Two dimensional experiments with VNMRF
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General remarks

This manuscript is designed to provide only the minimum information required to perform the most common routine two dimensional experiments with VnrmrJ in the NMR Facility at the University of Michigan. It doesn't include detailed information about optimization of parameters or about the NMR techniques. The user must be aware that further optimization, before and after acquisition is possible. However, Vnrmrj is very complex, with many more options and commands than a casual user would want to know, and making this manuscript more comprehensive would render it tedious for such kind of user. Extensive information can be found in the references. Please read this manuscript in its entirety before attempting to run or process your first 2D spectrum as some procedures may become clearer after you finish reading a whole section.

Acquire and optimize a proton spectrum before proceeding. If you are doing a $^1$H - $^{13}$C correlation it is also advantageous (but not required) to have a carbon spectrum. Setup your proton spectrum in workspace 1 (experiment 1), your carbon in workspace 2, and your 2Ds in workspaces 3, 4, etc. That way, you can easily switch from one spectrum to another for examination, referencing, etc. Please note that in general, in our Facility the Inova 500 is the preferred instrument for almost all 2D experiments. This is because the default probe in the Inova 500 is an “inverse” probe, i.e. it is optimized for proton detection and has better sensitivity and lineshape for protons. Nevertheless, it is still possible to get 2D correlations on the 400 and 300 MHz instruments, but they will take longer or require more concentrated samples. As a rule of thumb, at the same concentration you will require at least double the number of scans on the Inova 400 and probably four times on the Mercury 300 compared to the Inova 500. Note however that the probe on the Inova 500, tuned by default to $^1$H and $^{13}$C in CDCl$_3$, is somewhat sensitive to solvent changes. When doing a 2D on a solvent like D$_2$O, DMSO or methanol, it may be necessary to retune the probe to get the best results. Please ask the facility personnel about retuning the probe; do not attempt to do it yourself without authorization.

Acquisition

Start by running a quick 1D $^1$H spectrum in workspace 1. Narrow the spectral width to the region of interest leaving about 1 ppm margin on both sides (select the region using the cursors and type movesw) and take a new spectrum with the new parameters. Reference it with TMS or the standard that you use. You can save it to disk now. Copy it to workspace 3 (mf(1,3)). If desired, you can go to workspace 2 and get a $^{13}$C spectrum. Save it too.

Go to workspace 3 (jexp3). From the Experiments menu select the experiment that you want. In general, gradient experiments are preferred as they are faster to acquire and produce cleaner spectra, but they are also slightly less sensitive than the non-gradient versions. If your sample is concentrated enough to get a good proton spectrum with only 1 or 2 scans (at least 10 mg for a medium molecular weight organic compound) then choose a gradient experiment. If your sample is so dilute that you need 8 scans or more, then selecting a non gradient experiment may give you better sensitivity, but keep in mind that non gradient experiments require at least 8 scans to suppress unwanted signals. With less than 8 scans you will see artifacts in non gradient experiments. Gradient experiments start with the letter “G”, i.e. Gcosy, Ghsqc, Ghmqc, Ghmbc. The Mercury 300 does not have a gradients unit: select a non
gradient experiment. See the experiment selection table at the end of this manuscript for help in selecting an experiment.

In the Acquire, Defaults panel select an appropriate number of Scans per increment and a Number of increments. The minimum numbers should be 2 and 128 respectively. The number of scans should be determined by the concentration of the sample. With at least 10 mg of a medium molecular weight sample, a Ghsqc should give a decent spectrum in 15 minutes in the Inova 500 (double that time in the Inova 400). A gCOSY takes only about 5 minutes in the Inova 500. The Show Time button displays the total time the acquisition will take. If you want to increase the number of scans (to get better signal to noise), it may be advantageous to increase the number of increments instead. This will also improve signal to noise but additionally it will improve digital resolution in F1. So for example, if you have nt=2 ni=128, set ni=256 instead of nt=4. The acquisition time will increase by the same amount in both cases; it will double.

In the Acquire, Defaults panel you can also change the spectral width if not already done or, in the case of a heteronuclear experiment, the C13 Spectral Width, but the default value should be fine for most typical organic compounds. Set “Linear prediction in t1” to “default”. See Figure 1 for examples.

