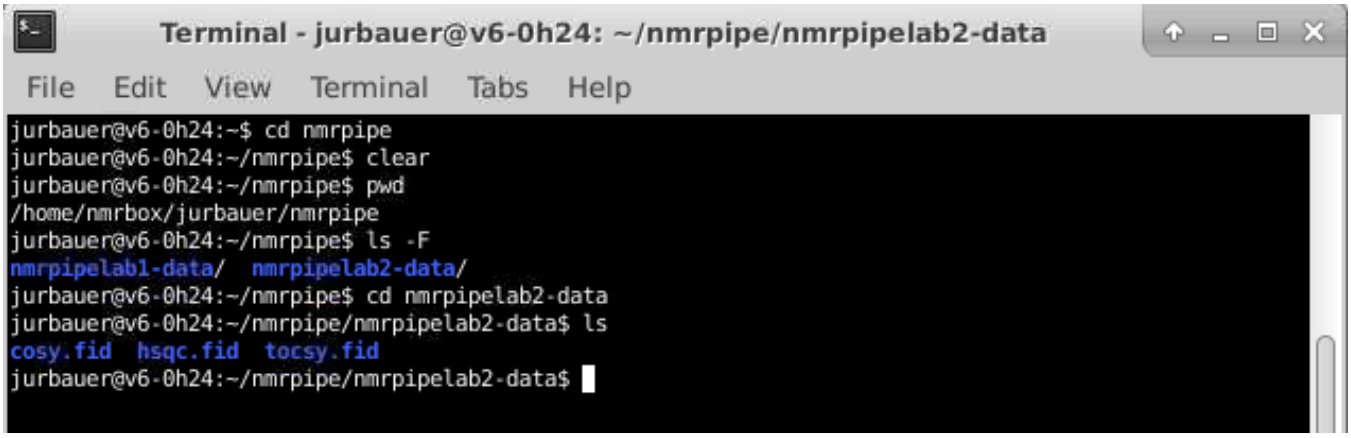
Introduction to Data Processing and Analysis using NMRPipe in NMRBox (Part 2)

This tutorial assumes that you have completed the 'Part 1' NMRPipe in NMRBox tutorial.

Thus:
- you have an NMRBox account
- you have the VNC Viewer installed on your computer
- you can log into your NMRBox account
- you know how to transfer files between your computer and NMRBox
- you have a directory structure on your NMRBox account that includes a folder/directory 'nmrpipe'
  /home/nmrbox/username/nmrpipe
- you have a subdirectory/folder in your 'nmrpipe' directory/folder named 'nmrpipelab1-data' (this folder holds the datasets that were used for the 'Part1' tutorial
  /home/nmrbox/username/nmrpipe/nmrpipelab1-data
- you have completed the 'Part 1' tutorial, so you are familiar with using NMRPipe/NMRDraw to convert, process, view and analyze 1D, and some 2D, data sets, including the use of macros for converting and processing NMR data

Before you begin, you must download the data for this lab ('data for part 2') from our course website. You need to follow the directions from the 'Part 1' tutorial to uncompress the data and transfer the data folder to your NMRBox account. You should put this folder in the 'nmrpipe' folder, along with the 'part 1' data. So, in the 'nmrpipe' folder/directory, there should be two subfolders/subdirectories, 'nmrpipelab1-data' and 'nmrpipelab2-data'. In the '**nmrpipelab2-data**' folder should be three subfolders, '**cosy.fid**', '**hsqc.fid**', and '**tocsy.fid**'.



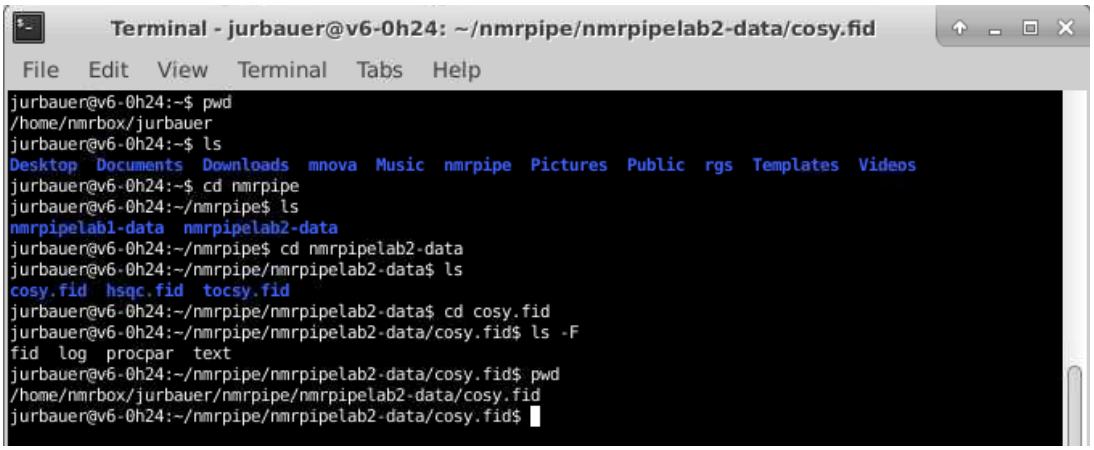
Finally, the "Demonstration Data" page at the NMRPipe website includes a very good introduction to NMRPipe about two pages down from the top ("NMRPipe: Introduction"), and the NIH still hosts two good tutorials. Here are the links: https://www.ibbr.umd.edu/nmrpipe/demo.html, https://spin.niddk.nih.gov/NMRPipe/doc1/, https://spin.niddk.nih.gov/NMRPipe/doc2/.

You should now be ready to begin the 'Part 2' tutorial.

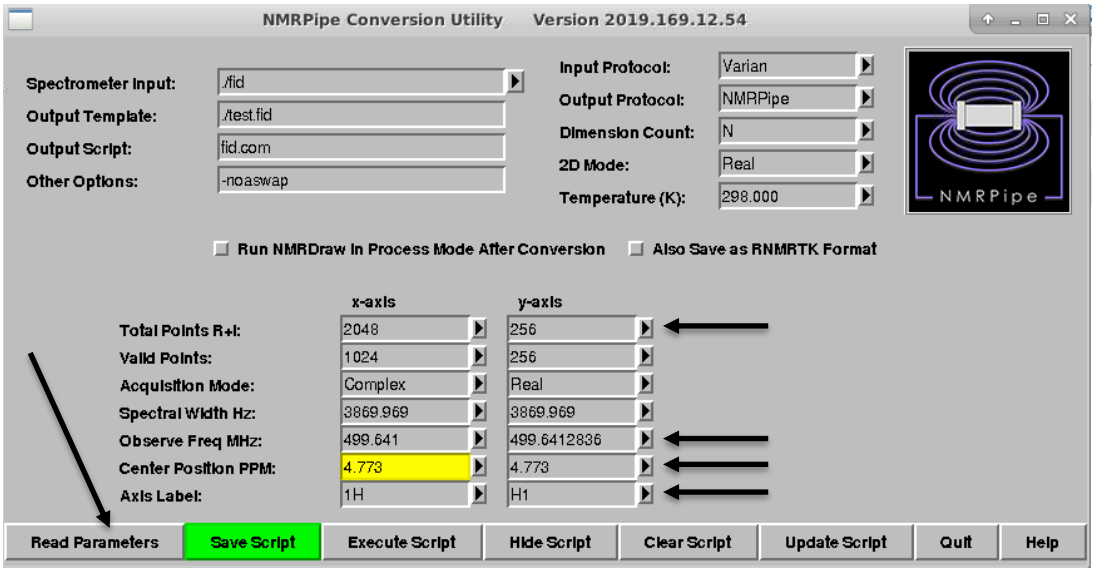
Converting the absolute-value or magnitude-mode COSY data to NMRPipe format:

The dataset was collected using a Varian spectrometer using the 'gCOSY' experiment, which is a gradient version of the traditional phase-cycled COSY experiment. This is not a phase-sensitive experiment, so the data are usually displayed in absolute value mode after application of a sinebell window (apodization) function to improve the poor lineshape.

