Introduction to Data Processing and Analysis using Mnova NMR in NMRBox

Modern NMR spectrometer systems include host computers with software provided by the manufacturers (mostly Varian/Agilent and Bruker) for acquiring, processing, displaying and analyzing NMR data. Nevertheless, there are many other software packages distributed by academic and commercial sources for processing, displaying and analyzing NMR data that may be used independently of the spectrometer ("NMRPipe", "NMRDraw", "Sparky" and "NMRFAM-Sparky", "NMRView", "Mnova NMR", "iNMR", "Felix NMR", "Nuts", to name just a few). These each have their strengths and weaknesses, and some were designed and are used for only very specific tasks ("Sparky", for instance, was designed for assigning resonances of proteins and other macromolecules).

Here we will use the very popular software package Mnova NMR for introducing NMR data processing, analysis and display as well as simple simulations. This program is a product of Mestrelab Research (http://mestrelab.com/). This company has a number of other software packages for various uses. It also has some nice free software for various NMR-related purposes, such as analyzing chemical exchange (EXSYCalc), predicting vicinal coupling constants (MestReJ) and for simulating spectrometer operation (MestReS). A number of helpful video tutorials are also available on the Mestrelab web site ("Resources"), some of which are reasonably useful.

The tutorial that follows includes elements specific to the use of Mnova in NMRBox. It assumes you have a NMRBox account, that you have installed the VNC Viewer (required for accessing the NMRBox), that you know how to transfer files to/from your NMRBox server, and that you know how to use a 'Terminal' window for using the UNIX/LINUX command line.

So, you need to do the following before beginning the Mnova tutorial:

1) The first page of the '**NMRBox - creating an account, logging in, transferring files**' tutorial tells you how to go about registering for an NMRBox account. You need an account before you can do the 'Mnova' exercises. Because it may take up to three business days for your account to be approved after you register, you need to register first. In the meantime, while you are waiting for your account to be approved, you can install the VNC viewer (see step 2).

2) Install VNC Viewer (see the '**Installing the VNC Viewer**' tutorial on the course website)

3) Once you have an NMRBox account, you can continue with the '**NMRBox - creating an account, loggin in, transferring files**' tutorial to learn how to log in to your NMRBox account using the VNC Viewer, and how you can transfer files and folders between your computer and your NMRBox account. You should complete that tutorial before proceeding to step 4.

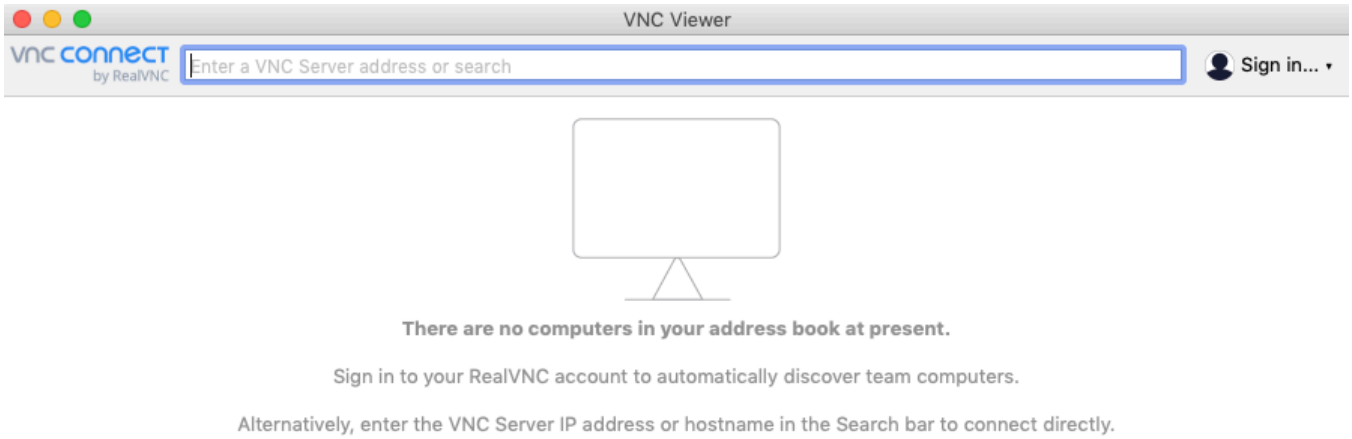
4) Once you've completed step 3, you can proceed (below) with the Mnova tutorial ('**Intro to data processing, weighting functions - Mnova**').

Finally, the complete manual for Mnova can be found at the following link:

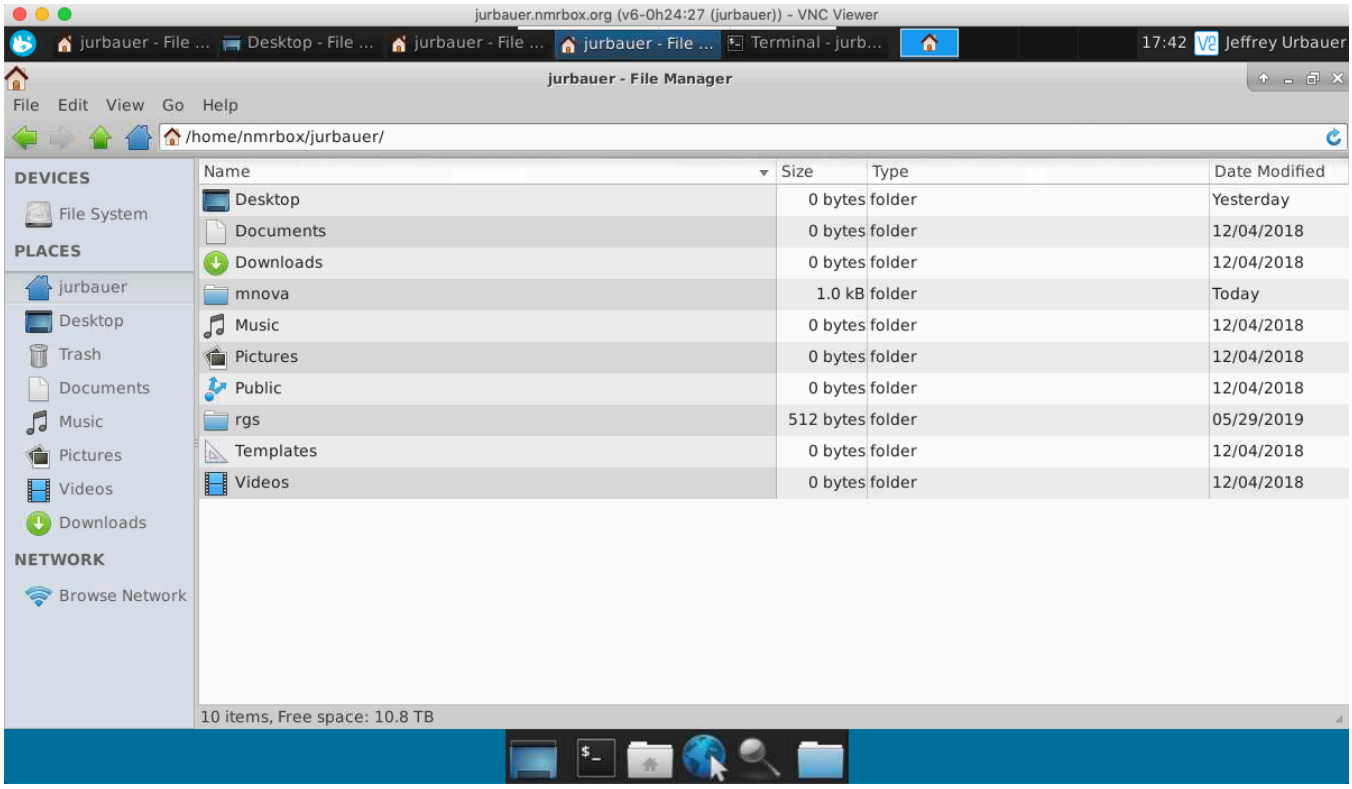
https://mnova.pl/files/download/MestReNova-12-0-0_Manual.pdf

Log into your NMRBox account and locate the data for the Mnova tutorial

Start the VNC viewer and enter the address of *your* NMR box account (username.nmrbox.org, for instance) in the window ('**Enter a VNC Server address or search**').

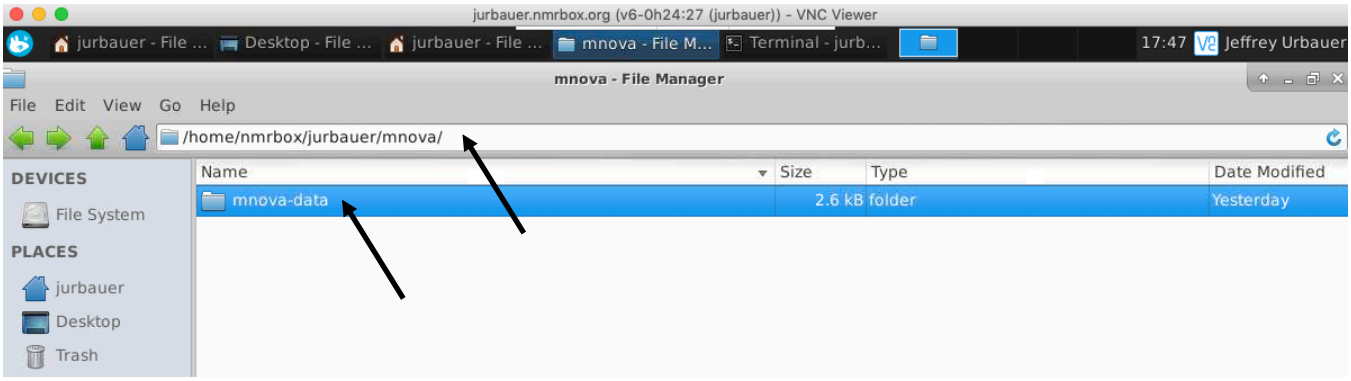


The main NMRBox window will then open. Here (below), is shown the home directory for the user 'jurbauer'.

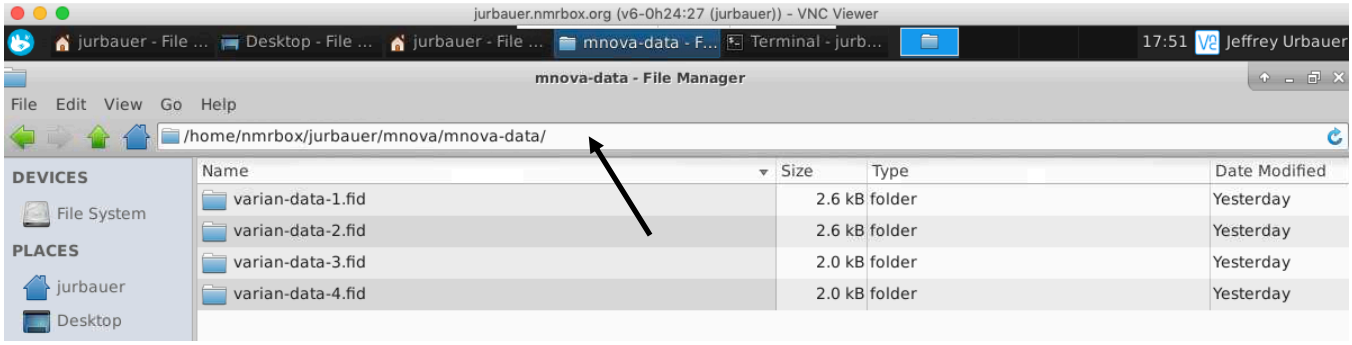


In the home directory we see the 'mnova' directory that we created in a previous tutorial for use here in the Mnova tutorial.

