

NmrPipe and nmrDraw: Processing and visualizing 2D data.

See 'Introduction to nmrPipe' for basic program description.

NmrPipe is really designed for multidimensional NMR processing; i.e. Executing repetitive operations on many FIDs. As described in the introductory laboratory for 1D data, the nD data is first converted from the spectrometer format to an nmrPipe format, then a processing script is created to fourier transform the dataset. Visualization can be done immediately with nmrDraw, or the processed data can be transported to other programs (NMRView and Sparky for example).

We will examine two different 2D datasets and their conversion and processing scripts. Next week we will continue with more data analysis tools, and examine a 3D dataset conversion and processing script.

Dataset #1. 2D Absolute-value or magnitude-mode COSY; 'cosy.fid'

Dataset #2. 2D phase-sensitive 'echo-antiecho' or 'Rance-Kay' C13-HSQC; 'hsqc.fid'

Dataset #3. 2D phase-sensitive 'States' proton-proton TOCSY; 'tocsy.fid'

Copy the datasets into your nmrPipe directory (see instructions in class).

Dataset 1. Absolute-value or magnitude-mode COSY.

Name: cosy.fid

The dataset collected on a Varian used the 'gCOSY' experiment, which is a gradient version of the traditional phase-cycled COSY, however the data is treated in the same way. This is not a phase-sensitive experiment, and so the data is usually displayed in absolute value mode after application of sinebell window functions to improve the poor lineshape.

1.1 Convert dataset using 'varian' tcl script

- cd your_nmrpipe_directory/cosy.fid, type 'varian'
- click 'read parameters' ; now have x (t2 direct dimension) and y-axis (t1 or indirect dimension).

- **Some of these values are incorrect and must be changed.**

- Change these y-axis parameters, using the pulldown lists:

Total Points R+I: 256

Acquisition Mode: Real

Observe Freq Mhz: 499.641

- this will also change the center position and the axis label for the y-axis.

NMRPipe Conversion Utility Version 97.027.12.56

Spectrometer Input: /fid Input Protocol: Varian

Output Template: /test.fid Output Protocol: NMRPipe

Output Script: fid.com Dimension Count: N

Temperature (K): 298.000

	x-axis	y-axis
Total Points R+I:	2048	256
Valid Points:	1024	256
Acquisition Mode:	Complex	Real
Spectral Width Hz:	3869.969	3869.969
Observe Freq Mhz:	499.641	499.6412836
Center Position PPM:	4.773	4.773
Axis Label:	H1	H1

Read Parameters Save Script Execute Script Hide Script Clear Script Update Script Quit Help

- You also need to edit the -aq2D parameter, and change it to Magnitude.

```
#!/bin/csh
var2pipe -in ./fid -noaswap \
  -xN 2048 -yN 512 \
  -xT 1024 -yT 256 \
  -xMODE Complex -yMODE Complex \
  -xSW 3869.969 -ySW 3869.969 \
  -xOBS 499.641 -yOBS 125.646 \
  -xCAR 4.773 -yCAR 97.870 \
  -xLAB H1 -yLAB C13 \
  -ndim 2 -aq2D Magnitude \
  -out ./test.fid -verb -ov
sleep 5
```

- Save the script, execute the script, then quit.

Lets examine the 'fid.com' script that was generated. If you are not in your_nmrpipe_directory/cosy.fid directory, go there now and look at the script using an editor, or type 'more fid.com'.

```
-----  
#!/bin/csh  
  
var2pipe -in ./fid -noaswap \  
-xN      2048 -yN      256 \  
-xT      1024 -yT      256 \  
-xMODE    Complex -yMODE    Real \  
-xSW      3869.969 -ySW      3869.969 \  
-xOBS      499.641 -yOBS      499.641 \  
-xCAR      4.773 -yCAR      4.773 \  
-xLAB      H1 -yLAB      H1 \  
-ndim      2 -aq2D      Magnitude \  
-out ./test.fid -verb -ov
```

```
sleep 5  
-----
```

We now have two dimensions and two axes, designated as x and y. The x dimension is the direct dimension and is always collected as complex data (quadrature detection). Thus the xMODE is complex, and the xN (real + imaginary points) parameter , i.e. the number of FIDs, is twice the xT (complex points) parameter, i.e. the number of sampled dwell times.

