Detailed Instructions for Some 2D NMR Experiments

Some Useful 2D NMR Experiments for Structural Elucidation of Organic Molecules

There are several NMR methods, other than simple one-dimensional proton NMR, that can help in the determination of molecular structures of small organic molecules. These are introduced below, along with their individual operating procedures using the Varian NMR spectrometers.

- **gCOSY**
- **TOCSY**
- **NOESY**
- **ROESY**
- **gHSQC**
- **gHSQCAD**
- **gHMBC**
- **gHMBCAD**
- **gHMQC**
- **gHMQCAD**
- **DOSY**

**COSY (COrrelated SpectroscopY):** This 2D NMR method produces a spectrum that correlated the peaks of scalar-coupled protons in the molecule. Along the diagonal line lie all the proton peaks, and the off-diagonal cross peaks connect the two protons that are scalar-coupled.

Example:  
**COSY spectrum of menthol**

How to run a COSY experiment?
1. In expn (e.g. exp1), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command `movesw` to fix this region, and `ga` to recollect a new spectrum.
2. Go to the next experiment number by `jexp(m)` (m = n + 1). If expm is not existent, type `cexp(m)` to create it.
3. Type `mf(n,m)` [ret] to copy the file in expn to expm. Type `wft` to see if the spectrum is exactly what is desired. Type `gCOSY` [ret] to load all parameters used in the gCOSY experiment. Type `su` [ret].
4. Check the total time needed for the experiment by typing `time` [ret]. Adjust `nt` (by default, nt is set to 1, which makes the total experiment time about 5 minutes), if needed, then start the experiment by typing `go`[ret].
5. The spectrum can be examined before the data acquisition is finished, if \texttt{proc1} is set to \texttt{ft} (if \texttt{proc1} is set to \texttt{lp}, you can temporarily change it to \texttt{ft}, and change it back to \texttt{lp} after the examination). To generate the spectrum, type \texttt{wft2d} \texttt{[ret]}. The resultant 2D spectrum will be displayed (command \texttt{dconi} is used to display the spectrum, if it is not displayed already).

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20\% and -20\% buttons.

7. If the chemical shift scales are not correct, follow the \textit{Spectral referencing procedure}.

8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the \textit{Expansion} button.

9. To plot the 2D spectrum displayed, type \texttt{plcosy('pos',i,j,k,m)} \texttt{[ret]}, where \textit{i} is the number of contour levels (e.g., 20) to be plotted, \textit{j} is the intensity ratio of every adjacent levels (e.g., 1.3), \textit{k} is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in both F1 and F2 axes, and \textit{m} is a multiplier to adjust the amplitude of the projections.

- \textbf{TOCSY (TOtal Correlated SpectroscopY):} This 2D NMR method produces a similar spectrum in COSY experiment, except that cross peaks will be observed among all protons in the same coupling family. For example, if \textit{A} is coupled to \textit{B} and \textit{B} is coupled to \textit{C}, but \textit{A} is not coupled to \textit{C}, there will still be a cross peak connecting \textit{A} and \textit{C}.

**Example:**

\textit{TOCSY Spectrum of Menthol}

\textbf{How to run a TOCSY experiment?}

1. In \textit{expn} (e.g. exp1), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command \texttt{movesw} to fix this region, and \texttt{ga} to recollect a new spectrum.
2. Go to the next experiment number by \texttt{jexpn} \textit{(m=n+1)}. If \textit{expm} is not existent, type \texttt{cexp(m)} to create it.
3. Type \texttt{mf(n,m)} \texttt{[ret]} to copy the file in \textit{expn} to \textit{expm}. Type \texttt{wft} to see if the spectrum is exactly what is desired. Type \texttt{TOCSY} \texttt{[ret]} to load all parameters used in the gTOCSY experiment. Type \texttt{su} \texttt{[ret]}. 
4. Check the total time needed for the experiment by typing **time** [ret]. Adjust **nt** (by default, **nt** is set to 1, which makes the total experiment time about 5 minutes), if needed, then start the experiment by typing **go**[ret].

5. The spectrum can be examined before the data acquisition is finished, if **proc1** is set to **ft** (if **proc1** is set to **lp**, you can temporarily change it to **ft**, and change it back to **lp** after the examination). Type **wft2da**[ret] to generate the spectrum. The resultant 2D spectrum will be displayed (command **dconi** is used to display the spectrum, if it is not displayed already).

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20% and -20% buttons.