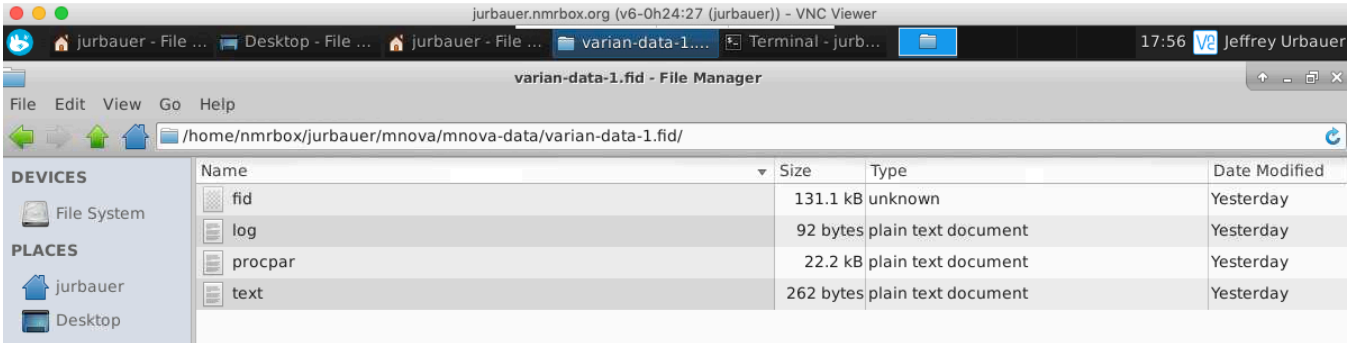
We can double-click on the ‘mnova’ directory to see a subdirectory ‘mnova-data’.



In the ‘mnova-data’ subdirectory are four folders/subdirectories that contain NMR data from a Varian instrument (‘varian-data-1.fid’, ‘varian-data-2.fid’, etcetera).

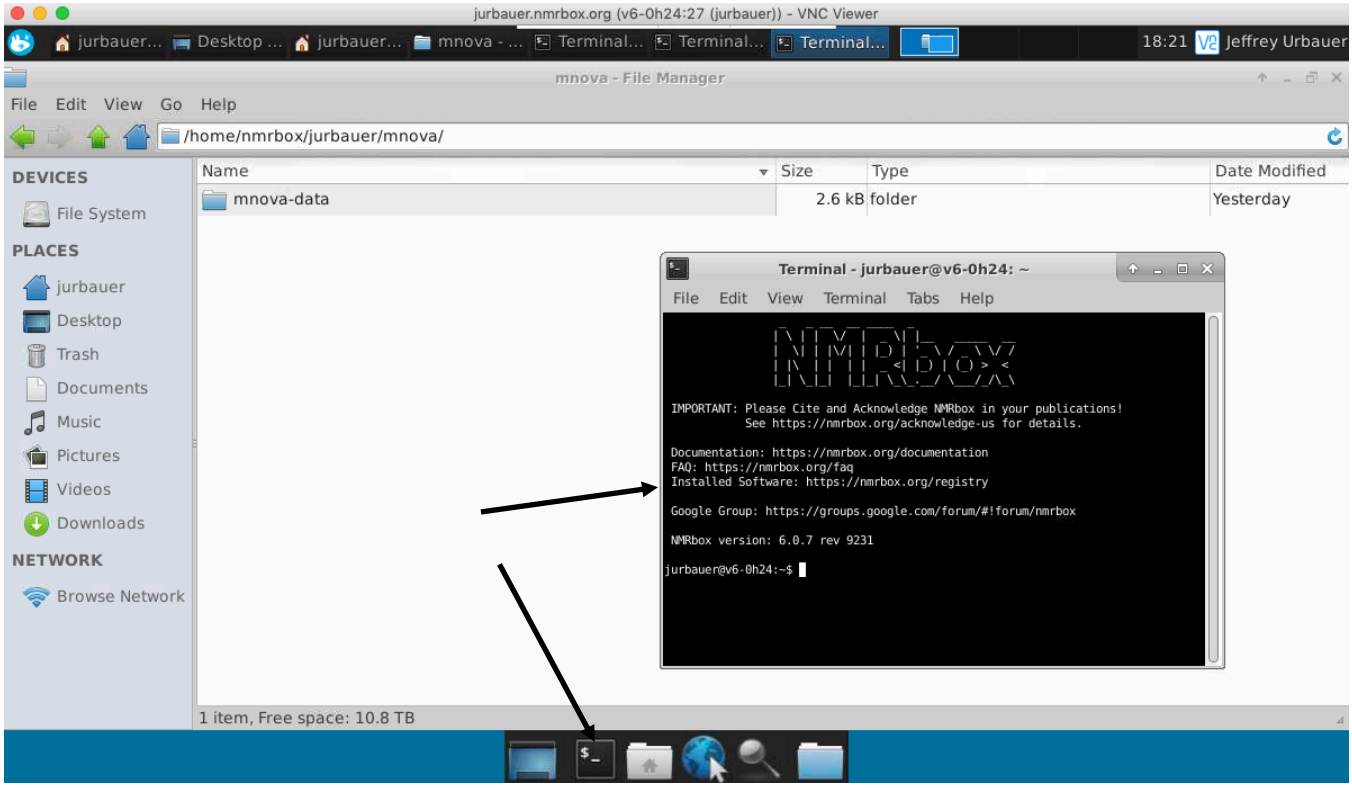


Each of the 4 ‘varian-data-x.fid’ folders/directories contains four files, ‘fid’, ‘log’, ‘procpar’, and ‘text’.

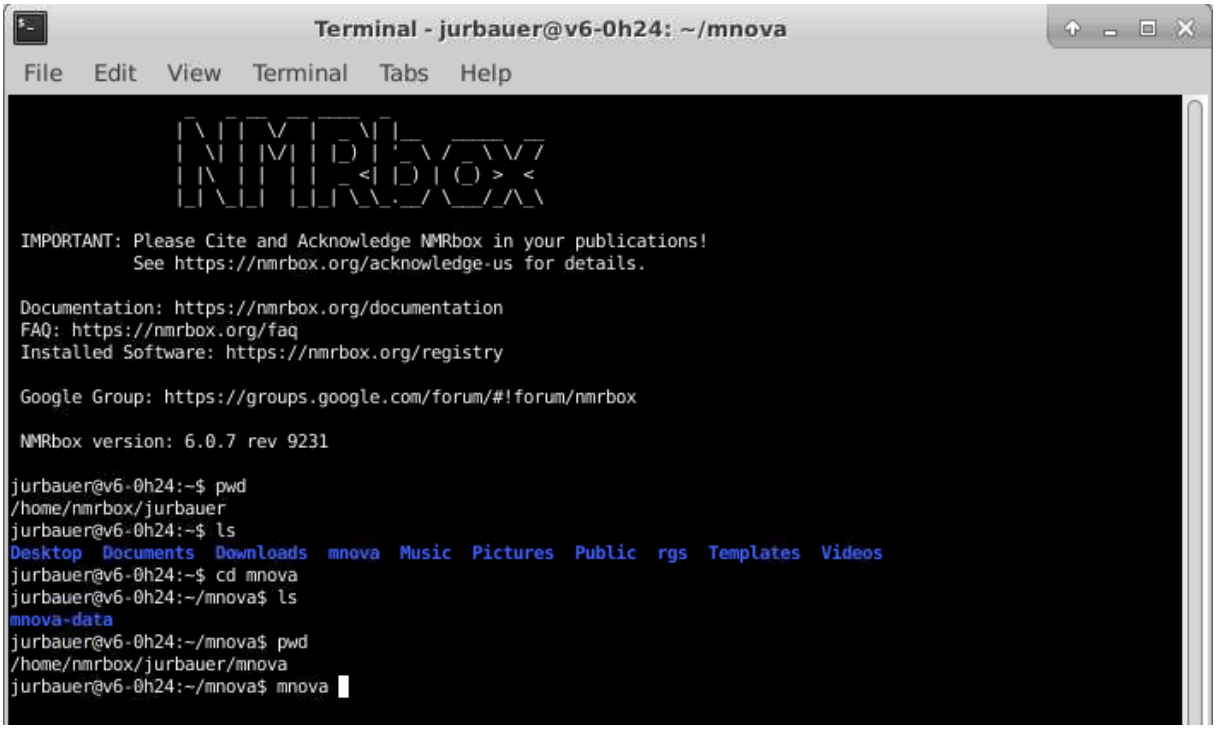


The “fid” file contains the data, and it is the file that we are interested in. The “log” file simply records a log of the experiment, and reports errors that occur during data acquisition. The “text” file exists so users can provide a description of the experiment or keep any notes they might want about the experiment, sample, etc. The “procpar” file contains a complete listing of all experimental parameters.

