MestReNova Manual

Last Revision: 26-2-2008

MestreNova 5.2

by MESTRELAB RESEARCH

This is the manual of MestreNova 5.2.

MestreNova Manual

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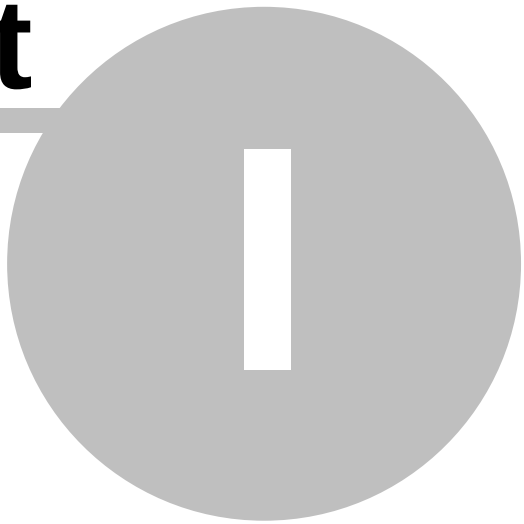

1 Introduction

Welcome to **MestReNova (Mnova)**. **Mnova** is the latest Nuclear Magnetic Resonance data processing, visualization, simulation, prediction, presentation and analysis software package available on the market. This **Help Manual** introduces you to this new software, ensuring that you can use it successfully and achieve good results right from the start. We strongly suggest that you print this manual and follow it when first using **Mnova**, as this will make your introduction to the software very simple and painless, and will make sure that you quickly identify all the main functionality in the software.

Mnova is the natural evolution of our very popular application **MestReC**. However, it is more than a new version of **MestReC**. Whilst including all the NMR processing and analysis functionality present in MestReC, it is a completely new development and a completely new concept which will open up a whole new range of possibilities when storing and sharing data within organizations, changing the way in which scientists process and report NMR data. **Mnova** has been designed to make NMR both more convenient and more powerful, and to give the user improved results with minimum effort.

The program provides a variety of **conversion facilities** for most NMR spectrometer formats and includes all the conventional processing, displaying, and plotting capabilities of an NMR program, as well as more advanced processing techniques with a very easy-to-use and intuitive graphical interface. To read a little bit more on the main features, and familiarize yourself with the principles behind the program, follow this guide.

1.1 System Requirements

Before installation, please read this chapter. In this section you will find information on which hardware and software requirements must be met before you can install Mnova. In any case, it is worth noting that Mnova is a **multiplatform** software which will run alternatively on Windows, Mac OS X, Linux and Unix-like systems. From now on, multiplatform laboratories will be able to allow each researcher to work on their Operating System of choice, and to seamlessly share data and processing capabilities with other researchers working on different platforms. There is no longer a need to have three different software packages, and three different file formats, for different Operating Systems.

The minimum and recommended system requirements for the software are:

Windows
Mac OS X
Linux

1.1.1 Windows System Requirements

The *minimum* recommended configuration for Mnova installation is at least Pentium 300 MHz, 128Mb RAM, a VGA color monitor with 800 x 600 pixels resolution, a compatible mouse and Windows 2000 as operating system. However, ideal system requirements for optimum operation of the software are Pentium 1,6 GHz or higher CPU with 512 Mb or more of RAM memory and Windows 2000/XP, Server 2003 or Vista. Mnova will not install under Windows 98 or lower. The default installation requires about 75 Mb of disk space.

If you are going to install (or uninstall) the program on a Windows 2000/XP system, be sure you have administrator privileges, because the installation procedure is going to install several files in your system folders. If you don't have such privileges, you won't be able to install or uninstall the program

correctly.

1.1.2 Mac OS X System Requirements

Mnova requires at least Mac OS X 10.4, with PowerPC or Intel architecture. The current version of Mnova is not compatible with Mac OS X 10.5 (Leopard), although the oncoming release version will be.

1.1.3 Linux System Requirements

Mnova requires at least Pentium 300 MHz, 128Mb RAM, Video Adapter Super VGA (800 x 600) with X Library (Xlib). Please, be sure to choose the correct setup file for your Operating System (Debian Etch, OpenSUSE 10.1, Fedora Core 6, Ubuntu 6.06-Dapper Drake, Red Hat Enterprise 3 or 4 and Mandriva Linux 2007)

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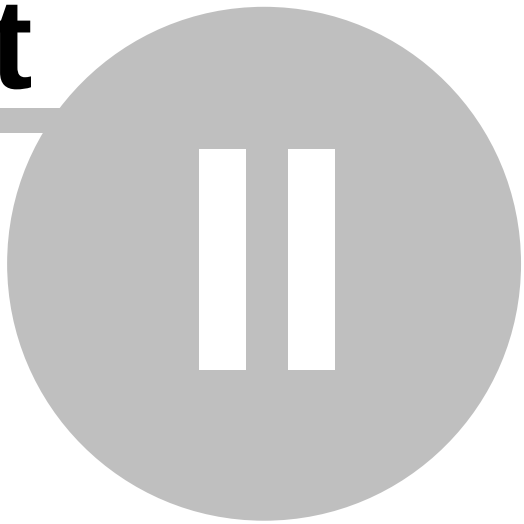

2 Mnova 5.2

Welcome to **Mnova 5.2**

In our case, the fact that this is a *commercial release* means the following:

1. We have tested **Mnova** in-house as far as possible to make sure that we deliver to you a version which is robust, already of high quality, and which does not present any apparent bugs. However, you may find a small number of bugs which we have not yet identified. Please tell us about any you discover by e-mailing us at support@mestrec.com.

2. There are some advanced functionalities which will be included in the forthcoming commercial releases, but which are not yet in this version. The reason for this is that, in order to bring you the advantages of MNova as soon as possible, we are bringing you the core functionality and more, while we complete some advanced and important but less-used functions. As a valued user of MNova you will be notified the moment these additional functions become available as upgrades. As a result of this you may find some features which were present in the final release of **MestReC**, our current application, are not yet implemented into this commercial version of **Mnova**. A list of such features, together with their planned time of inclusion in the software, can be found below.

3. Although we have now released the product commercially, we are, as ever, very interested in your feedback, bug reports and implementation suggestions. Please keep this coming, as we plan for continuous and rapid improvement of the application, to make forthcoming releases even more in tune with our customer needs.

What's New?

New Features

- Improved Full View feature
- Improved and customizable tables
- Customizable reports
- Zoom Out
- Best Algorithm for the prediction plugin
- Compatible with MacOS X 10.5 (Leopard)
- Multiplicity of the 13C signals
- 2D Resolution Booster
- Improved Contextual Menus
- Faster Script Engine
- Easier Script Interface
- Floating Toolbars
- Improved Bruker Converter
- Improved NUTS converter
- Exporting to SVG, TIFF, PS, EPS
- Importing SVG, TIFF + GIF, EPS
- Automatic solvent detection in 13C NMR

Bugs Fixed

- Problems with some Varian 1H-15N HMBC´s
- Problems predicting 3D molecular structures with explicit hydrogens
- Fist Point multiplication is now unlimited
- Problems selecting traces graphically

- Minor problems with the accurate of the assignments
- Problems with the panning
- Problems opening 2rr files of Bruker 2D-NMR spectra
- Problems with the 'Insert Object' feature
- Problems with the assignation of some Bruker spectra

Our plan is to distribute a new release approximately every four months. The functionalities you can expect to see added to the software during the intervening period are:

- Create Expansions
- Derivative Processing of Spectra
- Covariance NMR
- GEN-2D NMR, Processing to Improve Resolution in the Indirect Dimension.
- Line Fitting
- Spectral Editing
- DOSY Processing

We hope that you will find this version of Mnova to be one of the most complete NMR processing and analysis tools available in the market, combined with a revolutionary interface and many new, original and innovative features.

During the commercial release periods, we will welcome your **feedback**, both in the form of bug reports and also of implementation suggestions and feedback on current development. Please write with any of these to support@mestrec.com

Thank you once again for your support of our development effort!

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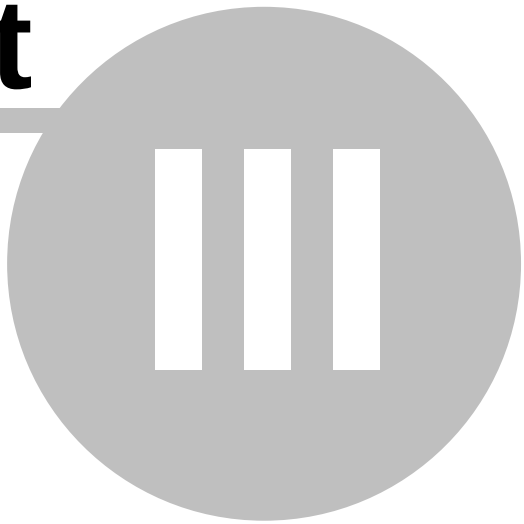


3 Installation Guide

Instructions to Install and Activate an Individual Copy of Mnova:

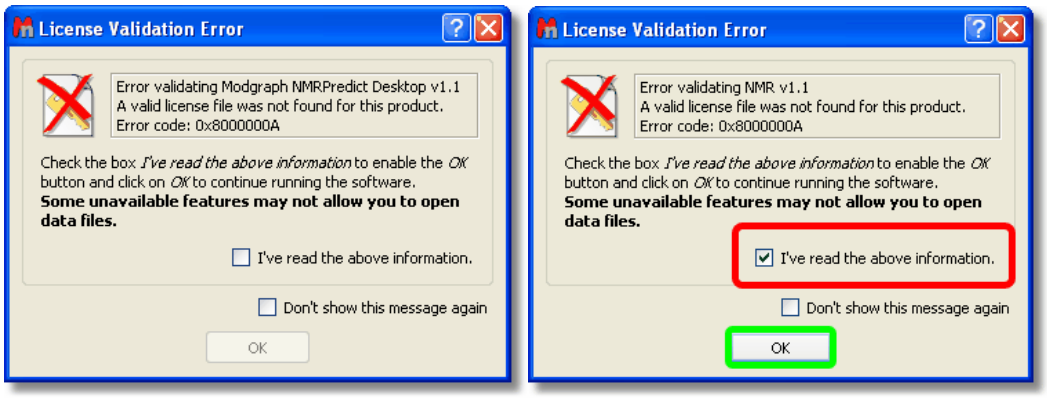
1. If you are going to install the program on a Windows 2000/XP system, be sure you have administrator privileges, because the installation procedure is going to install several files in your system folders. If you don't have such privileges, you won't be able to install or uninstall the program correctly.

2. On each host, download the Mnova SETUP file from our website at http://www.mestrec.com/descargas.php?idp=1&i18n=1. Then, double click on the Mnova SETUP file and follow the instructions as they appear on screen.

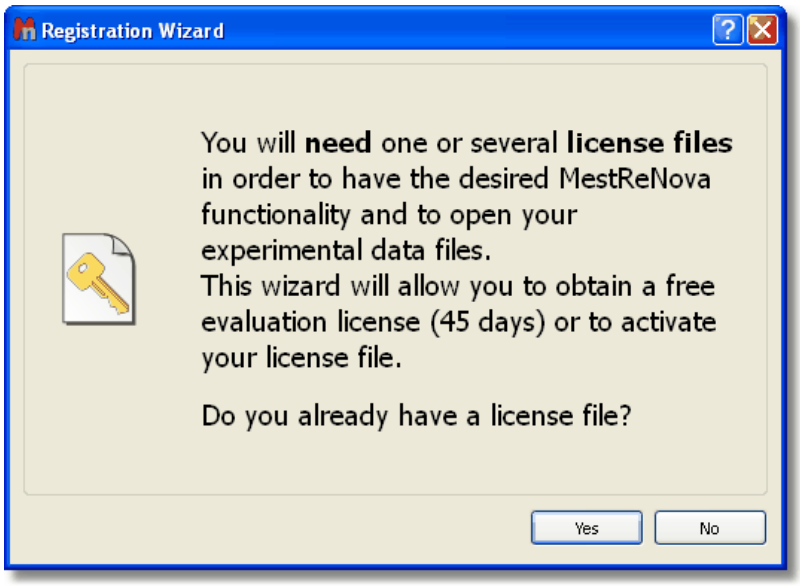
If you have had an alpha version of Mnova installed previously, the installer will inform you that it is necessary to uninstall the previous version of Mnova. Make sure that this version is closed and click OK and then OK again to confirm. This will uninstall Mnova from your computer. You will get a message to inform you that uninstall was successful: click 'Continue'.

Once you have read and accepted the terms of the License Agreement, click on "**Next**" and select the destination folder where the program files will be placed in your computer. We suggest that you accept all the installation defaults. Once you have chosen the Start Menu Folder as prompted by the installer, click on **"Install"**. This will install the application on your computer. On completing the installation, it will prompt you to select **"Finish"** to run the application.

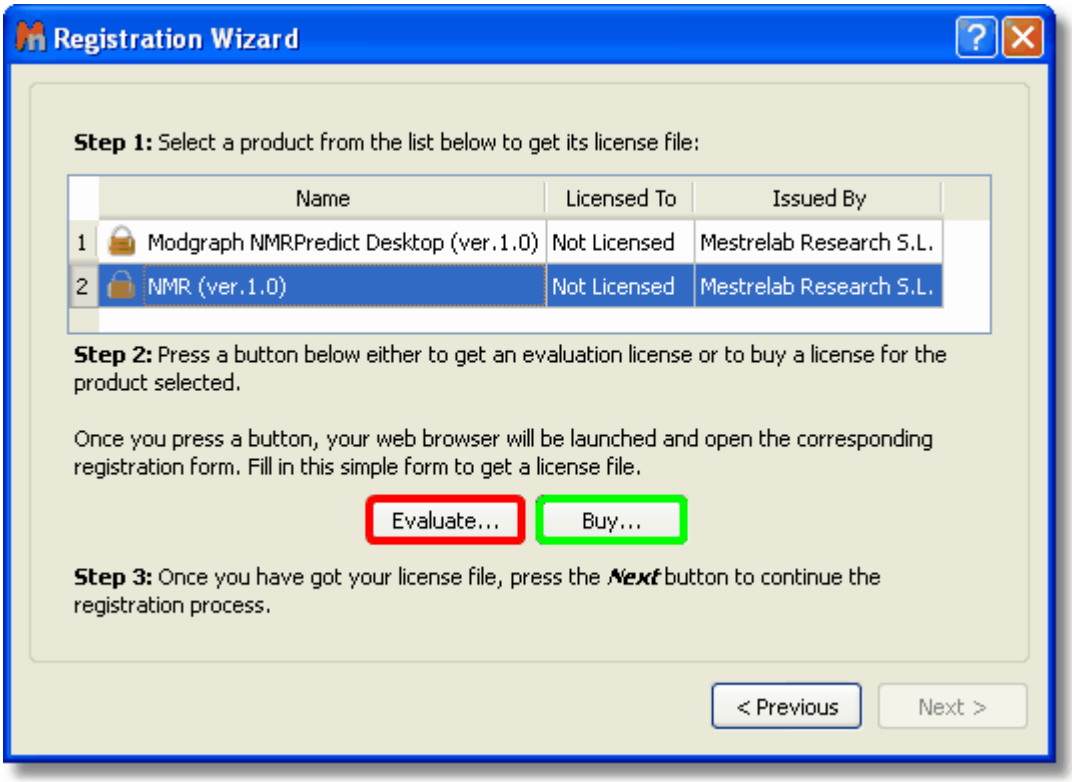
3. Run the application on each host. The following dialogs notify you that a license for the NMR plugin was not found will appear:



4. Check the 'I´ve read the above information' boxes, press the 'Ok' buttons and continue running the application. You will obtain the Registration Wizard dialog box, shown below:



5. If you have already a license file, click on 'Yes' and go to the step 8. If you do not have it, click on 'No' to display the following window:

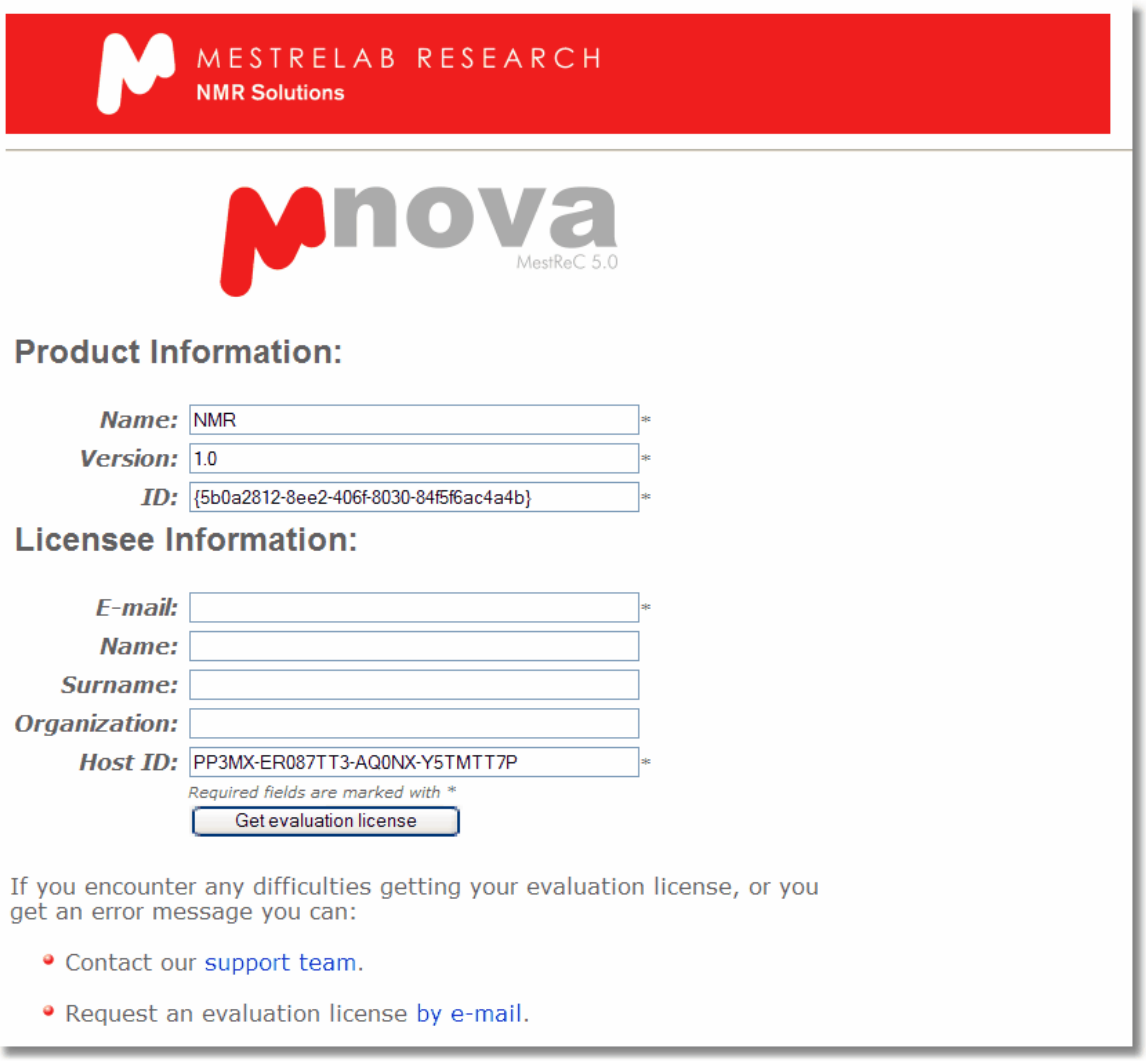


6. Select the desired *NMR* plugin item (which is the software you started to install in Step 1) and click on 'Evaluate' (red square in the picture above) or on 'Buy' (green square in the picture above).

If you Click on the 'Evaluate' button you will be redirected to a web page where you will be asked for a few registration details and your Host ID number, will be generated. An illustrative example appears below. Your Evaluation license will be generated automatically after having clicked on 'Get evaluation

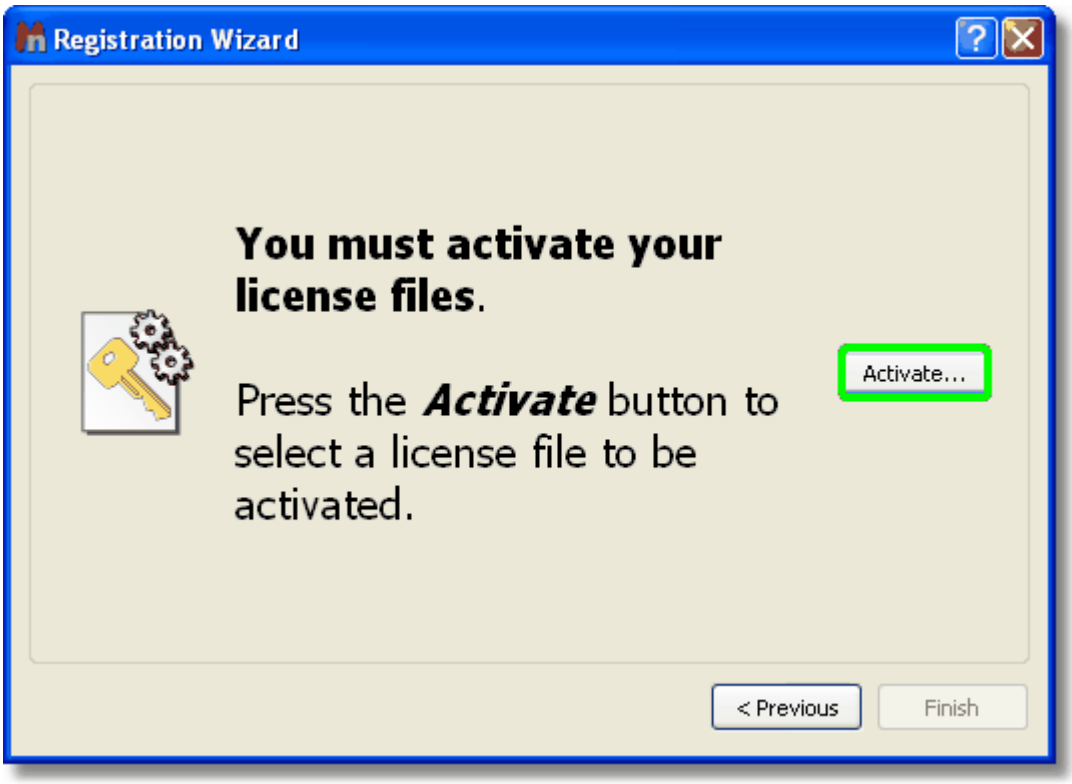
license'.

Alternately if you press the 'Buy' button you are again redirected to a web-page where a similar procedure occurs. However, instead of receiving a licence right away your registration information and your Host ID number are automatically sent to our Sales Department by e-mail. They will handle your purchase request and, once the financial formalities are complete, you will receive your licence file with installation and activation instructions by e-mail.

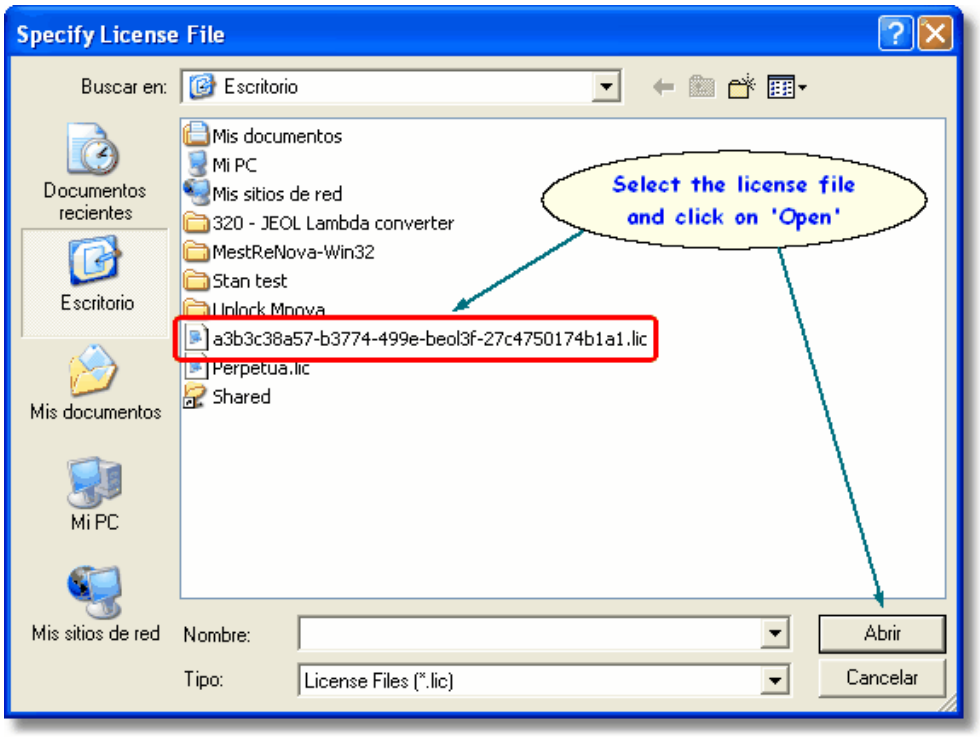


7. Save the 'Evaluation Licence' file or the 'Purchased License' file and click on 'Next'.

8. The picture below will be displayed.



9. Click on the 'Activate' button (the green square in the picture above), and this will open the 'Specify License File' dialog (see the picture below). Navigate on your Windows browser until you find the license file, and double click on it and select it (see example below). Now you should be able to run the application without receiving an error message dialog.



How to uninstall Mnova?

To **uninstall Mnova** on Windows; navigate to thenavigate to the Mestrelab Research S.L\MestReNova folder and double-click on **'Uninst'**  . Finally confirm by clicking 'Yes'.

To install Mnova on **Linux**: download the Mnova SETUP file from a web address provided by us, and:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -i namefile.deb
Other (*.rpm): type from the command line interface: rpm -i namefile.rpm

To update Mnova:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -i namefile.deb
Other (*.rpm): type from the command line interface: rpm -U namefile.rpm

To uninstall Mnova:

Debian, Ubuntu (*.deb): type from the command line interface: dpkg -r mestrenova
Other (*.rpm): type from the command line interface: rpm -e MestReNova

Part

4 Shortcuts

Mnova provides shortcuts for finding commonly sought tools. You can see a list of these commands in the table below:

Shortcuts in Windows or Linux/ Mac:	Action:
Ctrl+N / Cmd+N	Opens the 'New' dialog box.
Ctrl+ O / Cmd+O	Opens the 'Open' dialog box.
Ctrl+ F4 / Cmd+W	Closes the active document.
Ctrl+S / Cmd+S	Opens the 'Save' dialog box.
Ctrl+P / Cmd+P	Opens the 'Print' dialog box.
Ctrl+D / Cmd+D	Opens the 'Export to PDF' dialog box.
Ctrl+Z / Cmd+Z	Undoes the last action.
Ctrl+Y / Cmd+Y	Redoes the previously undone action.
Ctrl+X / Cmd+X	Cuts the selected item(s) to the clipboard.
Ctrl+C / Cmd+C	Copies the selected item(s) to the clipboard.
Ctrl+V / Cmd+V	Pastes the copied items(s) from the clipboard.
Delete	Deletes the selected item(s)
Ctrl+A / Cmd+A	Selects all the documents
Ctrl+M / Cmd+M	Creates a new page.
+	Increases intensity
-	Decreases intensity
Z	Activates the ‘Zoom in’ mode. Once ‘Z’: Horizontal Zoom; twice ‘Z’: Vertical Zoom and thrice ‘Z’: Zoom box.
SHIFT+Z	Activates the ‘Zoom Out’ mode.
Ctrl+Space Bar / Cmd+Space Bar	Temporarily activates the ‘Zoom in’ mode.

Shortcuts in Windows or Linux/ Mac:	Action:
X	Activates the ‘Cut’ mode. Once ‘X’: Horizontal Cut; ; twice ‘X’: Vertical Cut.
C	Activates the Crosshair.
V	Activates the ‘Restore Cut’.
P	Activates ‘Panning’ mode. Once ‘P’: Panning; twice ‘P’: Horizontal Panning and thrice ‘P’: Vertical Panning.
Space Bar	Temporarily activates ‘Panning’ mode.
ESC	De-selects a previously selected tool or closes an open menu.
Page Dn	Scrolls down on the page navigator.
Page Up	Scrolls up on the page navigator.
SHIFT+Up arrow key	In 1D and 2D NMR: Increases intensity In Stacked NMR: scrolls up to the previous trace.
SHIFT+Dn arrow key	In 1D and 2D NMR: Decreases intensity In stacked NMR: scrolls down to the next trace.
Up and Down arrow keys	Selects the adjacent item in the Page Navigator in the indicated direction, or moves a selected item on the main page up or down

Part V

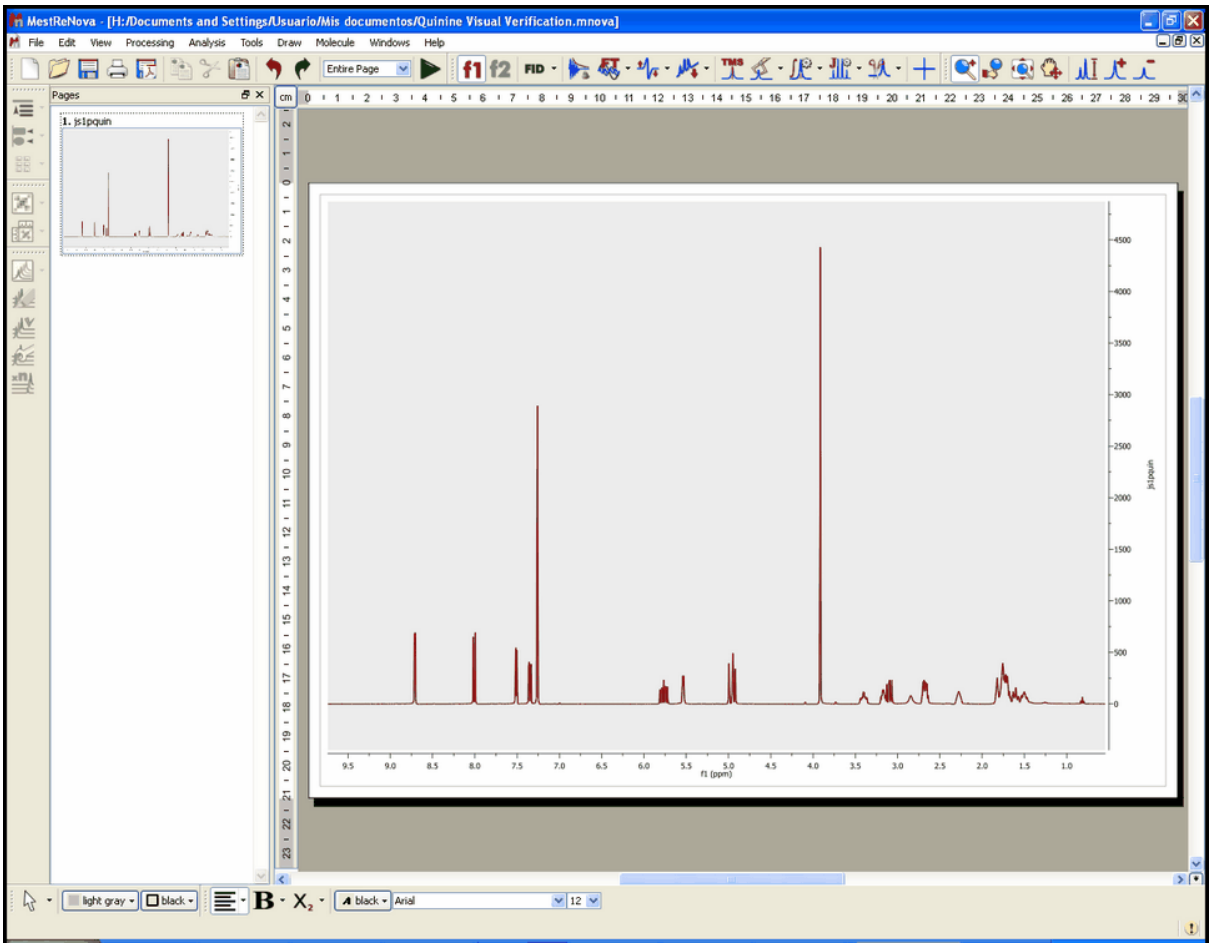
5 Fast Visual Guide to process routine 1D-NMR experimental data

Go through the following procedure to process a routine 1D-NMR spectrum:

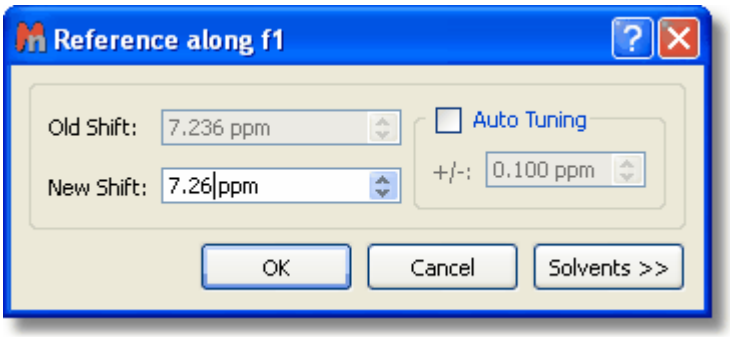
1. Run Mnova by clicking on the corresponding **'Mnova'** icon. 

2. Go to ***'File/Open'*** menu, use the keyboard shortcut **<Ctrl+O>** (Cmd+O in Mac) or click on the **'Open'** icon on the toolbar. You can also open your Navigator and just click and drag the NMR folder.

3. Locate the desired experiment on your hard disk, open the experiment folder and click on the FID file. The spectrum will automatically open **already processed.**

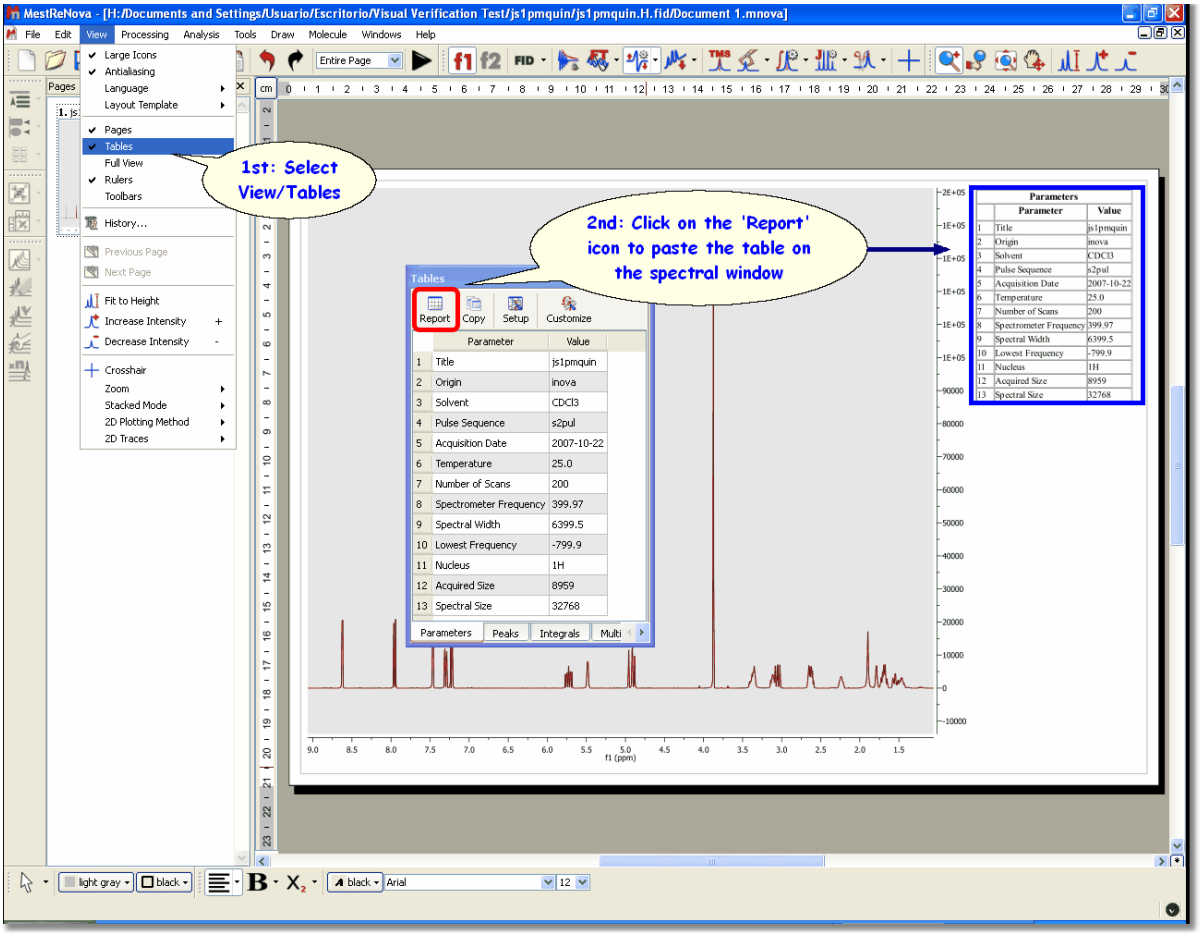


4. You can calibrate your spectrum by clicking on the '**Reference**'  toolbar button and then selecting the peak you want to be a reference point.

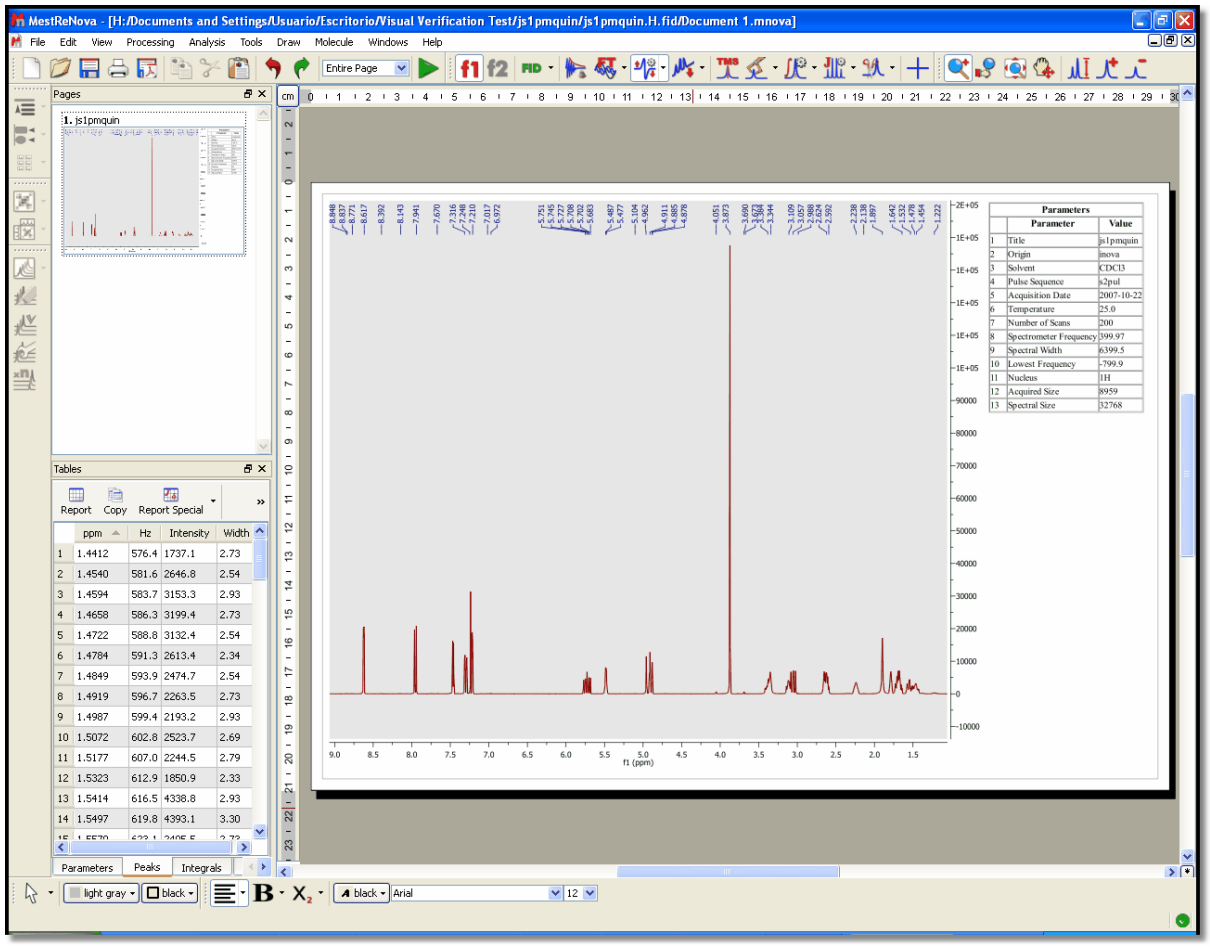


5. If your spectrum needs a **Phase Correction** or a **Baseline Correction,** click on the **'Phase Correction'** icon  or on the **'Baseline Correction'** icon  on the toolbar.

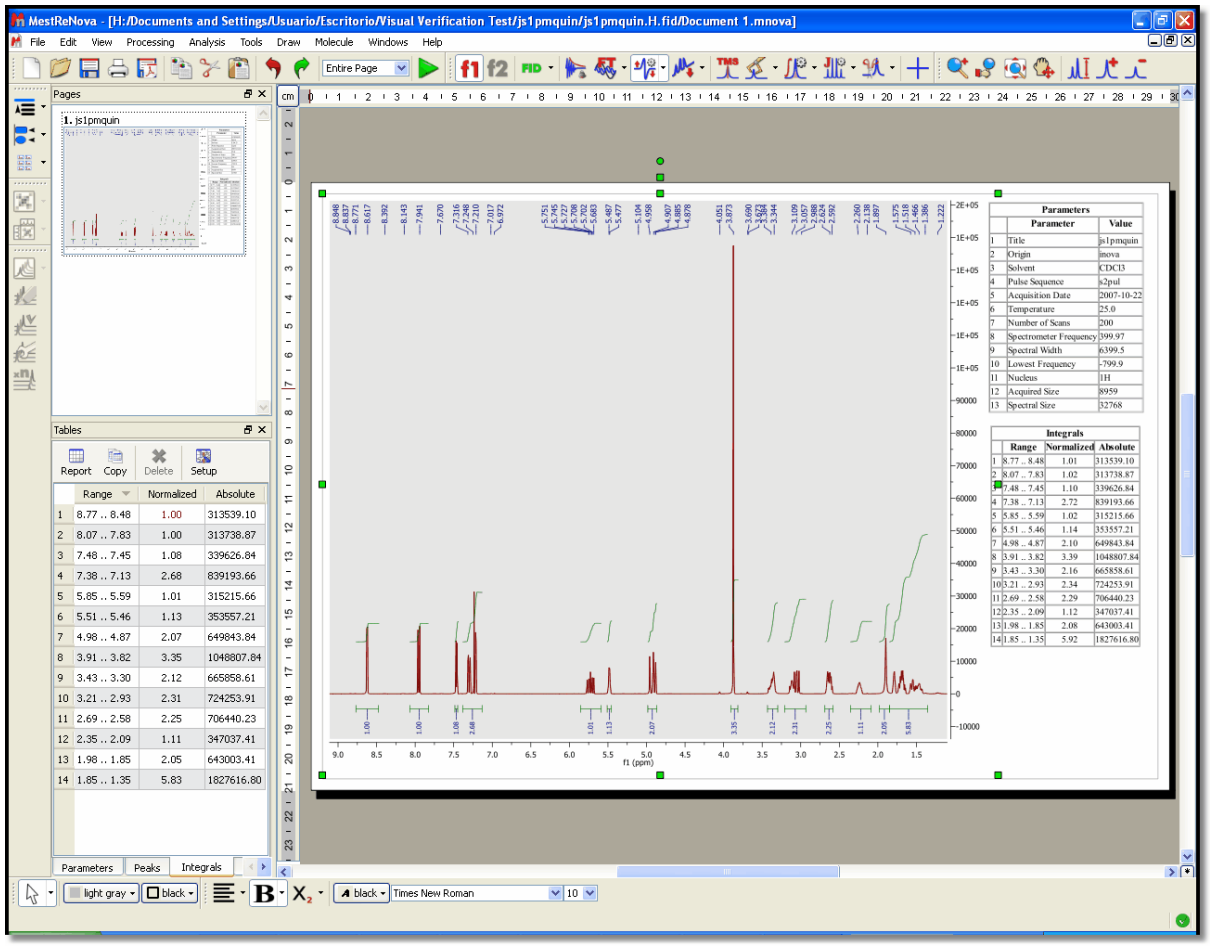
6. To paste the Table Parameters on the spectral window, just follow the menu: View/Table and click on the **'Report'** icon:



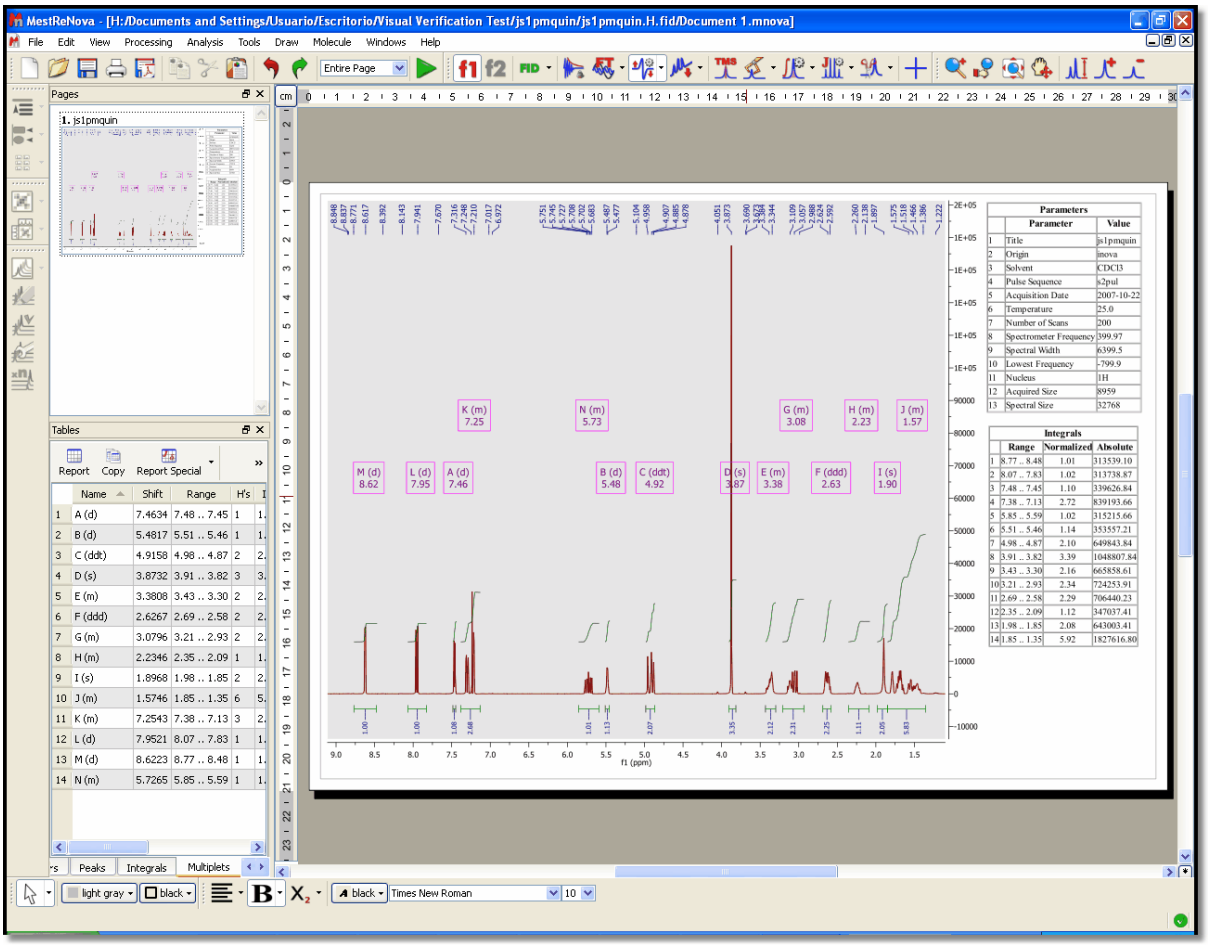
7. Click on the **'Peak Picking'** icon  to obtain an automatic Peak Picking analysis.



8. Click on the **'Integration'** icon  on the toolbar and your spectrum will be integrated automatically. To see the numerical value of the integrals look below each integral. To see a list of the integral values and the regions integrated go to View/Tables and select the 'Integrals' tab.

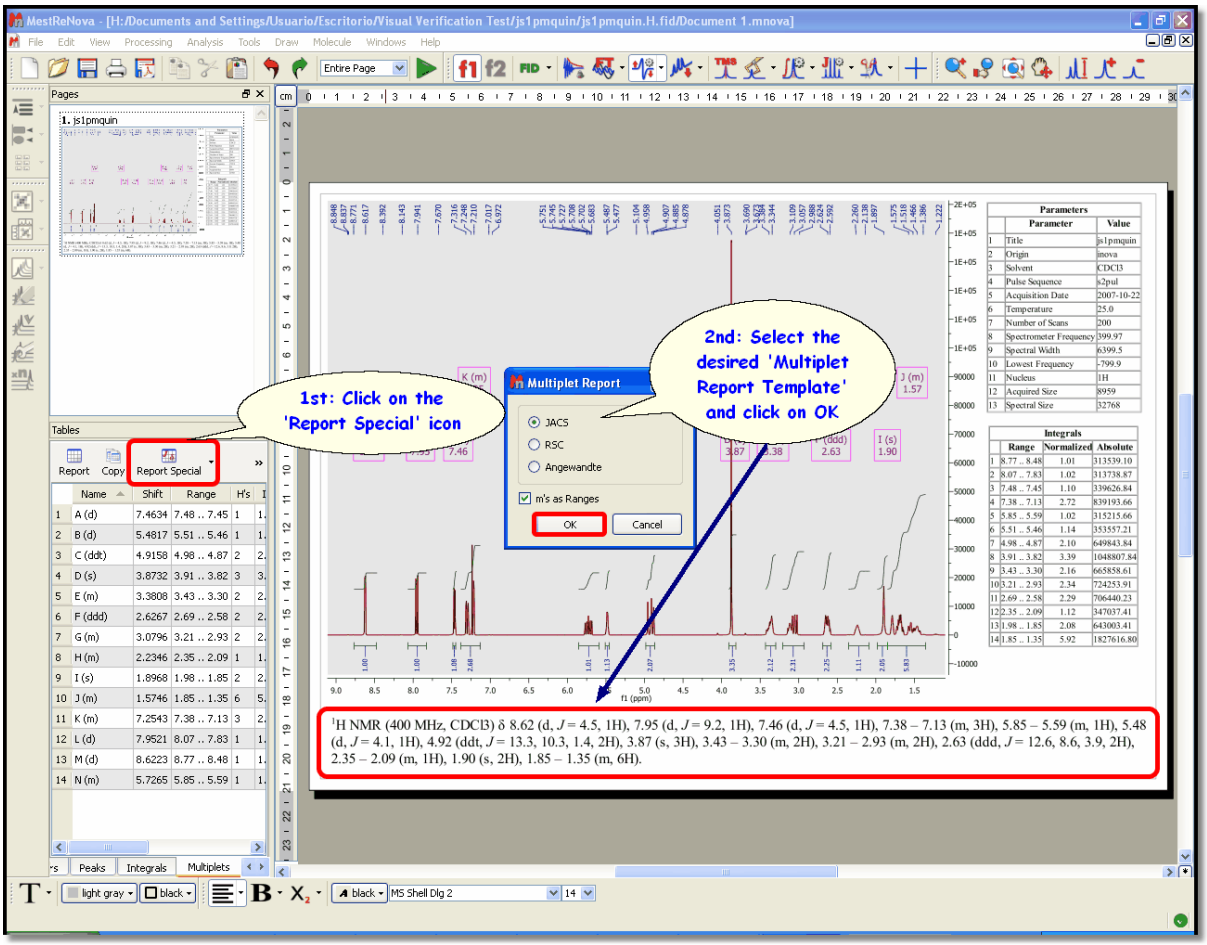


9. The user can also apply an automatic multiplet analysis by clicking on the **'Multiplet Analysis'** icon  .

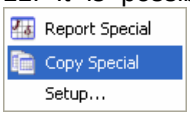


10. The user will be able to obtain the multiplet report list by clicking on the **'Report Special'** icon 

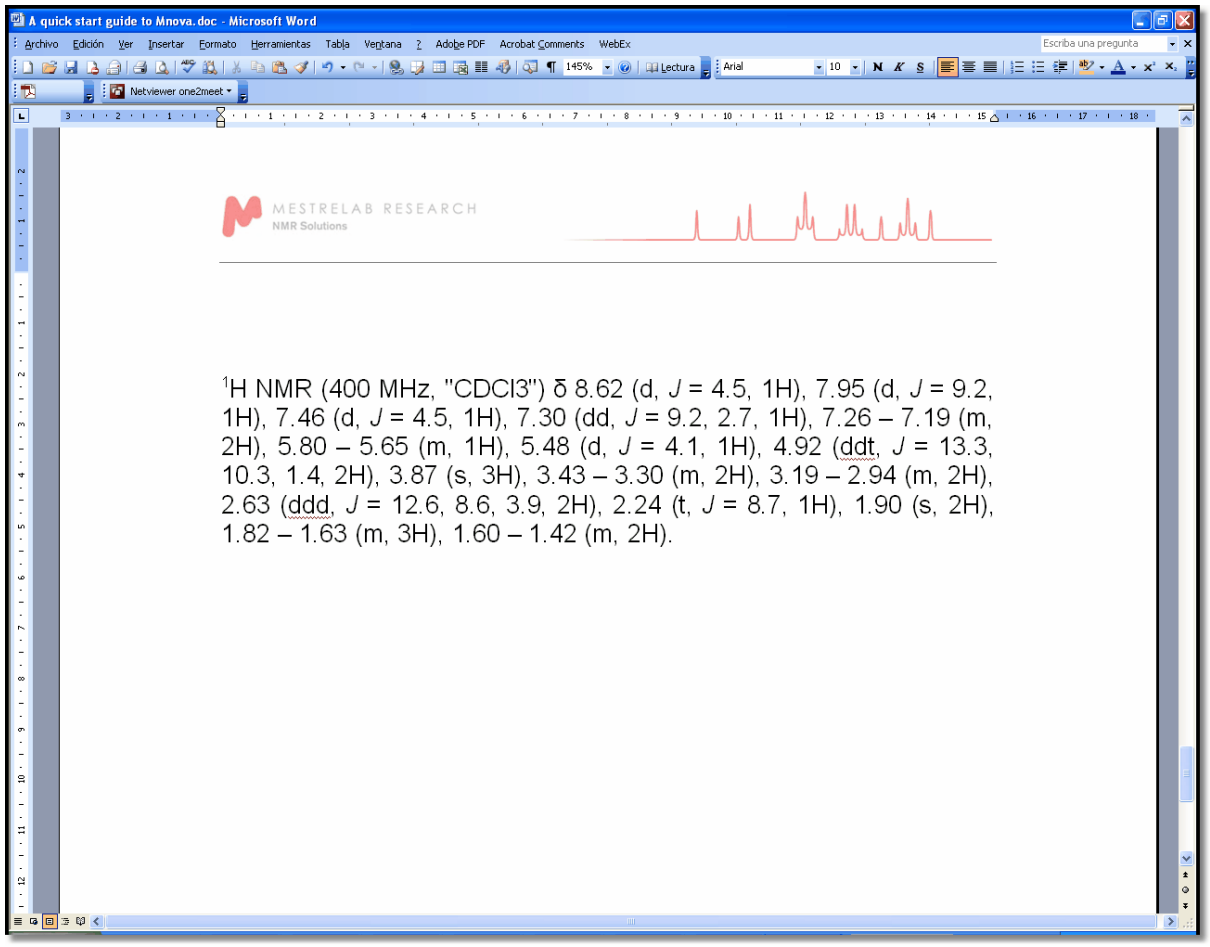
in the Multiplets Table. This will display the 'Multiplet Report' dialog box, which will allow the user to select the desired template (JACS, Royal Society of Chemistry and Angewandte).