The indirect y-axis is being read as 'Real' data. Here the number of FIDs, yN, is equal to the number of increments, yT. The data is also being processed as magnitude mode data (-aq2D parameter). This is particular to magnitude COSY data only.

The last line writes the output to 'test.fid' (-ov = overwrites old data) with some screen messages (-verb).

Note that you can specify the ppm reference of the center of each axis (xCAR, yCAR) if this is known. These values can be changed later.

1.2 Process the 2D COSY

We will use the 'macro' writing tool of nmrDraw as discussed in the previous introduction lab.

- a) Change directory ('cd') to your nmrpipe-directory/cosy.fid directory.
- b) type nmrDraw & to bring up the program window (see introduction)
- c) pull down File menu to 'macro edit' or just type 'm' to get new window.
- d) pulldown 'Process 2D' menu and select 'Basic 2D'
- e) Edit it to look like this: (comments at right indicate changes, don't include them).

The MC function can be added from Transforms – Modulus;

The REV function can be added from Transforms – Reverse;

The TP function can be added from Transforms – Transpose.

Make sure there are no empty lines, or spaces after the backslash ' \ ' .

```
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0 \ ## change SP -off 0.0
| nmrPipe -fn ZF -auto \ ## standard zero fill
| nmrPipe -fn FT -auto \ ## complex ft
                        ## deleted line with PS (phase) function.
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0 \ ## change SP -off 0.0
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
                        ## deleted line with PS function.
| nmrPipe -fn MC \ ## added MC function; gives abs val display.
| nmrPipe -fn REV -di -verb \ ## added REVerse function to flip y-axis.
| nmrPipe -fn TP \ ## added TP, puts f2 axis back on x-axis
  -ov -out test.ft2
```

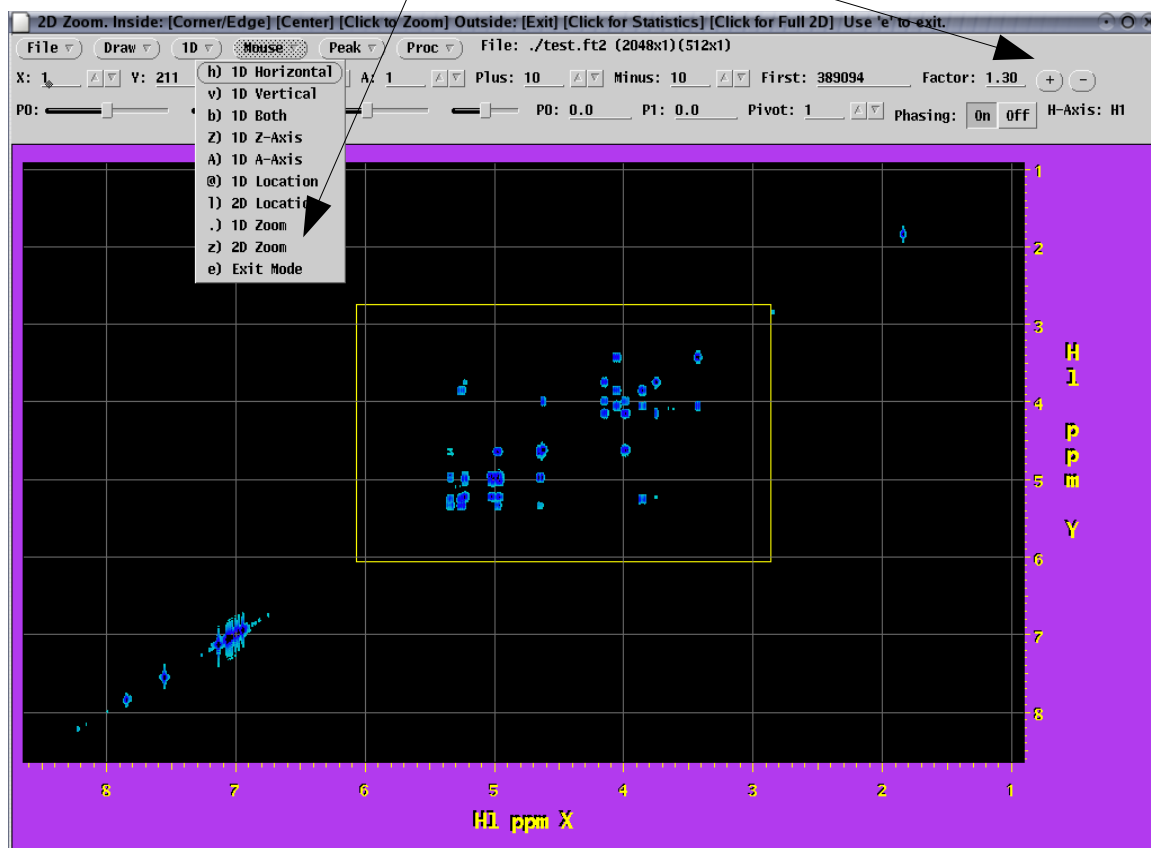
- f) Save, then Execute, then Done.