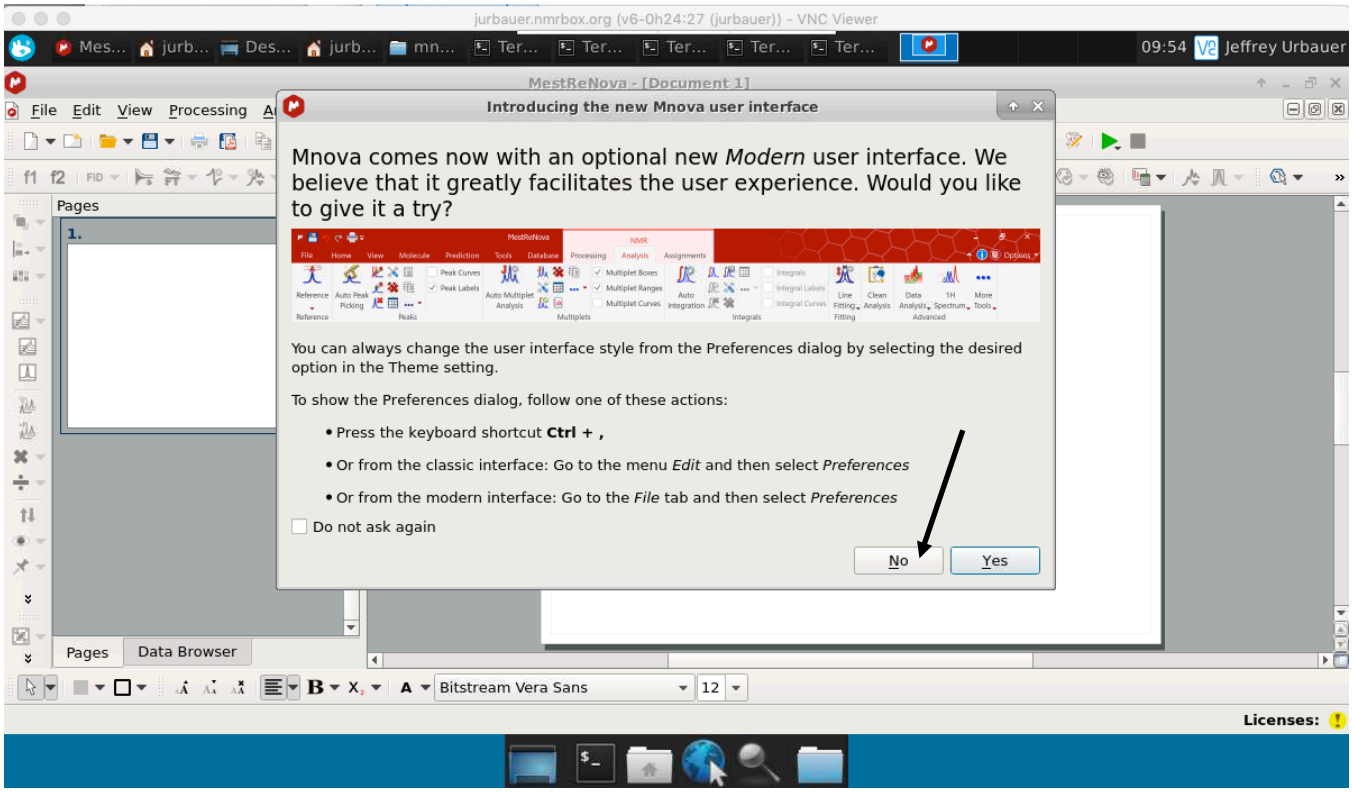
To start the Mnova program, we need to use the UNIX/LINUX command line. So, we need to click on the ‘Terminal’ icon at the bottom of the main page, and then a terminal window will open.



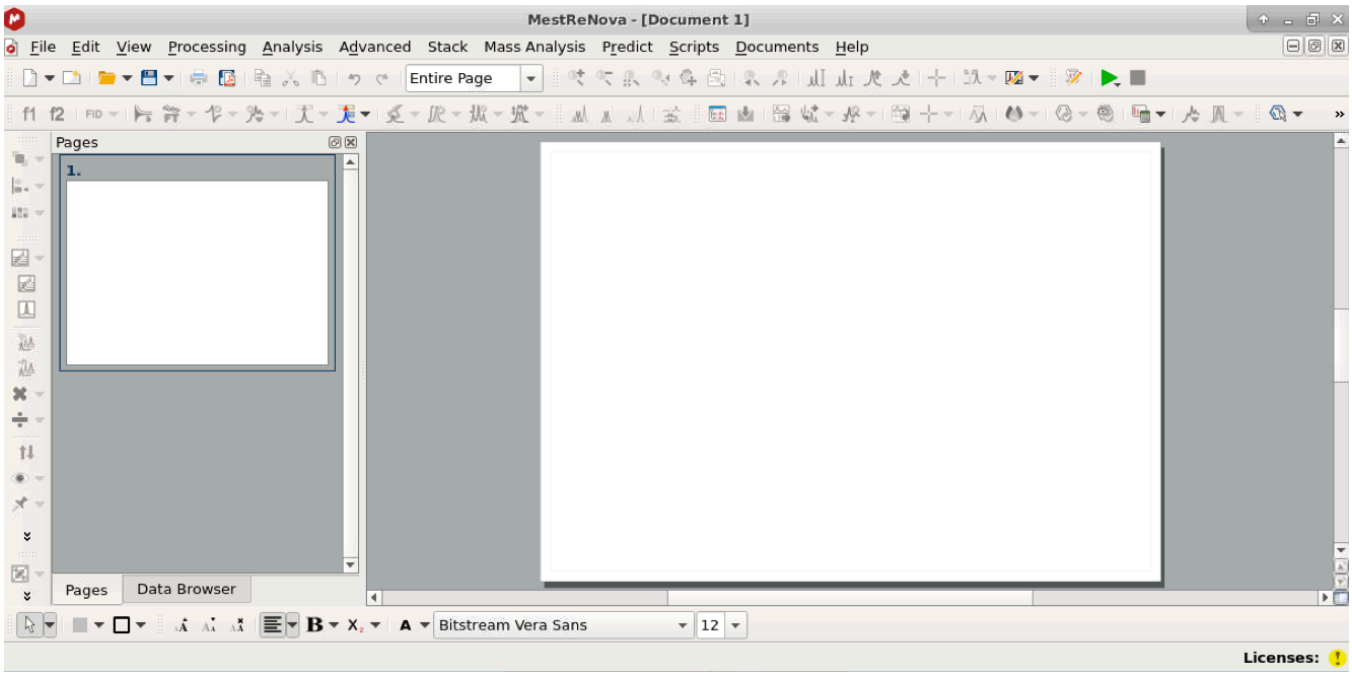
Using the terminal UNIX/LINUX command line, navigate to the ‘mnova’ folder. Then, to start the Mnova program, just type ‘mnova’ (all lower case) and hit ‘return’.



Once the ‘mnova’ (all lower case) command is entered on the UNIX/LINUX command line, the Mnova program main window opens. Most likely, a window will pop up asking what interface you want to use. We will be using, for this tutorial, the ‘classic’ interface. So, in this window, it asks if we want to try the ‘Modern’ interface. You should answer ‘No’.



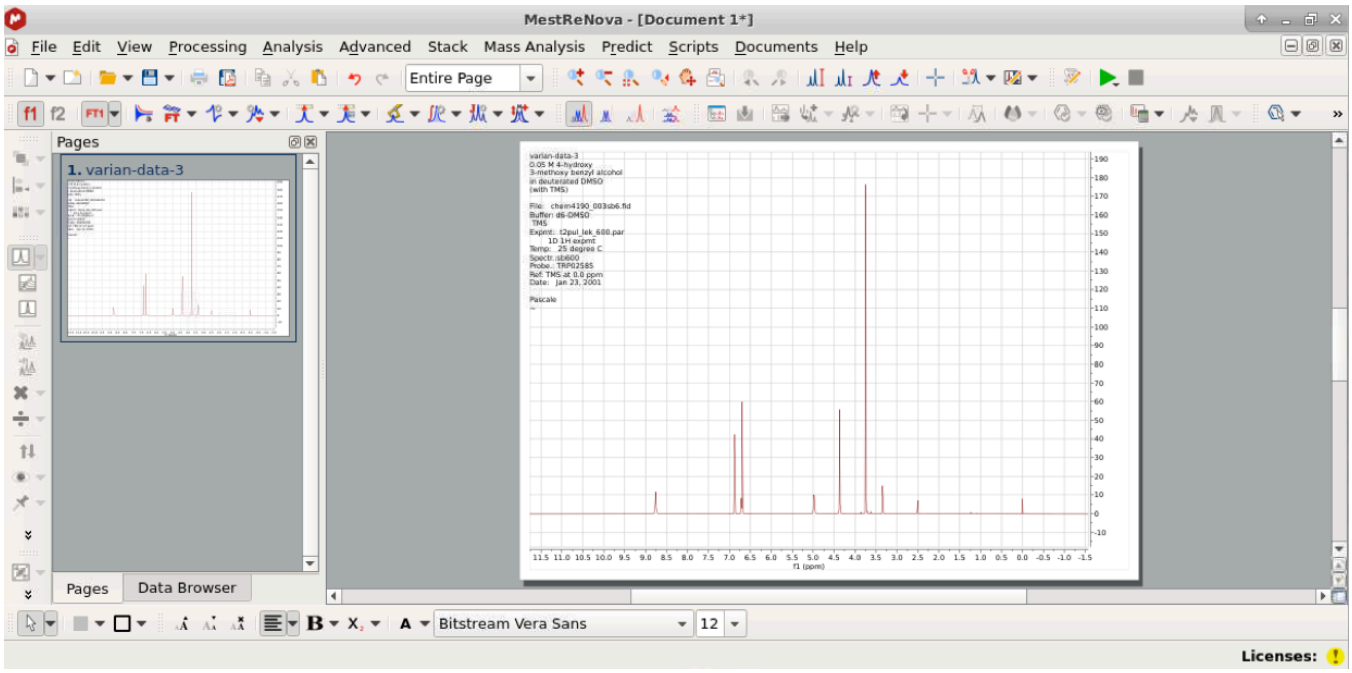
Now the main window is open, with the ‘classic’ interface, and you can proceed with the tutorial.



Opening a data file (Varian 'fid' file)

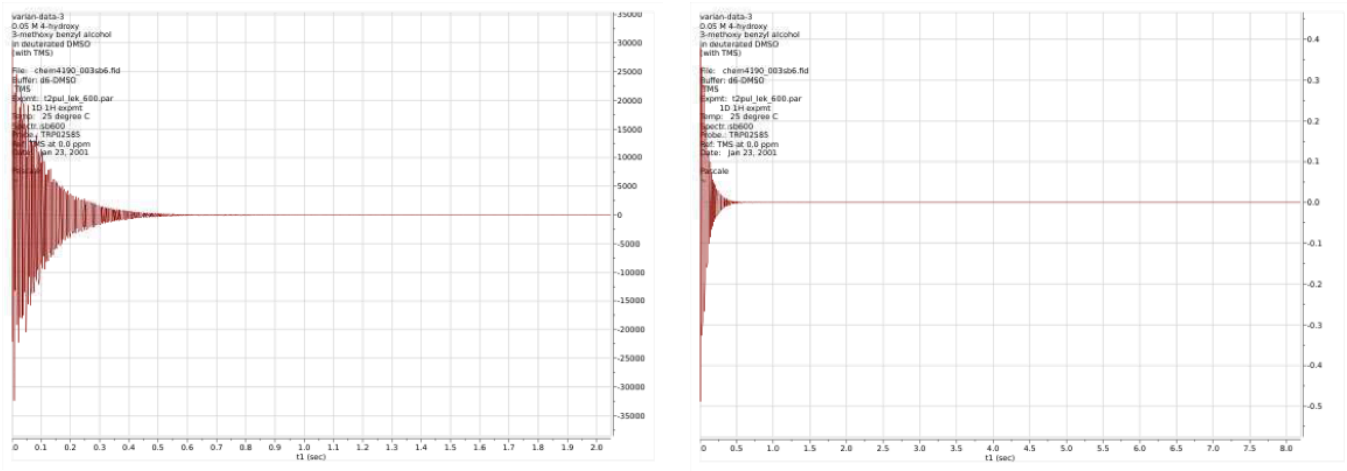
Here, we'll look at the spectrum of 3-methoxy benzyl alcohol. From the main menu on the MNova main window select **File/Open**. This will bring up a window that allows you to navigate through the files in your account to select the one you want. So, you should navigate to **/home/nmrbox/username/mnova/mnova-data/varian-data-3.fid/fid** and select/highlight the '**fid**' file, then click the '**Open**' button.

