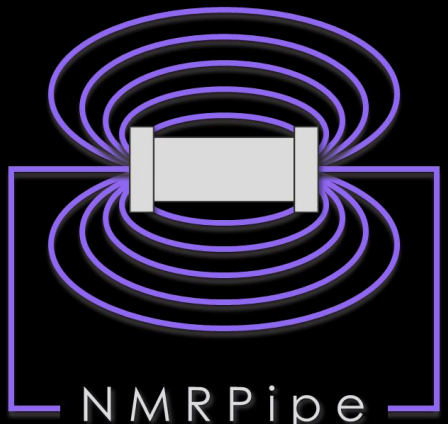
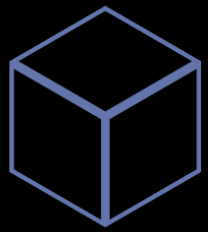
The NMRPipe System for Multidimensional NMR Processing and Analysis



www.nmrpipe.com



NMRbox
.org

SBGrid
CONSORTIUM

Key Steps of Spectral Processing

- Window Function and First Point Scaling
- Zero Fill
- Fourier Transform
- Phase Correction

Other Common Spectral Processing Tasks

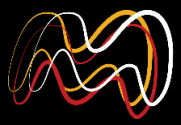
- Solvent Subtraction
- Baseline Correction
- Linear Prediction
- Hilbert Transform and Inverse Processing

Special Cases

- Gradient-Enhanced Data
- Interleaved Experiments and Spectral Series
- Non-Uniform Sampling and Alternatives to Fourier Transform
- Correction of Bad Points

Post-Processing

- Generating Projections and Extracts from 3D or 4D Spectra
- Viewing 2D Strips from 3D Spectra
- Peak Detection and Fitting of Spectra and Spectral Series
- Extracting Chemical Shift Evolutions from Spectral Series
- Backbone Structure from Chemical Shifts and Dipolar Couplings
- Multivariate Analysis of Spectral Series
- Structure Manipulation and Analysis



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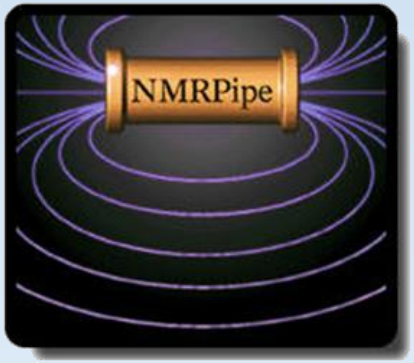
NIST
National Institute of
Standards and Technology
U.S. Department of Commerce

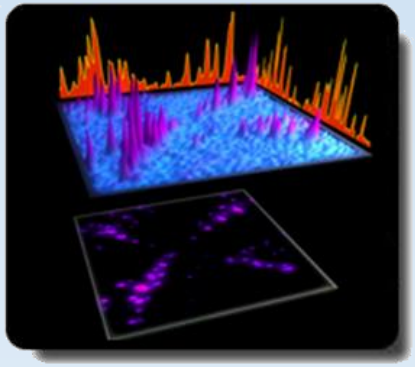
NIH
National Institutes
of Health

frank.delaglio@nist.gov
Mar 28 2018

With Thanks to Our NMRPipe Collaborators

frank.delaglio@nist.gov





Karen Allen • Luke Arbogast • Yves Aubin • Andrew Baldwin
Joseph Barchi • Ad Bax • Paul Bowyer • Robert Brinson
James Chou • Gabriel Cornilescu • Stefano Ciurli • Brian Coggins
Kathleen Farley • David Fushman • Michelle Gill • George Gray
Alex Grishaev • Stephan Grzesiek • Jeff Hoch • Sven Hyberts
Brad Jordan • Lewis Kay • Georg Kontaxis • Krish Krishnamurthy
John Kuszewski • Dong Long • Massimo Lucci • Mark Maciejewski
John Marino • Ryan McKay • Leszek Poppe • John Pfeiffer
Ben Ramirez • David Rovnyak • Dave Russell • Michael Shapiro
Adam Schuyler • Evgeny Tishchenko • Desi Tsao • Tobias Ulmer
Gerteen Vuister • Greg Walker • Gerard Weatherby • Justin Wu
Shen Yang • Jinfa Ying • Edward Zartler
Guang Zhu • Markus Zweckstetter

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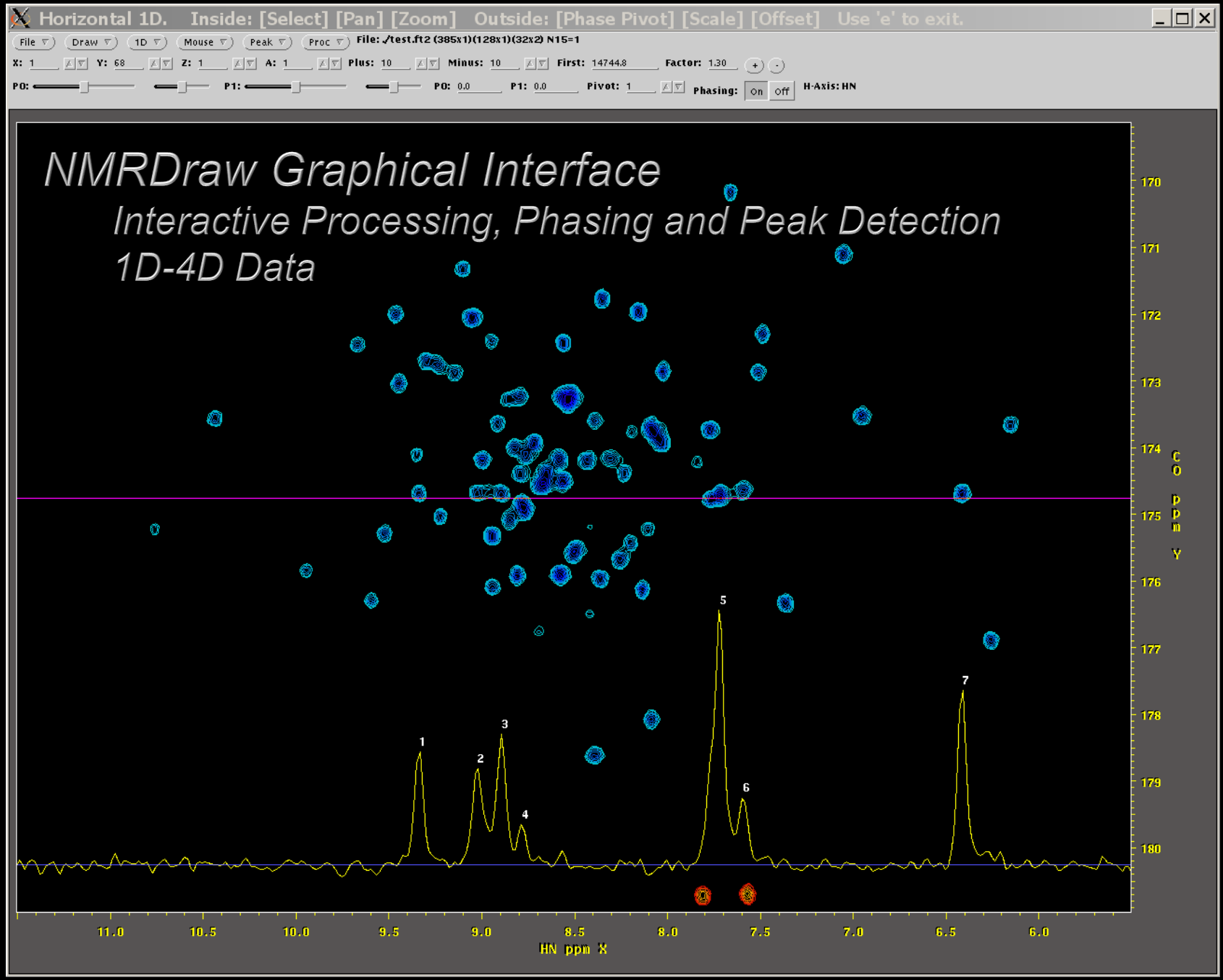
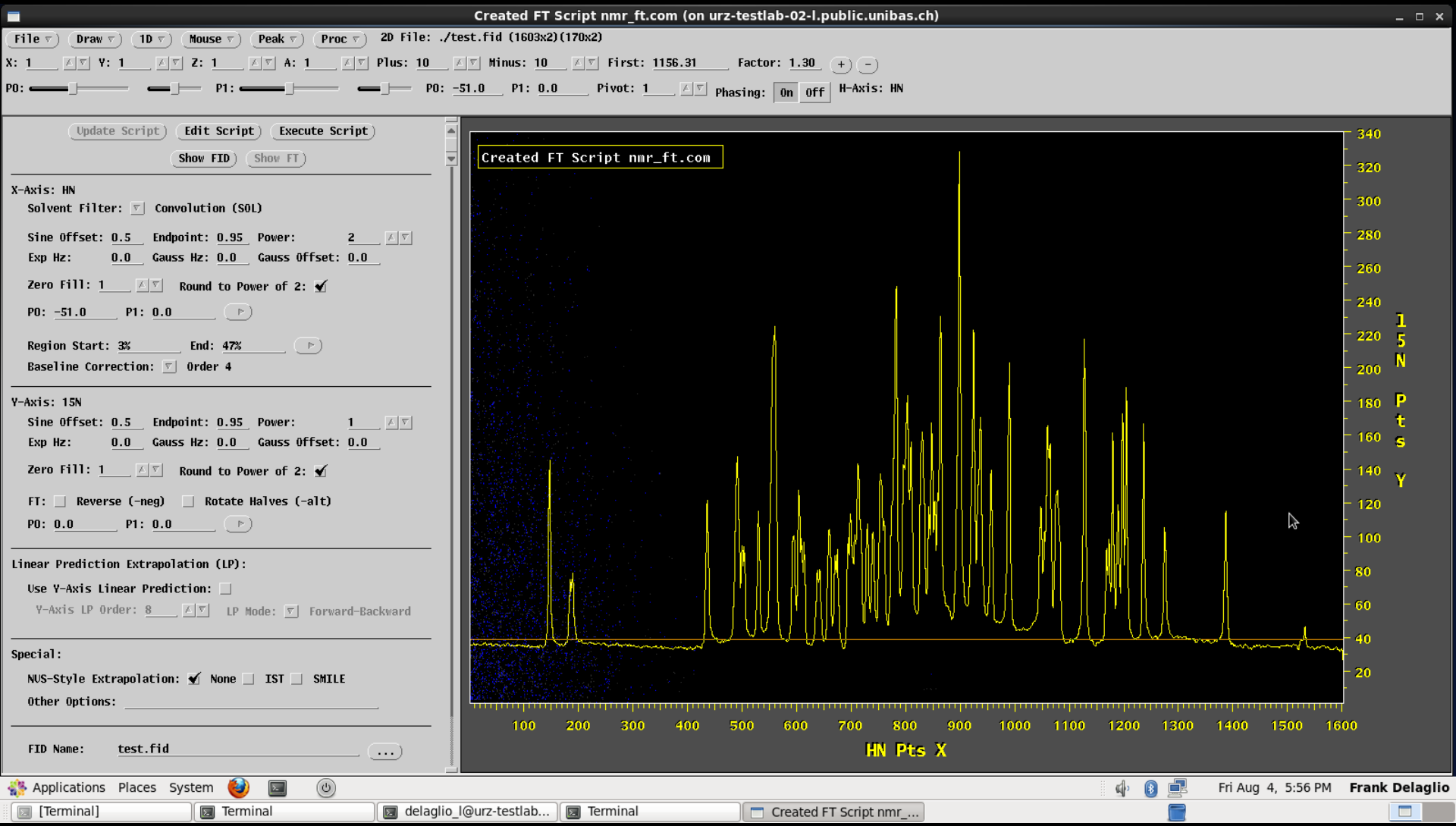
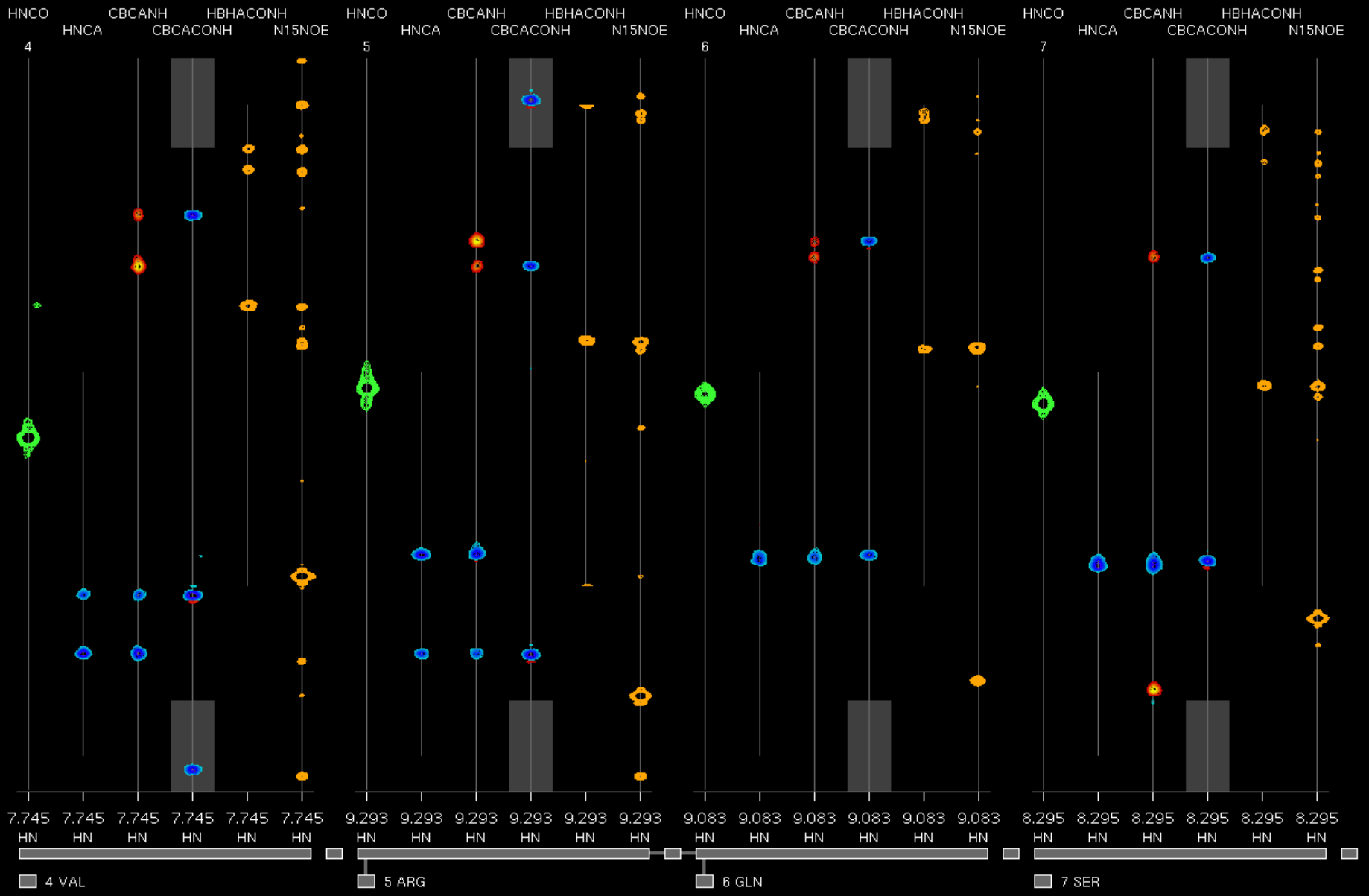

Horizontal 1D. Inside: [Select] [Pan] [Zoom] Outside: [Phase Pivot] [Scale] [Offset] Use 'e' to exit.
File Draw 1D Mouse Peak Proc File: ./test.ft2 (385x1)(128x1)(32x2) N15=1
X: 1 Y: 68 Z: 1 A: 1 Plus: 10 Minus: 10 First: 14744.8 Factor: 1.30
P0: P1: P0: 0.0 P1: 0.0 Pivot: 1 Phasing: On Off H-Axis: HN
NMRDraw Graphical Interface
Interactive Processing, Phasing and Peak Detection
1D-4D Data
1
2
3
4
5
6
7
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
HN ppm X
170
171
172
173
174
175
176
177
178
179
180
CO ppm Y

