

## Frequently Asked Questions (FAQ)

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The following notes are applicable to the current software and setup in our lab with RedHat Linux 4.8 and 5.4 and VnmrJ 2.2C and 2.2D.

### How can I import a spectrum into a Microsoft Word or PowerPoint document?

Choose one:

- 1) One possibility is to transfer the FID to a computer in the NMR Lab or in your own lab and process it with **MestReC** or its newer version **MestReNova**. MestReC or MestReNova is an NMR processing program for the Microsoft Windows XP operating system and also for Linux and MacIntosh OS. It is easy to annotate spectra and to paste chemical structures from ChemDraw. The transformed spectrum can simply be copied from MestReC and pasted into your Word or PowerPoint document. You will need to learn how to do some basic processing procedures with this program. Notice that a spectrum pasted in this manner can be very large as it actually contains all your nmr raw data and your document can become very big. If you need to insert several spectra, especially 2D spectra, a better size-efficient approach might be to convert the spectra to other formats like pdf, or jpg.

A single user license costs from \$165 to \$360 depending on its type. The University of Michigan's Chemistry Department has purchased a ChemOffice site license from CambridgeSoft, and users in the Department can download and install full versions of MestReC as well as ChemOffice. The NMR Facility has a single user license of MestReNova that can be used by any user of our lab. It is installed and available in one of our workstations (Copper) along with ChemOffice. MestReNova is a very nice alternative to vnmrj, with many different processing, displaying and plotting options and with a modern user interface. For example, the baseline correction routines for 2D spectra are simply excellent. If you anticipate doing many 2D spectra of diluted samples, or need more than just simple routine 1D spectra, the exploration of MestReNova will be worth your while.

- 2) Another possibility is to use Vnmrj to produce a postscript file or an image of your plot and import it into your document. The postscript file can be easily imported into Word or PowerPoint if it has the file name extension .eps (for encapsulated postscript). You can simply drag the file's icon into your Office document and drop it in the desired location. After that, you will probably have to change its size and other properties. The spectrum will look great when printed, but unfortunately it won't look that great on the computer's display (or on the projection screen). This is due to the way Microsoft's applications display embedded postscript files and to the way Varian produces its plots in postscript; other programs may differ. (Perhaps a better alternative is to produce an image of the plot file, keep reading.) You will have to create and transfer your postscript files, over the network via sftp (secure file transfer protocol) or with a flash drive, to your Windows computer. To help with these issues a new macro, **UMmailpage**, was developed. This macro was designed to be used as the page macro, i.e. first you build your plot with plotting macros like "pl pscale pir" or by clicking buttons on the **Process**, **Plot** panel, and then you type **UMmailpage**. The macro will save the postscript file, convert it to a png (portable network graphics) file and send both files via email to you. The png file is a bitmap file similar to the most common jpg format but with some additional advantages and can be imported into Microsoft documents in the usual fashion by dragging and dropping or selecting **Picture** from the **Insert** menu. The png file is created in high resolution and with reasonable compression and will look well both printed and displayed. It can

be edited with a graphics program like Photoshop to insert legends or chemical structures. When executed, `UMmailpage` will ask you for a file name for the pdf and png files, your email address and a line width for the plot. The default line width, 3 pixels, produces very thin lines which may be difficult to see from the distance or when the plot is reduced in size. For presentations, a line width of 5 or higher is recommended. If you are working with a 2D spectrum, use the commands `UMplcosy( )` or `UMplhxcor( )` instead of `plcosy( )` or `plhxcor( )` and then type the `UMmailpage` command. Notice that the plot may be in color or in shades of gray depending on the current palette being used.

## How can I plot in color?

Although the printer in the NMR Lab can only print in black and shades of gray, it is still possible to create file plots and images in color for printing in other printers and for presentations. Color can add information to a plot. For example, in edited gHSQC spectra, methyl and methine groups are positive while methylene groups are negative; and cross peaks arising from direct NOEs in NOESY spectra have the opposite sign as those arising from chemical exchange processes. It is customary to plot these spectra in different colors (or shades of gray) to show the relative peak's intensities.