By default, the Mnova NMR program will open the data file, process the data (apply an apodization function (described further below), phase and drift correction, zero-filling, followed by Fourier transformation), and display the processed spectrum.



Viewing the raw data (FID) and processed data and manipulating the display

From the main menu, select **Processing/Break Processing On**. A drop-down menu appears that allows viewing of the data or spectrum. The raw data ("Original FID"), the data after application of the apodization function and zero-filling ("Processed FID"), the data after Fourier transformation and application of phase and drift correction ("Spectrum after 1st FT"), and, for two-dimensional spectra, the spectrum after Fourier transformation of the second dimension ("Spectrum after 2nd FT") can all be viewed by selection the desired option. Below is shown the "Original FID" (left) and "Processed FID" (right) for our sample.



With the processed spectrum (“Spectrum after 1st FT”) displayed, **experiment with the buttons (shown below) on the main toolbar**.

If you place the cursor over the button (and wait), the function of the button will be displayed. For instance, the leftmost button (“Zoom In”) allows the user to select, with the cursor, a region of the spectrum and magnify it. The next button, (“Zoom Out”) allows the user to incrementally zoom out with subsequent clicks of the mouse. The third button from the left (“Full Spectrum”) displays the full spectrum. The buttons are toggles, so to deselect the tool you must click on the button again.

Experiment with each of the buttons on this toolbar, and try to familiarize yourself with each.

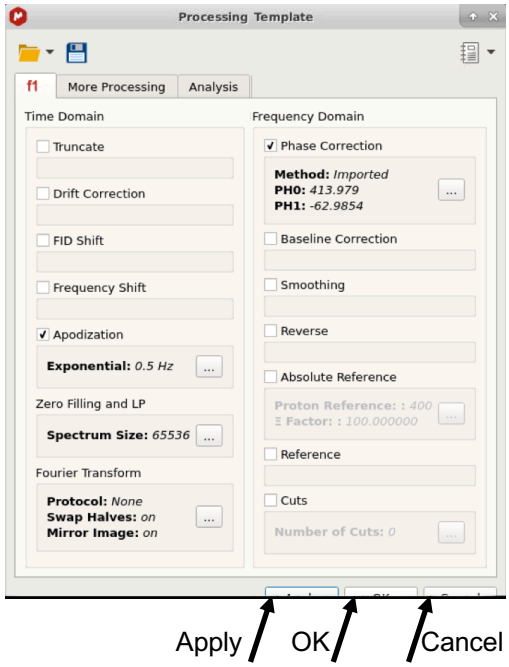
Before continuing on, exit the program, start it again and open the data file to show the processed spectrum (as shown on the previous page).

Manual data processing and Fourier transformation

Mnova is typicall set up, by default, to take raw data, process and Fourier transform the data automatically according to preset (default) parameters. We want to turn off all automation so that we can control how the data are processed.

Once again, load the **varian-data-3.fid/fid** data and display the processed spectrum. Then, from the '**Processing**' menu, choose the first option, '**Processing Template**'. The '**Processing Template**' window, shown here, will appear.

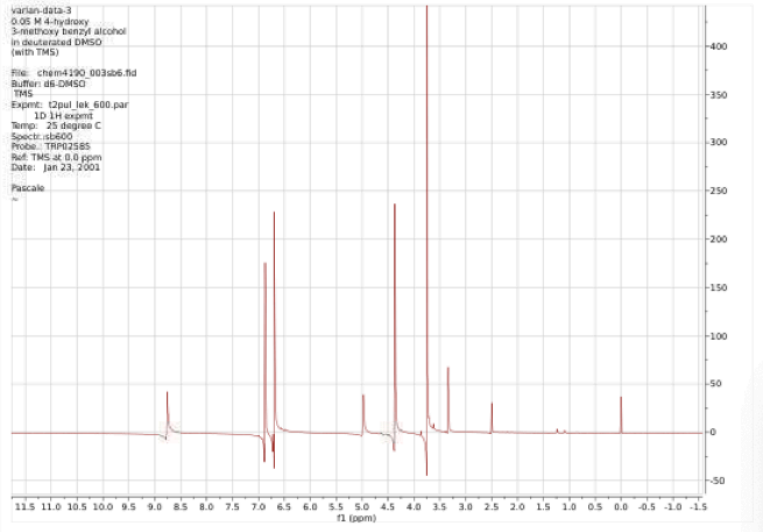
Please note that there are three buttons at the bottom of the window that are only partially visible. From left to right they are 'Apply', 'OK', and 'Cancel'. Apparently, there is no way to resize this window. In any case, they are active and you can click them and they do work.



***************A fix for this problem has been found. Please see pages 19-20 of this tutorial*************

There are three tabs ("f1", "More Processing", and "Analysis"). **The "f1" tab should be selected, but, if not, select it**. You'll see that "Phase Correction" is checked, as is "Apodization". **Uncheck all of these**. If there are any others checked, uncheck them as well. Also, you'll see that under "Zero Filling and LP" (LP stands for "Linear Prediction"), the "Spectrum Size" is reported as 65,536. **Click the small button** (with the three dots on it) to the right of "Spectrum Size". A new window ("Zero Filling and LP along t1") will appear. At the top, you'll see that the original size of the spectrum (the actual number of data points comprising the FID) is 16,384 (or "16k"). However, the current size is four times that (64k, or 65,536). **Readjust the current size to 16k** using the drop-down menu, then **click OK**. In the "Processing Template" window, you'll now see that the "Spectrum Size" is 16,384. You can click the other tabs ("More Processing" and "Analysis") to confirm that none of the other options are active. **Click OK**.

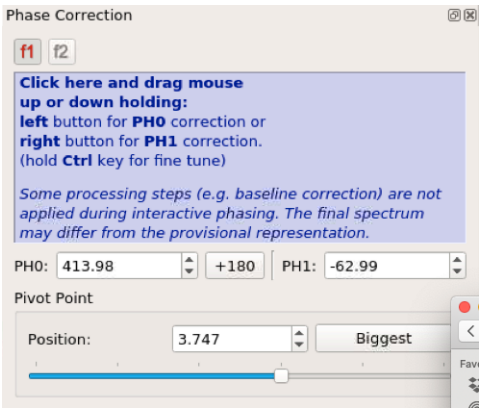
Now, if you select **Processing/Break Processing On/Original FID** and **Processing/Break Processing On/Processed FID** you'll see that the data (FIDs) shown in each case are essentially the same. Also, as shown here (right), if you look at the Fourier Transformed data (**Processing/Break Processing On/Spectrum after 1st FT**) you'll see that the signals are not in phase (no phase correction now being applied) and the line widths have changed (difficult to discern, but due to the lack of application of the default apodization function).



Manual phase correction

When Mnova automatically processes the data it calculates and applies phase corrections. Most of the time this works pretty well, but not all of the time. So, we need to learn how to phase the data manually.

With the default processing parameters turned off, the individual signals are somewhat out of phase (part of each signal is above, part below, the baseline). You can correct this automatically by selecting **Processing/Phase Correction/Automatic**, which works well most of the time. However, sometimes the automatic phase correction routines don't work very well, and it is important to know how to make the phase corrections manually.



Go back to **Processing/Processing Template** and **uncheck the Phase Correction box** (if it isn't already) and then **click OK**). The spectrum will now once again be out of phase. To manually fix the phase, go to **Processing/Phase Correction/Manual Correction**. The "Phase Correction" window will appear (right).

If you simply click with the right or left mouse button in the violet colored region of this window, an automatic phase correction will be applied (if you do this, go back and remove the phase correction as described above). There are a number of ways to apply manual corrections. We'll examine two of them.

a. In the "Phase Correction" window, **change the value of both PH0 and PH1 to 0**. Then **change the Pivot Point position to the smallest value** (drag the slider bar all the way to the right). If the values of PH0 and PH1 change when you change the Pivot Point, change PH0 and PH1 back to 0. Now, with the cursor in the violet colored region of the window, **hold down the left mouse button and move the mouse back and forth until the most upfield signals (at 0.0 and 2.5 ppm) are in phase** (signals "up" and baseline flat). **Then, hold down the right mouse button to adjust the phase of the most down field signals** (7 to 9 ppm). Now, you may have to iteratively re-adjust PH0 and PH1 until all signals are perfectly in phase. The first operation adjusts "PH0" (makes a "zero order", or frequency independent, phase correction). The second adjusts "PH1" ("first order", or frequency dependent, phase correction). You should find that values of (approximately) PH0 = 50 and PH1 = -55 (with the 'pivot' bar all the way to the right side) will give suitable phase corrections.

b. Close the "Phase Correction" window. Go back to **Processing/Processing Template** and **uncheck the Phase Correction box** (then **click OK**). The spectrum will now once again be out of phase. Open the "Phase Correction" window again (**Processing/Phase Correction/Manual Correction**). Now, adjust the "Pivot Point" to the biggest signal by **clicking the 'Biggest' button**. Change PH0 and PH1 to 0.0. You may have to click the 'Biggest' button again. You'll see a blue cursor aligned on the biggest signal (at ~ 3.75 ppm). Now, as you did above, with the cursor in the violet colored region of the window, **hold down the left mouse button and move the mouse back and forth until the most upfield signals are in phase**. **Then, hold down the right mouse button to adjust the phase of the most down field signals**. Again, you may have to iteratively re-adjust PH0 and PH1 until all signals are perfectly in phase. You should find that values of (approximately) PH0 = 57 and PH1 = -67 (with the 'pivot' bar at 3.75) will give suitable phase corrections.

For the remaining exercises, if the spectrum is ever out of phase, simply correct it automatically as described above.