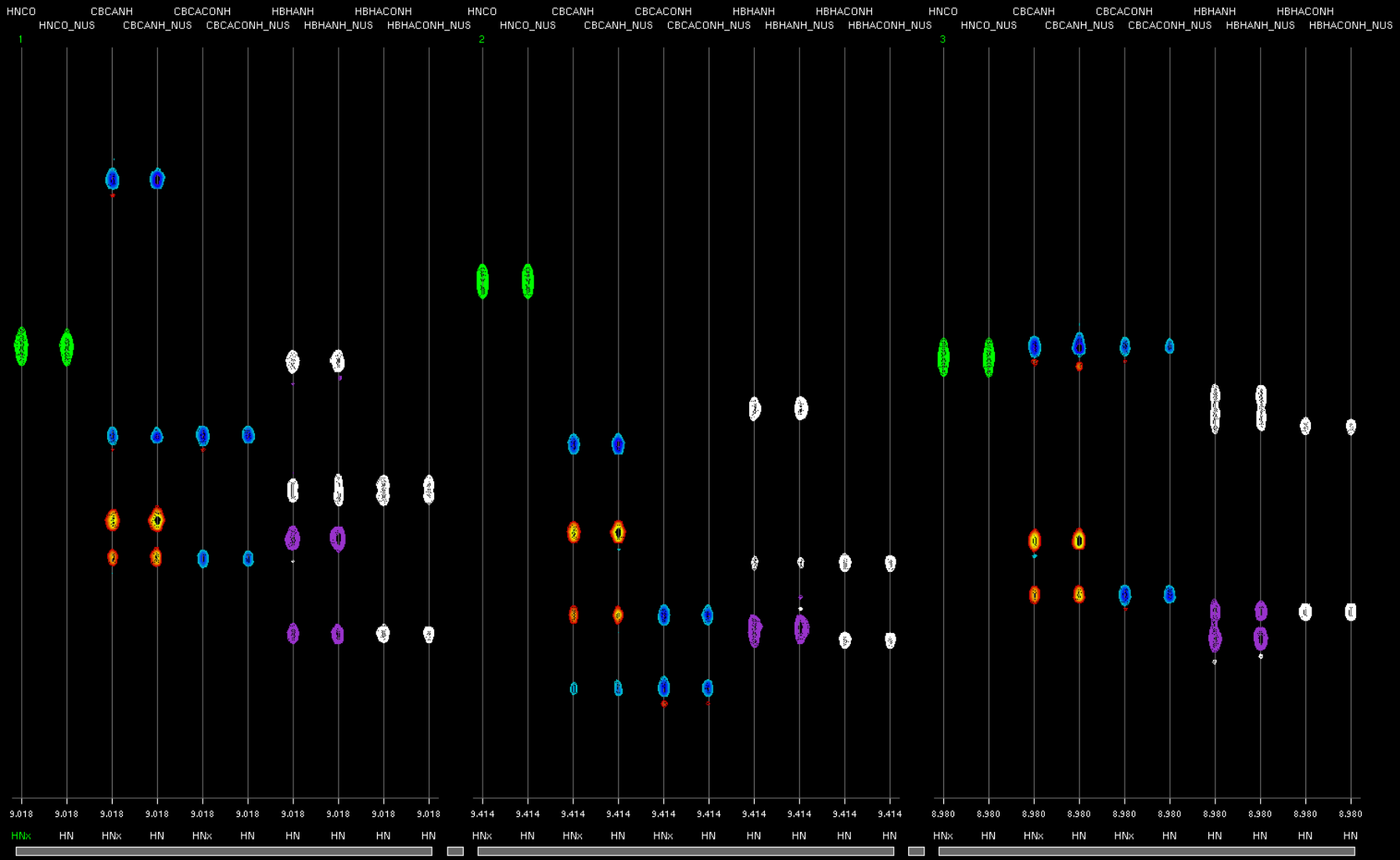
New: NMRDraw Graphical Interface for Processing Script Generation



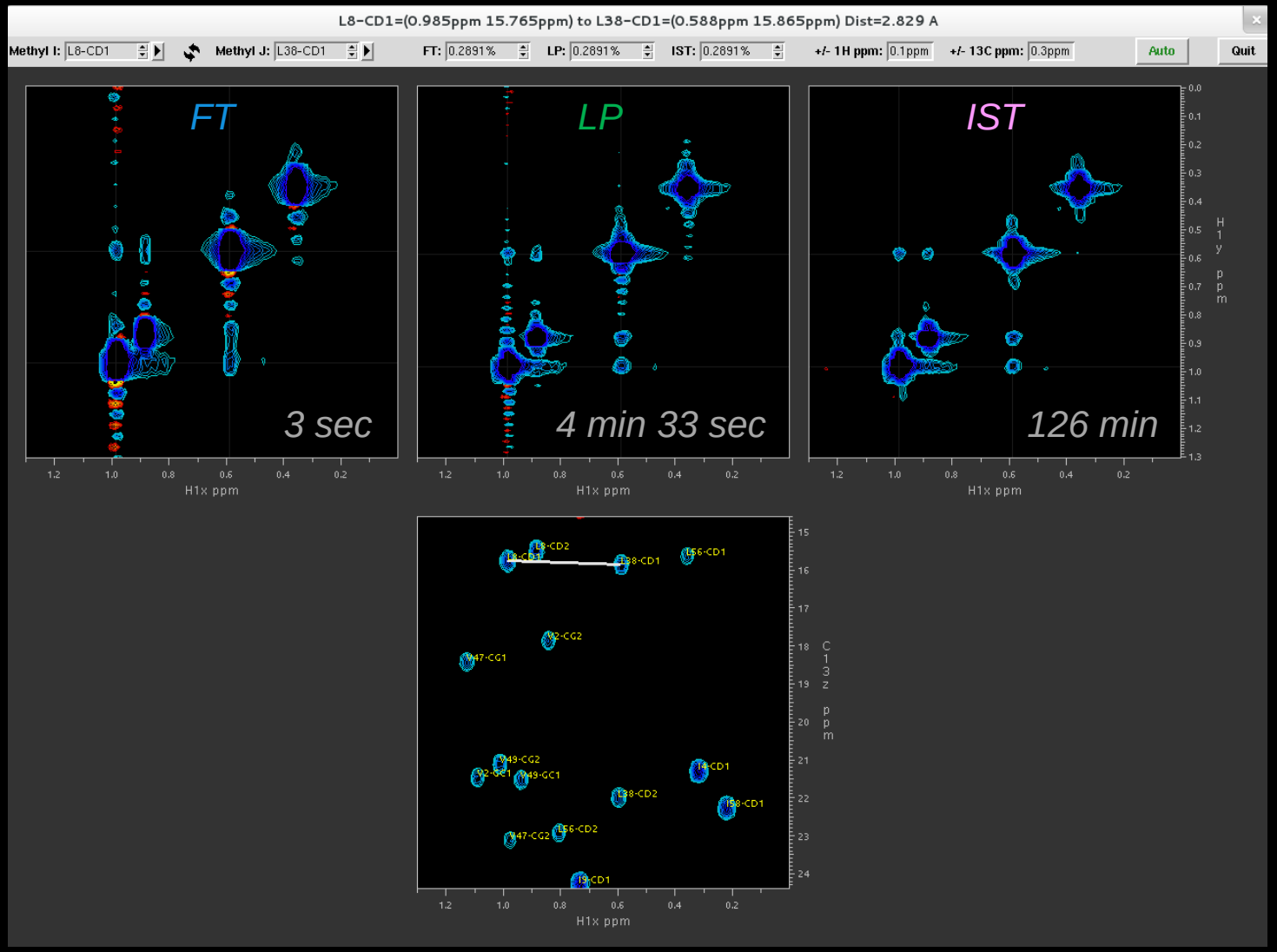
Multi-Spectrum Strip Manipulation



Uniformly Sampled Data and 33% NUS Reconstructed by NMRPipe's Iterative Soft Thresholding (IST)



NUS Zero Fill as an Alternative to Linear Prediction for Conventional NMR Data (4D Methyl-Methyl NOE)



Key Steps of Spectral Processing

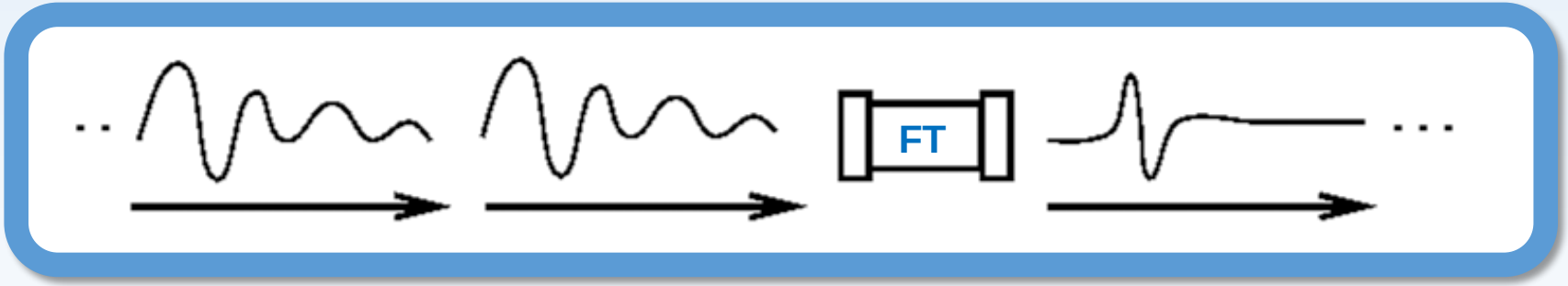
- Window function and first point scaling
- Zero Fill
- Fourier Transform
- Phase Correction

Spectral Processing Function as a UNIX Filter

```
nmrPipe -fn FT < test.fid > test.ft1
```

… or …

```
nmrPipe -fn FT -in test.fid -out test.ft1
```

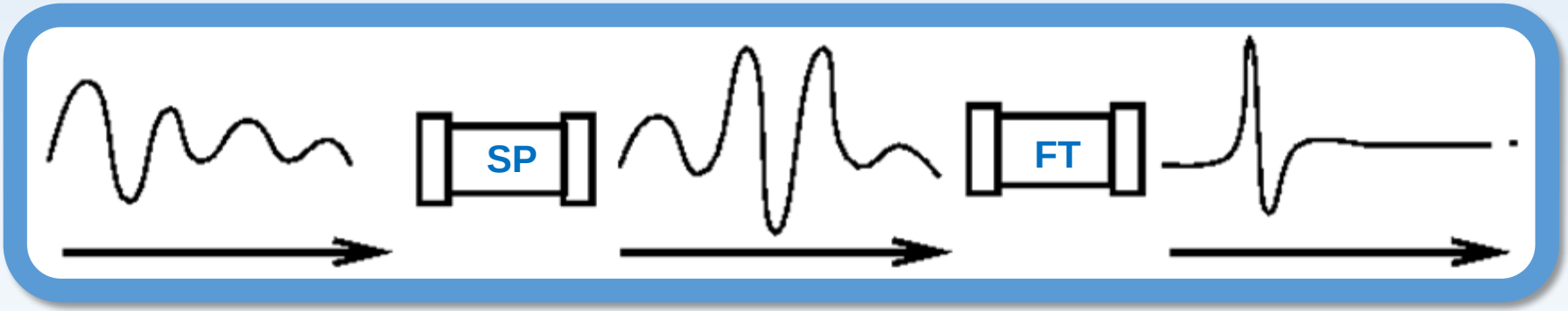


Spectral Processing Scheme as a UNIX Pipeline

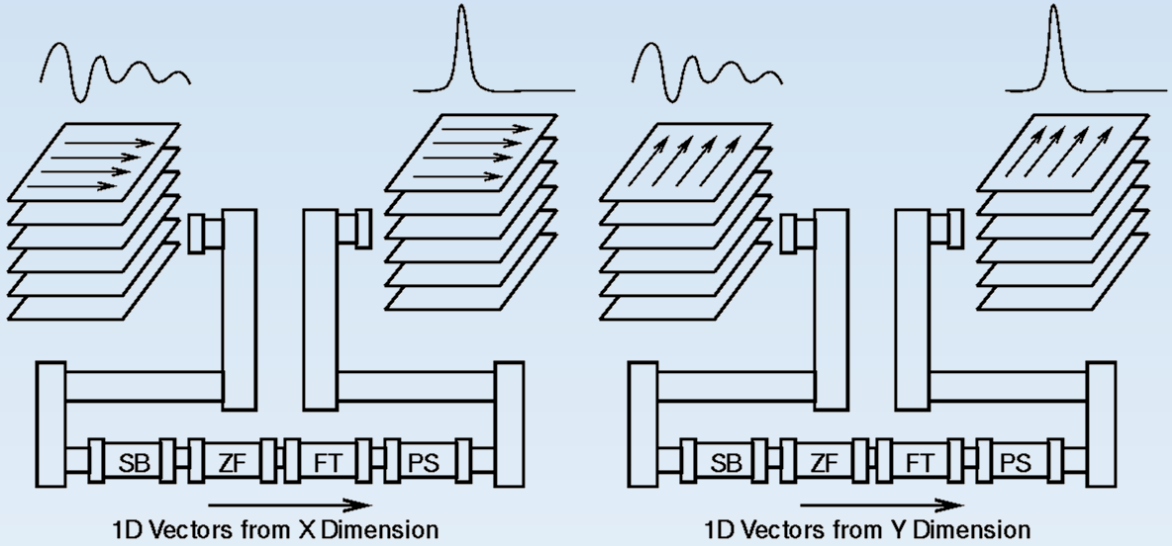
```
nmrPipe -in test.fid -fn SP | nmrPipe -fn FT -out test.ft1
```

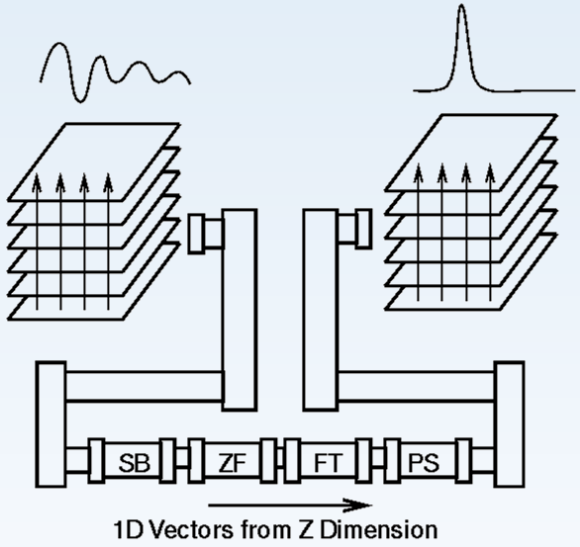
... or ...

