MddNMR

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Reconstruction of NMR spectra from non-uniformly sampled signal using multi-dimensional decomposition (MDD) and Compressed Sensing (CS)

The User manual

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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 multi-dimensional decomposition (MDD) and compressed sensing (CS) to replenishing missing data points in the full matrix followed by regular FT processing of the complete data.

Copyright

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 Multi--Dimensional Decomposition. Prog. Nucl. Magn. Reson. Spectrosc., 2011, in press, doi:10.1016/j.pnmrs.2011.02.002
2. Kazimierczuk, K. and V.Y. Orekhov, Accelerated NMR Spectroscopy by Using Compressed Sensing. Angew. Chem.-Int. Edit., 2011, 123, 5670-3, DOI: 10.1002/anie.201100370

Downloads and updates

The software is available upon request from:

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Additional information about the project can be found at http://pc8.nmr.gu.se/~mdd/Downloads . 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.

Installation

Note that to run the software you must have functioning nmrPipe package (Delaglio, F., et al., 1995, J. Biomol. NMR, 6, 277-293).

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 10.6 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

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.

General concept

Traditionally, multi-dimensional 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 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; viewing 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. 1). The software offers three general possibilities (i) direct Fourier transform, all missing points are set to zero; (ii) multi-dimensional decomposition (MDD); and (iii) compressed sensing (CS).

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 Appendixes. Description of underlying mathematical algorithms and processing protocols can be found in our papers listed above, and references cited therein.

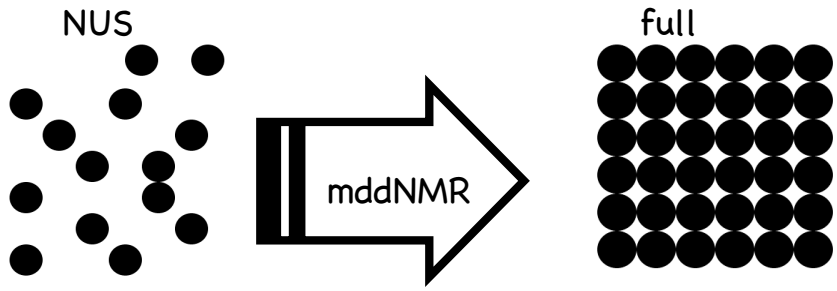


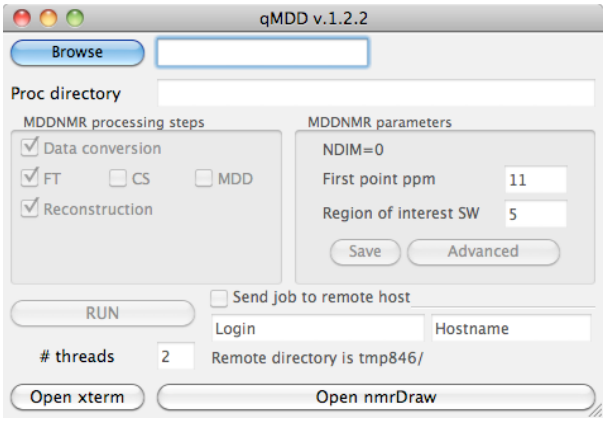
Figure 1. The software replenishes time domain data points in the indirect dimensions that are missing in the NUS set and produces the full data set amenable for regular Fourier transform

qMDD graphical user interface

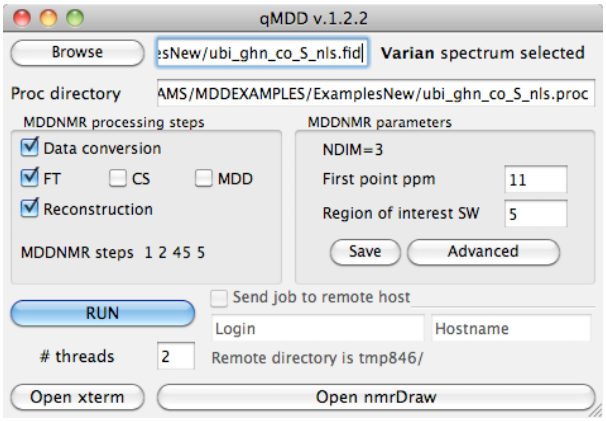
The primary mode of mddnmr usage, which gives access to the full set of the software functionalities, is by C-shell scripts. There is, however, a graphical user interface (GUI) that simplifies work with the program, and is especially recommended for beginners. It is started with command