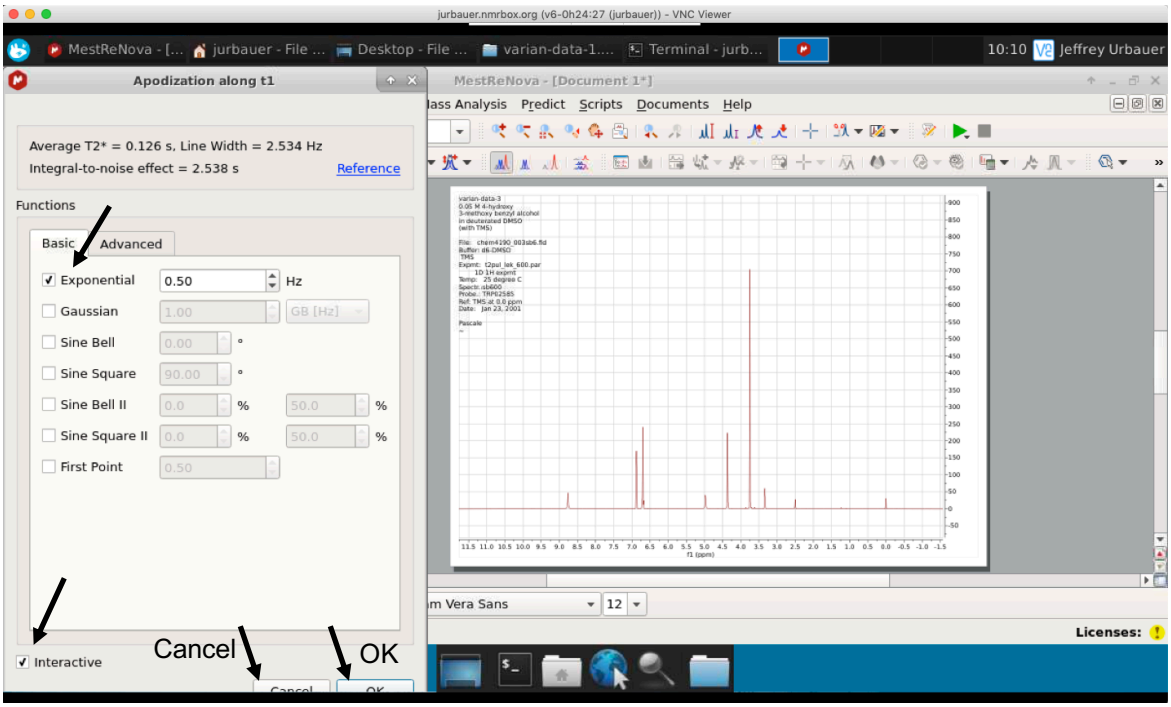
Apodization (applying window functions to the FID)

The word “apodization” (or “apodisation”) means “removing the foot”. In NMR, it is used to describe the process of applying a mathematical function, sometimes called a “window function”, to the acquired data (FID) in order to change the appearance of the signals/spectrum after Fourier transformation. Certain functions are used to improve signal intensity (at the expense of resolution), whereas others are used to improve or enhance resolution (at the expense of signal intensity). When the FID is truncated (the acquisition time is too short), Fourier transformation of the step function results in baseline artifacts described by a sinc ((sin *x*)/*x*) function (squiggly baseline), so apodization functions are applied, in these cases, to bring the decaying FID smoothly to zero amplitude to eliminate the truncation artifacts.

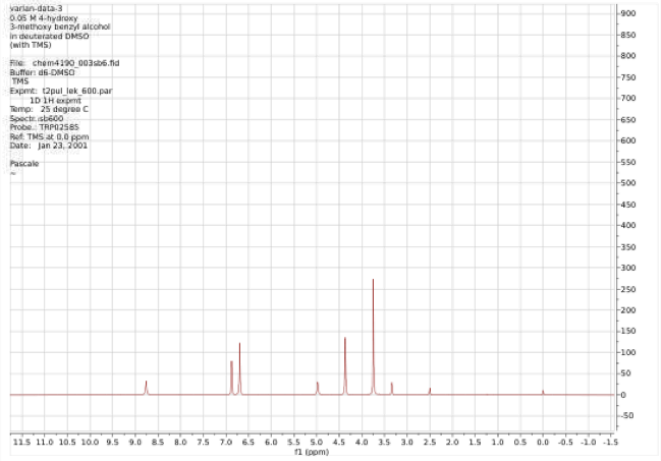
The Mnova manual (link to the manual is on the first page of this tutorial) has good descriptions of the mathematical forms of the apodization functions and, in some cases, how and why they are used.

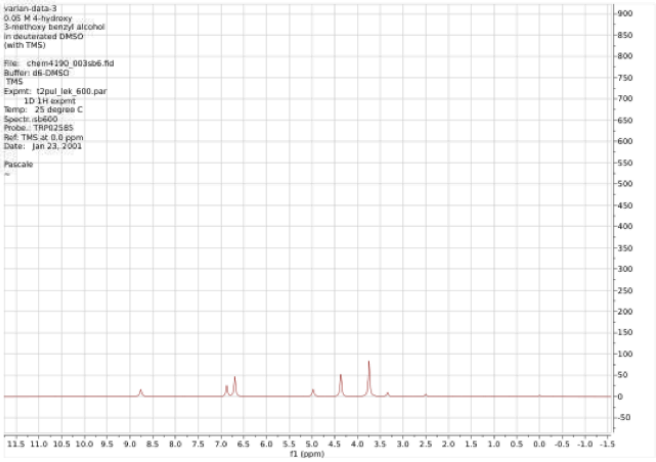
With the default parameters turned off (except for phase correction, see step V.1 above) and the “Spectrum Size” set equal to the “Original Size” (16384), use **Processing/Break Processing ON/Original FID** and **Processing/Break Processing ON/Processed FID** to verify that these are the same (no functions have been applied to the FID). Also, view the processed spectrum using **Processing/Break Processing ON/Spectrum after 1st FT** and note the peak widths.

Now, we’ll interactively apply an exponential function. The exponential function or “line broadening” function is often used to improve signal-to-noise (S/N) at the expense of resolution (lines get broader at the base). Select **Processing/Apodization** and the “Apodization along t1” window will open. **Move it away from the actual spectrum** so you will be able to interactively change the apodization function and watch the changes to the spectrum (note, the ‘Cancel’ and ‘OK’ buttons are again partially hidden, but they still function properly). Make sure that **the “Exponential” and “Interactive” buttons are checked** for this exercise. Then proceed as described below



Now, **change the value of the function (in Hz) from 0.5** (this is the spectrum on the previous page) **to 5** (this is the spectrum below, left) **and to 20** (this is the spectrum below, right), for instance, and observe how the spectrum is changed. As this value is increased, you'll see the signals become much broader. You also see the signal-to-noise (called '**Integral-to-noise**' in the 'Apodization along t1' window) increases substantially (2.5 at 0.5 Hz line broadening, 8.0 at 5 Hz line broadening, 16 at 20 Hz line broadening).

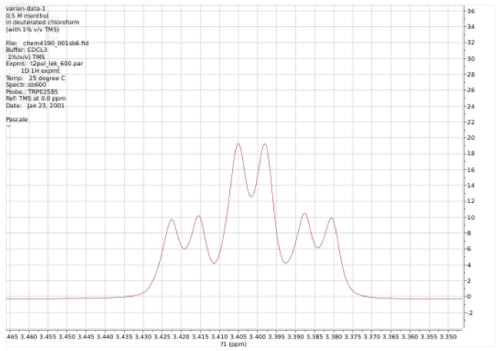


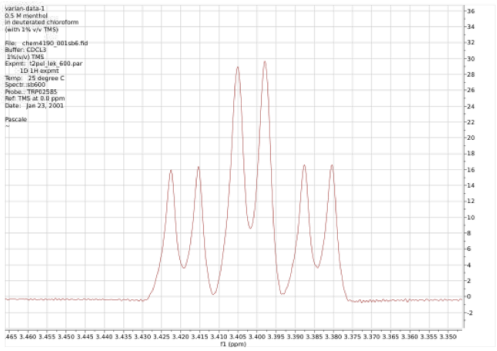


Go ahead and experiment a bit more with this function. What happens when you make the value of the function negative?

We'll now apply a Lorentz-to-Gauss function for resolution enhancement. We will use the ^{1}H NMR spectrum of menthol for this exercise (select **File/Open/home/your-user-name/mnova/mnova-data/varian-data-1.fid/fid**). Note that the window on the left has two data sets now ('varian-data-3' and 'varian-data-1'). So, Mnova allows you to have multiple data sets open, and the window on the left allows you to select the one you want to display and manipulate.

Zoom in tight on the signal at ~3.4 ppm, and **increase the intensity of the signal** so that the largest peak in the signal is about half of the height of the data window (below, left). Then, select **Processing/Apodization** and the "Apodization along t1" window will open. Make sure to **check the "interactive" box**. Now, **check BOTH the "Exponential" and "Gaussian" boxes**. **Make the value of the "Exponential" function -1.0 Hz, and the value of the Gaussian function 0.5 Hz**. A significant resolution enhancement (below, right) of the multiplet will result (S/N is lower).





Go ahead and experiment with some of the other functions.

Zero filling, linear prediction, drift correction, baseline correction

"Zero filling" is the process of adding additional data points, all with zero amplitude, to the end of the FID. This improves digital resolution (decreases the number of Hz/pt). The normal process is to double the total number of points in the FID by zero filling. By default, Mnova performs zero filling on the data. Thus, as we have seen above, the "Spectrum size", by default, is normally at least twice the "Original Size", due to the default behavior of zero filling to increase the number of points in the FID.

"Linear prediction" is a method of extrapolating (predicting) additional time-domain data points based on linear combinations of existing points. This process needs to be used with care, and it is turned off in Mnova by default.

"Drift correction" is used to remove a baseline offset of the spectrum resulting from a non-zero integral for the FID and zero-frequency spike in the spectrum. Mnova *sometimes* does this automatically, by default, and *sometimes* does not. When it does, it uses the common procedure of averaging the last 5% or so of the points in the FID and subtracting these from the rest of the FID.

"Baseline correction" is a general process of flattening or otherwise improving the appearance of the baseline. This can be important for accurate signal integration and for other reasons. The default behavior of Mnova is no baseline correction.

You can experiment with some of these if you like. For instance, you can access the "Baseline Correction along f1" by selecting **Processing/Processing Template** and then by **checking the Baseline Correction** box. The small button (with the three dots) brings up the "Baseline Correction along f1" window that permits the baseline "Method" and "Parameters" to be adjusted. Similarly, by selecting **Processing/Baseline**, the first of the three options again brings up the "Baseline Correction along f1" window, the second does an automatic correction based on parameters determined by the Mnova program, and the third permits the user selection of points that represent baseline (as opposed to points in signals), and uses these to define the baseline before application of the correction (usually polynomial) function. Feel free to experiment with baseline correction. Normally, it is a good idea to begin with a conservative, low order polynomial (i.e. 2) and a simple polynomial correction (see "Baseline Correction along f1" window). If that doesn't work, then higher order polynomials and other methods can be tried.

Likewise, you might try linear predicting some points. In the 'Processing Template' window, under 'Zero Filling and LP, click the button (with three dots) next to the 'Spectrum Size'. In the 'Zero Filling and LP along t1' window that appears, make sure the 'Backward' and 'Forward' are both checked, change 'To Spectrum Size' at the top ('Zero Filling' section) to 64k (the button below should read 'LP Filling'). Click OK, then 'Apply' and 'OK' in the 'Processing Template' window. See if you can detect any differences in your spectrum. You should also try zero filling without any linear prediction.

Chemical shift referencing

Observed chemical shifts are normally displayed and reported relative to the chemical shifts in accepted, standard compounds. In organic chemistry, for instance, the chemical shifts of hydrogen nuclei are referenced, either directly or indirectly, to the chemical shifts of the hydrogens in tetramethylsilane (TMS). The chemical shifts of the equivalent hydrogens in TMS are defined to be 0.00 ppm. Sometimes, small amounts of TMS are spiked into samples for the purpose of providing the reference for chemical shifts. Other times, common components of solvents can serve as a chemical shift reference. For instance, the hydrogen nucleus in residual $CHCl_3$ in samples of $CDCl_3$ (a common NMR solvent in organic chemistry) has a chemical shift of 7.27 ppm relative to TMS. So, this signal can often be used as a chemical shift reference, eliminating the need for added TMS. There are many such examples.

