4D_A.sh	Script for processing MDD shapes for A dimension
Proc4D_YZ	shapeProc4D_YZ.sh	Script for processing MDD merged shapes for YZ dimensions in a 4D spectrum
proc_out	./ft/tdrec%03d.dat	nmrPipe template for the final output spectrum
REC2FT	recFT.com	Script to process indirect dimensions in a 3D spectrum
RECHEAD	./XYZA/ft4sp.xyza	Reserved
ROISW	6	Size of the region of interest in the directly detected dimension, ppm

RUNQUE		Reserved
seed	2345	Random seed for MDD calculations
selection_file		NUS schedule file, typically comes with the experiment
SHAPEMAP		Co-processing: setting correspondence of dimensions between two experiments
SHAPEMAP_FILE	./MDD/regionMAP	Co-processing: file with the reference MDD shapes
soft_mode	p	Reserved
SPARSE		If set, overrides value in nls.in file
SRSIZE	0.18	Approximate size of sub-region in ppm
SW		If set, overrides value in nls.in file. Important for the deconv !
T2		Decay of sampling density for the nussampler program
XDimSize	1	Reserved

9 Parallel calculation for faster MDD and CS processing

MDD and CS calculations may be lengthy. The computation time rapidly increases with amount of experimental data, number of iterations, number of components (for MDD), and size of the final spectrum (for CS). Calculations for different sub-regions are independent and can be performed in parallel on several CPUs that are available on one computer or within a local network. On one computer, parallel calculations are organized simply by setting parameter MDDTHREADS to the number of CPU cores. In order to distribute calculations over a network, e.g. for a Linux cluster, step 3 may be performed off-line. First steps 1 and 2 are performed:

```
# Preparing input for sub-regions
mddnmr4pipeN.sh 1 2
```

This produces files *regions.runs* and *MDD/regionXX.mdd*, which are the only input for standalone MDD and CS solvers, *mddsolver* and *cssolver*, respectively. File *regions.runs* is a C-shell script. Each line in it contains a command for running calculations for one region. The commands may run in parallel on one computer or be distributed over the network together with *MDD/regionXX.mdd* for corresponding regions. When calculations are complete, the results, which are files *MDD/regionXX.res* or *MDD/regionXX.cs*, need to be collected to the original MDD directory followed by the spectrum reconstruction and final *nmrPipe* processing (steps 4 and 5):

```
# time domain spectrum reconstruction and final nmrPipe processing
mddnmr4pipeN.sh 4 5
```

For a quick test run, select (in *qMDD* or *proc.sh*) a narrow strip of about 0.2 ppm in the direct acquisition dimension.

10 Examples

Examples can be downloaded from http://mddnmr.spektrino.com/download and are described in the Appendix. For each example there is a compressed tar archive with two directories containing the spectrum and the script[s] for its processing (**.proc*). For large data sets, the spectrum may be in a separate tar archive, which allows skipping download of large examples data files. Scripts for the HNcoCA example can be used, after minor modifications, for most of the backbone experiments.

[3] Jaravine, V.; Ibraghimov, I.; Orekhov, V. Y. Nature Methods 2006, 3, 605.

Table 2: List of examples

	File name	Protein & citation	Experiment	Spectrometer	comment
1	gNhsqc_S.tgz	Azurin	2D HSQC	Varian	BioPack
2	ubi_ghn_co_S.tgz	Ubiquitin	3D HNCO	Varian	BioPack
3	284_hncoca.tgz	Ubiquitin	3D HNcoCA	Bruker	
4	57.tgz	Ubiquitin	3D HNCO	Bruker	
5	BPgnoesyNhsqc_S.tgz		3D 15N NOESY-HSQC	Varian	BioPack
6	az_HNCA_high_res.tgz	Azurin[3]	3D HNCA	Varian	BioPack*
7	Deconv_3D_HNCA.zip	Tau	high res. 3D HNCA	Bruker	

*The experiment was recorded in full, i.e. without NUS. This allows sparsifying of the data set and checking how quality of the spectrum degrades as fewer and fewer data are used for the reconstruction of the full fid matrix.