qMDD

A window is opened,



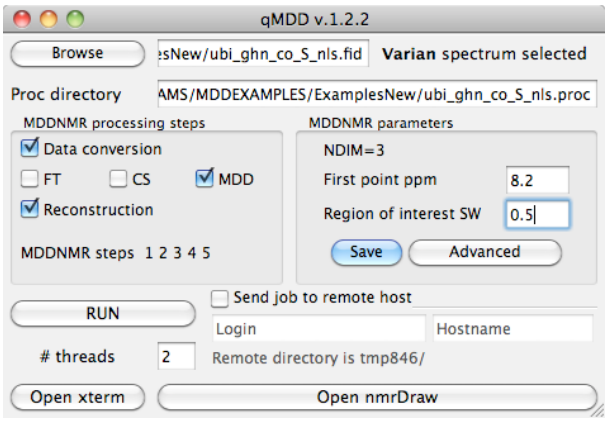
which invites to select a spectrum for processing. For example, you may select ubi_ghn_co_S_nls.fid, which is one of the example experiments in http://pc8.nmr.gu.se/~mdd/Downloads/mdd_examples/ (data type is recognized by directory name, e.g. .fid for Variant/Agilent). Directory named ubi_ghn_co_S_nls.proc is created, which is the place to 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".



A new terminal window opens (not shown), in which script *proc.sh* is executed. When the script is successfully finished, look at the resulting spectrum in nmrDraw by pressing "Open nmrDraw" or starting *nmrDraw* in a terminal window. Look at three projections of the 3D spectrum stored in *H1.C13.dat, 1H.N15.dat* and *N15.C13.dat* or the 3D spectrum located in directory *ft* . From the figure above, you may notice that the calculations have been performed with "FT*"* mode, which is the fastest and most robust method, albeit it provides the poorest results due to massive aliasing artefacts. Nevertheless, "FT*"* mode is useful since it allows fast look at the spectrum and adjustment of nmrPipe processing parameters (e.g. phases).

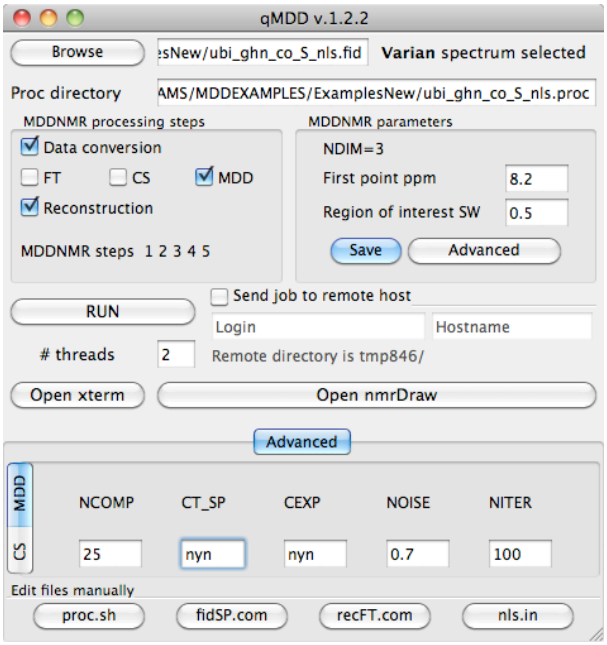
MDD calculation

MDD processing is activated by selecting "MDD" checkbox. Prior to pressing "RUN", you may define a small region of interest by setting "First point ppm" and "Region of Interest SW" (with "First point" as its



downfield border) in ppms followed by pressing "Save" button. 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. For modern computers, the number is 2-8 depending on how many processors and cores are available.