Start NMRBox, open a 'terminal' window, and 'cd' into the 'cosy.fid' directory.

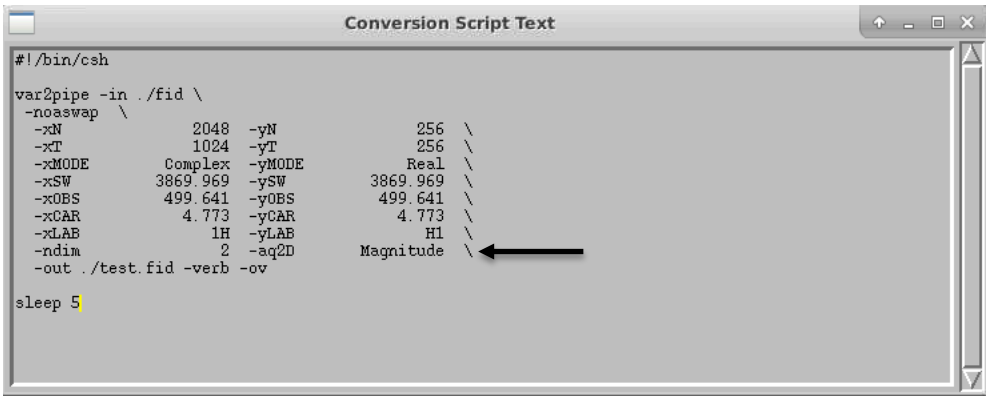


On the UNIX/LINUX command line, **type 'varian'** to open the 'NMRPipe Conversion Utility' window and the 'Conversion Script Text' window. Then **click the 'Read Parameters' button** to read the parameters for the cosy spectrum.



You need to make the following changes to the parameters: **'Total Points R+I' = 256**, **'Observe Freq MHz' = 499.641**, **'Center Position PPM'=4.773**, **'Axis Label=H1'**.

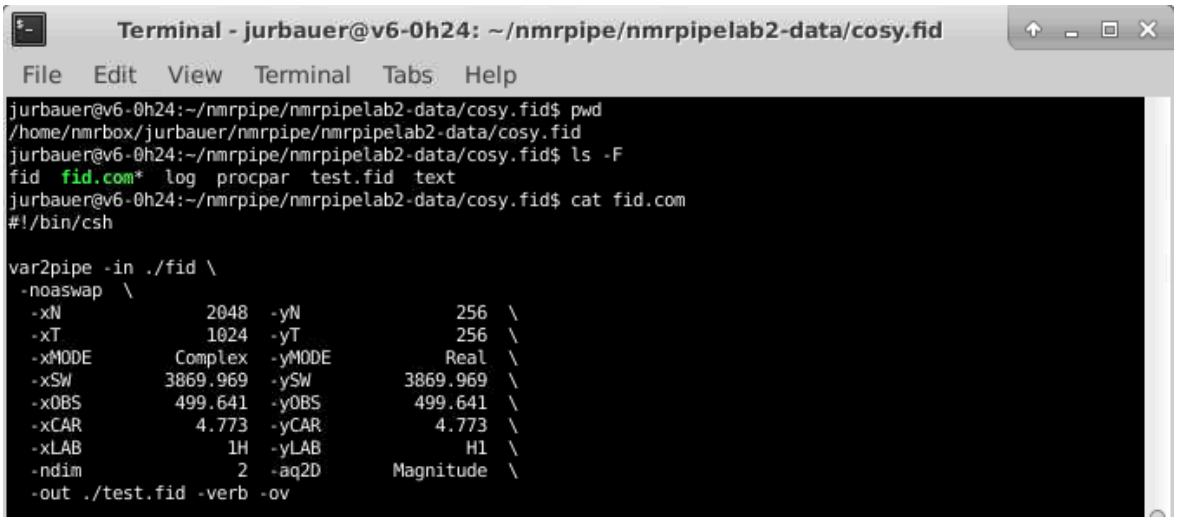
In addition, in the ‘Conversion Script Text’ window, you must **change ‘Real’ to ‘Magnitude’ for ‘-aq2D’**.



The **click ‘Save Script’**, followed by **‘Execute Script’**, and finally **‘Quit’**.

In the ‘cosy.fid’ directory now should be an executable script/macro ‘fid.com’ that converts the Varian format data to the NMRPipe format data, and a file ‘test.fid’ that is the NMRPipe format data.

Use the ‘cat’ command to list the contents of the ‘fid.com’ file (‘**cat fid.com**’).



This is a two-dimensional experiment, with two dimensions represented by ‘x’ and ‘y’. The ‘x’ dimension is the directly detected dimension, whereas the ‘y’ dimension is the second or indirectly detected dimension. In the first dimension, the data are collected as complex pairs (one real, and one imaginary point at each dwell time). So the total number of points (‘-xN’) is 2048, and the number of complex pairs (‘-xT’) is 1024 (so, points are sampled at 1024 dwell times). The ‘-xMODE’ parameter for the ‘x’ dimension indicates that the data are collected as complex pairs (‘Complex’). This particular magnitude COSY was collected as ‘real’ data (‘-yMODE = Real’), which is unusual. Therefore the parameters ‘-yN’ and -yT’ are equal. The second dimension is also being processed in the ‘Magnitude’ mode (‘-aq2D’ parameter) which is unusual. The ‘xLAB’ and ‘yLAB’ parameters just define the labels for the axes that will appear when the processed data are displayed in NMRDraw (you can edit them as you wish).

The parameters ‘-xCAR’ and ‘-yCAR’ are the positions of the carrier (reference) frequencies for the two dimensions, and are used for chemical shift referencing (can be changed if necessary). The last line outputs the converted data to the file ‘test.fid’ (‘-ov’ overwrites any existing file named ‘test.fid’).

Processing the absolute-value or magnitude-mode COSY data:

The dataset was collected using a Varian spectrometer using the 'gCOSY' experiment, which is a gradient version of the usual phase-cycled COSY. We will use the macro editing tool of NMRDraw to create a processing macro for this COSY data.

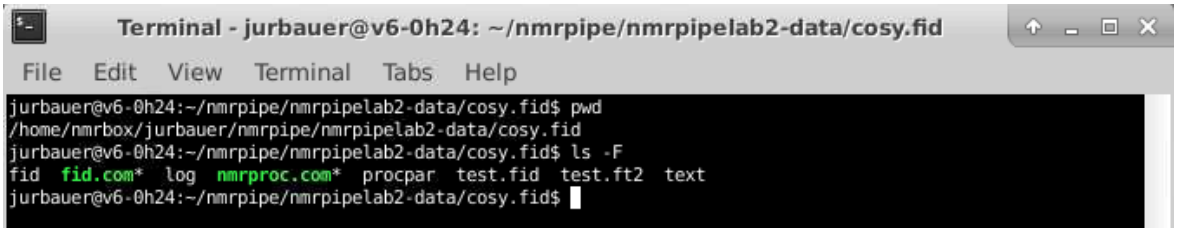
You should made sure you are in the 'cosy.fid' data directory ('cd' into that directory). Then start NMRDraw (you may see an interesting display of blue 'static', which a representation of the 2D data). From the 'File' menu, select the 'Macro Edit' option to bring up the 'NMRDraw Processing Macro' window (alternatively, with the cursor in the data window, just type 'm').