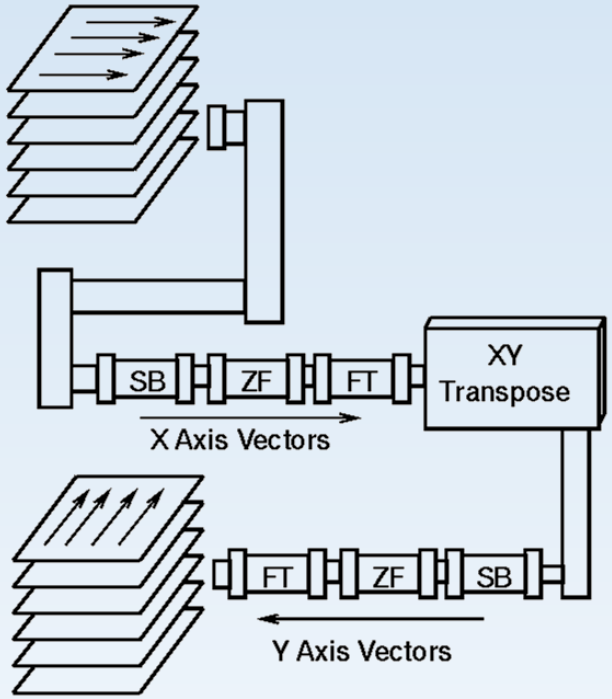
```
nmrPipe -in test.fid \
| nmrPipe -fn SP \
| nmrPipe -fn FT \
   -out test.ft1
```



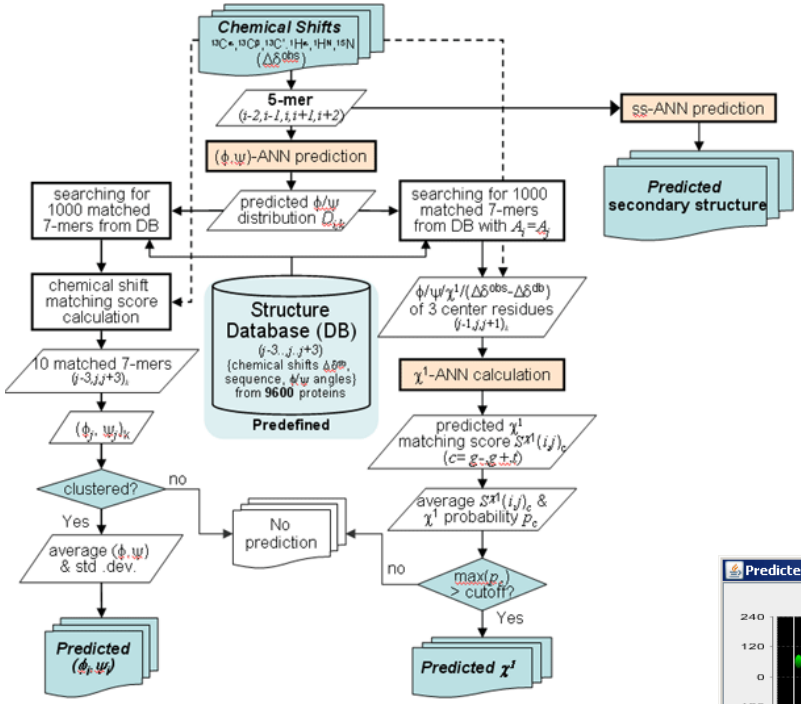
Multidimensional Spectral Processing Schemes as a UNIX Pipelines

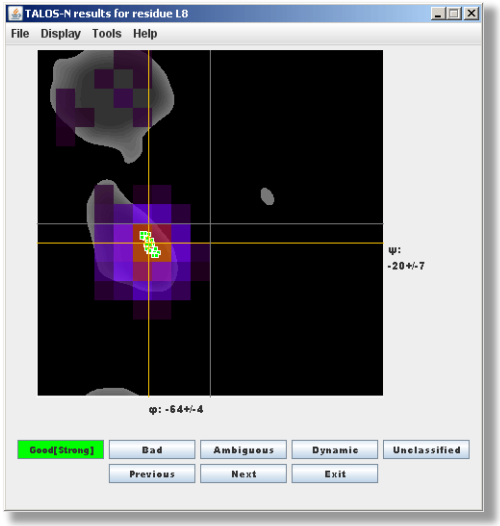


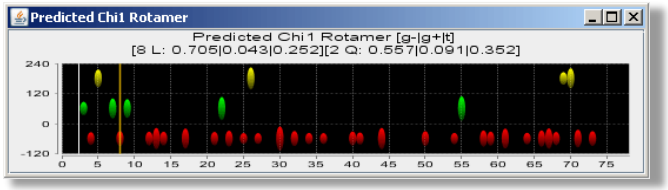




Related Software from the Bax Group at the NIH: TALOS-N Prediction of Protein Backbone and Sidechain Angles from Chemical Shifts

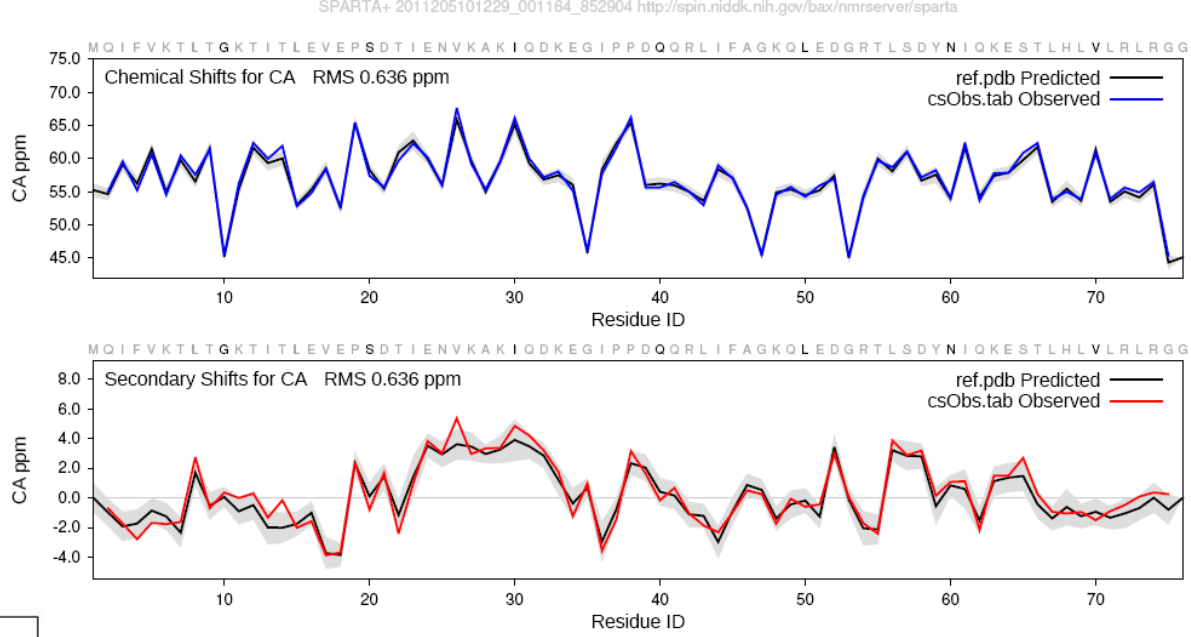


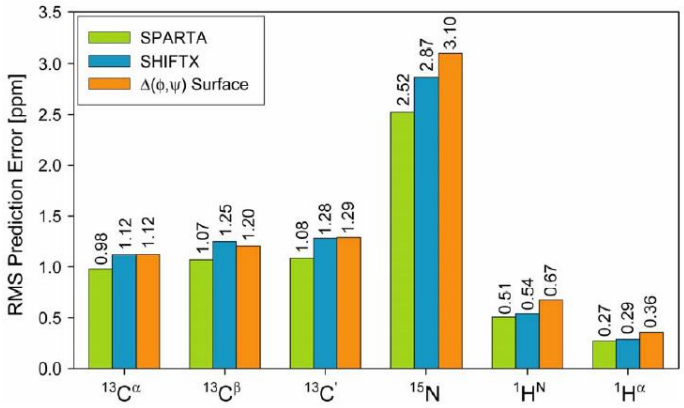


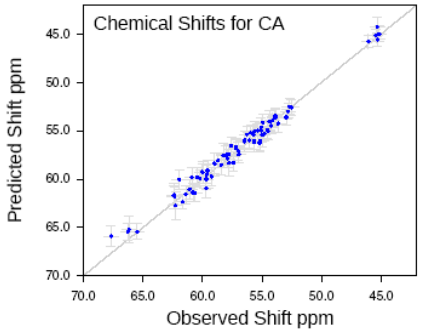


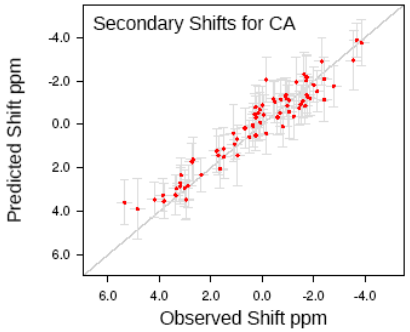
Yang Shen, and Ad Bax, J. Biomol. NMR, 56, 227-241(2013).

Related Software from the Bax Group at the NIH: SPARTA+ Prediction of Protein Chemical Shifts from Structure



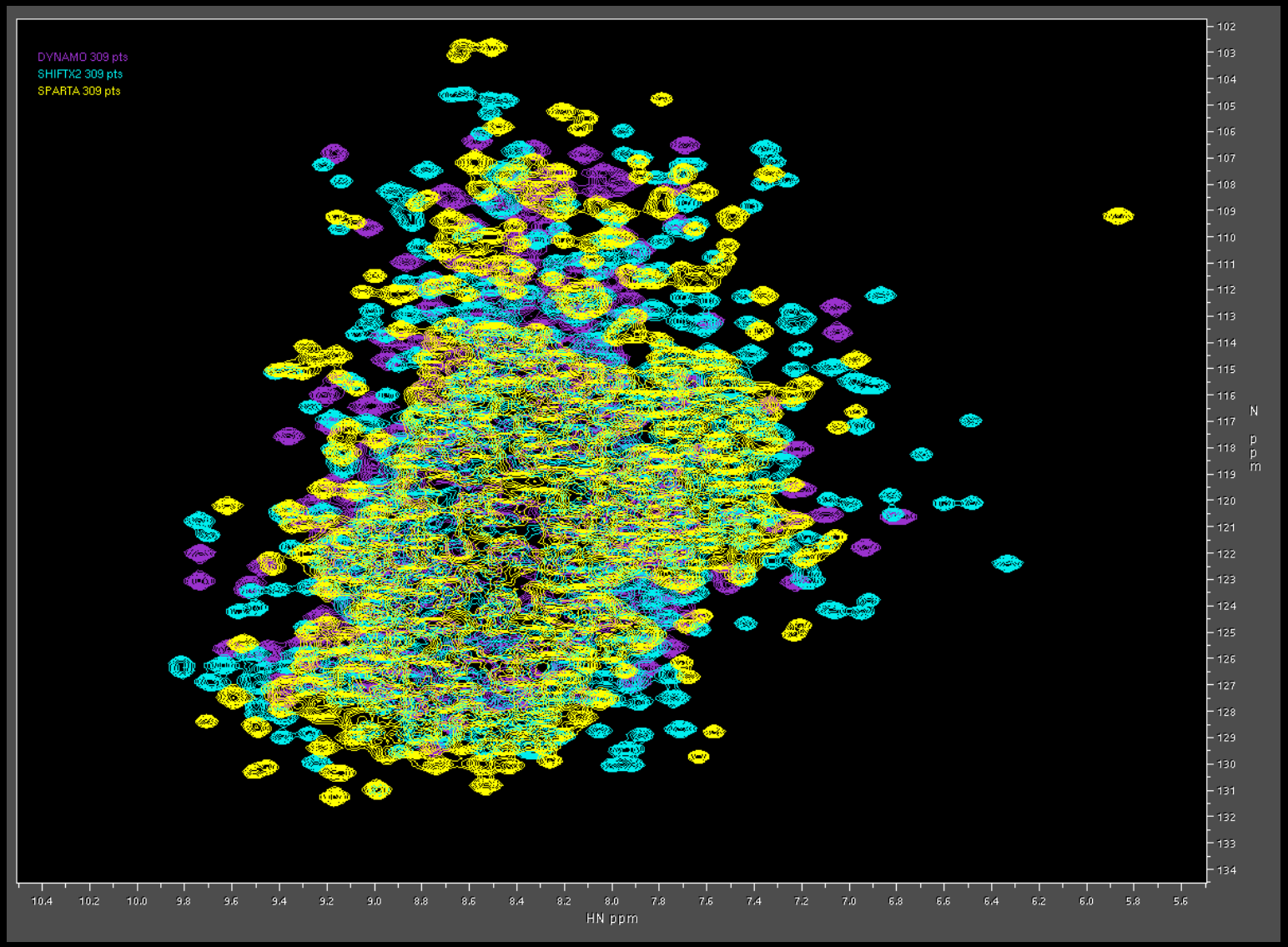




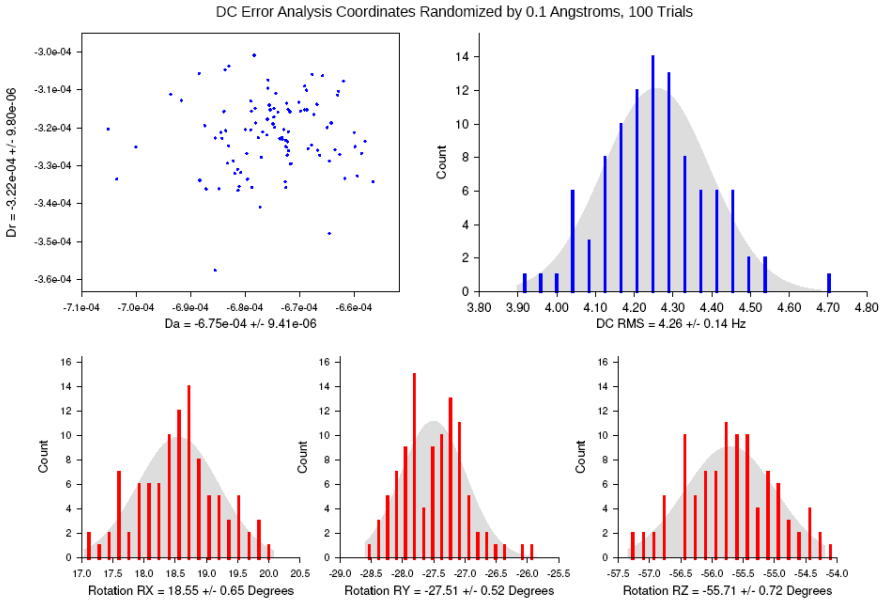


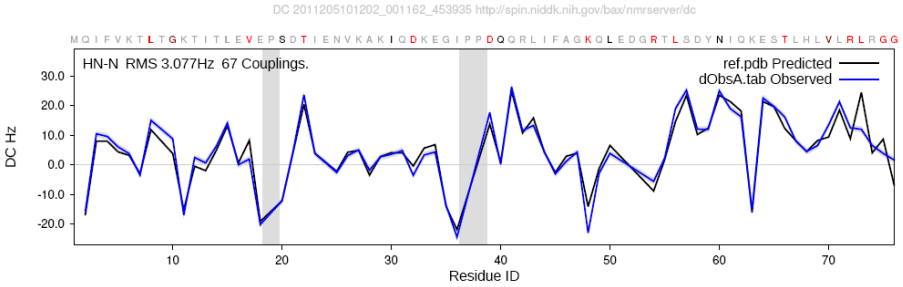
Yang Shen and Ad Bax, J. Biomol. NMR, 48, 13-22 (2010)

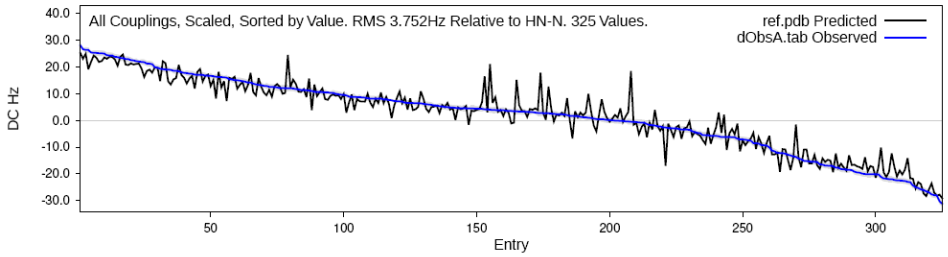
2D HN/N NMR of NISTmAb as Simulated via DYNAMO, SHIFTX2, and SPARTA+

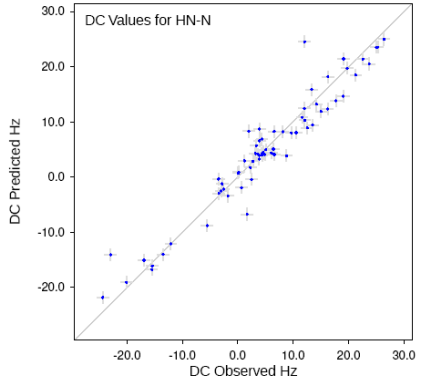


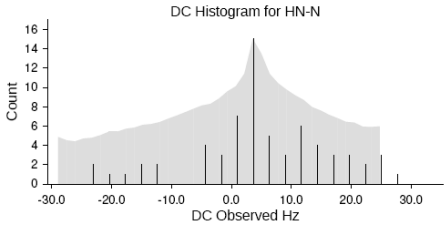
NMRPipe DC: Modules for Analysis of Dipolar Couplings







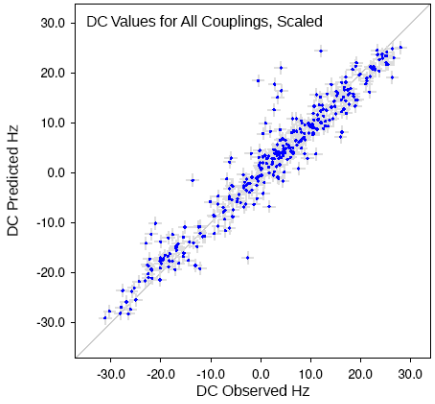


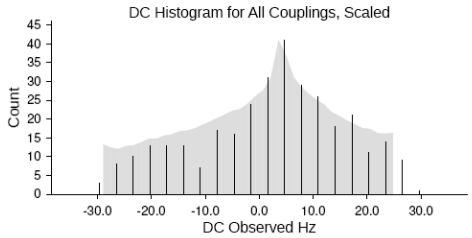


Tensor Parameters:
Da: -6.8589e-04 Dr: -3.2939e-04 GMag: 2.1011e-03 Rh: 0.480

Euler Rotations, Degrees: X: 18.53 Y': -27.60 Z'': -55.64

Order Matrix Values:
-8.7887e-04 -1.0887e-04 -6.7871e-04 -7.4130e-04 -2.5099e-04
sXX: 1.9180e-04 sYY: 1.1800e-03 sZZ: -1.3718e-03