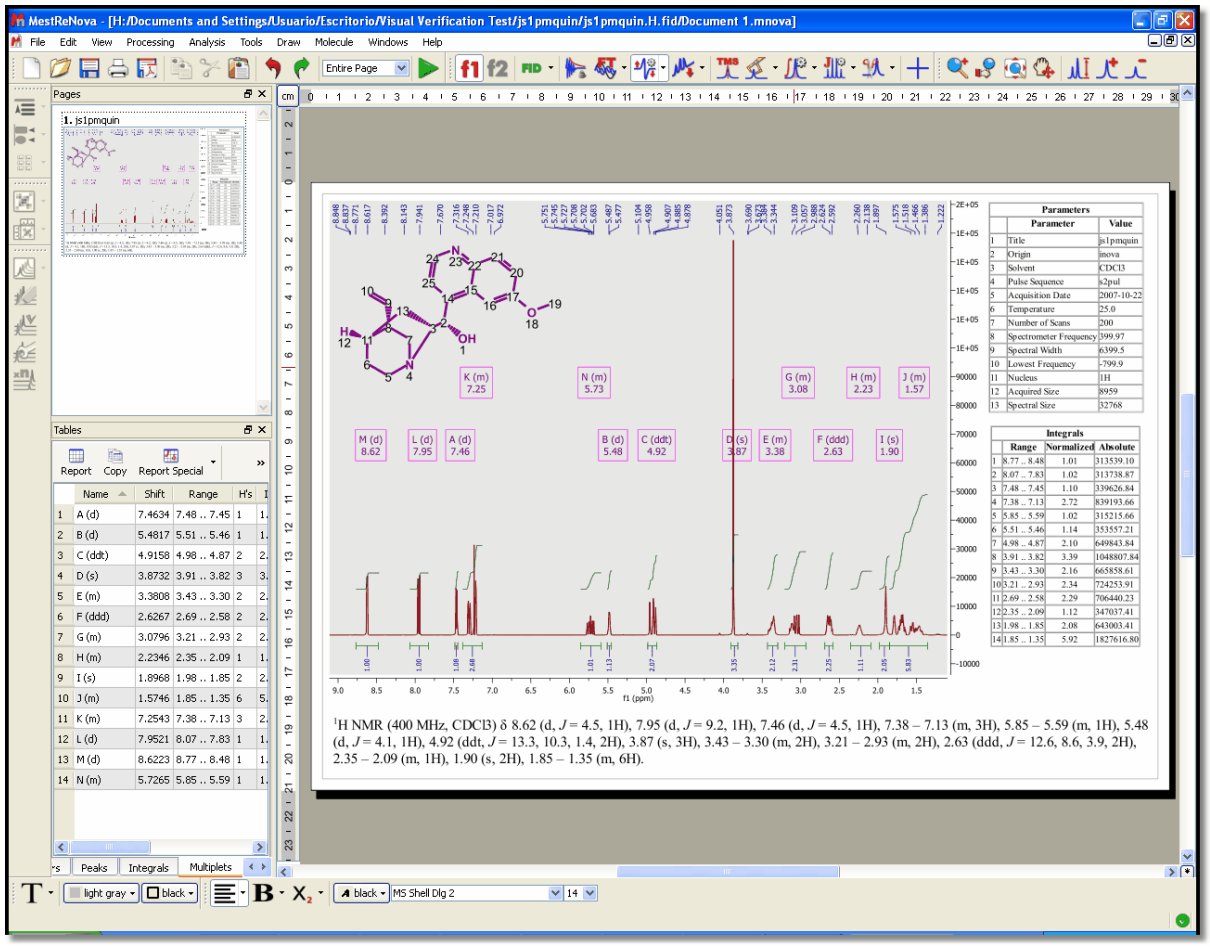
11. It is possible to copy the multiplet report to the clipboard just by selecting 'Copy Special'



on the 'Report Special' scroll bar menu and then paste the report in another document just by using Ctrl+V or Cmd+V.



12. The user will be easily able to copy & paste molecular structures from Chemdraw, IsisDraw or Chemsketch (by using Ctrl+C & Ctrl+V, or Cmd+C & Cmd+V).



13. Finally, the user will be able to save  , print  or export to PDF  , the document by clicking on the corresponding icon in the toolbar.

See also:

[1D Processing Tour]

[Fast Visual Guide to process routine 2D-NMR experimental data]

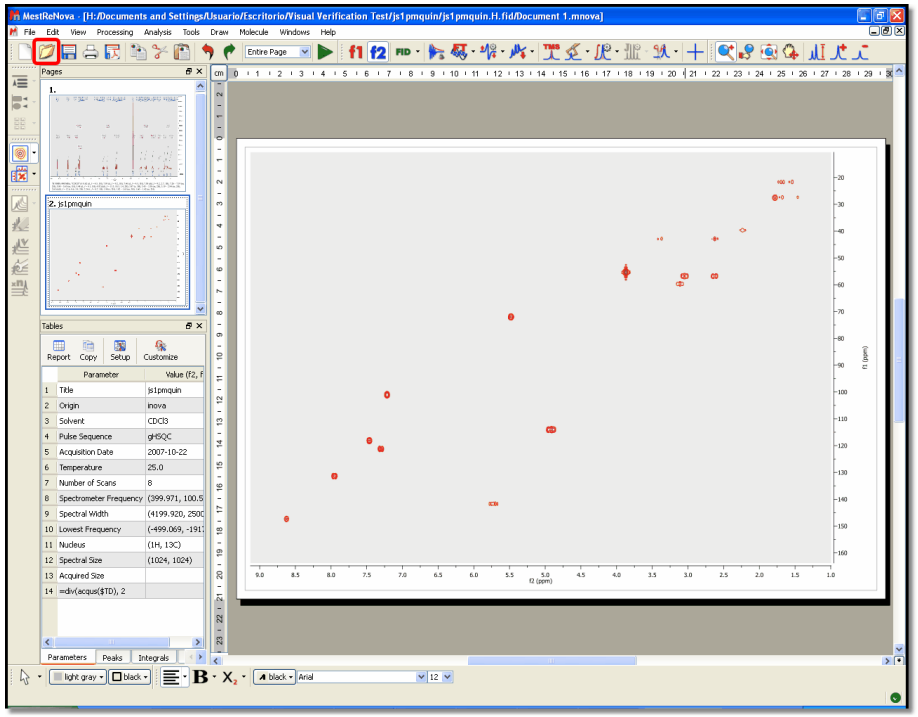
Part

6 Fast Visual Guide to process routine 2D-NMR experimental data

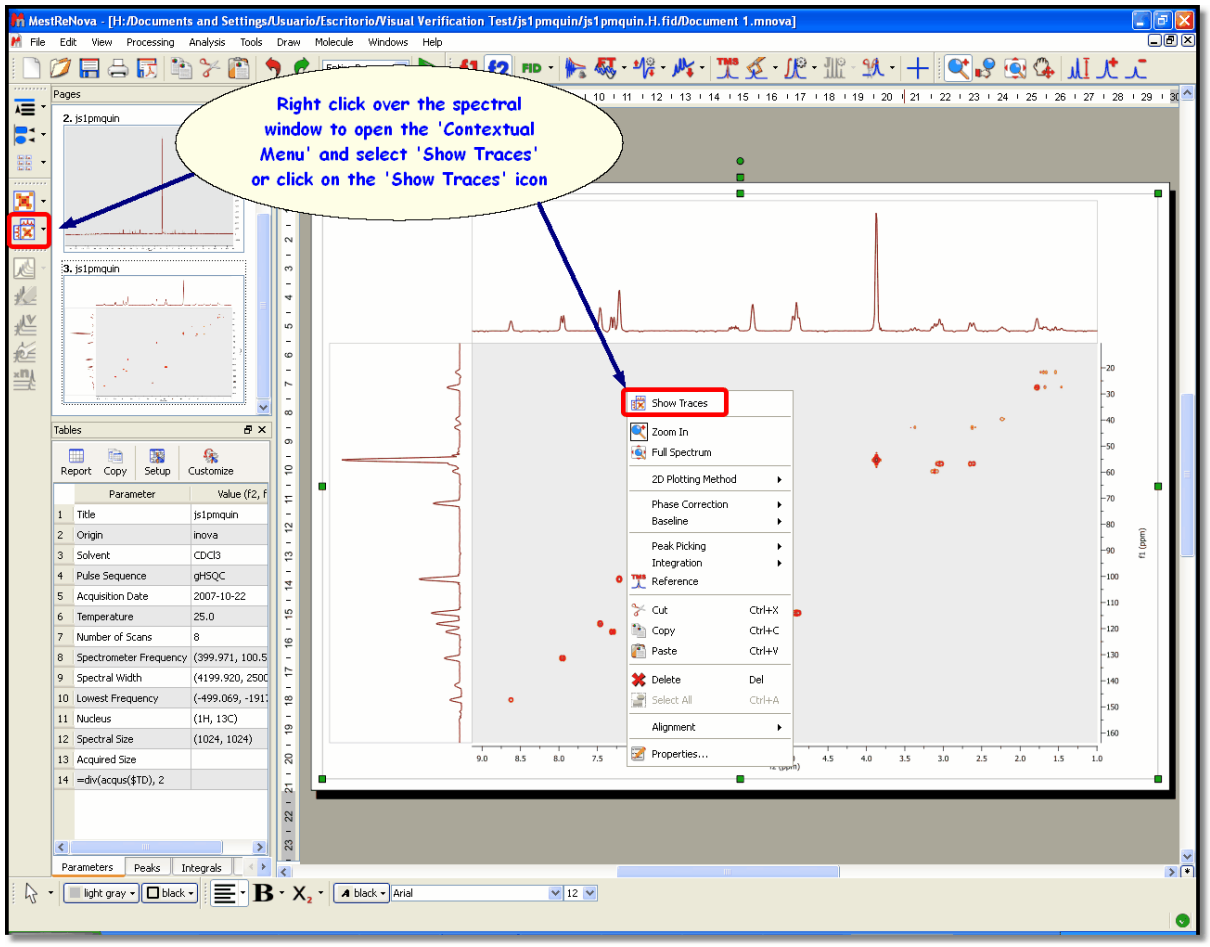
(Not available in Mnova Lite)

The procedure to process a **2D-NMR** spectrum is very similar with the monodimensional one:

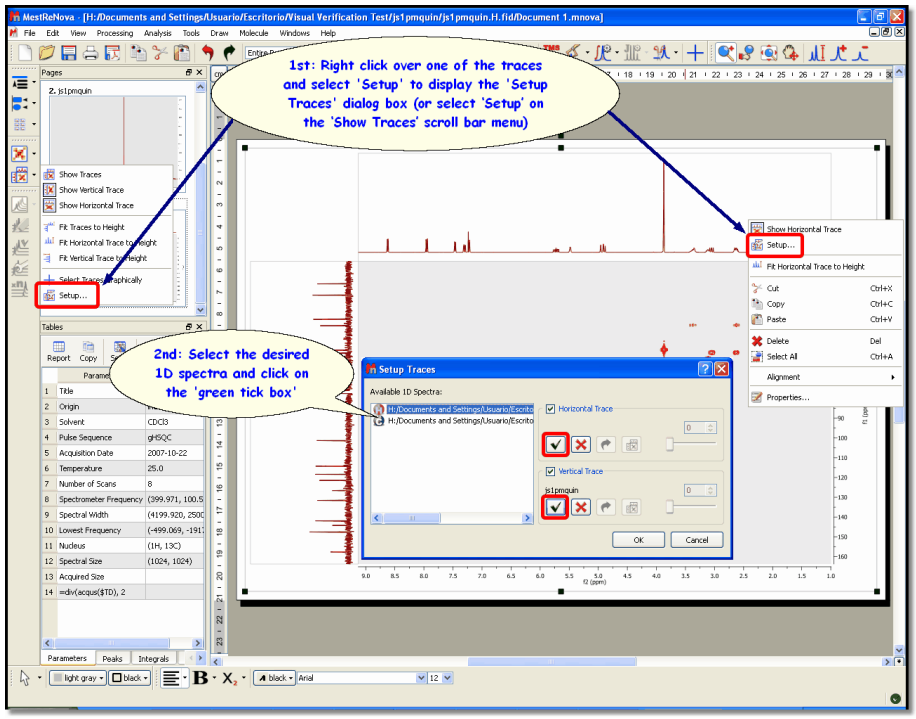
1. Click on the **'Open File'** icon  and select the FID file (or SER file in Bruker) to obtain the processed 2D-NMR spectrum. **Mnova** will select the processing functions for you (FT, Phase Correction, etc) but, of course, you may change and optimize any of them and then re-process the data if you wish.



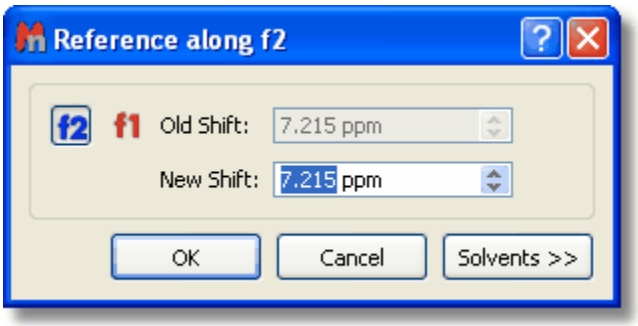
2. The user can show the traces of the 2D-NMR spectrum, just by clicking on the **'Show Traces'** icon  (or by using the contextual menu by right clicking over the spectral window).

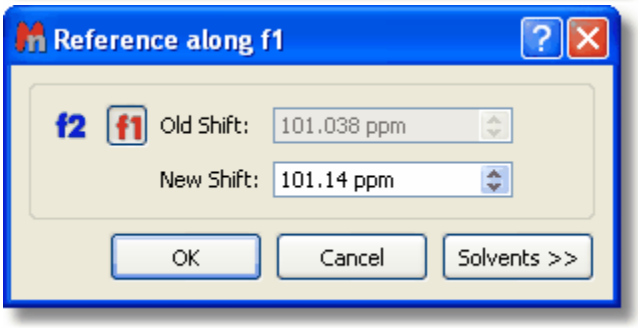


3. The user can select any currently opened 1D-NMR spectrum as a trace just by clicking on 'Setup' on the **'Show Traces'** scroll bar menu (or by using the traces contextual menu by right clicking over one of the traces).

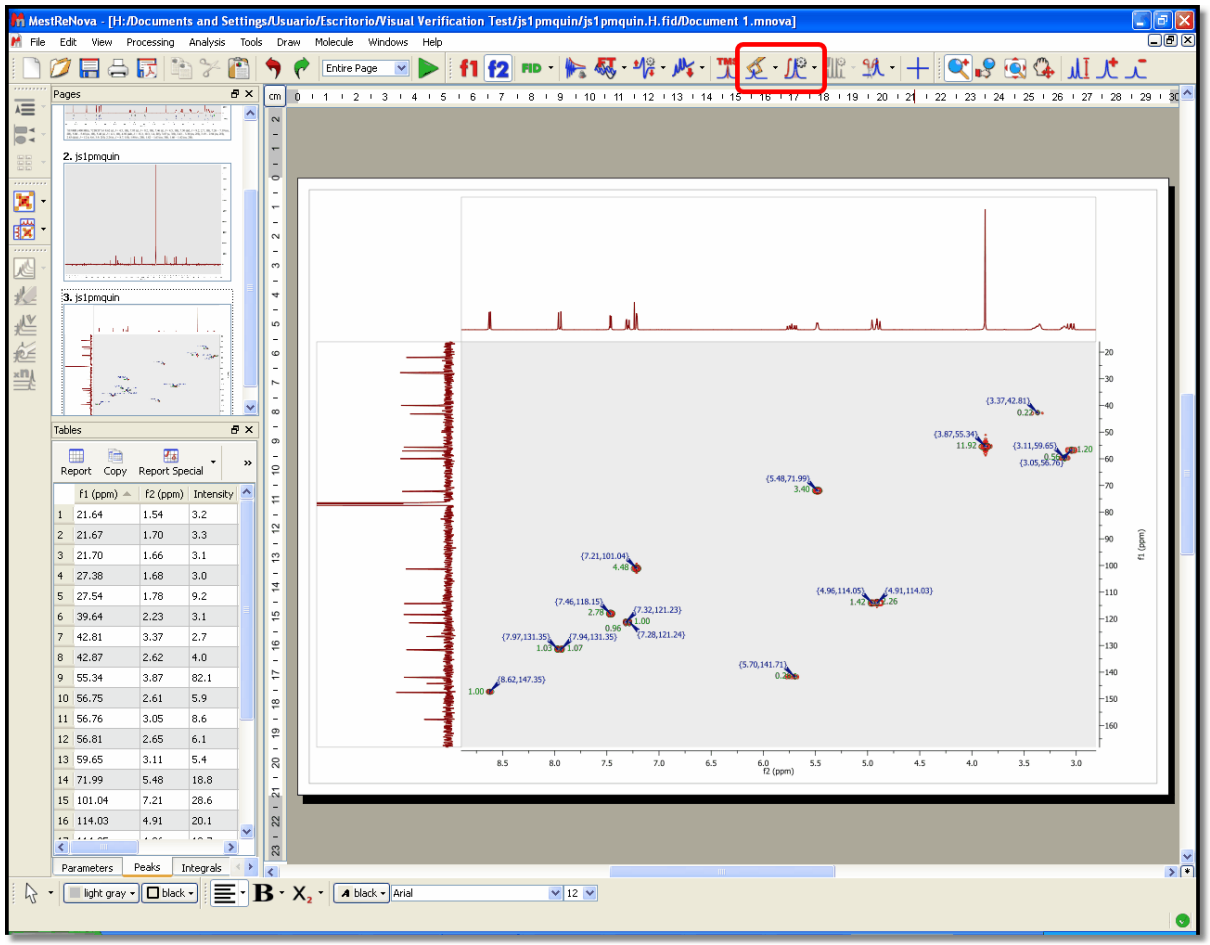


4. To calibrate your spectrum, just click on the '**Reference**' toolbar button  and then select the peak you want to be a reference point. Finally, select the desired reference along f2 and then along f1:





5. Click on the **'Peak Picking'** and on the **'Integration'** icons  to obtain a fully integrated spectrum with a Peak Picking analysis.



See also:

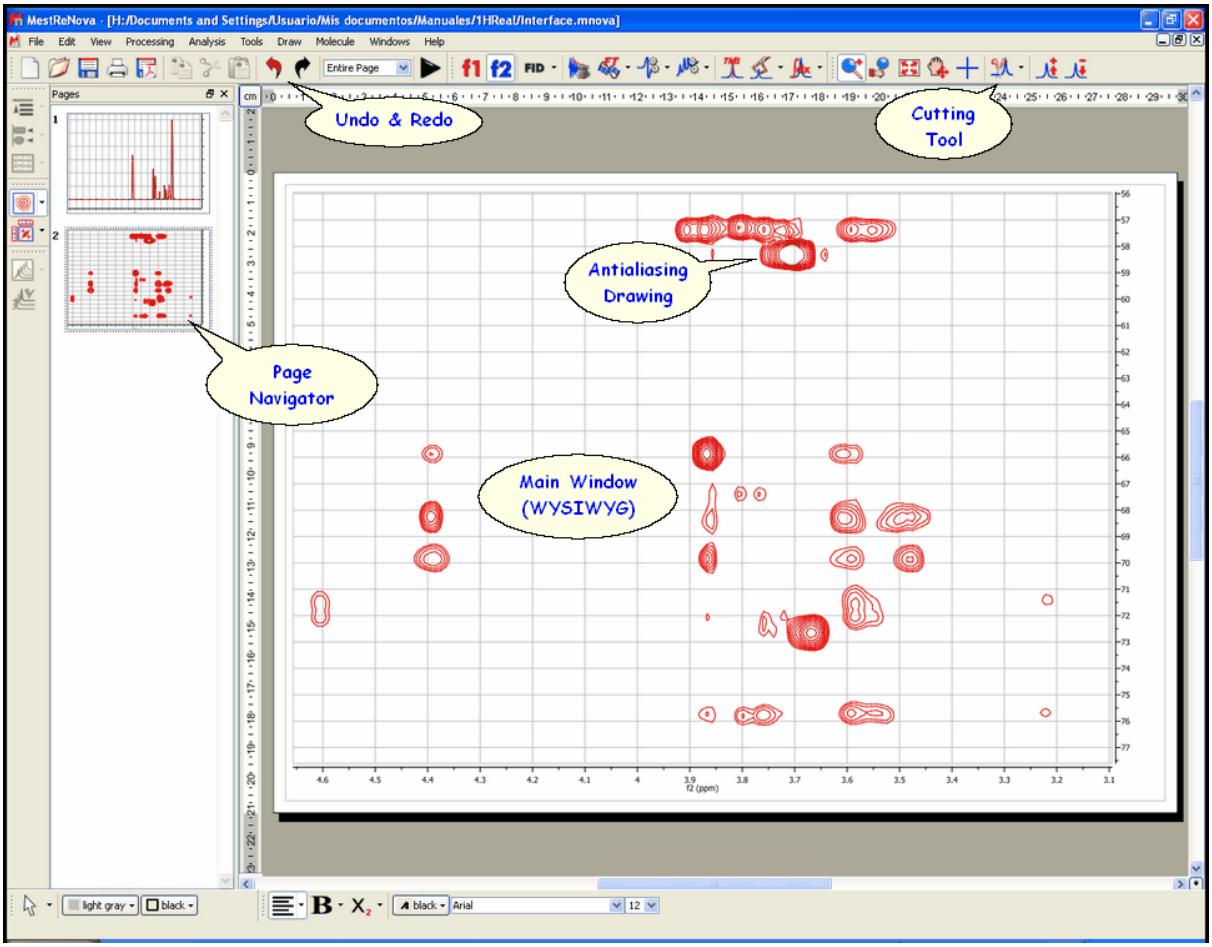
[Fast Visual Guide to process routine 1D-NMR experimental data]
[1D Processing Tour]
[2D Processing Tour]
[Manual Phase Correction in 2D-NMR]

Part

7 The Mnova interface

The **Mnova** interface has been designed to minimize the learning curve for the new user, by capitalizing on familiarity and commonality with some of the most commonly used general software packages on the market. The interface consists of a main page window with a menu bar, toolbars, dialog boxes, rulers, active window tabs, etc. giving users total flexibility in the organization of their data. With **Mnova** you will only need a couple of mouse clicks to open and process your NMR data automatically. When you first open **Mnova**, you will immediately notice a few things which represent the fundamental graphical principles of the program:

- First of all, the software is **multipage**, à la **Powerpoint.**
- The **Mnova** Graphical User Interface is based on the 'What You See Is What You Get' principle (**WYSIWYG**).
- The **Mnova** graphical engine makes extensive use of the anti-aliasing technique to produce very high quality spectral plotting.
- The program includes a powerful **Undo/Redo** mechanism within a standard toolbar.



In a few minutes and thanks to the intuitive interface you will gain a good command of the most powerful and modern tools provided by Mnova, such as:

- One single command to open and process FIDs in any file format
- Powerful drawing tools with advanced text editing capabilities.
- Cutting tool to exclude uninteresting regions from the spectrum.
- Powerful scripting engine.
- Molecular viewer with assignment capabilities.
- Prediction of 1H and 13C NMR from chemical structures.
- And much more...

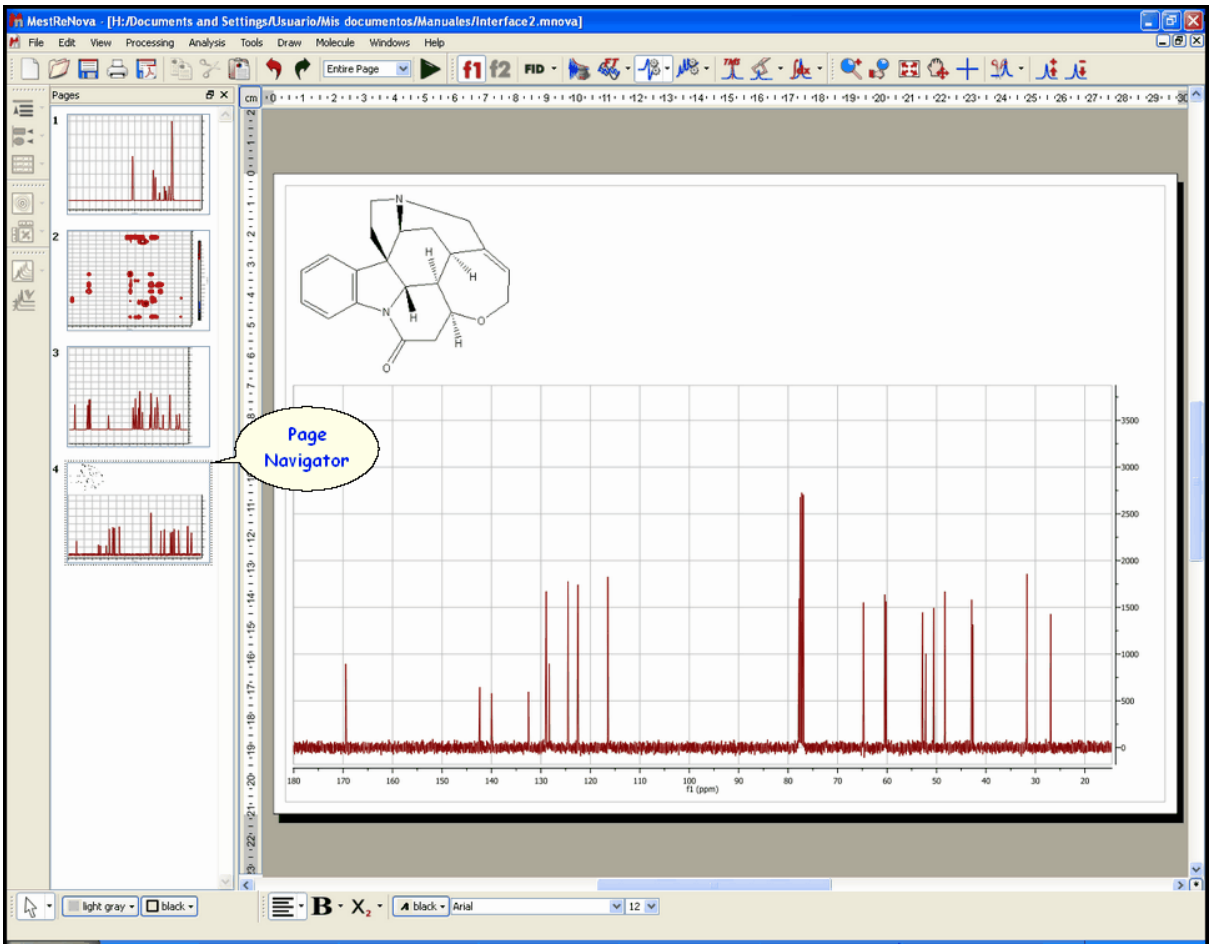
See also:

Multipage Software
Working on a single page
Zooming
Cutting
Undo & Redo
Graphics & Annotations

7.1 Multipage Software

The **Mnova interface** is designed to look and feel like **MS PowerPoint**. We think that the great majority of people in science are familiar with this presentation and reporting software, and that making our program behave in a very similar way to Powerpoint will ensure that even new users find it very easy to work with from the very start.

The first thing you will notice is that the software is **Multipage**, and incorporates a **page navigator**, which is placed, by default, as a column on the left hand side of the screen.



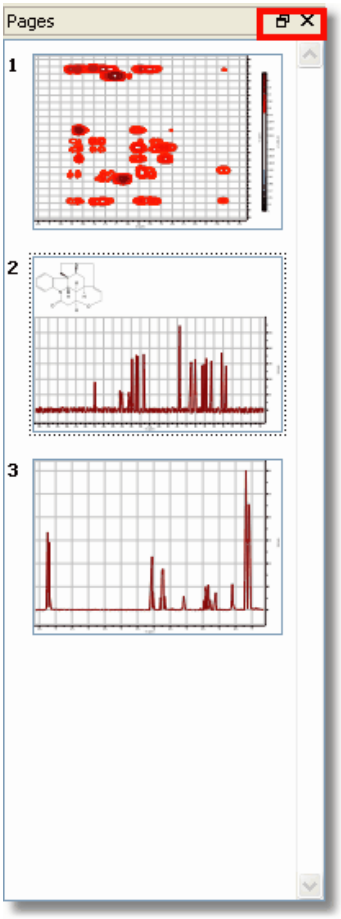
The **Mnova document** is a **Multipage Document**. This means that *all pages within a single document will be saved as a single document*. This will allow you to keep, for example, different experiments for a certain sample (^{1}H, ^{13}C, homonuclear and heteronuclear 2D correlations) as a single, unified document.

The active slide is highlighted in blue, and will appear in the main paper area of the document. This is the page to which all selected operations will be applied. You can choose the desired spectrum just by clicking on it on the page navigator or by following the menu 'View/Previous or Next Page'. In the same way, if you have highlighted any spectrum in the page navigator, you can move to the previous or the next spectrum by using the up or down arrow keys.

There are a number of things you can achieve by working on the page navigator:

- ✓ It is possible to alter the **order of the pages** by simply left clicking on a page, dragging it and releasing the mouse to drop it at the desired position.
- ✓ It is also possible to **Create a New Page** by selecting 'Edit/Create New Page', by right clicking on the page navigator window and selecting 'Create New Page' or by holding <Ctrl>+M (or Cmd + M in Mac).
- ✓ You can easily **Delete** an undesired spectrum by 'Edit/Delete'' or by right clicking on the spectrum or on the page navigator and selecting 'Delete'. A selected spectrum can also be deleted using the 'Del' key.
- ✓ It is possible to **select more than one page** by holding the <Ctrl> key (or Cmd in Mac) whilst clicking on the desired pages. When more than one page is selected, all operations (zooming, cutting, processing, etc.) will be applied to all selected pages.

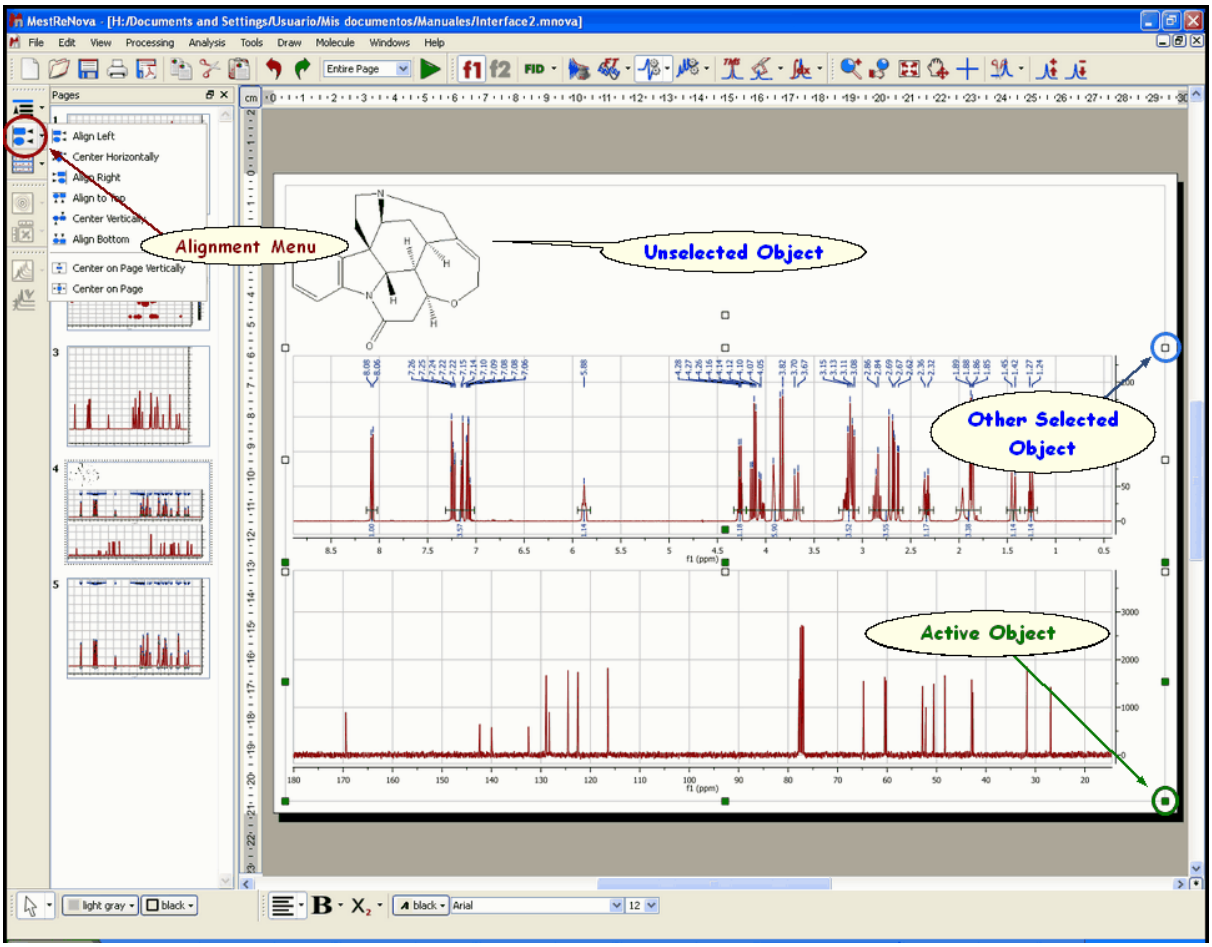
- ✓ It is possible to **copy** a page to the clipboard by simply pressing the <Ctrl> key (or Cmd in Mac) and then left clicking on the desired page, then dragging and releasing it whilst still holding <Ctrl> (or Cmd in Mac). Of course, a page can also be copied by selecting it and then choosing 'Edit/Copy', or <Right Click> and then Copy or by using the keyboard shortcut <Ctrl+C> (or Cmd+C for Mac). Any of these methods can be followed to copy one or several pages at a time, depending on what is selected when applying these operations.
- ✓ Conversely, a page or several pages can be pasted by using 'Edit/Paste', or <Right Click> and then Paste or by using the keyboard shortcut <Ctrl+V> (or Cmd+V for Mac).
- ✓ Finally, you can hide or move the page navigator, by clicking on the boxes highlighted on the picture below.



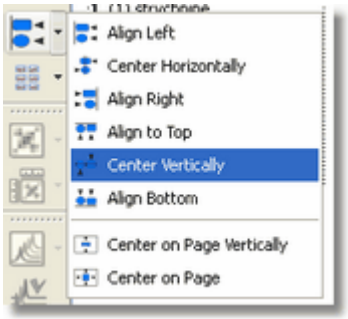
7.2 Working on a Single Page

Whilst **Mnova** is a multipage document, it also allows you to have several objects (e.g. spectra) within a single page. The easiest way of placing two spectra on the same page is to simply select one of them, copy it (**'Edit/Copy'**, <Right Click> and **Copy** or <Ctrl+C> (or Cmd+C for Mac)) and then paste it directly on the desired page (Select the desired page and press 'Edit/Paste', <Right Click> and **Paste** or <Ctrl+V> (or Cmd+V in Mac)).

Note that the **active object** is highlighted with **green squares** (in the green circle in the figure below). Any **Mnova** object can be **resized** and **moved** by just clicking and dragging on the corner boxes or on the object respectively. Note also that any other objects selected but not active will be highlighted with white squares (in a blue circle in the figure below) (To select more than one object, hold the <Ctrl> (or Cmd in Mac) key while clicking on the desired objects. To select all objects on a page, just choose 'Edit/Select All' or the keyboard shortcut <Ctrl+A> (or Cmd+A in Mac).



Once more than one object (spectrum or otherwise) has been placed on the same page, it can be manipulated and placed on the page by using the scroll menu shown in the figure below, accessible via the **'Alignment'** icon  .



This scroll menu will allow you to **Align Left**, **Right**, **Top** and **Bottom**, to **Center Horizontally** or **Vertically** (applying this last operation with multiple objects selected will Superimpose them). Of course, you can move the spectrum selected in a given direction using the arrow keys of your keyboard. Remember that multiple objects must have been selected for these commands to work effectively.

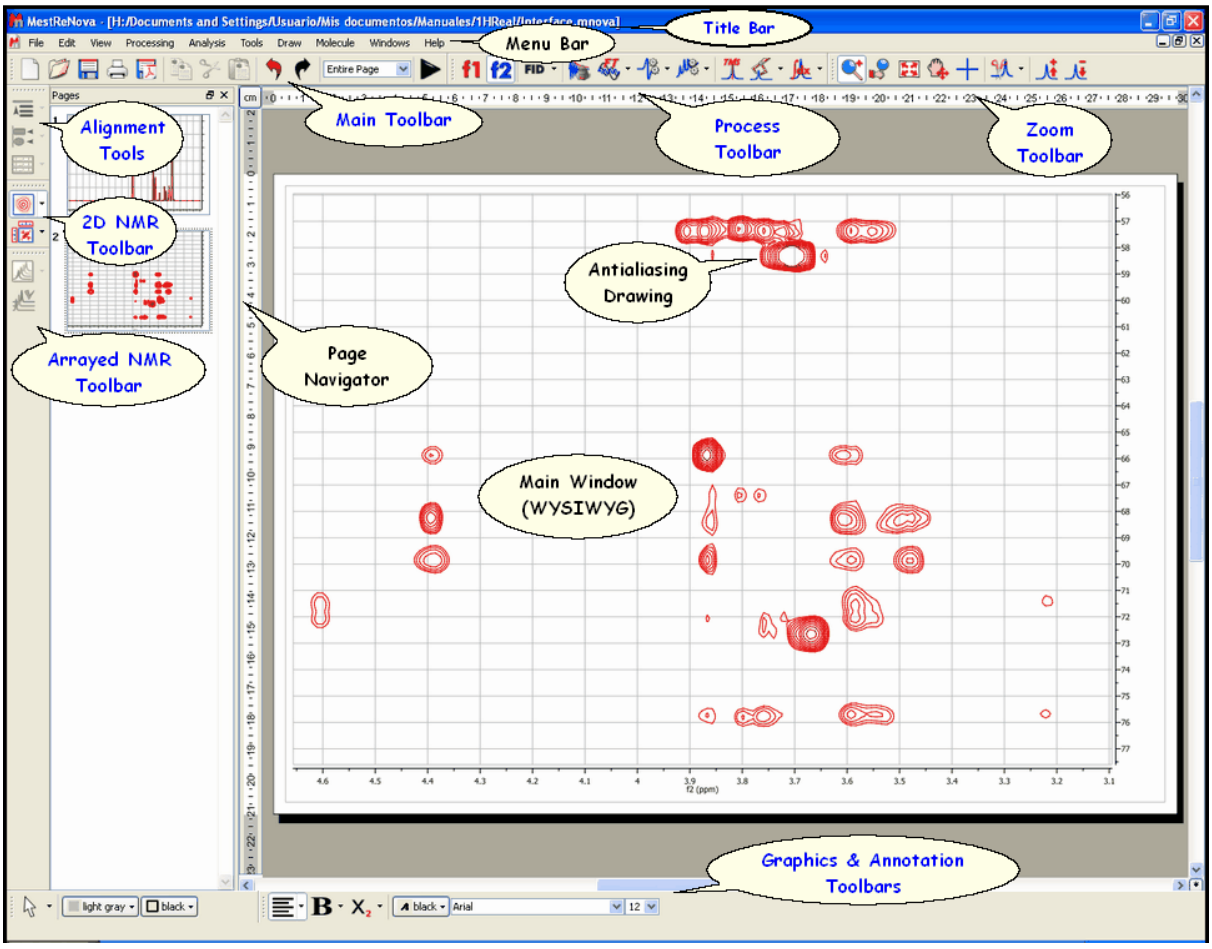
Utilities

The superimpose option is very useful when attempting to identify a known compound present in a complex mixture or when comparing similar compounds.

Any operations applied to the active object will also be applied to the other selected objects (zooming, panning, cutting, processing operations, etc) as was the case with multiple pages selected.

7.3 Mnova Menus & Toolbars

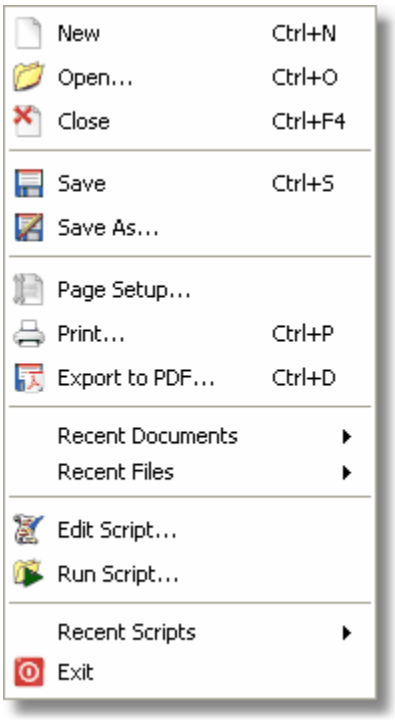
The graphical user interface is the part of Mnova that you interact with to perform tasks; it consists of a main page window, page navigator, menus, toolbars, dialog boxes... giving users total flexibility in the organization of their data.



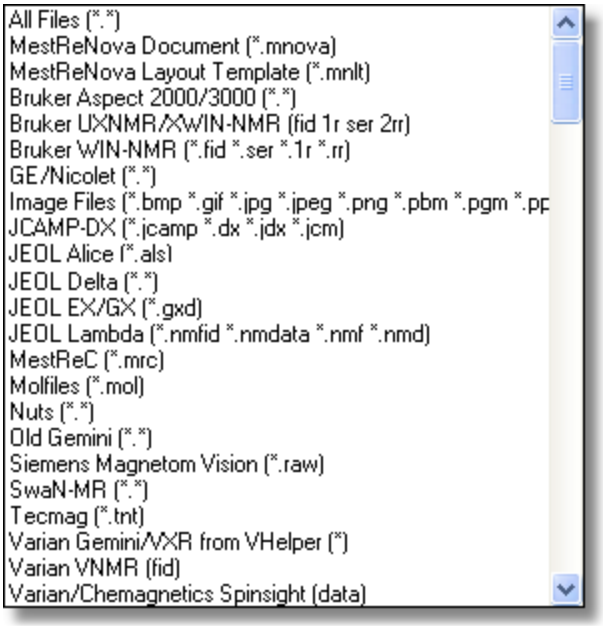
Title Bar: This blue bar shows the name of the active document

Menu and Toolbars: All Mnova commands and functions can be accessed from the menus and toolbars. The toolbars contain icons which offer shortcuts to many commonly used functions (you can select which ever toolbars you would like to display from the View menu). The user will be able to change the location of the toolbars to anywhere in the screen just by clicking on the border and dragging.

File Menu: In addition to the usual File commands (such as: 'New', 'Open', 'Close', 'Save', 'Save as', 'Page setup', 'Print' or 'Exit') you will find:



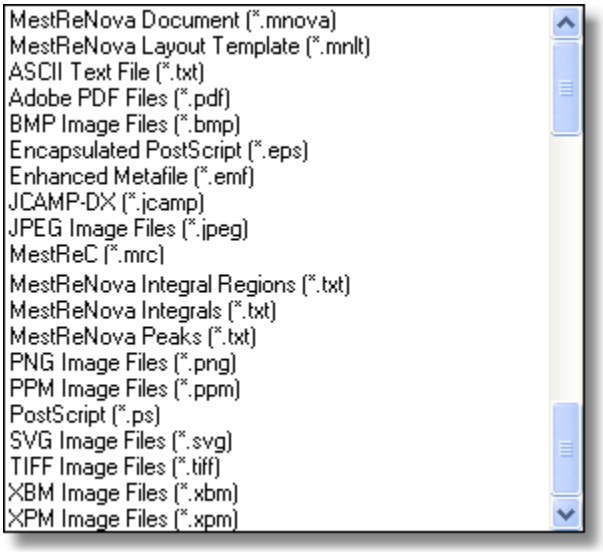
Mnova is able to import several spectral formats, image files and molecular structures (.mol) as you can see in the picture below:



Please bear in mind that the user will also be able to load any spectrum from the command line, just by typing the path where the Mnova exe file is located, followed by the path where the FID (or ser) file is stored. For example:

"C:\Programs Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Documents and Settings\User\My documents\1HReal\fid"

The user will be able to save the spectra in different formats by following the menu 'File/Save as', as you can see in the picture below:



Please bear in mind that you can also save the integral regions, the peaks and the template used in your document by using this command.

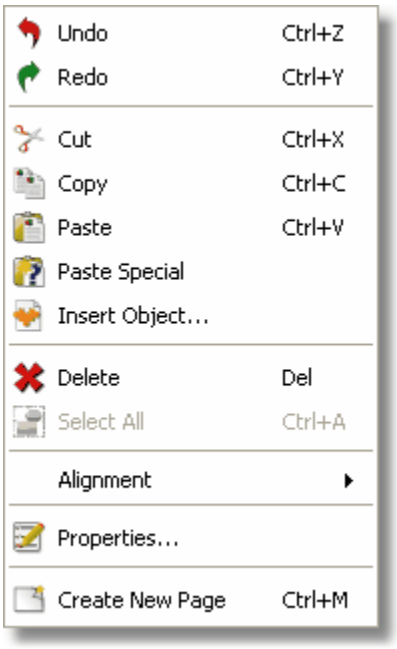
'Export to PDF': This is a useful option to convert your Mnova document to PDF.

'Recent Documents' & **'Recent Files'**: Dragging the mouse to these options you will able to select the most recent documents or files.

'Edit Script': Selecting this option, will open an 'Edit Script' dialog box. From there, you can create a new script or edit an existing one.

'Recent Scripts': Dragging the mouse to this option will enable you to select recently used scripts.

Edit Menu: In addition to the usual Edit Functions (such as **Cut**, **Copy**, **Paste**, **Paste Special**, **Insert Object**,...) you will find:



'Undo & Redo': Mnova implements very powerful Undo and Redo capabilities. If you select 'Undo', Mnova will reverse the last change done to the document, restoring it to its previous state. The Redo command reverses the undo. These tools are incorporated in the Standard Toolbar, so they will be fully described further on.

'Delete': This command is used to delete a spectrum. Alternatively, a spectrum can be deleted by right clicking on it and then selecting **Delete** on the pop-up menu or by using the 'Delete' key while the spectrum is selected.

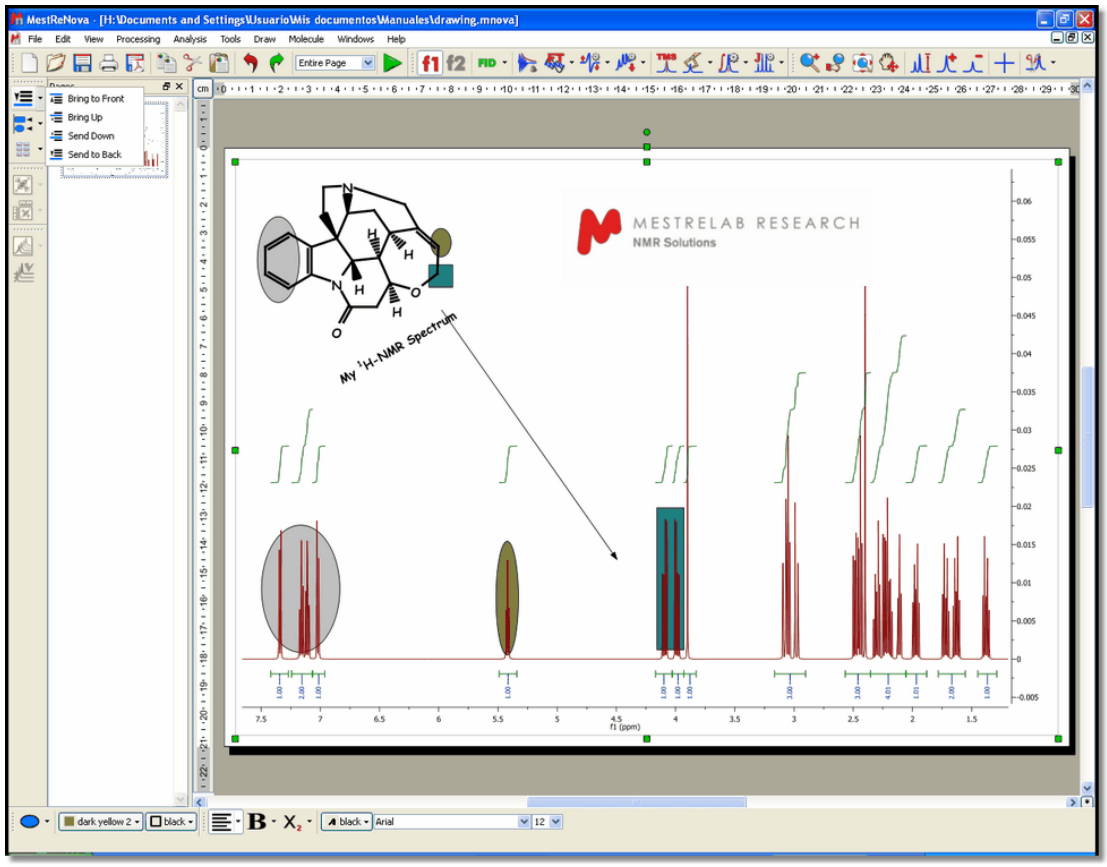
'Select All': Selects all spectra on the same page. This command is very useful when, for example, the user needs to apply the same processing to all spectra simultaneously.

'Alignment': The **Alignment** sub-menu is frequently used to neatly align spectra. To use the alignment toolbar, click on the spectra to be aligned and then press the alignment control desired. The alignment command will apply only to the selected spectra.

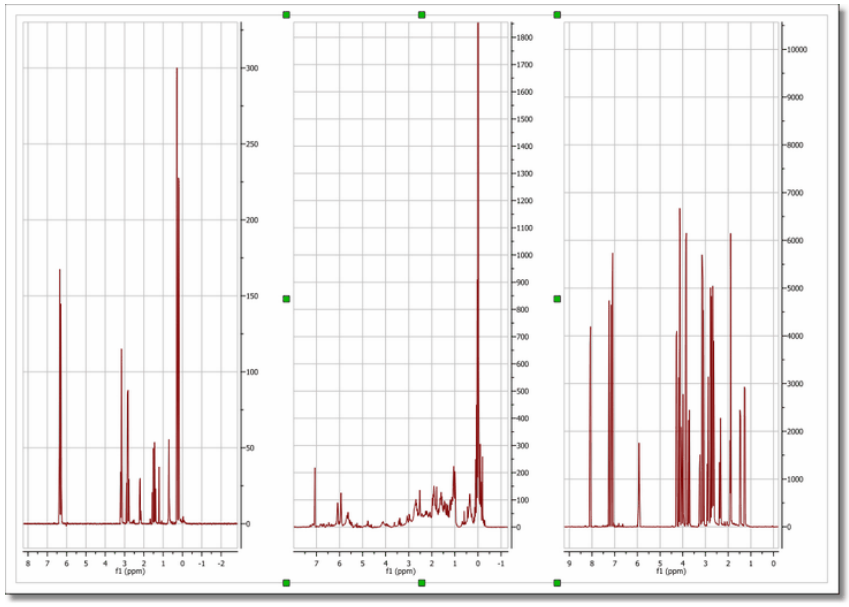
By clicking on this item, a sub-menu window will open and the user will be able to align (or otherwise manipulate) several selected spectra on the same page.

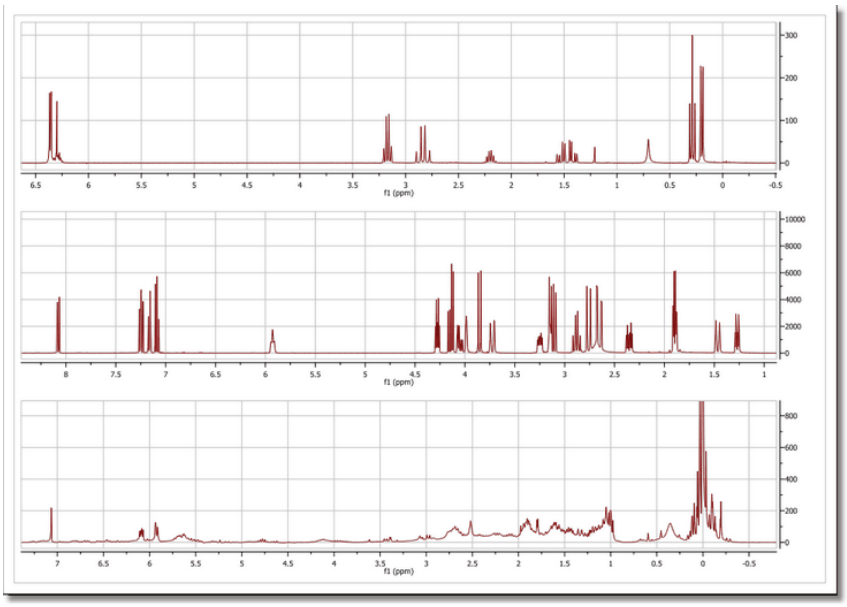
This menu will allow the user to **'Bring to Front'** or **'Send to Back'** a selected spectrum (or an object) as well as **'Bring Up'** or **'Bring Down'** it.

This menu allows the user to determine how items on a page (generally spectra) or items within a spectrum (chemical structures, titles, expansion spectra etc) are positioned with regard to overlapping.
The documents may have multiple layers, hence we have the commands **'Bring to Front'**, **'Send to Back'** as well as **'Bring Up'** (a level) or **'Send Down'** (a level). The tool **'Send to Back'** is also very useful to create 'semi-transparent' shapes, as you can see in the picture below:



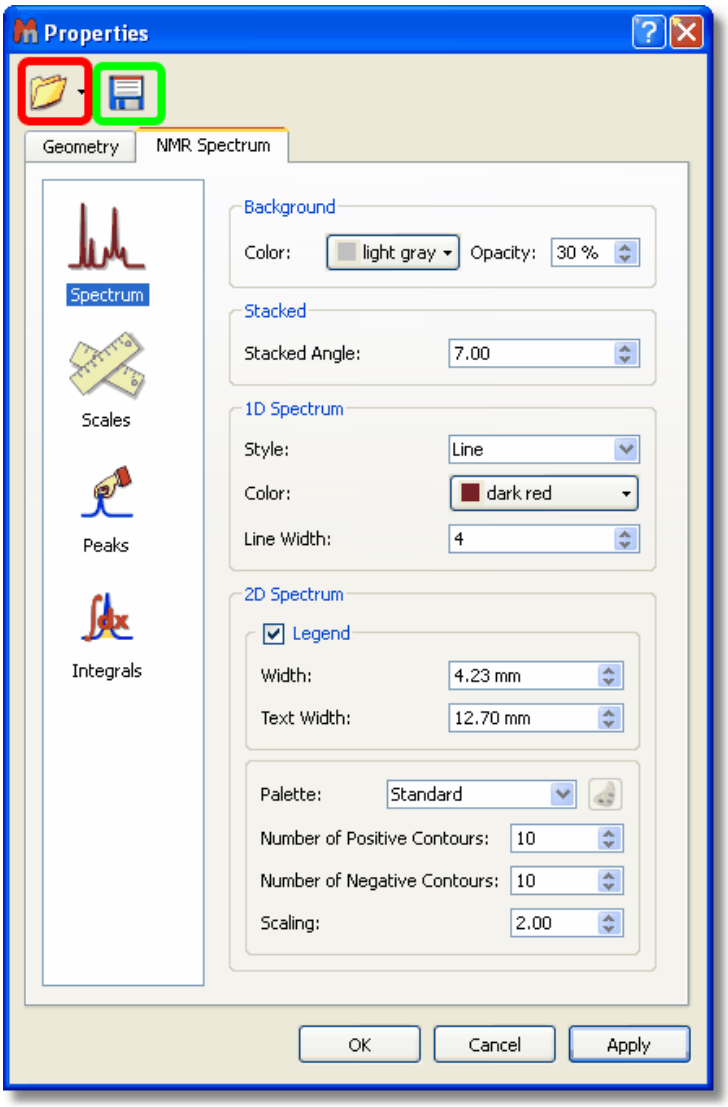
In the same way, the user can **'Align Left'**, Right, Top and Bottom, and **'Center Horizontally'** or Vertically (applying this last operation with multiple objects selected will Superimpose them). The user can also center the selected spectrum (or spectra) on the page (vertically and horizontally). Finally the user can apply the functions '**Tile Vertically'** and **'Tile Horizontally'** to separate the spectra on the same page (as shown below).





