3D SIFT processing

The user's manual

Developed by:
Yoh Matsuki, Osaka University
Judith Herzfeld, Brandeis University

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0. Preparation using nmrPipe

“SIFT in Matlab” program reads NMR data in the nmrPipe format. So, the first thing you do is as usual the conversion of the data. For Bruker data, for example, the script will look like:

```
#!/bin/csh
bruk2pipe -in ./ ser \
  -bad 0.0 -noaswap -DMX -decim 24 -dspfvs 12 -grpdly 0  \
  -xN 2800          -yN 4           -zN 608          \
  -xT 1400          -yT 2           -zT 304          \
  -xMODE Complex    -yMODE real     -zMODE real      \
  -xSW 62500.0      -ySW 3703.7     -zSW 3703.7      \
  -xOBS 126.188     -yOBS 50.851    -zOBS 126.188    \
  -xCAR 177.5       -yCAR 50.851    -zCAR 177.5      \
  -xLAB 13C         -yLAB 15N       -zLAB 13C        \
  -ndim 3           -aq2D States                     \
| pipe2xyz -x -out ./fid/test%03d.fid -verb -ov -to 0
```

Make sure that –zN is set to the total number of NUS points collected in the F1-F2 plane, 608 here. The number of NUS points is the number of lines in the NUS schedule file you used in the experiment. The y dimension has only 4 points corresponding to the quadrature modulations for two indirect dimensions, coscos, sincos, cossin, and sinsin. The acquisition mode for y and z dimensions are set to “real” to treat each vector individually, rather than a complex pair.

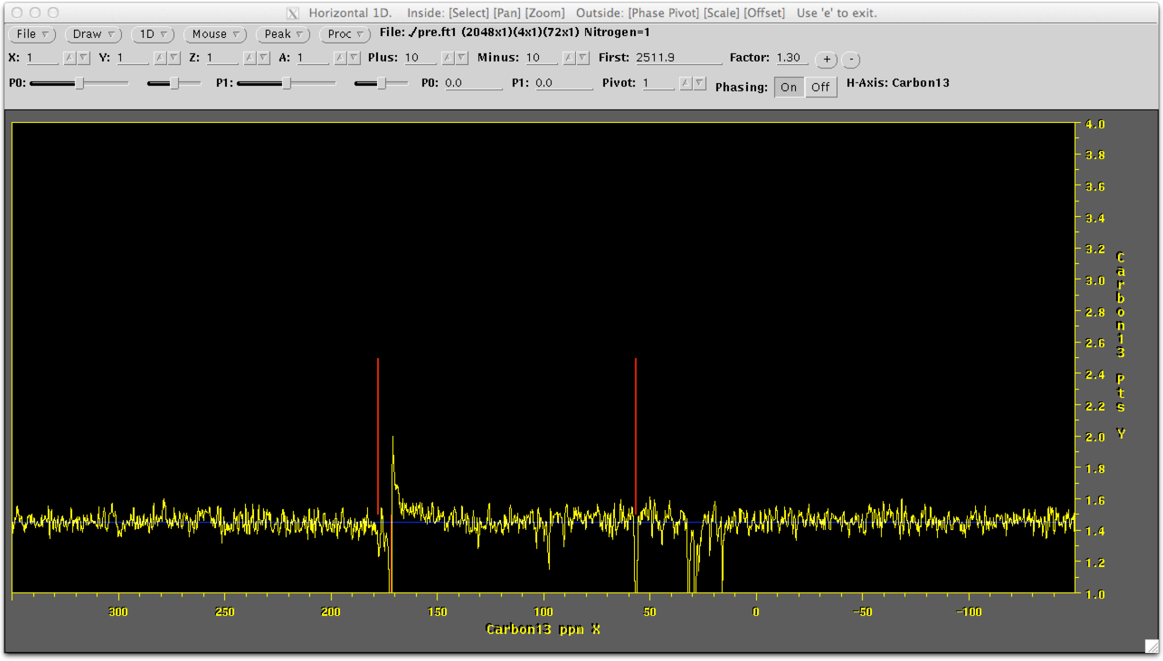
Next, determine the phase in the direct acquisition dimension. Use a script similar to this:

```
nmrPipe -in ./fid/test001.fid                      \
#| nmrPipe -fn SOL                                 \
| nmrPipe -fn ZF -auto -size 2048                  \
| nmrPipe -fn FT                                   \
| nmrPipe -fn PS -p0 -0 -p1 0 -di                  \
#| nmrPipe -fn POLY -auto -ord 0                    \
#| nmrPipe -fn EXT -x1 6.5ppm -xn 10ppm -sw        \
| nmrPipe -out ./test001.ft1 -ov
```