Right click the 'Process 2D' menu, then **left-click 'Basic 2D'** to select that option. Then, **edit the file so that it is identical to what is shown below** (except DO NOT include the comments that follow the backslash at the end of each line). Make sure that there are no characters or spaces after the backslash at the end of each line. Note the following:

- change **both instances** of 'SP -off 0.5' to 'SP -off 0.0'
- delete **both of the lines** with the '-fn PS' functions (phase corrections)
- add the line with the '-fn MC' function (you can add this manually, or select 'Modulus' from the 'Transforms' menu
- add the line with the '-fn REV' function (manually, or select 'Reverse' from the 'Transforms' menu), including the -di -verb options (you'll have to do this manually)
- add the second instance of the '-fn TP' function (manually, or select 'Transpose' from the 'Transforms' menu)
-make sure there are no empty lines, and no spaces or characters after the backslashes.

```
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0     \
| nmrPipe -fn ZF -auto                                \
| nmrPipe -fn FT -auto                                \
| nmrPipe -fn TP                                      \
| nmrPipe -fn SP -off 0.0 -end 1.00 -pow 1 -c 1.0     \
| nmrPipe -fn ZF -auto                                \
| nmrPipe -fn FT -auto                                \
| nmrPipe -fn MC                                      \
| nmrPipe -fn REV -di -verb                           \
| nmrPipe -fn TP                                      \
  -ov -out test.ft2
```

Click 'Save', then '**Execute**', the '**Done**'. You may get an error message when you 'save', just continue. In the 'cosy.fid' directory, you should see both the processing macro ('nmrproc.com*') and the file that corresponds to the processed spectrum ('test.ft2').

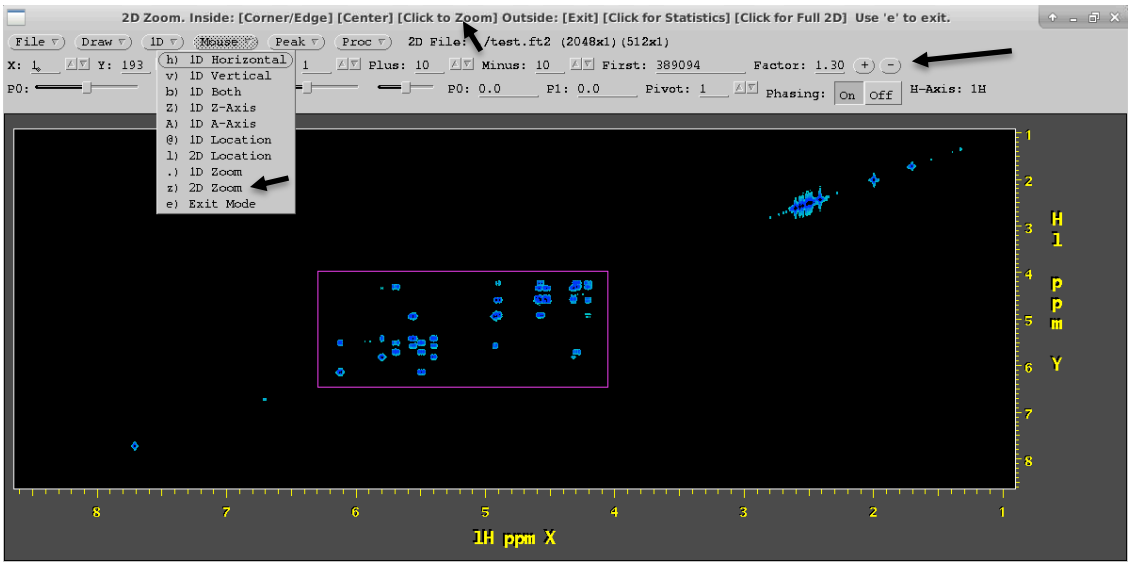


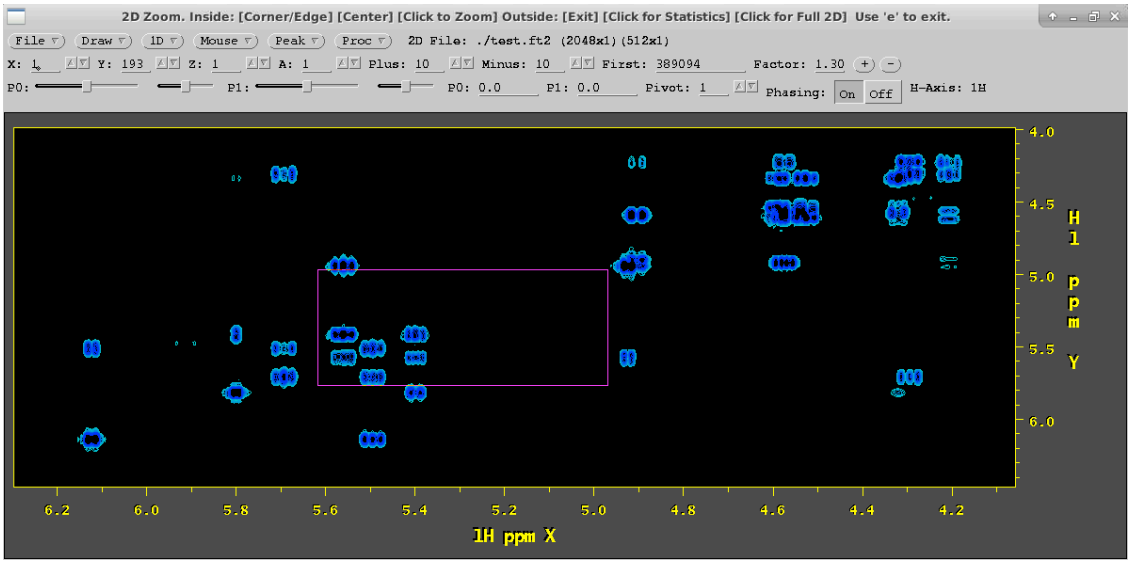
Displaying the absolute-value or magnitude-mode COSY spectrum:

Assuming you've started NMRDraw from the 'cosy.fid' directory, from the 'File' menu in NMRDraw select the 'Select File' option, then select 'test.ft2' and click the 'Read/Draw' button at the bottom, then click 'Done' (remember, you may have to resize this window to see the buttons at the bottom). You should see some small signals.

To adjust the vertical scale (the 'contour level'), the '+' and '-' buttons are used. To increase the signal intensity, click 4 or 5 times on the '-' button, then refresh the display (you can refresh the display many ways, including simply typing 'd' with the cursor in the data window, a single left-click on the 'File' menu, or by right-clicking on the 'Draw' menu, then selecting the 'Contours' option with a left-click).

Now, zoom in on the region shown below (upper figure, below) bounded by the purple box). To zoom, **right-click on the 'Mouse' menu**, then **left-click on '2D zoom'** to select that option (or, type 'z'), then **adjust the box with the cursor**, and, finally, **right-click in the spectrum window**. Once you are zoomed in (lower figure, below), you can select 'Exit Mode' from the 'Mouse' menu, or simply type 'e'.



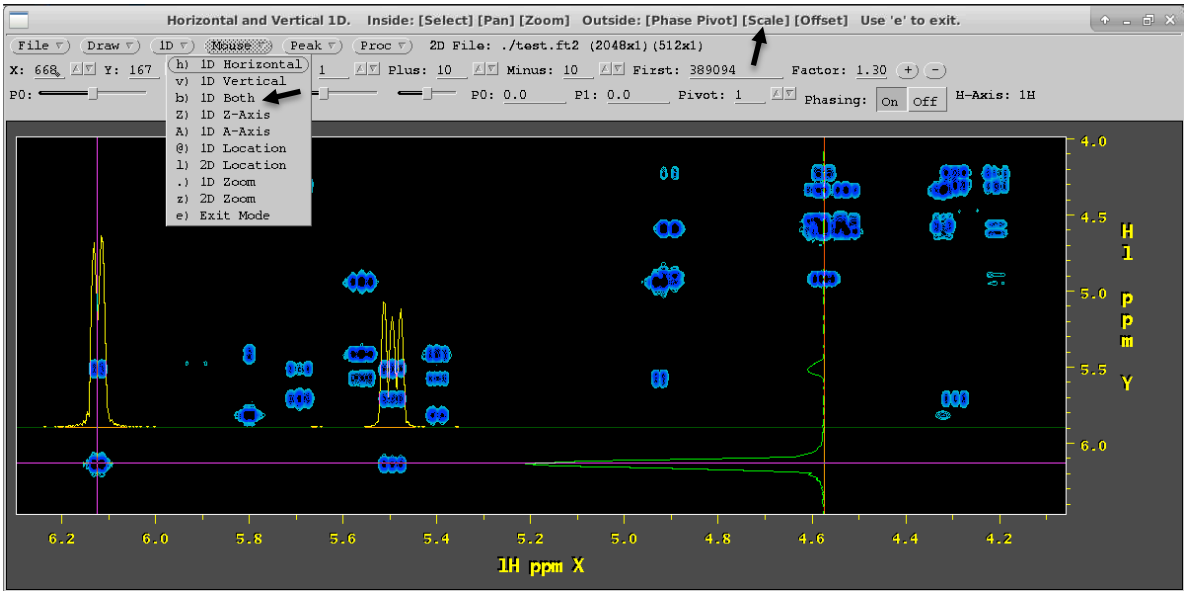


Referencing, determining chemical shifts, plotting:

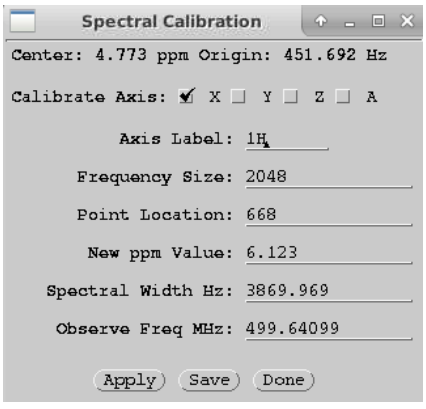
If you've not already, zoom in on the region shown in the lower figure on the previous page.

Right-click on the 'Mouse' menu. The top three options are '1D Horizontal' (or type 'h'), '1D Vertical' (or type 'v'), and '1D Both' or type 'b'). These options bring up horizontal, vertical, or both cursors, respectively. Bring up a horizontal cursor. As you move the cursor up and down in the spectrum window, you see 1D projections of the signals along the horizontal axis ('Mouse' then 'Exit Mode', or simply type 'e', to escape). Bring up a vertical cursor, and you'll see 1D projections of the data along the vertical axis ('e' to exit).

Bring up '1D Both' cursors. Place the crosshairs (purple) on the signal shown. You'll see yellow peaks showing the horizontal projection and green peaks showing the vertical projection (if the peaks are too big or too small, place the cursor in the gray area to the right of the spectrum and use the middle mouse button to scale).

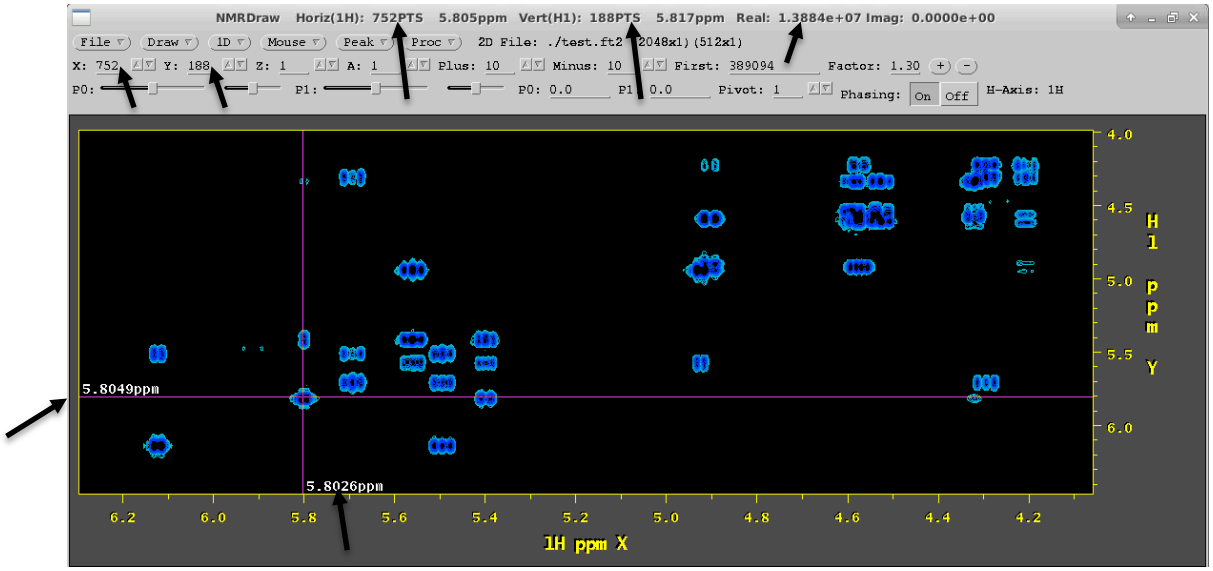


Once you have the crosshairs on the signal shown, type 'C' (upper case 'C', i.e. shift-c) and the 'Spectral Calibration' window will appear. The location of the crosshairs are shown as both ppm and point values. You can check either the 'x' or 'y' axis to see the values for that axis. You can change the values and click 'Apply' to see the changes. Don't click 'Save', but, if you do, the changes will be permanent.

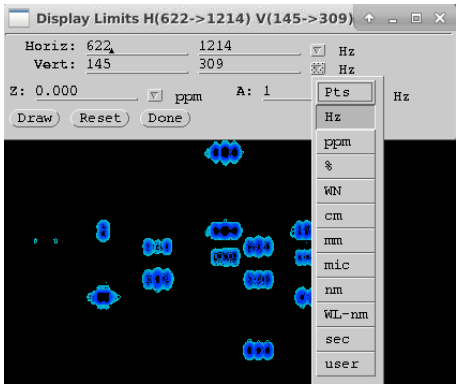


So, this tool allows you to get the position of a signal and to change the chemical shift referencing.

You can also get the position of a signal by typing ‘l’ (**lower** case L) with the cursor in the spectrum window (or select ‘2D Location’ from the ‘Mouse’ menu). Then, holding down the left mouse button brings up a crosshair cursor with the chemical shifts in each dimension. You can move it around, while holding down the left mouse button, to get chemical shifts of signals. You can also get the positions in points from the ‘X’ and ‘Y’ values in the main menu and from the display at the very top of the NMRDraw window. You can get the intensity there as well.

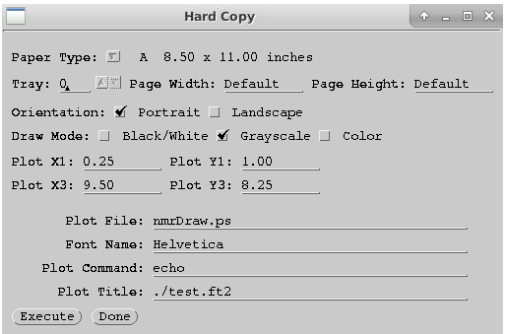


NMRDraw allows you to create a plot of a spectrum, or any region of a spectrum. First, type ‘e’ to exit from other operations. Then, if you type ‘L’ (upper case ‘L’, or ‘shift-l’, or, you can select the ‘2D Limits’ option from the ‘Draw’ menu) the ‘Display Limits’ window appears that allows you to select the region you are interested in plotting. Each value has a drop-down menu associated with it (‘right-click to show the options, left-click to select an option) to select the units.



Set the units for both ‘Horiz’ and ‘Vert’ (horizontal and vertical axes, respectively) to ‘ppm’. Then set the limits to 5.0 and 4.0 ppm for both ‘Horiz’ and ‘Vert’ (you can ignore the ‘Z’ and ‘A’ options). Once you’ve made the changes, click ‘Draw’ the ‘Done’. The region you’ve selected will then be displayed.