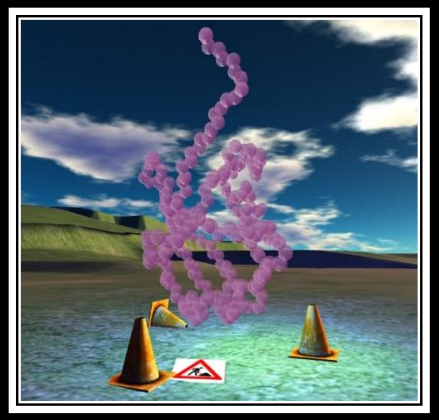


Tensor Parameters:
Da: -6.8589e-04 Dr: -3.2939e-04 GMag: 2.1011e-03 Rh: 0.480

Euler Rotations, Degrees: X: 18.53 Y': -27.60 Z'': -55.64

Order Matrix Values:
-8.7887e-04 -1.0887e-04 -6.7871e-04 -7.4130e-04 -2.5099e-04
sXX: 1.9180e-04 sYY: 1.1800e-03 sZZ: -1.3718e-03

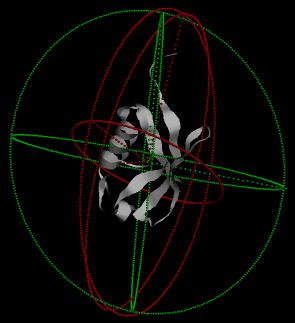
NMRPipe PDB Utilities

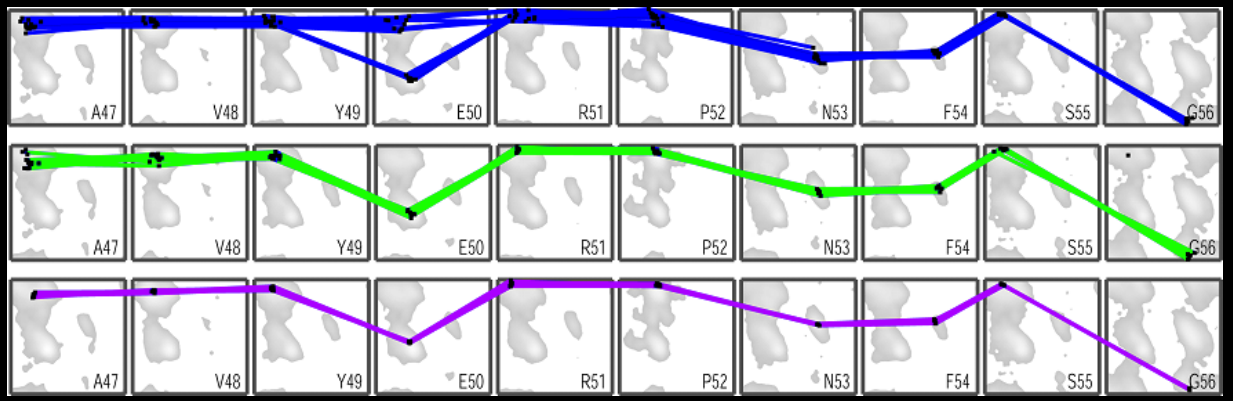


- Create Extended Structure
- Add Protons
- Align Coordinates of Two or More Structures
- Transformations of PDB Coordinates
- List Secondary Structure, H-Bonds
- Mass, Volume, Surface Area
- Simple simulated annealing

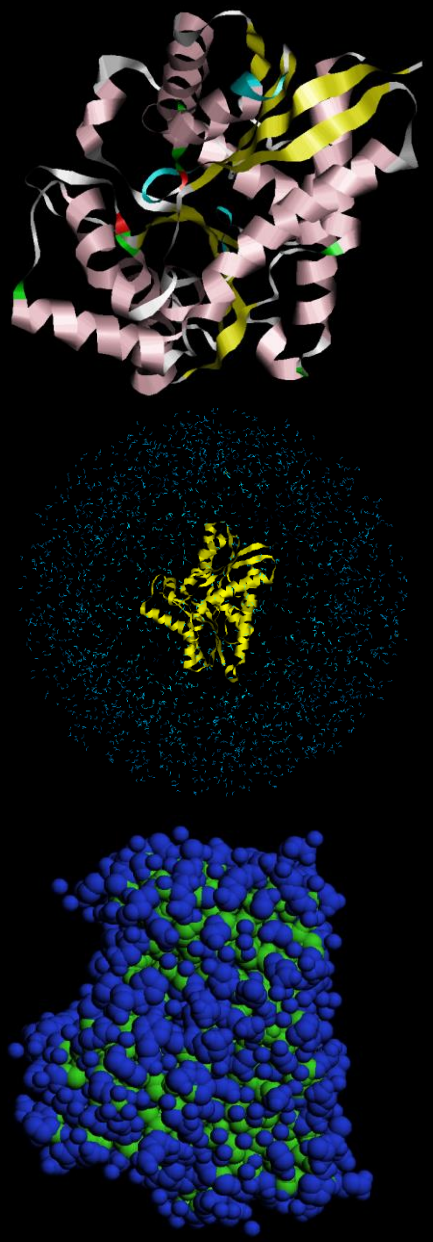
NMRPipe Homology Search Utilities

- Rotate PDB onto Dipolar Alignment Tensor Frame
- Molecular Fragment Replacement (NMR Fragment Homology)
- Amino Acid Type by Chemical Shift
- Estimate Dipolar Alignment Tensor from NMR Homology Search





NMRPipe Protein Structure Analysis and Report Generation



PDB SS Utility 2012026140916_031907_502954 http://spin.niddk.nih.gov/bax/nmrserver/pdbutil

3M4Z.pdb Residues 1 to 309

Atoms: 2716 Mass: 36383 Da Area: 14921.1 A^2 Volume: 40847.2 A^3 Radius of Gyration: 19.91 A

Distance Min: 1.20 A Max: 66.06 A Range X: -20.47 to 33.41 A Y: -35.39 to 22.92 A Z: -35.95 to 10.92 A Center XYZ: 7.16 -5.50 -13.60 A

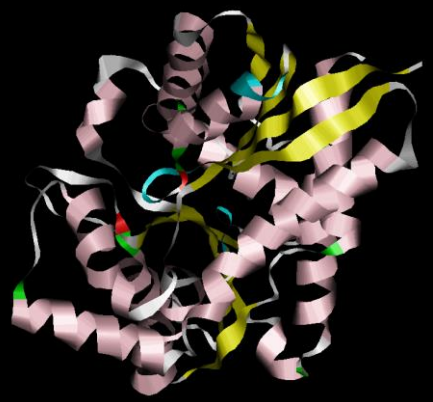
Structure Class
Sequence
Phi,Psi Class
Ramachandran Score
H-Bond Access to Solvent

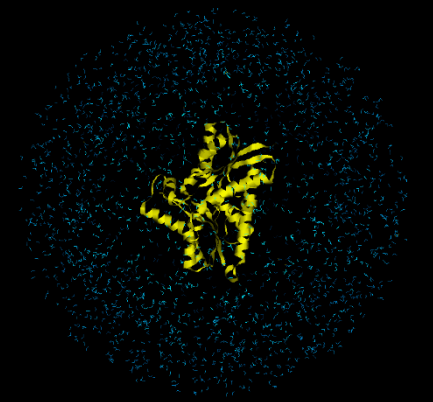
Phi,Psi Classes

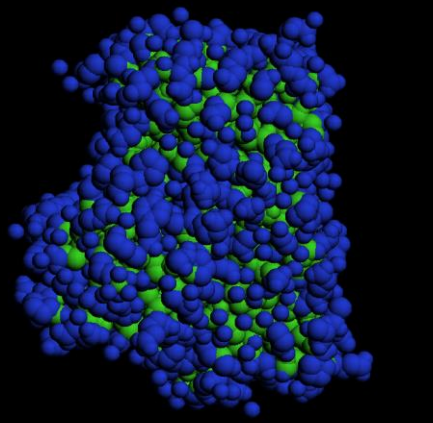
Ramachandran Scoring
1.0 Most Populated
0.8
0.6
0.4
0.2
Above 0.004

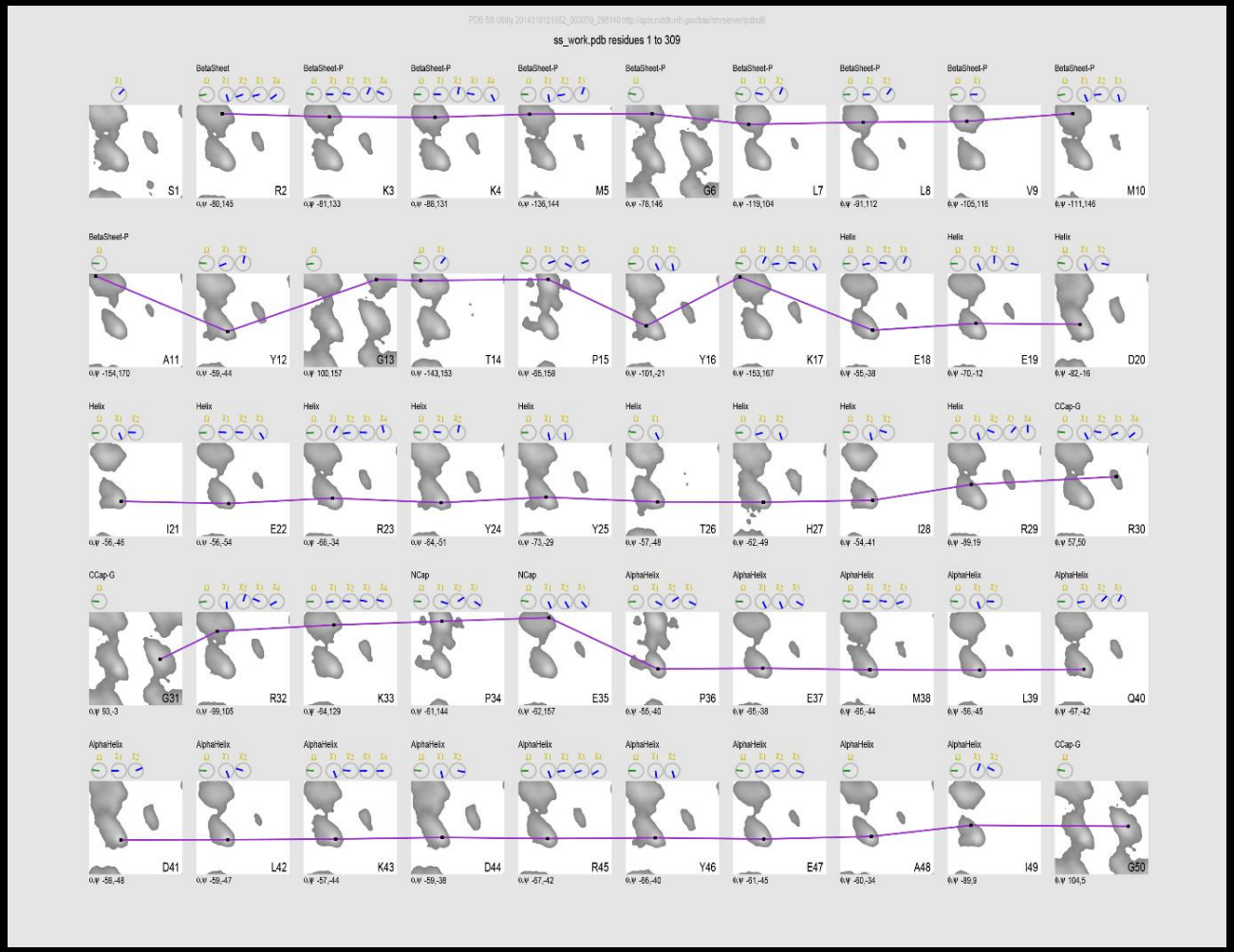
Solvent Access
CO HN SC
HN SC
CO SC
Side Chain (SC)
CO HN
HN
CO