`Vnmrj > 2.2` allows changing the colors of individual components in a plot. The “Styles and Themes” dialog window, accessed from the `Edit > Display options...` menu permits changing the colors in a plot. Unfortunately, this window controls both *display* and *plot* colors as well as many other attributes like the fonts, of `vnmrj`'s interface. In `vnmrj`, the spectrum's colors shown on the screen are the *display* colors and not the *plot* colors which are completely separate things. In other words, the colors you see on the screen are not the final colors of the plot. To avoid confusion, plot attributes should have been controlled separately from display preferences, but in Varian's wisdom they are controlled from the same place.

All colors and attributes can be saved together in a “Theme”. Perhaps the easiest way to change colors is to select a predefined theme. Besides Varian's supplied themes, two basic themes have been created in our lab: `UM_color` and `UM_grey`. They can be selected from the **Theme** drop down menu at the top of the window (select a theme and click on [Load]). `UM_color` plots 1D spectra with a black spectrum, blue scale and green integral and parameters; and positive cross peaks in 2D spectra are plotted in red while negative cross peaks are plotted in blue. `UM_gray` (the default) plots all elements in a 1D spectrum in black while positive cross peaks in 2D spectra are plotted in black and negative cross peaks are plotted in gray.

The colors can be viewed or modified in two of the 14 tabs in the “Styles and Themes” window. The colors used for plotting of 1D spectra are located in the “DisplayColors” tab and those for 2D spectra in the “2DColors” tab (see the two figures below). The drop down menu controls the context of the colors shown below it. When it is in “Display” the colors shown will be used for displaying the spectrum on the screen, and when “Plot Postscript” is selected, the colors will be used for plotting to postscript printers (our case at UM's NMR Facility). The other two choices, “Plot PCL” and “Plot HPGL” are for printers that don't speak postscript and for real pen plotters. You can change the color of any element in the plot by selecting from the corresponding drop down menu or by clicking the little color button on its right. The colors under the “Spectroscopy” and “Imaging” sections in the DisplayColors tab and under “Peak Color” on the 2DColors tab are used only on the screen display and not for plotting (!). In addition, for 2D spectra you also have to select with the buttons [Absolute] and [Phase] the type of spectrum that the colors will be used with. Absolute mode 2D spectra contain only positive peaks and phase sensitive spectra may contain both positive and negative. Common

examples of absolute mode spectra are COSY, gradient COSY, TOCSY, HMBC, gradient HMBC and CIGAR; examples of phase sensitive spectra are NOESY, ROESY, DQF-COSY, HSQC, HMQC and HSQCAD.



You can change the colors of individual contours for the cross peaks if you wish. And you can save your own set of colors in a new theme by typing a new name in the theme drop down menu and clicking on [Save]. Your new theme will be used in all further vnmrj sessions until you load a new theme or change the current one. A color plot file can then be produced with the UMmailpage macro as described in the previous section of this document. Notice that if you plot in color to a black & white laser printer like the one in our lab, the printer will decide how to translate your colors to shades of gray.

**NOTE:** The following procedure is applicable to vnmrj versions prior to 2.2. Since all our workstations have vnmrj 2.2 or higher, this is no longer applicable to our facility, but it is kept here to help users in other facilities.