7. If the peak phases are not right, follow the Phasing procedure to correct them. If the chemical shift scales are not correct, follow the Spectral referencing procedure.

8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the Expansion button.

9. To plot the 2D spectrum displayed, type **plcosy('pos/neg',i,j,k,m)** [ret], where the first argument is used to select either the positive peaks or the negative peaks (or both when it’s ignored), **i** is the number of contour levels (e.g., 20) to be plotted, **j** is the intensity ratio of every adjacent levels (e.g., 1.3), **k** is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in both F1 and F2 axes, and **m** is a multiplier to adjust the amplitude of the projections.

- NOESY (Nuclear Overhauser Enhancement Spectroscopy): This 2D NMR method produces a similar spectrum in COSY experiment, except that cross peaks will be observed between protons which have short internuclear distance, usually < 5 Angstrom.

**Example:**

NOESY Spectrum of Menthol

**How to run a NOESY experiment?**

1. In **expn** (e.g. **exp1**), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command **movesw** to fix this region, and **ga** to recollect a new spectrum.

2. Go to the next experiment number by **jexpm** (**m=n+1**). If **expm** is not existent, type **cexp(m)** to create it.
3. Type \texttt{mf(n,m) [ret]} to copy the file in \texttt{expn} to \texttt{expm}. Type \texttt{wft} to see if the spectrum is exactly what is desired. Type \texttt{NOESY [ret]} to load all parameters used in the NOESY experiment. Type \texttt{su [ret]}.

4. Check the total time needed for the experiment by typing \texttt{time [ret]}. Adjust \texttt{nt} (by default, \texttt{nt} is set to 4, which makes the total experiment time about 25 minutes) and mixing time \texttt{mix} (set to 0.5 sec. by default, which is adequate for small molecules), if needed, then start the experiment by typing \texttt{go [ret]}.

5. The spectrum can be examined before the data acquisition is finished, if \texttt{proc1} is set to \texttt{ft} (if \texttt{proc1} is set to \texttt{lp}, you can temporarily change it to \texttt{ft}, and change it back to \texttt{lp} after the examination). Type \texttt{wft2da [ret]} to generate the spectrum. The resultant 2D spectrum will be displayed (command \texttt{dconi} is used to display the spectrum, if it is not displayed already).

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20\% and -20\% buttons.

7. If the peak phases are not right, follow the \textbf{Phasing procedure} to correct them. If the chemical shift scales are not correct, follow the \textbf{Spectral referencing procedure}.

8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the \textbf{Expansion} button.

9. To plot the 2D spectrum displayed, type \texttt{plcosy('pos/neg',i,j,k,m) [ret]}, where the first argument is used to select either the positive peaks or the negative peaks (or both when it’s ignored), \texttt{i} is the number of contour levels (e.g., 20) to be plotted, \texttt{j} is the intensity ratio of every adjacent levels (e.g., 1.3), \texttt{k} is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in both \texttt{F1} and \texttt{F2} axes, and \texttt{m} is a multiplier to adjust the amplitude of the projections.

- \textbf{ROESY (Rotating-frame nuclear Overhauser Enhancement Spectroscopy):} This 2D NMR method produces a similar spectrum in COSY experiment, except that cross peaks will be observed between protons which have short internuclear distance, usually < 5 Angstrom. This experiment is needed when the molecular weight is about 1,000, which slows down the molecular motions to a point that the NOE is approaching zero.

Example:

How to run a ROESY experiment?

1. In \texttt{expn} (e.g. exp1), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the
interested region, and type the command `movesw` to fix this region, and `ga` to recollect a new spectrum.

2. Go to the next experiment number by `jexpm (m=n+1)`. If `expm` is not existent, type `cexp(m)` to create it.

3. Type `mf(n,m)` [ret] to copy the file in `expn` to `expm`. Type `wft` to see if the spectrum is exactly what is desired. Type `ROESY` [ret] to load all parameters used in the ROESY experiment. Type `su` [ret].

4. Check the total time needed for the experiment by typing `time` [ret]. Adjust `nt` (by default, `nt` is set to 4, which makes the total experiment time about 25 minutes) and mixing time `mix` (set to 0.2 sec. by default, which is adequate for small molecules), if needed, then start the experiment by typing `go` [ret].

5. The spectrum can be examined before the data acquisition is finished, if `proc1` is set to `ft` (if `proc1` is set to `lp`, you can temporarily change it to `ft`, and change it back to `lp` after the examination). Type `wft2da` [ret] to generate the spectrum. The resultant 2D spectrum will be displayed (command `dconi` is used to display the spectrum, if it is not displayed already).