Press “RUN” button and wait until output in the terminal window indicates successful completion of the calculations. Look at the spectrum in nmrDraw. The GUI allows modification of several most important parameters and C-shell scripts. This is done in the “Advanced” display. For example, you may set parameters CT_SP and CEXP to “nyn” in order to activate R-MDD mode for the 2nd indirect dimension (N15) of the experiment. You need to press “save” to activate the changes. Three scripts *proc.sh, fidSP.com* and *recFT.com* can be edited by pressing corresponding buttons. Meaning of these files and parameters is described in the next section.



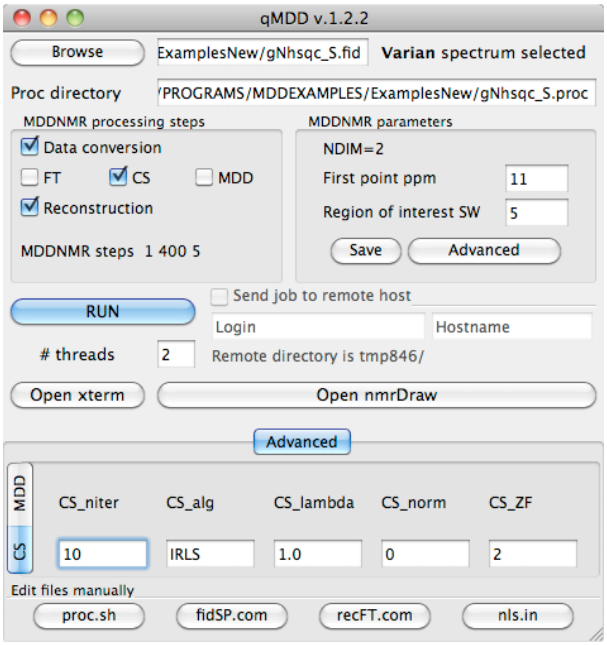
Adjusting conventional processing parameters

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” display by pressing the corresponding buttons. The procedure can be illustrated on *gNhsqc_S.fid* spectrum example. Load the spectrum using “Browse” button and answer “Yes” to the question (if any) to discard the existing processing scripts. Process the spectrum with “FT”. Disregard an error massage (if any) in the terminal window after the line “*test.dat ready*”. 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” 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
```

Compressed Sensing

CS processing is activated by selecting "CS" checkbox. You may try CS on *gNhsqc_S.fid* spectrum example. Note that in *mddNMR* version 2.0, CS can be used only for 2D and 3D spectra. 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" view (see figure below) 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. Meanings and recommended values for the parameters are indicated in contextual help, which appears when the mouse cursor is placed on the parameter field.



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 supports only 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" 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. For example, *proc.sh* script for MDD processing of *gNhsqc_S.fid* spectrum described in the previous section is

```
#!/bin/csh
        setenv FID ../gNhsqc_S
        setenv fidSP fidSP.com
        setenv REC2FT recFT.com
        setenv in_file nls.in
        setenv selection_file nls.hdr_3
        setenv FST_PNT_PPM 11
        setenv ROISW 5
        setenv proc_out test.dat
        setenv METHOD MDD
        setenv MDDTHREADS   2
#MDD related parameters
        setenv NCOMP  25
        setenv NITER  50
        setenv MDD_NOISE    0.7
        setenv lambda 0.01