To plot in color you have to load a color map. Two basic color maps have been created: "color1" and "gray1"; the default is gray1. To load a map, type the command `loadcolors('map_name')`. You will not see the difference on the screen until you refresh it by clicking on the Refresh button on the vertical tool bar or with the `df` or `dcon1` commands for 1D and 2D spectra respectively. Use the UMmailpage macro as described earlier in this document under "How can I import a spectrum into Microsoft Office documents" to generate a plot file (in postscript) and an image that you can use to print to your own printer or to import into your documents. Notice that screen colors are separate from plot colors in the maps; the colors on the plotted spectrum may not be the same shown on the screen. Use the `colors` command (see below) to see the colors used for plotting. Gray1 plots in shades of gray (black for positive cross peaks and gray for negative). Color1 plots in black and gray for 1D and magnitude mode 2D spectra (COSY, HMBC, etc) and in red (+) and blue (-) for phase sensitive 2D spectra (HSQC, HMQC, NOESY, etc). You can also edit and create your own maps with the `colors` command. For example, if you don't quite like the colors of the color1 map, type `colors('color1')`. The color editor will start with that map preloaded and you can modify it and save your map. This editor is not exactly very friendly and you will have to play with it a little to get used to its idiosyncrasies. Two important points to keep in mind are: 1- if you use the editor to load a map, it doesn't refresh the screen when you exit and you won't see the new map until you refresh it yourself. 2- If you run the editor without indicating a color map, i.e. with the `colors` command, it will always load the default map which in our lab is called `hp1j2300d_plot` (this map is identical to gray1). Therefore, if you made changes to the colors but didn't save them to a map file, the next time you run `colors` these changes will be lost. Also, every time you start Vnmrj, the default map is loaded; vnmrj will not remember what map you used last time. Evidently the use of colors is not very important according to Varian's wisdom.

## How can I transfer files to another computer in the Department?

For security reasons, file transfers are allowed only to or from other computers in the Chemistry Department and some other Departments in the UM campus. Use any secure file transfer protocol program (sftp). Your vnmr spectra are located in the `/home/your_login_name/vnmrsys/data` directory. Each spectrum is contained in a folder or directory with a name ending in ".fid". You must transfer the whole directory. These folders contain the raw (free induction decay or FID) data and will need processing by vnmrj or any other NMR processing software before you can use see the actual spectrum.

## How can I copy or read files to/from a USB Flash drive?

Insert your flash drive into a USB port. Linux should recognize it and open a File Manager window showing its contents. Open another File Manager and drag and drop files between the two windows.

## How can I copy files to a CD or DVD?

Each computer in the lab has a program called **k3b** that works like similar CD-burning programs in the MS-Windows environment. Essentially, you build a list of the files you want to copy by dragging and dropping, and then give the burn command. Ask the Facility's personnel if you need further assistance.

You can use CD-RW, CD-R, DVD+RW or DVD-R disks, but CD or DVD drives are sometimes picky and may not work with a particular brand or disk property (like its maximum writing speed).

## How can I read files from a CD or DVD in Linux?

Drop it in the CD drive (the lower tray) or the DVD drive (upper tray). Linux should recognize it and mount it in `/cdrom`, `/dvdrom` or on `/media`. To load a spectrum in vnmrj, use the *File, Open* menu and type either `/cdrom` or `/dvdrom` in the Directory input field of the dialog window that should appear.

## How can I increase the line width of a plot?

Instead of issuing the `page` macro at the end of your plot sequence, use `pagelw(n)`, where  $n$  is the line width in points. The default is 3. A value of 3 or 5 will be suitable for presentations and for bolder looking spectra. Notice that this macro was custom-made in our Lab and is not part of Varian's standard library. Also, our printer in vnmrj has been configured at a resolution of 600 points per inch.

## How can I plot an inset on a spectrum?

Unfortunately, this feature in vnmrj 2.2 is broken. In the mean time, you can do it the hard (manual) way. First, plot your spectrum as usual with `pl p scale pir`, etc, or by clicking buttons on the **Process, Plot** panel, but do not type the `page` command or click on **[Plot Page]**. Second expand the region that you want to plot as an inset. Now you will have to calculate and set the parameters `wc`, `sc`, `vp` and `vs`. `wc` is the width of the inset in mm; `sc` is the horizontal position of the right side of the inset relative to the right side of the already plotted spectrum in mm, `vp` is the vertical position of the

inset relative to the baseline of the already plotted spectrum in mm, and  $v_s$  is a number that is related to the tallest peak in the spectrum. The easiest way to set  $v_s$  is interactively by dragging a peak in the inset with the mouse's middle button. For example,  $w_c=50$ ,  $s_c=120$ , and  $v_p=80$  will make an inset 5 cm wide, starting at 12 cm from the right (and plotting towards the left of the page) and 8 cm above the full spectrum. Now type `pl p scale` to plot the inset. More insets can be made by repeating the procedure. Finally, the `page` command will print everything.