A Appendices

A.1 NUS schedule

NUS schedule is typically produced by spectrometer software and is saved in a text file together with the experiment. Major spectrometer software vendors use program *nussampler*, which is also part of the *mddNMR* distribution. *nussampler* can be evoked from a terminal command line:

```
nussampler nls.in
```

nussampler takes parameters from the input file *nls.in* (this name is fixed for most cases), generates a sampling scheme and writes it to file (*nls.hdr_3* on Varian or *nuslist* on Bruker). Both files *nls.in* and *nls.hdr_3* are needed for NUS spectral processing and must be stored together usually in the directory with *fid* or *ser* files. Typically the *nls.in* file is produced by executing the script from a GUI in spectrometer software, e.g. BioPack (Varian). The *nls.in* text file can be edited manually, and the above command for generating of a schedule can be typed from Unix command line.

Random number generation: now the program uses the 'Mersenne Twister' pseudorandom number generator developed by Takuji Nishimura and Makoto Matsumoto[4] (in previous versions it was function rand() from stdlib.h). The generator is tested to be platform independent; test generated the same nuslist on Linux 32-bit, Linux 64-bit, Windows 32-bit, Windows 64-bit.

A.1.1 File nls.in – setting NUS parameters

Each line of NUS parameter file *nls.in* starts with a keyword followed by a list of parameters values. All the keywords are mandatory, but the lines order is not important. Below several examples of input files are explained. Location of *nls.in* file needs to be specified by parameter in_file in the master script (*proc.sh*).

```
file        nls.in
NDIM        3
seed        54321
SPARSE      y
sptype      poissongap
f180        nnn
CT_SP       nyn
CEXP        yyn
```

[4] M. Matsumoto and T. Nishimura, "Mersenne Twister: A 623-Dimensionally Equidistributed Uniform Pseudo-Random Number Generator", ACM Transactions on Modeling and Computer Simulation, Vol. 8, No. 1, January 1998, pp 3-30. http://www.math.sci.hiroshima-u.ac.jp/~m-mat/MT/emt.html

```
NIMAX       40 30 1
NIMIN       0 0 0
NI          7 30 1
SW          1824.818 2112.825 8389.262
T2          1.0 0.05 1
Jsp         0 0 0
phase       0 0 0
PERMUTE_NUSTABLE 2 1
FIRST_POINT_ZERO 1
nholes -1
```

- NDIM – number of dimensions, e.g. 3 for 3D spectrum
- seed – seed for random number generator. If seed and other parameters are not changed output NUS schedule table will be always the same.
- SPARSE – y/n – during the processing, the flag toggles processing of a real sparse spectrum ('y') vs sparsifying of a full spectrum ('n'). When setting up a BioPack experiment on a spectrometer, it toggles between NUS and regular sampling.
- sptype – option for type of random selection (default:poissongap)
  - sptype = poissongap — reg — shuffle — norepeatshuffle (only the first char is checked)
  - poissongap [default] Poisson Gap sampling, all gaps in accordance with the Poisson distribution with exponentially and J-coupling matched options applicable.
  - reg - regular =uniform sampling, if selected other options may be ignored
  - reg - regular =uniform sampling, if selected other options may be ignored
  - shuffle - incremental matched sampling, no check for point repetition (so schedule may contain point repeats).
  - ovewriteshuffle - old name, which is now equal to shuffle (kept for back-compatibility).
  - norepeatshuffle - incremental matched sampling, points are checked for no repetition, takes longer due to that, and sometimes will not find schedule especially for very short T2, e.g. $<$ 1ms (increase T2, if so).
- f180 – flags to specify 180 degree linear phase. Set y or n for every dimension. Direct dimension is the last one. The flag is important only for dimensions with CT_SP equal 'y'.
- CEXP – y/n toggle R-MDD / MDD mode for a dimension, with 'y' time domain shape in the dimension is expected to be autoregressive. In other words, we assume that the FID in the dimension is a complex exponent. CEXP=y may be used, for example, for HNCO and HNcoCA experiments, but not for the NOESY's.
- CT_SP – n/y toggles mirror image processing for dimensions with CEXP='y' . Set 'n' for the first indirect dimension. CT_SP have to be stet to 'y' for the constant-time second indirect dimension (typically 15N) in the triple resonance experiments.
- NIMIN – min indices [for evolution increments]
- NIMAX – full indirect sizes of the spectrum (you may put 1 for the (last) direct dimension)
- NI – Multiplication of all NI values gives total number of (hyper-complex) points in the indirect dimensions. Put 1 for the direct dimension, which is the last number.
- SW – spectral windows for dimensions in Hz
- T2 – estimate of transverse relaxation times for each dimension for NUS. Use large value for dimension with constant-time (CT) evolution