![Acquisition panels for Gcosy and Ghsqc experiments.](image)

Other parameters that may be changed are found in the Acquire, Pulse Sequence panel. The parameters shown depend on the pulse sequence used (Figure 1). For example, you can turn on solvent presaturation, useful with non-deuterated solvents to reduce the huge solvent peak (notice that a critical parameter is missing in the panel, satfrq, the frequency of the solvent peak). The Steady State option enables a section in the pulse sequence that destroys residual transverse magnetization before each pulse. The purpose is to reduce t1-noise (the vertical ridges of noise in strong peaks). In practice, this technique sometimes helps but sometimes it makes the spectrum worse. In general, don’t enable it unless you already took a spectrum and it has strong t1-noise. In this case, it will also help if you increase the relaxation delay d1 and the number of increments.
In the case of heteronuclear experiments you will find other parameters in this panel that can be optimized if you want. Look in Varian's “VNMRF Liquids NMR” reference manual and in the tables at the end of this manuscript for more information.

**Figure 2.** Pulse sequence parameters for Gcosy

In general, the default parameters are good enough for most routine analyses. You are advised not to change them unless you have already done several 2D experiments and have a good idea of what you are doing or unless you already took a 2D spectrum of this sample and want to improve the results.

**Figure 3.** Pulse sequence parameters for Ghscq

Start the acquisition with go or by clicking the Acquire button. Don't forget to save your spectrum after it is finished. After the acquisition starts, you can go to a new workspace, setup a new experiment and start it. The new experiment will be placed in a queue and started right after the previous one is finished.

**Processing**

When processing on a workstation, be aware of a “feature” of vnrmr. Normally, when a user double clicks on a filename in Vnrmr, the FID is loaded and “autoprocessed”. During autoprocessing the FID is not only Fourier transformed but also automatically referenced according to the solvent. If the spectrum was properly referenced before it was saved, the new reference set by the program will probably be wrong! If you want the original reference to be kept, then autoprocessing must be disabled. You can do this with `doprocess[1] = 'n'` or by deselecting “Process data on drag-and-drop from locator” from the Utilities, System Settings, Display/Plot menu. Then when you double click on a filename or open a file from the File menu the FID will be loaded but not altered or transformed, so you will have to do the Fourier
transformation, phasing, etc, after loading it.

Before continuing, please take a few minutes to familiarize yourself with the display icons:

Most of the processing operations are controlled from the Process, Process panel (See Figure 4 for reference). Two-dimensional spectra are usually preprocessed to enhance their appearance. Linear prediction is applied along F1 to reduce FID truncation artifacts and a weighting function is applied to both dimensions to improve lineshape. Click on [Set default t1 Linear prediction] to calculate its parameters. Click on [Full 2D Transform] to do the Fourier transformation. The 2D spectrum should be displayed. If you get an error message similar to “scale outside boundaries…” go to the Process, Display panel and click on “Screen position” Full. Homonuclear spectra can be symmetrized along the diagonal with the foldt macro to help eliminate t1 noise but this can also reduce real peaks, so beware. If the baseline of the traces is not flat, clicking on [BC F2] or [BC F1] buttons in Process, Display panel may help to get a better looking spectrum by applying a baseline correction along the desired dimension.

Figure 4. Process panel for Ghsqc

Referencing. If you have the proton spectrum in workspace 1 and it is properly referenced, you can set the proton dimension of your 2D to use the same reference with \texttt{mref(1,3)}. The second dimension can then be referenced with \texttt{reff1}. Alternatively, you can choose a cross peak and use it to reference both dimensions. Expand the region around a cross peak whose chemical shifts in both dimensions are known and place the cursor in the center (Figure 5). It is better if you have real contour displayed (Process, Display, “2D Contour Display” buttons).
In the example below, the chemical shifts should be 5.030 ppm in F2 (proton) and 68.01 ppm in F1 (carbon). To set these chemical shifts type: rl(5.03p) rl1(68.01d). Or, enter the appropriate values in the left side column of the Process, Display panel.

Figure 5. Setting the reference in a 2D spectrum.