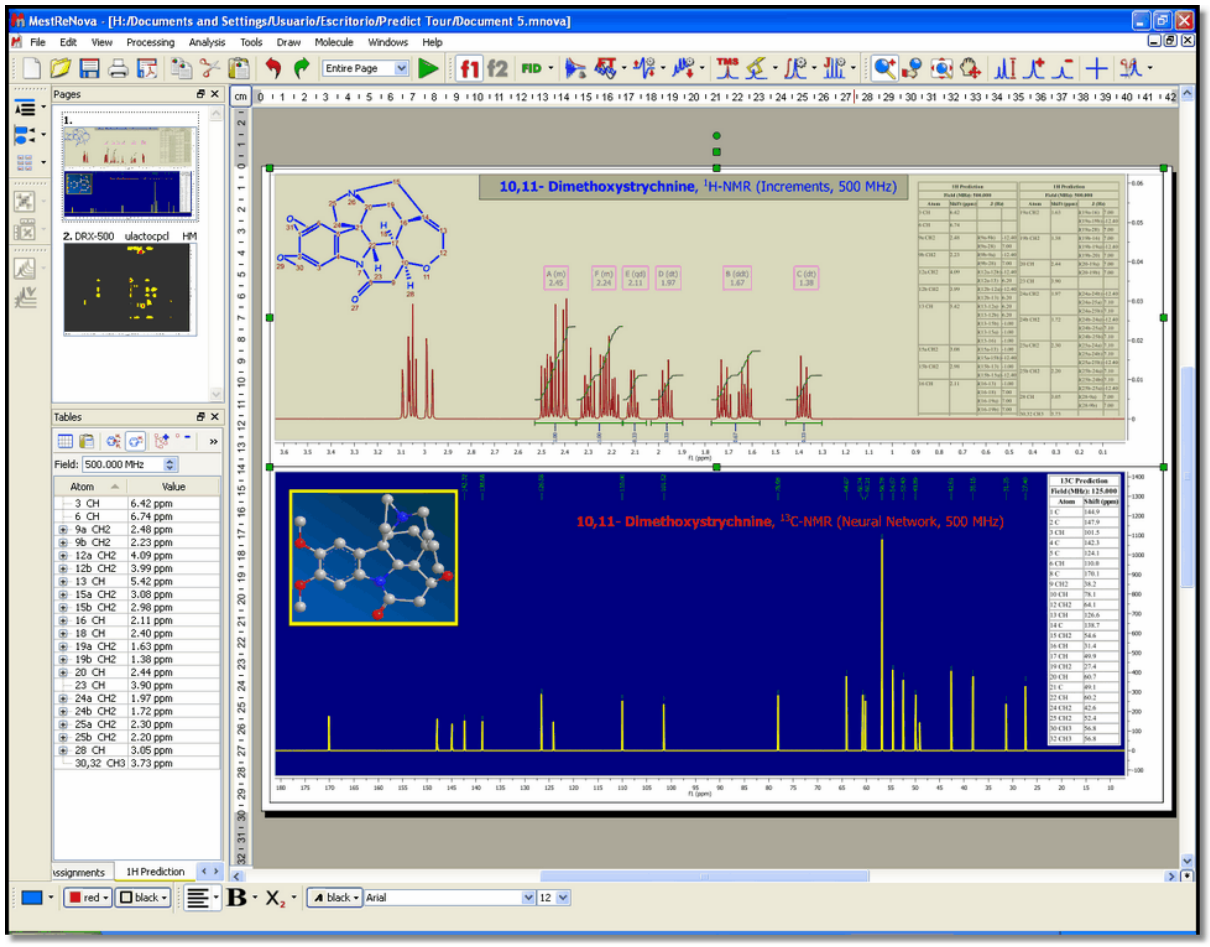
Alignment tools can also be found on the left vertical toolbar area.

Properties: Mnova will allow the user to customize nearly all the attributes of the spectrum. The user can select the properties of the spectrum by following the menu 'Edit/Properties' or by double clicking the left mouse button or pressing the right mouse button on the spectrum display and selecting '*Properties'* from the *pop-up* menu.

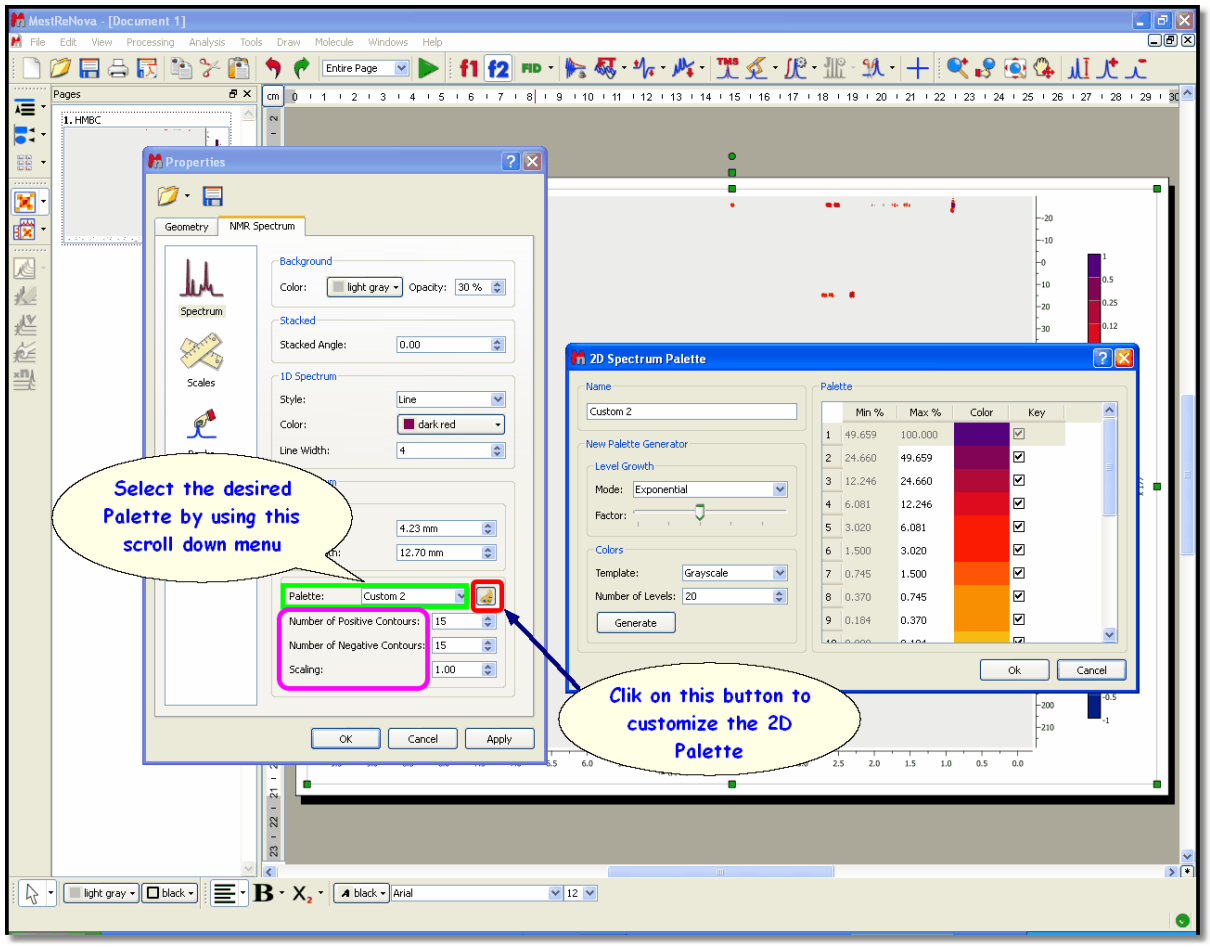


The user will be able to save his/her own properties just by clicking on the **Save Properties**' icon  (green square in the picture above). The Saved Properties will be opened by clicking on the '**Load Properties**' icon  (red square in the picture above).

From this set of tabbed pages you can change, for instance, the spectrum's geometry (position, angle, size), the **'Background'**, the **'Line-Style'** (line, crosses or circles), the **'Line Colour'** and the **'Line Width'**. Bear in mind that a '**Line Width'** of zero indicates a *cosmetic pen*. This means that the *pen* width is always drawn one pixel wide, independent of the transformation set on the painter**.** In 2D NMR spectra, you can set the legend, the number of contours and customize the palette. The user can also set the scales' properties, change the grids, the aspect of the axes and margins or the units (ppm, Hz, points, seconds), for a more convenient representation of the spectrum. It is also possible to easily change the color of the spectrum background to suit your eyesight, your working hours, or your mood. Enjoy complete freedom on how to present your data! Transparency is also supported! Here you can see an example:

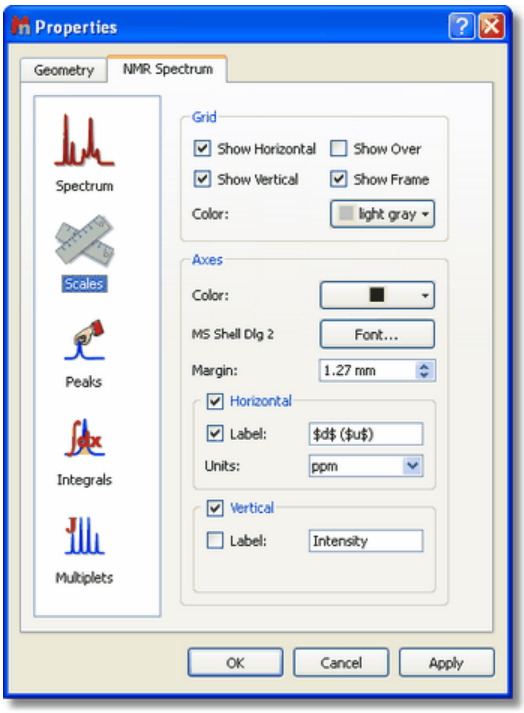


The user will be able to select the desired 2D spectrum palette and also customize his own palette by selecting for example 'Custom 2' in the 'Palette scroll down menu and then clicking on the '**Palette Editor**' button  to display the '**2D-Spectrum Palette**' dialog box, where the user will be able to customize the desired palette (Name, Level Growth, Colors, Number of Levels, etc..). It is also possible to select the number of the positive and negative contours and also the scaling (in the 'Properties' dialog box) as you can see in the picture below:



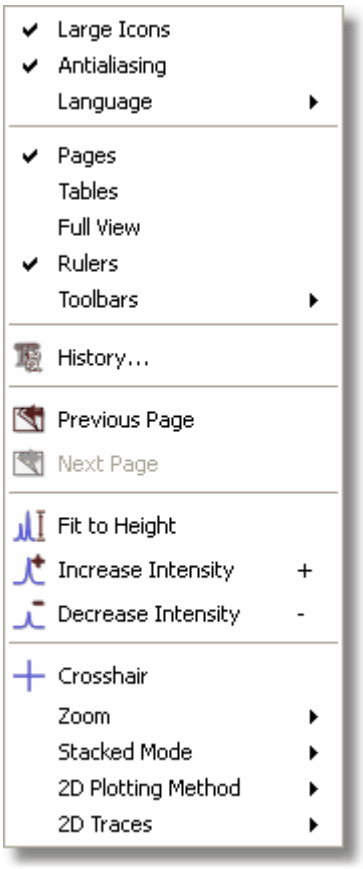
The user can include the spectrum title (the title set previously on the spectrometer at acquire time) on any axis label by typing 't' in its corresponding edit box. Bear in mind that, Mnova will only show the first line of the original title. This title appears by default on each page of the page navigator.

Hovering the cursor over the label edit box will reveal other commands for including information on the axis label, such as: u = Lowercase Units; U = Uppercase Units; d = Lowercase Dimension; D: Uppercase Dimension; or $1n$ = Observed Nucleus (e.g. 1H, 13C...).



In the same way, the user can modify many other features such as the parameters and properties of the peaks or integrals. We will not go into further detail here since all the property pages should be self-explanatory and a description of each one can be found in its corresponding section of this manual.

View Menu: The 'View' menu is where the user may select which toolbars, tables, rulers... are visible. In addition, it has other commands, such as:



'Large Icons': The user can choose between large (if 'Large Icons' is checked) or small toolbar icons.

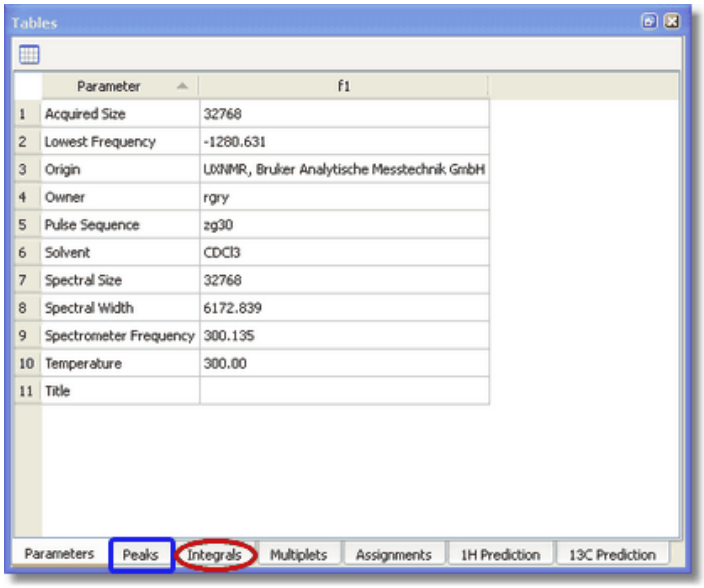
'Antialiasing': This is the technique used to minimize the distortion artifacts known as *aliasing* when representing a high-resolution signal at a lower resolution.

Aliasing is an effect that causes different continuous signals to become indistinguishable when sampled. *Aliasing* also refers to the distortion or artifact that is caused by a signal being sampled at high resolution (on the spectrometer) and then rendered on the PC video-display at lower resolution, violating the Nyquist Criterion which requires that the highest frequency present must be sampled at least twice per signal period.

The **'Antialiasing'** is checked by default in Mnova.

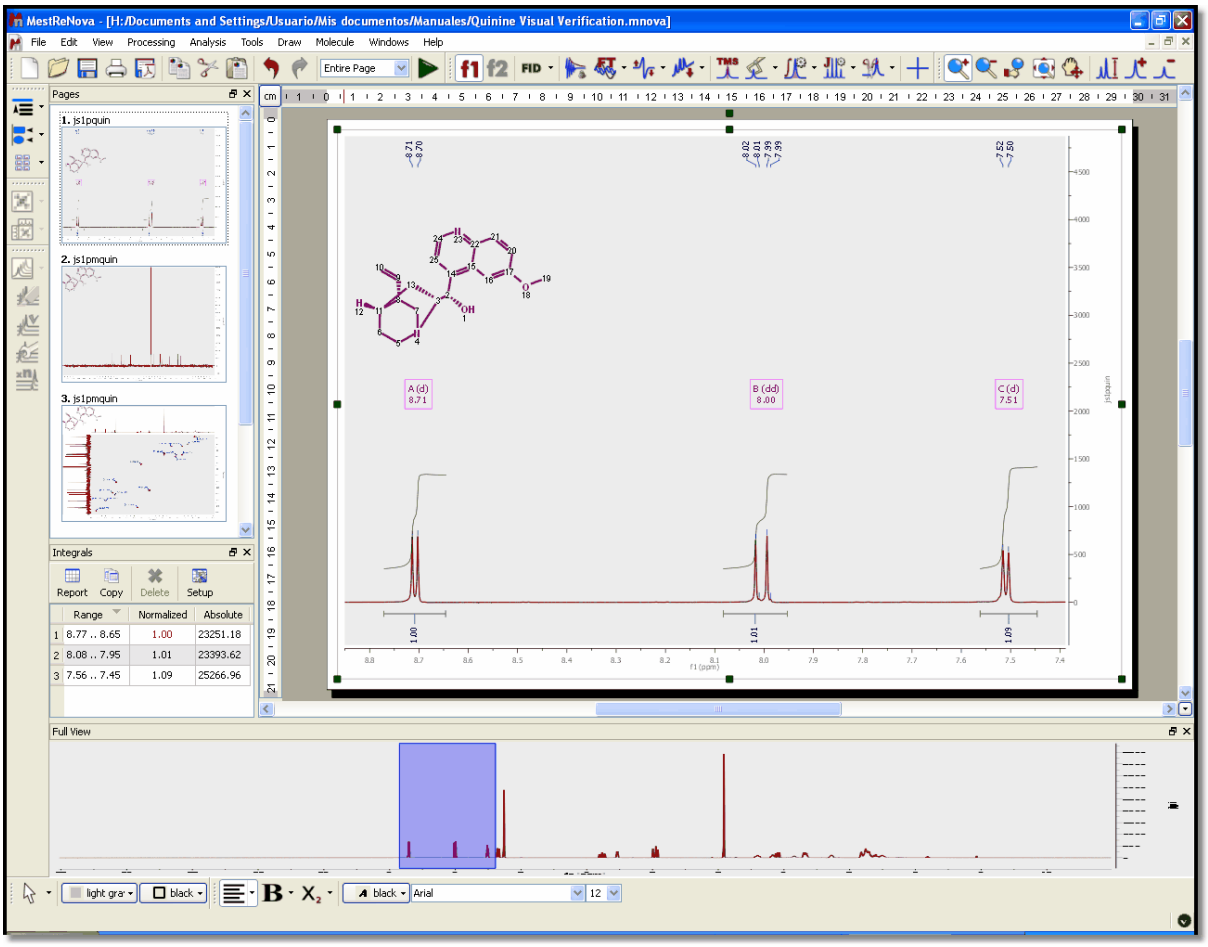
'Pages': This option allows the user to remove the page navigator (activated by default).

'**Tables**': **Mnova** groups all spectra-relevant information into the so-called '**Tables Window'**, available from the ***View*** menu. This option allows the user to see the spectral parameters (Acquisition Parameters are selected in the figure below), or Peaks (shown in the blue square), Integrals (in the red ellipse) or any of the other parameter classes as indicated on the tabs at the lower edge of the window.



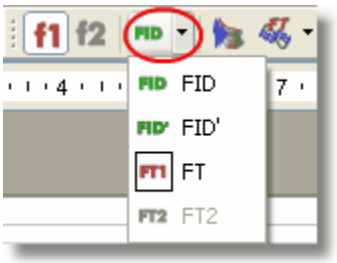
See also: Tables

'Full View': (Not to be confused with 'Full Spectrum) will bring up a miniature view of the entire working document with the active area of the spectrum highlighted in blue, as you can see in the picture below:

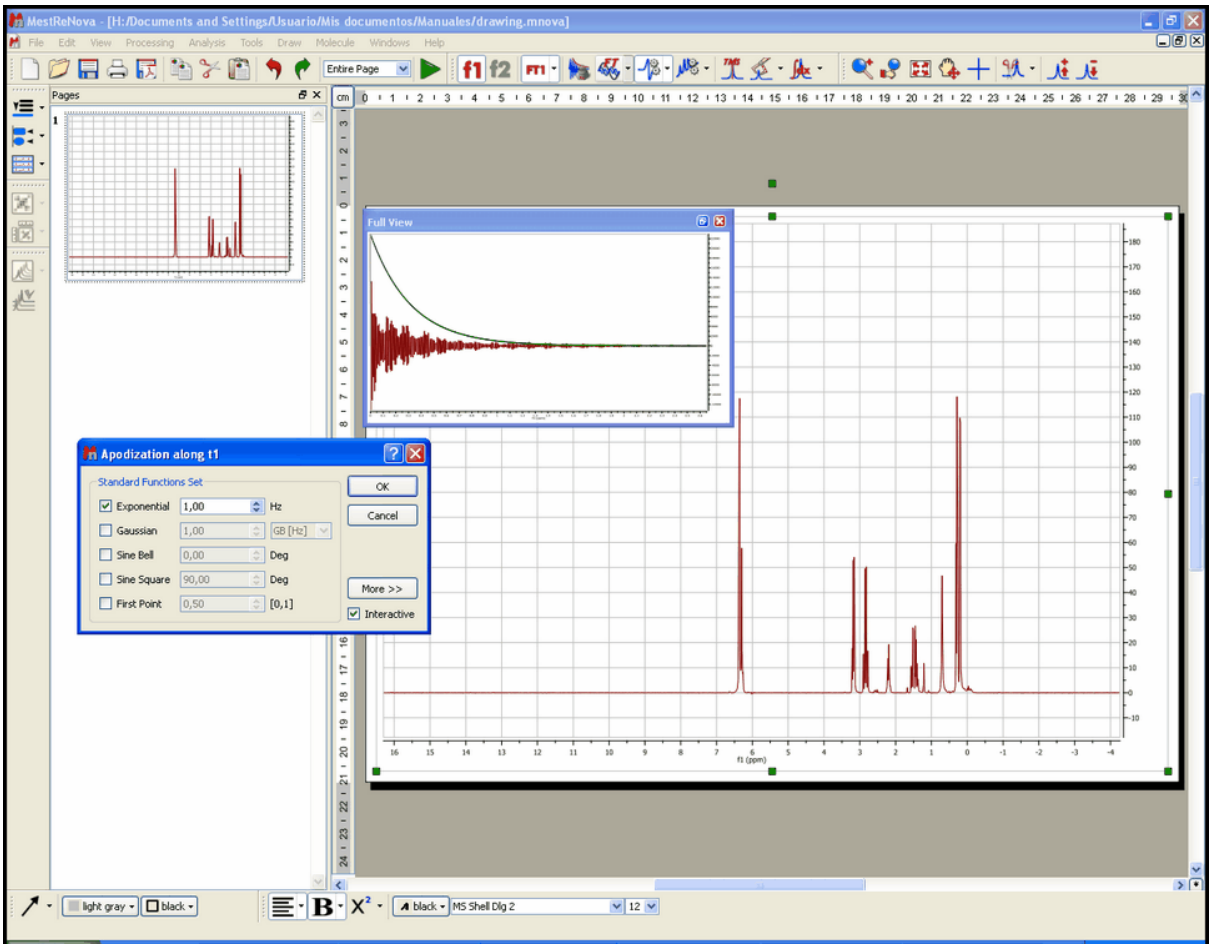


If **'Apodization'** is selected from the Processing menu then the **'Full View'** switches to show the FID and the effective window function.

To obtain the FID **'Full View'**, just click on the '**FID'** icon on the toolbar (in the red circle on the figure below) and then select the 'Full View' window on the 'View' menu.

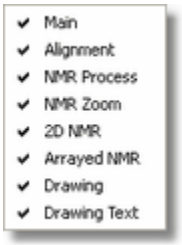


This will give you a miniature **'Full View'** of the FID and the FID on the main-page. You can zoom onto the main-page FID while maintaining a **'Full View'** as well. If you click on the side-bar if the FID icon and select **'FT1'** (spectrum after first FT) you can view and manipulate the spectrum while the FID is still in the Full View Window. This allows you to manipulate the FID (using apodization for example) and observe the effect on both the FID and the spectrum.



'Rulers': Click here to show or hide the rulers.

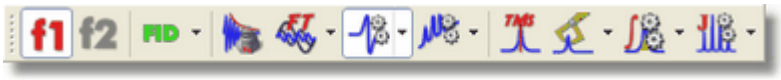
'Toolbars': This item of the 'View' menu allows the user to select which toolbars are visible on screen. Click the name of a toolbar to select it for display. Click again to deselect. You can attach a toolbar to any side of the graphical interface by dragging it to where you want it attached.



'Main': 

This toolbar contains the standard file operations 'New', 'Open', 'Save', 'Print Tools' and 'Export to PDF', the editing operations 'Copy', 'Cut', 'Paste', 'Undo' and 'Redo', and the 'Page Width' drop-down menu which will be fully described in the zooming chapter.

'Alignment':  Contains all the alignment tools. These commands are duplicated on the 'Edit' menu.

'NMR Processing':  This toolbar contains the most frequently used NMR processing tools..

The 'f1' and 'f2' commands are used to set the processing frequency dimension (f1 or f2).

'FID': It is possible to see the **original FID** or the **processed FID** (**FID'**) by clicking on the appropriate sub-menu item on the FID-icon side-bar. There are several further options in the scroll-down menus which will be fully described in the Processing Chapter.

'Apodization' (or *window function processing)*: Windowing is a technique for manipulating the FID to either remove the "sinc" wiggles from the peak bases resulting from the truncation of the FID (apodization) or optimize the spectral resolution or remove various other acquisition artifacts or any combination of the above. You can find a full description of this tool further on in the Fourier Transform chapter.

'FT' : This command transforms the time domain FID into the frequency domain spectrum. It will be described in detail in the Fourier Transform chapter.

'Phase Correction': This process adds constant (`Zero Order') and linear (First Order about the pivot point) phase to the spectrum in order to obtain pure phase line-shapes. If you click on this icon, Mnova will carry out an automatic phase correction. This process will be described further in its corresponding chapter (**'Phase Correction'**).

'Baseline Correction': This is a very important processing step to obtain good quality spectra, with all the signals connected by a flat line. Mnova will apply an automatic baseline correction if the user clicks on this icon. If you are interested in studying **'Baseline Correction'** in Mnova in depth, please refer to the corresponding chapter in this manual.

'Reference' : Click on this icon to calibrate your spectrum by assigning the position of an internal standard (such as TMS or a solvent peak). Mnova offers you a list of the most common deuterated solvent chemical shifts to calibrate the spectrum. Again, you can find a full description of this tool further on in the **'Chemical Shift Referencing'** chapter.

'Peak Picking': Clicking on this tool will select all the peaks in the spectrum using a default threshold, mark them, indicate their chemical shifts and tabulate the shifts. You can find an advanced description of this tool in the corresponding chapter.

'Integration': Integration is used to estimate the number of species (e.g. protons) associated with a peak in the spectrum. Manual and fully automatic routines are available. Please read the **'Integration'** chapter for more information about this tool.

'NMR Zoom' :  This toolbar contains the following

functions:

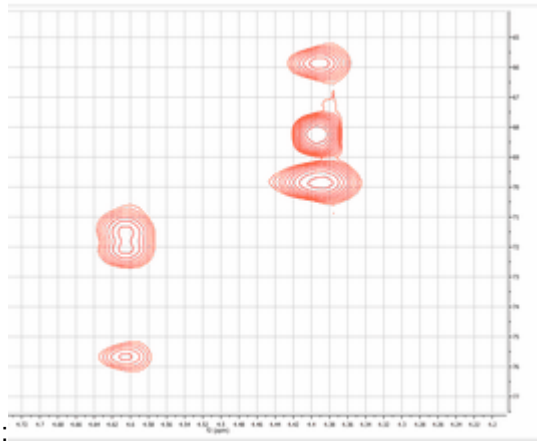
'Zooming': This tool is used to expand the spectral regions on screen. Mnova incorporates several ways of zooming, such as zoom in, manual zoom, full spectrum, and panning.

'Fit to Height' and **'Increase and Decrease Intensity'**: These tools scale the data of your NMR spectrum

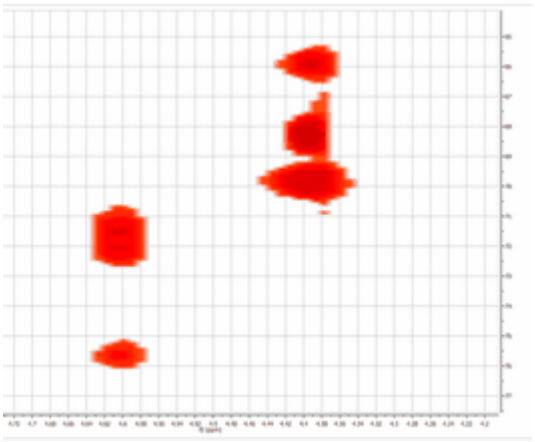
'**Crosshair**': These two orthogonal lines forming a crosshair are available on the 'View' menu (or by pressing the <c> key) for reporting positional information, and for help in visualizing peak alignments.

'**Cutting**': Allows the user to hide regions of the spectrum, when they are of no interest. These tools are fully described in later chapters.

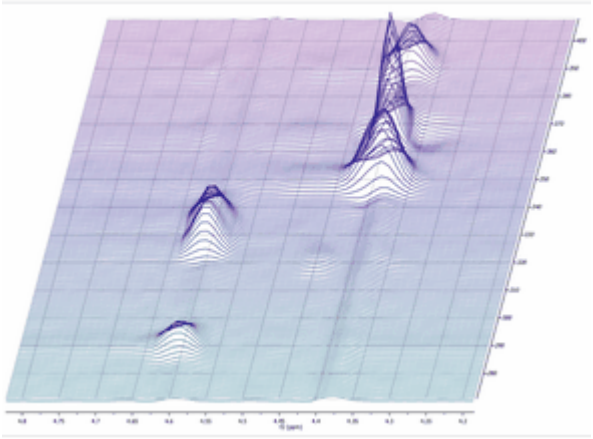
'2D NMR' : This toolbar contains the plotting methods for 2D NMR display. Mnova incorporates four 2D-NMR spectra representation modes. The most commonly used mode is the 'Contour Plot'. This representation provides a good global view of the data points and allows a precise location of all signals (except the weakest). Nevertheless, the relative intensities and the multiplet structure of many signals can be difficult to interpret. In the following picture, a section of a spectrum in Contour Plot is shown:



A faster alternative in computational terms is the 'Bitmap Plot'. Mnova uses this representation as a default mode; the intensity of each point is represented by a colored scale, thus making relative intensities easier to interpret. If only positive intensities are plotted, the color scale is from red (high intensity) to blue (low intensity). In the figure below, you can see a spectrum shown in Bitmap Plot:

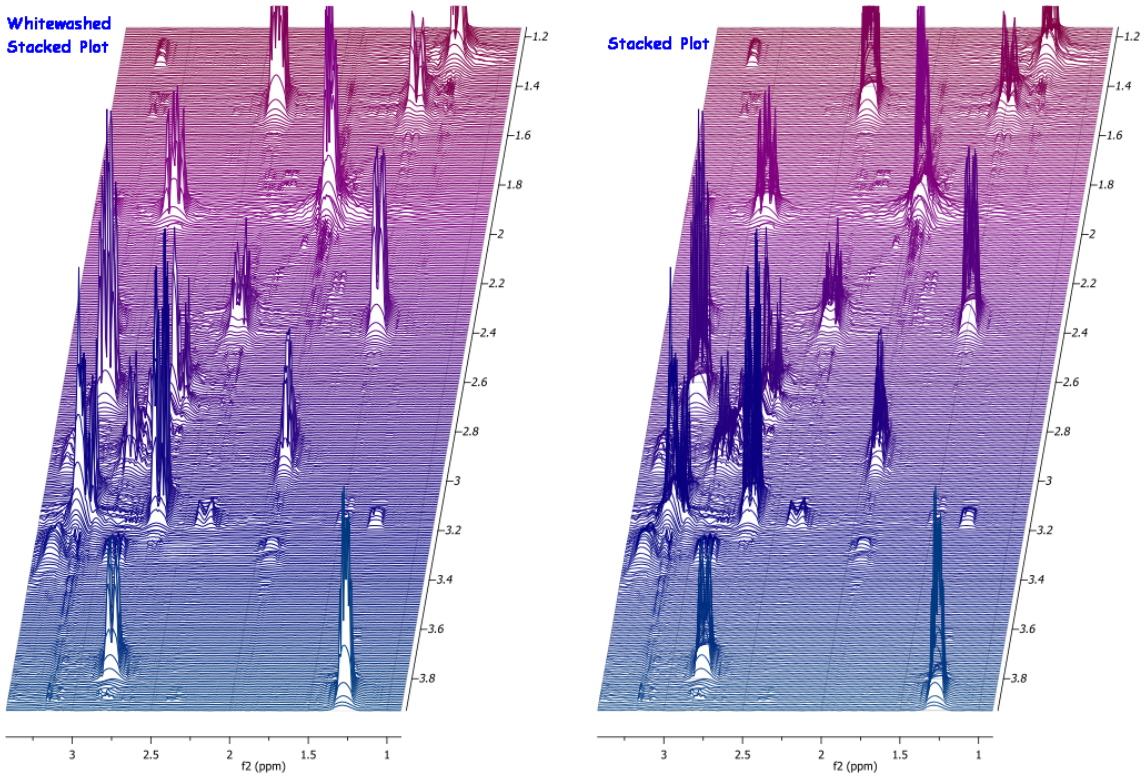


A '**Stacked Plot'** provides a *pseudo-3D* visualization of the data. This kind of representation is useful for revealing weaker signals and for comparing the intensities of different signals in the spectrum. A stacked plot is shown below:



'Whitewashed Stacked Plot': In the called 'Stacked Plot' mode, all the individual traces that compose a 2D experiment or an arrayed experiment are superimposed on each other, so it can be difficult to obtain a clear view. However, this problem can be avoided using the '**Whitewashed Stacked'** mode. In this mode all the individual traces are plotted, but the traces are not "transparent", so that the characteristic 3D look is obtained. The contrast between the 'Stacked Plot' and '

Whitewashed Stacked' modes can be appreciated in the figure below.

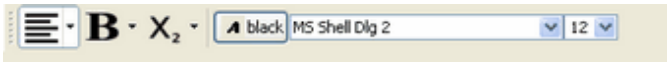


'Show traces': this tool allows the user to show and setup the vertical and horizontal traces of a bidimensional spectrum. The user can increase or decrease the intensity of the traces simply by scrolling the mouse wheel (windows only for the moment) after hovering the cursor over the desired trace. To move the trace hold the Shift key down while scrolling the mouse wheel over the trace.

'Stacked NMR':  Mnova includes a general-purpose fitting module designed to analyze stacked experiments such as relaxation, kinetics or DOSY experiments, using a robust but intuitive working environment. The user can find a full description of this tool further on in the **Stacked NMR** tutorial.

'Drawing' : 

You can draw an object by using these tools. You can choose the line color (default is black) and the fill color (default is light grey).

'Drawing Text': 

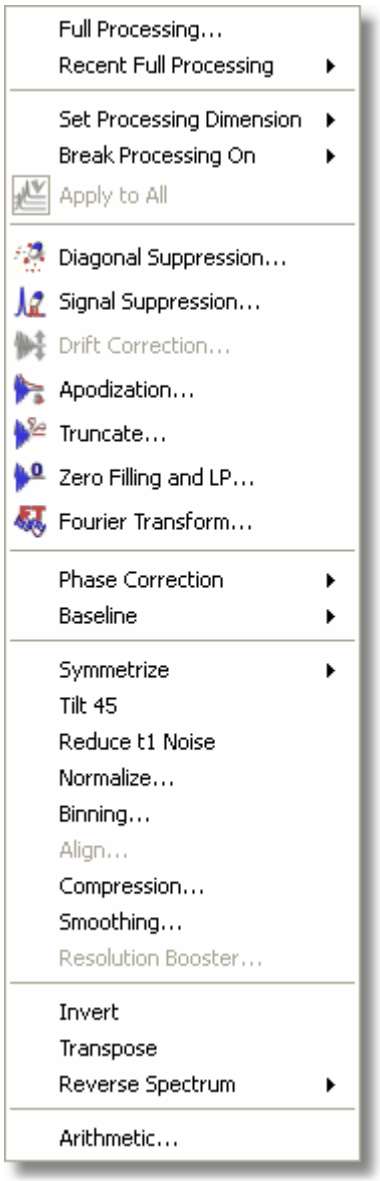
The text tool allows you to enter text with different formats. You can also find more information about drawing tools further on in **'Graphics and Annotations'** chapter.

'History': This tool opens a list of the previous operations applied to the spectrum. The document history saves a list with all the changes (with date and time) which the user has applied since the file was opened. This command is very useful if you have a saved file but you do not remember the steps you took in processing it.

'Previous and Next Page': These commands are used to scroll trough the page navigator. Of course, the user can use the 'Page Up' or 'Page Down' keys to obtain an identical result.

The user can also select the desired spectrum by simply clicking on it on the page navigator (and then, can navigate to the previous or the next by using the up or down arrow keys). The rest of the 'View' Menu has already been explained above.

Processing Menu: The Processing menu allows the user to select which tools should be applied when processing the NMR spectrum. The user can also find the most frequently used tools in the toolbar.



'Full Processing': This tool allows the user to automate the full processing of both 1D & 2D NMR data sets. The user can find a full description of this tool further on in the Automated Processing chapter.

'Recent Full Processing': You can find here the most recent Full Processing archives.

'Set Processing Dimension': The user can choose the dimension (f1 or f2) to apply the processing. This tool is also present in the NMR process toolbar. 

'Break Processing On': By default, when a new dataset is opened and time domain data (a 'fid' file for example) are selected, Mnova immediately applies appropriate fid pre-processing (Apodization) followed by Fourier transformation. If you wish to intervene in this process 'Break Processing On' allows you to halt the processing when, for example, the raw data are loaded, so that you can adjust apodization, zero filling, linear prediction or the Fourier transform parameters before continuing processing. (This is fully described further on in the 1D NMR Processing chapter)

The following commands are described in more detail in the Processing Basics chapter:

'Diagonal Suppression': This option is used to remove the signal on the diagonal from homonuclear spectra. Mnova applies this tool automatically prior to the FT.

'Signal Suppresion': This tool is used to remove any undesired signal such as a solvent resonance, from the spectrum.

'Drift Correction' : This tool is used to remove the spurious peak which may appear at the transmission frequency when the the integral of the FID is non zero. The transmission frequency is known as "o1" on Bruker spectrometers and as "sfrq" on Varian instruments. Some texts refer to this as "Zero Frequency" but quadrature detection ensures that we don't have to deal with negative frequencies.

'Apodization': This is used to remove the so-called "Sinc Wiggles" which can appear at the base of peaks when the FID has been truncated. Generally a decaying exponential "window function" is applied to the truncated FID to 'force' the FID to zero.

'Truncate': This technique may be used to shorten the acquisition data time series. If the FID, for example, decays to zero in 0.5 seconds and the acquisition time was 2 seconds, the FID consists of 25% signal and 75% noise. Since both 0 and a 90 degrees phase shifted spectra are collected a time series data length of twice the FID time is required for maximum information content. Truncating the FID at about 1.0 second will reduce the amount of storage required with no data loss. At a later time Zero Filling may be used to cosmetically adjust the spectrum's appearance.

'Zero Filling and LP': Zero Filling allows FID time series data which has just decayed to zero at the end of the acquisition time to be extend by one further "acquisition time" by appending zero data points equal in number to those in the FID. This avoids the data loss which normally would result when half the data points are assigned to the "imaginary" part of the frequency spectrum optimizes frequency resolution of the spectrum. Zero filling beyond this point only improves the digital resolution of the lines which can still be useful although no new data is revealed.

LP (Linear Prediction) can be used to approximate a complete FID when data are missing from the start or the end of the transient. Typically if the FID has been truncated and has not decayed to zero by the end of the acquisition time, LP may be used to predict, based on the existing data, the missing (truncated) data. This is an alternative to applying a severe window function to avoid the peak-base (sinc) oscillations or wiggles caused by a sudden change in the FID. The "synthetic FIDs" obtained along f1 in indirectly observed 2D experiments are almost always truncated and linear prediction can be applied to very good effect.

'Fourier Transform': Transforms the *time-domain signal (*acquisition data) into a frequency-domain representation (spectrum)

'Phase Correction': Mixes the real and imaginary components of the spectrum in order to obtain pure phase lineshapes. During the detection process the receiver reference signal's phase may differ from

that of the magnetization vectors by a fixed amount. This may be corrected by adding or subtracting a constant phase shift to the transformed data (the Zero Order phase correction).

The delay after the last transmitter pulse until the receiver becomes active allows the magnetization vectors to evolve slightly according to their chemical shifts, introducing a frequency dependant phase shift which may be corrected by the First Order phase correction.

'Baseline': This tool attempts to connect all the spectral signals by a horizontal line.

'Symmetrization': This function, used in homonuclear correlation spectroscopy, replaces all signals which are symmetrically positioned about the diagonal by the smaller of the two signals. In this way unsymmetrically positioned noise and artifacts are removed. This technique should be used with caution since any symmetrical noise or artifacts will be preserved and may be mistaken for genuine signals.

'Tilt 45': This technique is most frequently used with homonuclear J-resolved spectra where the effect of couplings in the f2 dimension is not required.

'Reduce t1 Noise': This function reduces the streaks of noise parallel to f1 which often accompany strong 2D signals and are due to instrument instabilities.

Normalize: The intensity of 1D NMR spectral signals is generally displayed in intensity units based on the instrument's receiver digitizer's maximum range in bits. Normalization allows the intensity to be displayed in terms of some spectral feature such as the height of the tallest peak or some arbitrary peak or the total spectral area. In most cases post-acquisition signal suppression (Processing/Signal Suppression) is needed to remove solvent signals.

Normalization can be used to investigate the manner in which the intensities of some signals are changing from one sample to another while other signals remain constant. Examples include chemical kinetics and pharmaceutical drug degradation studies.

'Binning': Chemical processes within cells are often tracked through the study (metabolomics) of the small-molecule metabolites produced. Typically many 1H spectra are taken and studied statistically. To render the many data points in a typical spectrum tractable they are "binned" into around 200 ins (or buckets) and each bin is integrated. Thus from the original spectrum a metabolic "finger print" is extracted for further analysis.

Binning is a technique used in metabolomics to reduce the many data points in a typical 1H spectrum to a small number of representative values.

'Compression': This tool is used to reduce the size of spectral data with minimal loss of information. This is useful when many spectra must be taken and stored for possible later analysis.

'Smoothing': This technique is used to reduce the effect of noise on a spectrum and possibly reveal signals embedded in, or distorted by, noise. Noise contains rapid fluctuations which are are generally faster than the rates of change of genuine signals. Smoothing applies a low-pass filter to the spectral data to remove these rapid fluctuations while having minimal effect on signals.

Resolution Booster: A new frequency domain post processing method which yields a considerable resolution enhancement.

'Invert, Transpose and Reverse': Invert effectively adds 180 degrees of constant phase to the entire spectrum.

Transpose switches the axes in a 2D experiment. In a heteronuclear 2D Bruker place f2 horizontally while Varian place it vertically. The user of one vendor looking at the other's data would be more comfortable with the spectrum transposed.

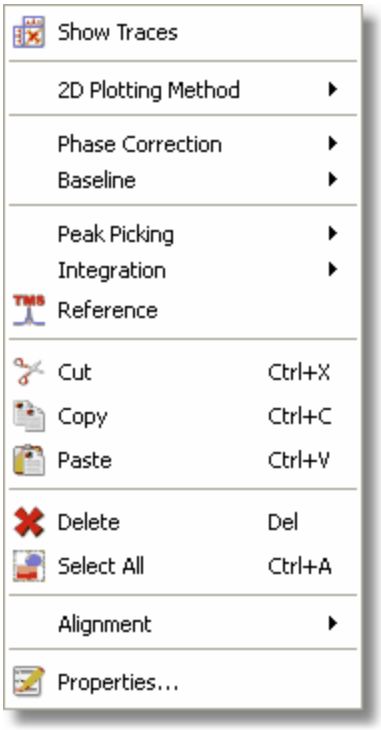
Reverse switches the spectral data around the transmitter (excitation) frequency. This might be required to correct data that had been incorrectly processed. A peak at x ppm below the transmitter frequency will appear at x ppm above the transmitter frequency after applying 'Reverse'.

'Arithmetic': to carry out arithmetic operations with or processed spectra.

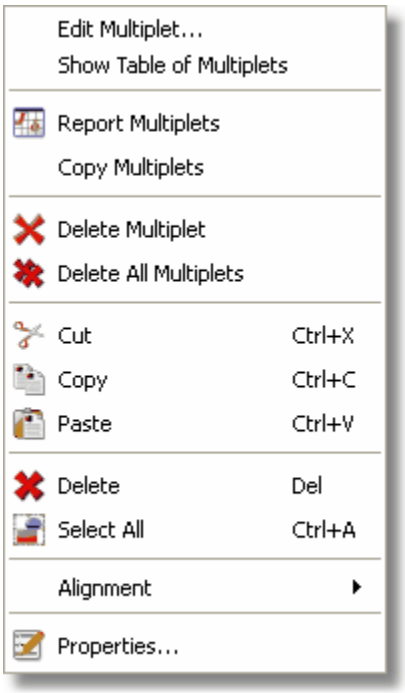
7.4 Contextual Menus

Contextual Menus

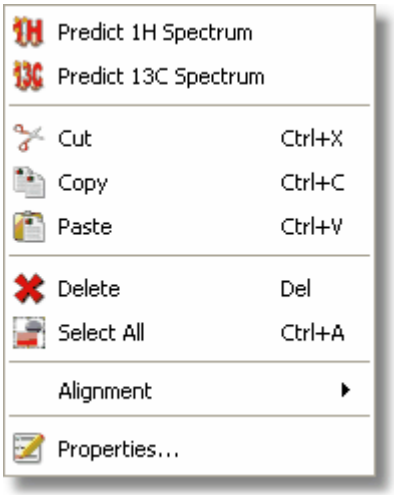
Mnova incorporates very useful contextual menus to make it easier to perform some common tasks. The contents of the contextual menu will depend on the window area clicked. For example if you right click (or Ctrl+Click in Mac) on a 2D spectral window, you will obtain the following contextual menu:



If you right click on a multiplet box, you will obtain:



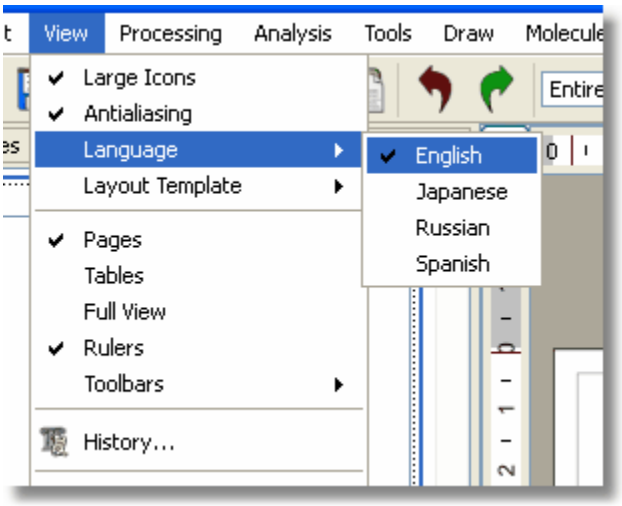
On a molecule:



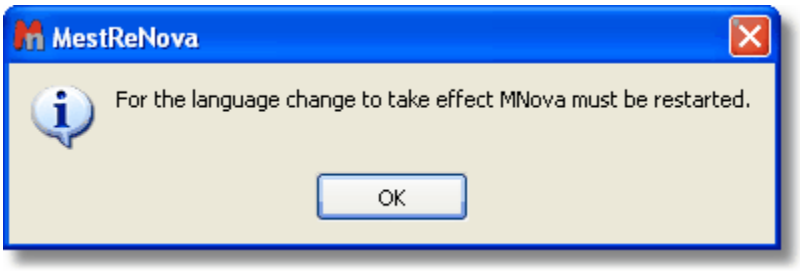
You will also find useful contextual menus on 1D spectral windows, page navigator, integral curves, traces, etc.

7.5 Localization

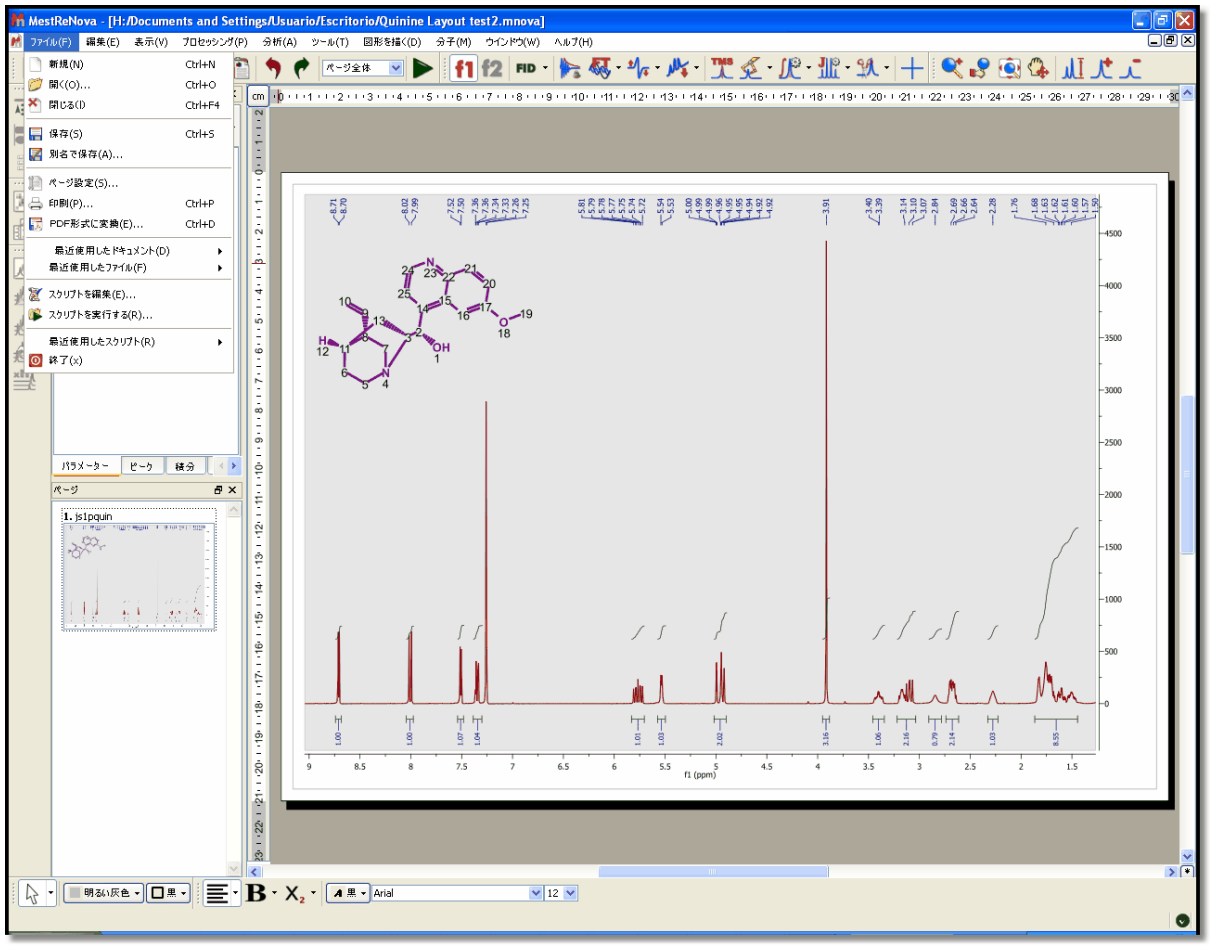
Localization:
English, Japanese, Russian and Spanish versions of Mnova are currently available. The user will be able to select the desired idiom by following the menu 'View/Language':



Once, the user had selected the desired language, a dialog box will be displayed to inform that it will be needed to restart Mnova in order to obtain the change of the language .



The Japanese version is shown in the picture below:

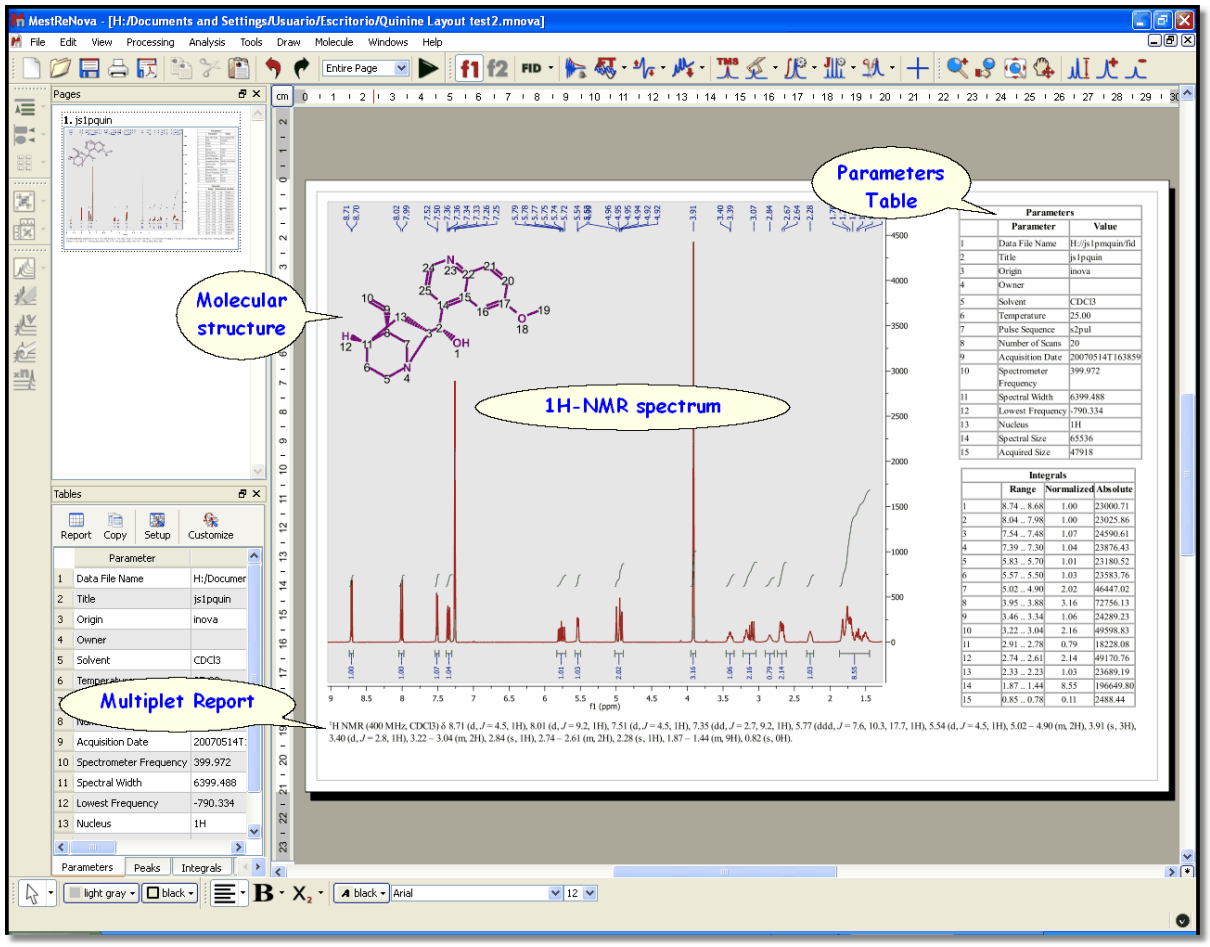


We plan to update once per year the different translations of the software, so you may find the newest feature menus without translating (in english).

