

NmrPipe and nmrDraw: Processing and visualizing 3D data.

See 'Introduction to nmrPipe' for basic program description, and previous section on processing 2D data.

NMRPipe is designed to process 3D and 4D data; nmrDraw can visualize 2D planes. Although the author (Frank Delaglio) has provide tools to analyze 3D data, other visualization programs (see NmrView labs coming up) are typically easier to use for multiple datasets.

In todays lab we will process a 3D dataset and look at the data.

A 3D dataset 'ghnco3d.fid' has been provided. This is a gradient HNCO experiment collected on a Varian Inova 800 Mhz spectrometer equipped with a triply tuned (H1, N15 and C13) probe. The data will have three axes, with 'xy' being the H1- N15 planes, and 'xz' being the H1-C13 planes. The H1-N15 planes give amide proton to nitrogen correlation, as in a typical HSQC. The H1-C13 planes give the i-amide proton to i-1 carbonyl correlation. In combination with other experiments, these data can be used to link peptide residues, but typically they provide a 'carbonyl-resolved' H1,N15-HSQC, in addition to giving the carbonyl assignments.

3D processing uses input/output functions that differ from 2D, but the concept of unix pipes and the other processing functions are the same.

The input function is 'xyz2pipe' and the output function is 'pipe2xyz'. These read and write one-dimensional vectors from data that has been converted using the appropriate conversion program (e.g. we use 'varian'). The initial Varian 3D data is converted into a series of 2D planes, numbered for example as test001.fid , test002.fid, etc. These 2D planes are then then sequentially read by the xyz2pipe function, which then feeds each vector into the processing pipe, just as we did with a regular 2D dataset. The processed vectors are then fed back into new 2D planes with pipe2xyz. The third dimension is done in the same way, only now specifying a new axis.

For more details type 'man xyz2pipe' to get the manual pages for these functions.

1. Convert the data from Varian to NMRPipe format.

1.1 Follow the directions in the previous lab notes. (i.e. Type 'varian' from within the ghnco3d.fid directory, read in parameters, etc.)

1.2 Edit the 'zMODE' parameter to be 'Rance-Kay', as you did with the 2D HSQC dataset.

1.3 Examine the fid.com file that is created. Note the number of dimensions, and hence columns is 3; the number of points (1024 X 32 X 16 complex points) for each separate dimension is now very small relative to a typical 1D.

1.4 Change directory to the new 'data' directory inside ghnco3d.fid. The original Varian single binary file 'fid' (~16Mb) has now become a series of 2D files labeled test001.fid, test002.fid, ... up to test032.fid. Each of these planes will be 1024 X 32 complex points, and there are now 32 of them (16 complex points in z or N15 dimension), each about ~0.5Mb. It is clear that you need plenty of disk space to process these.

2. Processing the 3D data with macros.

2.1 Take a look at a single HC plane or HN plane.

In most cases, one can collect the data with known initial t1 and t2 delays in order to predict the zero and first order phase corrections in the indirect dimensions. However, you can always process a plane to ensure the phasing is correct.

2.1.1 – Change directory to ghnco3d.fid, if you are not already there, and start nmrDraw.

2.1.2 Open the macro edit window from the File menu.

2.1.3 Choose Process 2D; XY plane of 3D. Edit all the phase values to be zero.

2.1.4 In the first line of the nmrPipe section, change nmrPipe -in **fid**/test001.fid to nmrPipe -in **data**/test001.fid. This reflects the default subdirectory 'data' which contains the converted data.

2.1.5 Change the Macro name from nmrproc.com to nmrproc_xy.com.

2.1.6 Save (it should look like below) and execute.

```
#!/bin/csh
#
# Processing of a 2D Plane from 3D States HN-Detected data:
# "POLY -time" subtracts solvent by polynomial fitting.
# "EXT -left" extracts left half of HN dimension.
```

```
nmrPipe -in data/test001.fid \
| nmrPipe -fn POLY -time          \
| 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 0.0 -p1 0.0 -di \
| nmrPipe -fn EXT -left -sw -verb \
| 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 0.0 -p1 0.0 -di \
-verb -ov -out test.ft2
```

There are a few additional functions from the previous 2D examples. Here POLY provides a default polynomial subtraction of the time domain data which serves to reduce the residual water signal. Here EXT extracts the left half of the spectrum. This is common with these data, since they were collected with the water at the center (~4.75ppm) and we only look at the amide proton region (~6-11ppm). The data are thus reduced in size by half.