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20% and -20% buttons.

7. If the peak phases are not right, follow the Phasing procedure to correct them. If the chemical shift scales are not correct, follow the Spectral referencing procedure.

8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the Expansion button.

9. To plot the 2D spectrum displayed, type `plcosy('pos/neg',i,j,k,m)` [ret], where the first argument is used to select either the positive peaks or the negative peaks (or both when it’s ignored), `i` is the number of contour levels (e.g., 20) to be plotted, `j` is the intensity ratio of every adjacent levels (e.g., 1.3), `k` is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in both F1 and F2 axes, and `m` is a multiplier to adjust the amplitude of the projections.

Phase Correction of Phase-sensitive 2D Spectra (e.g. NOESY, gHSQC, gHMQC, TOCSY, ROESY)

1. Display the 2D spectrum with command `dconi`.
2. Use the left mouse button to select a row across a large diagonal peak on the upper right corner.
3. Type `ds` to display this selected row and phase the diagonal peak with only the 0th order phasing parameter.
4. Type `dconi`, and select another trace across a diagonal peak on the lower left corner of the 2D spectrum.
5. Type `ds` to display the second row selected. Phase the diagonal peak with only the 1st order phase parameter (do not change the 0th order phase). – This is done by clicking on the **Phase** button, left-click on the peak region selected in step 3 above, don’t do any phasing, left-clicking on the region containing the diagonal peak and phase this peak.)

6. Type `dconi` and see if all the rows are phased.

7. To phase columns, rotate the 2D matrix by clicking on the **Return**, **More**, and **F2 Mode** (or **F1 Mode**) buttons; then follow the above steps to phase the new rows.

- If you cannot perform phase correction, check the parameter **pmode**, it should be set to **full**.
- For NOESY spectrum of small molecules, always phase the diagonal peaks to negative so that the NOE cross peaks are positive.

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- **HSQC (Heteronuclear Single Quantum Correlation):** This 2D NMR method produces a 2D spectrum with one dimension representing the proton chemical shifts and the other dimension representing the carbon-13 chemical shifts (or chemical shifts of the X-nucleus like N15 or P31, etc.).

Traditionally, when chemical shifts of the C13 nuclei are needed, one would run a one-dimensional C13 spectrum, which is very insensitive compared with proton NMR, due to the lower resonance frequency and the low natural abundance of the C13 isotope (1.1%). In addition, the C13 chemical shift range (200 ppm) is much wider than proton and peaks are usually very well separated from each other. Therefore, the spectral resolution is not as important as in proton spectra.

HSQC is an experiment that observes the proton signals that carry the information of their directly bonded carbons. When the spectrum displays a peak, it will provide the chemical shift of the proton on one axis and the chemical shift of the directly bonded carbon on the other axis. Since the sensitivity of observing proton NMR is much higher (10 times or more), the experimental time needed is much shorter than that for running a one-dimensional C13 spectrum.

In addition, the HSQC spectrum displays peaks in phase sensitive mode, which shows CH and CH3 peaks with same phase, and CH2 peaks in opposite phase, similar to the DEPT and APT spectra.

The **gHSQCAD** produces more uniform intensity for correlation peaks through a range of one-bond proton-carbon couplings.
Example:

**gHSQC Spectrum of Menthol**

How to run a HSQC experiment?