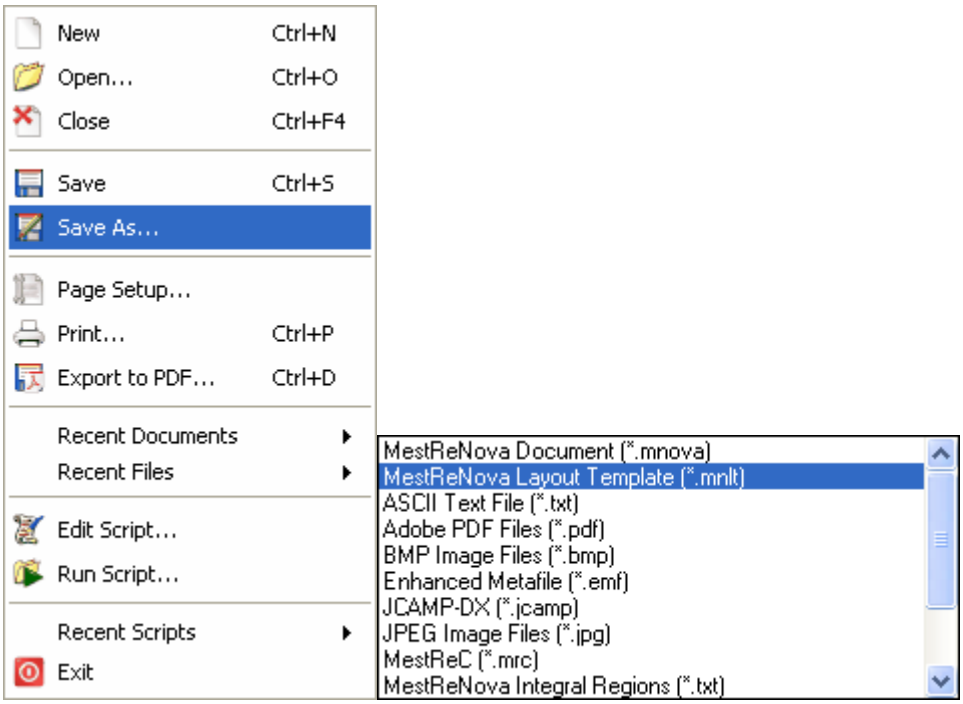
7.6 Layout Templates

Layout Templates:
It is now possible within Mnova to create **'Layout Templates'** for your NMR spectra**.** Please note that this feature is not completely finished in this version and that it will be greatly enhanced for the oncoming release versions.

The first version of this feature includes the capability to create templates with the location of the spectrum, molecular structure, tables and multiplet reports. It is very easy to create a template with Mnova, just open a document and select the desired location and properties for the different objects present in it. The picture below shows a 1H-NMR spectrum, with the corresponding molecular structure, multiplet report and the Parameters and Integral tables:

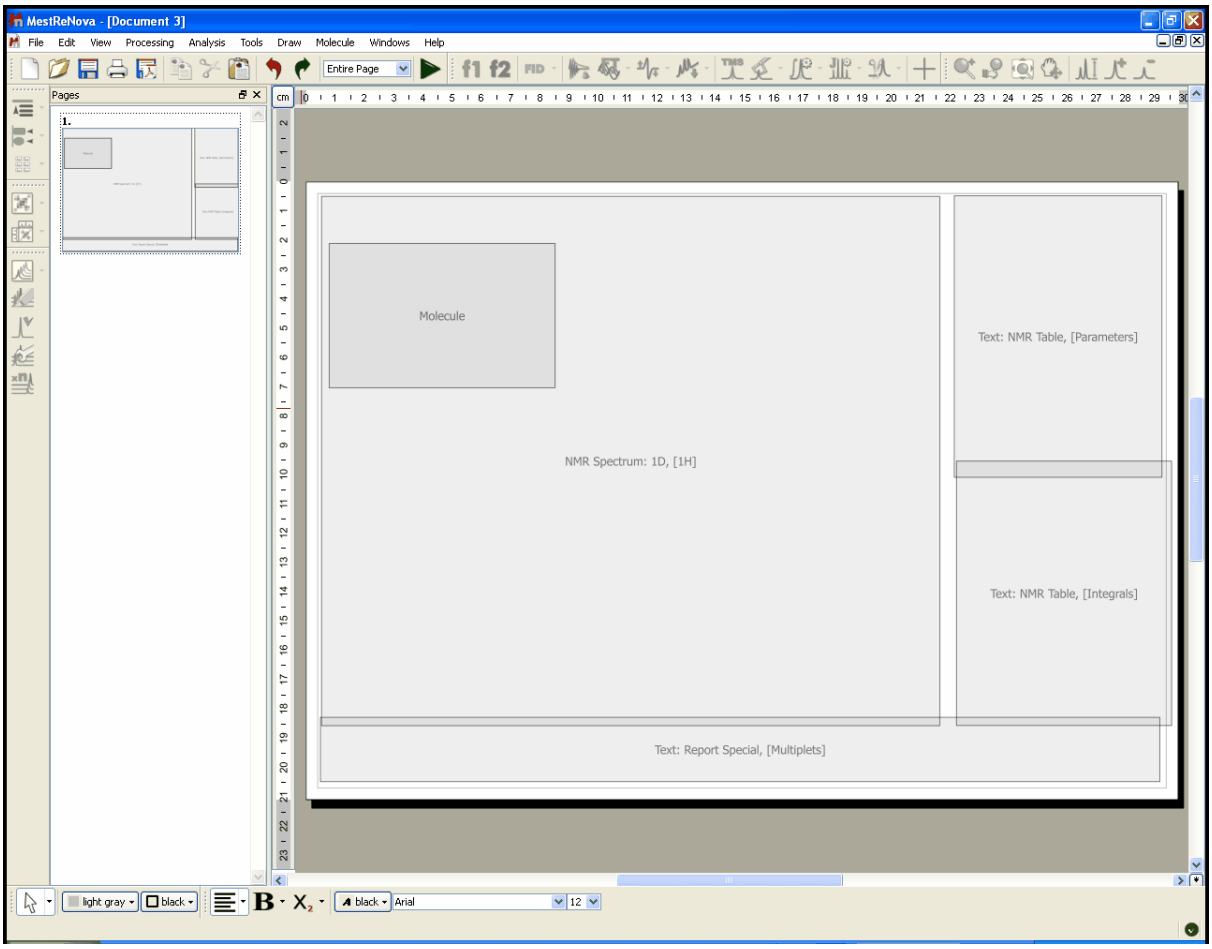


To save the created layout as a template, just follow the menu **'File/Save As'** and select 'MestReNova Layout Template (*.mnlt)

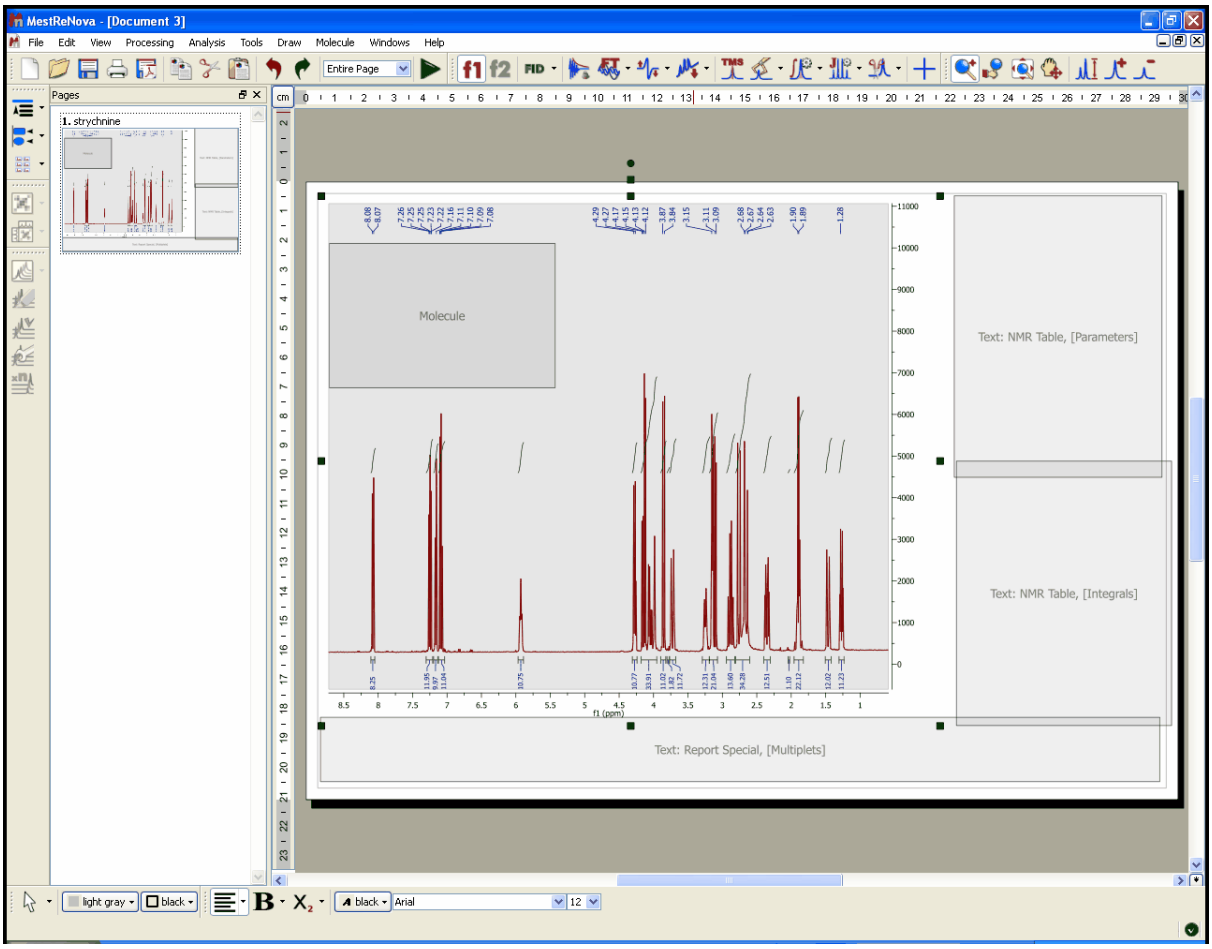


Once saved, a template can be easily opened by following the menu 'File/Open', by using the shortcut Ctrl+O (or Cmd+O) or by clicking on the 'Open File' icon , exactly in the same way as this would be applied to a spectrum. Please note that drag and drop also works in this instance.

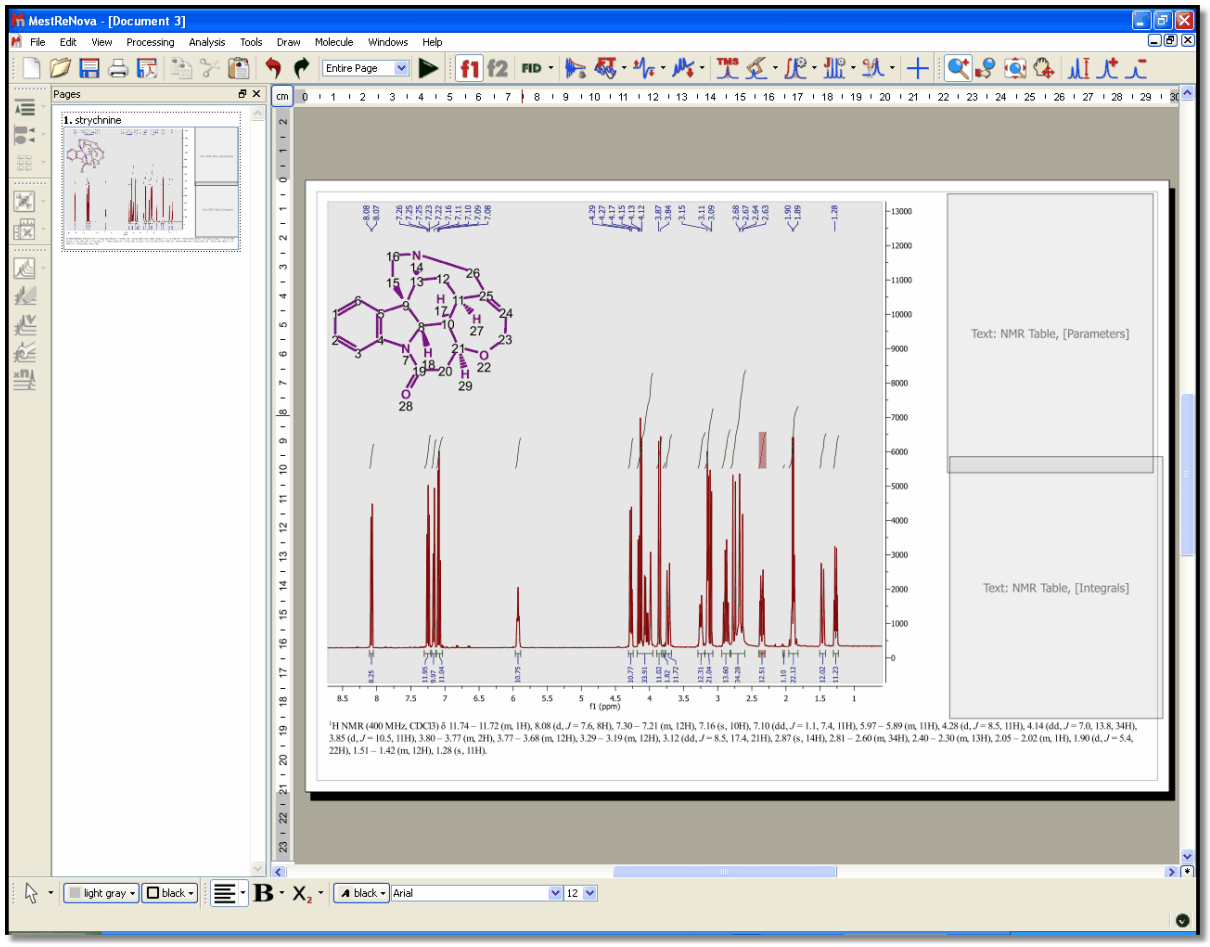
The picture below shows the layout created previously:



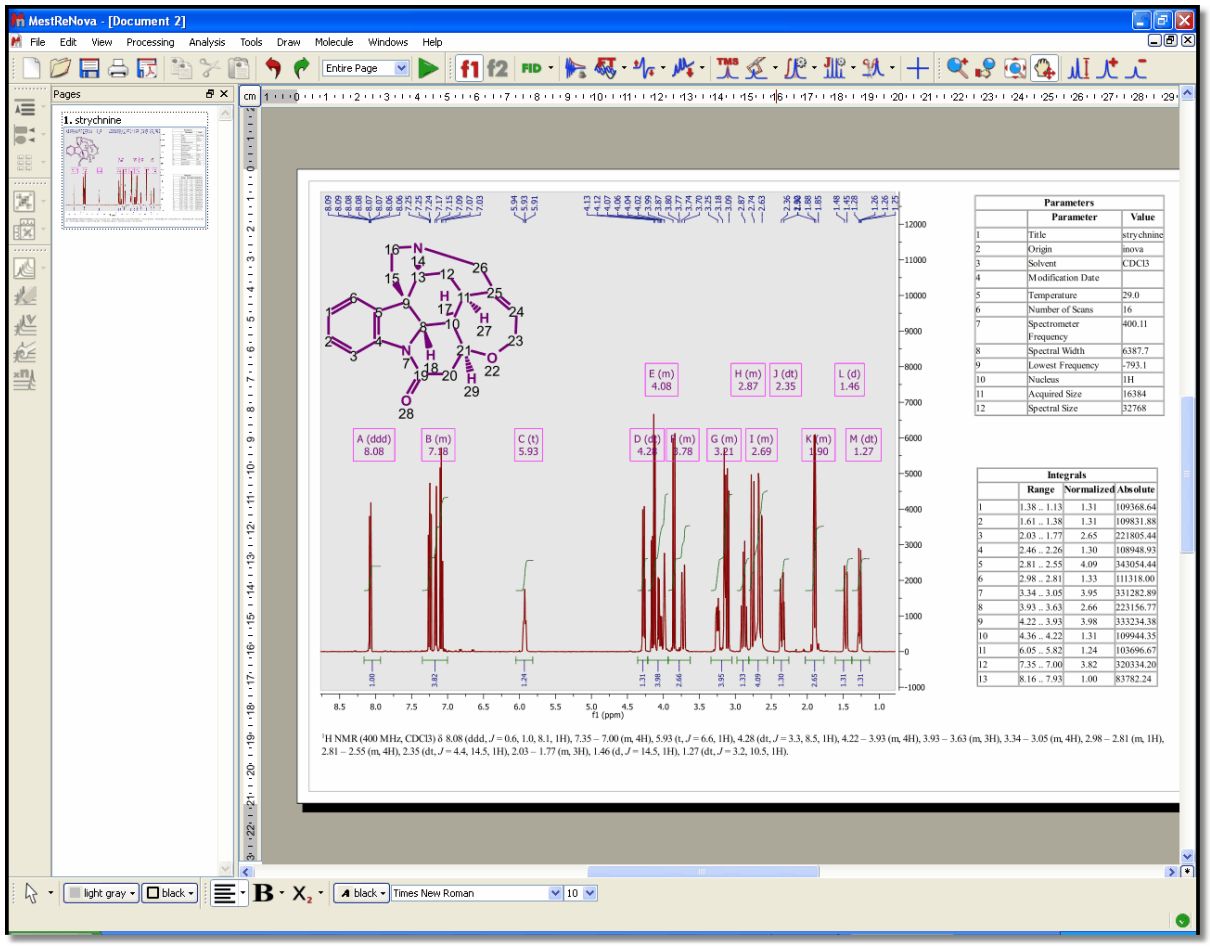
If the user opens a different 1D-NMR spectrum over this layout, it will be obtained in the corresponding location and with the corresponding object properties:



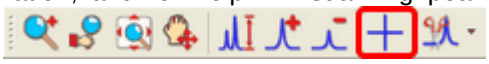
In the same way, when the user opens or pastes a molecular structure and reports the multiplets list, they will also be automatically displayed in the corresponding location:



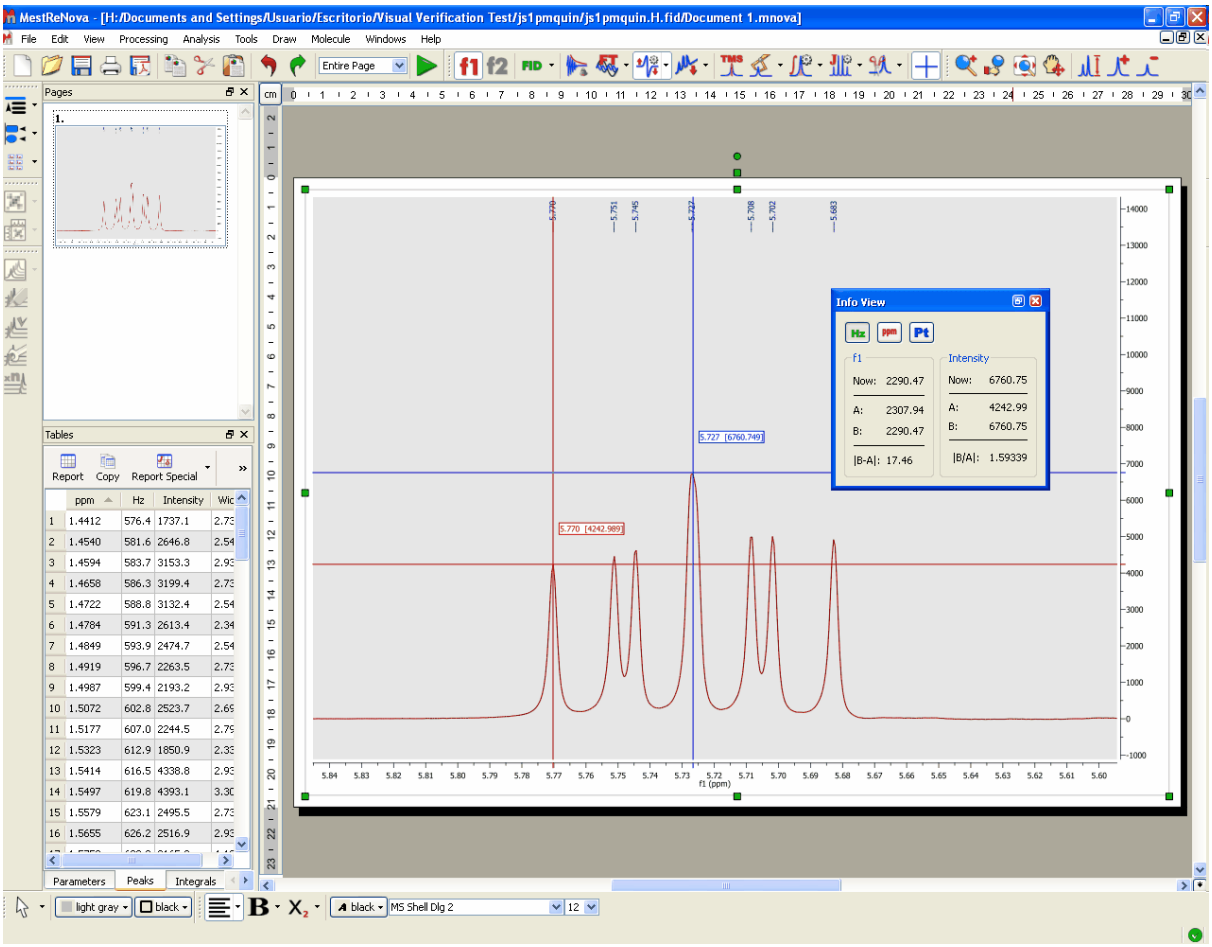
Finally, when the user reports the relevant tables, these are once again positioned on the relevant area of the paper, ready to report. Also note that our concept is designed to be extended to reports with more than one page, to allow for the placing of several spectra on the report.



7.7 The Crosshair Tool

The Crosshair:  These two orthogonal lines forming a crosshair are available on the 'View' menu (or by pressing the <c> key) for reporting positional information, and for help in visualizing peak alignments. This tool can also be found on the 'Zoom' toolbar 
.

The crosshair includes information about the chemical shift and the intensity of the peak (as you can see in the picture below) and also allows the user to measure distance between peaks (or coupling constants) by clicking on the left mouse button and dragging to the desired peak. This will open an 'Info View' dialog box, where the user will be able to change the units distance is expressed in (Hz, ppm or points). This dialog box will also display the distance between the desired peaks, as well as the intensity ratio.



The cursor will "ride" along the spectrum simplifying the location of peak maxima and minima by pressing the 'Shift' key.

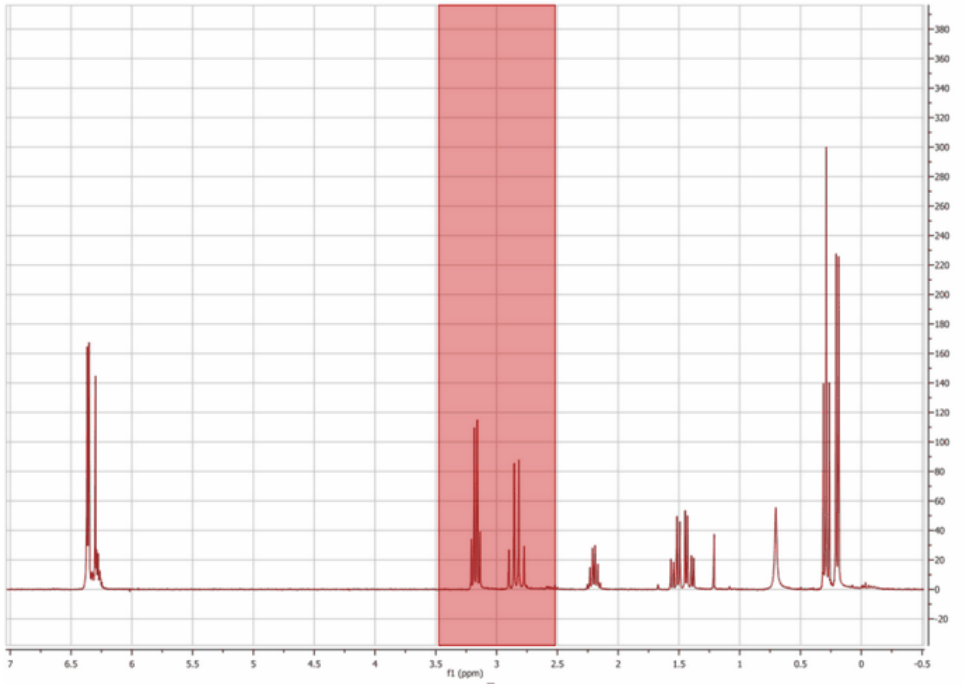
7.8 Zooming

A number of ways of zooming are available in **Mnova**.

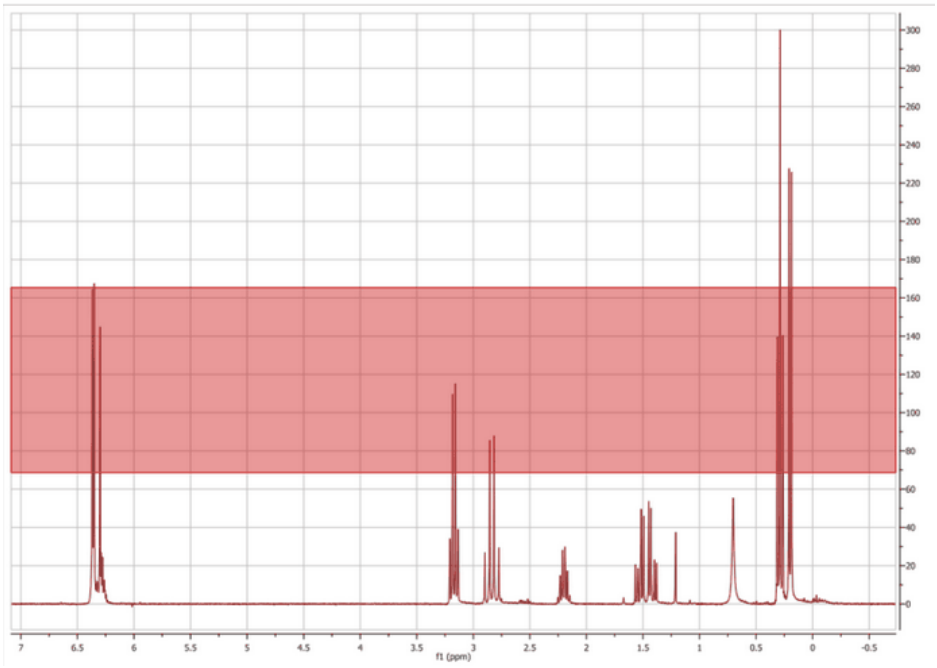
The most common type of zooming in spectroscopy applications is the **'Zoom In'** mode. This mode can be entered by following the menu 'View/Zoom/Zoom In' or by using the **'Zoom In'** icon on the toolbar.

In this area, Mnova presents a significant innovation, allowing the user to apply 'horizontal', 'vertical' and 'box' zooms, as illustrated by the figures below.

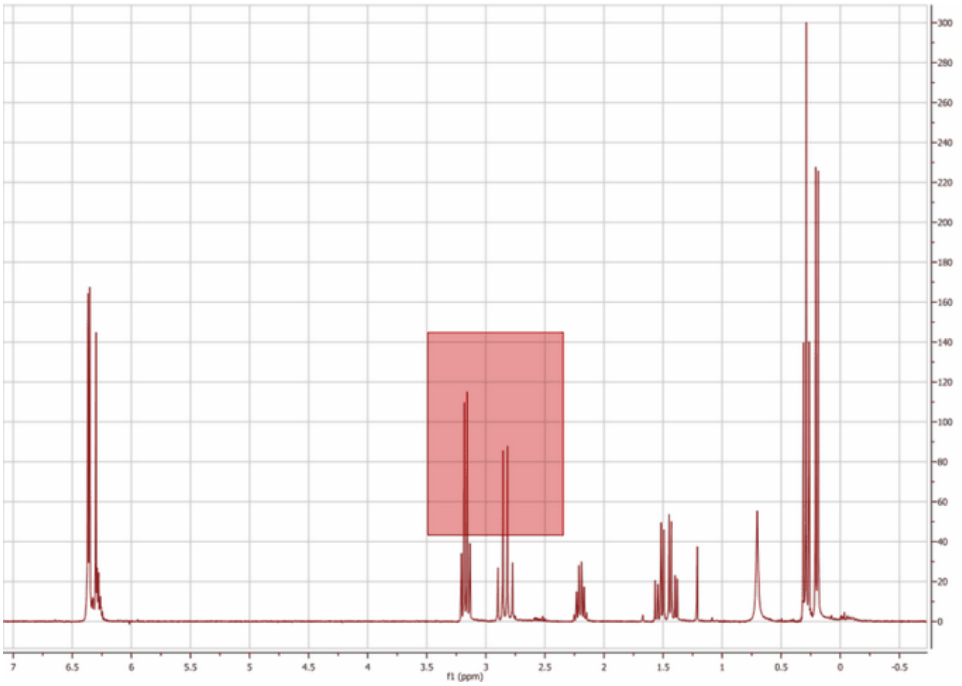
HORIZONTAL ZOOM. Click the **'Zoom In'** icon  on the toolbar (or press **<Z>** key), hold down the left mouse button on the spectrum display and drag the *magnifying glass cursor* (with a red horizontal segment) across the area you want to zoom in to; a highlighted rectangle indicating the new spectral region to be displayed once the mouse is released, will follow the cursor. Use 'Undo' to revert to the previous display. A zoom lens of a 20% will be obtained by clicking on the left mouse button.



VERTICAL ZOOM. Click the **'Zoom In'** icon on the toolbar, press **<Z>** key (you will see a red vertical line segment beside the mouse pointer) while the cursor is located on the spectrum display and then click and drag the cursor vertically over the area you want to zoom in on; once again you will see highlighted red rectangle.



BOX ZOOM. Click the **'Zoom In'** icon on the toolbar, press **<Z>** key twice with the mouse pointer on the spectrum display, then click and drag the cursor diagonally over the area you want to zoom in (highlighted rectangle in red). This zoom is very useful for 2D-NMR.



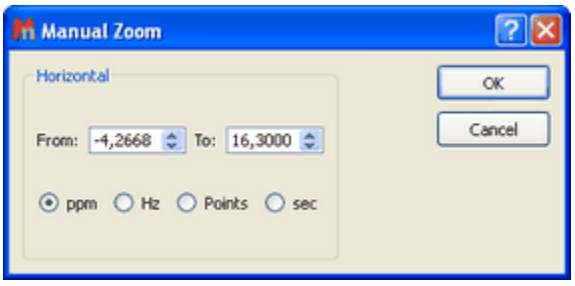
In order to switch between these three types of zoom, just press the **<Z>** key whilst in the **'Zoom In'** mode.

ZOOM OUT: Click the **'Zoom Out'** icon on the toolbar  and click on the left mouse button to obtain a zoom lens out of a 20%.

MANUAL ZOOM: Click 'Manual Zoom' on the toolbar, as you can see below:

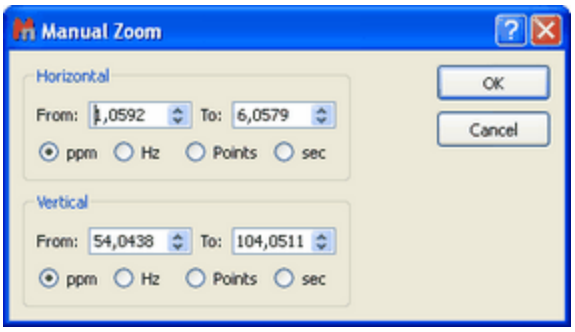
Manual zooming is also available from the menu 'View/Zoom/Manual Zoom'. The manual zoom is slightly different in 1D to that in 2D NMR spectra where you may select spectral spectral limits for both dimensions.

1. If you are working on a 1D-NMR spectrum, a dialog box like this will open:



You can select the limits of the spectral region of interest in ppm, Hz, points or sec and then click 'OK'.

2. If you are working on a 2D-NMR spectrum, the dialog box will look like this:



Select the horizontal and vertical limits of the region of the spectrum that you want to zoom in on, and click 'OK'.

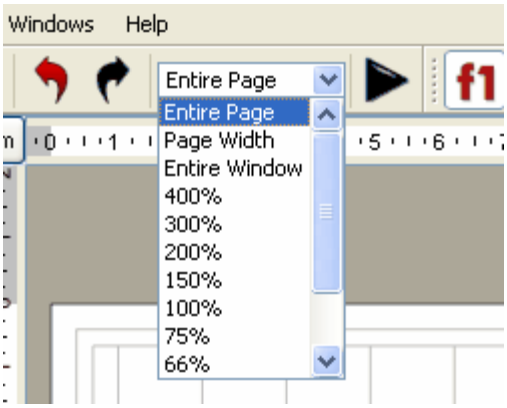
OTHER ZOOMING MODES

1. FULL SPECTRUM: This tool may be used to revert to a full spectrum from a zoomed view. This may be done from the menu 'View/Zoom/Full Spectrum' or by using the **'Full Spectrum'** icon on the toolbar.

2. FIT TO HEIGHT: The Fit to Height function adjusts the display of spectral intensity in both 1D and 2D spectra so that the strongest signal is optimally displayed.

3. PANNING: While the spectrum is in a zoomed state, click on the **'PAN'** icon on the toolbar (or hold down the space bar, or hover the mouse over an axis), hold down the left mouse button and drag the *hand cursor* in any direction to navigate throughout the spectrum. In order to select vertical or horizontal panning only, press the <P> key whilst in the **'Panning'** mode.

4. ZOOMING COMBO BOX: In addition to the traditional **'Zoom In'** mode, **Mnova** also allows the user to apply different zooms, including Zoom Out (from a zoomed state), by using the zooming combo box.

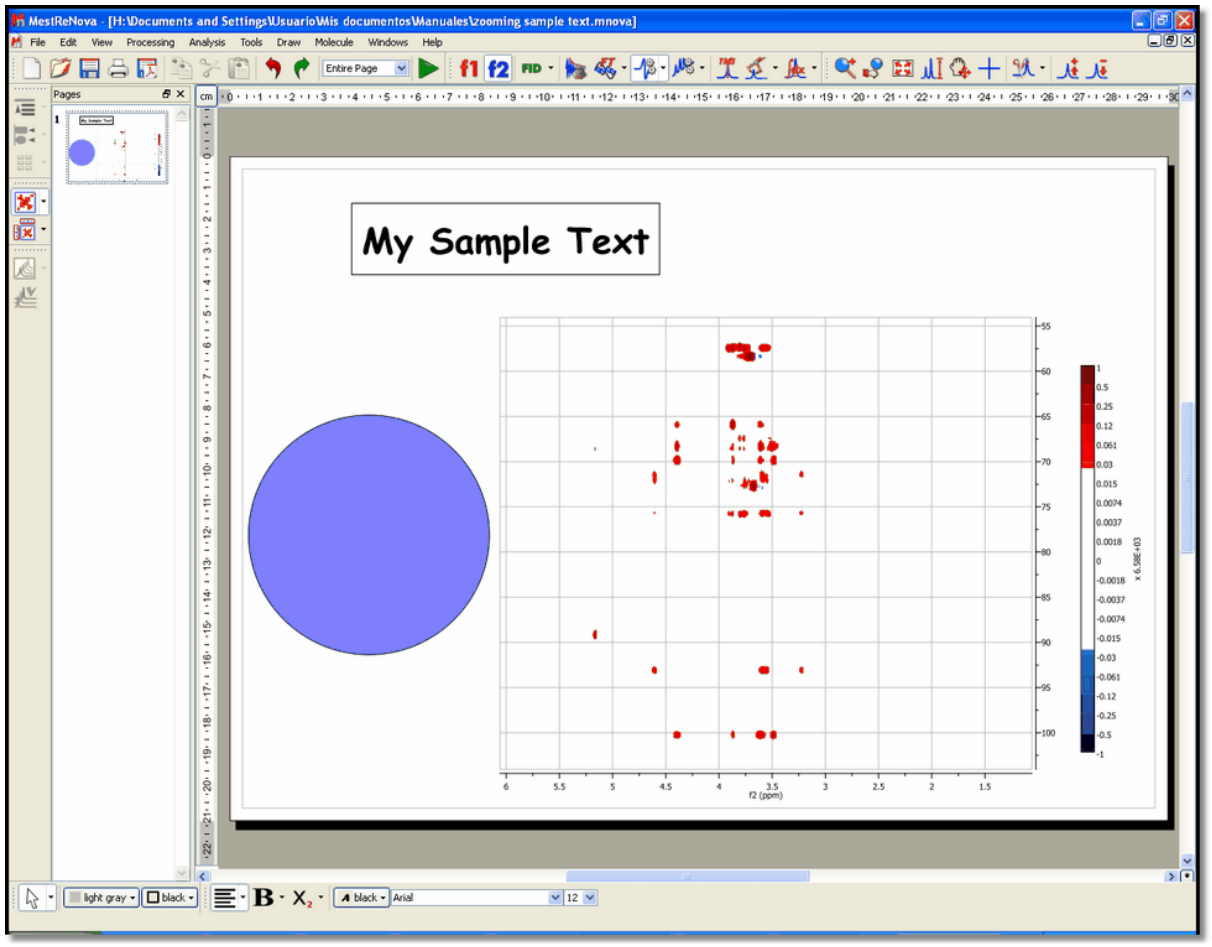


These zooming modes are:

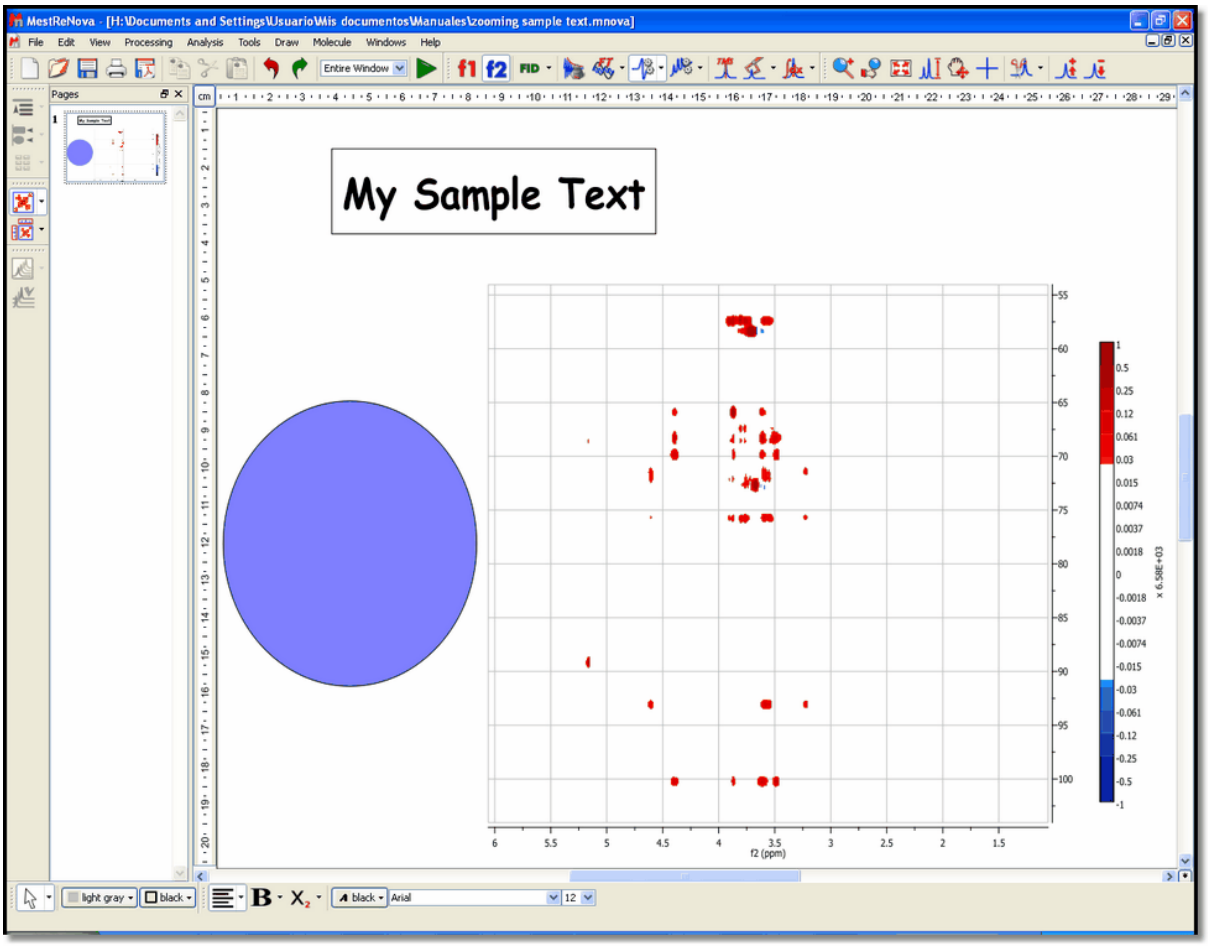
- ✓ **Percentage zooms**: These allow different size views of the object, by selecting the different percentages available (from 400 to 25%).
- ✓ **Entire Page**: This is the standard view, which can be seen in all the figures above.
- ✓ **Page Width**: In this view, the program will fit the size of the paper to the window width, whilst preserving the original paper proportions.

✓ **Entire Window**: This is a special view within Mnova. It will fit the size of the paper to the size of the available window, to optimize the available screen area. This view represents an exception to the WYSIWYG concept, since in this case the proportions of the screen paper may differ from the proportions of the printer paper and therefore of what you will get when printing the page.

The **Entire Window** concept deserves further comments. For example, consider the following scenario in which the page zooming mode is set to **Entire Page:**



As seen in the figure above, Mnova is a fully **WYSIWYG** application. This can be appreciated in the blue circle which was created with the **ellipse** tool and then its dimensions set to form an actual circle. However, in this case there is a significant space of the window which is not covered by the page (see gray regions surrounding the page) which can be considered as wasted space. In order to make the most of the available space, we can use the so-called **Entire Window** zooming mode. In this mode, the page stretches to fit all the available space. Of course, this means that paper proportions (i.e., width/height ratio) have to be changed and thus the WYSIWYG principle is broken. As always, a picture is worth a thousand words:



Note that all the objects are now distorted. However, this mode is very convenient to work with spectra on the screen as the graphical spectral proportions are rarely an issue. Nevertheless, if you print a document in this non WYSIWYG mode, you will get exactly the same results as when working on real WYSIWYG modes.

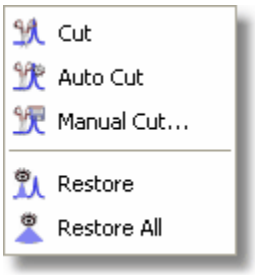
7.9 Cutting Spectra

THE SPECTRUM CUTTING TOOL

This is a tool that most **Mnova** users will not have seen before (with the notable exception of **iNMR** users). It is important to note that this tool is in no way related to the "copy", "cut" and "Paste" object manipulating tools found in this and most Windows programs as Icons and in the Edit drop-down window. The **'Cutting'** tool allows the user to create **dark** regions, i.e., to hide areas of the spectrum the user is not interested in seeing (these could be noise regions or other regions which are of no interest at a specific time). By hiding these regions, areas of interest become larger on the screen without a need to create numerous expansion windows. The Cutting Tool is basically a way of split zooming, as opposed to the more limited traditional zooming in a single block. The hidden areas are shown on the scale and on the grid. The software also implements an **Auto Cut tool**, which will hide all noise-only regions of the spectrum at the click of one button.

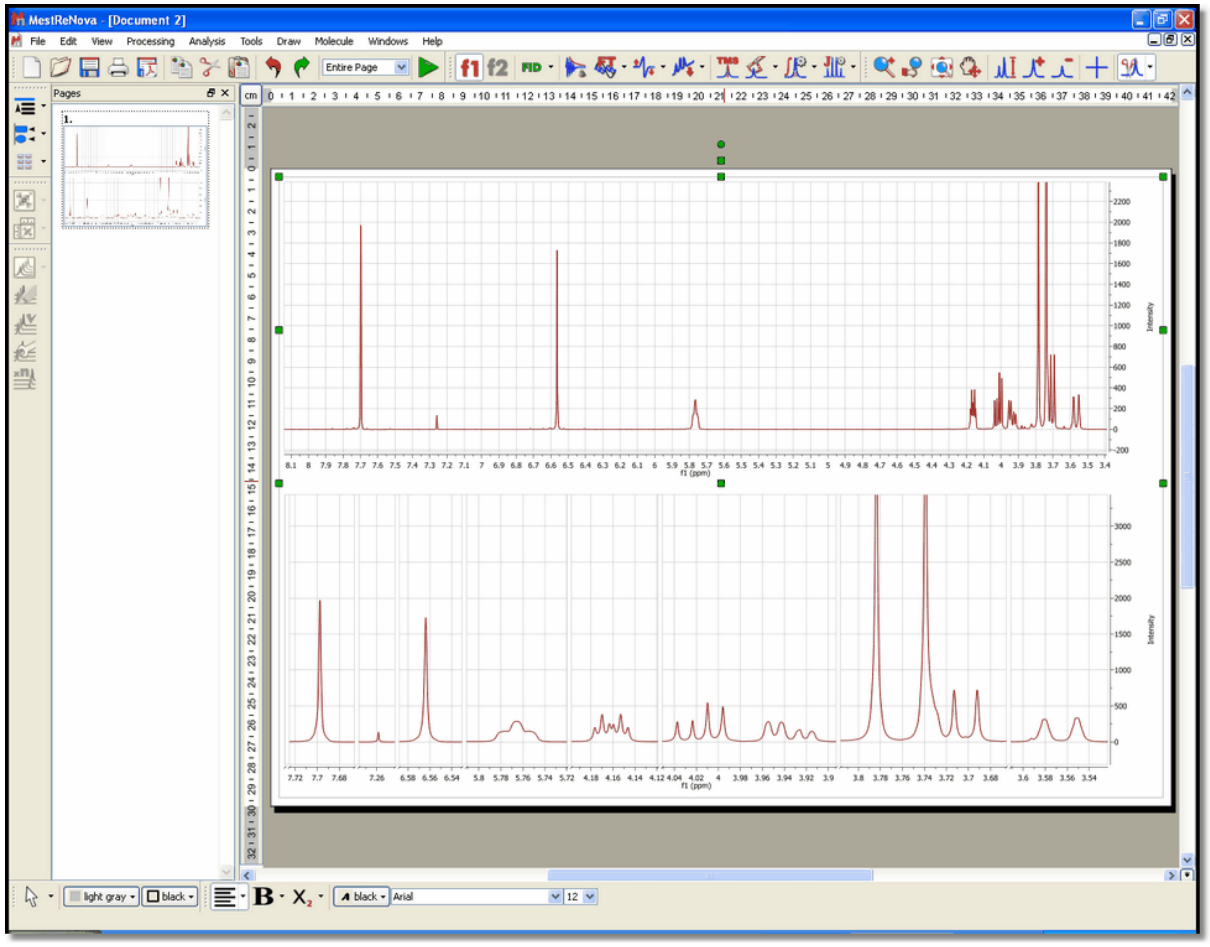
It is important to stress that this tool is not eliminating the information, but simply not displaying it. The 'Cut' and 'Auto Cut' options are available from following the menu item 'View/Cuts/Cut' or 'View/Cuts/Auto Cut'. Manual cutting can also be accessed by clicking on the **'Cut'** icon (or simply, by pressing the <X> key) as can be seen in the figure below. Auto Cut can be accessed via the

drop-down menu which can be displayed by clicking on the side-bar of the Cutting Icon.



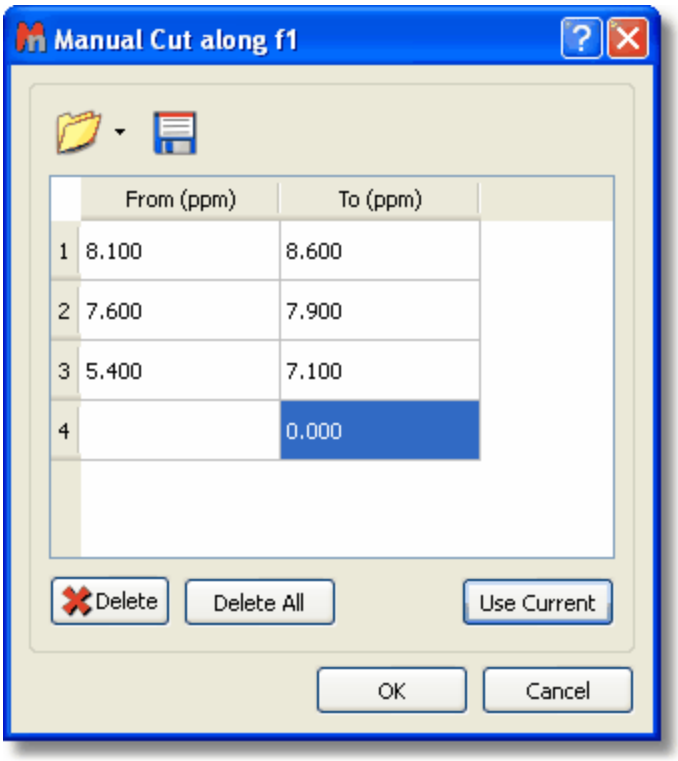
It is also possible to **restore** one specific region ('Restore', or <V> key), a limited region ('Manual Restore') or all regions ('Restore All'). These options can be selected from the menu 'View/Cuts/Restore', 'View/Cuts/Manual Restore' and 'View/Cuts/Restore All' or by selecting 'Restore', 'Manual Restore' or 'Restore All' in the scroll menu shown in the figure below.

By the way, you should also note that the 'Cut' mode is available in 2D, along direct, indirect or both dimensions. Once again, switching between modes is achieved by simply pressing the <X> key.



Manual Cutting

The user will be also able to use the 'Manual Cut' to select the limits of the dark regions.



The user will be able to predefine the cuts ranges to automate the cutting analysis. **Clicking on OK, will cut the current spectrum with the desired ranges and keep the regions to apply further cuts to additional spectra.**

The user will be able to save these regions for later uses, by clicking on the **'Save Cuts'** icon  which will be loaded by clicking on the **'Load Cuts'** icon 

7.10 Increasing and Decreasing Intensity

The two icons  towards the right of the standard toolbar allow the user to interactively scale the data of the NMR spectrum. This operation does not modify the acquisition or the MestreNova data file; it only magnifies or diminishes the representation of the data which you see on your screen. You can also find these options via the 'View/Increase Intensity' and 'View/Decrease Intensity' menus. Additionally, you can use the '+ key' to increase or the '- key' to decrease the intensity and the same effects may be obtained by rotating the scroll wheel of the mouse.

7.11 Undo & Redo

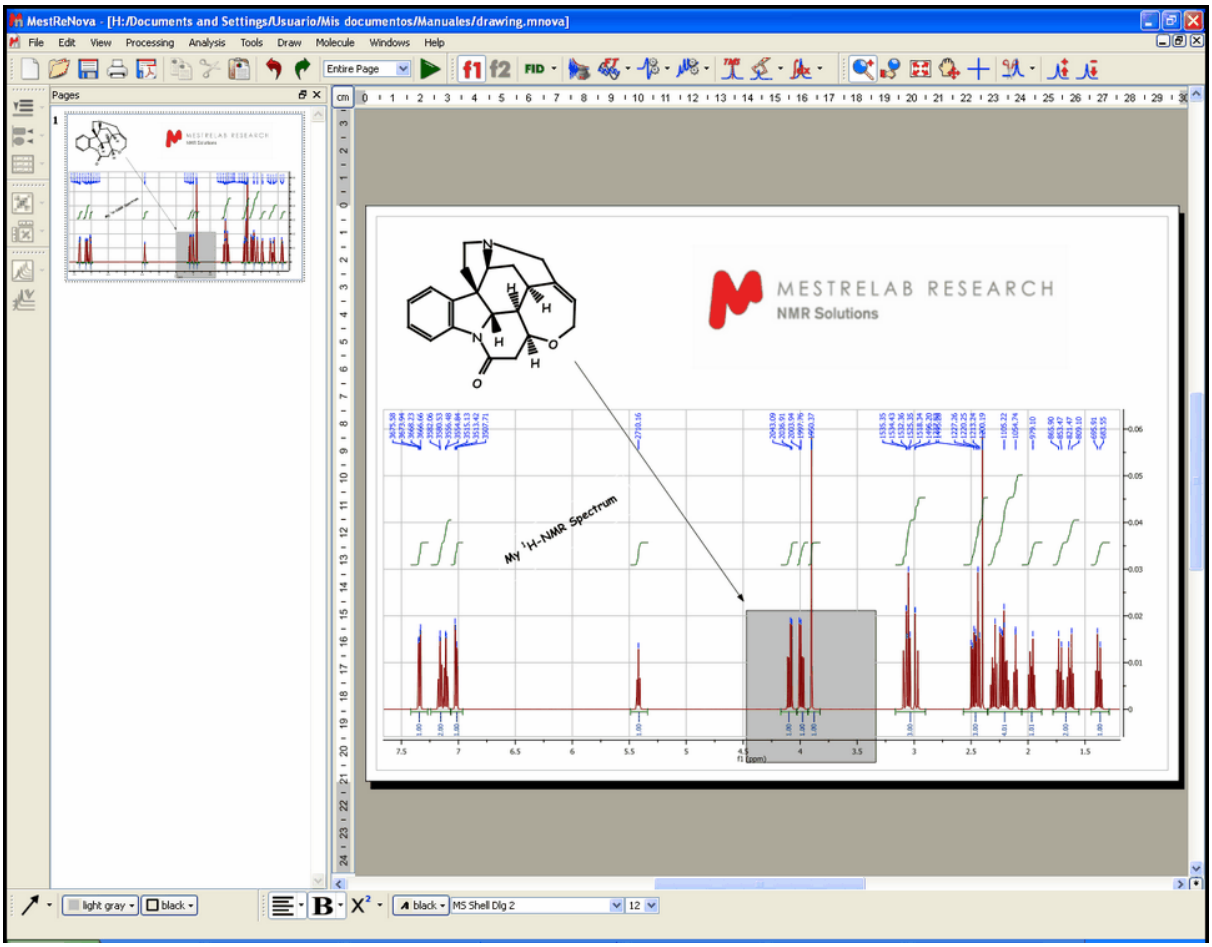
Undo is a very useful command; it erases the last change made to the document, causing the document to revert to an earlier state. The **Redo** command reverses the undo.
Mnova has very powerful **Undo** and **Redo** capabilities. These can be applied to virtually any operations carried out by Mnova, from creation of pages and opening of documents through zooming, panning, and annotating, to any processing or analysis operations. Undo & Redo in Mnova are virtually unlimited (RAM memory limitations may apply). These options can be found as the toolbar icons shown above or via the 'Edit/Undo' and 'Edit/Redo' menus. Additionally, there are keyboard shortcuts

for these operations, <Ctrl+Z> (or Cmd+Z for Mac) for Undo and <Ctrl+Y> (or Cmd+Y for Mac) for Redo.