1.3 Displaying 2D COSY spectrum.

a) You should still be inside the cosy.fid directory. Pull down (right button) File menu and select file. Highlight 'test.ft2' and click 'Read/Draw', then Done.

b) Adjust vertical scale with +/- buttons. Increase or decrease, then redisplay by typing 'd' (or Draw menu -Contours)

c) Expand with 2D zoom ('z'), then right click.



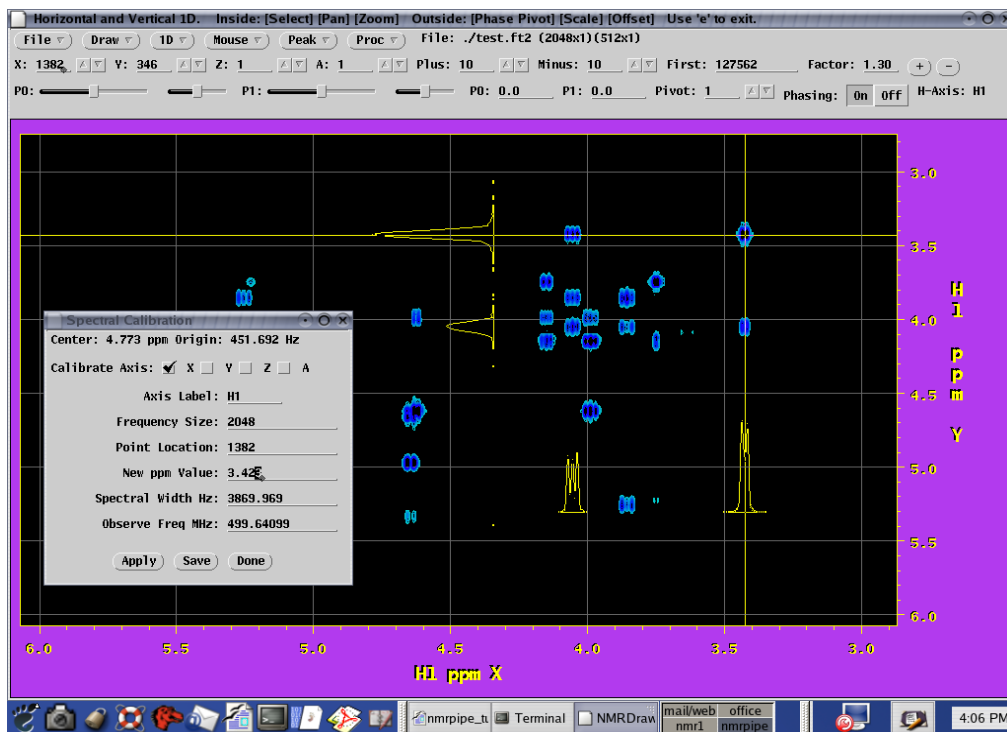
d) exit from the zoom mode with 'e' (or Mouse menu - Exit mode.)

e) you can return to the full display by typing 'f' (or Draw menu – 2D Full)

1.4 Using the 2D Display

1.4.1 Referencing

- Zoom into a region.
- Display crosshairs by typing 'b' (or Mouse menu -1D Both).
- Place on a peak and type 'C' ('shift c') (or File menu – Calibrate axis)



- choose axis, and change the 'New ppm Value'
- Click Apply; for permanent change, click Save.
- Repeat with other axis.

1.4.2 Getting peak positions.

- Zoom into a region of peaks. Exit zoom mode.
- Type 'l' (or Mouse menu- 2D Location). The holding down left button gives the location of the cursor in points and ppm, as well as the height of the peak at that position.