- Jsp – estimated resolved J-coupling for each dimension
- phase – zero order phase correction for indirect dimensions. Used for dimensions with CT_SP=y

Optional parameters

- PERMUTE_NUSTABLE 2 1 – for Bruker the table is permuted
- FIRST_POINT_ZERO – if 1 makes the point with zero evolution times at the top of NUS schedule
- nholes - if negative, no check for empty rows/columns in the R-MDD nD matrix.

Alternatively, the parameters are entered directly as arguments to the program, using option '-p', one by one, enclosed in '' (= sign can be omitted):

```
nussampler -p 'file=nls' 'NDIM=3' 'SPARSE=y' 'seed=4321' 'sptype=shuffle'
'f180=nnn' 'CT_SP=nyn' 'CEXP=yyn' 'NIMIN=0 0 0' 'NIMAX=120 224 1' 'NI=12 224 1'
'SW=1500 2500 10000' 'T2=0.035 1 1' 'Jsp=0 0 0' 'phase=0 0 0'
'PERMUTE_NUSTABLE 2 1' 'nholes -1'
```

A.1.2 NUS table files *nls.hdr_3* or *nuslist*

The number of entries in the NUS table is equal to product of NI values for all indirect dimensions, i.e. NI x NI2 [x NI3 ...]. Each line consists of indexes for all indirect dimensions, i.e. two numbers per line for 3D experiment. The points are selected from a regular grid, thus values of the indexes in the table are integers from zero to NImax-1, NI2max-1, [NI3max-1 ...], respectively. By default, the NUS schedule is given in random order, that is evolution time points are not ordered. This allows stopping an experiment at any time without losing digital resolution.

A.1.3 Algorithm for the generation of the NUS schedule

NUS or sparse schedule suitable for MDD/CS processing selects for detection only a fraction of points from a complete Nyquist grid. Sampling on the grid is a special case of more general NUS that allows sampling at arbitrary selected time points. The selection of points in *mddNMR* software (see *nussampler*) is governed by a multidimensional probability density function for all indirect evolution dimensions. For example for a 3D experiment, the function is defined on a two-dimensional grid (t1, t2) determined by spectral widths and maximal acquisition times (t1max, t2max) in the two indirect dimensions. The distribution is obtained as a product of the two envelopes, P(t1,t2) = P1(t1) x P2(t2). The envelope functions P1(t1) and P2(t2) are devised to match the signal intensity in the indirect dimensions for a particular system and experiment. Currently two possibilities are implemented:

(i) mono-exponential relaxation: P(t1) = exp(-t/T2)

(ii) modulation by the one-bond J-coupling: P(t2) = cos(t pi/J).

The J-modulation can be combined with the relaxation decay. The transverse relaxation time T2 and value of the J-coupling are parameters of the procedure and are defined in the "*.in*" file. For a given probability distribution, we use the following procedure to generate the NUS schedules. First, a pair of integer indices is randomly selected that corresponds to the acquisition times (t1, t2). Then the pair is added to the sampling schedule table if the corresponding value of the probability distribution P(t1,t2) is larger than a randomly generated number ranging between 0 and 1; otherwise, the index pair is discarded. This process is repeated until the sampling table contains the requested number of data points for each step. Thus, a NUS schedule is a table of evolution delays (t1, t2) spanning maximal acquisition times and spectral widths in the indirect dimensions.