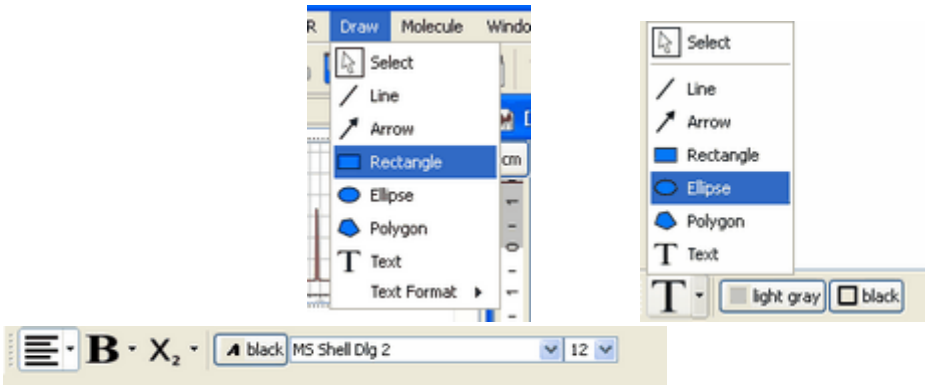
7.12 Graphics and Annotations

Mnova has the most powerful graphics and annotation capabilities available in any NMR software. Most of the annotations and drawing available in MS Powerpoint are available in Mnova via the graphics toolbar placed by default at the bottom of the screen, below the page navigator and the document area. The software supports Rich Text and so a wide range of fonts and font embellishments (sub- and superscript, bold, italic etc) are available.

Mnova can import objects such as **molecular structures** using Edit/Insert Object while **images** may be embedded using the standard Windows Copy and Paste tools.



You can draw a line, an arrow, a rectangle, an ellipse or a polygon by using 'Draw' in the menu or the graphic toolbar, as you can see below. Once you have selected the object you want to draw, hold down the left mouse button in the spectrum display and drag the cursor over the area where the line or shape is to be drawn. Once created the line/shape may be moved, rotated and resized by selecting it and using the object's moving, sizing and rotating "tabs". You can also choose the fill color (light grey as default) as well as the colour and linewidth of the border of the object (black as default). Text may be added to the document in much the same way. The text can be aligned (right or left justified or centred) and you can select the text features **Bold**, *Italic* and Underline, sub-and superscript and the font (color, type and point size).

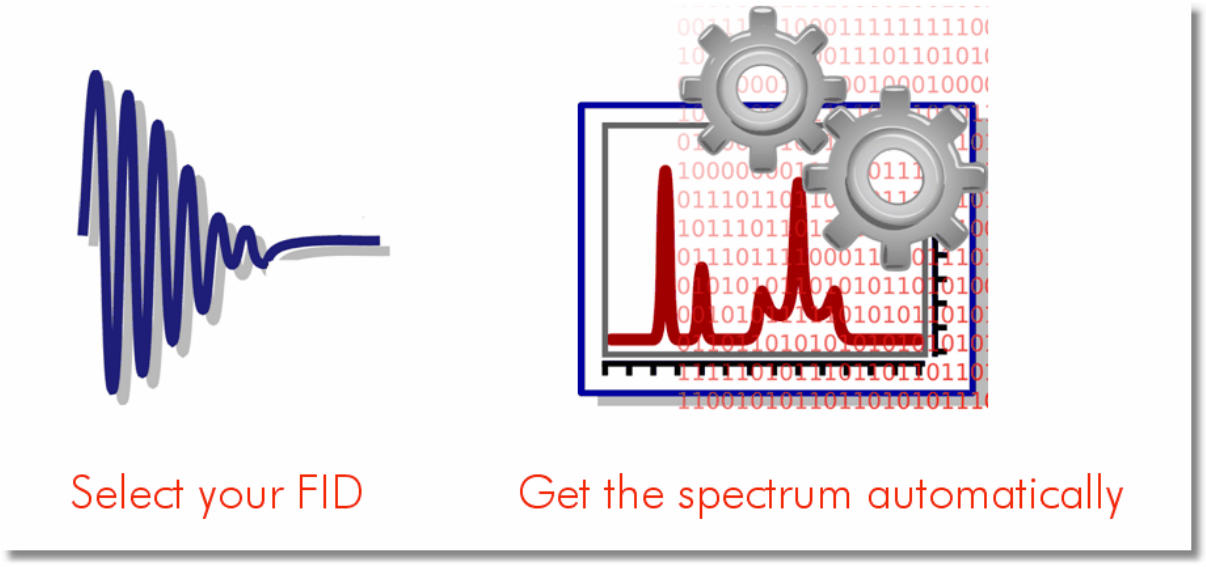

Draw
Molecule
Select
Line
Arrow
Rectangle
Ellipse
Polygon
Text
Text Format
Select
Line
Arrow
Rectangle
Ellipse
Polygon
Text
light gray
black
black
MS Shell Dlg 2
12

Part VIII

8 Processing Basics

Our aim in developing Mnova has been to make the opening, processing, handling, analysis, saving and printing of NMR data simpler than it has ever been before, so that even the NMR novice can enjoy the software and obtain excellent results from the very start.

Mnova introduces a brand new NMR data processing paradigm; when a raw, unprocessed spectrum (FID) is opened, you will get the fully processed spectrum instantaneously.



This new concept is very simple and consists of two fundamental points:

1. **Automatic File Format Recognition:** Once you select the FID, Mnova will automatically identify its origin (e.g. Varian, Bruker, Jeol, etc).
2. **Fully Automatic Processing:** Once the FID is opened Mnova will automatically and optimally process it using the information it discovered in Part 1, so you do not need to bother with processing details.

The two steps outlined above will successfully process most NMR data. However, if you are not completely satisfied with the result you can, at anytime, reprocess using your own choice of processing parameters, changing or adjusting the window function, the FT, the phasing and baseline correction to meet your requirements.

With this scheme, processing of 1D and 2D spectra is essentially identical as you will see in the following two tutorials:

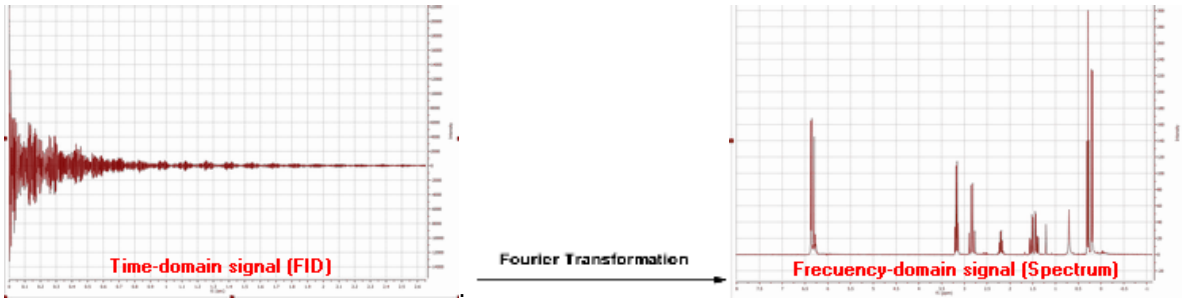
1D NMR Processing
2D NMR Processing

8.1 1D NMR Processing Tour

Opening the data set

In pulsed NMR spectroscopy we obtain what is called a *time-domain signal* since the measured signal is a function of time; all the nuclear resonances decay from the same starting time to form the Free Induction Decay or FID. This kind of signal is not readily interpretable by the human eye, and so it is necessary to transform it (into the frequency domain) using a Fourier transformation (FT) to display the

separate nuclear resonances as a frequency spectrum.



There is one single command for opening all types of files in **Mnova**. It is not necessary for the user to have any knowledge about the way in which the experiment has been acquired. **Mnova** will automatically recognize most common spectrometer file formats and acquisition modes and will apply the necessary processing operations.

Sample file

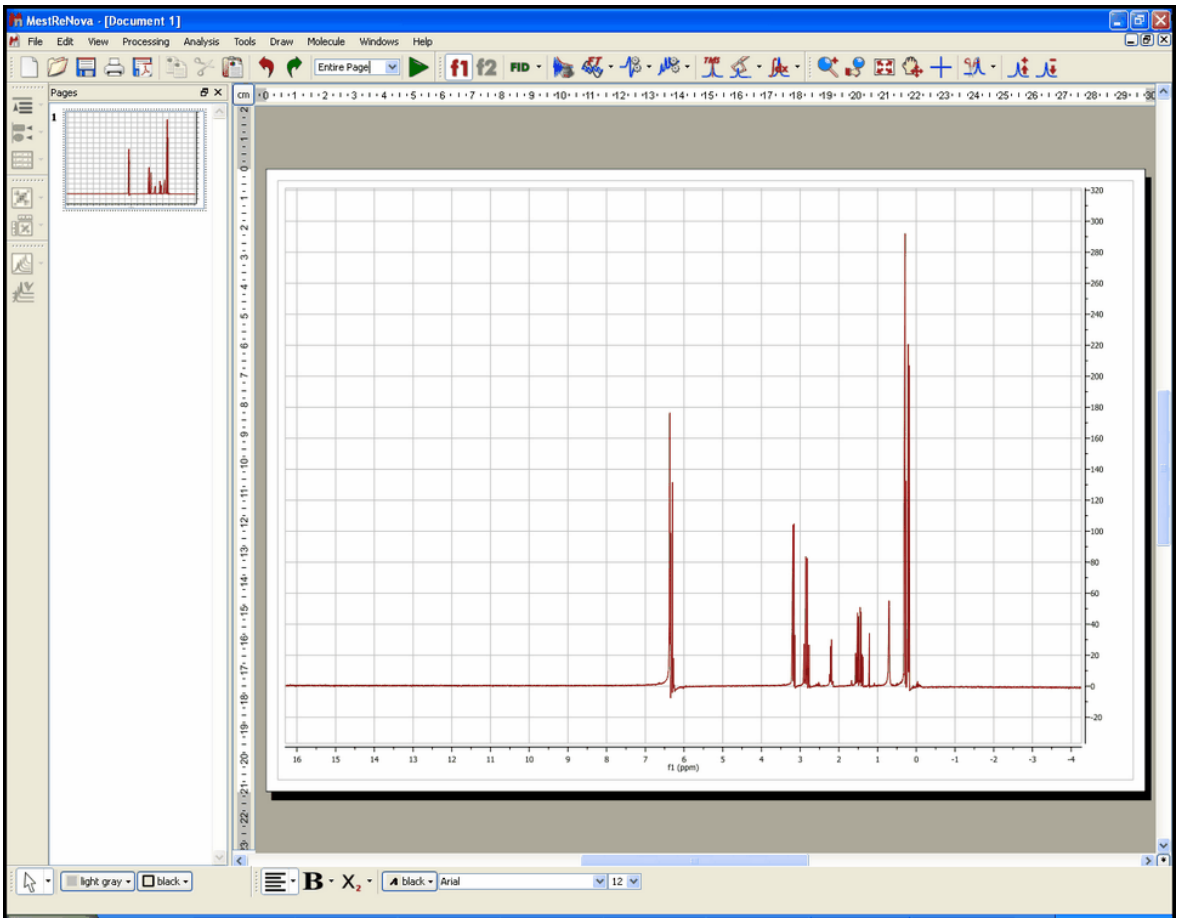
This tutorial has been designed using a 1D NMR spectrum available from the following link:

http://www.mestrec.com/samples/1HReal.zip

To open your data (including mol files and graphics files), just go to ***'File/Open'*** menu, use the keyboard shortcut **<Ctrl+O>** (or Cmd+O for Mac) or click on the **'Open'** icon on the toolbar (note that this is exactly the same for 1D and 2D data).

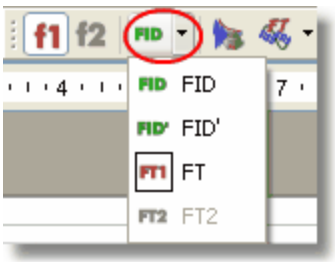
Once this command has been issued, navigate through your disk to find the NMR experiment of interest and select the desired FID file which is normally named **fid**; however in Bruker 2D experiments the time domain data is named ser (serial fid). After selecting this file, the first thing you will notice is that the spectrum will open **already processed**, despite the fact that you are opening raw data (FID). When opening files, Mnova will apply the necessary processing operations, selecting the correct processing parameters from the spectrometer files. If these parameters are not available (e.g. phase parameters are not available), Mnova will use automatic algorithms (e.g. automatic phase correction) and will apply them to the data set. Thus, even a completely inexperienced chemist, working with NMR for the first time, should be able to get good results when processing both 1D and 2D spectra.

In the figure below you will see what happens when the FID of our test example is opened:



As explained above, the FID is automatically processed. In this case, the native files contained values for phase correction and thus Mnova utilized them for phasing. However, as you can appreciate, the result is not good enough (most likely because the spectrum was processed in the spectrometer computer using an inefficient automatic phase correction algorithm). Later we will show how to improve the phase of this spectrum by applying first the automatic phase correction algorithm implemented in Mnova and finally using manual phase correction. Remember that if the native files do not contain values for phase correction, Mnova will apply an automatic phase correction.

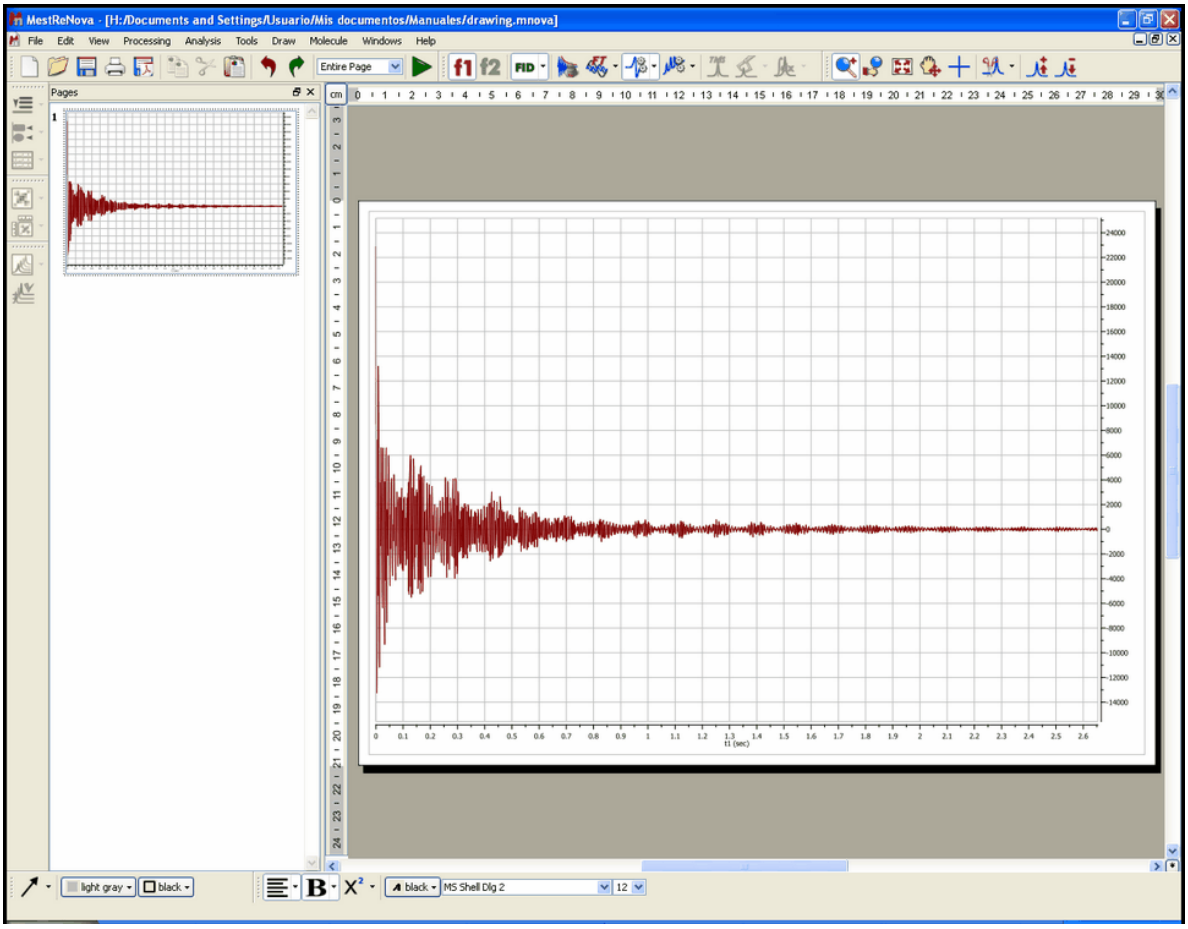
Even though you have opened a FID and you see only the processed spectrum in Mnova, it is possible to see the **original FID and the processed FID** (Processed FID or **FID'**). When we process the raw data on-the-fly, we do not discard these data, we just do not display them. The user can view the FID by just clicking on the '**FID'** icon on the toolbar (in the red circle in the figure below).



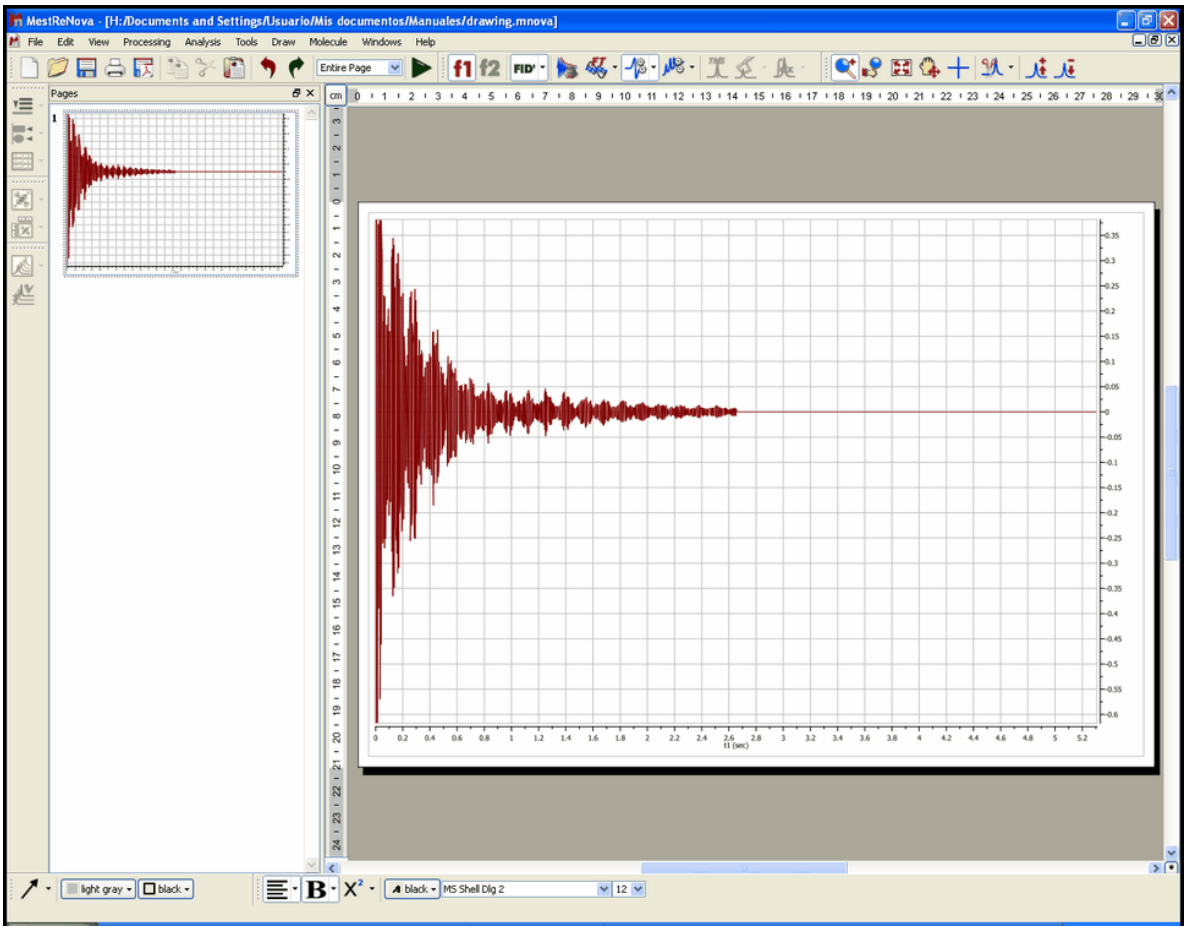
There are several options in the scroll down menu:

- **FID**: Clicking on this option will show the original *time-domain signal* (FID) as it was when ported from the spectrometer.
- **FID'**: Clicking on this option will show the FID after time-domain processing functions have been applied (i.e., immediately prior to FT)
- **FT**: Clicking on this option will show the data after FT along the direct dimension (spectrum for 1D data, interferogram in 2D data)
- **FT2**: Clicking on this option will show the data after FT along two dimensions (2D spectrum)

For example, this is the original FID of our test spectrum:



And this is the so-called **FID'**. In this case, prior to FT, Mnova applies a zero filling operation as can be appreciated in the FID' below. A zero fill increases the digital resolution by adding null data to the FID, increasing the size of the data array.



You can switch at any time between any of the displays (FID, FID' and processed spectrum).

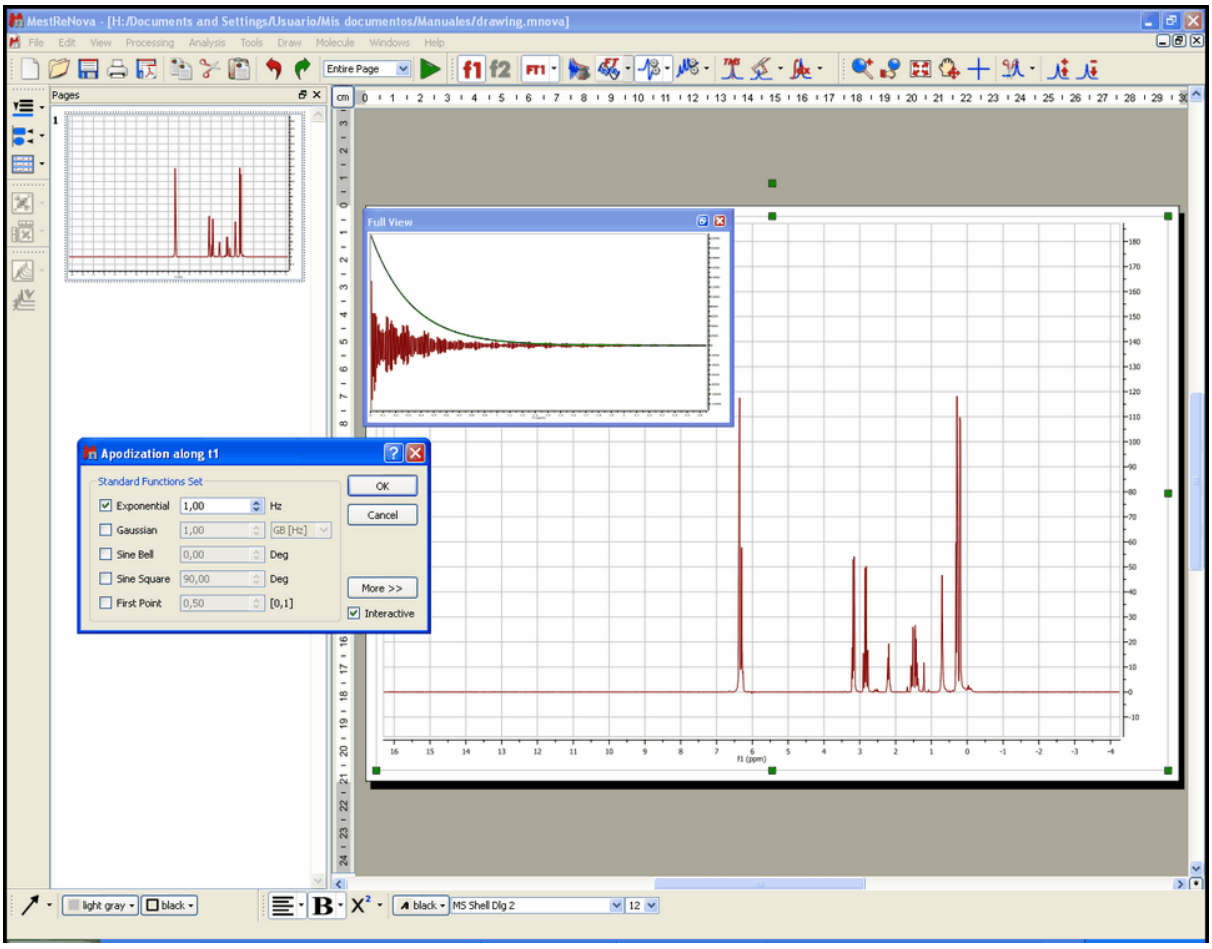
Interactive Processing: Real Time Frequency Domain Processing

As we saw in the previous section, Mnova will keep all pre, during and post processing information together with the spectrum we can see on the screen. This fact, combined with the extremely fast and optimized processing algorithms implemented in Mnova, allows us to introduce to NMR processing the concept of **'Real Time Frequency Domain Processing'**, designed to greatly simplify processing for the user and to ensure faster and better results even when complicated operations are applied to the data.

The way this concept works is very simple: Reprocessing of the spectrum is possible at any time, independently of the time domain and frequency domain operations which may have been applied to the data set (for example, it is possible to change the window function after having applied a FT, phase and baseline correction). This is possible because, in the background, Mnova is going back to the FID and reprocessing the spectrum by applying the new functions or parameters as well as all the other functions applied ad posteriori on the previous processing. The very important advantage for the user is that it is now possible to see, in **real time**, the effect of any processing operation (including time domain operations) on the resulting frequency domain spectrum (for example, you can apply a more drastic exponential apodization function and see in real time, on screen, what effect this is having on the resolution and sensitivity of the frequency domain spectrum).

In the figure below, you can see the frequency domain 1D spectrum, the FID with the window function which is being applied interactively (which is shown graphically in the 'Full View' window (go to the 'View/Full View' menu to activate), and also the Apodization dialog box - note that the 'Interactive' box

is ticked). The user can follow the exact effect of the function s/he is applying on the frequency domain spectrum. This can avoid repeated transformation and reloading of the data while the optimum processing parameters are sought and, of course, act as an excellent teaching and self-teaching tool for those who are not conversant with the effects of different processing parameters.



This same principle can be applied to all other processing operations, such as Zero Filling, Linear Prediction, Signal Suppression, etc. For example, if we want to increase the digital resolution of the spectrum, we can increase the number of points used in the FT operation by selecting **Zero Filling.**

Of course, standard frequency domain operations such as phase correction or baseline correction can be applied in the traditional way.

See also:
Phase Correction
Baseline Correction

8.2 2D NMR Processing Tour

(Not available in Mnova Lite)

1D experiments contain a wealth of information but this information is often obscured due to signal overlap. Homonuclear couplings are often more readily and rapidly determined by extension into a second dimension. Heteronuclear couplings (1H-13C for example) are much easier to determine using two frequency dimensions. 2D experiments which use one frequency dimension and some other variable on the other axis (the DOSY processed STE experiment for example with diffusion coefficient on one axis) exist but this tour will concern two frequency (chemical shift) dimensions.

These 2D spectra are collected as time domain data dependant upon two time variables (t_1 and t_2) and after two orthogonal Fourier transformations a frequency spectrum which is a function of two frequency variables results. The general scheme for these **two** dimensional experiments is shown in the pulse sequence below:

In the *Preparation Time*, the sample is excited by one or more pulses and the spin system is placed in a non-equilibrium state which will evolve later. During the *evolution* period, the resulting magnetization evolves freely. Each signal will evolve with its characteristic frequency. Then follows the *Mixing Time* containing a further pulse or pulses; it is in this period that, for example, magnetization, may be transferred from proton to 13C during indirect detection. Finally the signal is recorded (detected) as a function of the second time variable, t_2; the signal has, however, been frequency-labelled as a function of t_1.

After acquiring a bidimensional experiment, you will have a matrix of data; the rows contain data collected at fixed values of t_1 with t_2 varying; they are, in fact, normal 1D data FIDs. The columns contain data collected at fixed values of t_2 with t_1 varying and are "synthetic" 1D FIDs. This matrix will be fourier transformed twice, once for the rows and again for the columns. In this way, the time dimensions (t_1 and t_2) are converted into frequency dimensions (f_1 and f_2).

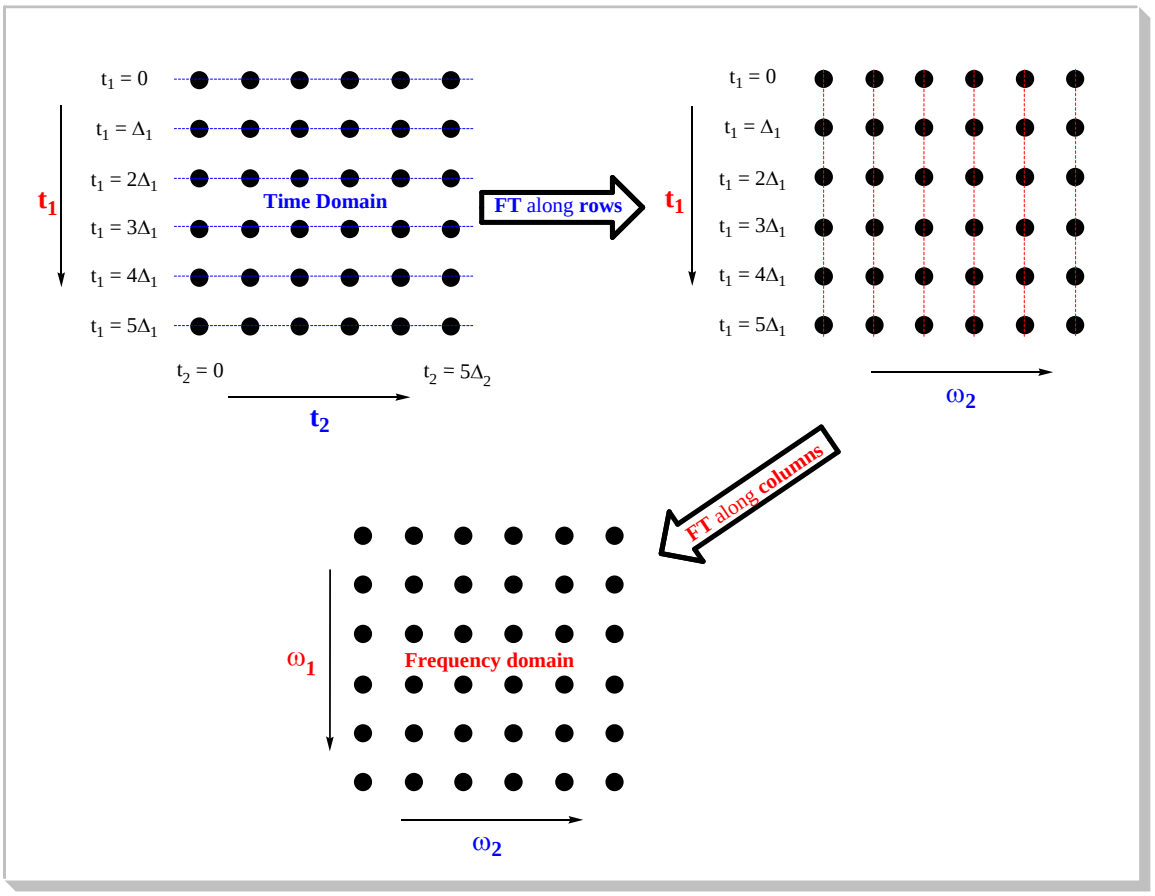
In a 2D-NMR experiment we can find any nucleus represented on either of the dimensions of the spectrum, so the spectrum can show correlations between the same nuclei ($^1H/^1H$) or different nuclei (such as $^1H/^{13}C$, $^1H/^{15}N$, $^1H/^{31}P$, etc...). In a 2D-NMR spectrum, the acquisition dimension (t_2) is the dimension in which we can obtain the greater number of data points without a considerable increase of the acquisition time. On the other hand, each point acquired in dimension t_1, multiplies the necessary number of scans, considerably increasing the total acquisition time. For this reason, in many cases the number of data points acquired along the t_1 dimension is much smaller than along t_2, although this number of points must always be must always be sufficient to provide adequate f_1 resolution. The "synthetic" f_1 FIDs are generally truncated and may benefit from forward linear prediction.

Processing of 2D NMR data sets with Mnova is almost as easy as processing 1D NMR data. You simply select the FID file (or SER file in Bruker) and the 2D spectrum appears on your screen without the need to touch a single button.

In this tutorial we will show how to process a 2D experiment. This tour will guide you through all the necessary steps to process, display, and print out two dimensional spectra with Mnova. Before you attempt to process a 2D spectrum, please read the 1D NMR Processing Tour since you need to be able to process 1D spectra also.

Multidimensional (or bidimensional) data processing requires only a few new concepts when compared with 1D NMR data processing. A 1D-NMR spectrum can be considered as a single vector of data points and its transformation into the frequency domain only requires only one FT. In the case of 2D spectra we have a two dimensional matrix of points and it is necessary to Fourier transform both rows and columns.

In 2D homo- and heteronuclear NMR the matrix of points results from an array of 1D FIDs which are functions of t_2 due to their detection (sampling of the FID at intervals of delta-t_2) and t_1 since each successive FID the value of t_1 which determined the evolution time, is incremented. The first row has t_1 =0, the second row has t_1=Δ_1, the third row has t_1=2Δ_1 and so on. The same happens with the data columns, although in this case the sample interval is different (Δ_2).



As you can see in the picture above, the first step is the application of the FT along the rows of the matrix; to obtain a new matrix (*interferogram*) where each row corresponds to an increasing value of t_1 and each column to a running frequency variable ω_2. Next the columns are subjected to a FT to obtain the final matrix (2D spectrum) where the frequency axes (ω_1) corresponds to the evolution in t_1, while ω_2 corresponds to the evolution in t_2.

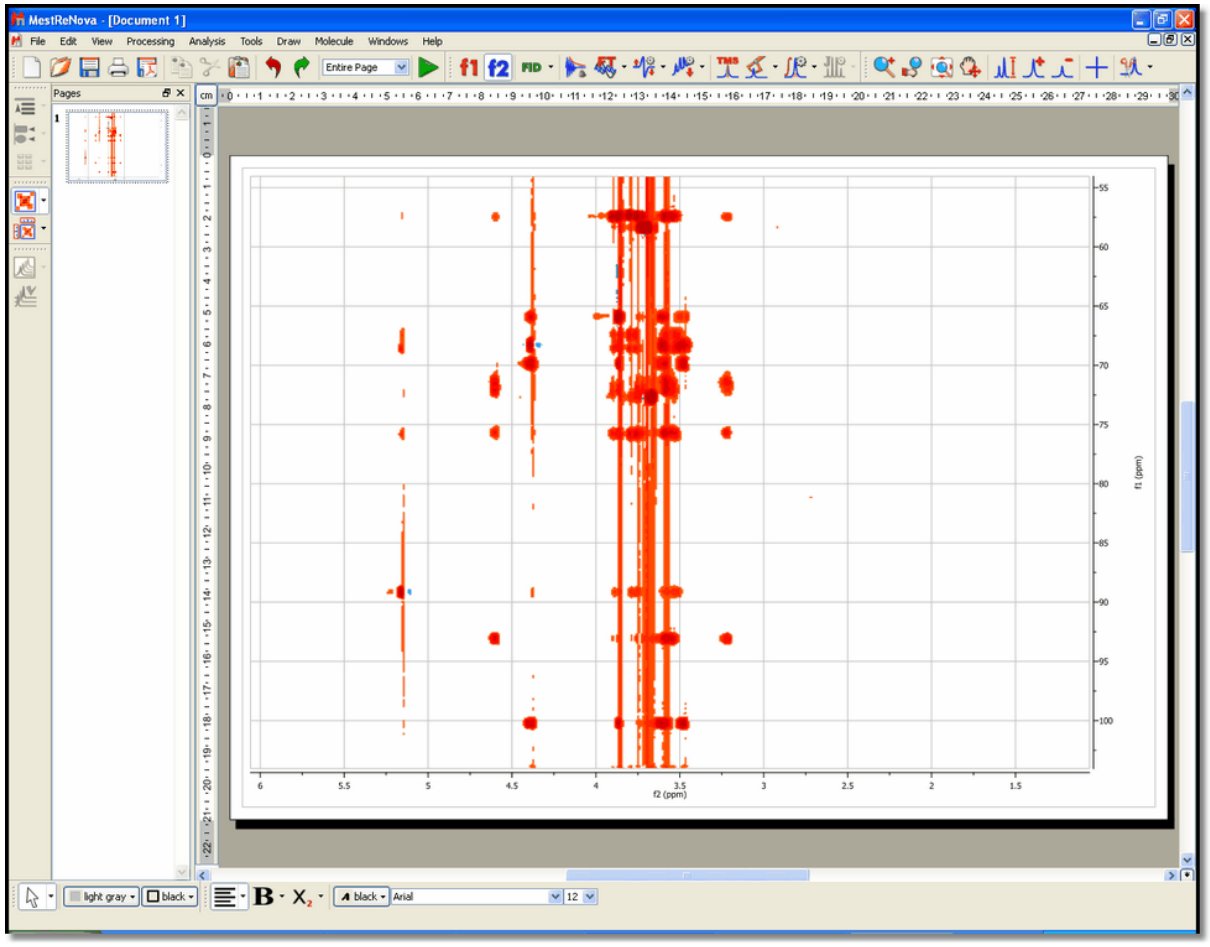
This 2D data can be processed using apodization functions and once you obtain the 2D spectrum, you can apply a phase or a baseline correction as with 1D processing. One important point is that processing operations need to be performed along each dimension. In Mnova, the processing is always started along the acquisition dimension, and finished along the indirect dimension.

Sample file

The data set used in this tutorial can be downloaded from the link below:

http://www.mestrec.com/samples/HMQCT_TOCSY.zip

In this example we will use a Bruker spectrum so you will need to select the **ser** file. Once you do so, you will immediately obtain the processed spectrum:

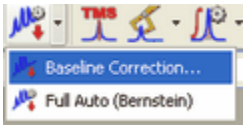


The program has performed the following set of operations on the fly:

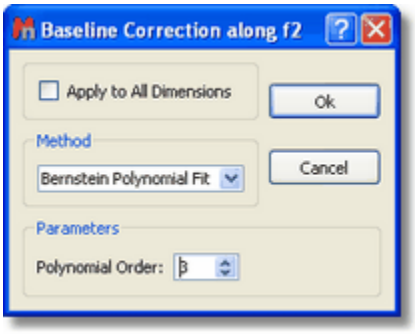
1. Automatic file format recognition: The program identifies this file as a Bruker file and uses the corresponding filter to decipher the data
2. For each dimension:
   - Weighting
   - Zero filling
   - Fourier Transform
   - Phase Correction

Baseline Correction

From the figure above, baseline artifacts along the indirect (f1) dimension are apparent. They can be easily removed by the baseline correction procedure. Select this option by clicking on the **'Baseline Correction'** icon  to obtain an automatic **'Baseline Correction'** or by choosing 'Baseline Correction' in the scroll bar menu:

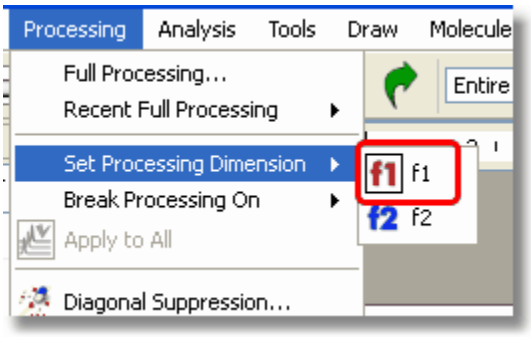


so that you get the following dialog box:



Baseline correction will be applied to the currently **active dimension** unless the 

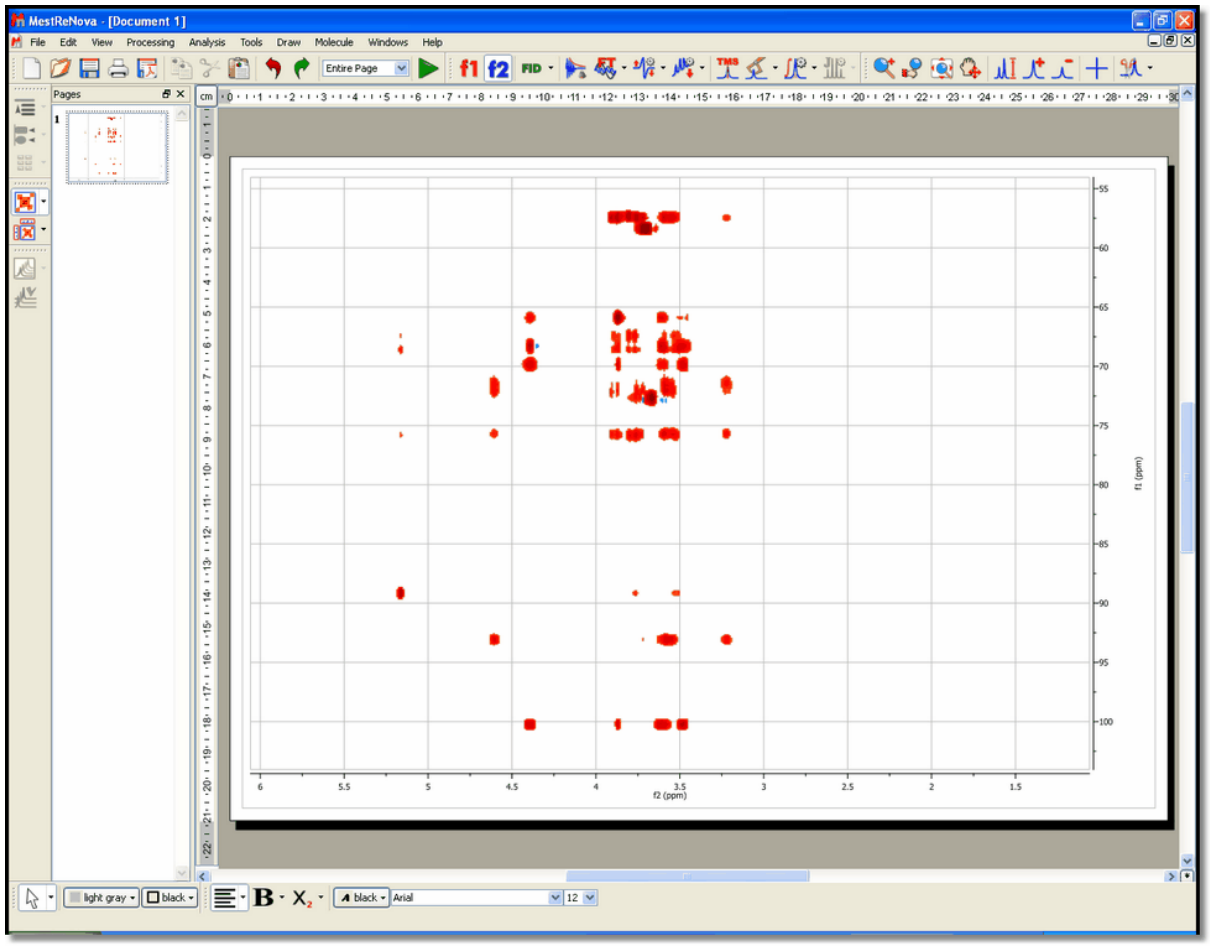
option is checked. What does **active dimension** mean? The active dimension is the dimension highlighted on the toolbar and is generally the direct dimension. With the exception of phase correction, processing operations in Mnova default to the active dimension only. For example, if we want to process along columns (f1) you just need to set the processing dimension to f1 as depicted below:



As a shortcut, you can use the corresponding toolbar buttons:

In our present situation, as the current processing dimension is **f2**, baseline correction will take place only along this dimension. This is not appropriate for our case as it is evident that baseline distortions are present mainly along the indirect **(f1)** dimension. We can simply set the processing dimension to **f1** , but it is even simpler to check the 

option in the **baseline correction dialog box** . After applying the correction using default values, this is the resulting spectrum:



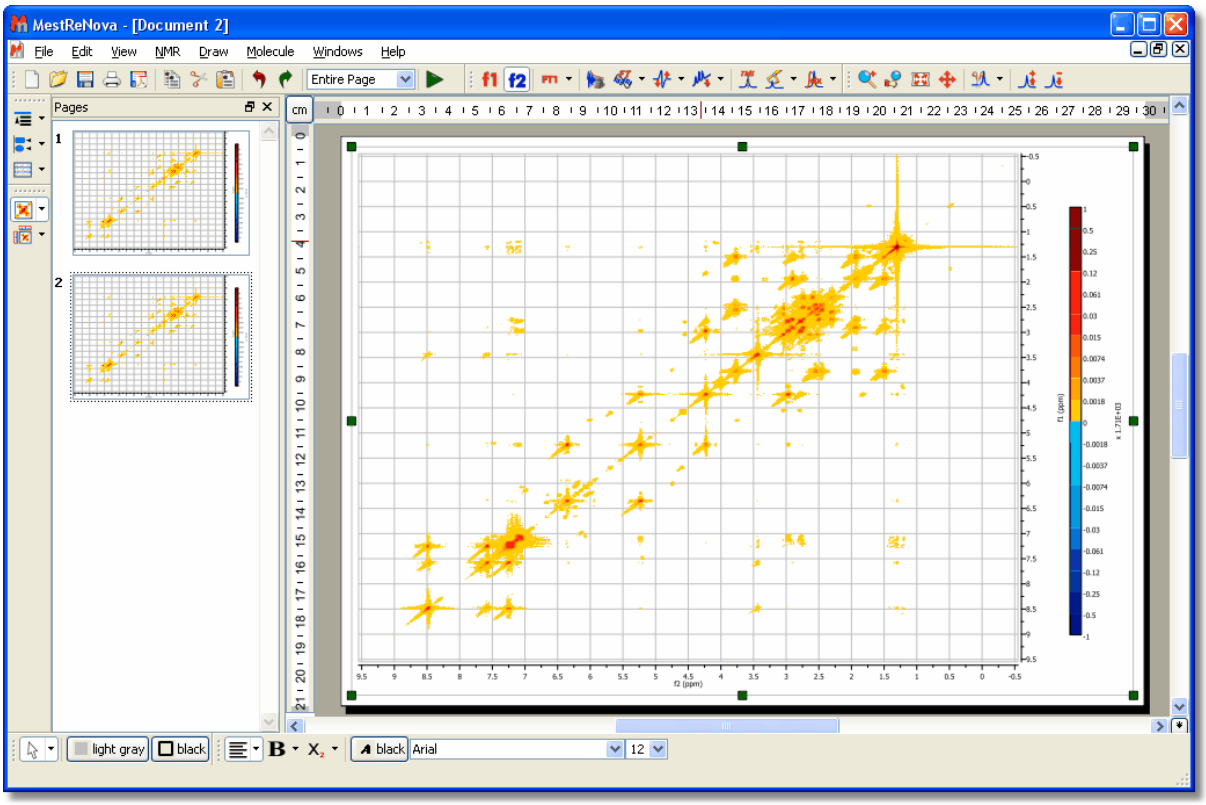
The user can show the traces of the 2D-NMR spectrum, just by clicking on **'Show Traces'** icon  . In the same icon, you will find a scroll bar menu to setup the vertical and horizontal traces of a bidimensional spectrum.

See also 'Show traces in 2D-NMR'

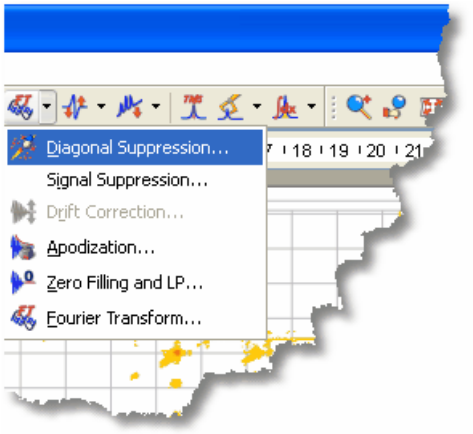
More on real time frequency domain processing

In the 1D NMR Processing section, we described the real-time frequency domain processing concept. **Diagonal Suppression** of homonuclear 2D experiments represents another example of this concept. This technique is typically carried out in the mixed time-frequency domain (interferogram). However, this is something you do not need to know in Mnova. You can suppress the diagonal while the transformed spectrum is on the screen.

For example, the figure below shows a 2D magnitude spectrum:

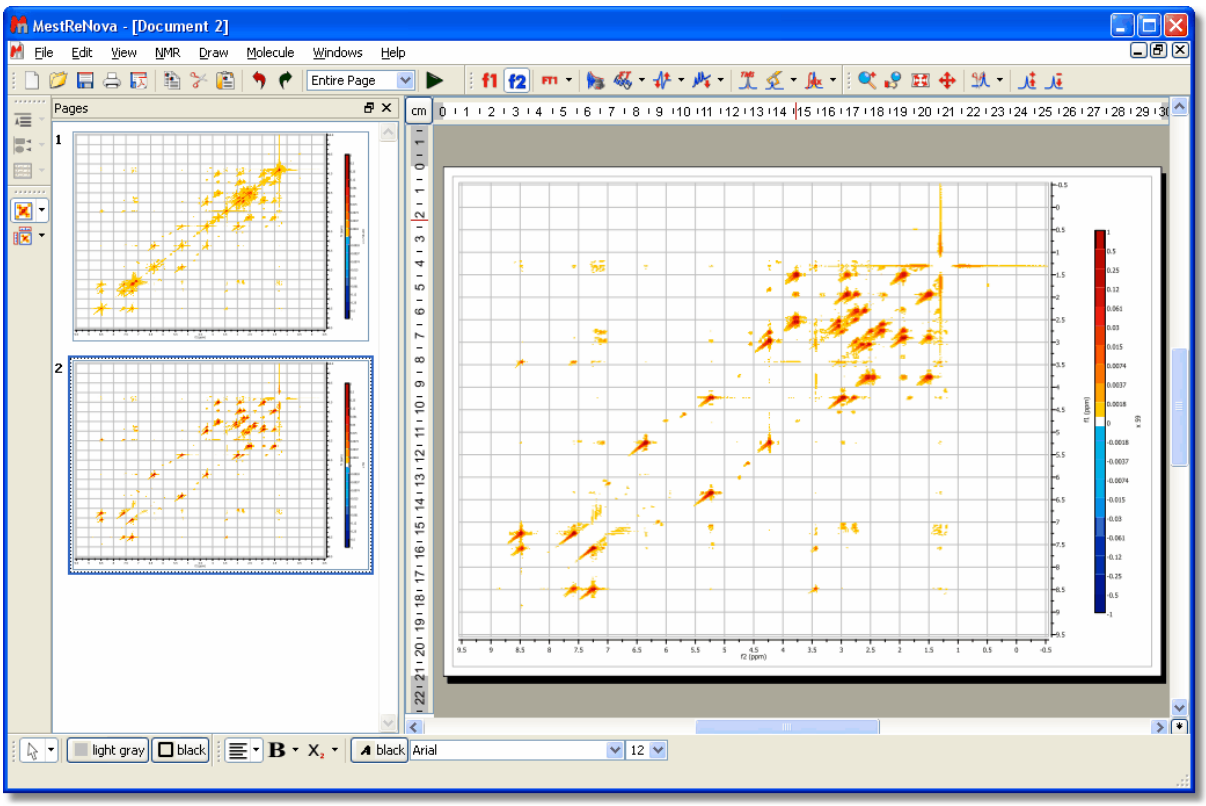


To suppress the diagonal, just click on the small arrow at the right-hand side of the FT toolbar button (see below) and select the **Diagonal Suppression** command:



Mnova provides different algorithms for this task. In this example we will select the Wavelet method

And this is the resulting diagonal-free spectrum.



Note that in the figure above there are two pages, the first one containing the 2D spectrum with its original diagonal, and the second one after the diagonal has been removed.

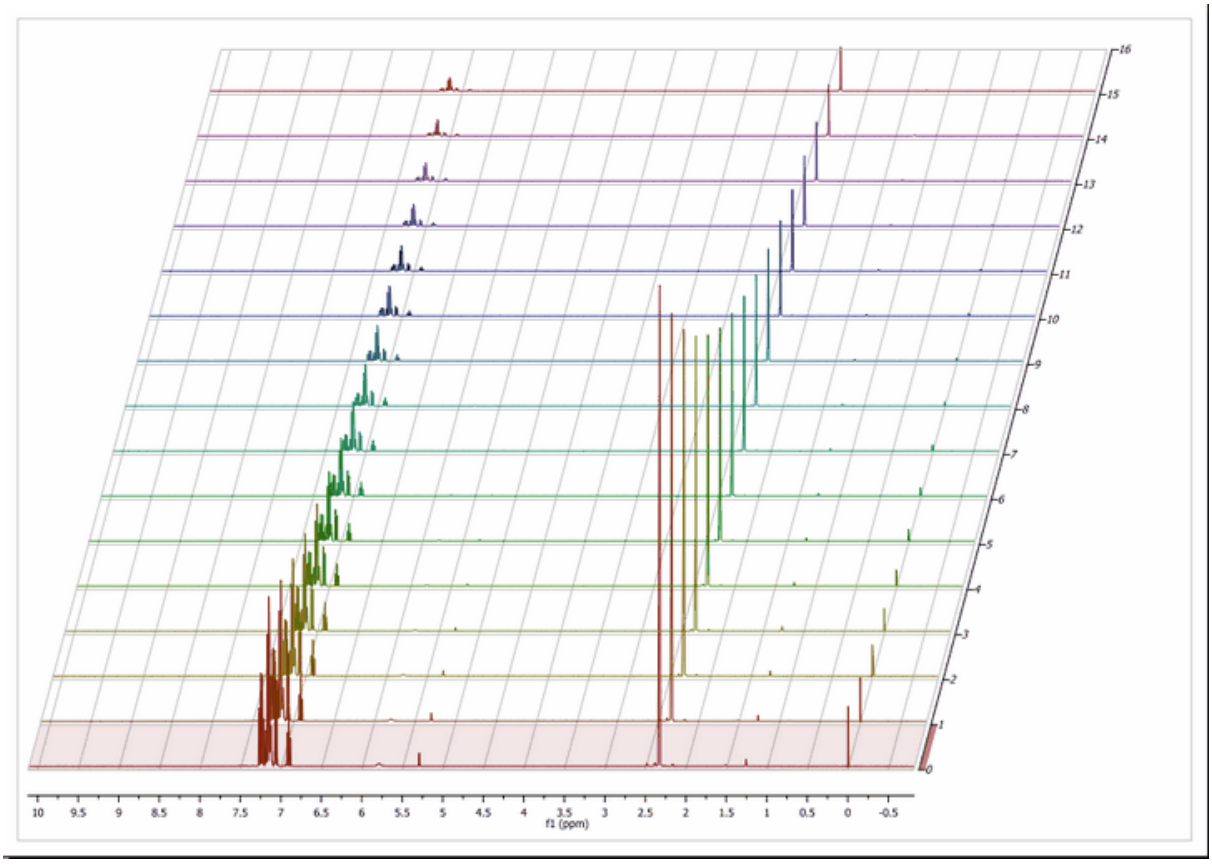
See also

Phase Correction
Baseline Correction

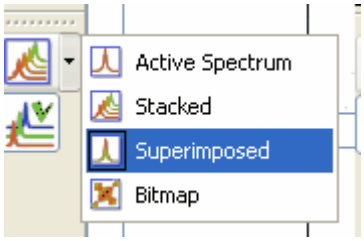
8.3 Stacked NMR Processing

Stacked NMR Processing (Not available in Mnova Lite)**:**

Mnova can detect that spectra have been acquired in the so-called arrayed-mode (or pseudo 2D), typically used in relaxation, kinetics or diffusion experiments. In all these cases, Mnova will only process the acquired dimension (t2). Basically, Mnova will process only the rows of the data matrix and, by default, will display the spectrum as a stacked plot (see below).