The following procedure is for previous versions of `vnmrj`.

1. Display your spectrum and plot it as usual (e.g. `pl p scale pir`) but do not include the `page` command yet. You can also go to the **Process, Plot** panel and click on the buttons [Plot Spectrum], [Plot Spectrum Scale], [Plot Parameters], etc., but do not click on [Plot Page].
2. Position your cursors around the region you want your inset to be plotted (not the region of the spectrum you want to expand) and type `inset`. (Or to the **Process, Cursors/Line Lists** panel and click on [Inset spectrum], [Plot inset], [Return from inset]) The full spectrum remains on screen but is inactive and your new inset will appear on top. Mouse buttons affect the inset: left button moves the inset horizontally, right button changes its width and middle button changes the spectrum's intensity. The inset's vertical position is controlled by the  $v_p$  parameter, whose value appears in the lower left corner of the spectrum's display. Type a new value if desired (distance in mm from the baseline of the full spectrum).  
You can also fine adjust the spectral region displayed in the inset. Click the *Pan & Stretch Mode* button (the icon with the little hand) on the vertical tool bar. Now the mouse's buttons have new (and unfortunately not so easy to use) properties. Left click and drag changes the spectral region displayed in the inset. If you want to move past the edge of the displayed spectrum, first drag the spectrum to the left or right edge of the inset, release the left button, right click (release the right button without dragging) on the other edge of the inset, and continue left dragging to the desired region. When you right click on the inset, a red horizontal line appears. Right dragging with the mouse below this line expands the spectrum, while dragging upwards contracts it. Move the mouse slowly as this operation is fairly sensitive to its movement. Practice a few times and you will get used to these procedures.
3. When all the parameters are the way you want, plot the inset either from the command line (e.g. `pl p scale`) or by clicking on the appropriate buttons on the **Process, Plot** panel.
4. You can plot more insets by clicking the *Display Full Spectrum* button [`<`] and repeating steps 2 and 3 but you won't be able to see the previous insets and will have to keep in mind their approximate positions so they don't overlap. For this reason, a practical limit is around 4 insets depending on your visual memory.
5. When you are finished, type the plot command (either `page`, `pagelw` or `UMmailpage`).

If you want to cancel the plot and start all over, type `page ('clear')`. If your display was also messed up, type `full f vp=12`.

### **How can I obtain accurate integrations in proton spectra?**

For accurate integration the digital resolution should be very good. Each peak should be covered by at least four acquired data points in order to properly represent its area. For this reason it is usually a good idea to increase the acquisition time to 5 or 6 seconds for  $^1\text{H}$  NMR. The recycle time (the time between pulses; usually  $d1+at$ ) is also important. When using 45 degree pulses (the default) the recycle time should be at least  $3T_1$  of the slowest relaxing nuclei. Medium size organic compounds have  $T_1$  values from 0.1 to about 3 seconds. Therefore you may need to increase the relaxation delay  $d1$  to increase the recycle time if slowly relaxing signals like methyl groups are important for your experiment. If you are using some kind of internal reference for integration, for example a known concentration of a small molecule like acetone, TMS or dioxane, it is especially important to use long recycle times since the relaxation times in these small molecules can be very, very long (perhaps 5 to

15 seconds). Use for example `at=5` with `d1=5` or even `d1=20` if a small molecule is present. For a quantitative integration, the spectrum should be centered in the region of interest and you should leave *at least* 15% of free baseline around both sides of the spectrum. For example, if the peaks in your proton spectrum go from 1.1 to 7.8 ppm, you won't need the region from 10 to 14 ppm or from 0 to -2 ppm that is present in the default parameters. Place one cursor on about 9.5 ppm and the other on -0.5 ppm and type `movesw` to select this region. Take a new spectrum to verify that all new parameters are fine.