mddnmr4pipeN.sh  1 2 3 4 5
```

The master script sets all parameters that have to be altered from defaults. This is done by setting C-shell environment variables using command *sentenv* . The calculations are started by command *mddnmr4pipeN.sh*

at the end of the script. As arguments, the command takes list of tasks to be done, which are defined by unique numbers (see next section).

Advanced processing

The most general mode of operation for mddnmr software is by using command line input. 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). 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 parameter *fidSP*).
2 – preparing input for MDD calculations.
3 – MDD calculations over all sub-regions of the ROI.
4 – full reconstruction is produced from MDD components and residuals (see MDD_NOISE).
5 – the full time domain reconstruction obtained in step 4 is processed using an nmrPipe script (see parameter *REC2FT*)
42 – 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 mode, intermediate results are stored in files in working directory MDD.

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 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 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 -x1 11ppm -xn 5ppm -sw -round 16        \
| 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 reconstructed spectrum 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*, which is located in the *${MDD_NMR}/com* directory.

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 and 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

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    25    # number of components per sub-region
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, which that are typed in bold, are needed to be set explicitly. The remaining parameters are there mostly for display.

Table 1. Parameters recognized by *mddNMR* software.

Parameter name	**Default value**	**Meaning and references for examples**
CEXP		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)
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_ZF	2	Frequency domain “over-digitization” in CS algorithm (best results for 2)
CT_SP		
DATAMAP_FILE		
DIM_MERGE		If defined, dimensions to merge. Used, e.g. to process 4D spectrum with 3D MDD
f180		If set, overrides value in nls.in file
FID		Directory with experimental data
FIX_FREQ		Reserved
FIX_FREQ_FILE		reserved
FST_PNT_PPM	10	Start (downfield) 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
in_file		Name and location of NUS parameter file. By default, name is obtained from the *selection_file* by changing the file extension
lambda	0.005	Tikhonov regularization for MDD: 0.001-0.1 with lower value for high S/N
MAP_FACTOR	1	Reserved
MDD_DIR	./MDD	Reserved
MDD_FILE	./MDD/region	Reserved
MDD_NMR	…./mddnmr1.8	Location of the software directory
MDD_NMR_COM	…./mddnmr1.8/com	
MDD_NOISE	0.85	A factor that scales residuals of the MDD 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
NCOMP	30	Number of MDD components per sub-region.
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
NLSpoints	0	reserved
NUS_POINTS		reserved
NUS_TABLE_ORDER		reserved
OVLP	3	Overlap between sub-regions in points
phase		If set, override value in nls.in file
PHASE_ORDER		If set, reshuffle FID’s inside each hyper-

		complex 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
SpecParFile	./XYZA/_t.hdr	reserved
SRSIZE	0.18	Approximate size of sub-region in ppm
SW		If set, overrides value in nls.in file
XDimSize	1	reserved

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.

MDD shapes

The MDD model looks for an approximation of a *M*-dimensional spectral matrix by the sum of a small number of tensor products of one-dimensional vectors:

$$\boldsymbol{S}_{MDD} = \Sigma_{\beta} \; {}^{\beta}a \, {}^{\beta}\boldsymbol{F}^{\boldsymbol{1}} \otimes \ldots {}^{\beta}\boldsymbol{F}^{\boldsymbol{M-1}} \otimes {}^{\beta}\boldsymbol{F}^{\boldsymbol{M}} \qquad (1)$$

where the model spectrum $\boldsymbol{S}_{MDD}$ is the sum of fixed number of components N_c enumerated by index $\beta=1...N_c$. Each component is given by the product of normalized vectors ${}^{\beta}\boldsymbol{F}^{\boldsymbol{m}}$ for every spectral dimension $m=1...M$, referred to below as shapes, and the component amplitude ${}^{\beta}a$. The term shape is introduced here in relation to the spectral line shape; its several synonyms are present in the literature, i.e. loads, modes, factors, etc. Symbol $\otimes$ denotes the outer product operation, which produces *M*-dimensional matrix from *M* one-dimensional shapes.

A simple approach is to think about a component as the representation of a cross peak in a multi-dimensional spectrum. The shapes then are traditional line-shapes of the peak in all dimensions. The actual situation, however, is more complex, since the components do not always have a one-to-one correspondence to peaks. In general, a peak showing complex structure, e.g. in an E.COSY spectrum, may require several components for its description. It also can be the other way around, as in 3D NOESY spectrum - one component may accommodate several cross peaks. It is important to emphasize that the MDD model does not make any assumptions about the shape vectors ${}^{\beta}F^{m}$. Thus it can be equally well applied to data in the time or frequency domains, as well as combination of both.

The reconstructed spectra are produced by summation over all components (Eq. 1). Thus typically, dealing with the individual components is not needed. However, the shapes can be stored in both time and frequency domains.