The user can select the mode of the stacked NMR by clicking on the scroll menu of the **'Stacked NMR'** icon:



The user can also apply changes only to one slice rather than "all together" (which is the case by default) by simply clicking on the corresponding **'Active Spectrum'** icon which will turn into (to indicate that the processing is only being carried out on the active spectrum). To navigate through the traces, press and hold down the 'SHIFT key' and use the up or down arrow key (or the mouse scroll wheel).

Creating a stacked (or pseudo 2D) spectrum with Mnova is very easy. Just select the desired spectra in the page navigator (by holding down 'Ctrl key' while clicking on each spectrum) and then issue the command 'Tools/Stack Spectra'.

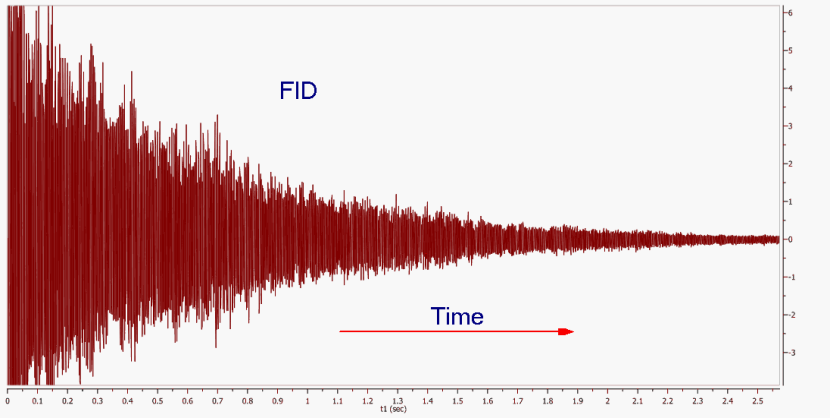
More information about this special mode will be available in the Stacked Spectra Tutorial (at the end of this manual). If you are interested in learning more about it, please contact us at support@mestrec.com.

8.4 Fourier Transform

Basic concepts on FT

Modern **NMR** involves pulse RF energy to excite all frequencies at once. Immediately after the pulse, the signal is detected as a time domain interferogram which contains the sum of all the damped-sinusoid signals emitted by the sample at the various nuclear resonance frequencies.

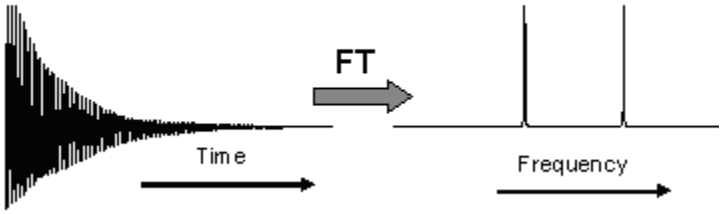
The signal will decay with time as various relaxation mechanisms either dephase the magnetization in the X-Y plane or return the magnetization to the Z axis. The resulting interferogram is called the *free induction decay* (FID, see figure below)



If you pay close attention to the FID shown in the figure above you can appreciate that it is composed of several frequencies. Moreover, if you were able to count the number of peaks and valleys in a given period of time (e.g. 1 second), you might be able to measure the periods and calculate the frequencies of some of the signals composing the FID.

In 'real' life, you don't need to do this conversion by hand. Fortunately, **Jean-Baptiste Joseph Fourier** suggested, while working a study of heat flow, that any function of a variable, can be represented by a sum of multiples of sinusoidal harmonics of that variable. This work was later developed into the Fourier Series.

Fourier's proposals led, in part, to the development of a linear mapping operator which maps a given function to other functions and in our particular case, functions of time to functions of frequency. The operator was named the Fourier Transform in Fourier's honour. In short (math details are beyond the scope of these documents), the Fourier Transform or FT is the mathematical process that converts the time domain function (the **FID**) into a frequency domain function (the spectrum) as illustrated below:



Thus, FT is a mathematical procedure which generates the spectrum from the FID. It searches through the FID for frequency information and allows a plot of signal intensity versus frequency to be generated. When the frequency information is well defined, the peaks will be sharp, but if the information is not precise, the peaks will be broader.

The Continuous Fourier Transform nitty-gritty involves the equation:

$$A(w) = \int_{-\infty}^{+\infty} A(t)[\cos(wt) - i\sin(wt)]dt$$

where $A(w)$ is the intensity of the signal as a function of frequency, $A(t)$ is signal intensity of the FID as a function of time and i is $(-1)^{1/2}$.

Separating the real from the imaginary part, we have:

$$A(w) = \int_{-\infty}^{+\infty} A(t)\cos(wt)dt - i\int_{-\infty}^{+\infty} A(t)\sin(wt)dt$$

The real part can be taken as the signal component which starts off in phase with the receiver reference oscillator, while the imaginary part is that which starts off 90 degrees out of phase with the receiver reference.

The original Fourier Transform is defined for functions of a continuous variable: To meet the needs of pulse NMR where the FID is sampled at short but discreet intervals, the so called **Discrete Fourier Transform** (**DFT**) is used. When the signal strength has been measured with discrete time-interval sampling, the Fourier integral is replaced by:

$$x(n) = \frac{1}{N}\sum_{n=0}^{N-1}\left[A(n)\frac{\cos(2\pi kn)}{N} - iA(n)\frac{\sin(2\pi kn)}{N}\right]$$

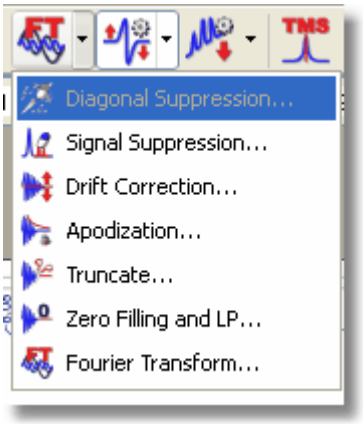
where N is the number of samples, n is the n^{th} sample and $2\pi k$ is a multiplier which converts point n, into the value corresponding to wt (in rad/s). If we have a collection (named A) of N signal points as a function of time, the calculation of the corresponding arrays of real and imaginary frequency points is quite straightforward and can be easily carried out with a computer.

The calculations with many points are obviously slow (though with current computers, they are extremely fast), but the more points, the smoother the plots will be. In order to reduce the computation time we can use the **Cooley-Tukey fast-Fourier algorithm** (**FFT**), reducing the number of

calculations from *N*N* for the **DFT** to $N log_2 N$ for the **FFT**. If *N* is 1024 samples the reduction is from 1048576 to 7098, a 93% time reduction. This algorithm has the special restriction that *N* must be a power of 2. (In cases where the number of points is not a power of 2, Mnova will extend the size of the FID by adding zeros to the next higher power of 2).

The Cooley-Tukey algorithm for the **FFT** achieves this stunning reduction in computation time in part by a process called "bit reversal" which avoids repetitions of internal vector component calculations. The algorithm also saves time by recognizing the orthogonality of certain calculations, allowing the one vector to be calculated from another just by changing an algebraic sign, or reversing sine and cosine terms. This process is an example of the general technique of *divide and conquer* algorithms apparent in many traditional implementations, in which the FT is divided in other more and more small FTs.

Although, as explained in the items dedicated to 1D and 2D NMR Processing, all processing parameters are applied by default by Mnova, prior to Fourier Transform and for Fourier Transform, the software also incorporates a significant number of algorithms which can be applied by the user to try to optimize the results obtained. Thus, on the scroll menu corresponding to the '**FT**' icon the user can find the following options to apply:



FT and Mnova

At first sight it might seem that a plain FFT would suffice to obtain the desired spectrum. However, this is not so in many cases. Although Mnova will adjust the number of samples by zero-filling to obtain a power of 2, the user may elect to increase the zero filling further to double the acquisition power of 2 value to obtain optimum 'frequency' resolution, or even further to improve the 'digital' resolution.

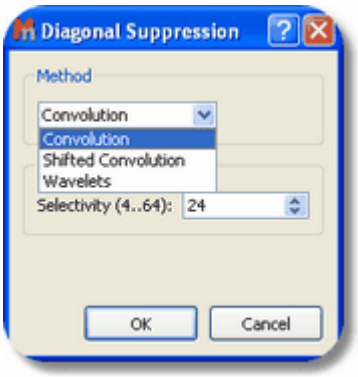
Other operations closely related to the FFT algorithm include:

- Quadrature Detection
- Drift Correction
- Digital Filtering
- Phase-sensitive Protocols

8.4.1 Diagonal Suppresion

Diagonal Suppression (Not available in Mnova Lite)**:**

This option offers a couple of well known traditional algorithms (Convolution and Shifted Convolution) as well as a new, in-house developed algorithm based on Wavelets to remove the diagonal from homonuclear spectra. Once you select the 'diagonal suppression' on the FT scroll menu, a dialog box like this will open:

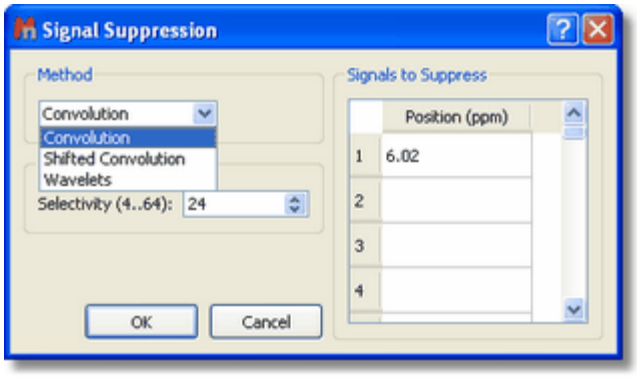


As you can see, you can select the desired algorithm and also the selectivity of the diagonal supression. In the case where you choose the in-house algorithm based on Wavelets, you will be able to set the 'Dyadic Levels' and the 'Daubechies Coefficients'.

8.4.2 Signal Suppresion

Signal Suppression (Not available in Mnova Lite)**:** Strong solvent resonances with solutes of low concentration can result in a lot of problems relating to spectrum quality. One of the primary challenges in obtaining High resolution NMR spectra of biological samples is the suppression of the water resonance to prevent it from overwhelming the signals of interest.

This option is intended to suppress the solvent signal but can also be used to remove any other signal from the spectrum. To suppress the signal, you can select the method (algorithm) of the suppression and the selectivity (or width of the filter; a higher value of selectivity will suppress a narrower band). You can choose the signal, or signals, by double clicking on the corresponding table titled 'Signals to Suppress'.



Utilities:

The 'Signal Suppression' tool is extremely useful when working with proteins, in which spectra are usually measured at very low concentrations in aqueous solutions.

8.4.3 Apodization

Apodization (or Weighting or Windowing): literally, apodization means *'cutting off the feet'* in the original Greek. In this case, the feet are the *leakage or wiggles* which appear when the signal decays rapidly to zero producing an abrupt truncation of the FID, as can happen for example when zero filling. Apodization is a very useful approach to enhance the S/N ratio (sensitivity) or the resolution, or even to remove truncation artefacts, after data has been collected.

The most commonly used apodization function is a decaying single-exponential curve. The magnitude of the decay constant determines how quickly the curve decays. In a regular FID, the resonances due to the sample are found in the earlier parts of the signal. When you multiply the FID by a decaying-exponential function, you favour the early parts of the time domain and attenuate the data at the end of the acquisition period, which is essentially all noise. A single exponential apodization function does not significantly affect the signal at short times, but it greatly reduces the noise at later times; at the expense of an increase in linewidth, that is to say, a decrease in the resolution. On the other hand, if you use an *increasing exponential curve* as apodization, you will obtain finer lines (increased resolution) but amplifying the noise (decreased sensitivity).

Utilities:

Windowing is particularly useful in ^{13}C-NMR spectra and extremely important in multidimensional-NMR to increase the signal/noise ratio or to improve the resolution.

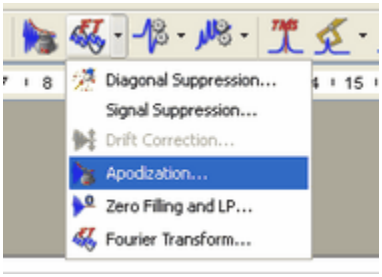
The Window function is applied by multiplying the data vector d, element by element, by a *vector* a. Thus element k of vector d is multiplied by element k of vector a to yield element k of vector d'.

$$d'_k = a_k d_k$$

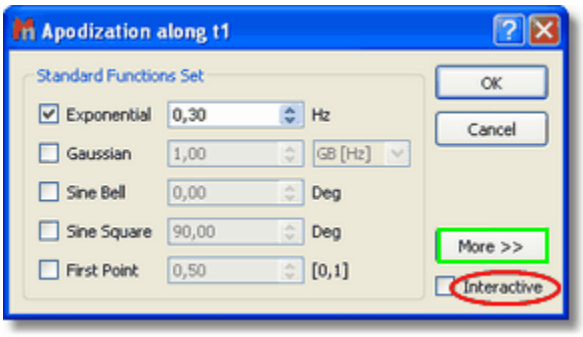
The challenge lies in finding a vector a, such that the noise in the tail of the FID is attenuated without excessive line broadening or, in the case of apodization, to smooth the truncated data exponentially towards zero, again without excessive line broadening. Theory shows that this is attained when the decay of vector a matches the decay of vector d; in this case we have what is known as a *matched filter*. Mnova makes this particularly easy by allowing the final spectrum to be observed while the decay parameter of the window function is adjusted.

Mnova incorporates many window functions (Exponential, Gaussian, Sine Bell, Sine Bell Square, TRAF, Trapezoidal, Parabolic, Hanning, Convolution Difference and Linear Ramp), which can be combined and can be used interactively (while viewing the FID as well as the resulting spectrum) to improve the sensitivity or the resolution. Unfortunately, it is very difficult to find a function which improves sensitivity and resolution simultaneously (an exception could be the Traficante function, as we will see later).

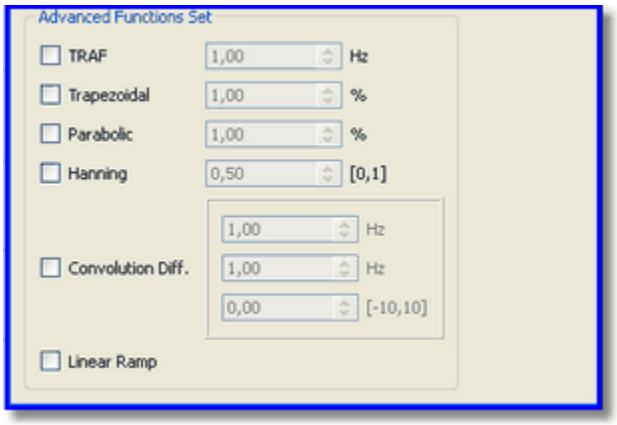
You can find this tool on the FT scroll menu on the toolbar or by following the menu 'Processing/Apodization':



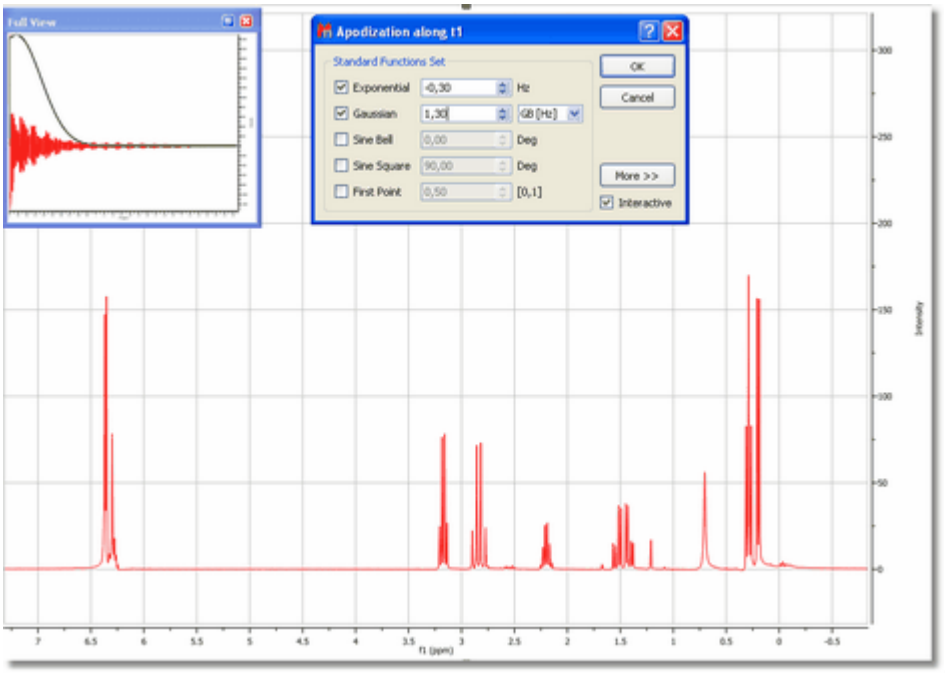
Once you have selected **'Apodization'**, a window like this will open:



In this dialog box you will find the type of window function which Mnova has applied prior to FT, as well as other Standard Functions (Gaussian, Sine Bell, Sine Square and First Point). If you click on 'More >>' (in the green square in the figure above), you can set other Advanced Functions, as shown below:



In order to assist in the process of finding the most suitable window function and its parameters, Mnova allows the user to interactively adjust the parameters of window functions, and to view the effect directly on the frequency spectrum. For this purpose, make sure the 'Interactive' box is ticked (highlighted in red in the figure), you can then follow the exact effect of the function you are applying on the frequency domain spectrum (or on the FID, if you are on the FID' view) by using the buttons  to change the desired parameter. If you have activated the 'Full View' window you will be able to see the window function displayed along with the FID.



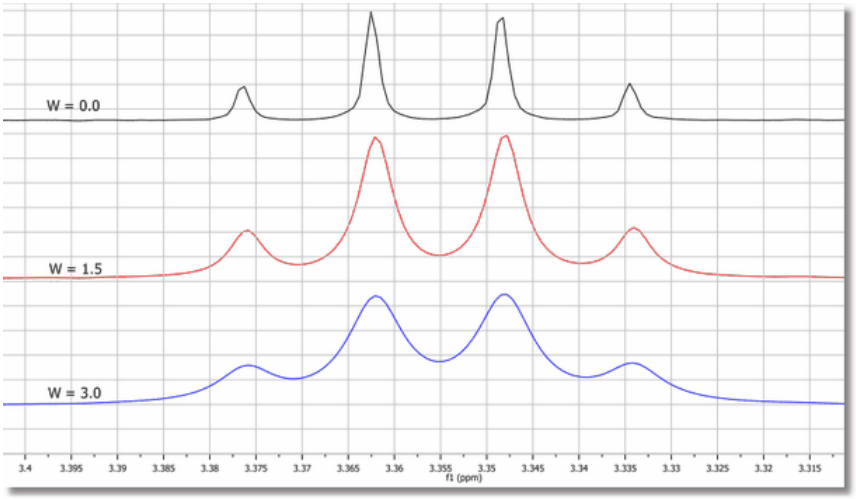
We will try to explain in a brief and easy way the applications and the effects of these functions:

Exponential: A exponential apodization function is defined as:

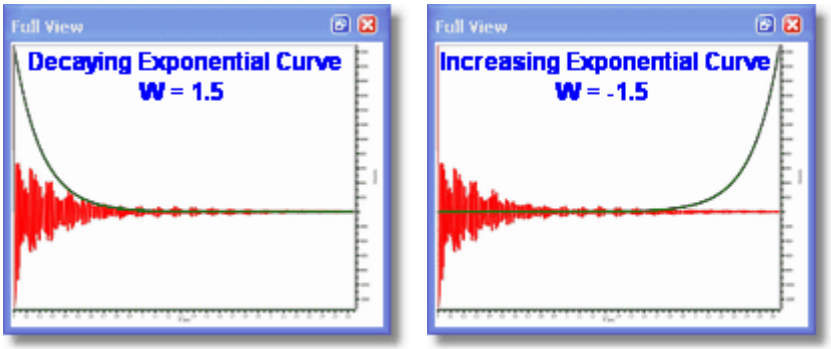
$$a_k = e^{-\pi W k \Delta t}$$

where *W* (the parameter to adjust) represents the the line broadening factor expressed in Hz. So, if the original width of the signal is '*L*', the final width of the signal after applying this window function would be *'L+W*'. If *W* is positive we will have a *decaying exponential curve* (and we are giving more weight to the points at the beginning of the FID and suppressing the data at the end of the time series, which is essentially all noise). As we explained previously, this will increase the **'sensitivity'** (Signal to Noise Ratio) of the spectrum at the expense of an increase in linewidth (decreased resolution).

The figure below shows the effect of *W* on the linewidth. The black trace has no window function applied. The red trace shows a taller signal due to application of an **Exponential Function** and also an increase of the Signal to Noise Ratio (matched filter), while the blue trace shows that if the **Exponential Function** is too severe the broadening of the lines reduces the signal size again.



If *W* is negative, we will have an *increasing exponential curve*, thus obtaining the opposite effect (increased **resolution**, with finer lines, and a decrease in sensitivity, i.e., a lower S/N ratio). You can see below the graphical difference between an *increasing and a decaying exponential curve* over the FID (shown in the 'Full View' window). Note that in this case an *increasing exponential curve* will not be a good choice (it is normally used in combination with other functions, such as Gaussian).



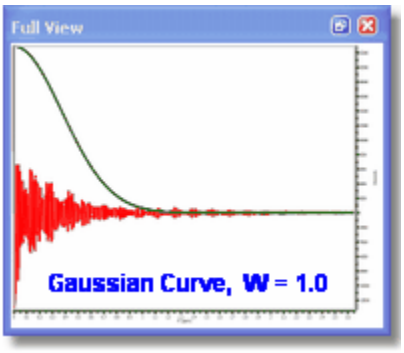
In most of the literature this parameter is symbolized by LB (*Line Broadening*).

Gaussian: In Gaussian window function resembles the Gaussian Probability Distribution function used in statistical analysis. The gaussian function rises quasi exponentially to its single maximum and then falls back to zero in the same manner and so may be regarded as almost two back-to-back exponentials.

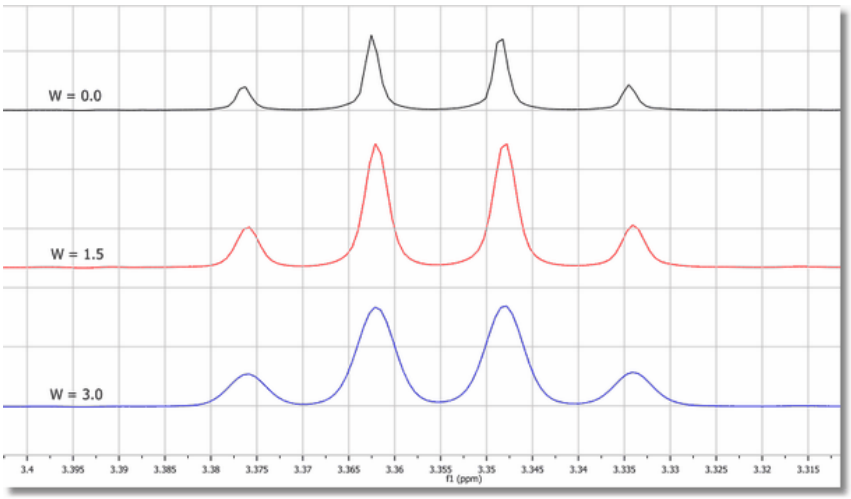
In Gaussian apodization, each data point of the FID is multiplied by the vector:

$$a_k = e^{-W(k\Delta t)^2}$$

where *W* is the same widening factor used in exponential apodization. This function is similar to the exponential but decays comparatively slowly at the beginning of the FID and then quite rapidly at the end. For this reason, it causes less line broadening than the exponential multiplication.



In the next picture, you can see the influence of *W* over the linewidth:



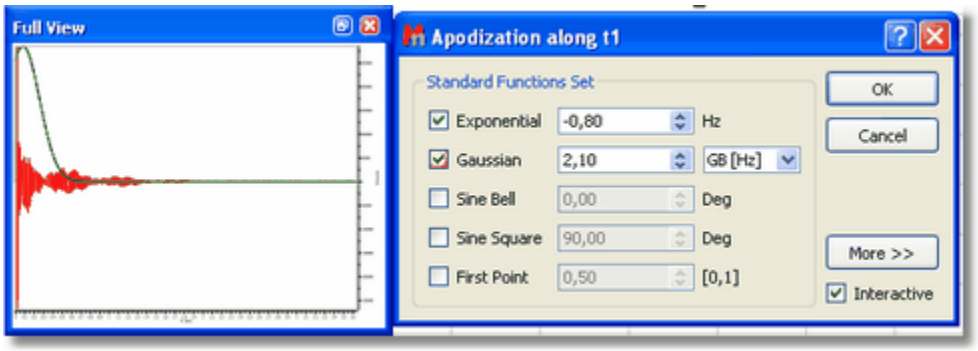
Gaussian plus Exponential: This function is also known as Lorentz-to-Gauss Transformation and is not directly implemented in Mnova, but you can apply it by ticking both exponential and Gaussian multiplication functions and introducing a negative value for the exponential line broadening (*W*) and a positive value for the Gaussian parameter (GB). The spectrum can be optimized for resolution by suitably adjusting these parameters.

Thus, this weighting function combines a rising exponential function with a decreasing Gaussian function; then, the resulting vector will be:

$$a_k = e^{\pi W k \Delta t} \, e^{W(k\Delta t)}$$

To perform Exponential/Gaussian apodization with Mnova, select the Apodization option in the FT scroll menu on the toolbar, then click on the Exponential as well as on the Gaussian resolution enhancement option and finally enter the appropriate values of W (or LB) and GB and click OK.

Usually, a process of trial and error is adopted with the parameters being adjusted until the best result is obtained. You can follow (by ticking on the 'Interactive' box) the exact effect of the function you are applying on the frequency domain spectrum or on the FID window (shown graphically in the 'Full View' window), as you can see in the screenshot below.



Sine Bell (Not available in Mnova Lite)**:** This function is very useful in those NMR experiments where the signals of interest are not maximal at the beginning of the FID and very convenient for magnitude spectra because it reduces the dispersive part of the line shape.
In COSY experiments the cross peaks are sine modulated while the diagonal peaks are cosine modulated. Sine bell windowing thus emphasizes the cross peaks and attenuates the diagonal signals. Unfortunately due to differing relaxation times for some signals possibly not all cross-peaks will be maximal at the same time; in this case the sine-bell may be shifted to yield the best compromise spectrum. The unshifted sine-bell also removes dispersive signals from the absolute value COSY, providing the sought for absorption line shape.

Utilities:

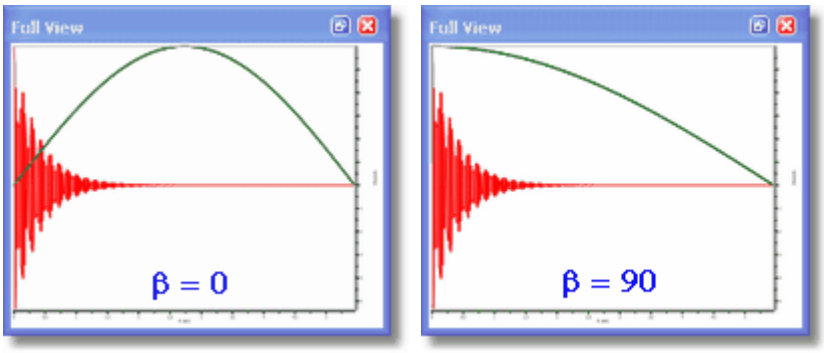
The **Sine Bell** window function is very useful for **COSY** experiments because it emphasizes the sine-modulated cross peaks compared with cosine-modulated diagonal peaks.

Avoid using this function with 1D spectra since it decrease the spectral intensity. If used with phase sensitive experiments the sine bell can result in signals with negative side lobes and near zero integrals.

This function consists in the multiplication of each data point of the FID by the next vector:

$$d_k = sin\left(\beta + \frac{(\pi - \beta)t}{t_{acq}}\right)$$

Mnova requires you to enter the β parameter, whose input is expressed in sessagesimal degrees. When $\beta = 0$; the equivalent function is a sine-bell, while when $\beta = 90$ the equivalent function is a cosine-bell. In the pictures below you can see the representations of the sine bell functions resulting from different values for β:

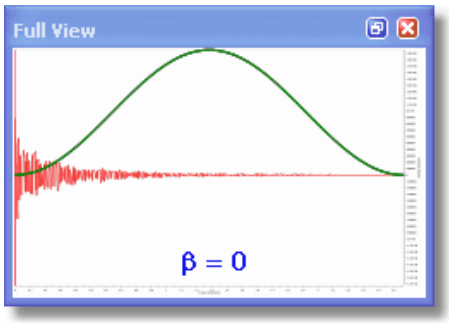


The cosine-bell apodization (β = 90) is very convenient in 2D-HSQC experiments:

Utilities:

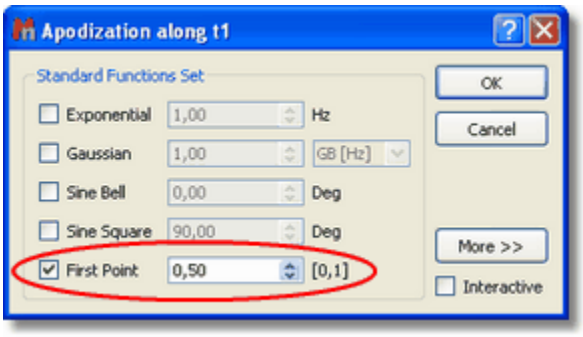
The **cosine-bell** weighting function (β = 90) is very convenient in 2D-HSQC experiments.

Sine Square (Not available in Mnova Lite)**:** The shape of the Sine Bell weighting function is altered by squaring it, resulting on the **Sine Bell Squared** function, which is more concentrated over the maximum. It is very useful to use a 90° shifted sine-bell squared to apodize 2D phase-sensitive experiments.



First Point Correction: There is an important difference between the *discreet* FT and the *continuous* FT. The *discreet* FT generates a constant vertical displacement of the baseline, which can be due to a distortion of the first point of the FID. It treats the FID as a function which is repeated periodically, thus the ordinate at t=0 would represent the algebraic average of the first and the last point. In most FIDs, the value of the last point is very close to zero, and thus the first real point would have to be divided by two, before introducing it in the FT algorithm. It is often necessary to multiply the first point of the FID by 0.5 before FT in order to ensure that the value of the integral over the spectrum is equal to the value of the first point of the FID.

You can alter this multiplication parameter in the range of 0 to 1 (it should be 0.5 by default, as you can see in the FT dialog box below).



The functions which follow are accessed via the [More >>] button in the Apodization dialog box.

Traficante (Not available in Mnova Lite)**:** The Exponential Multiplication Gaussian and the combination of both can improve spectral resolution at the expense of some loss in SNR. The Traficante window function endeavours to improve resolution without noticeable loss in SNR. Traficante enhances the middle part of the FID and then uses a matched filter to attenuate noise in the latter part of the time-series. The process involves the division of the FID by the sum of the squares of the FID and its reverse. If there are N data points, the k^{th} point in the original FID is point k while the corresponding point in the reversed FID is point (N-k). As the resulting FID is symmetrical (with respect to the midpoint), we can multiply it by a function to obtain a new desired FID which does not decrease.

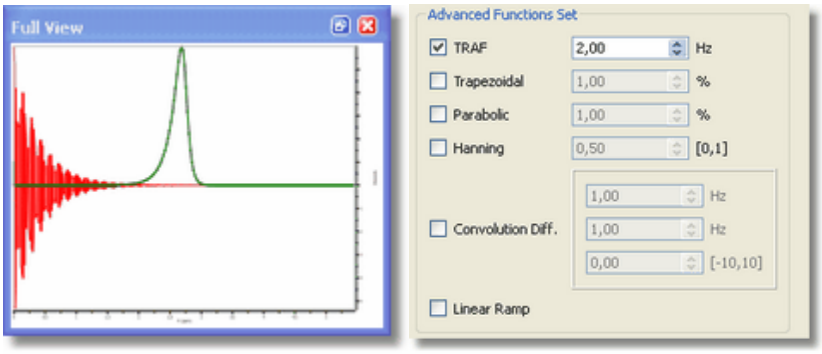
So, if we named the original FID *E*, and the reversed FID ε, we would get:

$$E \cdot g(t) + \varepsilon \cdot h(t) = 1$$

And Traficante apodization will consist of the multiplication of the FID by the following vector:

$$a_k = \frac{e^{-\pi w k \Delta t}}{(e^{-\pi W k \Delta t})^2 + (e^{-\pi W (N-k) \Delta t})^2}$$

In the picture below, we can see a Traficante function represented (with $W = 2$) applied to the FID



IMPORTANT:

Traficante apodization will be only useful if the FID decays exponentially and, moreover, the value of W must be chosen with care.

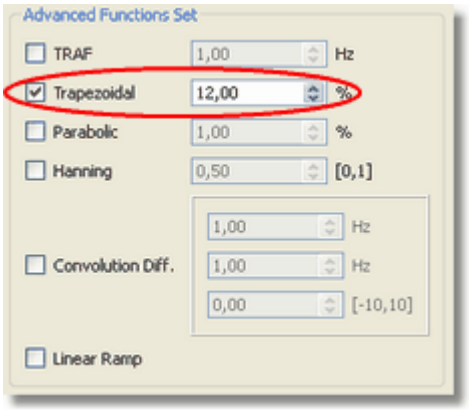
Trapezoidal (Not available in Mnova Lite)**:** This function multiplies the FID by a trapezoidal shaped function;

$$f(t) = 1 \cdots\cdots \text{when } t \le k$$

$$f(t) = 1 - \frac{t-k}{T-k} \cdots\cdots \text{when } \; t > \text{k}$$

where T is the acquisition time and *k* is the percentage of the total length of data

This option is useful to avoid the "sinc" artifacts resulting from truncation of the FID. You can select the percentage of the total length of data between 0% and 100% (*k*), in the advanced functions of the FT menu:



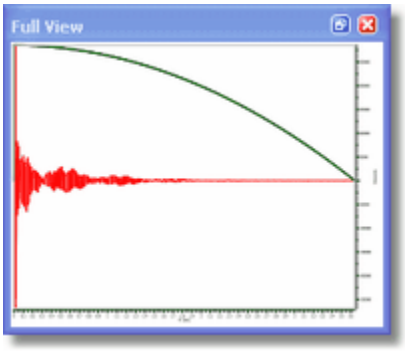
Parabolic (Not available in Mnova Lite)**:** This function multiplies the FID by a parabola shaped function, where

$$f(t) = 1 \cdots\cdots \text{when } \; t < b$$

$$f(t) = 1 - \frac{t-b}{[t(\max) - b]^2} \cdots\cdots \text{when } \; t > b$$

You can also set the length of the parabola by selecting (on the FT scroll menu) Apodization, click "More", select parabolic and then choose the desired value for the parameter (*b*, expressed as the

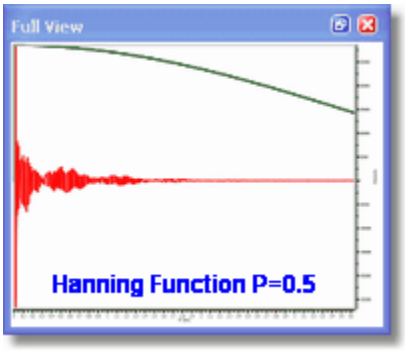
total length of data). Again, you can see the exact effect of the function you are applying on the frequency domain spectrum or the effect on the the FID by (pre-)selecting the Full View" window:



Hanning (Not available in Mnova Lite)**:** Mnova adopts the following generalized equation:

$$f(t) = p + (1-p)\cos\left(\frac{\pi t}{2T}\right)$$

where *T* is the acquisition time, and P can be a value from 0 to 1. You can set this parameter on the advanced Functions set of the FT dialog box. If ***P = 0.5*** we will have a Hanning function and if ***P = 0.54*** we will apply a Hamming function, producing 10 and 20% line broadening respectively. The Hanning window, more correctly called the Hann window and the Hamming window are part of a family of functions known as "Raised Cosines" and also includes the Gaussian Window. These functions, like a cosine bell, have a maximum value; Mnova generally implements these functions with their maxima at zero time. The Hanning Function is sometimes used to reduce aliasing. Aliasing arises when signals are insufficiently sampled (violating the Nyquist Criterion), resulting in spectral folding. It results in peaks appearing at incorrect positions and generally not phasing along with the authentic signals. This arises due to incorrect experimental set up (signals beyond the high frequency end of the spectral width), and the ability to correct for it after acquisition is useful indeed.



Convolution Difference (Not available in Mnova Lite)**:** This weighting function multiplies the FID by :

$$W(t) = 1 - e^{\left(-0.5t\pi\frac{LB}{SW}\right)}$$

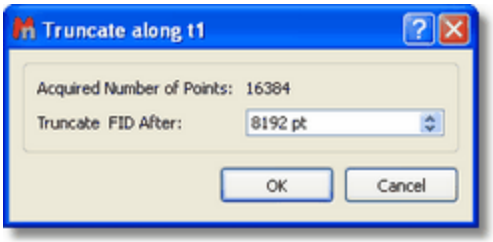
where *LB* is the line broadening and *SW* is the spectral width in Hz. You can also access this function from the scroll menu of the FT icon where you will be able to set the value of LB.

Linear Ramp (Not available in Mnova Lite)**:** This command is used to multiply the data points of the FID by an increasing linear ramp, resulting in the first derivative of the Fourier transformed spectrum { d(intensity)/d(frequency) }. This emphasizes the faster rising parts of the spectrum at the expense of SNR. If the initial spectrum had an excellent SNR this function can dramatically improve resolution. Mnova will soon offer derivative performed using wavelet processing which produces even better spectra.

This weighting function gives *more weight* to the end of the FID, amplifying the noise (decreasing the sensitivity), and for that reason we recommend its use only in combination with another function which gives more weight to the beginning of the FID (increasing sensitivity). In practical terms, add the ramp function to a spectrum that has already been windowed by, for example the EM function for 1D or the sine-bell function for a 2D in order to improve the selectivity.

8.4.4 Truncation

Truncation: This technique causes part of the time series, from a given time onward to be set to zero. For convenience Mnova truncates the time series after a given number of acquired points. The user may access this function either via the menu Processing/Apodization etc or via the FT icon scroll menu in the same manner.

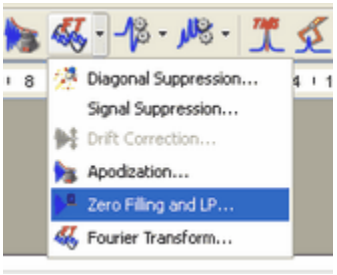


This technique may be used to shorten the acquisition data time series. If the FID, for example, decays to zero in 0.5 seconds and the acquisition time was 2 seconds, the FID consists of 25% signal and 75% noise. Since both 0 and a 90 degrees phase shifted spectra are collected a time series data length of twice the FID time is required for maximum information content. Truncating the FID at about 1.0 second will reduce the amount of storage required with no data loss. At a later time Zero Filling may be used to cosmetically adjust the spectrum's appearance.

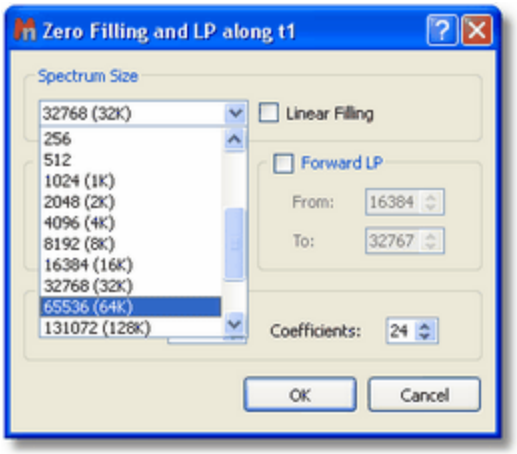
This tool is used when the acquisition time has been set much longer than the FID, resulting in the acquisition of a long period of signal-free noise. Truncation sets the signal free latter region of the time-series to zero. This does NOT amount to zero-filling, and the appending of zero-s to twice the original number of acquisition points will still reveal the intrinsic resolution.

8.4.5 Zero Filling and Linear Prediction

Zero Filling: This option can be used to set the size of the Fourier Transform. It can be used either to increase the digital resolution or to truncate the FID. When the time-domain signal is subject to a FT, the resulting spectrum is represented by a set of data points (by joining the points it is possible to plot the spectrum as a smooth line). As a Fourier transformation preserves the number of data points, if we take the original FID and add an equal number of zeroes to the end of it, the corresponding spectrum will have double the number of points, increasing therefore the 'apparent' digital resolution of the spectrum. The zero filling process is equivalent to a frequency-domain interpolation. This command is available in the **'FT'** icon scroll down menu, as shown below.



Just select the required number of points (only powers of 2 are allowed) and click 'OK':



In some cases, the FID does not fulfil the requirement of the number of data points corresponding to an entire power of 2. In these cases, Zero Filling must be applied to the immediately higher entire power of 2 for FT to be possible. Mnova will automatically carry out this Zero Filling when the number of data points is not an entire power of 2. By default, Mnova proposes a duplication of the original number of FID data points. For example, if the FID has 16384 data points (or a number of data points between 16385 and 32768), the Zero Filling open dialog would show us the value of 32768 (32K) data points, which indicates that Mnova will add up to 16384 data points at the end of the FID.

Linear Prediction (Not available in Mnova Lite)**:** Linear prediction is a mathematical procedure where a missing part of a FID can be constructed in order to increase the digital resolution of the spectrum; it is a tool which examines the free induction decay, extracts a set of coefficients, and extrapolates, either forward or backward, to predict what the data would have been had it been collected. This is a potentially valuable technique for extraction of useful information from marginal data.

Linear prediction is based in this fundamental principle: each value in the time series can be represented as a linear combination of the immediately preceding values. Mathematically, the basic LP equation postulates:

$$d_m = \sum_{p=1}^{p} a_p d_{m-p}, m = p,....., N-1$$

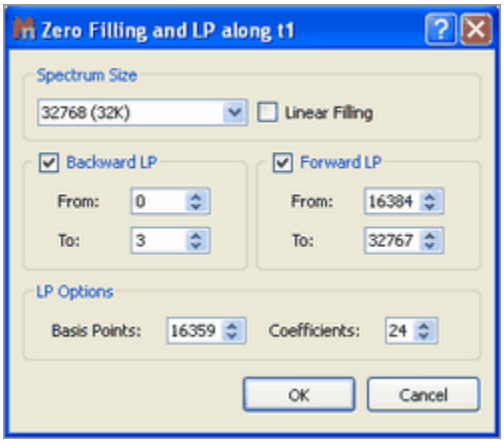
where a_p are the LP Coefficients and the number 'p' corresponds to the order of the prediction. Thus, it is possible to extrapolate the series beyond the point *N*. This equation is known as *Forward Linear Prediction* (extrapolates forward, predicting the new values from the older ones).

Backward Linear Prediction will be represented in the following equation:

$$d_m = \sum_{p=1}^{P} b_p d_{m+p}, m = 0,.....,N-P-1$$

This command is available in the same menu as zero filling. If you need to repair the baseline distortions (due generally to the first points of the FID) you have to tick Backward LP and select the limits; this will predict the first points of the FID.

Forward Linear Prediction is a better alternative to correct the last part of the FID than *zero filling;* this will extrapolate the FID in the direction of the advance, predicting the new data points from the older. If you select 'Linear Filling', a Forward LP from the end of the original FID up to the number of points defined in the 'Spectrum size' combo box, will be executed. You can also set the number of Basis Points and Coefficients of the algorithm that you want to use for Linear Prediction.



Coefficients: number of theoretical sinusoids. The number of the coefficients should be equal to the number of peaks in the FID in an ideal case. By default Mnova sets the number of coefficients according to:

Number Of Coefficients = points in the spectrum/16

Basis Points: they are the number of 'good' experimental points to be entered in the calculations. The number of basis points should be at least twice or three times greater than the number of coefficients.

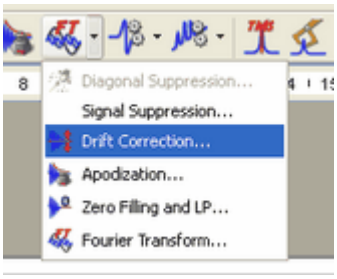
Linear Prediction is extremely useful in 2D NMR as a way of reducing the acquisition time or increasing the quality of already existing data sets. Also, in 1D NMR it is useful when the FID has not decayed to zero before experiment completion. This often happens when working with slowly relaxing nuclei (such as quaternary carbons, in ^{13}C-NMR) and in experiments with a wide ppm area (spectral width).

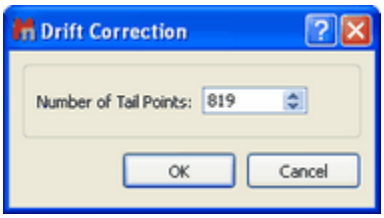
One of the most common causes of the corruption of the first data points of the FID is the security time or *pre-scan delay* (time which exists between the pulse and the beginning of the acquisition) which in many cases may not be enough to allow the detector to recover fully from the effect of the pulse.

8.4.6 Drift Correction

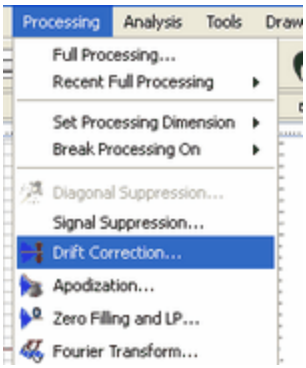
Drift Correction: The DC (Drift Current) component is the zero frequency (F_0) of the spectrum, which is proportional to the sum of all data points in the FID; if F_0 is different to zero; a glitch signal (spurious peak) will appear in the centre of the spectral window. Both real and imaginary parts of the FID should decay to zero at the end of acquisition, but sometimes this "zero" is different and the offset may not be

the same for both channels. Mnova corrects this spurious peak by default prior to Fourier Transform, although you can find and customize this tool (by selecting the number of tail points of the FID to determine the mean DC level) in the open dialog of 'Fourier Transform'; as you can see below:



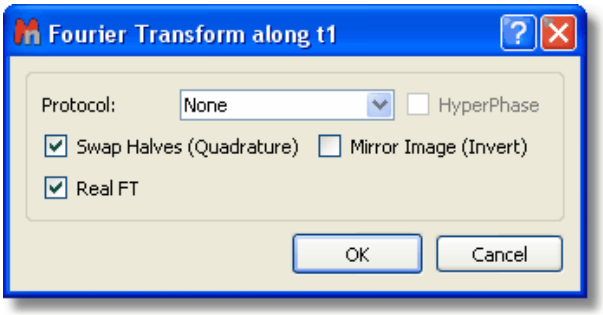


You can also find this option by following the menu item 'Processing/Drift Correction':



8.4.7 The FT Command

Fourier Transform: The FT command transforms the acquired data of the current data set, which may already have been processed using a filter function. Depending on the way a spectrum was recorded (e.g. simultaneous vs sequential sampling, quadrature settings, etc) the FT can be real or complex and some additional pre-processing may be required. All these options can be set in the Fourier Transform dialog box, which will appear by clicking on the '**Fourier Transform'** icon  , by selecting 'Fourier Transform' on the scroll menu shown in the figure above, or by following the main menu item 'Processing/Fourier Transform'.



Protocols: This option corresponds to the data shuffling algorithm required to process phase sensitive 2D spectra

If your spectrum is not phase sensitive, then the correct choice is: **none** protocol.

Phase sensitive: this kind of experiments are used to get higher resolution and more

information out of a 2D dataset. You can obtain better resolution and better S/N ratio by generating quadrature phase detection in the second dimension, but at the expense of longer acquisition times and larger datasets.

Echo-antiecho: You should select this option for phase modulated experiments with gradient pulses (used for coherence pathway selection), where pairs of slices are added and subtracted to generate complex "fids" in the indirect dimension. Each slice contains both sine and cosine terms in t_1. This means that the data must be handled differently from hypercomplex data, in which odd numbered slices contain only cosine t_1 terms, and even numbered slices contain only sin t_1 terms.

Hyperphase: this option can be chosen in conjunction with 'phase-sensitive' or 'echo-antiecho'. When 'Hyperphase' is ticked, the imaginary part is not deleted and phase correction is performed first along f1 and then along f2 (at the expense of doubling processing time and RAM requirements, because you keep the imaginary part of the spectrum). If you do not tick this option, the protocol will perform a reduction of the data points (deleting the imaginary part) and you can phase correct along f1 but no more along f2. If you do not plan to do a phase correction, there is no need to select this option.

Swap Halves (Quadrature): the negative frequencies are distinguished from the positive ones by the detection and summation of the in-phase and 90º out-of-phase magnetizations (two-channel mode).

Mirror Image (Invert): this command is used to generate a mirror image of both the real and the imaginary parts of the spectrum, with respect to a vertical line positioned in the middle of the spectrum; i.e., the spectrum is reversed.

Real FT: it allows to execute a real Fourier Transform as opposed to the conventional Discreet Fourier Transform (normally, this is only necessary for Bruker spectra acquired by sequential mode). Modern spectrometers acquire spectra in a way which avoids the use of this option.

8.5 Phase Correction with Mnova

NMR spectra are usually represented in absorption mode, which offers a greater resolution and proportionality between the integral of peaks and the number of nuclei which cause the signal, in comparison with other representations such as dispersion mode, magnitude or power of the spectrum. Nevertheless, the absorption and dispersion curves are often mixed, giving rise to curves which lose the original symmetry. This problem, combined with other deficiencies, such as delays between the initial pulse and the beginning of data acquisition, cause a phase shift which varies linearly with frequency. This phase errors can be easily rectified in software by applying a lineal combination of the real and imaginary components of the spectrum in order to obtain pure phase lineshapes.

This correction is composed by a *frequency independent* parameter, or zero phase correction (α) and a first order phase correction (β) or *linear dependent* on the frequency parameter:

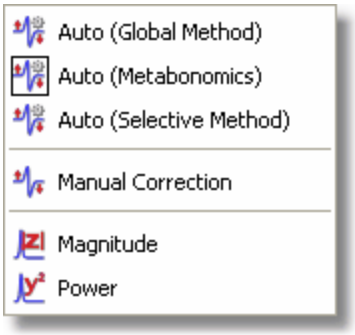
$$\mathrm{Re'}_k = \mathrm{Re}_k \cos(\alpha + \frac{k}{N}\beta) - \mathrm{Im}_k \sin(\alpha + \frac{k}{N}\beta)$$

$$\mathrm{Im'}_k = \mathrm{Re}_k \sin(\alpha + \frac{k}{N}\beta) + \mathrm{Im}_k \cos(\alpha + \frac{k}{N}\beta)$$

where k = 0,.....,N-1; Re_k and Im_k are the real and imaginary components of the data points (k), $\mathrm{Re'}_k$ and $\mathrm{Im'}_k$ are the new components after correction and N is the total number of points.

Mnova implements both manual and automatic **Phase Correction algorithms.**

The Phase Correction scroll menu is located on the arrow immediately to the right of the **'Phase Correction'** icon.



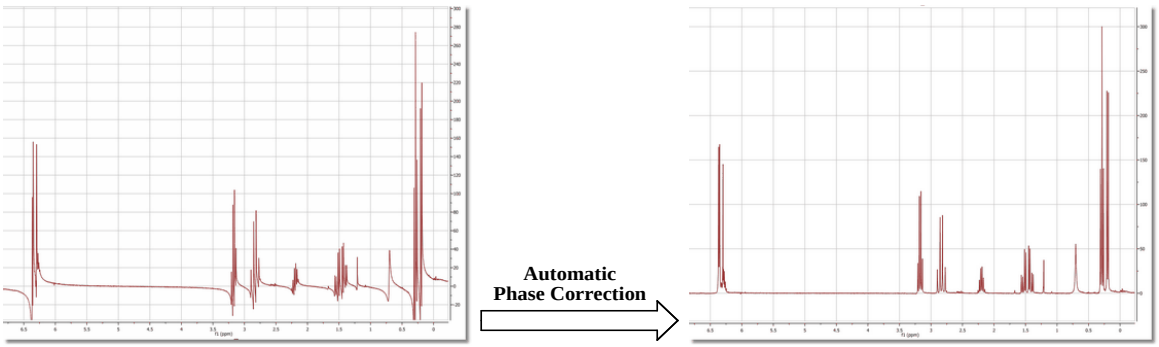
The software implements two algorithms for automatic phase correction, which can be applied both to 1D or to 2D data sets, and a manual algorithm, which allows for very easy, eye driven, real time zero order and first order phase correction. Once the user selects a Phase Correction method, Mnova will store it for future uses, highlighted with a black square.

Automatic Phase Correction

Mnova gives the user the choice between two auto-phasing algorithms for higher flexibility.

Global method: This method automatically finds phasing parameters (α and β or PH0 and PH1) using an iterative process in which the intensity of the lowest point in the spectrum is optimized. This method is not appropriate for experiments like DEPT or APT, where we can find both positive and negative peaks, but is highly efficient for most ^{1}H spectra. However, it is sensitive to bad baselines and low Signal/Noise ratios. Mnova will automatically execute this Phase Correction.