The phase is still set to all zero. Then, draw the processed data:

```
nmrDraw ./test001.ft1 &
```

Issue an nmrPipe shortcut “SHIFT+T” and click the “Draw” button to plot the 2D area with higher contour levels. This helps to see the slices more clearly. Hit “H” to enable the horizontal slice mode, and see the 1D slice in the first row by pointing the lowest part of the spectral area, or setting “Y:” equals to 1 in the tool area of nmrDraw. Then you will see something like this:



Horizontal 1D. Inside: [Select] [Pan] [Zoom] Outside: [Phase Pivot] [Scale] [Offset] Use 'e' to exit.

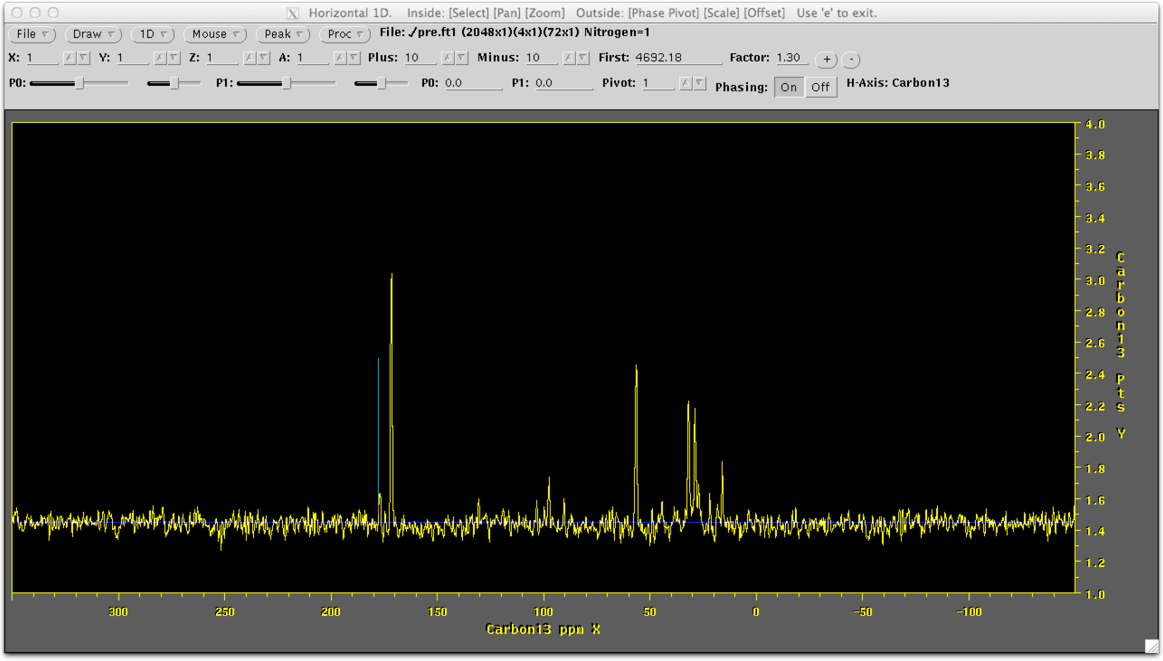
File: ./pre.ft1 (2048x1)(4x1)(72x1) Nitrogen=1

X: 1 Y: 1 Z: 1 A: 1 Plus: 10 Minus: 10 First: 2511.9 Factor: 1.30

P0: 0.0 P1: 0.0 Pivot: 1 Phasing: On Off H-Axis: Carbon13

Looking at the 1D slice, phase it as usual. It is important to take note of the phase correction, `[ph0,ph1]=[-4.7, 29.0]` found here, for later use.

Now set the phase values, and execute the script again. Check if the data is correctly phased:



Horizontal 1D. Inside: [Select] [Pan] [Zoom] Outside: [Phase Pivot] [Scale] [Offset] Use 'e' to exit.