For this part of the exercise, we'll use the menthol data set (select **File/Open/home/your-user-name/MNova/data/varian-data-1.fid/fid**). By default, the menthol spectrum will appear, processed, baseline corrected, and so on. Notice the large signal at 0.00 ppm. This is the signal from TMS used for chemical shift referencing. **First, expand tightly around the TMS signal**. Then, select **Analysis/Reference/Reference**. This will enable a vertical, red cursor that is used to identify a signal to be used for chemical shift referencing. **Move this cursor near the TMS signal**, and the cursor will automatically find the center of the signal. Once done, **click the left mouse button**, and the "Reference along f1" window will appear. You'll notice that the chemical shift is close to 0.000 ppm (so, Mnova must automatically guess that the large, upfield signal is TMS, and properly reference the spectrum to it). Let's incorrectly reference the spectrum. In the "Reference along f1" window, **change "New Shift" to 0.1 ppm**, then **click OK**. You'll now see that the TMS signal is at 2.0 ppm.

You can also reference the signal using **Analysis/Reference/Graphic Reference**. When you select this option, a cursor will be enabled. Move the cursor to the center of the signal you want to reference, and select this position with a click. Then, move the cursor to the chemical shift (0.00 ppm for TMS) that you want to reference the signal to and click again. Your signal will now be referenced to the position you selected.

In the menthol spectrum, look carefully at the tiny signal at about 7.3 ppm. **Zoom in tightly around this signal**, and **increase its intensity** so you can see it more clearly. The solvent used for this sample of menthol was $CDCl_3$, and the tiny signal at ~7.3 ppm is the ^1H signal from the residual $CHCl_3$. You'll notice that, by selecting **Analysis/Reference/Reference** you can put the cursor on this signal and see its chemical shift is approximately 7.28 ppm from TMS, just a bit different that the normally-quoted value of 7.27 ppm. In any case, you can use this signal, with a value of 7.27 ppm, as a reference for a sample in $CDCl_3$ that has no TMS.

Picking peaks

Identifying and selecting signals and peaks in spectra and recording their chemical shifts is often called "peak picking". Here, we'll again use the menthol data set (select **File/Open/home/your-user-name/MNova/data/varian-data-1.fid/fid** if you do not yet have this data open).

Expand tightly around the signal at ~3.4 ppm. There are six peaks in this signal. To "manually" determine their chemical shifts ("pick" them), select **Analysis/Peak Picking/Peak by Peak**. This will enable a cursor (similar to the one used for referencing) that will select the centers of peaks when moved onto the peak. **Once on a peak, click the left mouse button** (the peak is now "picked") and **move to the next peak**. When you pick all six, **press the "Escape" (Esc) button to exit** the peak picking mode. At the top of the spectrum window, you'll see the chemical shifts of the picked peaks (in blue, very small and impossible to read).

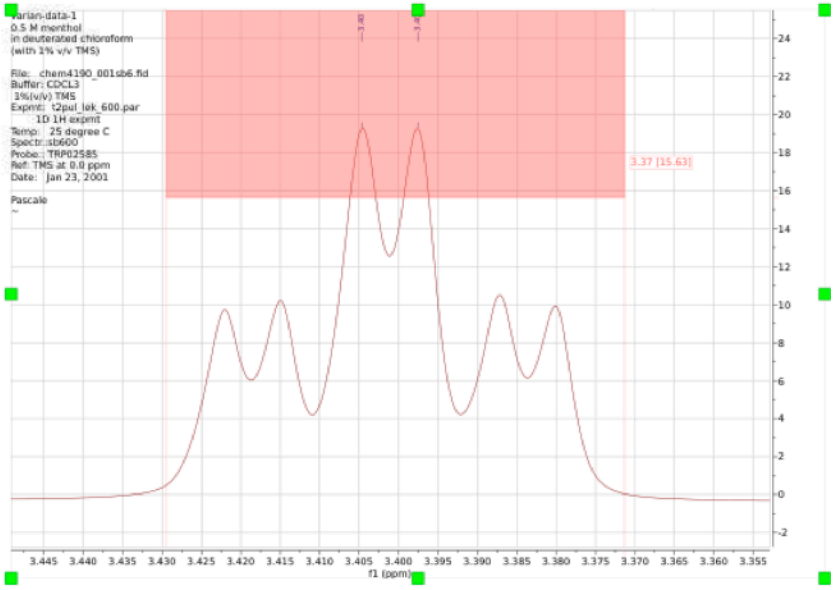
If you make a mistake and want to delete the peaks you've picked and start over, select **Analysis/Peak Picking/Delete All**.

If you select **Analysis/Peak Picking/Copy Peaks**, this will copy these chemical shifts into the "copy/paste" buffer, and you can then, for instance, paste them into other applications, like Microsoft Word, for instance, or any text editor, or even a terminal window. Give it a try.

Now select **Analysis/Peak Picking/Delete All**. This will delete all of the picked peaks and their chemical shifts from the buffer. Now we will pick only the largest two peaks of these six using a different method.

Select **Analysis/Peak Picking/Manual Threshold**. **Put the cursor at the left edge of the spectrum and press and hold the left mouse button**. **Drag the cursor to the right**. A red rectangular region will appear. **Place this red rectangle below the tips of the largest peaks, and above the tips of the other four peaks**. Once you **release the mouse button**, only the two largest peaks will be picked. **Press the Esc key to exit this mode**.

Experiment with the peak picking routines. For instance, expand around all of the menthol signals and try using the automatic peak picking routine, etcetera.



Integrating peaks/signals

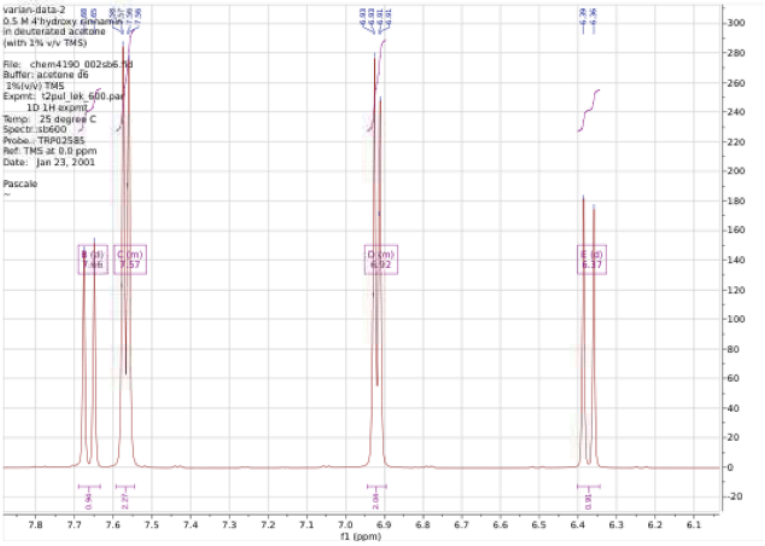
The areas under the signals (i.e. their integrals) in one-dimensional ^{1}H NMR spectra are proportional to the number of hydrogen nuclei that give rise to them. So, we can determine these relative numbers by integrating the signals. Before integrating NMR signals, it is important that the spectrum is phased well and the baseline is corrected. Mnova does a good job of this automatically, so all you have to do for this exercise is open the data (the processed spectrum will be displayed) and these conditions are met.

Use the menthol data set again. **Zoom in around the menthol signals (0.5 - 3.6 ppm or so, make sure the TMS signal at 0.0 ppm is NOT included)**. Select **Analysis/Integration/Autodetect Regions**. The green integral curves are displayed above the signals, and the blue integral values are displayed below the signals (and below the short horizontal green bars defining the width of each signal). The blue integral values are measures of the relative integral areas. If we know the number of hydrogen nuclei giving rise to a particular signal, we can normalize the integral values. For instance, it is known that the signal at ~1.47 ppm results from a single hydrogen, so we can normalize the integrals based on this knowledge. **Click on the green integral curve above the signal at 1.47 ppm**. The "Integral Manager" window will appear. **In the "normalized" box, enter 1.0**. Then close this window ('x' in the upper right hand corner). Now, the integral values represent the (relative, approximate) numbers of hydrogen nuclei giving rise to the signals.

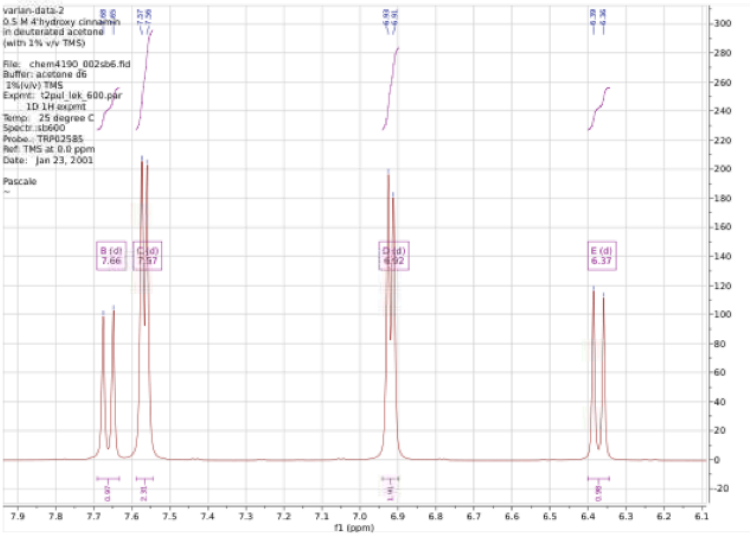
Multiplet Analysis

For simple spectra, deciding if a multiplet is a doublet, triplet, quartet, etc., is easily done by eye, and calculating coupling constants is easy (once the peaks are picked and chemical shifts determined). Mnova will automate this process, so, for simple spectra, these tasks can be performed quickly.

For this exercise, use the 4-hydroxy cinnamin data (select **File/Open/home/your-user-name/MNova/data/varian-data-2.fid/fid**). Zoom in on the region of the spectrum from ~6.0 ppm to ~8.0 ppm. There are four signals here, all doublets. Now, select **Analysis/Multiplet Analysis/Automatic**. Mnova will identify each of the four signals, and will draw to the screen a violet colored horizontal line below each signal indicating the width (beginning and end) of each signal and a violet rectangle on each signal with very small letters and numbers indicating the multiple type (d=doublet, t=triplet, etc.) and a value for the coupling constant (always in Hz).



In this example, Mnova believes that two of the doublets are actually doublets-of-doublets (it 'finds' four peaks instead of two in each of these signals). Zoom in on one of these signals (for instance, the signal at 6.9 ppm). You'll see that the peaks are not perfectly shaped, and appear to have 'shoulders', and Mnova has decided that these are real. They may or may not be, but we'll get rid of them with a little line broadening. First, go to **Analysis/Clean Analysis** and this will clear the analysis, so we can start over. Next use '**Processing/Apodization**' and use a line broadening (exponential) of 2.0 Hz (remember to click 'OK'). Then try the automatic analysis again. Now, Mnova recognizes each multiplet as a doublet.