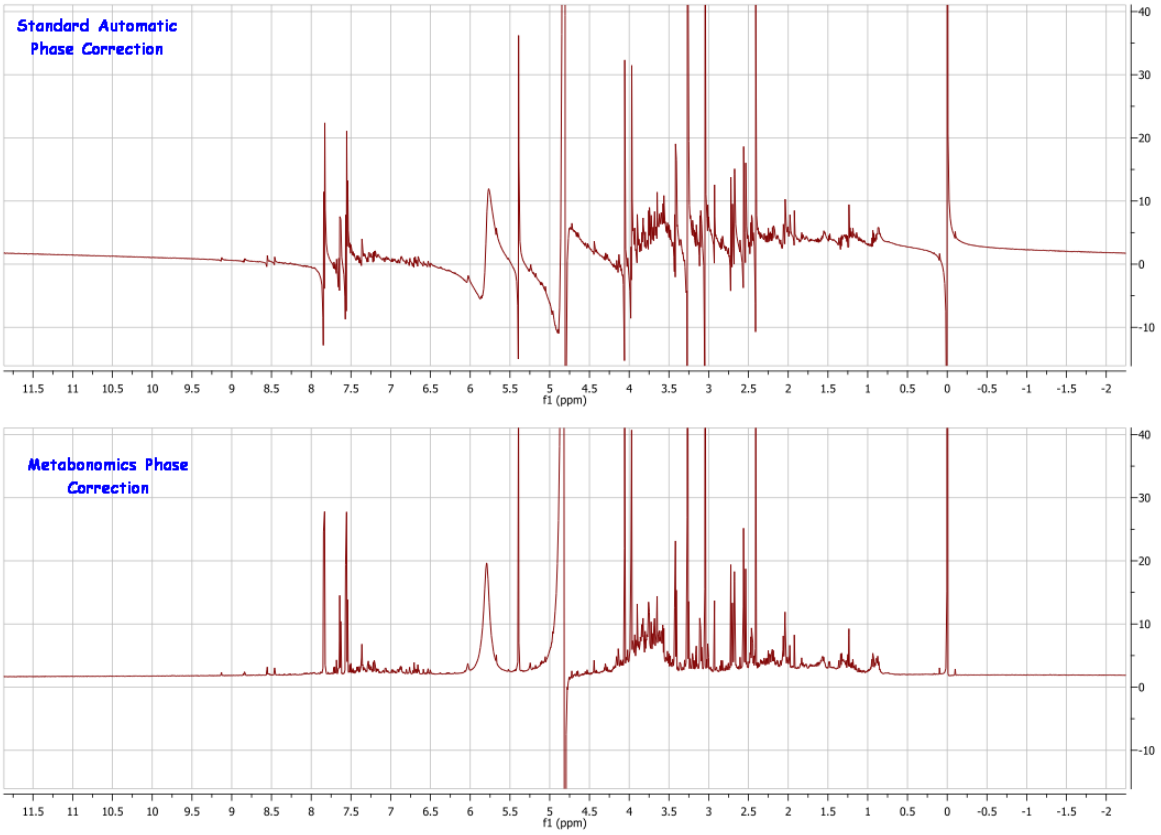
You can see below the effect of an automatic phase correction on a ^{1}H-NMR spectrum with a very bad phase:



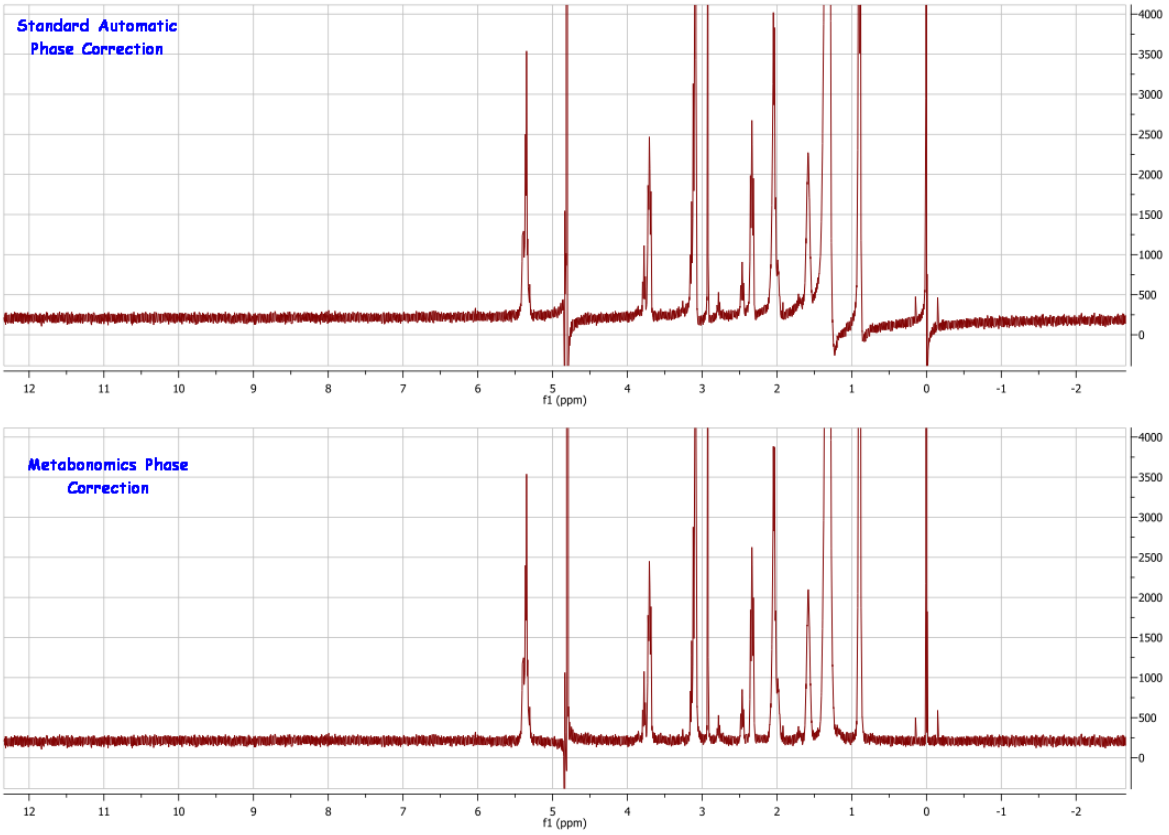
Metabonomics. A new Automatic phase correction algorithm optimized for biofluid-like spectra (Not available in Mnova Lite).

This algorithm is an extension of the standard automatic phase correction algorithm optimized to deal with Metabonomics-like spectra in which most of the signals are concentrated towards the center of the spectral width. In addition, it can also efficiently deal with situations in which a dispersive peak is present in the center of the spectrum, as is usually the case with water in urine samples. It is also tolerant to small first order baseline distortions (including V-shaped baselines).

The figure below shows a spectrum of rat urine with the standard automatic phase correction algorithm applied to the top spectrum and with the new automatic phase correction algorithm applied to the bottom spectrum.



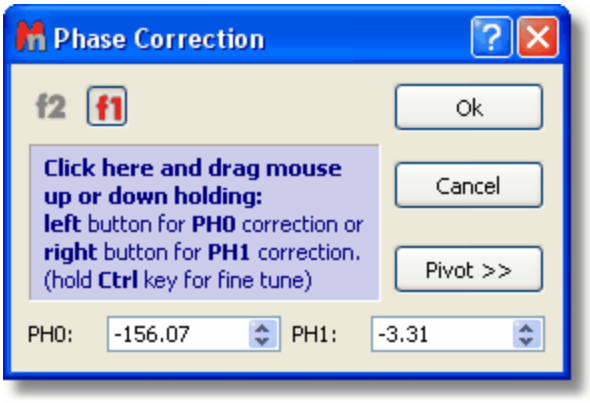
This algorithm is not restricted to Metabonomics spectra, as it is also good for any other spectra satisfying the two conditions outlined above. For example, the figure below shows the difference in performance between the standard automatic phase correction algorithm (top) and the new one (bottom). In this case, the water signal cannot be perfectly phased and thus a standard automatic phase correction fails. The new method yields a much better result as can be seen in the figure.



Selective Method (Not available in Mnova Lite)**:** This automatic method is intended for spectra containing negative and positive peaks (e.g. DEPT, APT, etc) or in the presence of baseline distortions. Mnova creates a list of the highest peaks in the spectrum and then uses symmetrization criteria to obtain the optimal phasing parameters (α and β).

Mnova will automatically execute the better Phase Correction in each case prior FT.

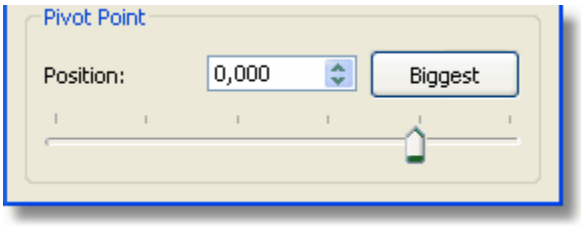
Manual Phase Correction: The manual phase correction is based in finding the appropriate α and β phase correction parameters. This option is very easy to use. Just open the 'Phase Correction' dialog box by clicking on the 'Phase Correction' icon  or via the 'Process/Phase Correction/Manual Correction' menu.



Once the dialog box is open (as can be seen in the figure above), establish a pivot for the adjustment

(by clicking on the **'Pivot >>'** icon 

) and then a pivot dialog box like this will open:



Pivot placement is also very easy and intuitive, by just using the scrolling bar provided. The pivot will maintain an invariable phase in the peak where it is located, while a first order correction is carried out. Click on the *Biggest* button to put the pivot point on the biggest peak of the currently displayed spectrum region. You can also place it in a position of your choosing by clicking and dragging the pivot point marker or by selecting the desired position in the edit box.

Next, correct the zero order phase (by clicking with the left mouse button anywhere on the *Phase Correction* dialog box and dragging the mouse up or down without releasing to correct the phase of the reference peak) and finally do exactly the same whilst right clicking to correct the phase of the remaining peaks (*First Order Phase Correction*). For a fine tuning, hold the 'Ctrl' (or cmd key in Mac) key while dragging the mouse.

You can use the 'Ctrl' key in a Mac machine to simulate the right mouse button in order to phase PH1.

The Phase Correction dialog box incorporates two edit boxes to introduce the values of a (PH0) and (PH1) β for the correction.

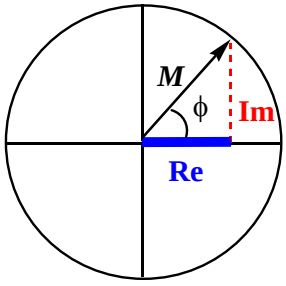
The power and speed of the manual phase correction algorithm implemented in Mnova becomes obvious when correcting the phase of 2D experiments. This can be done directly on the 2D matrix, and the phase can be quickly corrected along one and then the other dimension (Note that for all operations in 2D NMR, the active dimension is indicated by the **'f1'** and **'f2'** icons in the upper toolbar).

Alternatives to Phase Correction. Magnitude and Power.

There are other easier alternatives if you only need positive peaks: representations in 'Magnitude' or in 'Power'.

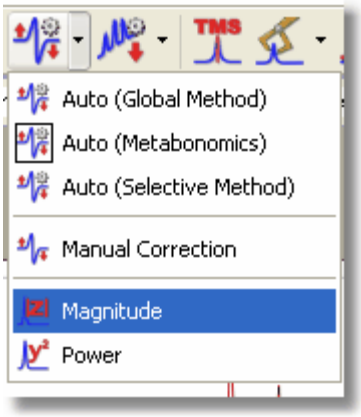
Magnitude: It determines the representation of the final spectrum. If this option is checked you will see the absolute part of the spectrum; if it is unchecked you will only see the real part. As a spectrum is composed of complex vectors, its magnitude or module ***M*** can be calculated by the following expression:

$$M = \sqrt{\mathrm{Re}^2 + \mathrm{Im}^2}$$



To represent the spectrum in *Magnitude* with Mnova, just open the 'Phase Correction' dialog box by

clicking on the 'Phase Correction' scroll menu  and then select 'Magnitude' (as you can see in the capture below) or via the 'Process/Phase Correction/Magnitude' menu.



Mnova will replace the real part of the spectrum by the magnitude (***M***) of each complex vector and the imaginary one by the phase angle of the vector ϕ, thus allowing reversal of this option. That is to say:

$$\mathrm{Re}'_i = M = \sqrt{\mathrm{Re}_i^2 + \mathrm{Im}_i^2}$$

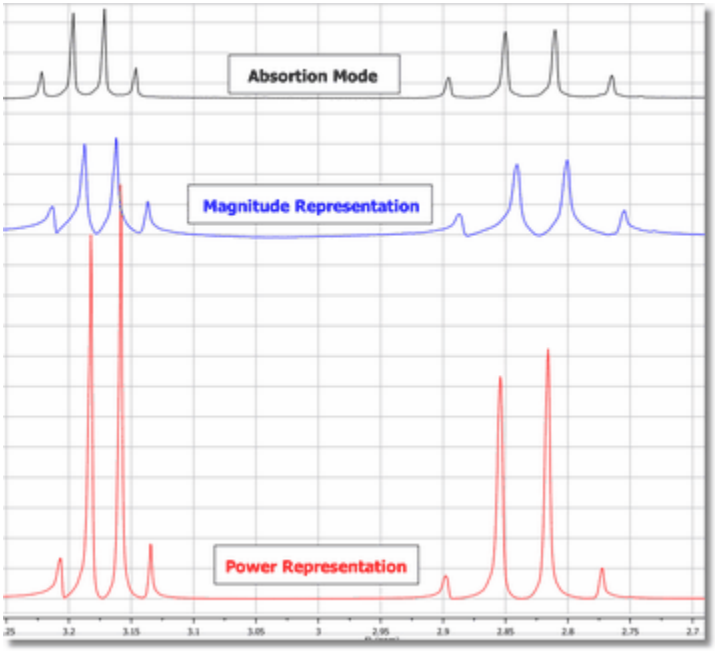
$$\mathrm{Im}'_i = \arctan\left(\frac{\mathrm{Im}}{\mathrm{Re}}\right)$$

Power: the power of the spectrum (***P***) is defined as the square of the magnitude:

$$P = M^2 = \mathrm{Re}_i^2 + \mathrm{Im}_i^2$$

To represent the power of the spectrum, open the **'Phase Correction'** dialog box by clicking on the **'Phase Correction'** scroll menu  and then selecting **'Power'** or via the 'Process/Phase Correction/Power' menu. The **'Power'** as well as the representation of the **'Magnitude'** are reversible options.

The **'Magnitude'** representation produces an increase in linewidth, whilst the **'Power'** representation distorts the intensities of the peaks:



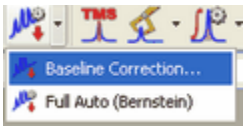
8.6 Baseline Correction with Mnova

The baseline of a monodimensional spectrum is the theoretical line which connects the spectrum points which are not peaks (nor artefacts). When this baseline is not flat and/or it is offset from zero, many problems arise. Quantitative measuring of high resolution NMR demands a precise signal integration. These integrals are very sensitive to slight deviations of the spectrum baseline. The deviations are often due to a distortion of the first points of the FID (see First Point Correction and Linear Prediction). The origin of this kind of distortion is often the *transmitter breakthrough* effect (the detector needs a period to recover from the pulse effect in spite of being switched off when the pulse is applied). This could distort the first points of the FID producing a vertical displacement of the baseline, or even undulations, and, consequently, errors in the signal integrations. You can use a ' Backward Linear Prediction' to solve this problem on the time domain, or a 'Baseline Correction' as a better alternative.

Modern spectrometer hardware uses oversampling and digital signal processing to improve the baseline, but some undesirable broad signals arise from real sources. Thus, a more general solution would employ an efficient post processing baseline correction in the frequency domain. In fact, this is the most common approach found in NMR literature.

2D spectra suffer from baseline problems which may cause difficulties in the visualization of the full data set at once, since the signals of interest may be smaller than the baseline distortions; specially for phase-sensitive experiments and when a large residual solvent signal distorts the baseline. Thus, it turns out that baseline correction is a very important processing step to obtain good quality spectra.

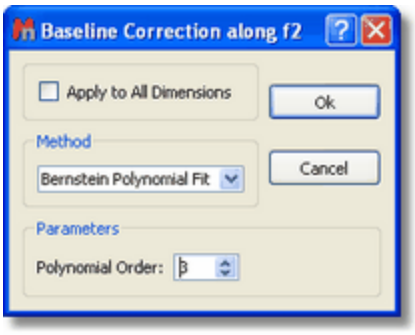
Baseline correction is an area in which Mnova is particularly strong. The software implements Manual Baseline Correction and several automatic Baseline Correction algorithms (Polynomial Fit, Bernstein Polynomial Fit and our in-house developed algorithm, the Whittaker Smoother, which gives exceptional results in 1D and 2D data sets, is extremely fast and in the vast majority of cases fully automatic and is tolerant to varying linewidths along the same spectrum and to poor signal to noise ratios).
The Baseline Correction interface is also very simple. Just click on the **'Baseline Correction'** icon  and this will execute a 'Full Auto' Baseline Correction, if you click on the scroll menu, the Baseline Correction dialog box will open and give the user a choice between the automatic algorithms available, allowing control of certain parameters depending on the algorithm.



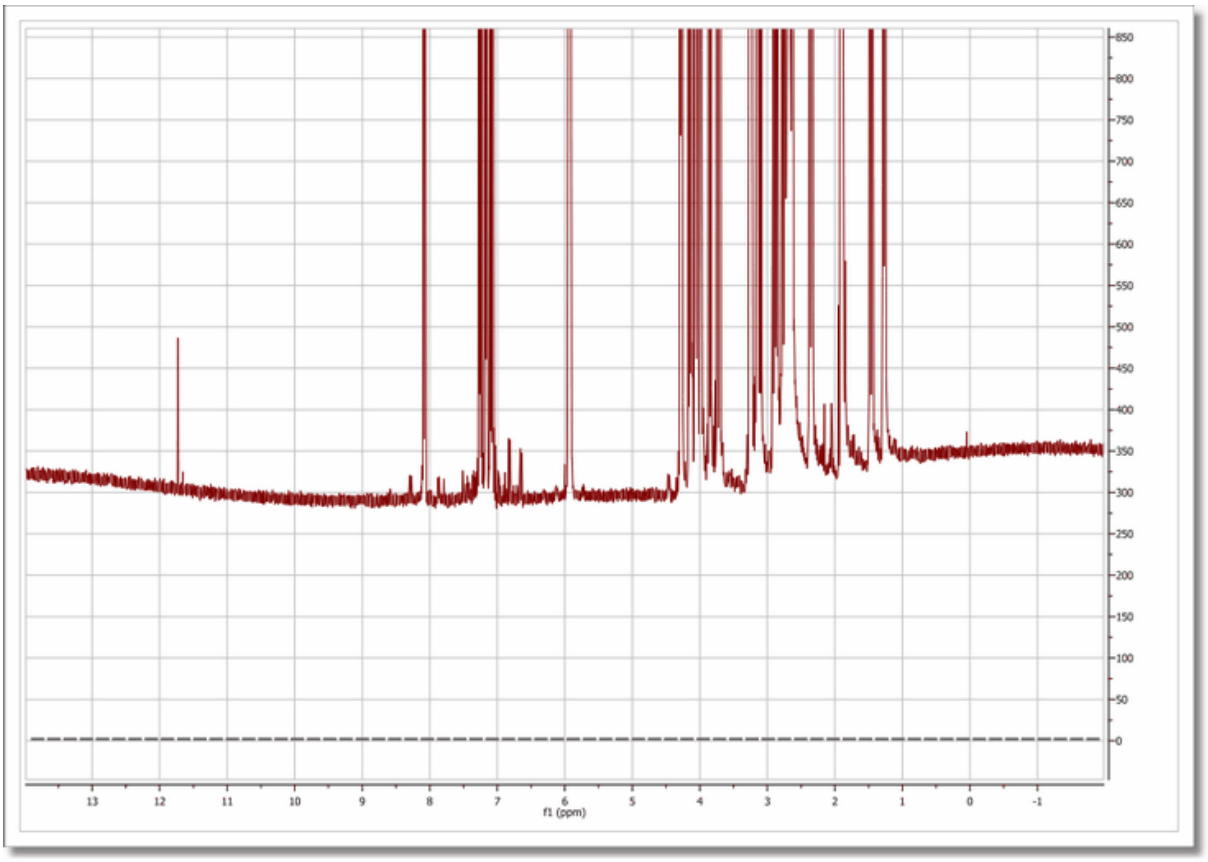
Novelty:

Using **Mnova**, you will able to adjust the phase after baseline correction, because Mnova will not discard the imaginary part of the spectrum after a baseline correction.

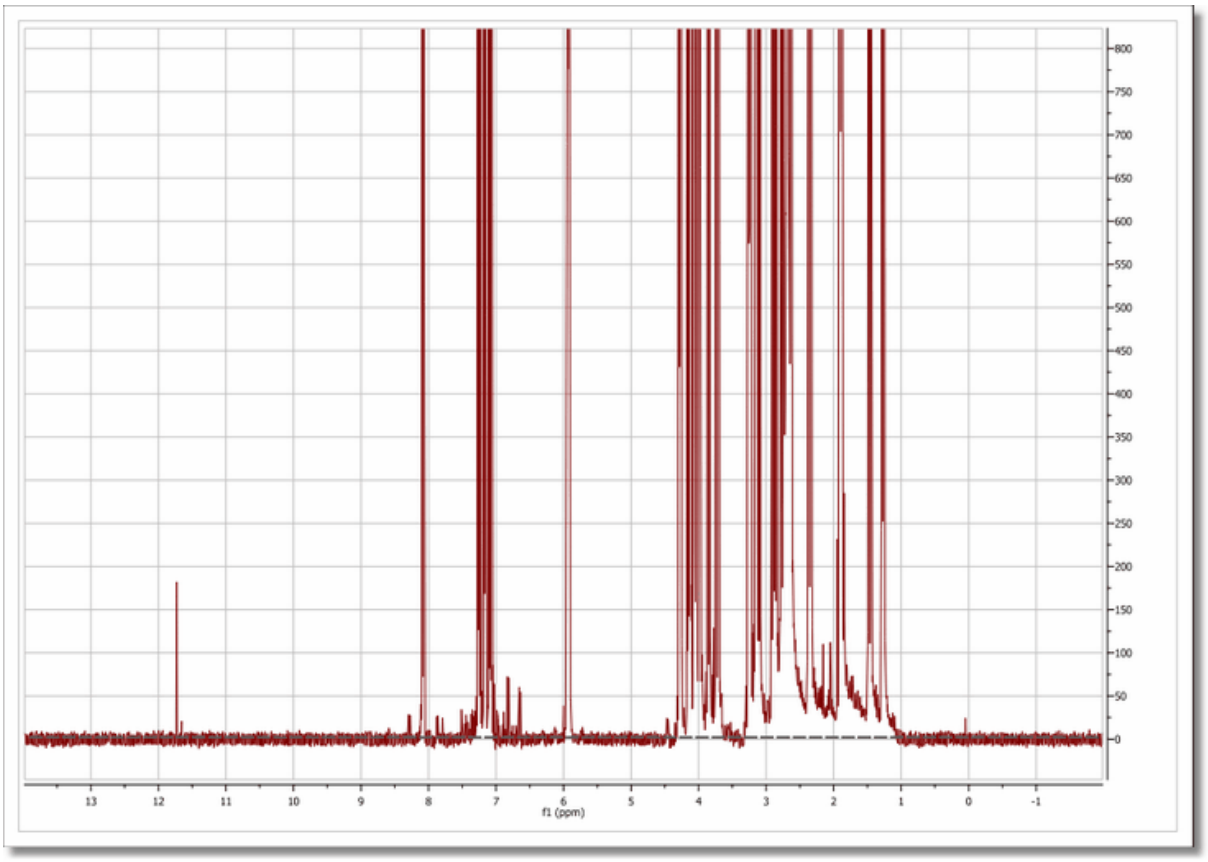
THE BASELINE CORRECTION DIALOG BOX



As a simple example of baseline correction, in the figure below we show a 1D spectrum in which the baseline distortions are evident. Note that the baseline not only deviates from the 0 level (represented by the gray dotted line), but it does also contain a significant rolling. You can choose the parameters of the baseline correction by dragging the parameter control (in the red circle in the 'Baseline Correction' dialog box above) or by introducing the values of the parameters directly into the edit boxes. You can also select 'Autodetect' if you prefer Mnova to calculate the best parameters for your spectrum.



After baseline correction (fully automatic), using the **Whittaker Smoother** algorithm, the baseline becomes perfectly flat as depicted in the figure below



Whittaker Smoother Algorithm: it is a new procedure for automatic baseline correction of frequency-domain NMR data sets which we show to be highly effective on 1D and 2D spectral datasets, and which preserves the range of component line widths which are present in the sample.

The algorithm consists of two independent processes:

1. Automatic baseline recognition (signal-free regions) based on a Continuous Wavelet Derivative transform (CWT) followed by iterative threshold detection in the Power mode domain.

2. A baseline modelling procedure based on the Whittaker smoother algorithm.

Overall, the algorithm has been designed to afford perfect baselines in NMR data sets spanning a wide range of possible baseline topographies and signal-to-noise ratio (SNR) conditions. In most cases, it can be applied successfully without any operator interaction, but further versatility is assured by two parameters which could be adjusted to guarantee an optimum outcome. The procedure can therefore be ‘‘tuned’’ to achieve more accurate baseline recognition of signal-free regions, or to increase smoothness at the expense of spectral fidelity, or vice versa.

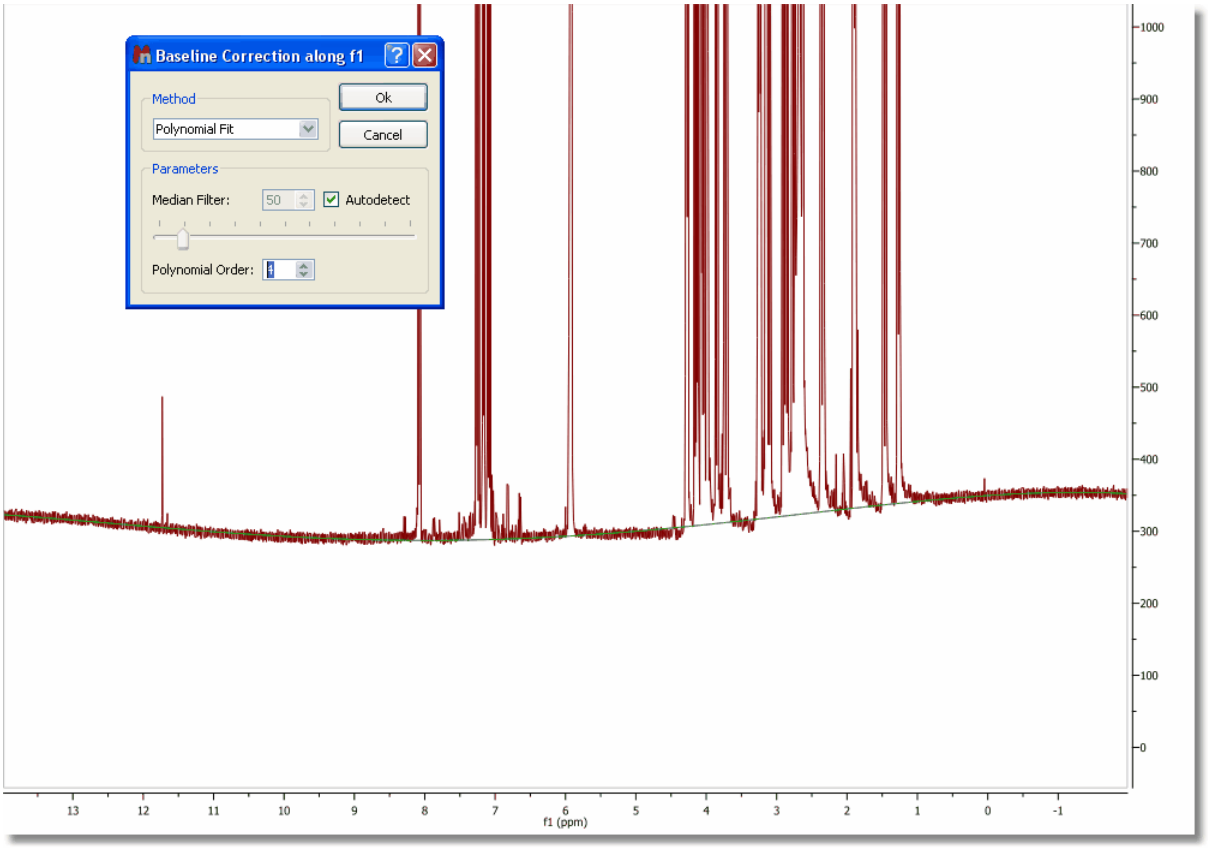
You can find more information about this 'in-house' developed algorithm in this reference: Cobas, J.C. *et al*. *J. Mag. Res*. *183*, **2006,** 145.

Polynomial Fit: In some cases, the employment of polynomial interpolations offers the advantage of smoothing the corners of each section. The user can adjust the polynomial from first to 20th order as well as the so-called 'Median Filter' (the user can see in **real time** the exact effect of the algorithm by a green line drawn on the spectrum display).

The polynomial used in this interpolation is:

$$Y = a_1 + a_2 x + a_3 x^2 + a_4 x^3 + \cdots\cdots + a_N x^{N-1}$$

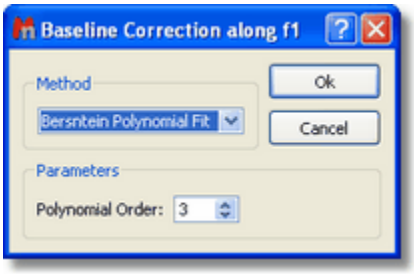
where a_1-a_N are the coefficients to be fitted to the baseline and x the coordinates along the spectral axis.



Bersntein Polynomial Fit: This interpolation is defined by the following polynomial:

$$Y = a_1(1-x)^{N-1} + a_2 x(1-x)^{N-2} + a_3 x^2(1-x)^{N-3} + \cdots\cdots + a_N x^{N-1}(1-x)^1$$

The user only needs to select the order of the polynomial (a_1-a_N) and then click 'Ok', as shown in the capture below:



For a 2D example, see the 2D NMR Processing Tutorial.

8.7 Symmetrization

Symmetrize (Not available in Mnova Lite):

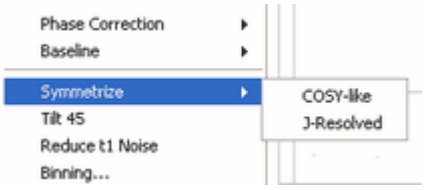
This function, used in homonuclear correlation spectroscopy, replaces all signals which are symmetrically positioned about the diagonal by the smaller of the two signals. In this way unsymmetrically positioned noise and artifacts are removed. This technique should be used with caution since any symmetrical noise or artifacts will be preserved and may be mistaken for genuine signals. The data can be cleaned up considerably by using this tool in square data sets (homonuclear experiments). There are two modes available:

Cosy-like mode

The most common type of symmetrization is performed on COSY type data where cross-peaks are symmetric on either side of a diagonal. This type of symmetrization is performed by following the menu 'Process/Symmetrize/Cosy-like'. The command replaces the larger of two symmetrical points with a point that has the same magnitude as the smaller one. The user must know that this kind of symmetrization must not be used on phase sensitive data (because of the potential introduction of distortions to the cross peaks).

J-Resolved mode

For J-Resolved type data where the spectrum is symmetric on both sides of a horizontal line running through the middle of the matrix. The data point with the larger magnitude is replaced by one which has a magnitude equal to that of the smaller data point. This command is available by following the menu 'Process/Symmetrize/J-Resolved'.



8.8 Tilt 45

Tilt 45 and Homonuclear *J*-resolved 2D-NMR spectroscopy (Not available in Mnova Lite)**:**

Homonuclear *J*-resolved two-dimensional NMR spectroscopy is used to generate one frequency dimension representing chemical shifts and another with coupling constant information. This technique is most frequently used with homonuclear J-resolved spectra where the effect of couplings in the f2 dimension is not required.

The sequence:

$$d1 \cdots 90^{o} \cdots \frac{t_1}{2} \cdots 180^{o} \cdots \frac{t_1}{2} \cdots t_2$$

(where $d1$ is for relaxation and $t_1/2$ and t_2 are the two time dimensions for the two-dimensional experiment) uses the 180º pulse to refocus chemical shifts during t_1.

In a *J*-resolved spectrum, the ^{1}H frequencies are found on the horizontal axis (f2) with the normal 1D spectrum displayed at the top while the vertical axis (f1) contains only proton-proton coupled multiplets,

each centered about a zero frequency point. Then, the 2D *J*-resolved spectrum may be 'tilted 45º' (a projection at an angle of 45º) along a horizontal line through the center of the matrix, so that all the lines of each multiplet line up in the f1 dimension. Each row of the 2D matrix is shifted by an amount proportional to its distance from the middle of the spectrum. If all the individual spectra are now added together, the result is a ^{1}H decoupled NMR spectrum which is normally shown above the 2D spectrum.

Finally, the user could clean up the spectrum by symmetrizing it about the f1 = 0 Hz row. As it is explained in the previous chapter, this consists of comparing the intensities in the spectra equally spaced above and below the f1 = 0 Hz row and taking the smallest value.

This command is available by following the menu 'Process/Tilt 45'.

8.9 Reducing t1 Noise

Reduce t_1 noise (Not available in Mnova Lite)**:**

t_1 noise is a particularly troublesome type of artifact which appears as streams of signals or ridges. The ridges (which are associated with strong signals in a contour plot), run parallel to the f1 axis and are found at their chemical shifts along the f2 axis. They can seriously interfere with the observation of real cross peaks from the signals.

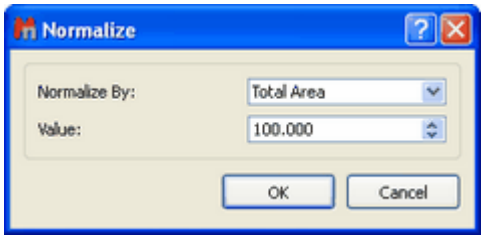
The t1 command allows the user to clean up the spectrum along the f1 dimension by a factor proportional to the r.m.s. noise of that trace. It takes advantage of the fact that t1 noise is normally associated with the intense peaks which can be reduced without a significant loss of information.

This command is available by following the menu 'Process/Reduce t_1 noise'.

8.10 Normalization

Normalization:

The user is able to normalize the intensity of the spectrum by using this tool located in the 'Processing Menu'. The user can normalize the spectrum (or the spectra) by three different methods: Total Area, Highest Peak or by a desired Peak. The user is also able to introduce the intensity value in its corresponding edit box.



Utilities:

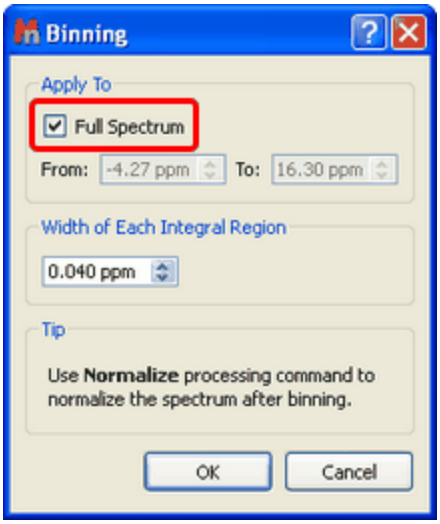
This tool is extremely useful when the user wants to stack spectra with different relative intensities and also after applying a binning.

8.11 Binning

Binning:

Spectral binning is a widely-used technique where the spectrum is subdivided into several regions (bins), and the total area within each bin is used as an abstracted representation of the original spectrum. The area encapsulated by a bin would ideally capture all of the area associated with a given resonance across all spectra in the dataset, thereby mitigating the effect of minor peak shift and line width variations for a compound across samples.

This command is available by following the menu 'Process/Binning'. Then, the 'Binning' dialog box will open and the user can choose the desired limits of the spectrum to apply the binning (or select Full Spectrum, in the red square in the figure). The user can also select the width of each integral region. A typical 64k point NMR spectrum would be reduced using bin widths of 0.04 ppm, resulting in ~250 bin integral values.



Binning (also called bucketing) is a tool used in the multivariate analysis of NMR-based metabonomics data to address the NMR peak misalignment issue. In NMR metabonomics, small variations in the resonance position of the individual peaks caused by experimental and instrumental variations can adversely impact PCA results.

Fixed-width binning (usually at 0.04 ppm) is commonly used to alleviate the impact of the peak misalignment by averaging up the data points falling inside the bin width. However, since it drastically reduces the data resolution, it makes it more difficult to interpret the PCA results such as identifying the changed metabolites from the loadings plot.

The goal of binning a spectrum is to produce a new spectrum in which each new spectral data point corresponds to the integral of a bucket with a given width **(**e.g. 0.04 ppm**).** In other words, this is simply a data reduction operation analogous to bucketing integration. Once the spectrum has been binned, you can export it in ASCII format in order to use it for further PCA analysis.

It is often convenient to use the Normalize processing command to normalize the spectrum after applying a binning.

8.12 Compression

Compression (Not available in Mnova Lite)**:**

A typical 2D experiment could be as large as 64Mb, whereas the final size of a processed 4D spectrum reaches easily the gigabyte range, resulting in a considerable burden on the data storage and backup systems and in low processing efficiency. Furthermore, these data must be transmitted

and stored on computer networks. It is clear that advances in technology for transmission or storage are not sufficient to solve this problem. These considerations clearly raise the issue of efficient data compression.

Mnova incorporates a fast in-house developed wavelet-based compression algorithm, which is generally applicable to 2D NMR data sets (obtaining high compression rates; up to 800:1). We have tested this compression methodology with some examples of 2D spectra focusing on keeping the NMR signal information intact either on cases of qualitative purposes of signal assignment or on quantitative analysis as commonly required for solution structure calculations derived from nuclear Overhauser effects.

This command is available by following the menu 'Process/Compression'. Then an 'Open' dialog will be shown:

The user can select the Compression Ratio or tick the Lossless Compression (in the red square in the figure). Lossless data compression allows the exact original data to be reconstructed from the compressed data.

8.13 Smoothing

Smoothing (Not available in Mnova Lite)**:**

The signal to noise ratio (S/N) of a spectrum can be enhanced by smoothing (or filtering) techniques. This technique is used to reduce the effect of noise on a spectrum and possibly reveal signals embedded in, or distorted by, noise. Noise contains rapid fluctuations which are are generally faster than the rates of change of genuine signals. Smoothing applies a low-pass filter to the spectral data to remove these rapid fluctuations while having minimal effect on signals.

Mnova incorporates some of the more used signal smoothing algorithms.

This command is available by following the menu 'Process/Smoothing. Then, a dialog box will open:

The user can select the smoothing algorithm (Moving Average Filter, Whittaker Smoother, Savitzky-Golay or Wavelets) in the Method scroll bar. The user can also apply the smoothing to All Dimensions (by ticking the corresponding box).

'Moving average algorithm' : The simpler software technique for smoothing signals consisting of

equidistant points is the moving average. An array of raw (noisy) data (y_1, y_2, …, y_N) can be converted to a new array of smoothed data. The "smoothed point" $(y_k)_S$ is the average of an odd number of consecutive 2n+1 (n = 1, 2, 3, ..) points of the raw data y_{k-n}, y_{k-n+1}, …, y_{k-1}, y_k, y_{k+1}, …, y_{k+n-1}, y_{k+n}, i.e.

$$(y_k)_s = \sum_{i=-n}^{i=n} \frac{y_{k+i}}{2n+1}$$

The odd number 2n+1 is usually named filter width (or span in Mnova). The greater the filter width the more intense the smoothing effect.

The moving average algorithm is particularly damaging when the filter passes through peaks which are narrow compared to the filter width.

The user can select the desired filter width (Span) in the dialog box.

Whittaker Smoother: A smoothing algorithm is proposed by Whittaker in1923. If y is a series of m data points and z is the smooth series which should approximate y, we minimize:

$$Q_2 = \sum_{i=1}^{m} (y_i - z_i)^2 + \lambda \sum_{i=2}^{m} (z_i - z_{i-1})^2$$

The first term measures the fit of z to y, the second term is a so-called penalty: it discourages changes in z. The influence of the penalty is tuned by the parameter λ; the larger a λ is chosen, the smoother z will be, at the cost of a worse fit to the data.

The user can select the Smooth Factor in the dialog box or let Mnova detect the best one automatically.

'Savitzky-Golay algorithm' : A much better procedure than simply averaging points is to perform a least squares fit of a small set of consecutive data points to a polynomial and take the calculated central point of the fitted polynomial curve as the new smoothed data point.

Savitzky and Golay showed that a set of integers (A_{-n}, $A_{-(n-1)}$ …, A_{n-1}, A_n) could be derived and used as weighting coefficients to carry out the smoothing operation. The use of these weighting coefficients, known as convolution integers, turns out to be exactly equivalent to fitting the data to a polynomial, as just described, and it is computationally more effective and much faster. Therefore, the

smoothed data point $(y_k)_s$ by the Savitzky-Golay algorithm is given by the following equation:

$$(y_k)_s = \frac{\sum_{i=-n}^{n} A_i y_{k+i}}{\sum_{i=-n}^{n} A_i}$$

Sets of convolution integers, instead of the smoothed signal, can be used to obtain directly, instead, its 1^{st}, 2^{nd}, ..., m^{th} order derivative, therefore the Savitzky-Golay algorithm is very useful for calculation of the derivatives of noisy signals consisting of discrete and equidistant points.

The smoothing effect of the Savitzky-Golay algorithm is not as aggressive as in the case of the moving average and the loss and/or distortion to vital information is comparatively limited. However, it should be stressed that both algorithms are "lossy", i.e. part of the original information is lost or distorted. This type of smoothing has only cosmetic value.

The user can select the 'order' and the 'Width' as the dialog box below shows:

Wavelets: Wavelet thresholding is the basis of wavelet based noise reduction. For a function *f* with Gaussian noise

$$y_i = f(t_i) + \sigma\varepsilon_i \cdots (i \in \mathrm{N})$$

this means that the function *f* is restored.

Hard thresholding is a simple “keep or kill” selection. All wavelet coefficients below a threshold λ are zeroed.

$$c_{j,k}^{hard} = \begin{cases} 0, & |d_{j,k}| \le \lambda \\ c_{j,k} & |c_{j,k}| > \lambda \end{cases}$$

Soft thresholding shrinks the coefficients towards zero:

$$c_{j,k}^{soft} = \begin{cases} c_{j,k} - \lambda, & c_{j,k} > \lambda \\ 0, & |c_{j.k}| \le \lambda \\ c_{j,k} + \lambda & c_{j,k} < -\lambda \end{cases}$$

The most important step is now a proper choice of the threshold λ.

A *universal threshold* consists of:

$$\lambda = \frac{\sigma\sqrt{2\log n}}{\sqrt{n}}$$

where *n* is the sample size and σ the scale of the noise on a standard deviation scale.

The overall procedure of noise suppression consists of a wavelet transform (WT), which yields the wavelet coefficients *cj;k,* followed by thresholding of these coefficients and by an inverse wavelet transform (IWT), which restores the original spectrum.

The user can select the scale (σ) and the fraction threshold (λ%) or also the soft or the Universal Threshold (by ticking the corresponding box).

8.14 Resolution Booster

Resolution Booster (Not available in Mnova Lite)**:**

Resolution is a key concept in high resolution NMR and considerable effort (e.g. shimming) is usually devoted to ensure optimum resolution. High spectral resolution is important for the measurement of NMR parameters, especially for signal intensities, chemical shifts, and coupling constants. However, in many areas of high-resolution NMR the observed resonance lines are broadened in some undesirable way that may complicate, if not preclude, the accurate analysis of e.g. scalar couplings. Moreover, it is

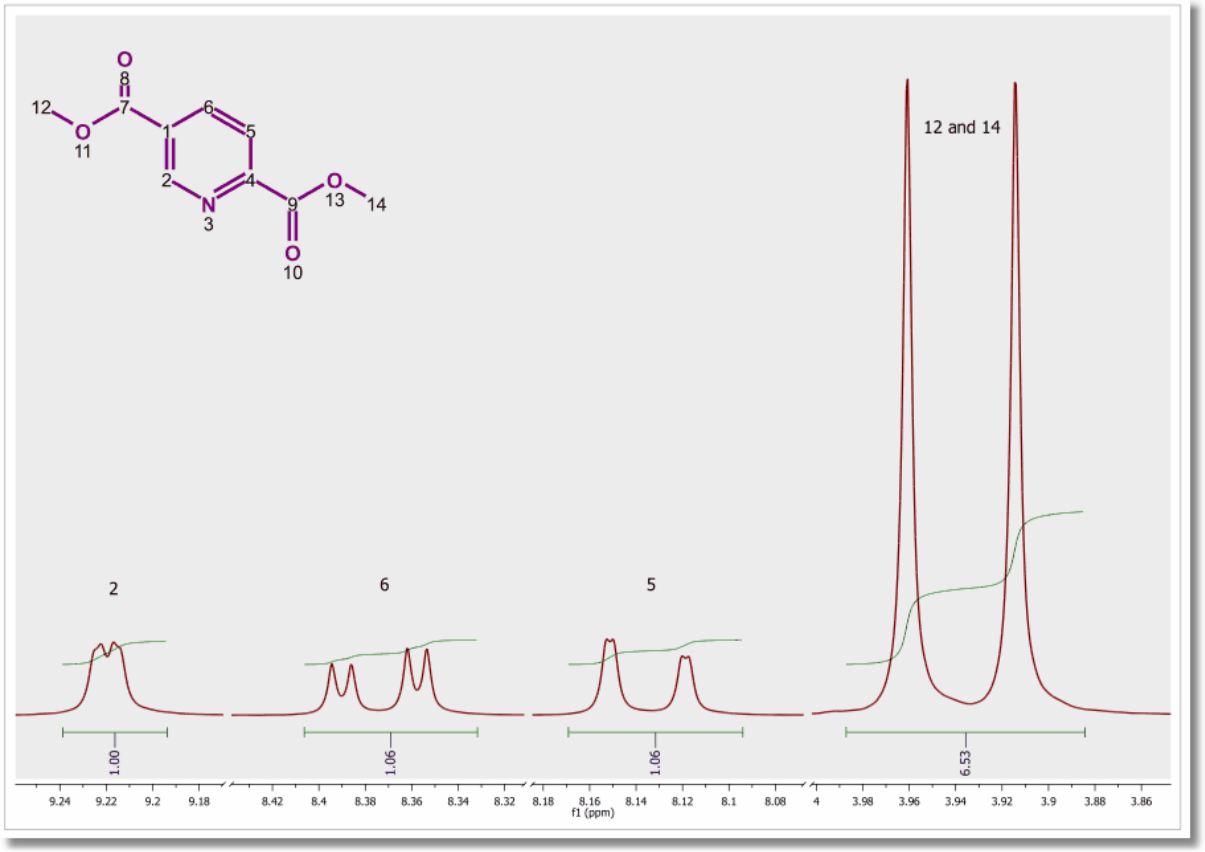
possible to directly measure accurate values of *J* only when the splitting is much larger than the linewidth. The classical solution to the line broadening problem, other than using higher magnetic fields and assuming proper shimming, is multiplication of the FID by a resolution-enhancement function. More sophisticated procedures involve nonlinear data-processing methods such as the much-discussed maximum entropy method. The Filter Diagonalization Method (FDM) and Reference Deconvolution are other well known processing procedures that can be used for resolution enhancement.

The most frequently used methods for resolution enhancement are those which involve appropriate mathematical treatment of the FID (weighting). For example, one popular, routinely used weighting function is the Lorentzian/Gaussian transformation. Other well-known weighting functions include the TRAF, convolution difference, and sine-bell functions. Most of these methods have in common that they alter the FID such that its beginning is deemphasized, relative to the latter parts. Although these methods can improve resolution significantly, caution must be taken because information can be destroyed or artefacts created by extreme enhancement, such as that which results in large negative lobes on both sides of a signal.

The **Resolution Booster** is a new frequency domain post processing method which yields a considerable resolution enhancement with a fast calculation, while avoiding the negative artefacts of the other resolution enhancement functions.

Real Example:

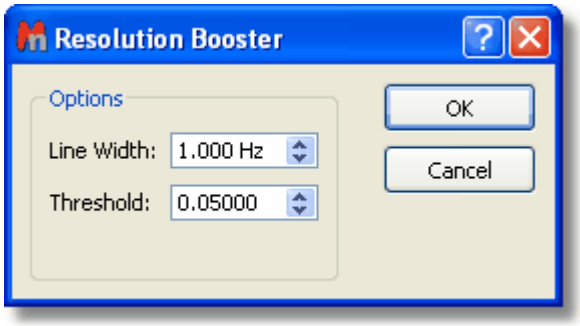
In order to illustrate the power of our Resolution Booster method, we will use as an example the spectrum of ***dimethyl pyridine-2,5-dicarboxylat***e acquired at 250 MHz:



This spectrum has been processed without applying any weighting function and the resolution is **0.13 Hz/pt**. If we look at the signals corresponding to proton 2 in the structure, we can appreciate a small splitting due to 4 bond coupling with proton 6. Proton 6 shows a large splitting due to 3 bound coupling with proton 5 and a small splitting due to the 4 bound coupling with proton 2. Proton 5 appears as a

double doublet because of the 3 bond coupling with proton 6 and a small 5 bond coupling with proton 2. The latter is barely appreciated in the figure because of the lack of resolution. In fact, the same splitting should show in proton 2 but this can not be seen at this resolution level. It is important to remember that it is possible to measure couplings constants accurately when when the splitting is much larger than the line width.

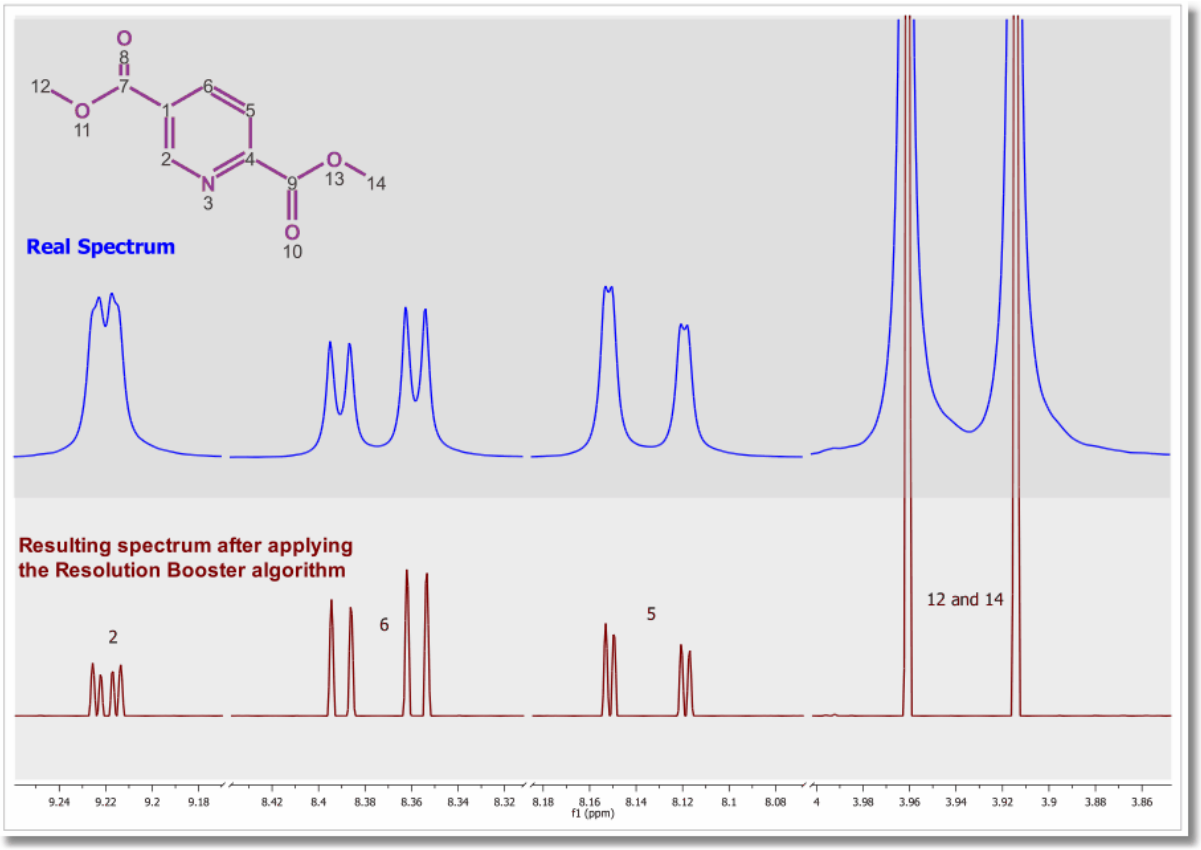
Following the menu 'Processing/Resolution Booster' will display the 'Resolution Booster' dialog box, where the user will be able to select the 'Line Width' and the 'Threshold' of the resolution function:



The Resolution Booster algorithm requires two parameters to be defined, but only the **Line Width** is important (the *Threshold* parameter can be safely ignored).

The **Line Width** should correspond, approximately to the natural line width. It does not need to bee very precise: making it smaller increases resolution and noise, making it larger goes in the opposite direction. However, this value does not need to be very precise, significant deviations from the natural line widths are perfectly tolerable.

In the below picture you will be able to see the result of applying the **Resolution Booster** method by using the default values of Line Width (1.000 Hz) and Threshold (0.05000).

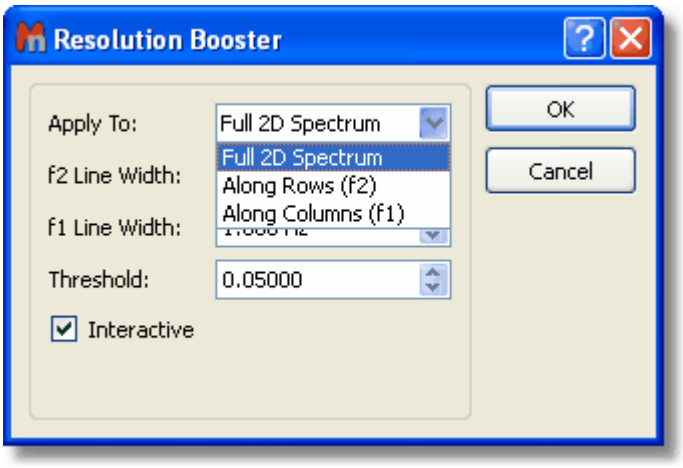


In this case, resolution has been increased by **~230%** making now possible the calculation of the weak, long range coupling constants.

Note:

It is important to notice that this procedure may change relative intensities. On isolated lines, in principle, it is approximately proportional to the second derivative which, when all lines have the same line width (as they often do) is proportional to the line height. However, broad lines can get suppressed and unresolved humps and shoulders get resolved (+) but their intensities and, to some extent, positions can not be trusted (-).

It is also possible to apply the Resolution Booster to 2D-NMR spectra (full spectrum or only along f1 or f2):



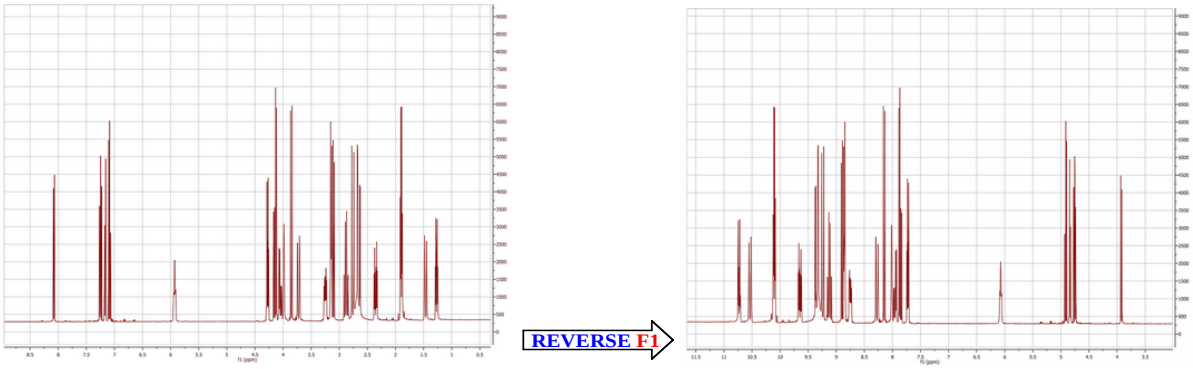
See also:
http://nmr-analysis.blogspot.com/2007/11/resolution-booster.html
http://nmr-analysis.blogspot.com/2007/11/selective-resoluton-booster.html
http://nmr-analysis.blogspot.com/2007/12/introducing-2d-resolution-booster-rb.html

8.15 Inverting, Transposing and Reversing Spectra

Invert: The command 'Process/Invert' multiplies all spectrum data points by -1. It is equivalent to applying a zero order phase correction of 180º It is very useful for DEPT spectra.

Transpose (Not available in Mnova Lite)**:** This command allows the user to transpose (by interchanging the rows and columns of the matrix) a spectrum for more convenient representation. This command is available by following the menu 'Process/Transpose'.

Reverse Spectrum: The commands ('Process/Reverse Spectrum') on this submenu allow you to reverse the spectrum along **F1**, **F2**, (horizontally or vertically) or **both axes** to obtain a more convenient representation.