Before Fourier transformation, set `lb=0.5` and `fn=np*4` to slightly decrease the spectral noise and to increase the digital resolution so the peak's shape and area are more accurately defined. For accurate integration, a very straight baseline is essential and some sort of baseline correction is usually needed. To do this, Fourier transform the spectrum with `wft` and adjust its phase carefully. Display the integral trace and define integral regions on **all** peaks in the spectrum, even on impurities and other peaks you are not interested in. Apply a 5<sup>th</sup> order polynomial correction with the command `bc(5)`. If you have an array of spectra, use `UMbc(5)` instead. Sometimes a spline function gives better results; try for example `bc(1,20,8,2)` or just plain `bc`. Consult the *Command and Parameter Reference* manual and experiment with the parameters a little until you get a straight baseline. After this, if you want you can reset all integral regions with `cz` and define new regions for only the peaks of interest. Increase the intensity of the spectrum until you can clearly see the noise and greatly expand the region of interest. Set the start and end points for each region just before and after the base of the peak starts to raise from the baseline. MestReNova, and maybe MestReC has a very nice baseline correction routine that works very well.

### **How can I correct the baseline of my spectrum?**

If the baseline distortion is mild, use the `bc` command as described under “How can I obtain accurate integrations in proton spectra?”.

If the baseline distortion is severe, as it frequently appears in some heteronuclear spectra like <sup>195</sup>Pt or <sup>199</sup>Hg spectra, backward linear prediction is more appropriate.

### **Why some computers in the lab appear to be frozen when opening Vnmrj?**

Vnmrj uses a database that is updated when Vnmrj is loaded. Since there are thousands of files in all the users directories, this updating may take a long time. There is no good reason for this database to be hard wired into Vnmrj, but that is the way Vnmrj was designed by Varian and we have no choice. It will help if you remove unneeded files from your data/ directory.

**Update:** Vnmrj versions 2.2 and higher allow the user to disable the use of this annoying database. All user accounts in our lab have this option disabled, allowing much faster start up.

### **Why does my spectrum look different on the spectrometer's display than when I load it in a workstation?**

Autoprocessing is probably enabled. Autoprocessing is a “feature” that automatically processes and displays the spectrum when you open an FID in vnmrj. It is nice because you see the resulting spectrum immediately, but unfortunately it not only does the Fourier transformation but also some other potentially undesirable and completely unneeded processing. Among these, it creates integral

regions (usually inappropriately) and performs a baseline correction based on them. It also readjusts the chemical shift scale, which, if the spectrum was properly referenced before saving, may lead to completely wrong chemical shifts!. For this reason, automatic referencing has been disabled permanently in the macros in our NMR Lab. It is therefore recommended to disable autoprocessing in every computer that you use in the NMR Lab and in your own lab. The only disadvantage is that you have to manually do a Fourier transformation and maybe other processing after opening a file but your spectrum won't be changed in any way.

To disable this feature, select the *Edit, System settings* menu, click on the *Display/Plot* tab and disable the "Process data on drag and drop from locator" option.

### **How can I change my password?**

In a terminal window type `passwd` and follow the instructions. You will need to do this in all computers in the lab.

### **I forgot my password, can it be recovered?**

No. So please don't forget it! However, we can reset it to a new password. **Update:** University of Michigan users can now use their UMICH (kerberos) passwords in the NMR lab's computers.

### **Are my spectra safe? Are there backups of my spectra in case of a hard disk crash?**

Since September 2009, we have implemented an automatic backup system where all the users' data in every spectrometers in the lab is backed up daily to a dedicated hard disk in one of our workstations. But if your data is very valuable, it is a good idea to keep a copy with you. It is also an excellent practice to hand in a copy of your spectra to your supervisor on a CD or DVD when you are about to leave the University. Search in this document about how to copy files to CD or USB flash drives.

After a period of inactivity of one or two years, it is assumed that a user is gone from the University and his/her account is removed from our computers. However, his/her data is usually stored in a CD before destroying the account. Ask the staff for assistance if old data is required.

### **I erased a file or spectrum by mistake, can it be recovered?**

If you dragged it into the Trash Can on the desktop, it is probably still there. Open the Trash Can (double click) and look inside. If you removed it from the command line, it is gone forever... But if the file stayed in the computer for at least a day after you acquired it, there is a good chance that a copy may have been saved by our daily backup system. Ask the staff for help.