File: ./pre.ft1 (2048x1)(4x1)(72x1) Nitrogen=1

X: 1 Y: 1 Z: 1 A: 1 Plus: 10 Minus: 10 First: 4692.18 Factor: 1.30

P0: 0.0 P1: 0.0 Pivot: 1 Phasing: On Off H-Axis: Carbon13

If the data has been digitally filtered, the number of points in the x dimension at this stage is changed from the one you collected in the experiment. Confirm the actual number of x points for the later use, using the `showhdr` commands:

```
showhdr ./fid/test001.fid
```

To complete the preparation in nmrPipe, concatenate the set of 2D planes into a single file as:

```
xyz2pipe -in ./fid/pre%03d.fid -x > pre.fid
```

1. SIFT processing in Matlab

To start SIFT processing in Matlab, download the 3D SIFT archive from the website (http://people.brandeis.edu/~herzfeld/SIFT/), and unpack it. Do not mix the scripts with older versions. The archive includes the run script `sift3d_v2_4.m`, a folder for the SIFT subroutines (`SIFT_suite_3D`), and `example` folder involving demo data (`mlf_3d_ncocx.bruker`).

A stepwise instruction for SIFT processing in Matlab is provided directly in the Matlab run script, but here are some tips and recommendations:

Section 1-2 ________________________________

The NUS table is a text file that consists of two columns of integers starting from 1, rather than 0, specifying recorded NUS points in the F1 and F2 dimension, respectively:

```
NUStab =

     1     1
    64    32
    29     8
     8    26
     1     3
    54    18
     9    17
     2     6
    …
```

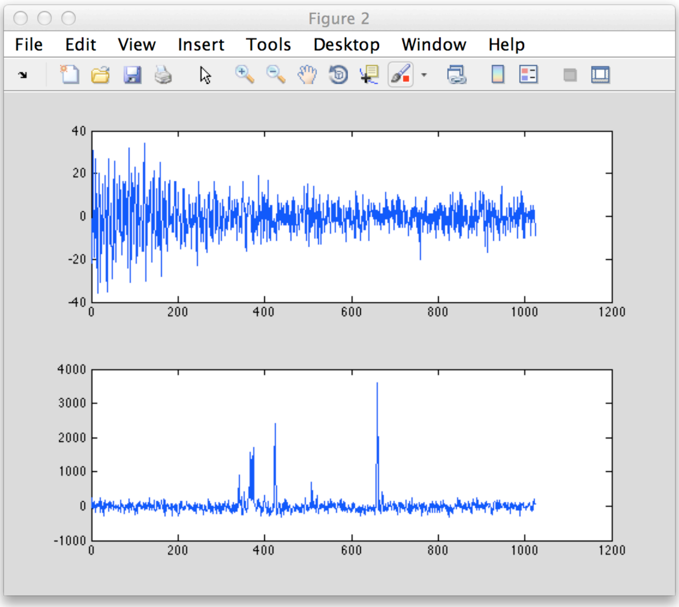
Section 1-3 ________________________________

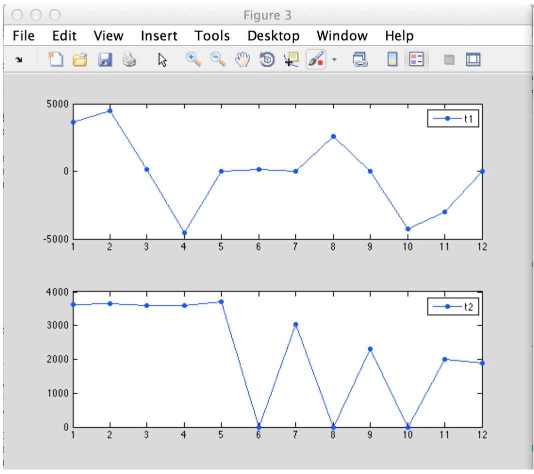
The number of grids in the indirect dimensions (`nt1, nt2`) refers to the number of full grids after the SIFT reconstruction. The number of points in the direct acquisition dimension `nt3` should be the actual number of points after the treatment of the digitally filtered data. The number of points after zero filling (`nzf1, nzf2, nzf3`) is normally set to the power of two.