NMRPipe Protein Structure Analysis and Report Generation

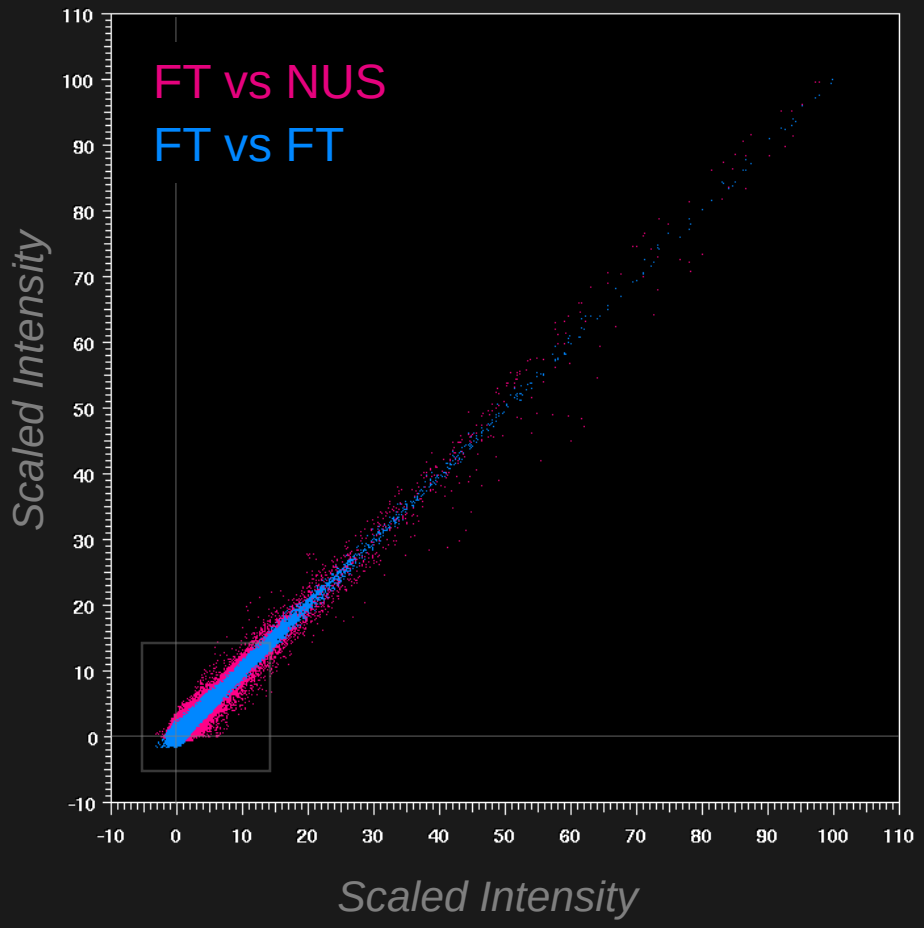


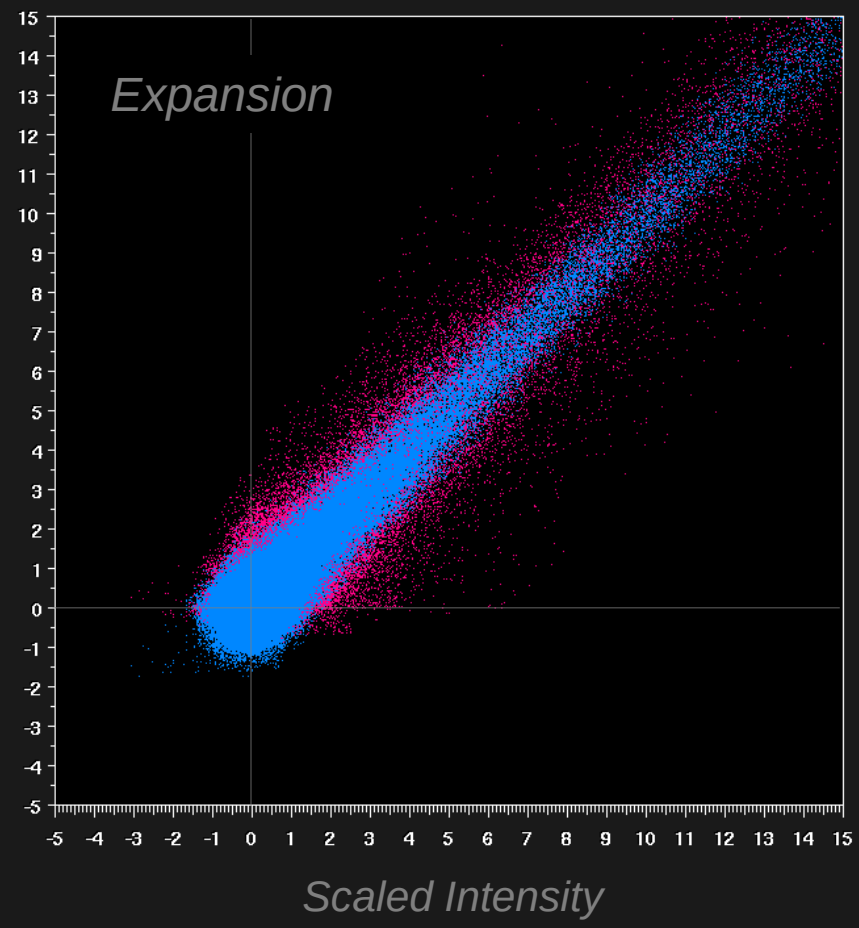




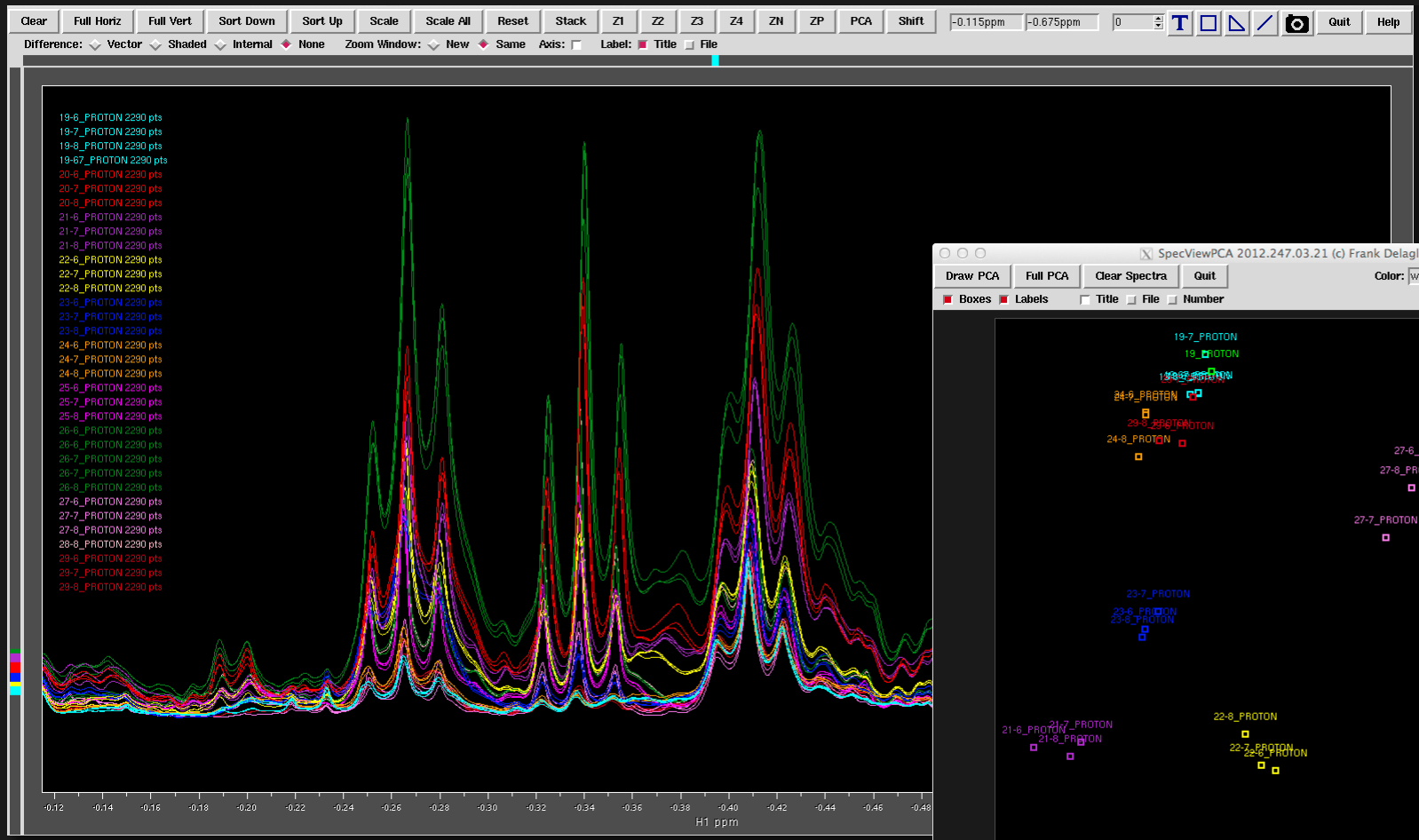


NMRPipe Spectrum versus Spectrum Correlation and Statistics



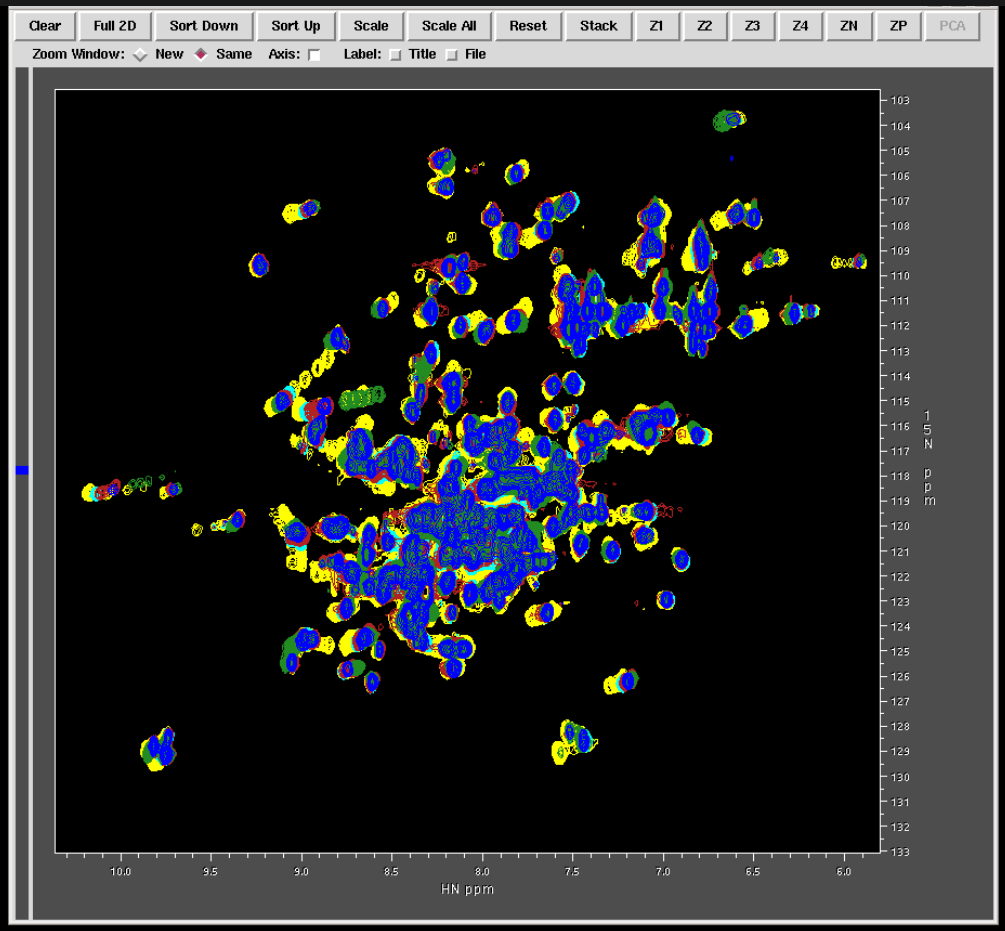


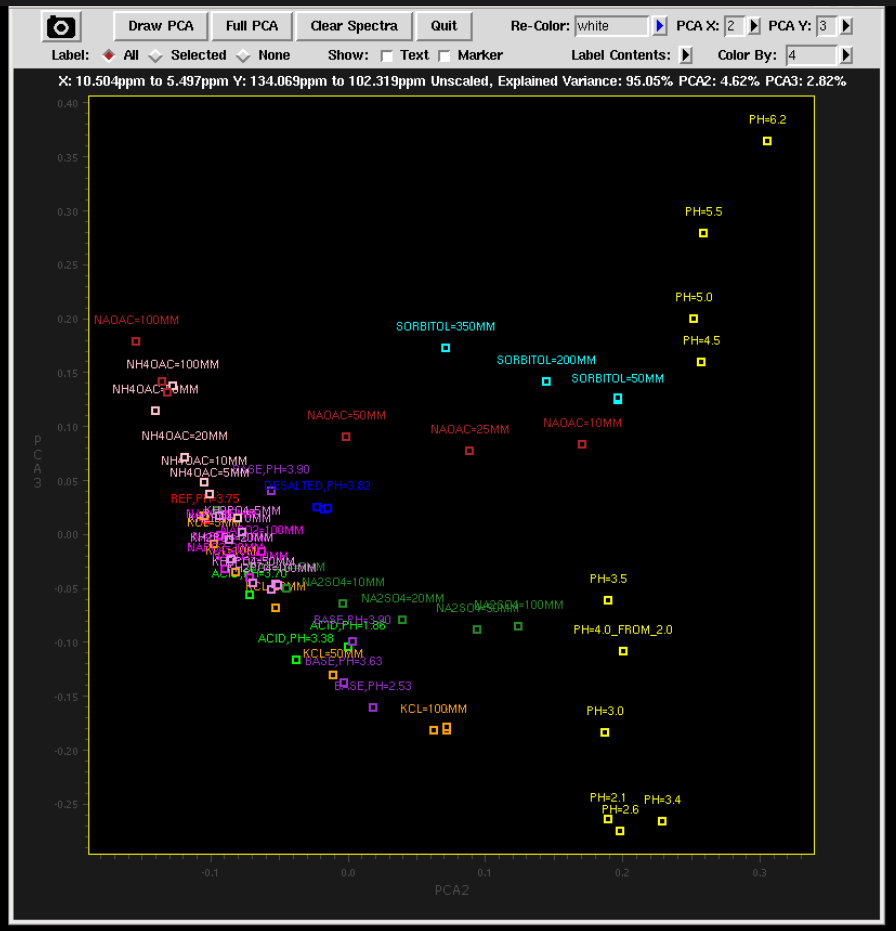
NMRPipe 1D Spectral Series Viewing with Principal Component Analysis (PCA)



Exploratory Analysis of 11 Varieties of Tea

NMRPipe 2D Spectral Series Viewing with Principal Component Analysis (PCA)

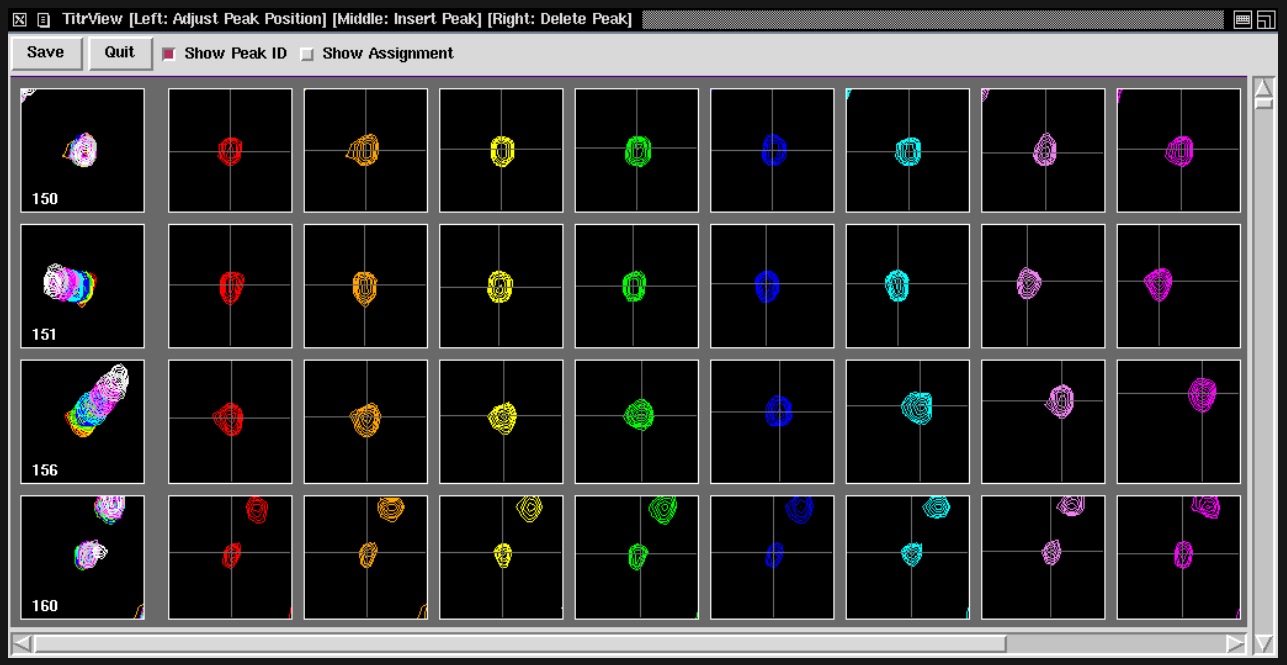


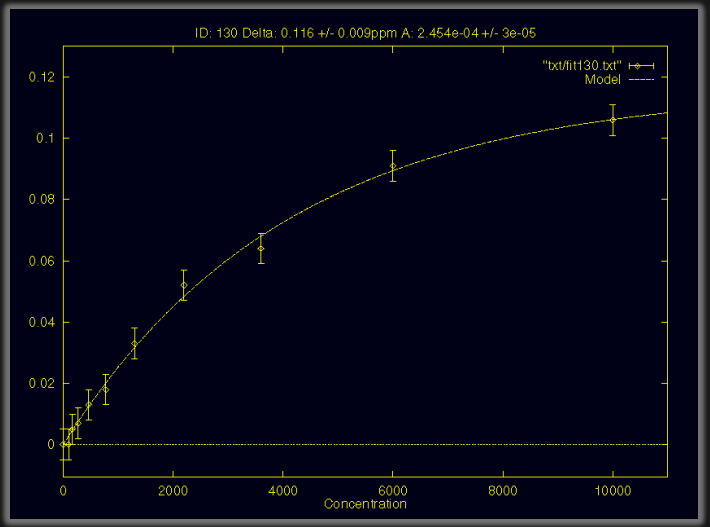


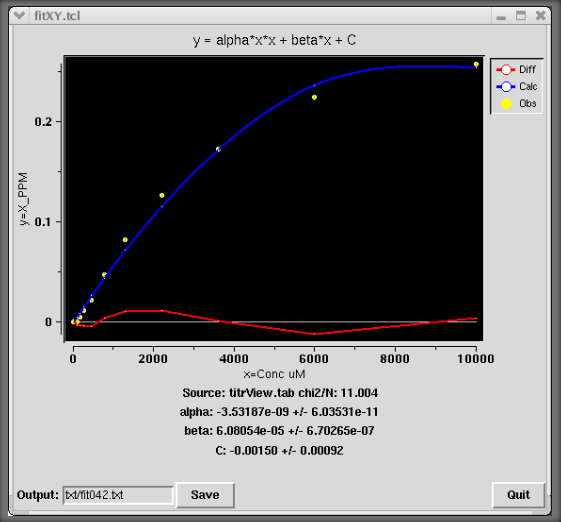
Monitoring Effects of Excipients, Formulation Parameters and Mutations on the High Order Structure of Filgrastim by NMR. Pharm Res, DOI 10.1007/s11095-015-1713-3 (2015)

Yves Aubin, Derek J. Hodgson, William B. Thach, Geneviève Gingras, and Simon Sauvé