1. In exp\text{n} (e.g. exp1), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command \texttt{movesw} to fix this region, and \texttt{ga} to recollect a new spectrum.
2. Go to the next experiment number by \texttt{jexp \{m\}} (\texttt{m}=n+1). If exp\text{m} is not existent, type \texttt{cexp(m)} to create it.
3. Type \texttt{mf(n,m)} [ret] to copy the file in exp\text{n} to exp\text{m}. Type \texttt{wft} to see if the spectrum is exactly what is desired. Type \texttt{gHSQC} or \texttt{gHSQCAD} [ret] to load all parameters used in the gHSQC or the gHSQCAD experiment. Type \texttt{su} [ret].
4. Check the total time needed for the experiment by typing \texttt{time} [ret]. Adjust nt (by default, nt is set to 4, which makes the total experiment time about 25 minutes), if needed, then start the experiment by typing \texttt{go} [ret].
5. The spectrum can be examined before the data acquisition is finished, if proc1 is set to \texttt{ft} (if proc1 is set to \texttt{lp}, you can temporarily change it to \texttt{ft}, and change it back to \texttt{lp} after the examination). Type \texttt{wft2da} [ret] to generate the spectrum. The resultant 2D spectrum will be displayed (command \texttt{dconi} is used to display the spectrum, if it is not displayed already).
6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20% and -20% buttons.
7. If the peak phases are not right, follow the **Phasing procedure** to correct them. If the chemical shift scales are not correct, follow the **Spectral referencing procedure**.
8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the **Expansion** button.
9. To plot the 2D spectrum displayed, type \texttt{plhxcor(‘pos/neg’,i,j,k,l,m,n)} [ret], where the first argument is used to select either the positive peaks or the negative peaks (or both when it’s ignored), \texttt{i} is the number of contour levels (e.g., 20) to be plotted, \texttt{j} is the intensity ratio of every adjacent levels (e.g., 1.3), \texttt{k} is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in the F2 axis, \texttt{l} is the experiment number (e.g., 2) where the 1D carbon NMR spectrum is stored and to be used as the projection in the F1 axis, \texttt{m} is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection, and \texttt{n} is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection.
   To plot the actual projections of the 2D spectrum, use buttons **Proj**, **Hproj(Max)** or **Hproj(sum)**, **Vproj(max)** or **Vproj(sum)**, and **Plot** to plot the projections first (the amplitude of the projections can be adjusted by using the...
middle mouse buttons before plotting), then use the command \texttt{pcon(‘pos|neg’,i,j)} and \texttt{page} to plot the 2D spectrum.

**Spectral Referencing:**

Reference Assignment of homonuclear 2D spectra (e.g. gCOSY, gTOCSY, NOESY)

1. Select a peak that its chemical shifts on both axes are known by using the left mouse cursor.
2. Type \texttt{rl(mp) rl T(np)}, where \texttt{m} is the chemical shift of the peak on the F2 axis and \texttt{n} is that on the F1 axis, and \texttt{p} designates the unit to be in ppm.

Reference Assignment of Heteronuclear 2D spectra (e.g. gHSQC, gHMQC, gHMBC)

1. Select a peak that its chemical shifts on both axes are known by using the left mouse cursor.
2. Type \texttt{rl(mp) rl T(nd)}, where \texttt{m} is the chemical shift of the peak on the F2 axis and \texttt{n} is that on the F1 axis. Note that \texttt{d} has to be typed after \texttt{n}.

- HMOC (Heteronuclear Multiple-Quantum Correlation): This 2D NMR method provides similar information as in the HSQC experiment. In many cases, the HSQC experiment is a better experiment to run. In addition, HSQC experiment can be designed to provide different peak phase for CH, CH3 carbons as opposed to CH2 carbons. Also, the peaks in the HSQC spectrum are generally sharper due to the absence of proton homonuclear couplings. However, the HSQC requires more RF pulses and is more sensitive to instrument calibration errors.

Example:

**gHMOC Spectrum of Menthol**

How to run HMOC experiment?

1. In \texttt{expn} (e.g. \texttt{exp1}), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command \texttt{movesw} to fix this region, and \texttt{ga} to recollect a new spectrum.
2. Go to the next experiment number by \texttt{jexp} \texttt{(m=n+1)}. If \texttt{expm} is not existent, type \texttt{cexp(m)} to create it.
3. Type \texttt{mf(n,m)} [ret] to copy the file in \texttt{expn} to \texttt{expm}. Type \texttt{wft} to see if the spectrum is exactly what is desired. Type \texttt{gHMOC} or \texttt{gHMQCAD} [ret] to load all parameters used in the gHMOC or gHMQCAD experiment. Type \texttt{su} [ret].
4. Check the total time needed for the experiment by typing `time` [ret]. Adjust `nt` (by default, nt is set to 1, which makes the total experiment time about 5 minutes), if needed, then start the experiment by typing `go`[ret].

5. The spectrum can be examined before the data acquisition is finished, if `proc1` is set to `ft` (if `proc1` is set to `lp`, you can temporarily change it to `ft`, and change it back to `lp` after the examination). Type `wft2da`[ret] to generate the spectrum. The resultant 2D spectrum will be displayed (command `dconi` is used to display the spectrum, if it is not displayed already).

6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20% and -20% buttons.

7. If the peak phases are not right, follow the Phasing procedure to correct them. If the chemical shift scales are not correct, follow the Spectral referencing procedure.

8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the Expansion button.