A.1.4 Algorithm for 'Poisson Gap' random number generation

The algorithm for Poisson Gap random number generation is adapted from Knuth. It ensures that the gaps between points in the nD distribution follow Poisson distribution[5]:

algorithm poisson random number (Knuth):
    init:
        Let $L \leftarrow \exp^{-\lambda}$, $k \leftarrow 0$, $p \leftarrow 1$
    do:
        $k \leftarrow k + 1$
        Generate uniform random number u in [0,1] and **let** $p \leftarrow p \times u$
    while $p > L$
    return $k - 1$

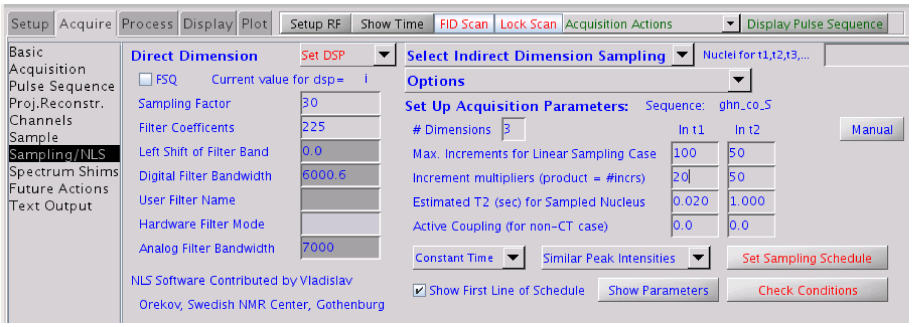
A.2 NUS Implementations on NMR spectrometers

The flexibility of the pulse programming languages of VnmrJ and TopSpin has allowed straightforward and generic implementations of acquisition of NUS nD data. See vendor software manuals for exact details, e.g. documentation for BioPack or TopSpin of version 3.0 and higher.

The NUS scheme is generically applicable for most, if not all, of existing pulse sequences. Uniformly incremented evolution delays in the pulse sequence are substituted by the values from the NUS table for every FID. New evolution delays are produced by multiplying the indexes by corresponding dwell time (i.e. 1/sw). For every combination of evolution times all FID's comprising one hyper-complex point must be recorded as one block. Thus, the block contains 2, 4, and 8 FID's for 2D, 3D, and 4D spectra respectively. This corresponds to standard Varian/Agilent convention, but is different for old Bruker pulse sequences.

A.2.1 BioPack: Varian/Agilent spectrometer

The BioPack implementation (see Varian/Agilent documentation for details) features automatic creation of a new NUS version of any multidimensional pulse sequence by selecting *Sparse NLS Sampling* (figure below). After specifying the number of increments, etc., a single button is used to generate all the NUS files, including **.in* file, table of increments (*hdr_3) via the *Set Sampling Schedule* button in the same page. Acquisition is performed in the normal manner. Saving of the data using the BioPack macro **'BPsvf'** also saves the NUS files and a script to permit processing by the MDD software.



In the VnmrJ command line, NUS version of any experiment can be produced using macro *BP_NLSinit(<dims>)*, where *<dims>* stands for the number of dimensions in the experiment, thus for HNCO in the command line type

BP_NLSinit(3)

[5] https://en.wikipedia.org/wiki/Poisson_distribution

The macro prepares NUS version of the pulse sequence (look at *ghn_co_S.c*) and adds few additional parameters, which can be viewed in the "text output" tab using command *dgnls*.

Set *SPARSE='n'*, *phase=1,2*, *phase2=1,2* and use parameters *nt*, *ni*, *ni*2 and command *time* to adjust time of the experiment.

Note that parameters *ni*, and *ni*2 define only total duration of the NUS experiment, the size of the sampling grid is defined by parameters *nimax* and *ni*2*max*. Set *SPARSE='y'*and calculate the sampling schedule by using macro

BP_NLSset

The macro creates two files in the experiment directory *~/vnmrsys/expXX*: *nls.in* and *nls.hdr_3*. The former contains parameters that are used for the generating the NUS schedule.

```
nls.in
    file /home/bcbp/vnmrsys/exp4/nls
    NDIM 3
    SPARSE y
    seed 4321
    sptype shuffle
    nholes 0
    f180 nnn
    CT_SP nyn
    CEXP yyn
    NIMAX 50 50 1
    NIMIN 0 0 0
    NI 5 50 1
    SW 2500 3000 10000
    T2 0.02 1 1
    Jsp 0 0 0
```

File nls.hdr_3 contains the sampling schedule, i.e. the list of selected points from the 2D grid (13C, 15N).

```
nls.hdr_3
    0 0
    36 45
    11 47
    3 26
    9 44
    8 38
    5 4
    ...
```

Files *nls.in* and *nls.hdr_3* are needed to run and process the experiment.