Once you’ve displayed the region you want to select, type ‘P’ (upper case ‘P’, or ‘shift-p’, or you can select the ‘Hard Copy Plot’ option from the ‘File’ menu). The ‘Hard Copy’ window will appear. There are lots of options that you can experiment with here. But, for the time being, check the ‘Color’ box (as opposed to the ‘Grayscale’ box) so the plot will be in color. Then click ‘Execute’ and ‘Done’. A file named ‘nmrDraw.ps’ will appear in your ‘cosy.fid’ directory. This is a postscript file that can be read and displayed by many applications, including vector graphics applications such as Adobe Illustrator. Transfer this file from NMRBox back to your computer to see what it looks like.



Converting the phase-sensitive ^{1}H, ^{13}C-HSQC data to NMRPipe format:

The dataset 'hsqc.fid' is from a gradient-selected, ^{1}H, ^{13}C-HSQC spectrum, where the frequency discrimination and phase-sensitive results are obtained by the 'echo-antiecho' or 'Rance-Kay' method of data collection.

If you have not already, quit the NMRDraw program.

The procedure for converting this Varian spectrometer data to NMRPipe format follows the same procedures laid out for the COSY experiment in the preceding section.

- open a terminal window
- 'cd' into the 'hsqc.fid' directory ('/home/nmrbox/username/nmrpipe/nmrpipelab2-data/hsqc.fid')
- type 'varian' to start the 'NMRPipe Converstion Utility'
- click the 'Read Parameters' button
- make the following three changes for the 'y-axis': change 'Observe Freq MHz' to 125.644, change 'Center Position PPM' to 82.861, change 'Axis Label' to 13C.
- click 'Save Script', then click 'Execute Script', then click 'Quit'

The contents of the 'fid.com' macro/script generated (and now present in the 'hsqc.fid' directory) should be as shown below. You need to make one correction (using a text editor, such as 'gedit'). You need to **change the option for '-aq2D' from 'Complex' to 'States'** (the correction has been made below).

```
#!/bin/csh

var2pipe -in ./fid \
 -noaswap  \
  -xN             1394  -yN              128  \
  -xT              697  -yT               64  \
  -xMODE       Complex  -yMODE      Rance-Kay  \
  -xSW        3869.969  -ySW        20100.503  \
  -xOBS         499.641  -yOBS         125.644  \
  -xCAR           4.773  -yCAR         82.861  \
  -xLAB              1H  -yLAB            C13  \
  -ndim               2  -aq2D         States  \
  -out ./test.fid -verb -ov

sleep 5
```

In addition, the file 'test.fid' (data in NMRPipe format), should be present in the 'hsqc.fid' directory.

As opposed to the 'COSY' spectrum in the preceding section, note here that, for the y-axis (indirectly detected dimension, ^{13}C), the '-yN' parameter is twice as big as the '-yT' parameter. This indicates that both a 'real' and 'imaginary' point are being collected in the indirectly detected dimension, which essentially allows for quadrature detection in that dimension. Note also the frequency and carrier position for 'y-axis' correspond to ^{13}C.

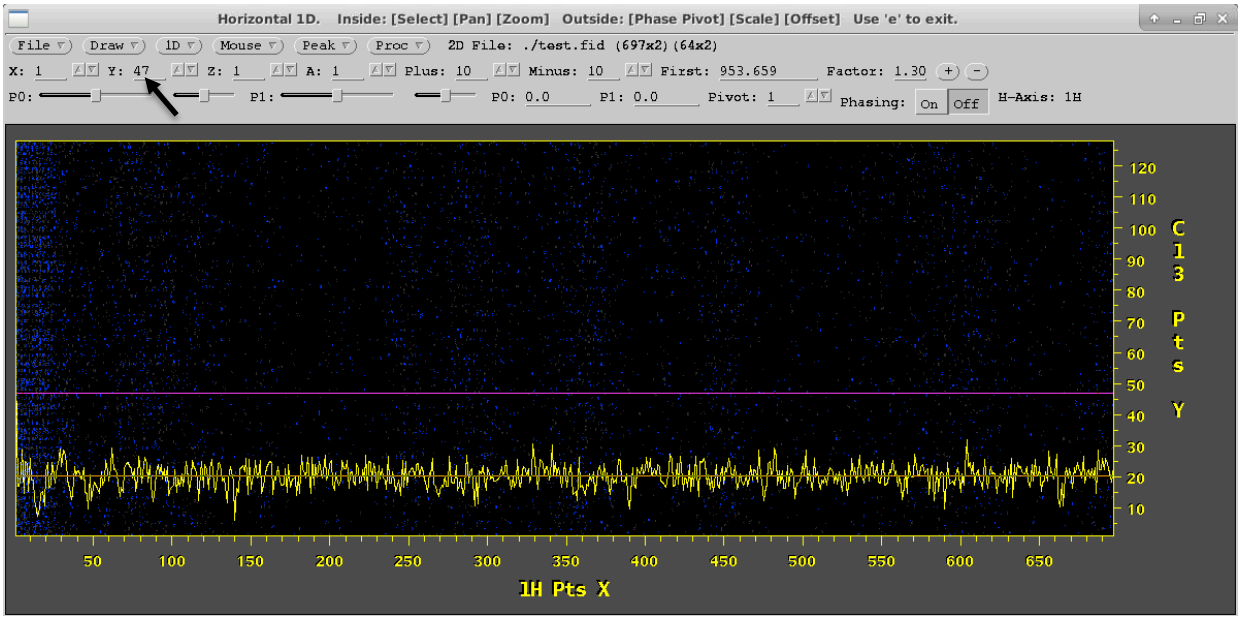
The value for '-aq2D', 'States', refers to one method for collecting complex data in the indirect dimension for quadrature detection. There are a number of ways to accomplish this, and a number of options, therefore, for '-aq2D'. In this particular experiment, the 'States' method was used.

Processing the phase-sensitive ^{1}H, ^{13}C-HSQC data:

Because this is a phase-sensitive data set, there will most likely be phase corrections for both dimensions. For the first dimension (directly detected dimension, in this case, ^{1}H) is often convenient to do this before actually processing the 2D data set.

Make sure you are in the 'hsqc.fid' data directory and the 'fid.com' file is present. Then start NMRDraw. You'll see a blue 'static' in the display, which represents the unprocessed data (typically, NMRDraw automatically opens the 'test.fid' data file if it is present). If you do not see the data, then from the 'File' menu select the 'Select File' option, select the 'test.fid' file, then click 'Read/Draw', then click 'Done'.

To get a horizontal cursor, type 'h' (or from the 'Mouse' menu select the '1D Horizontal' option). A horizontal purple cursor will appear. With the mouse, you can move the cursor up and down in the data window. As you do, you'll see the fids that comprise the 2D data set appear. If you scroll from top to bottom, the display will change 128 times. There are 128 fids, one pair for each of the 64 complex points in the second (^{13}C) dimension. You can tell which fid is in the display by the 'Y' parameter in the NMRDraw menus (below, fid #47 is being displayed).