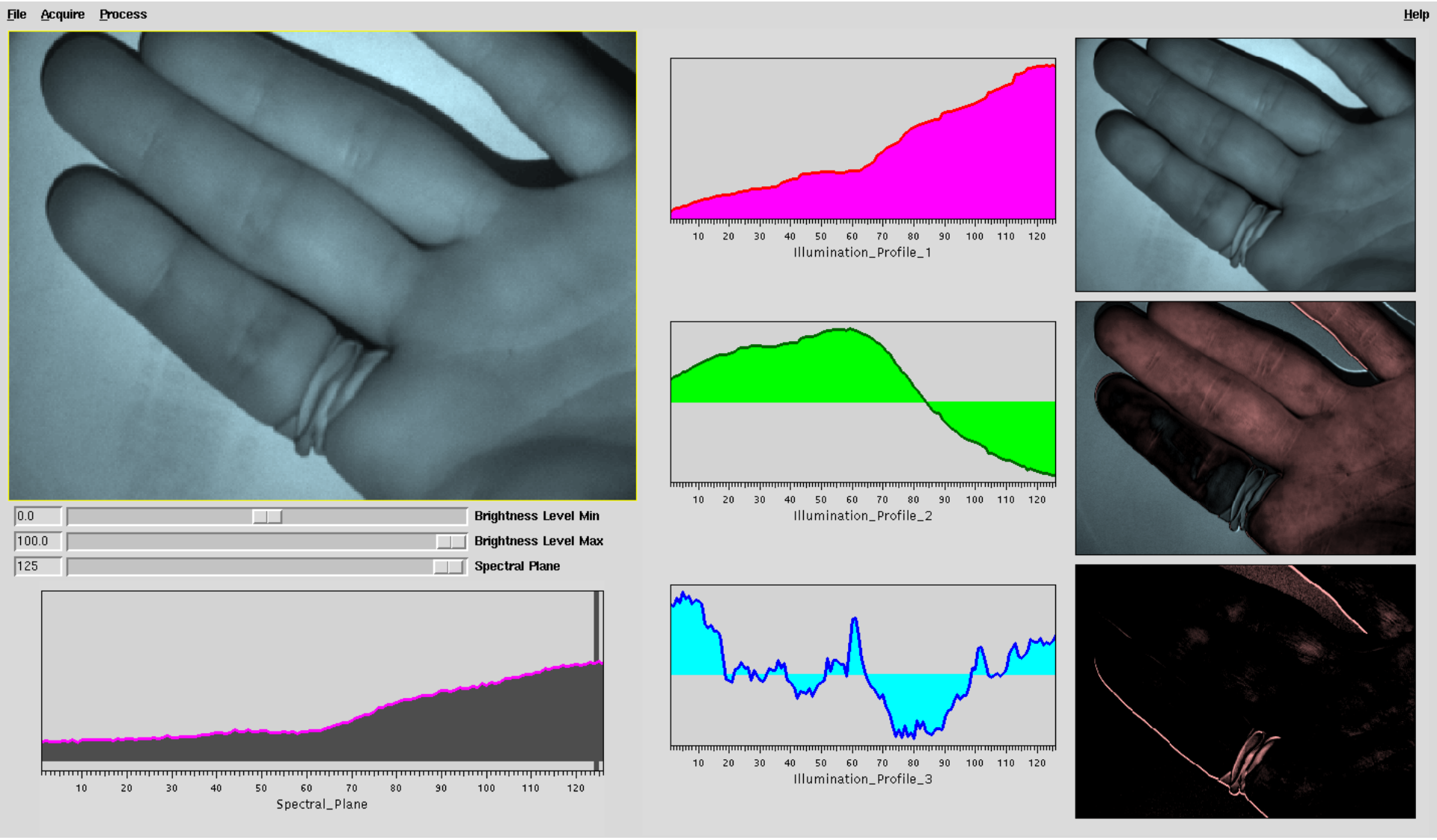
NMRPipe Extraction of and Fitting of Chemical Shift Titration Curves



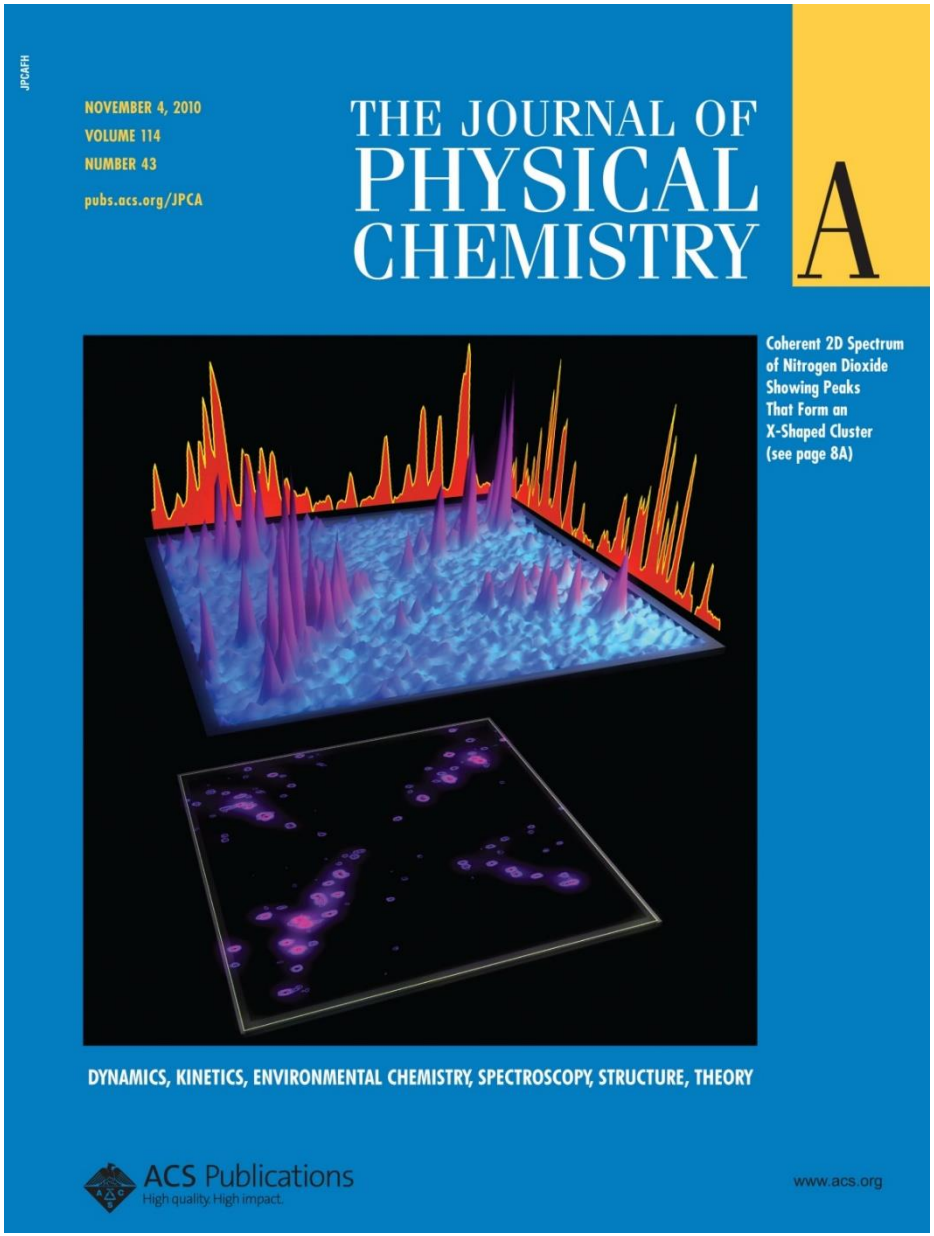


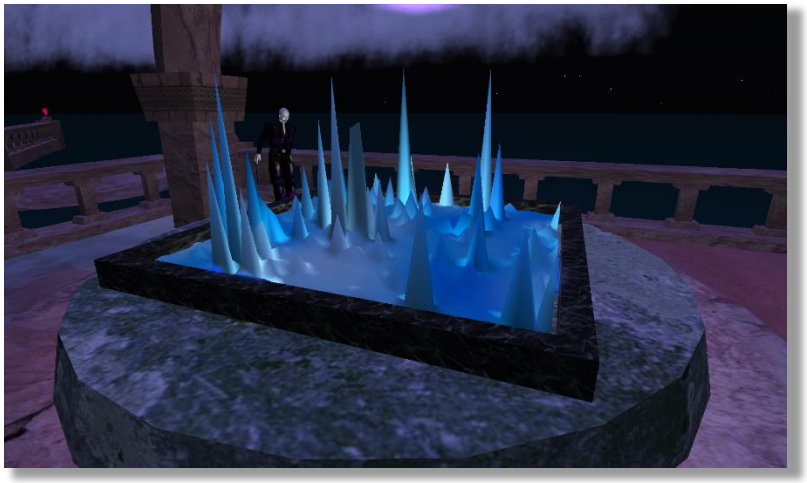


An NMRPipe Prototype Application: PCA Applied to Hyperspectral Optical Imaging



Importing Spectral Data into a Virtual Reality Environment





About the Cover

November 4, 2010: Vol. 114, Iss. 43

Conventional (1D) electronic spectra of NO_2 are largely patternless, but coherent 2D spectra of NO_2 show numerous X-shaped clusters such as that shown here. This spectrum is displayed in 2D contour form (below, in purple and black) and as a corresponding 3D intensity surface (above, in purple and blue). Orthogonal 1D projections (orange) of the surface resemble conventional 1D spectra. The spectral data was preprocessed using a pipeline-based software system for multidimensional spectroscopy (Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277—293), which was also used to generate images used to build the 3D scene. The figure was produced by Frank Delaglio (www.nmrscience.com), who used the interactive virtual reality environment Second Life to render this image (see page 11365).

Typical NMRPipe Workflow

The typical NMRPipe processing workflow involves frequent alternation between the UNIX command line and the **nmrDraw** graphical interface:

1. Prepare a script to convert spectrometer format data to NMRPipe format via an interactive interface. Commands: **bruker** / **varian** / **delta**
2. Execute the conversion script, and inspect the result via the nmrDraw graphical interface. Use the **Proc/Auto Process 1D** menu option to process the first 1D vector, and use the graphical interface to find a phase correction value.
3. At the UNIX command-line, use a text editor to create or adjust a processing script containing the needed phase corrections etc. Commonly, the processing script is copied from a previous case. Processing scripts can also be created via new general-purpose scripts: **basicFT2.com** / **basicFT3.com** / **basicFT4.com**.
4. Execute the processing script and read the processed result with **nmrDraw**. View vectors from any of the dimensions, and choose new phase correction values as needed.
4. Repeat steps 3 and 4 to adjust processing parameters as needed.
5. In the case of 3D data, create and inspect projections and view strips plots of the 3D data to confirm that the data is processed and phased properly. For example:

```
proj3D.tcl -in ft/test%03d.ft3 -abs
peakHN.tcl -in ft/test%03d.ft3 -out hn.proj.tab -proj -hi Full
scroll.tcl -in ft/test%03d.ft3 -tab hn.proj.tab -pair -hi 8%
```

Processing Functions for `nmrPipe -fn ...`

SOL	Time-domain Solvent Subtraction by Convolution and Subtraction
POLY -time	Time-domain Solvent Subtraction by Polynomial Fitting and Subtraction
SP	Window Function and First Point Scaling (**-c 0.5 or -c 1.0**)
ZF	Zero Fill (**-auto** rounds to power of two)
FT	Fourier Transform
PS	Phase Correction (**-di** deletes imaginaries after)
EXT	Extraction of a Chemical Shift Range
POLY -auto	Automated Baseline Correction by Polynomial Fitting and Subtraction
HT	Hilbert Transform (reconstruct previously deleted imaginary data)
TP	Exchange X-Axis and Y-Axis (also called YTP) for 2D, 3D, or 4D Data
ZTP	Exchange X-Axis and Z-Axis for 3D and 4D Data
ATP	Exchange X-Axis and A-Axis for 4D Data
LP	Linear Prediction (extend or replace time-domain data)
MEM	Maximum Entropy Reconstruction
ML	Maximum Likelihood Frequency Map
SMILE	SMILE Reconstruction
COADD	Weighted Co-Addition of Adjacent Vectors for Interleaved Data
MAC	NMRPipe's Macro Language for Simple Custom Processing Functions

Listing Command Line Options: ***nmrPipe -help -fn ...***

```
% nmrPipe -help -fn ZF

ZF: Extend By Zero Filling;
Use only one of the following:
 -zf   zfCnt   [1] Number of Times to Double the Size.
 -pad  padCnt      Zeros to Add.
 -size finSize     Final Size.

Other Flags:
 -mid              Zero Fill in Middle.
 -inter            Zero Fill by zfCount Between Points.
 -auto             Round Final Size to Power of 2.

Removing Previous Zero Filling:
 -inv              Extract Original Time Domain.

Set Time-Domain Size for Zero Fill Extrapolation:
 -td    Set Time-Domain Size to Zero Fill Size.
 -notd  Do Not Change Time-Domain Size (Default).
```

Typical NMRPipe 3D Spectral Processing Scheme

Commonly Adjusted Parameters / X-Axis Processing / Y-Axis Processing / Z-Axis Processing

The Script Provides Reproducible Processing

```
#!/bin/csh

xyz2pipe -in fid/test%03d.fid -x -verb                \   Read vectors from X-Axis
| nmrPipe  -fn SOL                                    \     Solvent Subtraction
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5    \     Window and First Point Scale
| nmrPipe  -fn ZF                                     \     Zero Fill
| nmrPipe  -fn FT                                     \     Fourier Transform
| nmrPipe  -fn PS -p0 43.0 -p1 0.0 -di                \     Phase Correction
| nmrPipe  -fn EXT -x1 10.5ppm -xn 5.7ppm -sw         \     Extract PPM Range
| nmrPipe  -fn TP                                     \   X/Y Transpose
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 1.0    \     Window and First Point Scale
| nmrPipe  -fn ZF                                     \     Zero Fill
| nmrPipe  -fn FT                                     \     Fourier Transform
| nmrPipe  -fn PS -p0 -90.0 -p1 180.0 -di             \     Phase Correction
| nmrPipe  -fn TP                                     \   X/Y Transpose
| nmrPipe  -fn POLY -auto                             \     Baseline Correction
| pipe2xyz -out ft/test%03d.ft2 -x                      Write vectors to X-Axis

xyz2pipe -in ft/test%03d.ft2 -z -verb                 \   Read vectors from Z-Axis
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 0.5    \     Window and First Point Scale
| nmrPipe  -fn ZF                                     \     Zero Fill
| nmrPipe  -fn FT                                     \     Fourier Transform
| nmrPipe  -fn PS -p0 0.0 -p1 0.0 -di                 \     Phase Correction
| pipe2xyz -out ft/test%03d.ft3 -z                      Write vectors to Z-Axis
```

New General-Purpose Processing and Script Generation:
basicFT2.com basicFT3.com basicFT4.com

The latest version of NMRPipe includes several new general-purpose commands for simplified spectral processing:

- Default input file is based on the contents of the current directory, so you can get a quick and easy trial result just with a command like:

```
basicFT2.com -xP0 43
```

- Default parameters change automatically for amide-detected data.
- There are options for Linear Prediction and NUS Zero Fill (IST reconstruction applied to conventional data, described later).
- First-point time-domain scaling is adjusted automatically according to first-order phase correction.
- The commands can perform processing directly, or generate the NMRPipe script so that you can edit it yourself.
- Command `basicFT2.com` can process a 2D spectrum, extract and process a 2D XY or XZ plane from 3D or 4D data, a 2D XA plane from 4D data, or a process an entire pseudo-3D series of 2D spectra.
- `basicFT3.com` and `basicFT4.com` process 3D and 4D spectra.