1.4.3 Plotting a specific region.

- Type 'L' (shift-l) to bring up a small window that allows entry of axis limits. The default units are points, but you can pull down the units list and select ppm. Enter the limits, e.g. 5.5 , 3.0 for both Horiz. and Vert., then 'Draw', and 'Done'.
- Type 'P' (or File menu-Hard copy plot) to bring up the plotting window. Execute will create a postscript plot file named nmrDraw.ps in the current directory. This can be sent to a printer or further manipulated in a drawing program.

2. Dataset #2: phase-sensitive HSQC.

This dataset is called 'hsqc.fid' and is a gradient HSQC experiment, where the frequency discrimination and phase-sensitive results are obtained by the 'echo-antiecho' or 'Rance-Kay' method of data collection.

For simplicity, quit the nmrDraw program, and we'll restart it later.

2.1 HSQC data Conversion.

The same protocol is used as for the COSY described above.

Change directory to hsqc.fid directory, and type 'varian'. The only change to the conversion script is to make the yMODE parameter Rance-Kay (or echo-antiecho) rather than complex. This mode invokes a shuffling of data, and the final converted data can now be processed with a standard 'States' phase-sensitive processing macro.

```
-----
#!/bin/csh
var2pipe -in ./fid -noaswap \
  -xN      1394 -yN      128 \
  -xT      697 -yT      64 \
  -xMODE    Complex -yMODE  Rance-Kay \  ## Note the change in yMODE
  -xSW     3869.969 -ySW     20100.503 \
  -xOBS     499.641 -yOBS     125.644 \
  -xCAR     4.773 -yCAR     82.861 \
  -xLAB     H1 -yLAB     C13 \
  -ndim     2 -aq2D     States \
  -out ./test.fid -verb -ov
sleep 5
-----
```

Note that the -yN parameter is twice the -yT parameter, indicating that we are collecting two datasets to allow for 'quadrature' in the indirect dimension. Also the y axis now correctly has the C13 (decoupler) parameters.

2.2 HSQC dataset processing

This processing macro is quite different than the COSY macro, so we will use the macro edit command and write it from scratch. Since it is phase-sensitive data, we will have to 'phase' the data.

- Start nmrDraw from the hsqc.fid directory.
- File menu-select file – read and draw the raw dataset, test.fid.
- Type 'h' (Mouse menu – 1D horizontal). Bring the horizontal line down to the bottom, so that it is showing FID #1 – the Y: point should be 1.
- Autoprocess the 1D (Proc menu).
- turn on phasing, and phase spectrum with P0. Note the value of PO (e.g. -136). Type 'e' to escape from the horizontal display.

If S/N is too low, ignore the phasing step. You can always adjust phasing later.

2.2 HSQC dataset processing cont'd.

f) Type 'm' to open macro editor, and select Basic 2D from the Process 2D menu.

g) edit the first PS function line so the value of -p0 is -136.

Also add lines at the end to REVERSE the y axis, then to transpose so H1 is on x-axis.

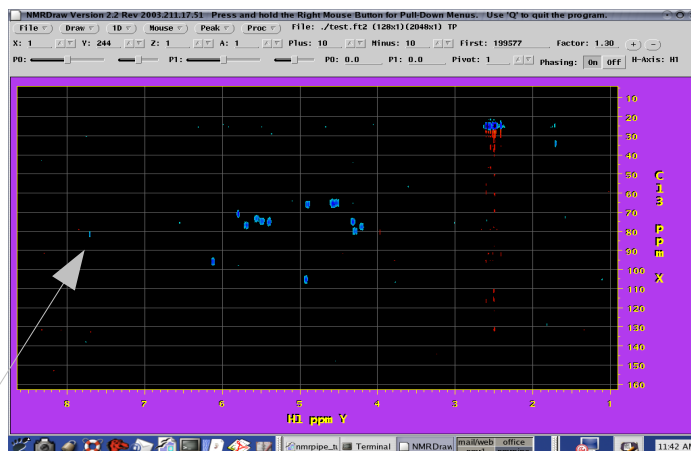
```
-----
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0 \ ## SP -off 0.5 is Cosine window.
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -136.00 -p1 0.00 -di -verb \ ## Change -p0 value
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 0.00 -p1 0.00 -di -verb \ ## see below
| nmrPipe -fn REV \ ## Add REV function
| nmrPipe -fn TP \ ## Add TP function
-ov -out test.ft2
-----
```

h) Save and Execute.