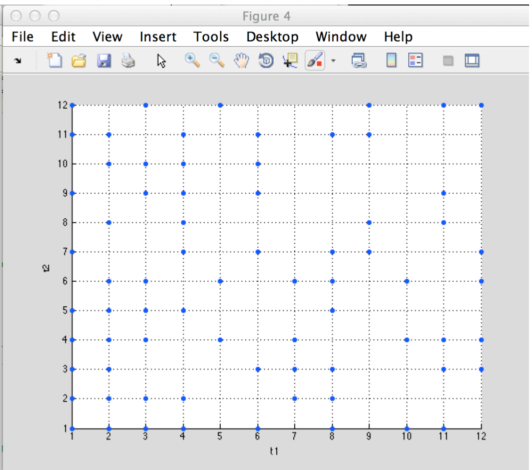
The phase for the direct acquisition dimension, `ph3`, is the set of values found during the nmPipe processing above.

Section 2-2 ________________________________

Executing this section will plot the initial FID, and its transform. Also shown is the initial interferogram along t1 and t2, and the scatter plot of your NUS schedule. Confirm that the data look descent, and is properly reshuffled according to the NUS schedule, i.e. the data have zeros only at the samples skipped recording. The “data cursor” tool in the tool bar of each figure will be useful here. Do not worry about the axis unit of the spectrum and its direction at this point.





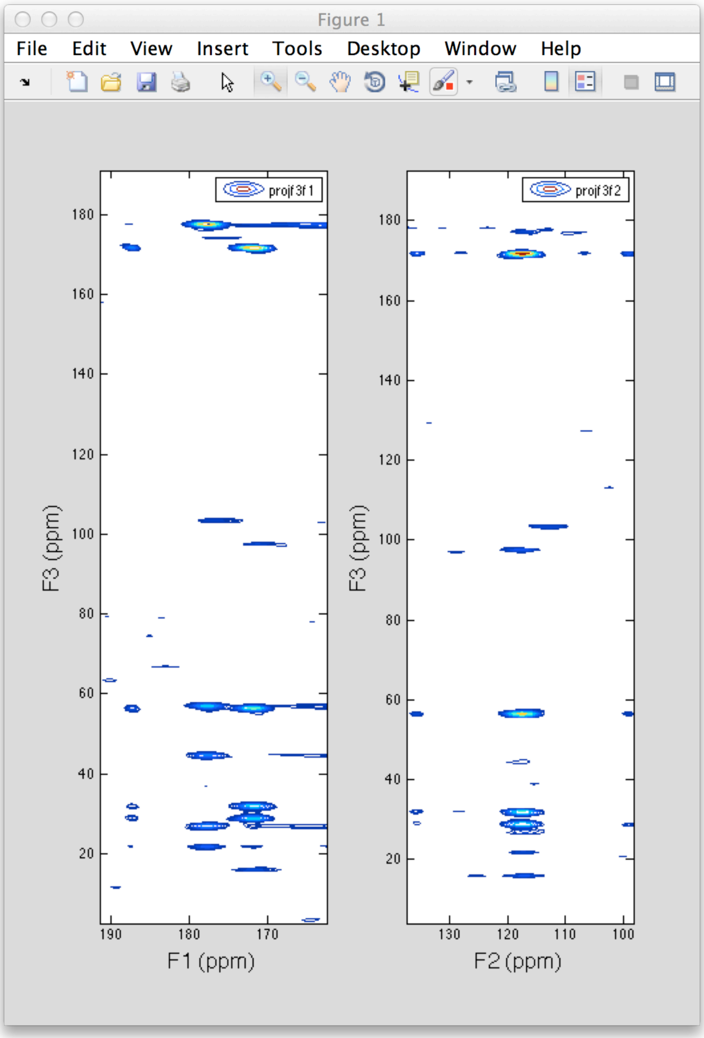


Section 2-3 ____

You can skip this section in the beginning. If you see the axis swap or inversion of the spectrum in the later part of the processing, come back to this section and change the flag.

Section 3-1 ____

Upon execution of this section, a 2D contour plot of F3-F1 and F3-F2 projections such as below will pop up that you can zoom in/out and drag using the tools in the top of the figure; use it to scout for the bright spectral regions in the following section.