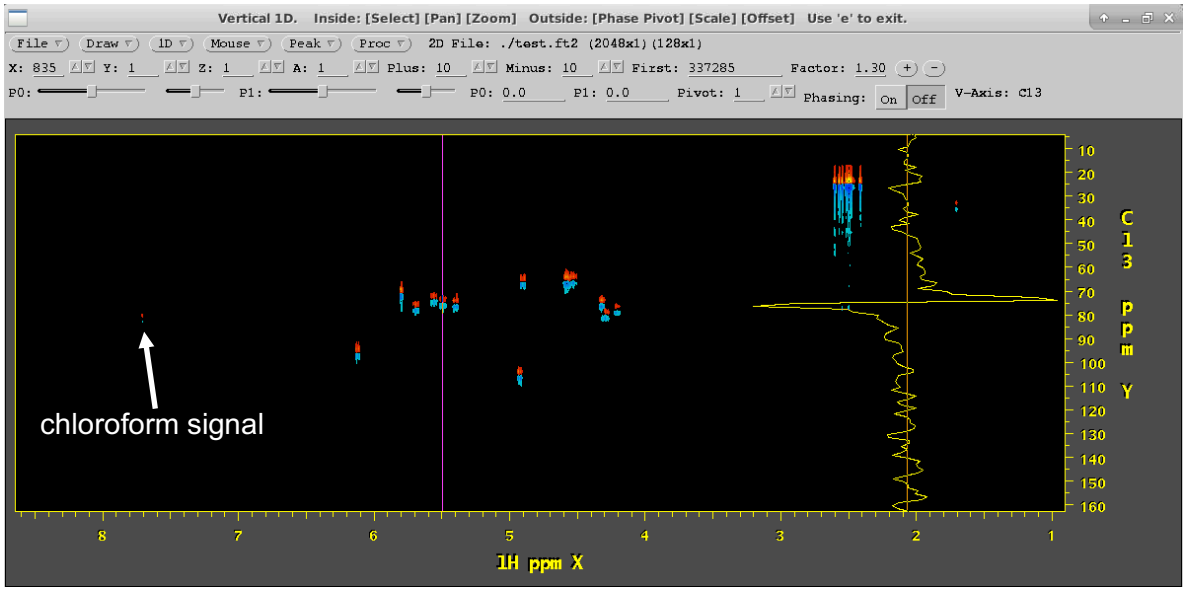
Move the horizontal cursor to the bottom so that fid #1 is being displayed (check the 'Y' parameter). Then **left-click on the 'Proc' menu** and **right-click 'Auto-Process 1D'** to select this option. The first fid will be Fourier transformed, and the spectrum will appear in the display. Then turn **Phasing 'On'**, make **'Pivot' equal to '1'**, then **adjust 'P0' to phase the spectrum** (the zero-order **phase correction P0 should be -136.0**). Then turn **Phasing 'Off'**. Type 'e' to end the horizontal cursor display.

We'll use the NMRDraw macro editor to create a processing macro. Just type 'm' (or from the 'File' menu select 'Macro Edit') and the 'NMRDraw Process Macro' window will appear. From the 'Process 2D' menu select 'Basic 2D'. In the first line that includes the 'PS' function, change the value of '-p0' from 0.0 to -136.0. After the line that includes the second 'PS' function, add a line with the reverse ('REV') function ('Transforms', 'Reverse'), and then add a line with the transpose ('TP') function ('Transforms', 'Transpose'). Then 'Save', 'Execute', 'Done'. The 'REV' function reverses the axis direction for the 'y' axis, and the 'TP' function changes the display so 1H is on the 'x' axis.

The processing macro ‘nmrproc.com’ should appear in the ‘hsqc.fid’ directory and should look like that shown below.

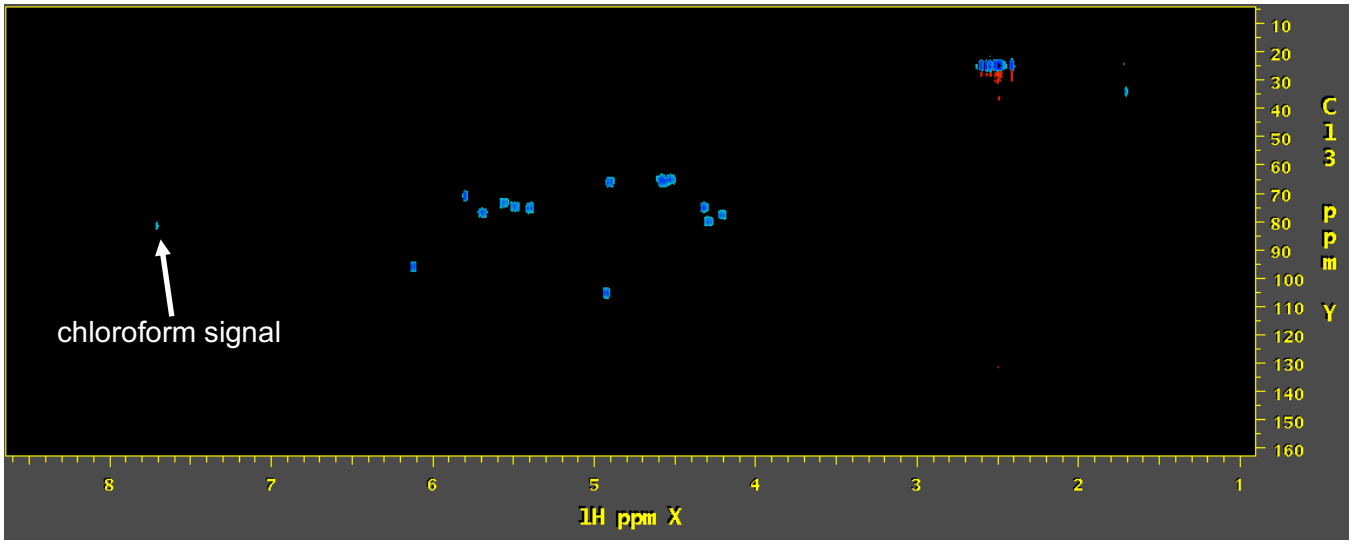
```
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -auto                               \
| nmrPipe  -fn FT -auto                               \
| nmrPipe  -fn PS -p0 -136.0 -p1 0.00 -di -verb       \
| nmrPipe  -fn TP                                     \
| 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         \
| nmrPipe -fn REV                                     \
| nmrPipe -fn TP                                      \
  -ov -out test.ft2
```

The file ‘test.ft2’ should be in your ‘hsqc.fid’ directory, so select it (‘File’, ‘Select File’, select ‘test.ft2’, ‘Read/Draw’, ‘Done’) and display it (you may have to use the ‘+’ and ‘-’ buttons to adjust the intensity). Your display should look like that below



You see that the signals are not phased properly in the ^{13}C dimension (the red and blue colors indicate negative and positive phases, or dispersive shapes). To determine the phase corrections for the ^{13}C dimension, type ‘v’ to get a vertical cursor (or from the ‘Mouse’ menu select the ‘1D Vertical’ option). Move the cursor horizontally onto one of the signals in the spectrum. Turn phasing ‘On’, make ‘Pivot’ equal to ‘1’, change P0 to phase the signal (should be about -94.0). Turn phasing ‘Off’. Quit NMRDraw.

Then, using a text editor (you can use ‘gedit’ in NMRBox), edit the ‘nmrproc.com’ macro. In the second line that includes the ‘PS’ function (fourth line from the bottom, as shown above), **change ‘-p0’ to 94.0** (because of the ‘TP’ and ‘REV’ functions, the correct phas correction is 94, not -94). Then run the macro (‘./nmrproc.com’). Start NMRDraw again and load the spectrum. Both dimensions should now be properly phased (see figure on next page).



For this 2D HSQC data set, there were only 64 complex points collected in the second (^{13}C, indirect) dimension. This results in a relatively marginal resolution in that dimension. In such cases, it is often advantageous to increase the number of points using the method known as 'linear prediction'. Linear prediction can be relatively complicated mathematically, but, in essence, the algorithm determines the frequencies and decay rates for the signals in an FID or interferogram, and then extends them mathematically. It is relatively robust, and, when used properly and responsibly, can reliably improve resolution, and thus provide time savings or the use of instrument time to improve signal-to-noise.

Here we will edit the 'nmrproc.com' file to include linear prediction. First, quite the NMRDraw program. Then, using a text editor ('gedit' is good for the terminal application in NMRBox) edit the 'nmrproc.com' macro to include the line containing the 'LP' command as shown below. The 'LP' command will increase the number of complex points in the ^{13}C dimension to 128, and the 'ZF' command will zero-fill to 256. So, there now will be 256 complex points in the ^{13}C dimension.