A.2.2 Varian/Agilent spectrometer: default sparse sampling

The data acquired in Agilent default NUS mode (available in VnmrJ 4.0 and higher) that can be set up by clicking "Sparse" checkbox in the Sampling tab can also processed with *mddNMR*. Note, that this approach does not modify the pulse sequence code and is available also outside Biopack.

To make such data accessible with *qMDD*, a few modifications are required:

- set SPARSE parameter on (SPARSE='y')
- after the experiment, set *ni* in *proc.sh* script to the actual number of NUS points, not the full grid size
- rename *sampling.sch* to *nls.hdr_3* in the data folder
- create *nls.in* file manually according to the template above

A.2.3 TopSpin: Bruker spectrometer

Consult the user manual for TopSpin 3.0 and later versions.

A.3 Examples of MDD/CS processing

The examples include: (i) spectra with relatively small number of signals (up to 100-300) and limited dynamic range (up to ca 100), examples are 2D 13C HSQC, 3D HNcoCA and HNCO; (ii) spectra of NOESY-HSQC or TOCSY-HSQC type.

A.3.1 2D 15N HSQC (ubiquitin, BioPack)

This example illustrates MDD and CS processing of a 2D spectrum. Extract files from tar archive *gNhsqc_S_30Jun2011.tgz* and change directory to *gNhsqc_S/gNhsqc_S.proc*. Run master script *proc.sh* and look at the resulting spectrum (*test.dat*) using *nmrDraw*. Phase in the directly detected dimension requires correction, which can be done by changing one number in file *fidSP.com* . Namely, change line

```
| nmrPipe -fn PS -p0 0 -p1 0 -di \
```

to

```
| nmrPipe -fn PS -p0 70 -p1 0 -di \
```

and rerun *proc.sh* script. Open *proc.sh* in a text editor and change parameter METHOD to MDD or CS in order to compare results for different methods.

To shorten the calculations you may reduce the region of interest in ppm's by changing parameters FST_PNT_PPM and ROISW. The spectrum has been recorded with random NUS 38%, i.e. 96 points were recorded out of 256. You may check how quality of the spectrum degrades as fewer data points are used for calculations. For this, set parameter NI in *pros.sh* to a value smaller than 96, e.g. for 25% NUS add line

```
setenv NI '64 1'
```

By setting variable NI in *proc.sh* we override the values stored in file *nls.in*.

Processing of the spectrum can also be performed using GUI *qMDD*. Start *qMDD* in directory *gNhsqc_S* and (if asked) choose to overwrite existing files, open spectrum named *gNhsqc_S.fid*, process the spectrum by pressing button "Run", and look at the resulting spectrum in *nmrDraw* (press corresponding button in the GUI). In order to correct the phase in the directly detected dimension, you need to edit *fidSP.com* script, which can be done in "advanced parameters" by pressing *fidSP.com* button. You may, for example change line

```
-xP0 156.1 -yP0 0.0 \
```

to

```
-xP0 226.1 -yP0 0.0 \
```

A.3.2 3D HNCO (ubiquitin, TopSpin 3.0)

This example illustrates MDD and CS processing of a 3D triple-resonance spectrum. Extract files from tar archive *57hnco_30Jun2011.tgz* and change directory to *57hnco /57.proc*. Run the master script *proc.sh* and check the resulting spectrum in *nmrDraw*. The spectrum was recorded with 25% NUS. Results for less data can be checked as described in the HSQC example above. The spectrum can also be processed using GUI *qMDD*. Note that phase for directly detect dimension is set to 45° in *fidSP.com*, which is different from the value provided by *qMDD*.