Phasing. Some 2D spectra like COSY, CIGAR and HMBC are transformed in “magnitude mode”. The peaks in these spectra are always positive and do not require further adjustment. Other spectra, like HMQC, HSQC and NOESY, are acquired in “phase sensitive” mode and may contain both positive and negative peaks. If your spectrum contains cross peaks that look like comets, i.e. the cross peaks have tails on one or two of their sides, it probably requires phasing (See Figure 6).

NOESY spectra generally require phase correction. To phase a NOESY spectrum, first make sure F2 (the vertical trace in Varian’s default display mode) is accurately phased. Click on the [FT 1D-1st Increment] button on the Process, Transform panel and phase this 1D spectrum carefully; increase the intensity a lot and make sure the baseline around the peaks is phased (if the baseline is not flat, correct it with “bc”). Retransform the 2D spectrum. Additionally, NOESY spectra usually require a phase correction in F1 (the horizontal trace). Apply the correction with rp1=rp1+45 and then redisplay the spectrum with the “redraw screen” icon.

To phase a other 2D spectra like HMQC or HSQC or to fine adjust a NOESY you must select two horizontal slices, one containing a peak on the right side of the spectrum and the other containing a peak on the left side. Notice that, due to a bug, with version 1.1D of Vnmrj the procedure that follows works only when the complete 2D spectrum is displayed; i.e. it does not
work with expansions. Vnmrj 2.1B does not have this problem. If you get the error “Cannot phase this data”, type pmode='full', retransform the spectrum and start the phase correction procedure from the beginning.

In the procedure that follows, it is imperative that you understand the results expected for a given experiment and phase your spectrum accordingly. For example, individual cross peaks in both HSQC or HMQC experiments should be completely positive or completely negative but some cross peaks can be positive while others may be negative in the same spectrum (Fig 6); cross peaks in Dqcosy experiments will have both positive and negative components (for example, a peak in a doublet will be positive while the other may be negative); and in a NOESY experiment the diagonal should be negative, positive cross peaks indicate positive NOE’s and negative cross peaks are either negative NOE’s or exchange correlations. For NOESY spectra, the opposite convention is also used, i.e. the diagonal is phased positive. In this case, what is important is the relative sign of the cross peaks, their absolute sign is irrelevant.

![Figure 6. A gHSQC spectrum that is out of phase (left) and the properly phased spectrum (right). Warm colors represent positive cross peaks and cold colors represent negative ones.](image)

You should also know that the weighing functions normally applied to these experiments can result in artifacts that appear as negative or positive ridges on both sides of diagonal and cross peaks. While these artifacts are unavoidable, they may be minimized by proper adjustment of the weighing functions.

1. Click on the phase correction icon
2. Move the cursor until you get to a horizontal trace with a strong peak upfield
3. Click on the “select upper right peak” icon
4. Move the cursor until you get to a horizontal trace with a strong peak downfield
5. Click on the “select lower left peak” icon
6. Click on the phase correction icon again. If you get the error “Cannot phase this data”, type pmode='full', retransform the spectrum and start the phase correction procedure from the beginning.
7. Click on the “select upper right peak” icon and click and drag on the rightmost portion of the spectrum to phase it. If there are no peaks downfield do not click or attempt to phase the left side of the spectrum.

8. Click on the “select lower left peak” icon and click (but do not drag) the rightmost portion of the spectrum (this prevents VnmrJ from changing the phase correction found in step 7). Then click and drag on the leftmost side of the spectrum to phase this side. You can repeat steps 7 and 8 to verify (or fine adjust) that whole spectrum is in phase.

9. Click on the return icon twice to get to normal 2D display mode.

10. Check that your spectrum is properly phased along the horizontal and repeat the procedure to make adjustments if necessary.

11. Check the phase correction along the vertical axis. If it shows signs of being out of phase, click on the “rotate along diagonal” icon and repeat this procedure. When you are done, click on the rotate along diagonal icon again to go back to the usual (Varian's) display convention.