i) Display the spectrum by selecting test.ft2 . Note that the phase along the y-axis is wrong.

j) Zoom if necessary, and display the vertical cursor by typing 'v' (or Mouse menu-1D vertical) and place it over a peak. Make sure the phasing is on and adjust p0. Note the value (e.g. -94). Exit cursor mode by typing 'e'.

k) return to the macro editor and change the second PS function line so p0 is **94**, i.e. **negative of the recorded value** (due to TP and REV functions). Save and execute. Re-read and display test.ft2 – it should look like this:



Chloroform peak

2.2 HSQC dataset processing cont'd.

For nD data where the indirect dimension(s) is collected with few points, it is often advantageous to increase the number of points and digital resolution by 'Linear Prediction'. This calculation determines the frequency and decay rate of the peaks in an FID or interferogram, and extends them mathematically. It is very useful for spectra like 2D HSQC, where experimental time is needed to improve signal-to-noise, so the number of increments is limited.

a) Edit the nmrproc.com file, and add the Linear Prediction function in the second dimension.

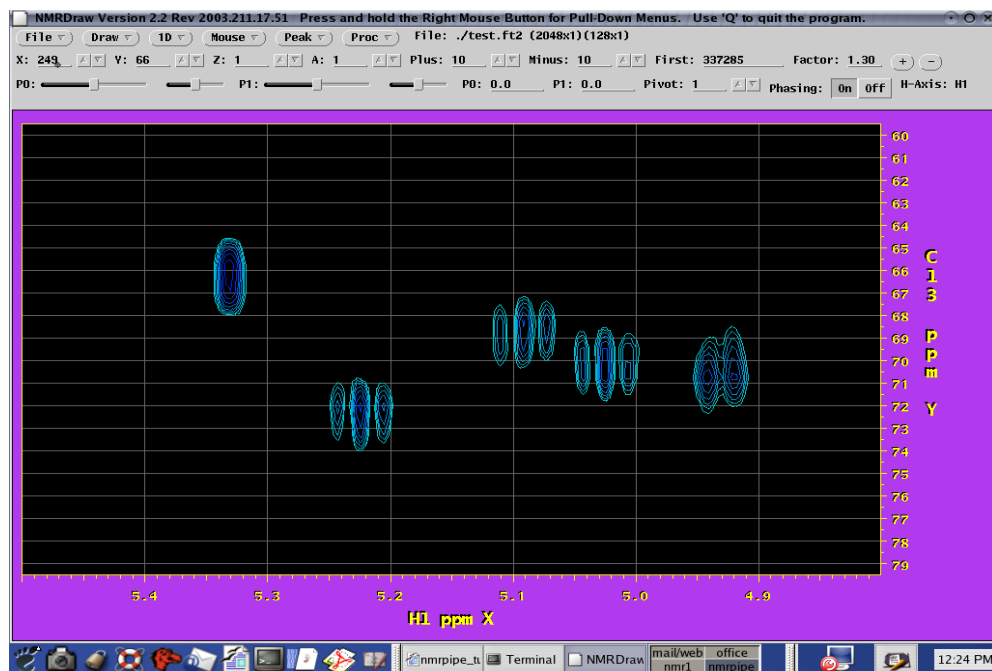
```
-----
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0 \
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 -136.00 -p1 0.00 -di -verb \
| nmrPipe -fn TP \
| nmrPipe -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0 \
| nmrPipe -fn LP -fb \          ## Add LP function here ##
| nmrPipe -fn ZF -auto \
| nmrPipe -fn FT -auto \
| nmrPipe -fn PS -p0 94.00 -p1 0.00 -di -verb \
| nmrPipe -fn REV \
| nmrPipe -fn TP \
  -ov -out test.ft2
-----
```

b) Rerun the new nmrproc.com macro and see the result – notice it takes a lot longer to process.