```
# Storing MDD shapes using step 42
mddnmr4pipeN.sh  1 2 3 42
```

Upon completion of step 42, two files in XML format (see also USF3 format) are produced, which contain shapes in frequency and time domain. The shapes are also stored in nmrPipe format in directory SHAPES and can be viewed using nmrDraw. For example, columns in file SHAPES/sh_Y_03.dat contain the processed shapes for first indirect dimension from the 3rd sub-region of the spectrum.

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 CPUs. In order to distribute calculations over a network, e.g. for a Linux cluster, step 3 is 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 is 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
```

GUI *qMDD* provides a simple possibility to distribute calculations using password-free *ssh* access and shared file system. If box "*Send computations to remote host*" in GUI is checked, the following lines are added to the master script *proc.sh* .

```
mddnmr4pipeN.sh  1 2
ssh login@host "mkdir -p tmpxxx/"
scp -C -r MDD regions.runs login@host:~/tmpxxx/
ssh login@host "cd tmpxxx/; queMM.sh regions.runs"
scp login@host:"~/tmpxxx/MDD/*.[rc]*" MDD/

mddnmr4pipeN.sh  4 5
```

The procedure runs script *queMM.sh* (part of *mddNMR* package) on a remote host, which distributes calculations over specified set of computers in the network. If the master script is ran from a directory, which is shared with other nodes in the network, *ssh* is not needed and the lines above are simplified to:

```
mddnmr4pipeN.sh  1 2
queMM.sh regions.runs
mddnmr4pipeN.sh  4 5
```

Note that the header of *queMM.sh* should be edited for every new network.

Examples

Examples, can be downloaded from http://pc8.nmr.gu.se/~mdd/Downloads 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.

Table 1. 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
4	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 [1]	3D HNCA	Varian	sparsifying of full spectrum*
7	HD384_plasma_gChsqc.tgz	Blood plasma	2D 13C HSQC	Varian	BioPack, sparsifyingof full spectrum*

(1) Jaravine, V.; Ibraghimov, I.; Orekhov, V. Y. *Nature Methods* **2006**, *3*, 605.
*examples cannot be ran using qMDD, see description in the Examples section in the Appendix

To speed up calculations and save disk space in the examples, the master scripts (*proc.sh*) are set for narrow strips of about 0.2 ppm in the direct acquisition dimension. Scripts in the examples may serve as templates for processing of spectra of similar type. For example, scripts for the HNcoCA example can be used, after minor modifications, for most of the backbone experiments.

APPENDIX

I. NUS schedule

NUS schedule is typically produced by spectrometer software and is saved together with the experiment. The schedule can be also produced by running program *nussampler* from terminal command line:

nussampler nls.in

nussampler takes parameters from the input file *nls.in* (this name is used for most cases), generates a sampling scheme and writes it to file *nls.hdr_3*. Both files *nls.in* and *nls.hdr_3* are needed for 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). Alternatively, 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.

File nls.in – setting NUS parameters

Each line of NUS parameter file *nls.in* start with a keyword followed by a list of parameters values. All the keywords are mandatory, but the lines order is not important. Below and in Appendix 2. 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_setup3d.in
NDIM    3
seed    54321
SPARSE  n
sptype  shuffle
f180    nnn
CT_SP   nyn
CEXP    yyn
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