### Plotting

After you have selected and zoomed in the region of interest, you must choose the intensity threshold. Use the “increase intensity” or “decrease intensity” icons to set an intensity just above the noise level. You can also use the color scale on the right of the spectrum for this purpose (click with the middle button). After this you must select how many contours you want displayed and their separation. Use the command `dpcon` to do it. `dpcon` takes two arguments; the first is the number of contours and the second is the separation (a number, usually between 1.0 and 2.5). If you are plotting a large area of the spectrum the cross peaks will be very small and you won't be able to see too many contours (try 5); if you want to expand a small region you will get a better looking spectrum if you use many contours (try 12 to 16). Start with `dpcon(5, 1.5)` and change the parameters until you get a spectrum in which all your peaks are well defined, with contours that go from the base (just above the noise level) all the way to the top. You will use these parameters in the plot command.

In order to differentiate positive from negative cross peaks, spectra are plotted in black and gray. The default is black for positive peaks and a shade of gray for the negative ones; the exact “color” or shade is selected with the `color` command. If you get everything in black type “maxpen=8” and try again.

For homonuclear experiments the plot command is, for example, `plcosy(5, 1.5, 1)` or `UMplcosy` (see below) where the first two parameters are the ones you found with `dpcon` and the third is the workspace number where the high resolution 1D proton spectrum is located.

For heteronuclear experiments the plot command is, for example, `plhxcor(5, 1.5, 1, 2)` or `UMplhxcor` (see below) where the first three parameters are the same as in the `plcosy` example and the fourth is the workspace where the high resolution 1D $^{13}$C spectrum is located. If you don't have a carbon spectrum omit the fourth parameter. It is also possible to “extract” a low resolution carbon spectrum from the 2D (a projection) and use it to plot it alongside the 2D.

Notice that the `plhxcor` macro supplied by Varian requires that all spectra had been acquired on the same spectrometer, otherwise the chemical shifts won't match. This makes sense as a 1D spectrum measured at a different field may look different. It will certainly be the case for proton spectra because their splitting patterns are so sensitive to field strength. In our lab this is inconvenient as the most sensitive spectrometer configuration for C13 is the Inova 400 while the most sensitive (and recommended) for heteronuclear 2D correlations is the Inova 500. To
provide more flexibility, the macro plhxcor was modified in all the computers in the lab so that it is now possible to plot a C13 spectrum collected on the Inova 400 along a 2D collected on the Inova 500. Keep in mind that this is not formally correct as you are displaying and comparing spectra at two different fields. However, in the case of natural abundance C13 spectra and other nuclei lacking homonuclear coupling, and for appearance purposes, it may be acceptable.

There are other problems with Varian’s macros plcosy and plhxcor. They do not allow saving the 2D plot as an image, to easily import it into a presentation software like PowerPoint for example. They do not allow to adjust the intensity of the high resolution 1D plots. This is a problem when the region of interest contains both very tall (like methyl groups) and short peaks; the intensity is automatically adjusted to the tallest peak and the other signals may appear very weak. And if the spectral region of the 2D plot is not completely covered in the high resolution spectrum, the scale of the latter will not match that shown in the 2D plot. (This may be considered a bug; the 1D spectrum is still plotted, but the scale will be wrong. It happens for example when the 2D spectrum goes from 10 to 0 ppm but the 1D spectrum only goes from 8 to 0 ppm.) All these problems have been corrected in the macros UMplcosy and UMplhxcor and they work the same way as plcosy and plhxcor with the following 2 differences:

- Before using them, go the workspace(s) containing the high resolution spectra and adjust the vertical intensity the way you want it to appear along the 2D.
- The UM macros require the page command to send the plot to the plotter as usual or the UMmailpage command to capture the plot as an image file and send it to you via email. This command can also adjust the thickness of the plot, if desired, to make it look bolder. When used without arguments, UMmailpage will ask the required parameters (a file name for the image, an email address where to send the image to, and the linewidth of the plot in pixels, try 2). Otherwise, indicate the parameters in the command: UMmailpage('filename', 'email_address', 2).

If you want to plot a grid in your spectrum, use the plgrid command after the UMplhxcor or UMplcosy commands, for example: UMplhxcor(5, 1.5, 1, 2) plgrid UMmailpage.