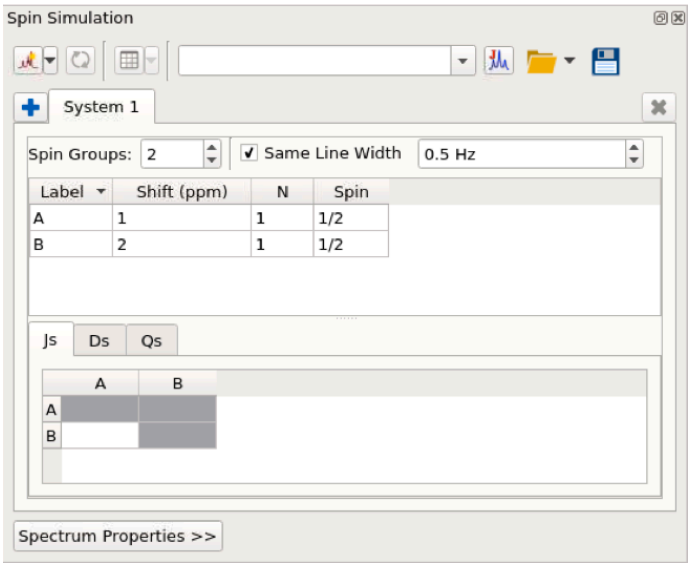


Now, **move the cursor over one of these violet boxes** and you'll see the cursor changes and allows you to highlight the box. Double-click on the box and the "Multiplet Manager" window will appear. In the window, information at the selected multiplet will appear. For instance, for the signal at approximately 6.9 ppm, the chemical shift is actually 6.92 ppm, it is a doublet ("d"), the coupling constant is 8.3 Hz (*J*=8.3 Hz), etc. The "*J*-List" will list all coupling constants in the multiplet (in this case, only one).

Experiment with the multiplet analysis tool. For instance, use it to analyze multiplets from some of the other data sets. Instead of using the "Automatic" option, try the "Manual" option, etc.

Spin Simulation

Mnova has a built-in utility that one can use to simulate simple systems and their spectra.



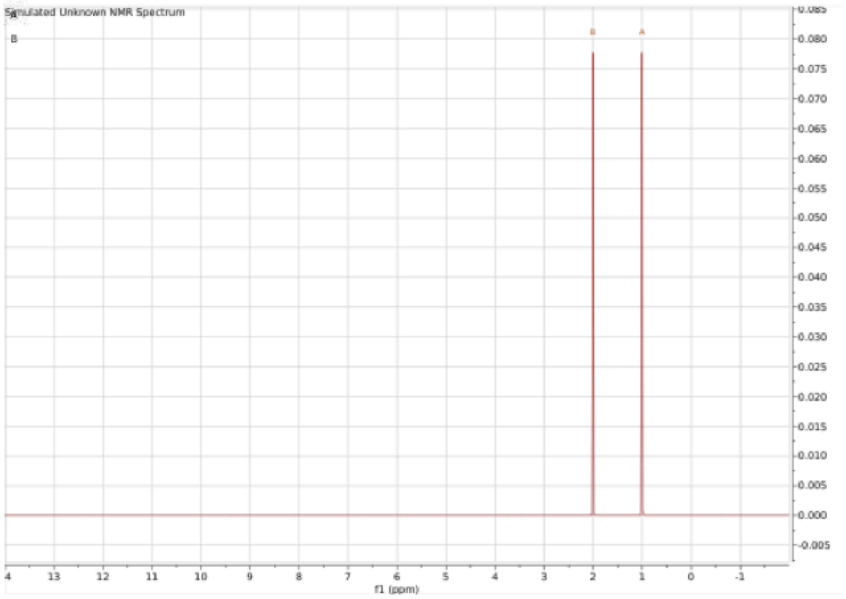
For this exercise, first exit and end your Mnova session, and then restart the program.

Select **Advanced/Spin Simulation** (on older versions of Mnova, the 'Advanced' menu is called 'Tools'). The "Spin Simulation" window will appear. We will simulate a very simple system of two nuclei, initially not scalar coupled to one another, and will add in the coupling later.

One note, the 'Spin Simulation' window CAN be resized, etcetera. Sometimes, when opened, it appears as a very small window with most everything below 'Spin Groups' hidden. Just know that you can move it around and resize it so that you can see the whole window.

Set the parameters as shown in the example above: for "**Spin Groups**", **select 2** (two nuclei). Make sure the '**Same Line Width**' box is checked and use **0.5 Hz as the line width**. Make the chemical shift of spin 'A' 1 ppm, and the chemical shift of spin 'B' 2 ppm. The **value 'N' (number of nuclei) should be 1 for both A and B**. Both A and B are spin ½.

At the bottom of the window is a 'Spectrum Properties' menu. Click on that and then scroll down. Keep the default values (Frequency = 500.13 MHz, Points = 64K (64,000 points in the FID), From = -2.0 ppm, To = 14.0 ppm). Close the 'Spectrum Properties' menu.

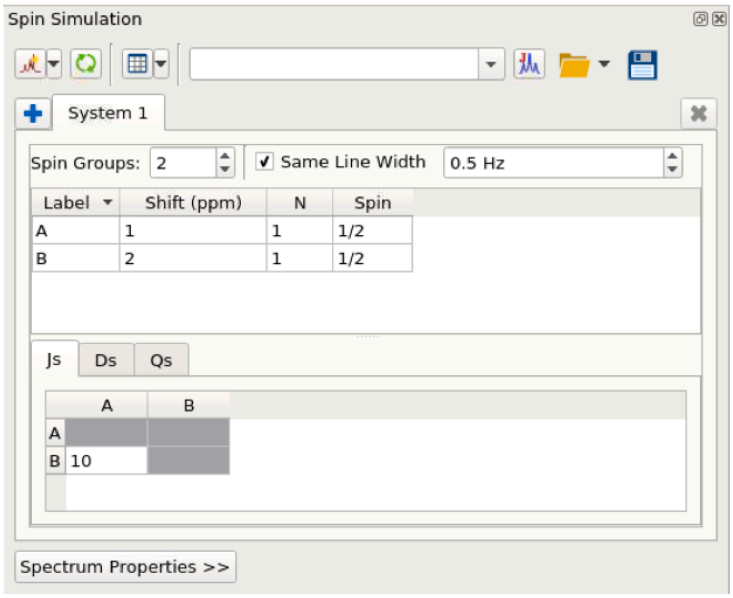


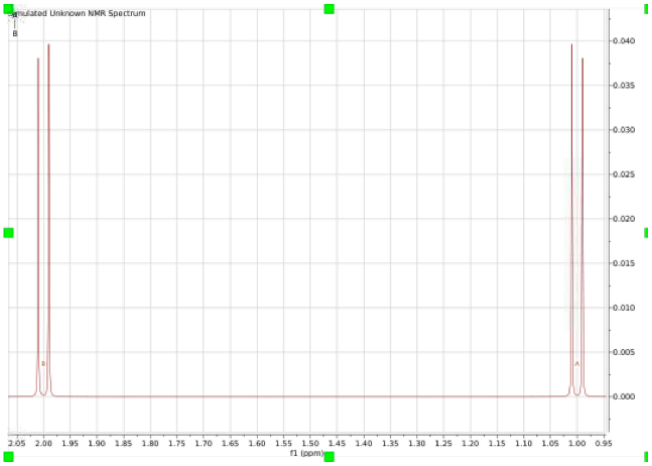
For now, we'll not include coupling between the nuclei.

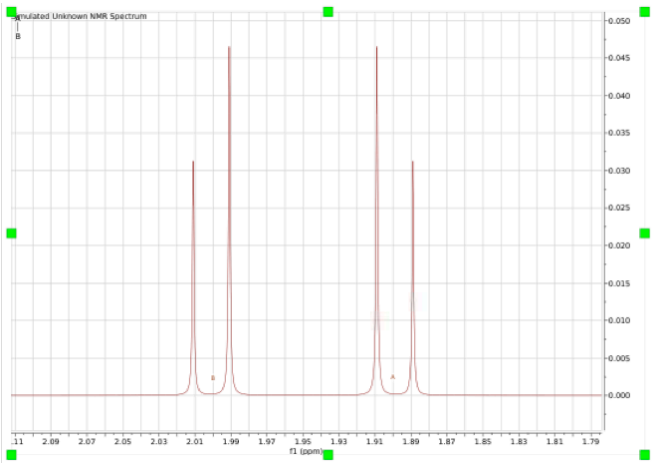
Click the 'New Simulation' button (this is the multi-color button in the far upper left-hand corner). When you do, the spectrum will appear. It will be two singlets, one at 1.0 ppm, and one at 2.0 ppm.

Now, **change the value of the coupling constant (J_{AB} = J_{BA}) to 10 Hz** and click **'New Simulation'** again. You'll now see two doublets with chemical shifts of 1.0 and 2.0 ppm (below, left). You'll notice that within each doublet, the heights of the two peaks are not quite identical, indicating some non-first order behavior ($\Delta\nu \rightarrow J$, strong coupling).

Now, **change the chemical shift of the "A" spin to 1.8 ppm** and **click "Recalculate"**. The signals will now be at 1.8 and 2.0 ppm, and they will display more pronounced non-first order behavior (below, right)







Output

We'll not say too much about output. However, whatever spectrum, and other information, you have displayed in the spectrum window, you can output to a .pdf file by selecting **File/Export to PDF…** .