Section 3-2 ________________________________

If you have run the NUS experiment with a slight oversampling as demonstrated in Y. Matsuki et al. *JACS* **131**, 4648- (2009) and *Angew. Chem. Int. Ed.* **49**, 9215- (2010), it is easy to find spectral limits by looking at the 2D projections. You could also rely on the BMRB statistics for the expected bright region in your spectrum. For example, a single bright region covering all peaks in a NCoCx type spectrum, the spectral limits in ppm would be:

```
brightLimits_F1=[102 135]; (15N dimension)
brightLimits_F2=[170 183]; (13Co dimension)
brightLimits_F3=[0   200]; (13Co + 13Cx dimension)
```

To define more regions, just add another row in all `brightLimits_F1`, `brightLimits_F2` and `bright_Limits_F3` as:

```
brightLimits_F1=[102 125;
                 115 135];
brightLimits_F2=[170 183;
                 170 183];
brightLimits_F3=[0   100;
                 120 200];
```

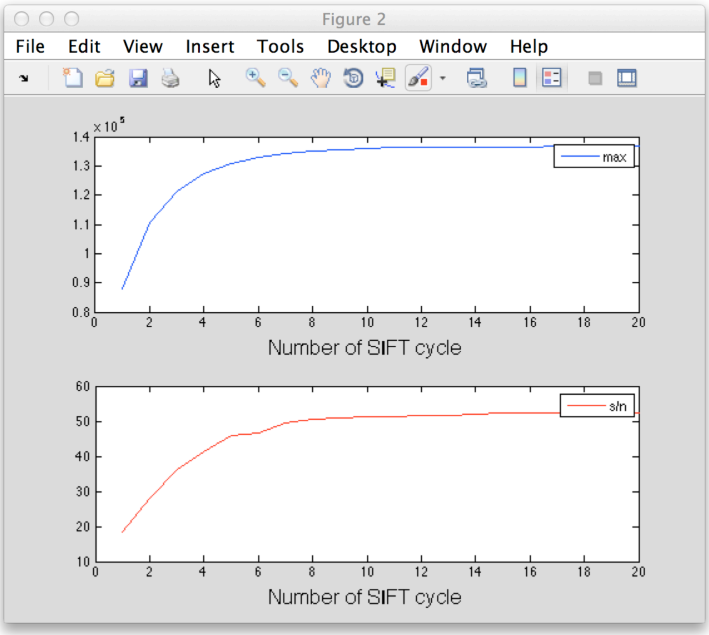
Each corresponding rows define together a single 3D bright region. There is no problem if two 3D bright regions partly overlap to each other as in this example. You can define indefinite number of bright regions.

Keeping the bright region in the direct acquisition dimension as small as possible will help accelerating the SIFT processing. Also important is to exclude solvent peak from the bright region since the tallest peak in the bright area will be used to keep track of the S/N ratio during the SIFT cycle.

Section 4-1

A recommended protocol is to do an exploratory SIFT processing with relatively large number of cycles, say cyc=20, look at how does the S/N ratio improve along the cycle. Then re-run the final SIFT processing with the best number of cycle. With cyc=20, the whole processing took only a couple of minutes on my Mac Book Pro.

Once the exploratory run is completed, a figure showing the peak intensity, and S/N ratio along the SIFT cycle will pop up. The shortest cycle that makes the S/N ratio leveled off will usually give the best result, i.e., cyc=10 is good enough.



Section 5-1

SIFT process takes data in the time domain, and outputs the SIFTed data in the time domain. Executing this section will output the data into Bruker format for subsequent standard 3D NMR processing.

Section 5-4

Data processed in Matlab can be plotted in Matlab. The run script as is provided allows you to plot the following spectra:

a. 2D F3-F1 and F3-F2 projections

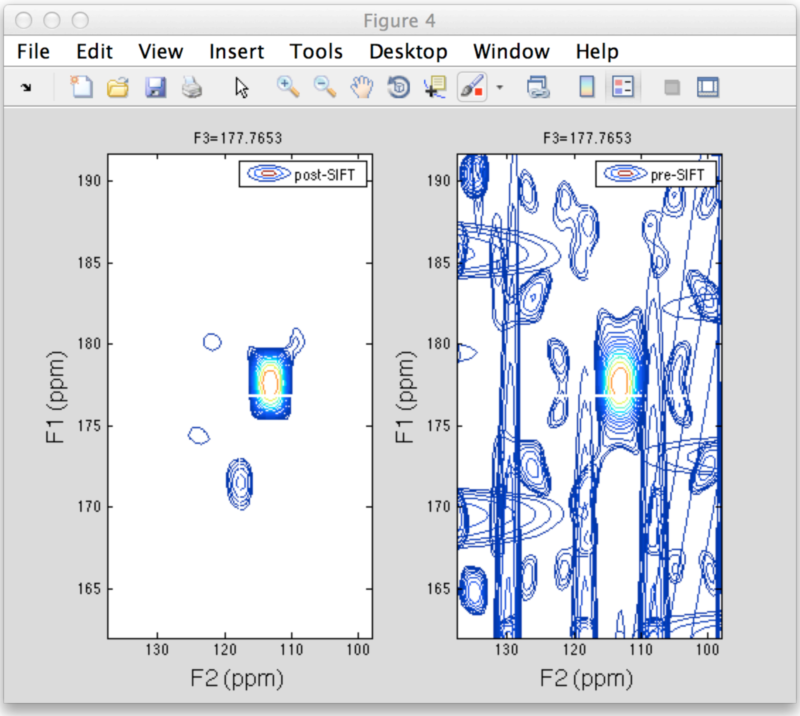
b. Comparison of pre and post SIFT spectra in 2D F1/F2 planes taken at the tallest cross peak