New General-Purpose Processing and Script Generation: basicFT2.com basicFT3.com basicFT4.com

This:

```
basicFT2.com -xP0 13 -xP1 0 -xEXTX1 10.4ppm -xEXTXN 5.4ppm -yP0 -90 -yP1 180
```

takes the place of this:

*Input (**-in**) determined automatically*

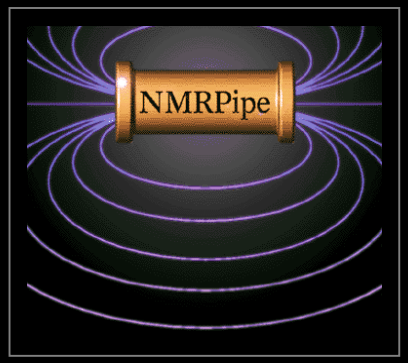
```
nmrPipe -in fid/test001.fid \
| nmrPipe  -fn SOL                                          \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5          \
| nmrPipe  -fn ZF -auto                                     \
| nmrPipe  -fn FT                                           \
| nmrPipe  -fn PS -p0 13  -p1 0.0 -di                       \
| nmrPipe  -fn EXT -x1 10.4ppm -xn 5.4ppm -sw               \
| nmrPipe  -fn TP                                           \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 1.0          \
| nmrPipe  -fn ZF -auto                                     \
| nmrPipe  -fn FT                                           \
| nmrPipe  -fn PS -p0 -90 -p1 180 -di                       \
| nmrPipe  -fn TP                                           \
| nmrPipe  -fn POLY -auto                                   \
   -out test.ft2 -verb -ov
```

***SOL** default for HN data*

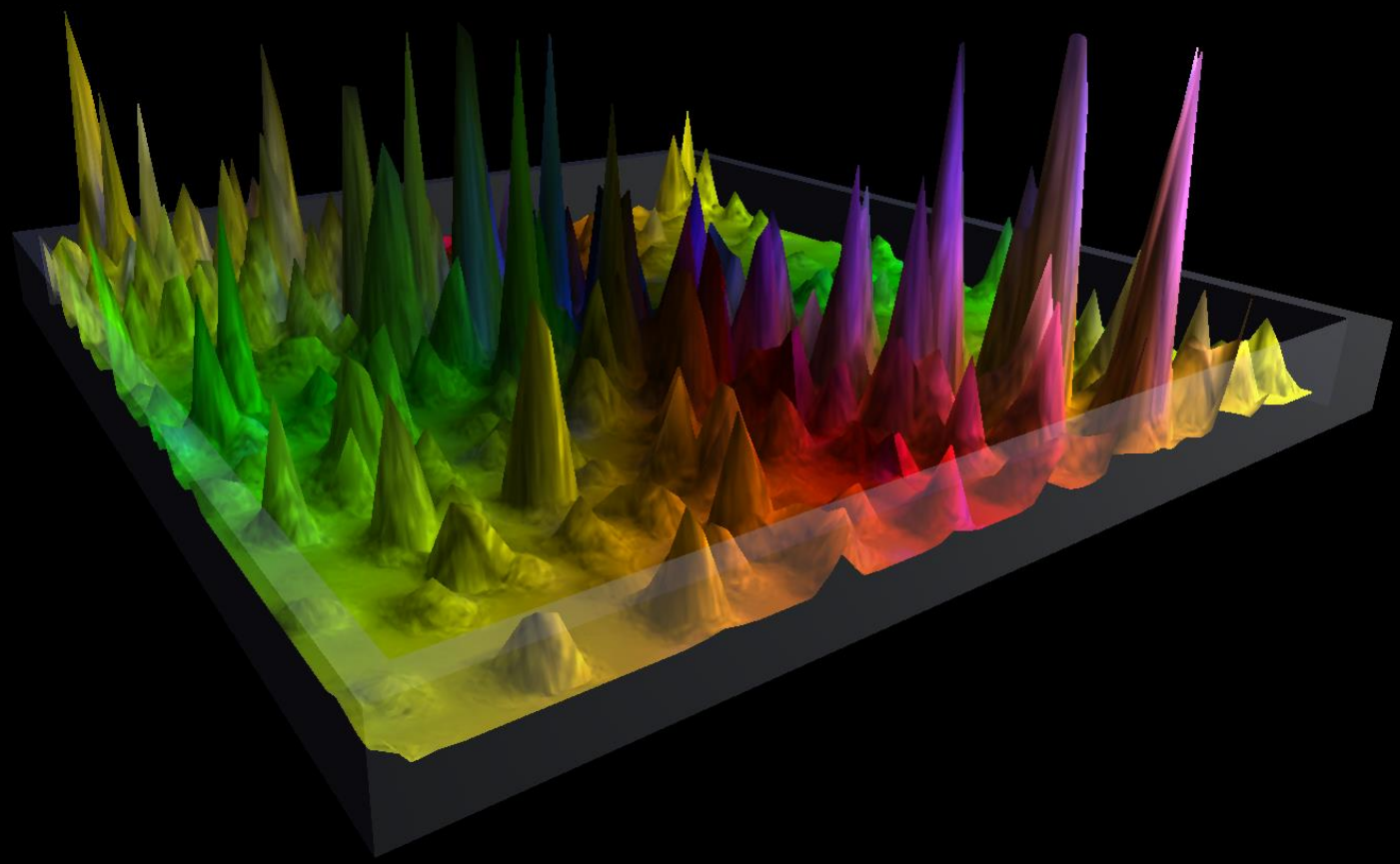
First-Point Scale Factors (-c values) automatically calculated from P1

POLY -auto default for HN data

*Output (**-out**) set by default*



Non-Uniform Sampling in NMRPipe



About Non-Uniform Sampling

Non-Uniform Sampling (NUS) is an acquisition method for multidimensional NMR spectra that works by skipping some fraction of the data that would be acquired in a conventional measurement, which is uniformly sampled (US).

The goal of NUS is to improve the spectral resolution (or other metric of spectral quality) obtained with a given amount of measurement time.

Because some of the data is skipped in a NUS acquisition, the usual Fourier transform processing used for conventional data is not ideal, and so other reconstruction methods are required to take best advantage of NUS.

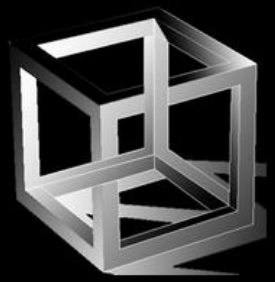
There are many tools for reconstructing NUS data, including Matrix Decomposition (MDD software, Orekhov), Maximum Entropy (RNMRTK, Hoch et al.), Iterative Soft Thresholding (IST) (hmsIST, Hyberts and Wagner, and applications from Stern, Donoho, Hoch et al.), SCRUB (Coggins and Zhou), and NESTA (Sun, Gill, Byrd et al.). Many of these tools use NMRPipe as part of the reconstruction workflow.

NMRPipe includes its own implementation of IST, so that it is easy to perform all the steps needed for NUS reconstruction. This presentation explains NUS and IST, and shows how to use NMRPipe to convert and process 2D and 3D NUS NMR data with IST. It also shows how to apply IST to conventional data as an alternative to Linear Prediction.

NUS Software from Our Friends

Many NUS Reconstruction Systems Use NMRPipe Workflows

SMILE spin.niddk.nih.gov/bax/software/smile

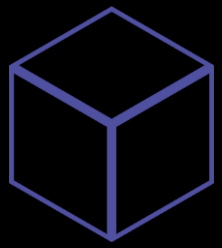


NESTA nestanmr.com

SCRUB coggins.biochem.duke.edu/scrub

hmsIST gwagner.med.harvard.edu/intranet/istHMS

Find these methods and more on NMRbox



NMRbox.org

Non-Uniform Sampling in NMRPipe

The NMRPipe NUS tools expect the following:

- NUS schedules are plain text files with the same number of space-separated values on each line, and with no blank lines or other content. Increment numbers are always given as integers.
- 2D NUS schedules have one increment number per line.
- 3D NUS schedules have two increment numbers per line, one for the Y-Axis, and one for the Z-Axis.
- 4D schedules have three increment numbers per line, for the Y-Axis, Z-Axis, and A-Axis.

There is more than one convention for recording NUS sampling schedules. NMRPipe can directly accommodate the following:

- Some schedules will use increment numbers which start at one for the first increment rather than zero.
- Some schedules will include floating-point weighting factors along with the integer increment numbers.
- By default, NMRPipe assumes that 3D and 4D schedules have the Y-Axis increment value listed first on each line. In some conventions, this order is reversed, so that the Y-Axis increment is last on each line.

NMRPipe Workflow for NUS Data

1. Prepare a conversion script with the usual tools, including options which sort and expand the NUS time-domain data with zeros. Commands:

   ```
   bruker -nus / varian -nus / delta -nus
   ```

2. Perform trial processing via ordinary Fourier processing on the NUS fid, to confirm phase correction values, etc. This can be done via the usual NMRPipe scripts, or via new general-purpose processing scripts:

   ```
   basicFT2.com / basicFT3.com / basicFT4.com
   ```

3. In the case of 3D or 4D data, inspect the ordinary Fourier transform result by preparing projections. Commands:

   ```
   proj3D.tcl / proj4D.tcl
   ```

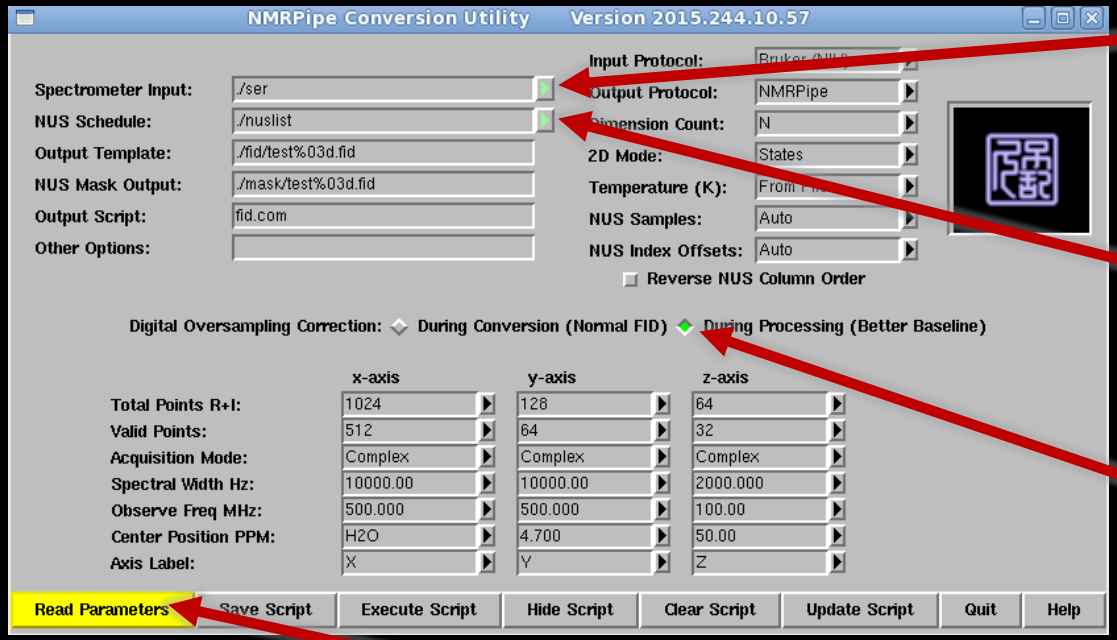
4. Use the ordinary Fourier transform result to estimate the convergence parameter used by NMRPipe's NUS reconstruction tools:

   ```
   specStat.com -stat istMaxRes -in ...
   ```

5. Use NMRPipe's NUS reconstruction scripts to generate a final spectrum:

   ```
   ist2D.com / ist3D.com / ist4D.com
   ```

How to Convert NUS Data in NMRPipe



1. Select the binary spectrometer-format input data as usual.

2. Select the NUS Sampling Schedule Input File.

3. Choose options for best baseline.

4. Read Parameters

   This will also read the NUS schedule, and extract information about sample count, dimension sizes, etc.

Bruker 3D NUS Conversion

```
#!/bin/csh

nusExpand.tcl -mode bruker -sampleCount 2048 -off 0 \
 -in ./ser -out ./ser_full -sample ./nuslist

bruk2pipe -in ./ser_full \
  -bad 0.0 -aswap -AMX -decim 1680 -dspfvs 20 -grpdly 67.9866027832031  \
  -xN              2048  -yN                64  -zN               320  \
  -xT              1024  -yT                32  -zT               160  \
  -xMODE            DQD  -yMODE  Echo-AntiEcho  -zMODE         Complex  \
  -xSW        11904.762  -ySW         3846.154  -zSW         11904.762  \
  -xOBS         950.204  -yOBS          96.294  -zOBS          950.204  \
  -xCAR           4.773  -yCAR         118.579  -zCAR            4.773  \
  -xLAB              HN  -yLAB             15N  -zLAB               1H  \
  -ndim               3  -aq2D          States                          \
  -out ./fid/test%03d.fid -verb -ov

xyz2pipe -in ./fid/test%03d.fid \
| nusExpand.tcl -mask -noexpand -mode pipe -sampleCount 2048 -off 0 \
  -in stdin -out ./mask/test%03d.fid -sample ./nuslist
```

Expand the Spectrometer-format Data / Convert Expanded Data / *Create Mask*

Varian 3D NUS Conversion

```
#!/bin/csh

nusExpand.tcl -mode varian -sampleCount 455 -off 0 \
 -in ./fid -out ./fid_full -sample ./sampling.sch

var2pipe -in ./fid_full \
 -noaswap -aqORD 1 \
  -xN              2404  -yN               140  -zN                52  \
  -xT              1202  -yT                70  -zT                26  \
  -xMODE        Complex  -yMODE         Complex  -zMODE       Rance-Kay  \
  -xSW        12019.231  -ySW         7000.000  -zSW          1320.000  \
  -xOBS         599.422  -yOBS         599.422  -zOBS           60.746  \
  -xCAR           4.770  -yCAR           4.770  -zCAR          118.178  \
  -xLAB              HN  -yLAB              1H  -zLAB              N15  \
  -ndim               3  -aq2D          States                          \
  -out ./data/test%03d.fid -verb -ov

xyz2pipe -in ./data/test%03d.fid \
| nusExpand.tcl -mask -noexpand -mode pipe -sampleCount 455 -off 0 \
  -in stdin -out ./mask/test%03d.fid -sample ./sampling.sch
```

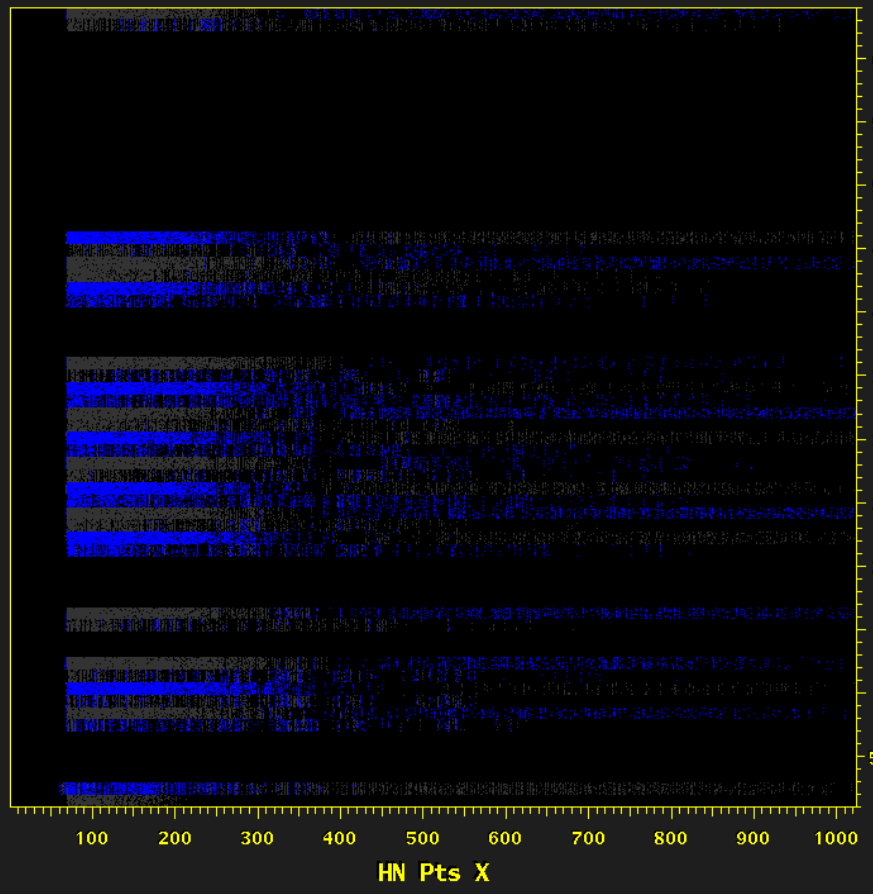
Expand the Spectrometer-format Data / *Convert Expanded Data* / *Create Mask*

JEOL 3D NUS Conversion

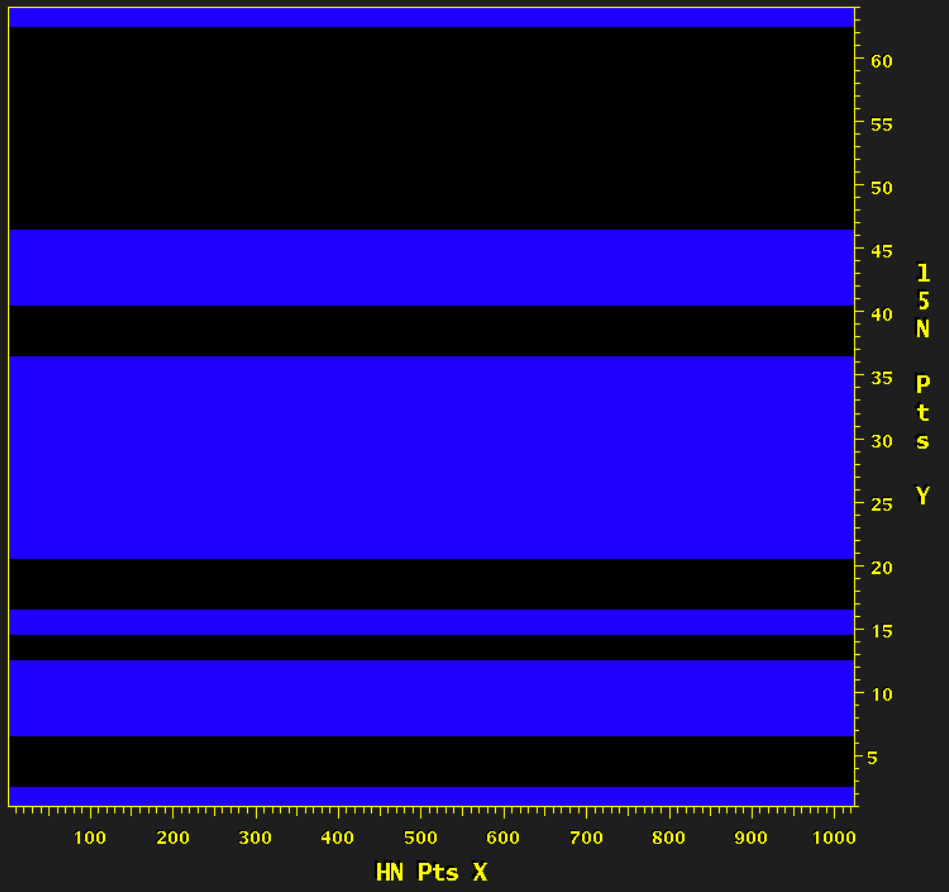
```
#!/bin/csh

delta2pipe -in ./3C15N_Ubq_hnco_20NUS-1-1.jdf -nusDim 3 \
  -nodf -dfVal 19.687500 -trVal 8.000000e-01  \
  -xN              2048  -yN              1004  -zN                1  \
  -xT              1024  -yT                50  -zT               25  \
  -xMODE        Complex  -yMODE        Complex  -zMODE          Real  \
  -xSW        11261.261  -ySW         3018.594  -zSW         3041.363  \
  -xORIG      -2815.707  -yORIG      25428.789  -zORIG       5783.051  \
  -xOBS         600.172  -yOBS         150.913  -zOBS          60.815  \
  -xCAR           4.681  -yCAR         176.000  -zCAR         120.000  \
  -xFT             Time  -yFT            Time  -zFT             Time  \
  -xLAB              HN  -yLAB              CO  -zLAB              15N  \
  -ndim               2  -aq2D        Complex                         \
  -out nus.fid -verb -ov

nusExpand.tcl  -in nus.fid -out fid/test%03d.fid  \
               -sample hnco_NUS.txt -sampleCount Auto

nusExpand.tcl  -in nus.fid -out mask/test%03d.fid \
-sample hnco_NUS.txt -sampleCount Auto -mask
```