If you want to plot in color see the “Frequently Asked Questions” document in the documentation section of our web site (http://www.umich.edu/~chemnmr/docs/faq.pdf). Plotting in color not only produces better looking spectra, but also displays important information. For example positive and negative enhancements have different signs in NOESY spectra, and methyl and and methylene groups also have opposite signs in Ghsqc spectra. Although our laser printer only prints shades of gray, you can produce an image in color and either display it in your presentation, insert it in a document or print it in a color printer.

Experiment selection guide

**Homonuclear correlations**

<table>
<thead>
<tr>
<th>Name</th>
<th>Remarks</th>
<th>Adjustable parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosy, Gcosy</td>
<td>Classical correlation experiments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Magnitude mode. Very easy to run.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variations are possible: COSY-45, COSY-LR.</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Remarks</td>
<td>Adjustable parameters</td>
</tr>
<tr>
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<td>--------------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Dqcosy,</td>
<td>Double Quantum filtered Correlation Spectroscopy. Phase sensitive.</td>
<td>Mixing time can be 0.1 s to 0.3 s for large</td>
</tr>
<tr>
<td>Noesy</td>
<td>Dipolar (through space) correlation experiments. Phase sensitive.</td>
<td>Mixing time can be 0.1 s to 0.3 s for large</td>
</tr>
<tr>
<td>Roesy</td>
<td>Rotating Frame Overhauser spectroscopy. Phase sensitive.</td>
<td>Mixing time 30 - 500 ms.</td>
</tr>
<tr>
<td>Tocsys</td>
<td>Total Correlation Spectroscopy (HOHAHA; homo-nuclear Hartmann Hahn).</td>
<td></td>
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</tbody>
</table>

**Heteronuclear correlations**

<table>
<thead>
<tr>
<th>Name</th>
<th>Remarks</th>
<th>Adjustable parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsqc,</td>
<td>Heteronuclear single bond correlation. Phase sensitive. Cross peaks in</td>
<td>The average value of the one bond coupling</td>
</tr>
<tr>
<td>Ghsqc,</td>
<td>Hsqc are multiplicity edited by default; CH and CH$_3$ are positive and</td>
<td>constant can be optimized if necessary. The</td>
</tr>
<tr>
<td>Hmqc,</td>
<td>shows correlations through 2 and 3 bonds (C-C-H) and (C-C-C-H). Useful</td>
<td>default is 140 Hz.</td>
</tr>
<tr>
<td>Ghmqc</td>
<td>for assignment of quaternary carbons or tertiary amines.</td>
<td></td>
</tr>
<tr>
<td>Hmhc,</td>
<td>Heteronuclear Multiple Bond Correlation. Phase sensitive. Shows</td>
<td>The average value of the 2,3 bond coupling</td>
</tr>
<tr>
<td>Ghmhc</td>
<td>correlations through 2 and 3 bonds (C-C-H) and (C-C-C-H). Useful for</td>
<td>constant can be optimized. The default is 8 Hz.</td>
</tr>
<tr>
<td>Cigar2j3j</td>
<td>assignment of quaternary carbons or tertiary amines.</td>
<td></td>
</tr>
<tr>
<td>Hsqctocsy</td>
<td>Heteronuclear multiple bond correlation with tocsy coherence transfer.</td>
<td>Same as hsqc and tocsy.</td>
</tr>
<tr>
<td>Hmqctocsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Remarks</td>
<td>Adjustable parameters</td>
</tr>
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<tr>
<td>CH–H</td>
<td>Useful for interpretation of crowded proton spectra.</td>
<td></td>
</tr>
<tr>
<td>Hetcor, coloc</td>
<td>Heteronuclear correlation. Uses direct $^{13}$C detection and it is therefore much less sensitive than newer methods. Obsolete, do not use unless you have a very good reason.</td>
<td></td>
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<tr>
<td>Inadequate</td>
<td>Homonuclear $^{13}$C–$^{13}$C correlation. Extremely insensitive. Use only with $^{13}$C-enriched compounds.</td>
<td></td>
</tr>
</tbody>
</table>

References

6. Varian, Inc. VNM RJ online documentation. [http://www.umich.edu/~chemnmr/docs.html](http://www.umich.edu/~chemnmr/docs.html)