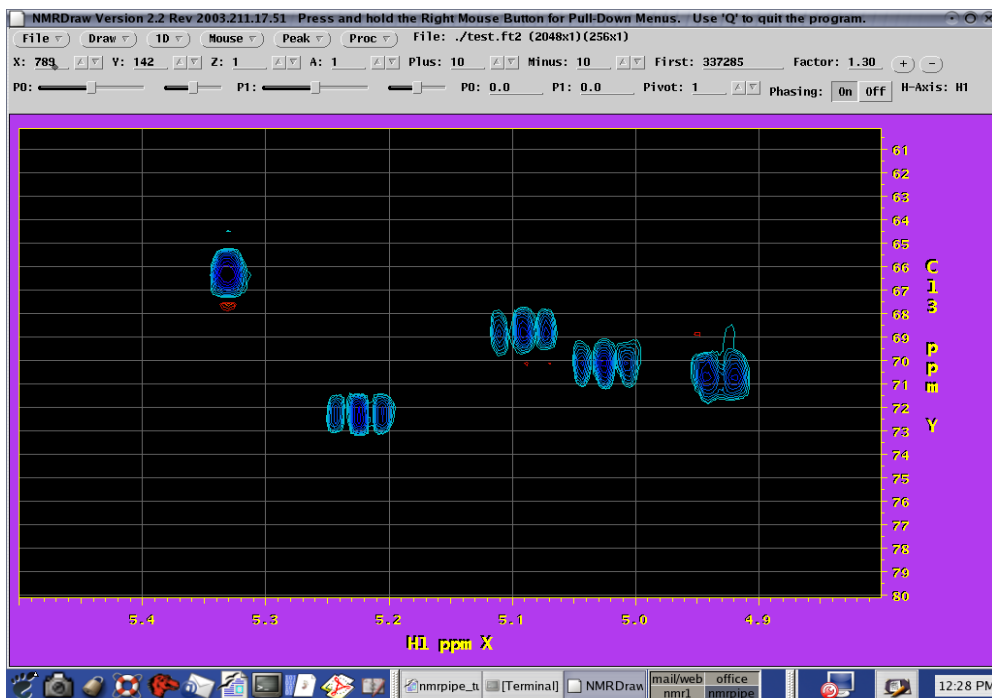
Note: There is a mistake in this example. The line with the 'LP' command needs to be placed BEFORE the line with the 'SP' command above it (between the 'TP' line and the 'SP' line):

TP
LP
SP

Without Linear Prediction.



With Linear Prediction.



2.3 HSQC dataset analysis

2.3.1 Referencing.

The chemical shift axes are not correct, since we used the default values in the conversion script. The peak at the left (see plot above) is chloroform and we can use it to reference the spectrum.

- a) zoom around the chloroform peak area by typing 'z' ,adjusting box dimensions, followed by right click. Type 'e' to exit zoom mode.
- a) type 'b' to get crosshairs (vertical and horizontal cursors), place on peak
- b) type 'C' to get calibration box, choose x, and enter 7.24, then Apply. Choose y, enter 77.0, Apply, then Save, then Done.
- c) exit crosshair mode with 'e', and type 'f' to get full spectrum.

The spectrum should now be referenced correctly, and any redisplay will give the updated values. However, the original data still retains the wrong values, and reprocessing will give the old values. To permanently correct these values, you must edit the fid.com file and change xCAR and yCAR. The spectrum is 2048 points along x and 128 points along y. Therefore, the ppm values at 1024 and 64 points for x and y respectively, will be the correct xCAR and yCAR values. Then rerun the fid.com script and the converted data will now reflect the new referencing values.

2.3.2 Automatic peak picking

- a) zoom around a few peaks. (e.g. 4.3 to 5.4, 63 to 77)
- b) Pull down Peak menu, select Peak Detection. This will bring up a box.
- c) Click on Clear, then Detect, then Done.

The spectrum should show number labels on each peak, and a text file called 'test.tab' is created in the current directory. You can examine this file, which contains all the information about peak positions, peak heights, and peak volumes.

Exercise: 2D phase-sensitive ('States') TOCSY dataset

Convert and process the 'tocsy.fid' dataset. It is a H1-H1 correlated, phase-sensitive experiment; that is collected with quadrature in f1 and the data is complex in both dimensions. You will have to edit the conversion macro so that x and y axes are both H1.

The processing macro will be a standard 2D macro; cosine-bell window functions are appropriate, and both dimensions will have to be phased. Linear prediction is not necessary.