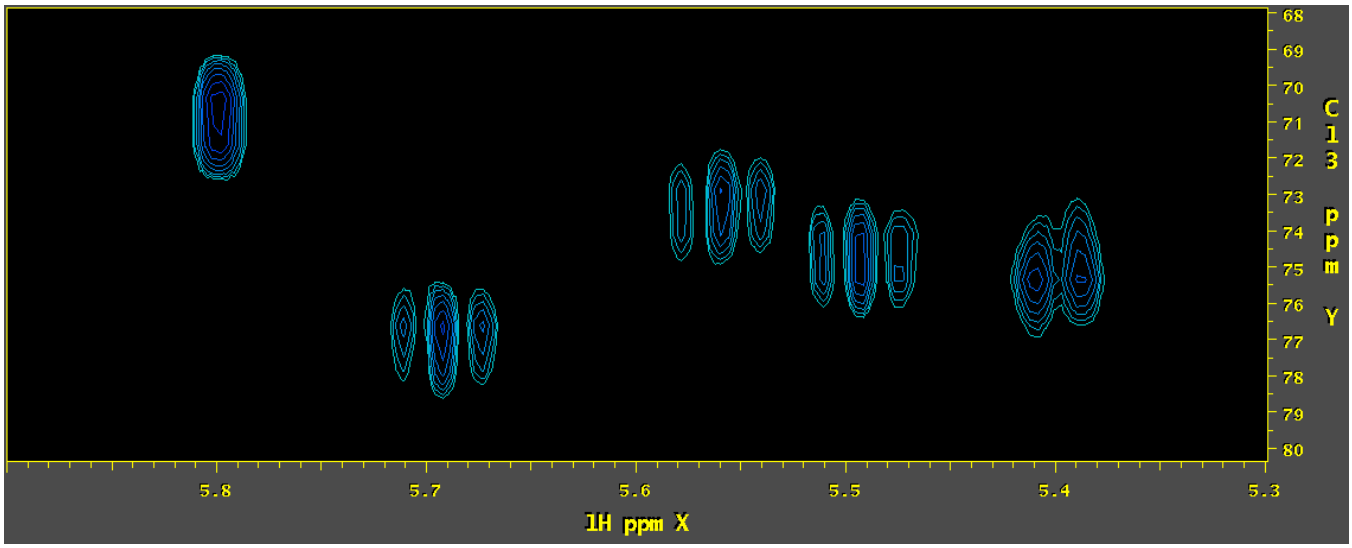
```
#!/bin/csh
nmrPipe -in test.fid \
| nmrPipe  -fn SP -off 0.5 -end 1.00 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -auto                               \
| nmrPipe  -fn FT -auto                               \
| nmrPipe  -fn PS -p0 -136.0 -p1 0.00 -di -verb        \
| nmrPipe  -fn TP                                     \
| nmrPipe  -fn LP -fb                                 \
| 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 94.0 -p1 0.00 -di -verb        \
| nmrPipe -fn REV                                     \
| nmrPipe -fn TP                                      \
  -ov -out test.ft2
```

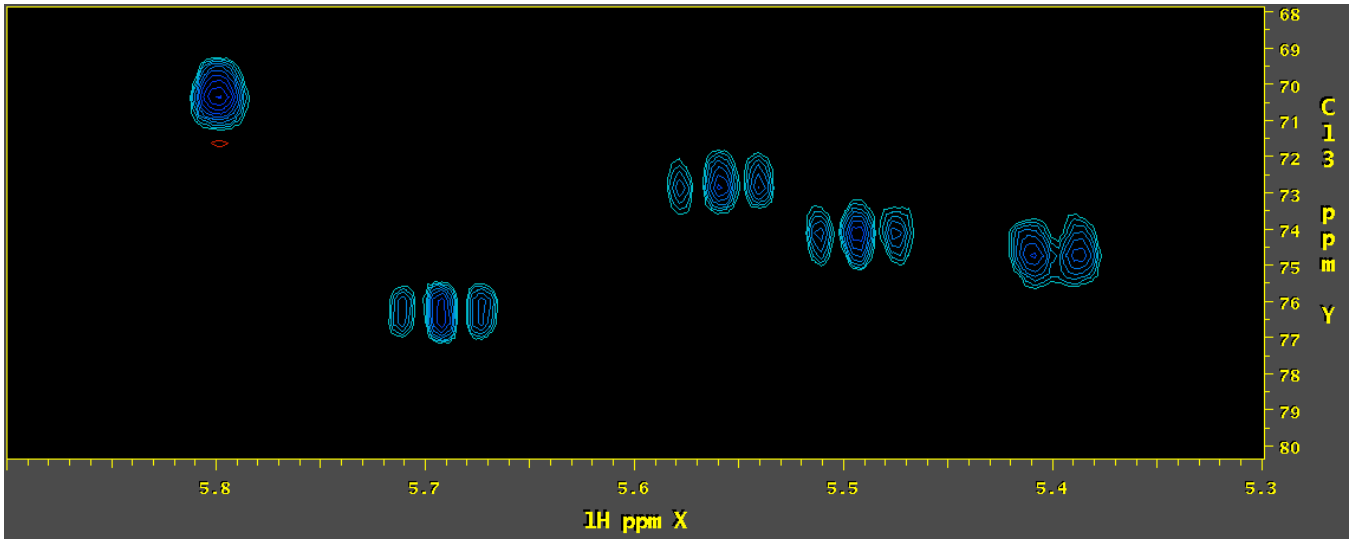
Once you have edited and saved the 'nmrproc.com' file, **execute the file ('./nmrproc.com')**. Then, start the NMRDraw program and load the new (linear predicted) spectrum.

Below, the top figure shows an expansion of the spectrum without linear prediction.

The bottom figure shows an expansion of the spectrum WITH linear prediction.

So, linear prediction, in this case doubling the number of points in the indirect (13C) dimension from 64 to 128, and zero-filling to 256, resulted in a significant improvement in resolution in the ^{13}C dimension.





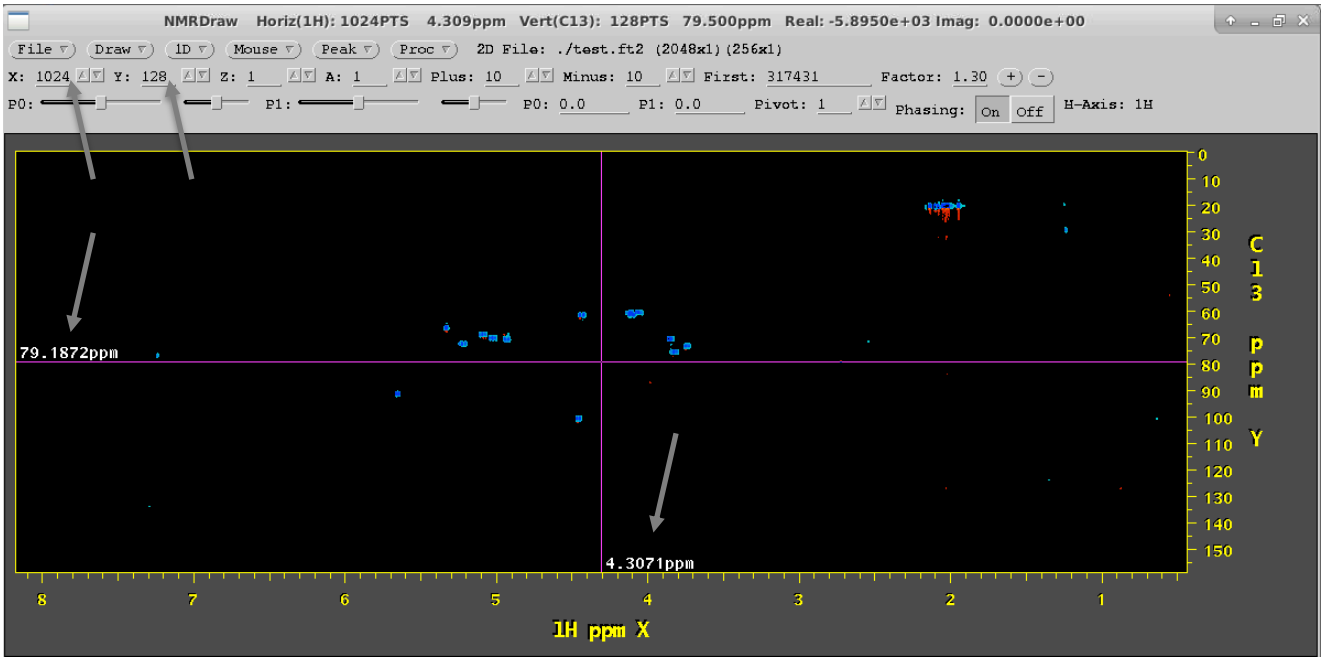
Chemical shift referencing the phase-sensitive ^{1}H, ^{13}C-HSQC spectrum:

The chemical shift referencing is not correct for this spectrum, because default values were used in the conversion script. In the spectrum there is a signal corresponding to chloroform, so the known chemical shifts of chloroform can be used to reference the spectrum.