Convert 3D Data as NUS 2D / Expand the Converted Data to 3D / Create Mask

Output of NMRPipe NUS Conversion



Sorted, expanded time-domain data, arranged like conventional data but with the increments that were skipped filled in with zeros



Corresponding mask of ones and zeros, used as additional input for NMRPipe reconstruction tools

How to Process NUS Data in NMRPipe

It is recommended to apply trial processing of the expanded time-domain NUS data using ordinary Fourier transform schemes. This often allows confirmation of details such as phase correction before more time-consuming reconstruction methods are used. General-purpose scripts `basicFT2.com` `basicFT3.com` `basicFT4.com` are provided to help with this step …

```
#!/bin/csh

basicFT3.com \
 -in fid/test%03d.fid -out ft/test%03d.ft3 \
 -xP0 -75 -xP1 0 -xEXTX1 10.4ppm -xEXTXN 5.4ppm \
 -zFTARG alt

proj3D.tcl -in ft/test%03d.ft3 -abs
```

Process complete 3D

Generate and Inspect 2D projections from 3D

How to Process NUS Data with NMRPipe's Iterative Soft Thresholding (IST)

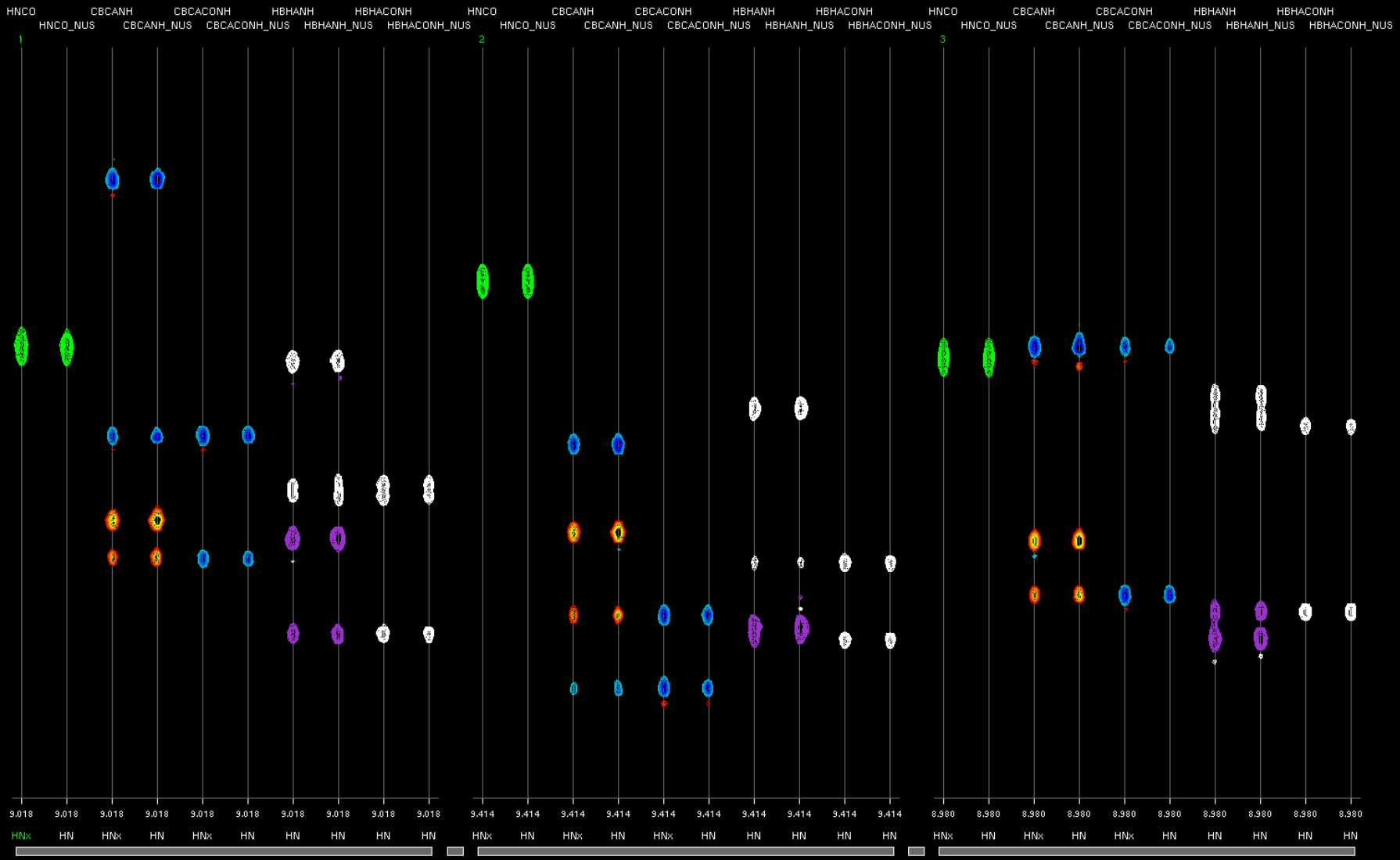
After processing parameters have been confirmed using ordinary Fourier transform schemes, use the NMRPipe IST script (`ist2D.com` `ist3D.com` `ist4D.com`) to reconstruct a spectrum.

For convenience, the NMRPipe IST scripts have similar arguments to scripts `basicFT2.com` etc. NMRPipe's IST takes the expanded time-domain data and corresponding mask as input, and produces a spectrum as output.

The parameter `istMaxRes` defines the convergence condition for IST, specified as a percentage of the largest value in the starting spectrum. Convergence is achieved when the largest value in the residual is below this percentage. By default, this parameter is determined automatically, and all the examples in the NMRPipe demo data archive use the automated setting.

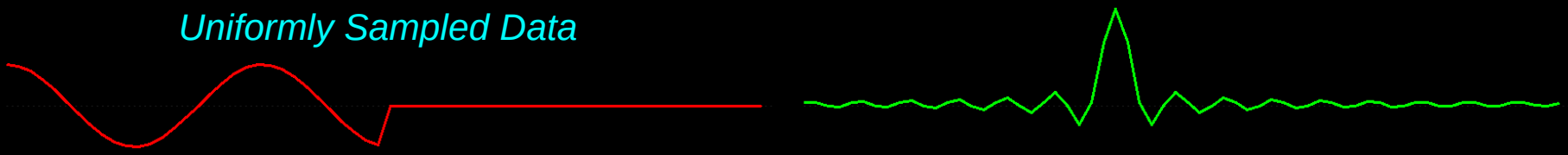
```
ist3D.com -istMaxRes Auto \
  -in fid/test%03d.fid -mask mask/test%03d.fid -out ist/test%03d.ft3 \
  -xP0 -75 -xP1 0 -xEXTX1 10.4ppm -xEXTXN 5.4ppm \
  -zFTARG alt
```

Uniformly Sampled Data and 33% NUS Reconstructed by NMRPipe's IST

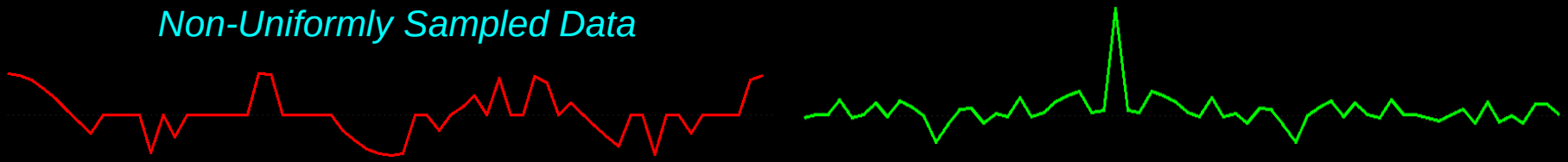


NUS Zero Fill as an Alternative to Linear Prediction

Uniformly Sampled Data



Non-Uniformly Sampled Data



- Conventional Fourier transform, Conventional Uniform Sampling Schedule:
  Broad lines and periodic truncation wiggle artifacts

- Conventional Fourier transform, Non Uniform Sampling Schedule:
  Narrow lines and random-noise-like artifacts

All peaks for a given schedule have the same artifacts, and the size of the artifacts is proportional to the size of the peaks.

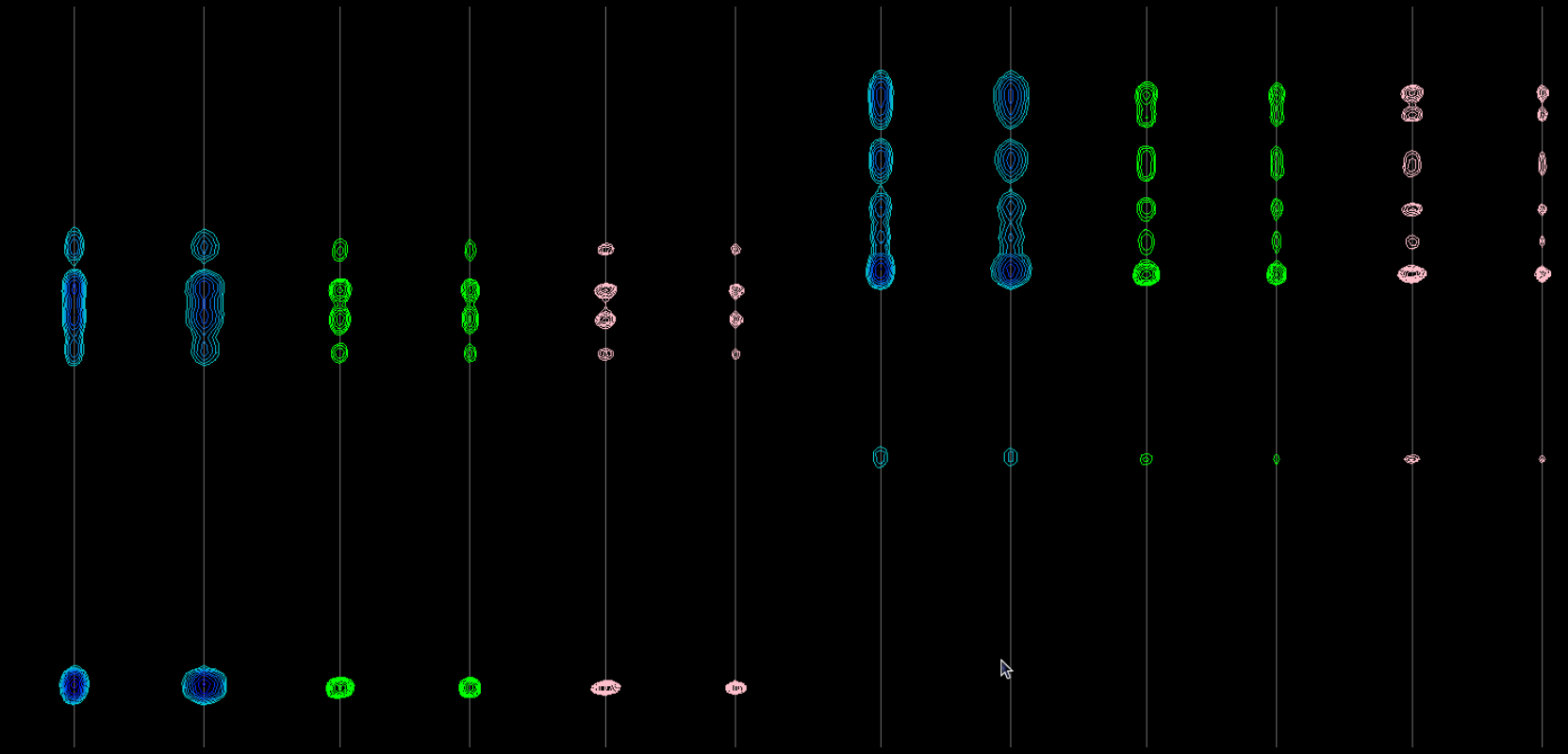
As noted by Hoch and coworkers, IST can be used with Non-Uniform schedules, and also with conventional Uniform Schedules. This means it is possible to apply IST to conventionally-sampled data as an alternative to extrapolating data via Linear Prediction. We call this approach NUS Zero Fill.

NUS Zero Fill using IST as an Alternative to Linear Prediction for Conventional NMR Data (15N-NOE)

Conventional FT
4 sec

Linear Prediction
2 min 30 sec

NUS Zero Fill via IST
20 min 21 sec



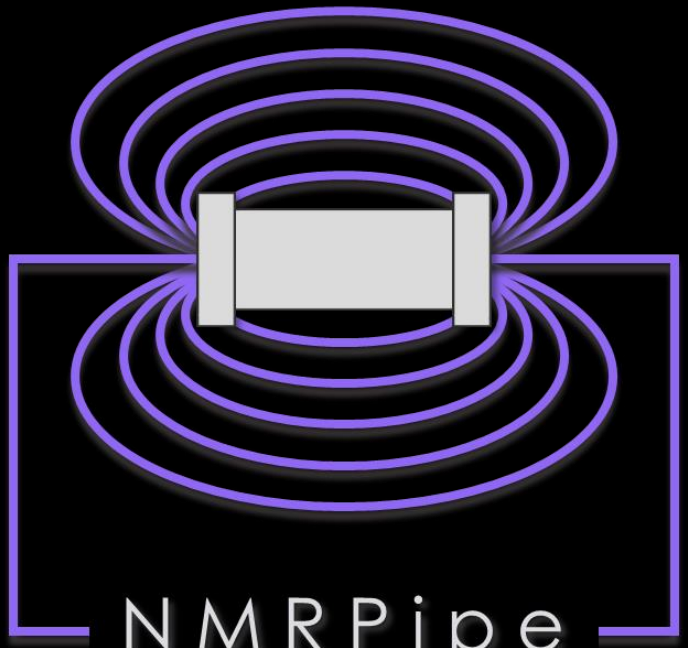
HN
8.929 ppm

N
122.2 ppm

HN
9.138 ppm

N
121.9 ppm

NMRPipe is Provided by the National Institute of Standards and Technology in Partnership with the National Institutes of Health



www.nmrpipe.com

NIST
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NMRPipe: a multidimensional spectral processing system based on UNIX pipes. Frank Delaglio, Stephan Grzesiek, Geerten. W. Vuister, Guang Zhu, John Pfeifer, and Ad Bax, *J. Biomol. NMR*. **6**, 277-293 (1995).

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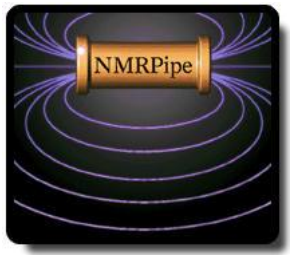

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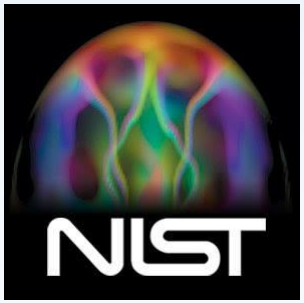

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