A.3.3 3D HNCO (ubiquitin, BioPack)

This example illustrate MDD and CS processing of a 3D triple-resonance spectrum. Extract files from tar archive *ubi_ghn_co_S_nls_30Jun2011.tgz* and change directory to *ubi_ghn_co_S_nls/ub_ghn_co_S_nls.proc*. Run the master script *proc.sh* and check the resulting spectrum in directory *ft* and 2D projections in *1H.C13.dat*, *1H.N15.dat*, *C13.N15.dat*. The spectrum was recorded with 6% NUS. Note the fid reshuffling from *phase2,phase* to *phase,phase2* in the master script[6]:

```
if(! -f fid) fid_shuffle ../ubi_ghn_co_S_nls.fid/fid fid 4 1 3 2 4
```

A.3.4 3D HNcoCA (ubiquitin, TopSpin 3.0)

This example illustrate MDD and CS processing of a 3D triple-resonance spectrum. Extract files from tar archive *284_hncoca_30Jun2011.tgz* and change directory to *284_hncoca/284.proc*. Run the master script *proc.sh* and check the resulting spectrum in directory *ft*, file *284.tf3* and 2D projections in *1H.C13.dat*, *1H.N15.dat*, *C13.N15.dat*. The spectrum was recorded with 9% NUS. Note that scripts *fidSP.com* and *recFT.com* have been adjusted relative to the setting provided by GUI *qMDD*. Thus, phase in the directly detected dimension is corrected in *fidSP.com*.

A.3.5 3D NOESY (BioPack)

This example shows processing of 3D 15N NOESY-HSQC spectrum of a 15 kDa protein. Extract files from tar archive *BP_gnoesyNhsqc_S_30Jun2011.tgz* and change directory to *BP_gnoesyNhsqc_S/ BP_gnoesyNhsqc_S.proc*. The experiment has been run in sparse level of 20%. Run master script *proc.sh* and check the resulting spectrum in directory *ft*, file *284.tf3* and 2D projections in *1H.C13.dat*, *N15.1H.dat*, *N15.C13.dat*. The spectrum can be also processed using GUI *qMDD*.

A.3.6 3D HNCA (azurin, BioPack, full spectrum)

This example shows MDD processing of a 3D spectrum, which was recorded in full. Thus, spectrum reconstructed from a small fraction of data points can be compared with the full spectrum processed using traditional DFT. A high resolution HNCA experiment[7] was recorded for globular 14 kDa protein azurin. Extract files from tar archive *az_HNCA_high_res_30Jun2011.tgz* and change directory to *az_HNCA_high_res.proc*.

Since the spectrum was recorded without NUS, GUI *qMDD* processes it as a regular full spectrum. In order to "sparse" the spectrum, in *qMDD* choose not to overwrite the existing files. In "advanced parameters" display, check script *proc.sh* (also shown below) and file *nls.in*. Script *proc.sh* produces NUS schedule (10%) "on the fly" by running program *nussampler* on *nls.in* file. Note that parameter SPARSE in *nls.in* file is set to "n". This tells *mddNMR* that sparse data are to be extracted from a full spectrum. For spectra recorded in real NUS mode, the parameter must be set to "y". Below you see content of commented script *proc.sh*:

```
#!/bin/csh
setenv FID ../az_HNCA_high_res  # input experiment (without .fid )
setenv in_file nls.in           # local copy of nls.in with NUS schedule parameters
setenv selection_file nls.hdr_3 # NLS schedule file to be produced by nussampler

setenv fidSP fidSP.com          # nmrPipe script for fid conversion
                                # and DFT of the direct dim
setenv REC2FT recFT.com         # nmrPipe script to process reconstruction
                                # after mdd calculations

# MDD related -------------------------
```

[6] Since processing of the directly detected dimension is performed by *nmrPipe* in 2D mode (script *Tx.sh.2D*, this reshuffling cannot be performed by *var2pipe*. It cannot be done either by setting *mddNMR* parameter PHASE_ORDER to 1 3 2 4, because decoding of the Echo-Anti-Echo signal is performed by *nmrPipe* prior to the processing by *mddNMR*.