9. To plot the 2D spectrum displayed, type `plhxcor('pos/neg',i,j,k,l,m,n)` [ret], where the first argument is used to select either the positive peaks or the negative peaks (or both when it's ignored), `i` is the number of contour levels (e.g., 20) to be plotted, `j` is the intensity ratio of every adjacent levels (e..g., 1.3), `k` is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in the F2 axis, `l` is the experiment number (e.g., 2) where the 1D carbon NMR spectrum is stored and to be used as the projection in the F1 axis, `m` is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection, and `n` is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection.

   To plot the actual projections of the 2D spectrum, use buttons `Proj`, `Hproj(Max)` or `Hproj(sum)`, `Vproj(max)` or `Vproj(sum)`, and `Plot` to plot the projections first (the amplitude of the projections can be adjusted by using the middle mouse buttons before plotting), then use the command `pcon('pos|neg',i,j)` and `page` to plot the 2D spectrum.

   - HMBC (Heteronuclear Multiple-Bond Correlation): The HSQC and HMQC experiments provide chemical shifts of those carbons that have directly bonded protons. But, there are many quaternary carbons that are also important in identifying the molecular structure. They can be obtained by running HMBC experiments, which produce peaks that correlate protons with those carbons that have long-range scalar coupling through 2, 3, or 4 chemical bonds. Therefore, by combining HSQC or HMQC with HMBC experiments, the chemical shifts of all the carbons can be obtained. It takes less machine time to run these two 2D NMR experiments than a 1D C13 spectrum. The gHMBCAD produces more uniform intensity of correlation peaks through multibond carbon-proton couplings with wider range.
Example:

**gHMBC spectrum of menthol**

How to run a HMBC experiment?

1. In exp$n$ (e.g. exp1), take a simple 1D H1 spectrum. If the spectrum shows a large regions on both sides that contain no peaks, expand the spectrum to show only the interested region, and type the command *movesw* to fix this region, and *ga* to recollect a new spectrum.
2. Go to the next experiment number by *jexpm* ($m=n+1$). If exp$m$ is not existent, type *cexp(m)* to create it.
3. Type *mf(n,m)* [ret] to copy the file in exp$n$ to exp$m$. Type *wft* to see if the spectrum is exactly what is desired. Type *gHMBC* or *gHMBCAD* [ret] to load all parameters used in the gHMBC or gHMBCAD experiment. Type *su* [ret].
4. Check the total time needed for the experiment by typing *time* [ret]. Adjust *nt* (by default, nt is set to 1, which makes the total experiment time about 5 minutes), if needed, then start the experiment by typing *go*[ret].
5. The spectrum can be examined before the data acquisition is finished, if *proc1* is set to *ft* (if *proc1* is set to *lp*, you can temporarily change it to *ft*, and change it back to *lp* after the examination). Type *wft2d*[ret] to generate the spectrum. The resultant 2D spectrum will be displayed (command *dconi* is used to display the spectrum, if it is not displayed already).
6. The amplitude of the spectrum can be adjusted with the middle mouse button in two ways: (1) by moving the mouse cursor to the spectral region and click the middle mouse button, (2) by moving the mouse cursor to the intensity scale bar located on the right hand side of the spectral region and click the middle button. Finer adjustment can be done by using the +20% and -20% buttons.
7. If the chemical shift scales are not correct, follow the Spectral referencing procedure.
8. The expansion of the spectrum can be done with the left and the right mouse buttons, which define the lower left and the upper right corners of the selected region. Once the region of expansion is selected, click on the Expansion button.
9. To plot the 2D spectrum displayed, type *plhxcor*('pos/neg', *i,j,k,l,m,n*) [ret], where the first argument is used to select either the positive peaks or the negative peaks (or both when it’s ignored), *i* is the number of contour levels (e.g., 20) to be plotted, *j* is the intensity ratio of every adjacent levels (e..g., 1.3), *k* is the experiment number (e.g., 1) where the 1D proton NMR spectrum is stored and to be used as the projection in the F2 axis, *l* is the experiment number (e.g., 2) where the 1D carbon NMR spectrum is stored and to be used as the projection in the F1 axis, , *m* is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection, and *n* is a multiplier to adjust the amplitude of the proton 1D spectrum used as the projection.

To plot the actual projections of the 2D spectrum, use buttons *Proj*, *Hproj(Max)* or *Hproj(sum)*, *Vproj(max)* or *Vproj(sum)*, and *Plot* to plot the projections first (the amplitude of the projections can be adjusted by using the
middle mouse buttons before plotting), then use the command \texttt{pcon(‘pos\neg’,i,j)} and \texttt{page} to plot the 2D spectrum.