Finally, the complete manual for Mnova can be found at the following link:

https://mnova.pl/files/download/MestReNova-12-0-0_Manual.pdf

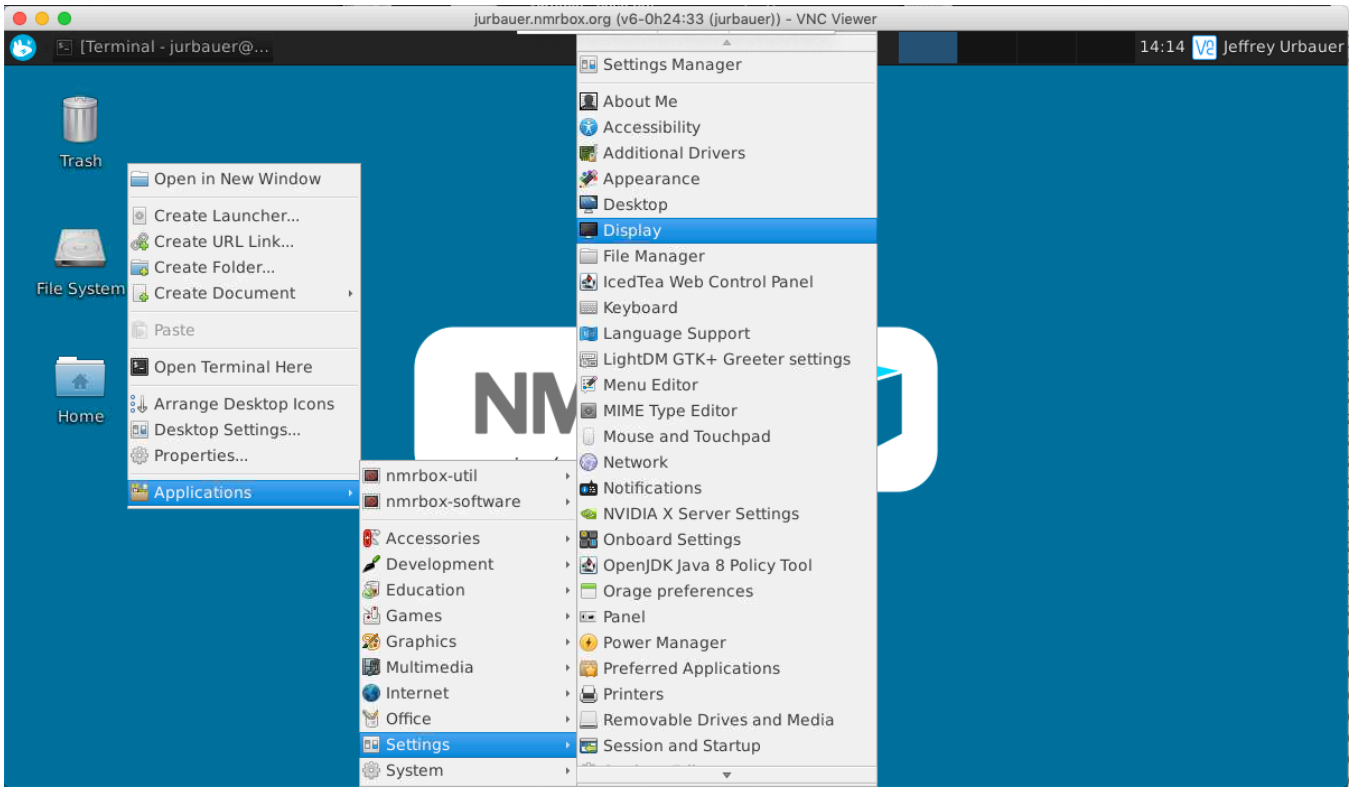
Among other things, the manual has very good descriptions of the apodization functions available in Mnova, as well as detailed descriptions of the many other functions and uses of this program.

Setting the display resolution for NMRBox

On page 8 of this tutorial, there is an issue noted regarding the inability to 'resize' menus in Mnova running in NMRBox, resulting in an inability to see attributes (buttons, for instance) at the bottoms of the menus.

This problem is typically the result of the default display resolution used by NMRBox (1200 x 675). Resetting the display to larger values (presumably) more consistent with the display you are using will correct the problem.

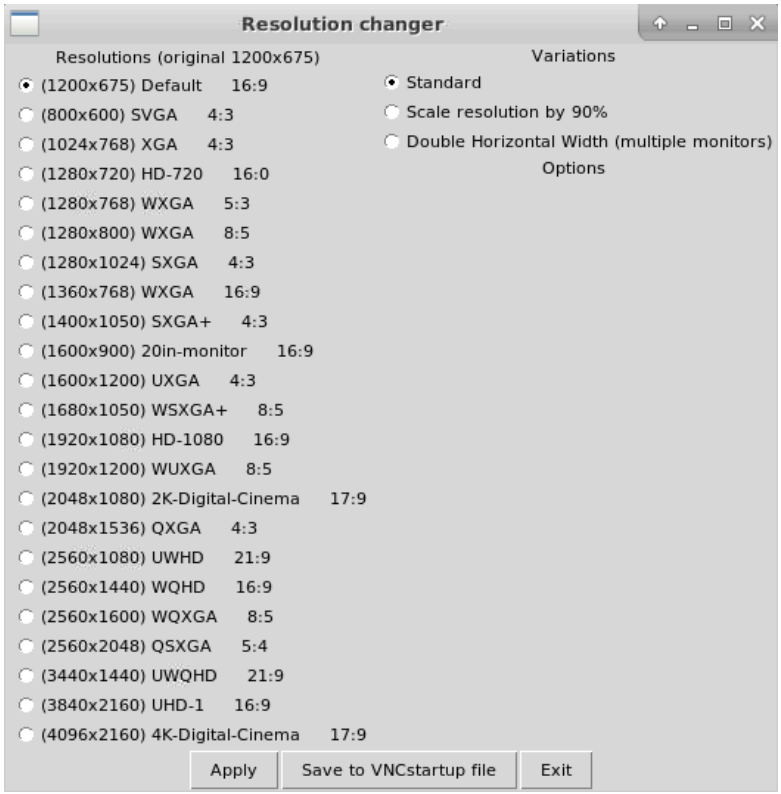
In order to change the resolution to something more suitable, right-click in the NMRBox window and then select "Applications" → "Settings" → "Display

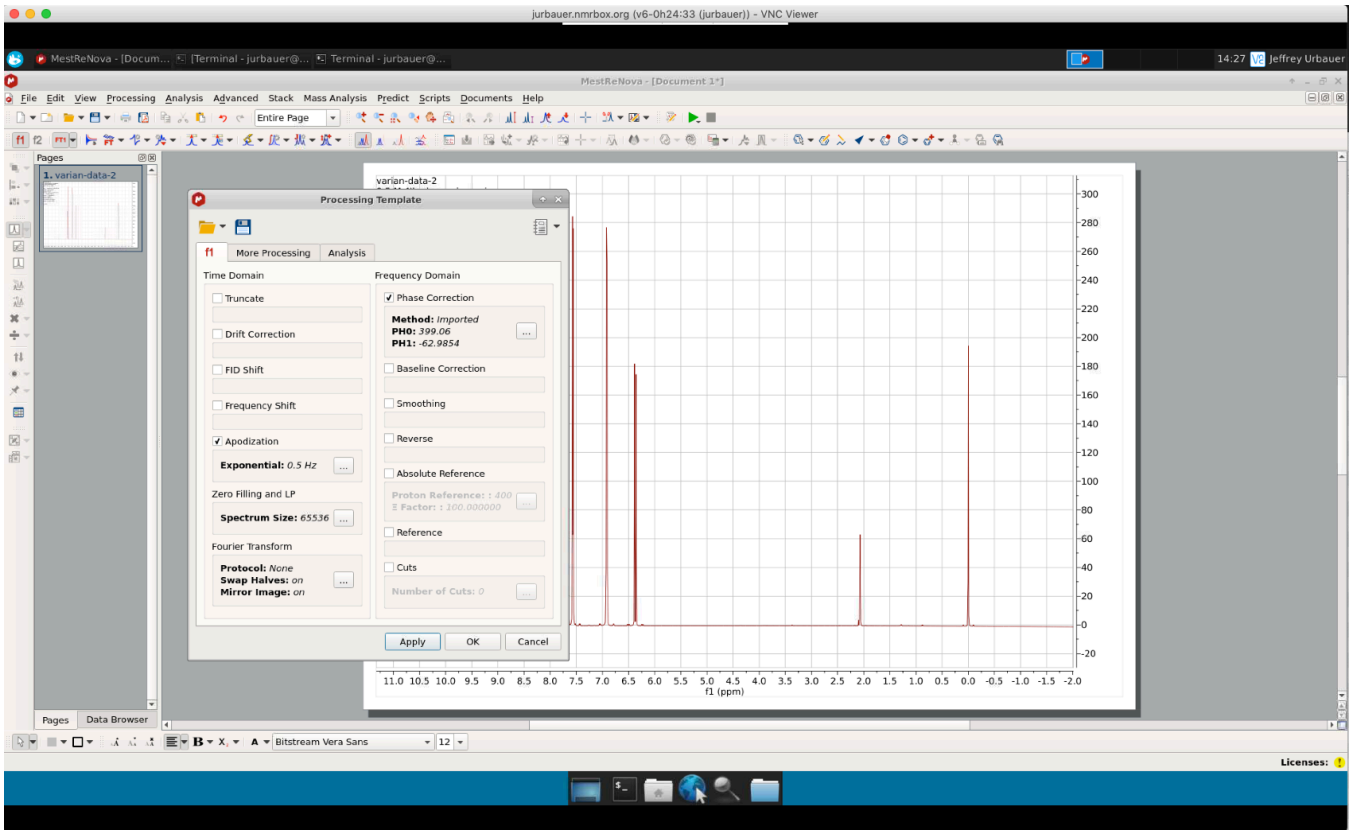


This will open the 'Resolution Changer' window (see below, next page). In this window, you can select an alternate resolution. If you have a 'normal' (not terribly high resolution) display, you might try "(1600x900) 20 in monitor 16:9" or "(1920x1080) HD-1080. 16:9". If you have a much higher resolution display, you might try one of the higher resolution options.

Once you choose an option, you can click 'Apply' at the bottom, and then click 'Exit'. Your NMRBox window will be resized accordingly.

You may have to experiment a while to find a good resolution for your screen. Once you do, you can also click 'Save to VNC startup file' so you won't have to change the resolution each time you use NMRBox. On the next page (bottom), you'll see the Mnova display with the 'Processing Template' shown, and all buttons are observable after the display resolution was changed appropriately. Remember, you may have to experiment a bit to find a good resolution for your particular display.


Resolution changer
Resolutions (original 1200x675)
(1200x675) Default 16:9
(800x600) SVGA 4:3
(1024x768) XGA 4:3
(1280x720) HD-720 16:0
(1280x768) WXGA 5:3
(1280x800) WXGA 8:5
(1280x1024) SXGA 4:3
(1360x768) WXGA 16:9
(1400x1050) SXGA+ 4:3
(1600x900) 20in-monitor 16:9
(1600x1200) UXGA 4:3
(1680x1050) WSXGA+ 8:5
(1920x1080) HD-1080 16:9
(1920x1200) WUXGA 8:5
(2048x1080) 2K-Digital-Cinema 17:9
(2048x1536) QXGA 4:3
(2560x1080) UWHD 21:9
(2560x1440) WQHD 16:9
(2560x1600) WQXGA 8:5
(2560x2048) QSXGA 5:4
(3440x1440) UWQHD 21:9
(3840x2160) UHD-1 16:9
(4096x2160) 4K-Digital-Cinema 17:9
Variations
Standard
Scale resolution by 90%
Double Horizontal Width (multiple monitors)
Options
Apply
Save to VNCstartup file
Exit


jurbauer.nmrbox.org (v6-0h24:33 (jurbauer)) - VNC Viewer
MestReNova - [Document 1*]
Processing Template
Time Domain
Frequency Domain
Truncate
Drift Correction
FID Shift
Frequency Shift
Apodization
Phase Correction
Baseline Correction
Smoothing
Reverse
Absolute Reference
Reference
Cuts
Zero Filling and LP
Fourier Transform
Apply
OK
Cancel
f1 (ppm)