- zoom in around the chloroform signal ('z', adjust box with cursor, right-click, 'e' to exit), which is the most downfield signal in the 1H dimension (apparent ^{1}H chemical shift of approximately 7.7 ppm, and an apparent ^{13}C chemical shift of 80 ppm).
- get the crosshairs cursor (type 'b') and place the crosshairs on the center of the peak (NOTE: with the NMRBox version of NMRDraw, sometimes the crosshairs cursor does not move smoothly, especially in the 'zoom' mode, so that it can be impossible to place the crosshair cursor exactly where you want it. For this tutorial, don't worry about it. Just put the cursor as close as you can to the center of the peak. Even if you just get the cursor anywhere on the peak, that will be good enough for this tutorial).
- display the 'Spectral Calibration' window by typing 'C' (upper case 'c')
- in the 'Spectral Calibration' window, for the 'X' axis, enter '7.24' for 'New ppm Value', and then click the 'Apply' button
- in the 'Spectral Calibration' window, for the 'Y' axis, enter '77.0 for 'New ppm Value', and then click the 'Apply button
- click 'Done', then type 'e' to exit the cursor mode
- if you type 'f' you can once again view the full spectrum

The spectrum is now referenced correctly. However, the referencing may be lost upon redisplay, and will be lost if the spectrum must be regenerated. The referencing can be retained by changing the values of '-xCAR' and '-yCAR' in the conversion macro ('fid.com'). In order to do so, the chemical shift values for the center points of the spectrum have to be determined.

The processed spectrum has 2048 complex points in the ^{1}H dimension (after zero-filling), and 256 complex points in the ^{13}C dimension (after linear prediction and zero-filling). We need to determine the chemical shifts at the center of each dimension (i.e. the carrier, 'CAR', positions), which are at point 1024 in the ^{1}H dimension and 128 in the ^{13}C dimension. In order to do so, we use the method



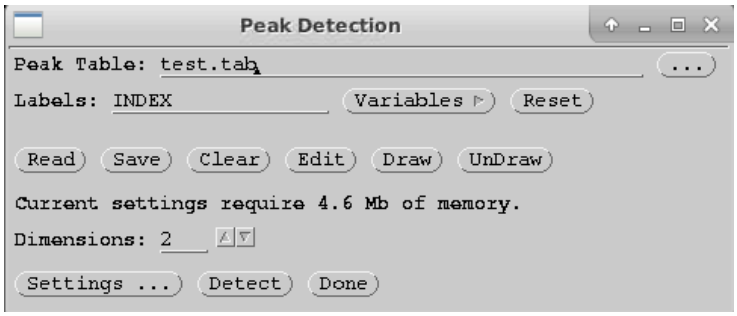
introduced earlier. First **type 'l'** (**lower** case L) with the cursor in the spectrum window (or select '2D Location' from the 'Mouse' menu). Then, holding down the left mouse button brings up a crosshair cursor showing the chemical shifts in each dimension. Move it around, while holding down the left mouse button, to get chemical shifts at the crosshair. Move the cursor so that the 'X' and 'Y' values in the main menu read '1024' and '128', respectively, then write down the chemical shifts. In this case, for ^{1}H, the chemical shift at point 1024 is 4.3071, and for ^{13}C the chemical shift at point 128 is 79.1872 (see figure on previous page).

Finally, in the processing macro 'fid.com', change '-xCAR' to 4.3071, and change '-yCAR' to 79.1972. Now, you can reconvert and reprocess the data, and the spectrum should be properly/correctly referenced.

"Peak Picking" the phase-sensitive ^{1}H, ^{13}C-HSQC spectrum:

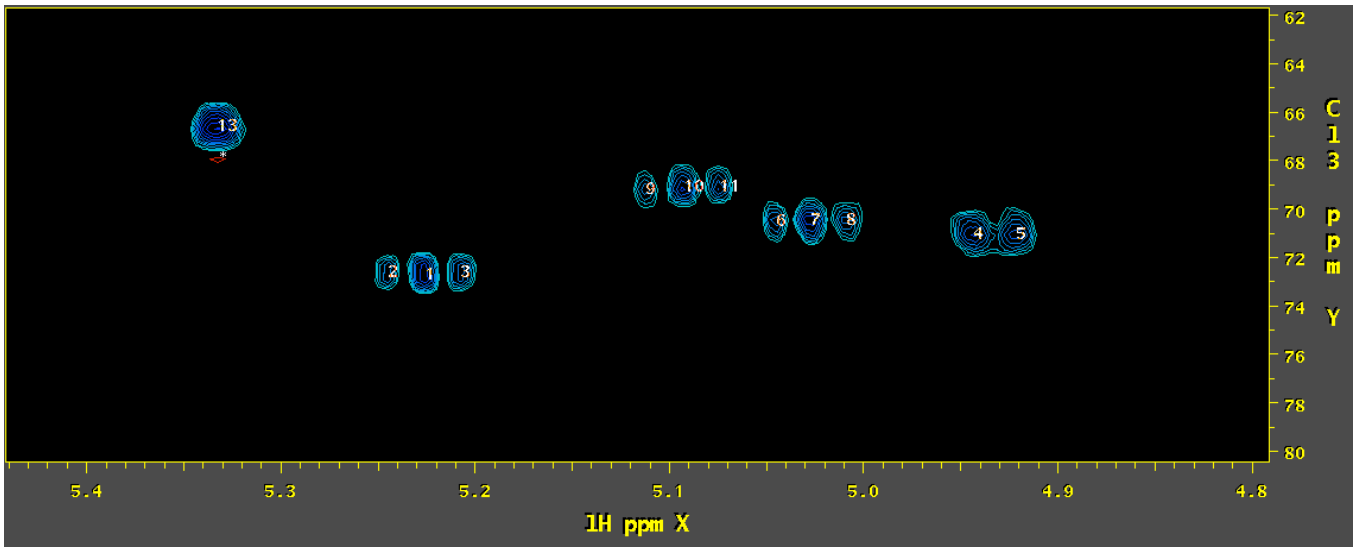
Deciding what is a signal, and determining the chemical shifts of signals, is usually referred as "peak picking". Here we'll demonstrate a way to automatically "pick some peaks" in NMRDraw.

First zoom in around some signals (you should know how to do this by now). Then, from the 'Peak' menu, select 'Peak Detection'. This will bring up the 'Peak Detection' window.



Although there are some options here, just use the defaults. **Click 'Clear'**, then **click 'Detect'**, then **click 'Done'**.

Each signal detected should now be labeled by an integer. In the case below, 13 signals/peaks were detected. A file 'test.tab' was also created and is stored in the 'hsqc.fid' directory. You can examine this file, which includes information about the peak positions, heights, volumes, etcetera of the peaks/signals that were detected.



Exercise: convert and process the 2D phase-sensitive ('States') TOCSY dataset:

In the 'nmrpipelab2-data' directory is a third dataset named 'tocsy.fid'. This is a 1H-1H correlated, phase-sensitive TOCSY experiment. The data is complex in both dimensions, with quadrature detection in the indirect dimension.

See if you can convert the data to NMRPipe format, and then process the data to give the spectrum. For the processing macro, you can start with the basic 2D macro, cosine-bell window functions are appropriate, and both dimensions will have to be phased. Linear prediction is not necessary.