c. Comparison of pre and post SIFT spectra in 2D F1/F2 planes taken at a user-defined F3 position (specified in ppm)

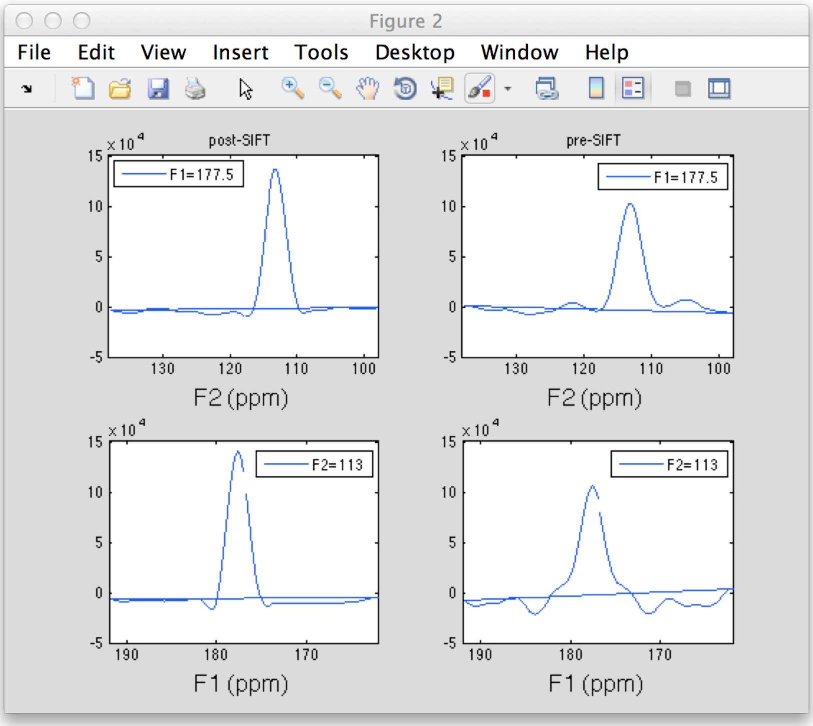
d. 2D F3/F2 plane taken at a user-defined F1 (ppm) position

e. 2D F3/F1 plane taken at a user-defined F2 (ppm) position
f. 1D slices in the above 2D planes

Here are example plots:



for Spectra generated in “c”.



and for 1D slices taken at the indicated positions, generated in “f”.

2. Processing of post-SIFT data

The section 5-1 outputs post-SIFT data in the Bruker format. Convert it to the nmrPipe format using a script similar to the following. Now, you should use the number of full grids for `-yT and –zT`, and the acquisition mode all "Complex".

```
bruk2pipe -in post.bruker \
  -xN 2800          -yN 128          -zN 64           \
  -xT 1400          -yT 64           -zT 32           \
  -xMODE Complex    -yMODE Complex   -zMODE Complex   \
  -xSW 62500.0      -ySW 3703.7      -zSW 3703.7      \
  -xOBS 126.188     -yOBS 50.851     -zOBS 126.188    \
  -xCAR 177.5       -yCAR 50.851     -zCAR 177.5      \
  -xLAB 13C         -yLAB 15N        -zLAB 13C        \
  -ndim 3           -aq2D States                      \
| pipe2xyz -x -out ./fid/post%03d.fid -verb -ov -to 0
```

Subsequent nmrPipe processing will just be the conventional 3D processing: phasing in the xy plane, phasing in the xz plane, and followed by full 3D processing. Any standard procedures can be performed, including the linear back/forward prediction, windowing, zero-filling, FT/iFT, baseline correction etc.