[7] The experiment was used for our publication Jaravine, V., et al. Nature Methods 2006, 3, p 605

```
setenv METHOD MDD                # toggle MDD mode
setenv MDDTHREADS 2              # allows up to 2 simultaneous calculations
setenv FST_PNT_PPM 8.75          # leftmost point of region of interest (ROI)
setenv ROISW 0.15                # full ROI size (ppm)
setenv SRSIZE 0.1                # recommended size of sub?region
setenv NITER 50                  # number of iteration
setenv NCOMP 30                  # default number of components for one sub-region
setenv lambda 0.002              # Tikhonov regularization parameter

setenv MDD_NOISE 0.2             # scales residuals as they are added
                                 # to the reconstructed spectrum
setenv proc_out ft/test%03d.dat # nmrPipe template for the final 3D spectrum

########################################################################
############### end of definitions ####################################

nussampler $in_file      # the spectrum has been recorded in full and is 'sparsed'
                         # for processing; so calculate NUS table here
                         # check/edit file nls.in for the NUS schedule parameters

# process spectrum with mdd
mddnmr4pipeN.sh 1 2 3 4 5

# make 2D projections of the final 3D spectrum
proj3D.tcl -in $proc_out

## uncomment the following lines to process the full spectrum for comparison
# FTx.sh XYZA/FTx.xyza                    # make FT for directly detected dim
                                          # for ref spectrum

# recFT.com XYZA/FTx.xyza ft/ref%03d.dat  # FT of Y and Z dimensions
# cd ft
# proj3D.tcl -in ref%03d.dat              # make 2D projections of reference spectrum
```

A.3.7 3D HNCA (Tau protein, TopSpin 3.5)

This example demonstrates the possibility of deconvolving J-modulation in the first indirect dimension (CA) of the HNCA spectrum. The upper border of the Gly singlet region is set to 45 ppm and the region does not undergo deconvolution. The deconvolution part of the script is:

```
setenv CS_lambda '1 45' # 45 defines the upper border of the singlet region
setenv FT_FLAGS '3 0 0'   # FT -neg -alt for CA
setenv deconv              'nnn cos:J=35'
setenv SW                   '6031.36 2918.86 9615.385'
```

In this example, we use 400 points from the NUS schedule with J-modulated sampling with assumed J-modulation of 35 Hz. The total size of the dataset is 700 NUS points, so the sampling level can be increased for tests.

A.4 Additional tools in the package

A.4.1 Programs

fid_shuffle
Program to shuffle (=re-order) 1D FIDs in Varian fid
Use: fid_shuffle <input fid> <output fid> <array size> <n1> <n2> ... < arr size>}

- <input fid> – data file
- <array size> <n1> <n2> ... <n arr size> – size of reshuffled block and new order of 1D's

Example 3D phase2,phase to phase,phase2 : <input fid> <output fid> 4 1 3 2 4
Example 4D phase3,phase2,phase to phase,phase2,phase3 : <input fid> <output fid> 8 1 5 3 7 2 6 4 8

ser_shuffle
Program to shuffle (=re-order) 1D FIDs in Bruker ser
Use: ser_shuffle <input ser> <output ser> <FID size in 4 byte words> <NF -FID's in block> <n1> <n2>...<n NF>
change order of FID's within block; initial order is 1 2 3 4 5 6 7 ...
Example: ... 4 1 3 - only 1st and 3rd FID's out of 4 are passed to the output
Example 3D phase2,phase to phase,phase2: ... 4 1 3 2 4
Example 4D phase3,phase2,phase to phase,phase2,phase3 : ... 8 1 5 3 7 2 6 4 8

mddsolver, cssolver
Standalone MDD and CS solvers respectively.

A.4.2 Shell scripts

queMM.sh
Allows to do parallel calculations of step 3 on multi-CPU localhost or a network cluster (over passwor – free ssh). Edit parameters in this script setup for your local network.

recFT.com
Default template *nmrPipe* script, for processing of YZ dimensions. It is normally copied to each **.proc* directory and manually edited, e.g. to set indirect phases if different from defaults (0 0).

nussampler
NUS generator; described above

nus2pipe
A python script that converts Agilent and Bruker parameters to *nmrPipe* conversion script *fidSP.com*. Run the script without arguments to get usage info.

A.5 Copyright and Legal Information

Date: 12 September 2020

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