2.1.7 Display the output file 'test.ft2'. You may have to lower the threshold to see any peaks (clicking on the '-' button). Note that the residual water signal will be at the right of the spectrum. The axes should be roughly correct.

2.1.8 Type 'h' to get a horizontal line and select some peaks. If you don't see much, increase the height of the trace, by putting the cursor outside of the spectrum in the border region, hold down the middle button and slide up.

Turn phasing on and adjust the P0 phase; note the value and edit your nmrproc_xy.com macro to include that value in the first dimension phase correction.

Type 'e' to exit the horizontal trace mode.

2.1.9 Type 'v' to get a vertical trace, and do the same for the y direction. This will be harder to see because of the poor digital resolution. Only make a change if it is obvious.

2.1.10 Repeat 2.1.2 to 2.1.7, except now choose the XZ plane. Change the name of the macro to nmrproc_xz.com, and change the output file name from test.ft2 to testxz.ft2.

You can edit the phase values to be zero, except the P0 value you determined already.

After processing and looking at the plane, you can also repeat 2.1.9 to see if any phase adjustment is necessary for the z direction.

These two macros can let you try different processing functions, such as window functions and linear prediction, before applying it to a possibly lengthy 3D calculation. In this example, the data have high signal to noise, so it is easy to see something in one plane.

2.2 Creating the 3D macro.

2.2.1 Now go back to 'macro edit' ,clear any existing macros.

2.2.2 Select 3D ; 3D HN in place

This does all the processing directly into a final set of 2D planes, with no permanent formation of an intermediate dataset. The alternative provide is '3D HN detected' which, if you examine it, has an intermediate dataset which remains on your disk. This is not necessary, but can allow for different processing schemes. The '3D HN with LP' is more complicated, and includes linear prediction in the indirect y and z dimensions. Ultimately we would want to do this, but it is very slow computationally and would expand the space requirements.

2.2.3 Edit the macro to replace the **fid/test%03d.fid** with **data/test%03.fid** as before; if this is not done, you will not be able to read the data.

Edit the phase values – at least the first dimension -p0 should have some value. All the others can be zero if no major distortions were seen with the 2D planes.

Comment out the ZF zerofill lines – this is only to conserve space and time. If possible, try with the lines left intact.

2.2.4 Save and execute the macro.

```
#!/bin/csh
```

```
#
```

```
# 3D States HN-Detected Processing, in-place version:
```

```
xyz2pipe -in data/test%03d.fid -x -verb          \  
| nmrPipe -fn SOL                               \  
| nmrPipe -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5 \  
#| nmrPipe -fn ZF                               \  
| nmrPipe -fn FT                               \  
| nmrPipe -fn PS -p0 -22.0 -p1 0.0 -di         \  
| nmrPipe -fn EXT -left -sw                    \  
| nmrPipe -fn TP                               \  
| nmrPipe -fn SP -off 0.5 -end 0.98 -pow 1 -c 1.0 \  
#| nmrPipe -fn ZF                               \  
| nmrPipe -fn FT                               \  
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di          \  
| pipe2xyz -out ft/test%03d.ft3 -y -ov
```