____
```

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 the same on the same computer architecture.

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.

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.

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

File nls.hdr.3 – NUS table

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.

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 *nussanpler*) is governed by a multi-dimensional 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.

II. Unified Spectral Format (usf3)

Unified spectral format (USF3) is an XML format for compact storage and handling of spectra. It was originally intended to present results of spectra decomposition by programs *MddNMR* and *PRODECOMP*; it is also suggested as a general frame for compact storing of regular multidimensional and hyper-dimensional spectra of any dimensionality. USF3 is a standard data storage format for CCPN. The latest formal description of the format (2011-05-13) can be found at http://www.ccpn.ac.uk/ccpn/projects/extendnmr/shape-data-format or requests from Rasmus Fogh (CCPN), Vladislav Orekhov (Swedish NMR Center), or Martin Billeter (University of Gothenburg).

III 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 on BioPack. The NUS scheme is generically applicable for most if not all 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.

BioPack: Varian/Agilent spectrometer

The BioPack implementation (see Varian/Agilent documentation for details) features automatic creation of a new NUS version of any multi-dimensional pulse sequence by the use of a checkbox button in the DigitalFilter page in the Acquire Folder of VnmrJ. 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 easy processing by the MDD software.

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)

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, ni2* and command *time* to adjust time of the experiment. Note that parameters *ni,* and *ni2* define only total duration of the NUS experiment, the size of the sampling grid is defined by parameters *nimax* and *ni2max*. 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
       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.

TopSpin: Bruker spectrometer

Consult the user manual for TopSpin 3.0 and later versions.

IV. Examples of MDD/CS processing of spectra recorded with NUS

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. Notably, requirements for number of signals and dynamic range for these spectra refer to diagonal signals, but not the cross-peaks. The peaks, which share line shape with diagonal signals, may be close to the noise level.

Example of backbone experiment

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*.tgz* and change directory to *gNhsqc_S/gNhsqc_S.proc.* Run master script *proc.sh* and look at the resulting spectrum 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.

3D HNCO (ubiquitin, TopSpin 3.0)

This example illustrate 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.

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 */ubi_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 N15.1H.dat N15.C13.dat . The spectrum was recorded with 6% NUS. Note the fid reshuffling from *phase2,phase* to *phase,phase2* in the master script:

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

Since processing of the directly detected dimension is performed by nmrPipe in 2D mode (script *FTx.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* has to be performed by nmrPipe prior to the processing by *mddNMR.*

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.da,t N15.1H.dat, N15.C13.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.*

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 SPARESE (20%) mode and saved in BioPack using macroses BP_NLSinit, BP_NLSset, BPsvf. Run master script *proc.sh* and check the resulting spectrum in directory *ft,* file *284.tf3* and 2D projections in *1H.C13.da,t N15.1H.dat, N15.C13.dat.*

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, which was used for our publication Jaravine, V., et al. Nature Methods 2006, 3, 605, 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 not recorded in NUS mode, it cannot be processed using *qMDD* GUI. 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  --------------------
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
```

2D 13C HSQC (fish blood plasma, matabolomcs)

This a high resolution 2D 13C HSQC is recorded for a fish blood plasma sample in a metabolomic study by Drs. L. Samuelsson and J. Larsson, Dept. of Physiology/Endocrinology The Sahlgrenska Academy at Gothenburg University. The data set, which is recorded in full, can be used to illustrate quality of the spectrum obtained using different NUS schedules. Extract files from tar archive HD384_plasma_gChsqc _30Jun2011.tgz and change directory to HD384_plasma_gChsqc.proc . Since the spectrum was not recorded in NUS mode, it cannot be processed using *qMDD* GUI. Script *proc.sh* produces NUS schedule "on the fly" by running program *nussampler* on *nls.in* file. Note that parameter SPARSE in *nls.in* file is set to "n". The fully sampled spectrum is 'sparsed' to 15% (ni/nimax = 180/1200). Both 15% MDD sparse spectrum and reference-full (100%) spectrum are written for comparison.

V. Tools in the package

Programs

fid_shuffle

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

Shell scripts

queMM.sh

allows to do parallel calculations of step 3 on multi-CPU localhost or a network cluster (over password-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

VI. Copyright and Legal Information

Date: Jul 3, 2011

DESCRIPTION
MddNMR is a program for processing uniformly and non-uniformly sampled (sparse) NMR spectra.
The following is legal information pertaining to the use of the MddNMR program. It applies to all MddNMR source files, executable (binary) files, configuration and documentation files contained in the official MddNMR archives. (Certain portions refer to custom versions of the software, there are specific rules listed below for these versions also.) All of these are referred to here as "the software".

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When results obtained by MddNMR are used in lectures, publications or other similar occasions, then a reference to the authors and at least one of the following papers is to be made:

(1) Orekhov, V.Y. and V.A. Jaravine, Analysis of non--uniformly sampled spectra with Multi--Dimensional Decomposition. Prog. Nucl. Magn. Reson. Spectrosc., 2011, in press, doi:10.1016/j.pnmrs.2011.02.002

(2) Kazimierczuk, K. and V.Y. Orekhov, Accelerated NMR Spectroscopy by Using Compressed Sensing. Angew. Chem.-Int. Edit., 2011, 123, 5670-3

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