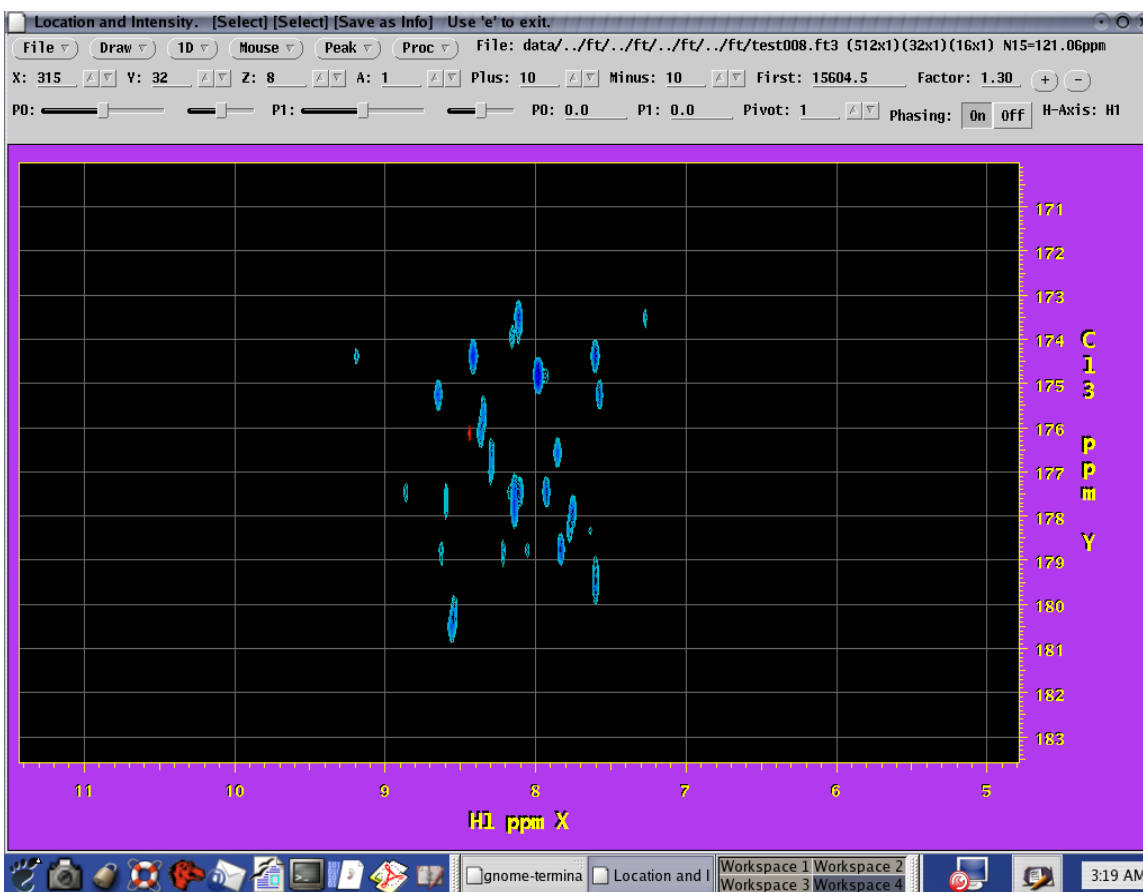
```
xyz2pipe -in ft/test%03d.ft3 -z -verb          \  
| nmrPipe -fn SP -off 0.5 -end 0.98 -pow 1 -c 0.5 \  
#| nmrPipe -fn ZF                               \  
| nmrPipe -fn FT                               \  
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di         \  
| pipe2xyz -out ft/test%03d.ft3 -z -ov -inPlace
```

3. Viewing the 3D dataset.

3.1 Note that the processed data now resides in a new subdirectory in ghnco3d.fid called 'ft', as 2D planes from test001.ft3 to test016.ft3. When you open the select file window from the File menu in nmrDraw, you must then choose the ft directory. In the center panel will be displayed 'test%03d.ft3 1 16' ; double click on this to choose the full set of planes for viewing. Click Done.

3.2 You can now choose the planes by clicking the Z: arrows up or down. It should read 1, and so if you click up, you will walk through the planes until you get to 16. If you don't see anything, get to plane 8 and adjust the contour level.

3.3 Notice that in addition to the Z number of the plane, the value in ppm of the nitrogen frequency is also indicated. This would allow you to coordinate each plane with the appropriate trace through a higher resolution 2D H1,N15-HSQC for example.



2D amide H-CO plane #8 from processed ghnco3d dataset, corresponding to nitrogen frequency of 121 ppm.