8.16 Arithmetic

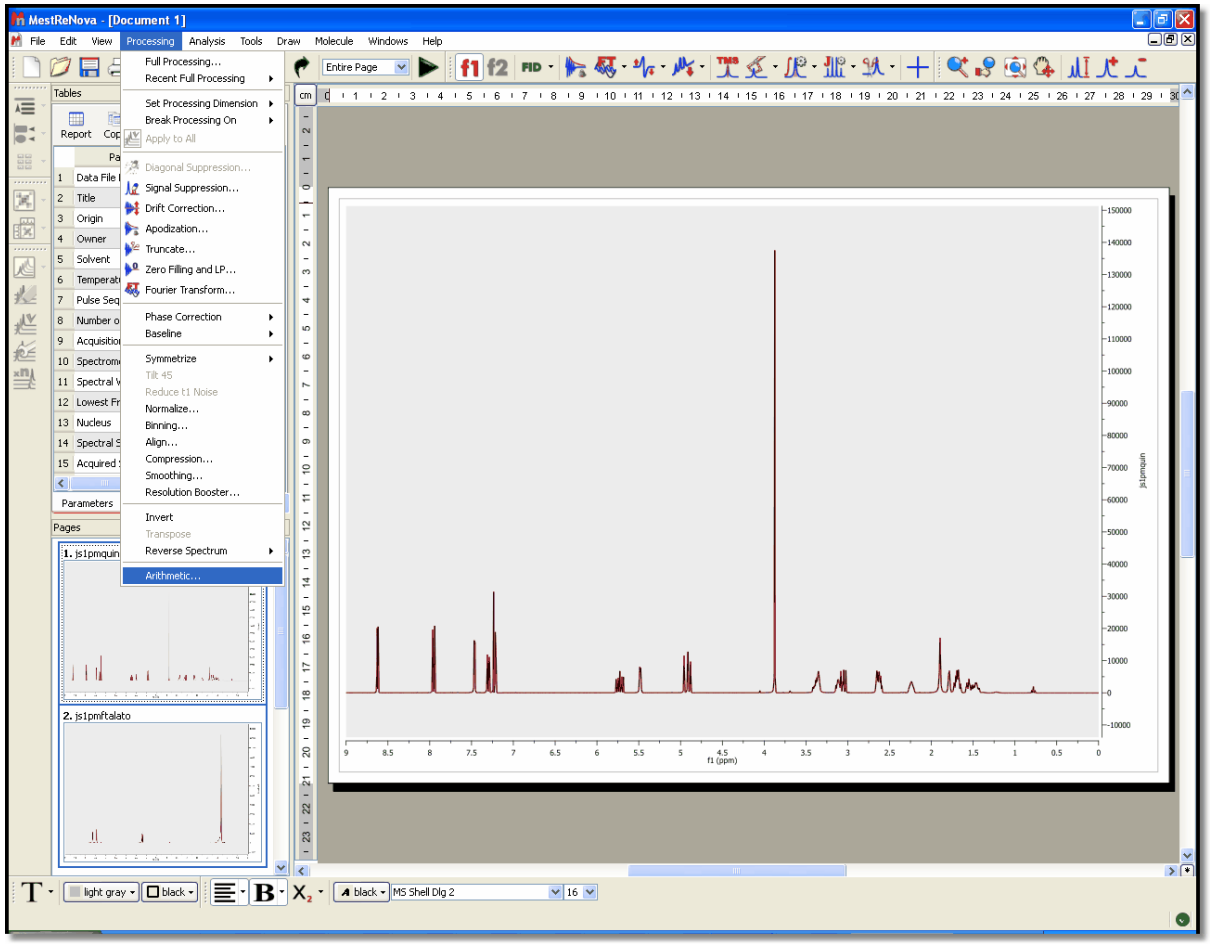
Arithmetic (Not available in Mnova Lite)**:**

This feature is used to carry out arithmetic operations with 'fid''s or processed spectra. Typically, you will use the arithmetic module implemented in MestReNova to sum 'fid''s acquired under the same measurement conditions in order to improve the signal-to-noise ratio.

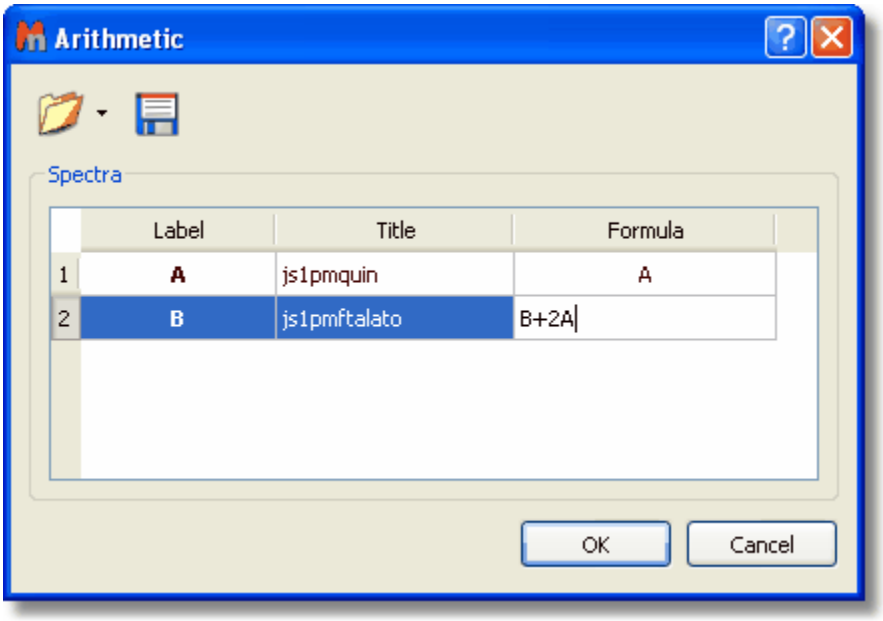
This tool is also very useful to **remove impurities** or solvent signals from a spectrum, to **analize DEPT experiments** and also to **subtract FIDs of Selective Experiments** (such as NOE or ROE).

How to sum or substract spectra?

Application of Linear Combinations (sums, subtraction and multiplication) to spectra in the MestRe Nova interface is extremely simple. Just select the desired spectra on the Page Navigator (by holding down 'CTRL key' or 'Cmd key' while clicking on each spectrum) and follow the menu 'Processing/Arithmetic':

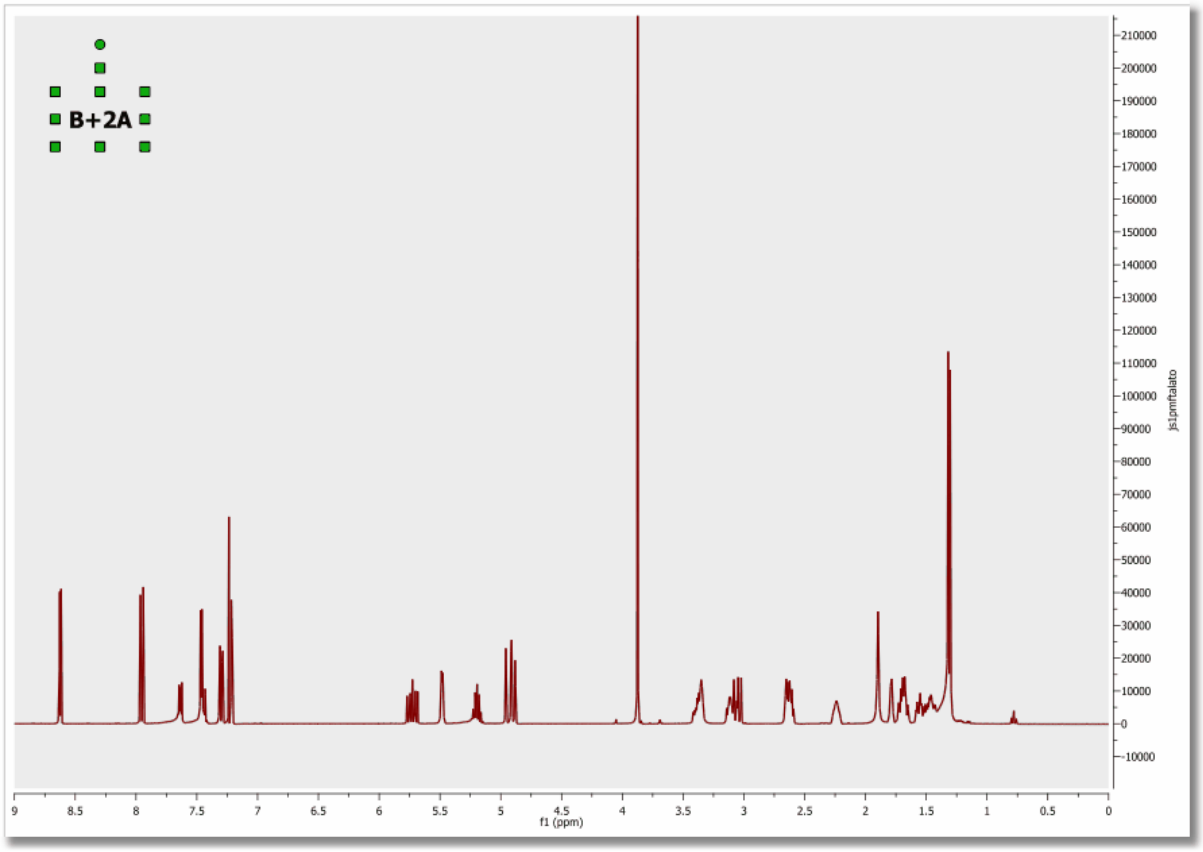


This will display the 'Arithmetic' dialog box on screen. In this example, we want to add two times spectrum A to spectrum B, so we shall type the formula 'B+2A' in the corresponding cell.

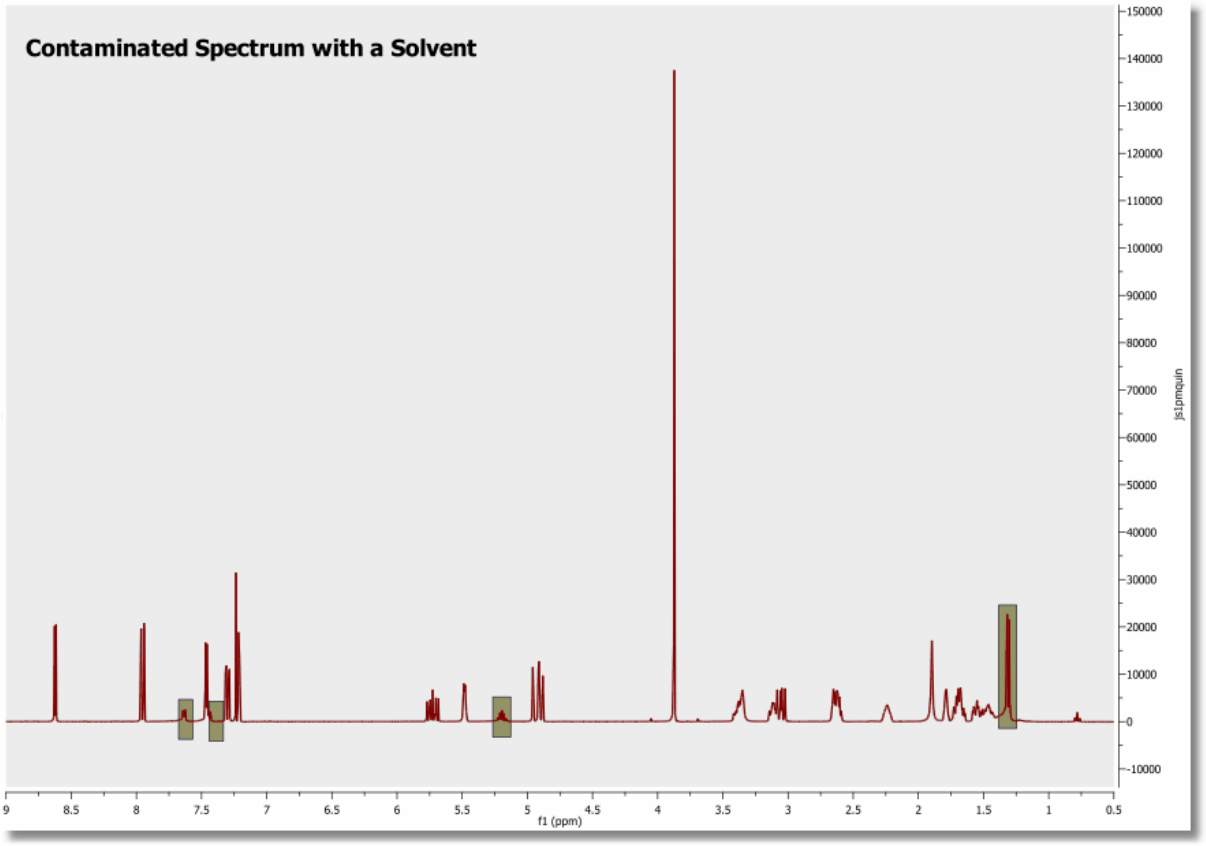


The '**Save Formulas**' icon will allow the user to save formulas, which can then be loaded by using the '**Load Formulas**' icon .

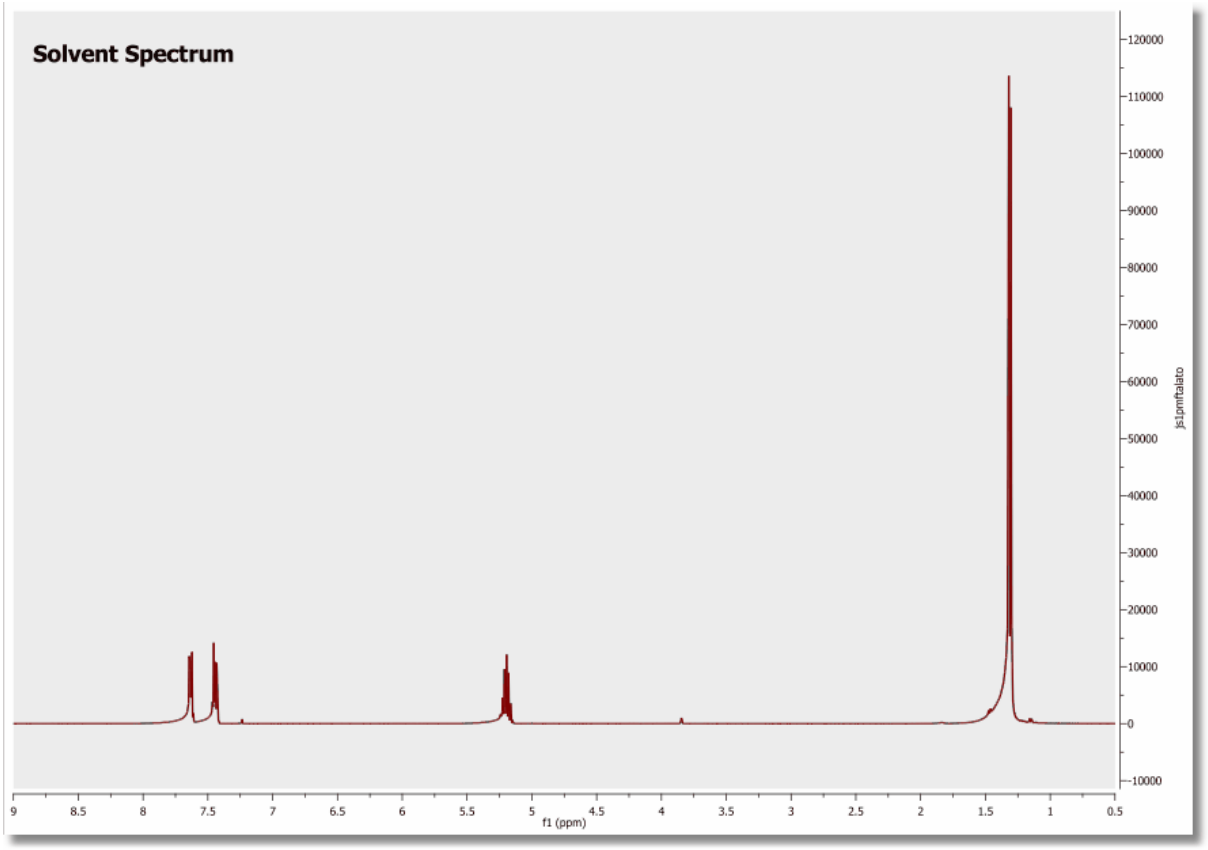
On pressing 'OK', the result is a new spectrum which will consist of the sum of spectrum B plus two times spectrum A.



It is also possible to subtract spectra, but in this case it is important that the user calculates the intensity ratio of both spectra. For example, in this spectrum we have a sample contaminated with a solvent.

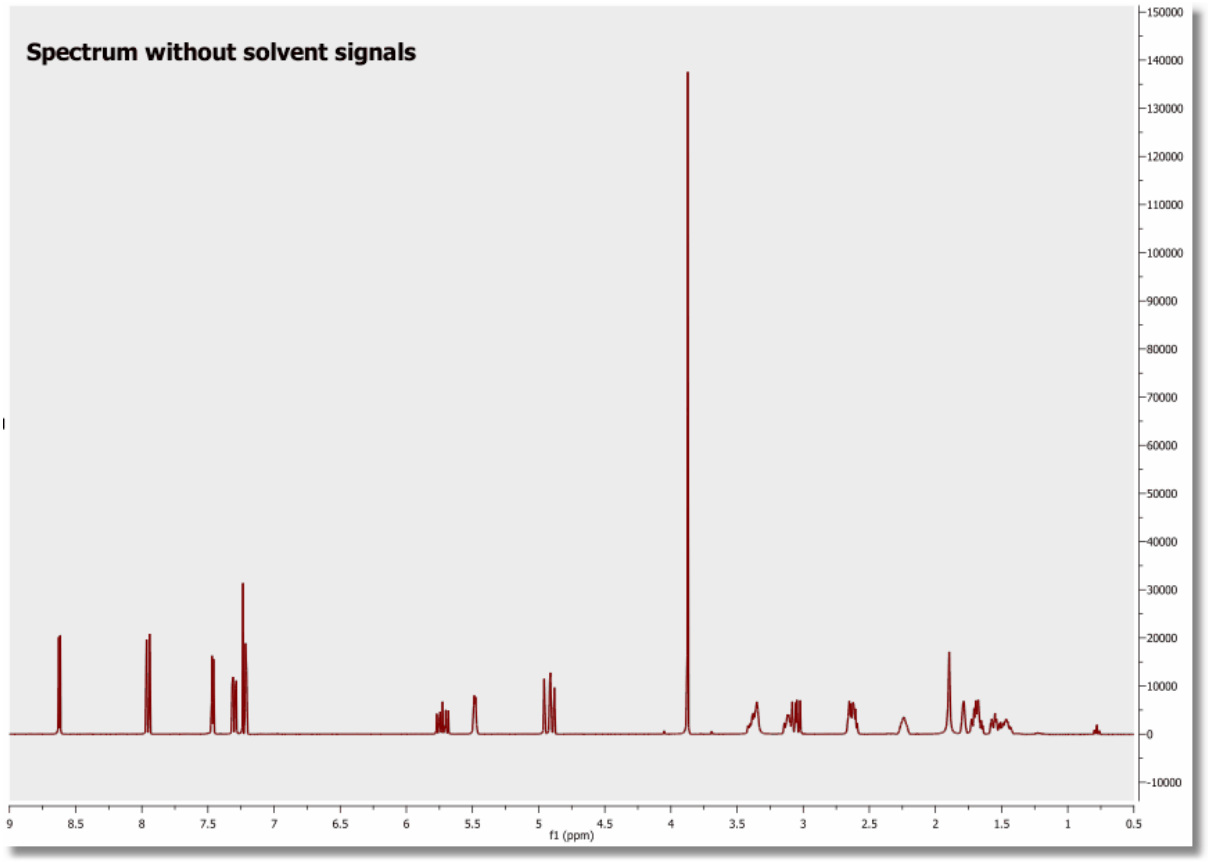


Whilst the spectrum below corresponds to the solvent which we need to eliminate from the sample.



To successfully carry out the subtraction, we need to calculate the intensity ratio between the contaminated spectrum and the solvent spectrum. To obtain this ratio we can measure the intensity of a signal present in both spectra (by applying the command ‘Peak by peak’ and getting the result on the corresponding table). For example, we measure the intensity of the singlet which appears at 1.32 ppm. For the first spectrum, we obtain an intensity of 22617 and for the second, 115031, indicating the the intensity ratio to be 1/5, or 0.2.

Typing the formula **'A-0.2B'** (or 'A-0.2*B') in the 'Arithmetic' dialog box, we shall obtain the spectrum below, where the solvent signals have been removed:

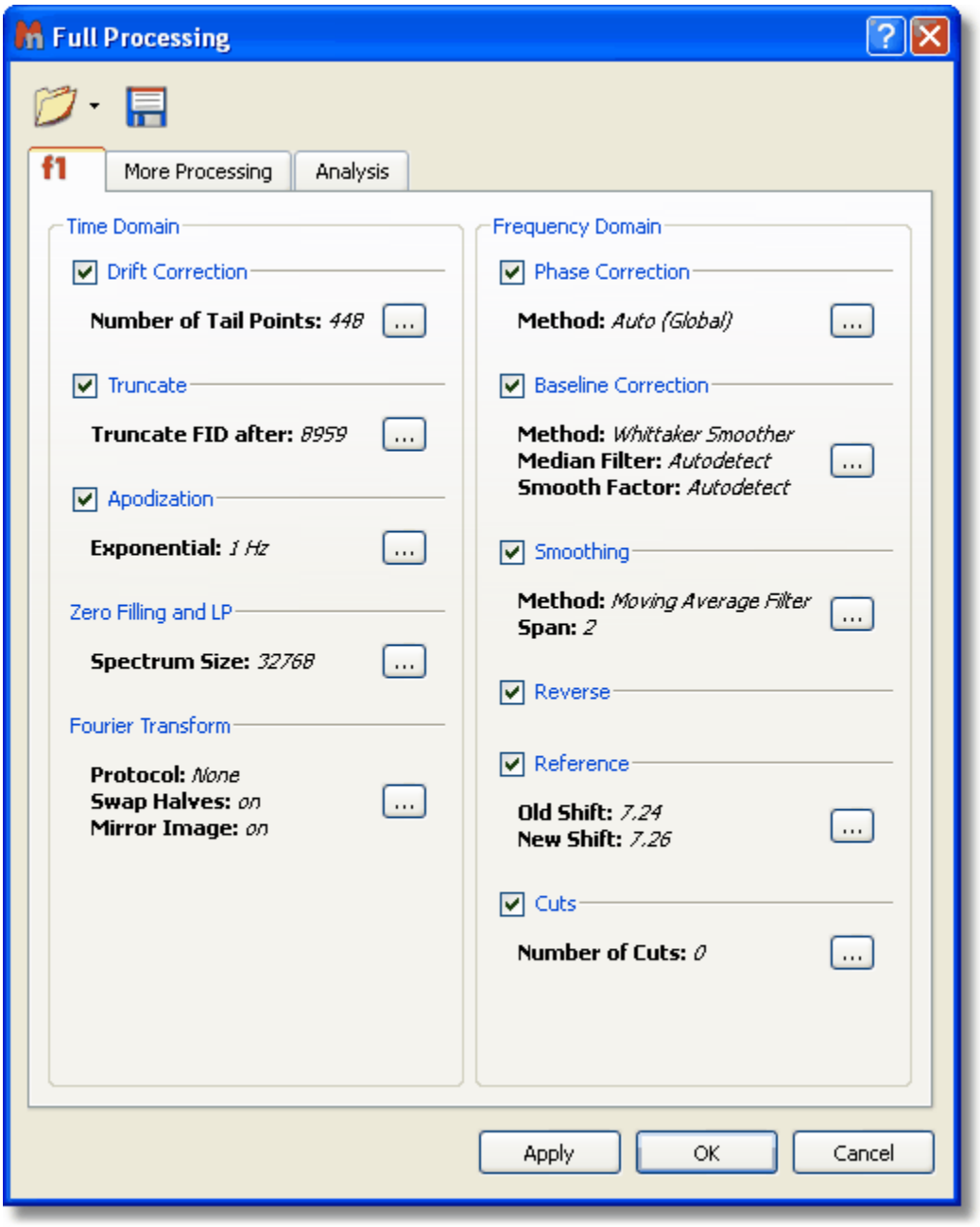


8.17 Automated Processing

Mnova includes a powerful and flexible feature which allows the user to easily automate the full processing of both 1D & 2D NMR data sets. This is ideal for the batch processing of spectra of the same type, and can also incorporate analysis operations.

For example, let's say you have acquired 20 proton spectra which you want to process using the same processing operations. The procedure is very simple:

1. Load one spectrum and process it as desired.
2. Go to ***Process/Full Processing***. The following dialog appears:



This dialog includes all the processing and analysis features implemented in **Mnova**. You can choose the required options by selecting the check boxes and, if necessary, specify options by clicking the button  next to the corresponding command.

3. Once you are happy with the result, **save** the script file to a Mnova Processing file (*.mnp) by clicking on the save button in the dialog box. 

4. You can now apply this processing script file to any other spectrum/spectra by selecting the spectra you wish to process following this procedure (one or several spectra), and then opening the previously

saved file by using the open command in the Full Processing dialog box. 

A few helpful tips ...

1. If you want to apply a processing script to all the spectra opened in one document, first select all the spectra in all the pages.
2. Next you can simply go to ***Processing/Recent Full Processing*** and select the appropriate processing file script.
3. Remember that only spectra acquired using the same conditions should be processed using a processing script file. For example, if you have acquired a spectrum using a sequential mode and another one using simultaneous quadrature detection, you will get erroneous results.

Part

9 Analysis Tools

The oncoming release version of Mnova will implement a very extensive and advanced suite of NMR analysis tools. This section will give you an outline of the features already implemented, and which will have full or at least partial capabilities in this version:

Chemical Shift Referencing
Peak Picking
Molecular Structure
Molecular Structure Assignment
Prediction of NMR Spectra
Multiplet Analysis (Fully Automatic and Multiplet by Multiplet)

There are a number of analysis capabilities which are currently in implementation, and which will be available either in the oncoming commercial release versions (see the Mnova 5.1 section of this Manual):

9.1 Chemical Shift Referencing

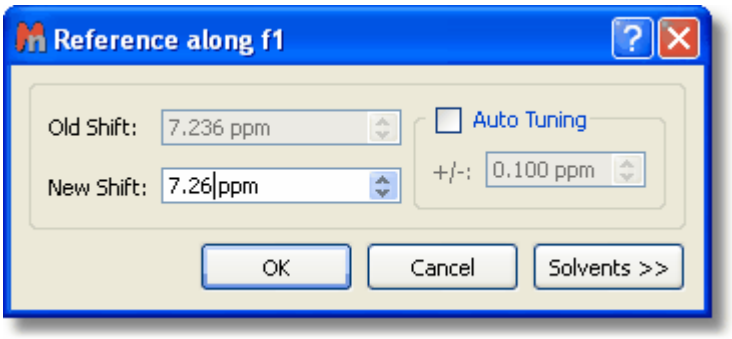
In NMR, the frequencies are usually measured relative to a frequency standard. As these frequencies are strictly measured relative to a frequency standard proportional to the magnetic field, it is convenient to define the chemical shift as:

$$\delta = 10^6 \cdot \frac{(\nu - \nu_{ref})}{\nu_{ref}} = 10^6 \cdot \frac{\sigma_{ref} - \sigma}{1 - \sigma_{ref}} \approx 10^6 \cdot (\sigma_{ref} - \sigma)$$

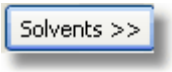
Thus, in order to establish a chemical shift scale, it is necessary to choose some substance as a reference and define its chemical shift. In non-aqueous solvents, the most used reference substance is **TMS** (tetramethysilane) which is defined to have a chemical shift of **0.0 ppm**. For biomolecules, slightly different compounds (e.g. TSP, $(CH_3)_2SiCD_3CD_2CO_2Na$, Sodium-salt of trimethylsilyl-propionic acid) are used, since TMS is not soluble in water.

You can calibrate your spectrum by selecting ***Analysis/Reference*** on the main menu or by clicking on the  toolbar button and then following this procedure:

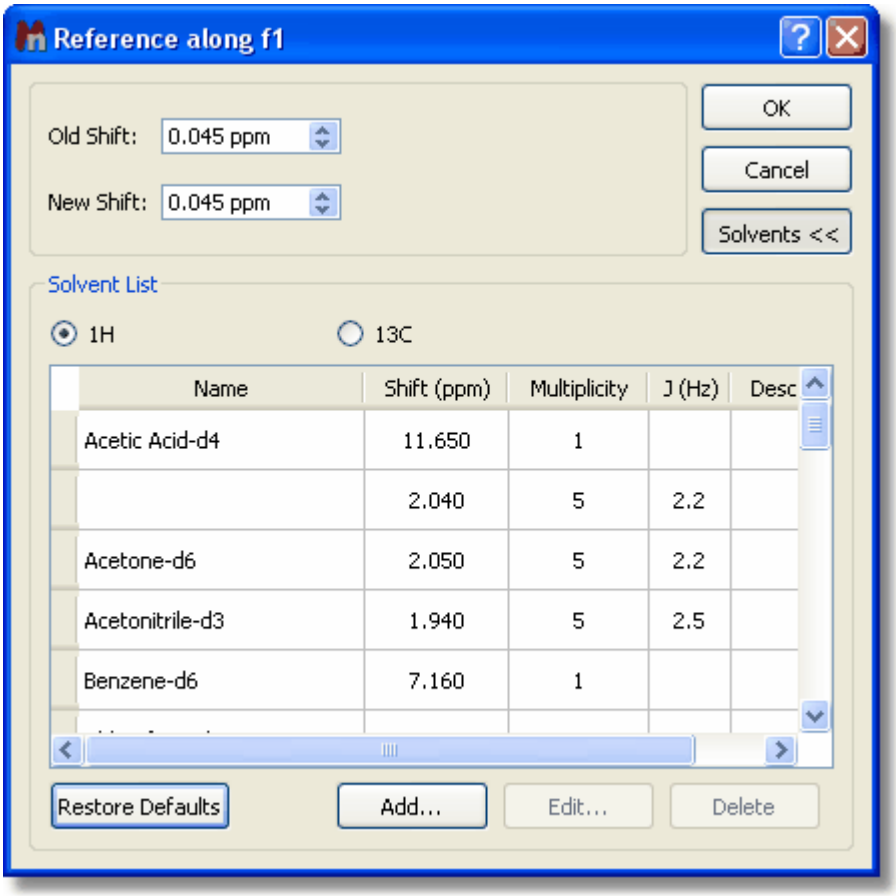
1. Zoom-in on the spectrum region containing the reference peak
2. Select the peak you want to be a reference point. You will notice that the mouse cursor automatically *jumps* to the peaks maxima. To select a peak at an arbitrary position which is not a local extreme (e.g. the shoulder of a peak), hold down **SHIFT** and click.The **following** dialog box appears:



The 'Auto Tuning' option finds the maximum peak inside a limit and references it. This is very useful for example if you need to reference automatically a large amount of metabonomic spectra, where the solvent peak could slightly change its shift with the pH of the sample.

You can type the new value for that peak or you can click on the 

button to get a list of solvent chemical shifts.



You can add new entries or edit the list of standard reference chemical shifts by using the buttons at the bottom of the dialog box.

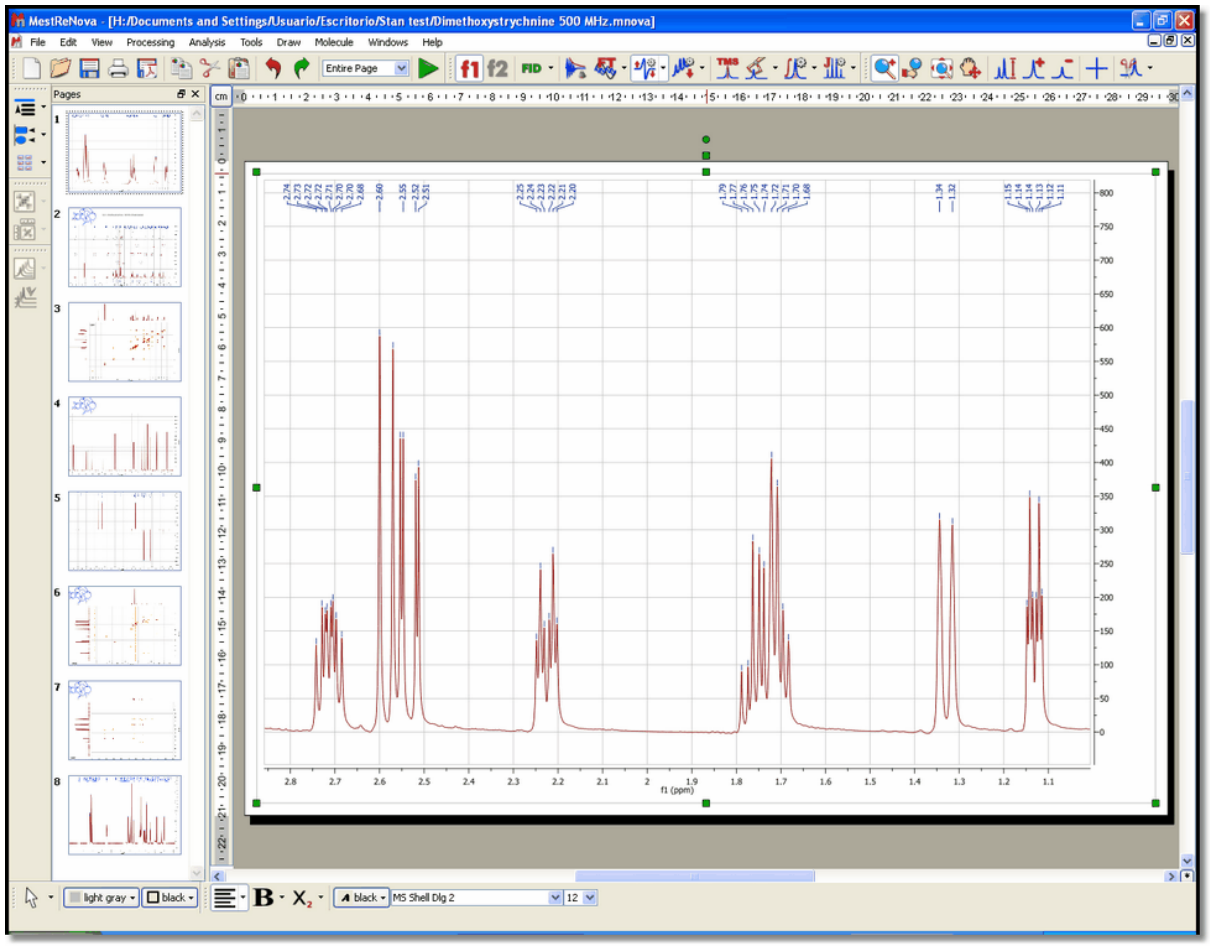
9.2 Peak Picking

Mnova implements a series of peak picking algorithms for 1D and 2D. These algorithms can be accessed via the menu item 'Analysis/Peak Picking' or via the scroll menu immediately to the right of the **'Peak Picking'** icon  on the toolbar. This scroll menu will allow you to use Automatic Peak Picking, Manual Peak Picking, Peak by Peak Peak Picking, to show or not show peaks on screen, to delete all peaks or delete peaks one by one and to set Peak Picking Options.

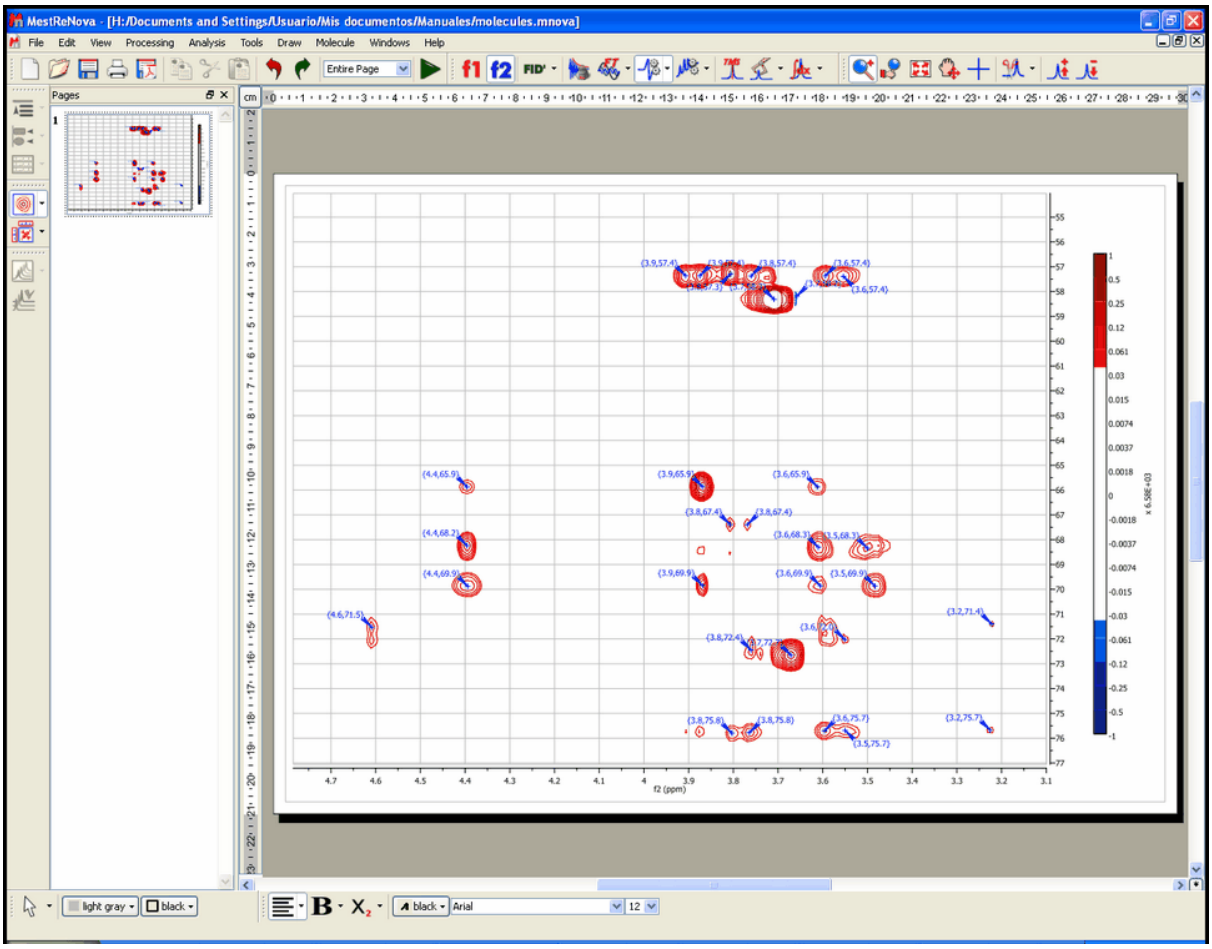
Automatic Peak Picking

The Automatic Peak Picking option can be launched by simply clicking on the **'Peak Picking'** icon  on the toolbar. This algorithm will peak all the peaks in the spectrum, and, by default, show them on screen. This algorithm can be applied indistinctively to both 1D and 2D spectra.

Example 1: 1D Peak Picking

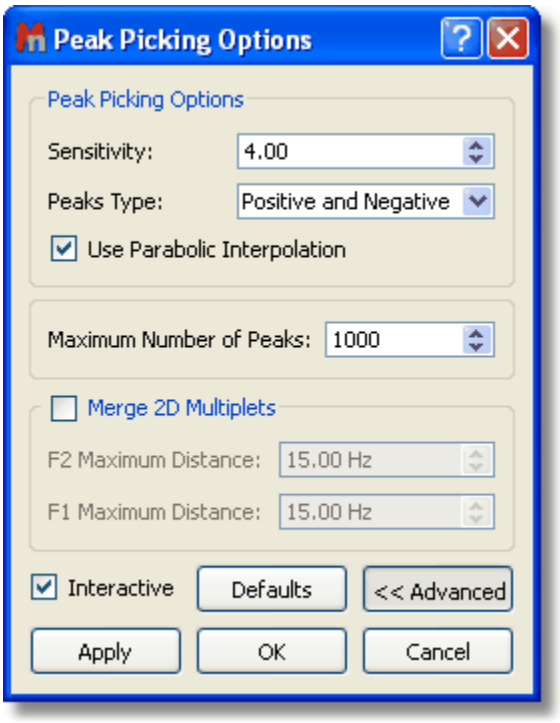


Example 2: 2D Peak Picking



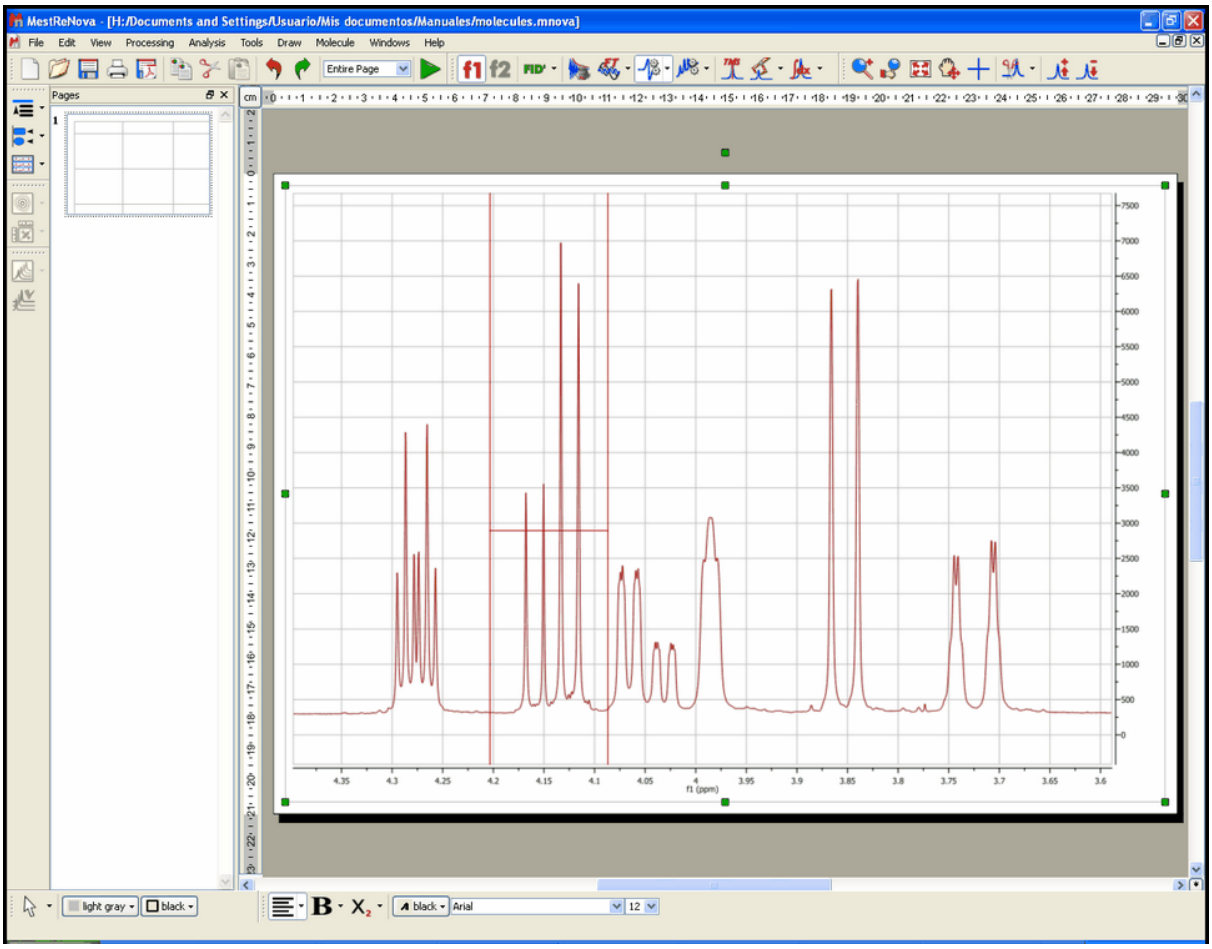
Peak Picking Options

A series of Automatic Peak Picking Options can be controlled on the 'Peak Picking Options' dialog box, which can be accessed via the Peak Picking scroll menu. The user can choose the sensitivity (the higher the value the less peaks will be picked), the peak type, whether to use parabolic interpolation, merging options for 2D multiplets, maximum number of peaks, etc. All these options can be evaluated interactively, so that their results can be viewed on screen in **real time**, by making sure the **'Interactive'** box is selected.

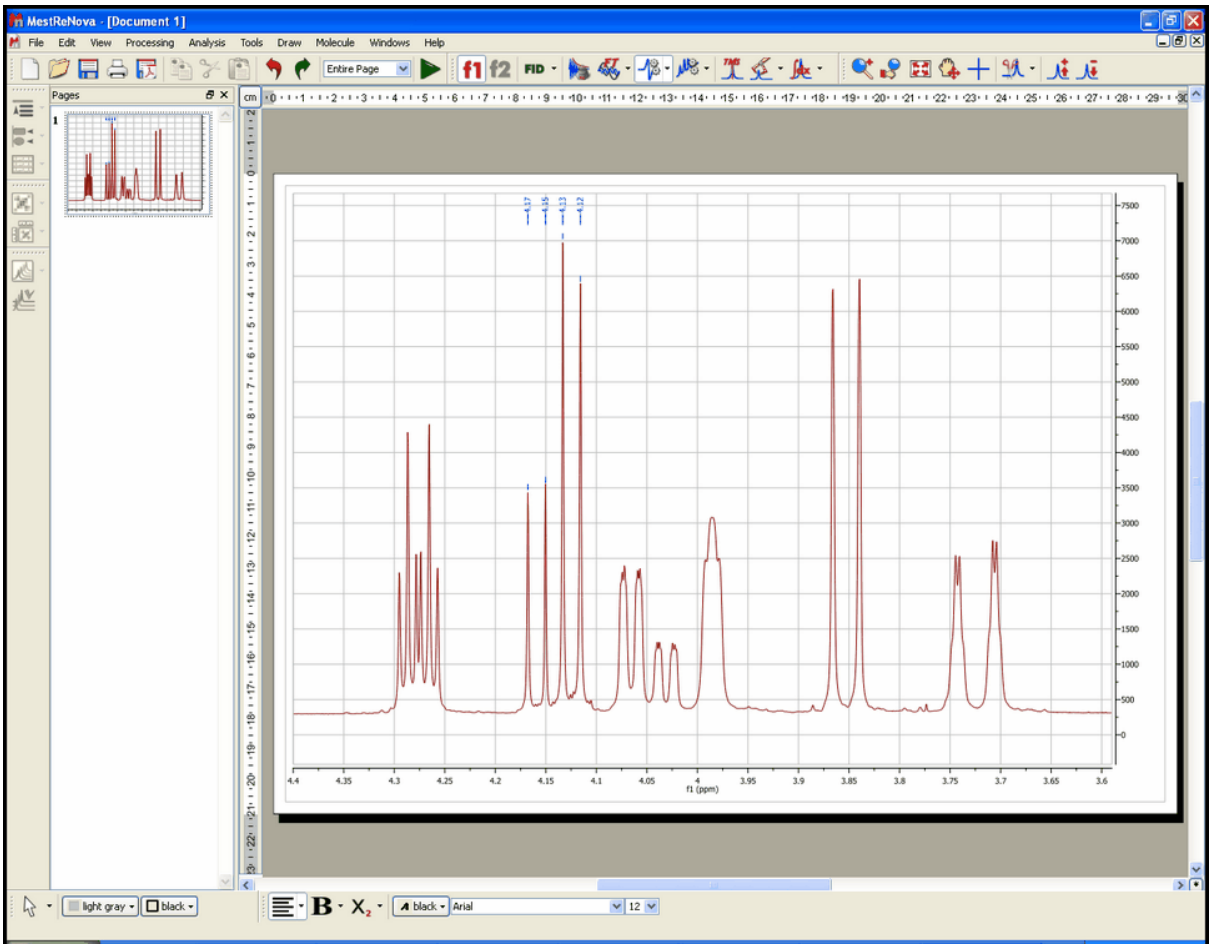


Manual Peak Picking

The Manual Peak Picking option allows the user to set an area of the spectrum where the software will carry out the Peak Picking, by simply left clicking and dragging the mouse over the area.

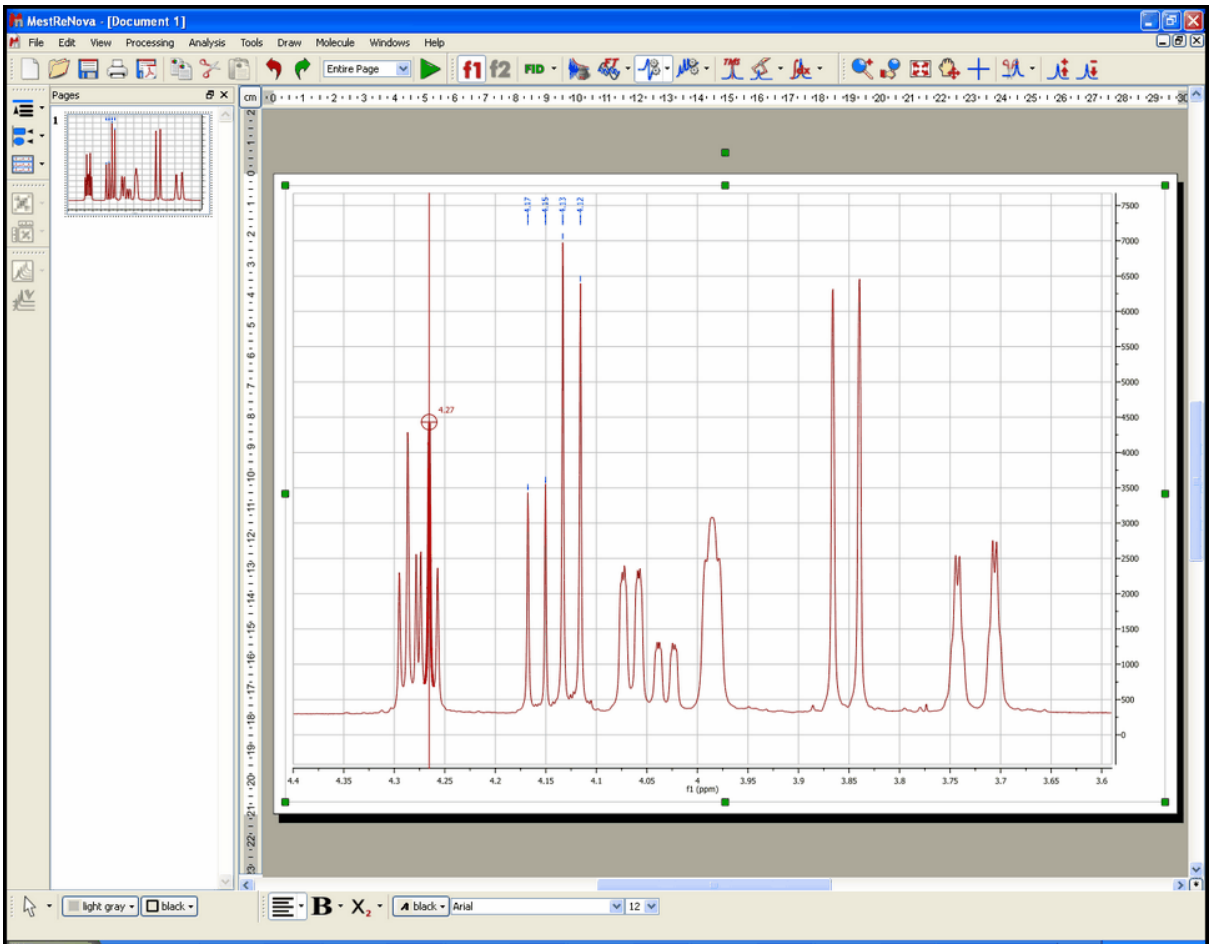


This will generate a bucket, and all peaks with chemical shifts between the left and right hand sides of the bucket and with intensities above the bottom of the bucket will be picked.



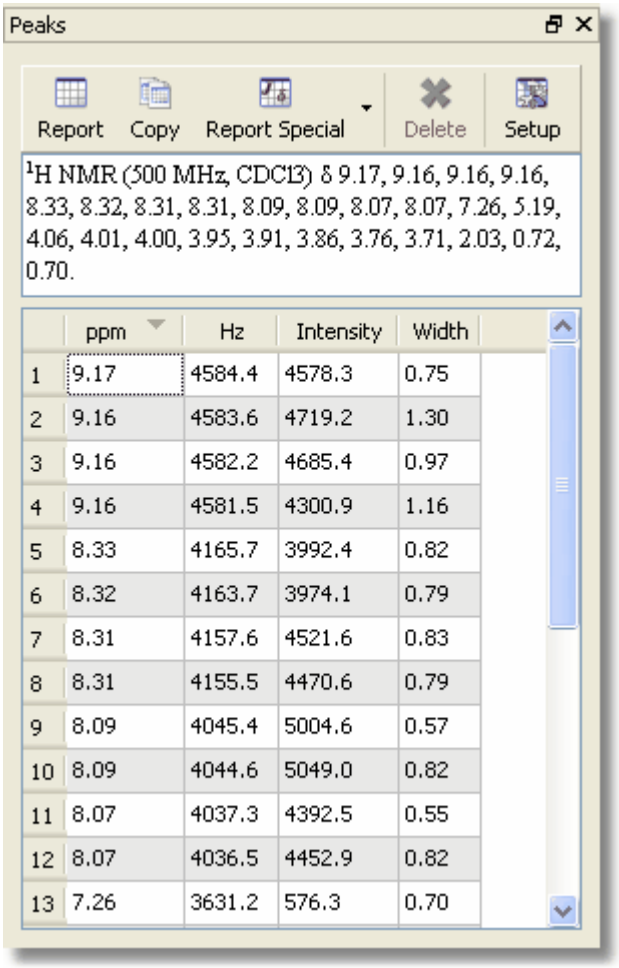
Peak by Peak

The Peak by Peak option will allow you to hover the mouse over specific peaks of interest and to only select those for the peak picking output, by just left clicking on each one at a time. The procedure is very simple: just point to the peak you want to pick so that it is highlighted and then left click on the mouse.



Note that, by default, Mnova will find the peak top by using parabolic interpolation. To pick a peak at an arbitrary position (e.g. not at a local extreme), hold down <Shift> and click.

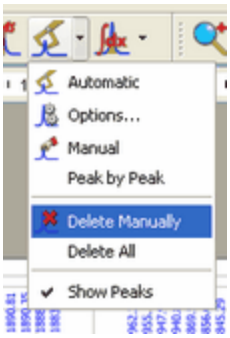
Peak Table: The user will obtain the peak table by following the menu 'View/Tables/Peaks'.



	ppm	Hz	Intensity	Width
1	9.17	4584.4	4578.3	0.75
2	9.16	4583.6	4719.2	1.30
3	9.16	4582.2	4685.4	0.97
4	9.16	4581.5	4300.9	1.16
5	8.33	4165.7	3992.4	0.82
6	8.32	4163.7	3974.1	0.79
7	8.31	4157.6	4521.6	0.83
8	8.31	4155.5	4470.6	0.79
9	8.09	4045.4	5004.6	0.57
10	8.09	4044.6	5049.0	0.82
11	8.07	4037.3	4392.5	0.55
12	8.07	4036.5	4452.9	0.82
13	7.26	3631.2	576.3	0.70

This table displays the list of peaks, including the following information: chemical shift in ppm and Hz, intensity (height) and width. The user can report the Peak table directly on the spectrum by clicking on the **'Report'** icon  .

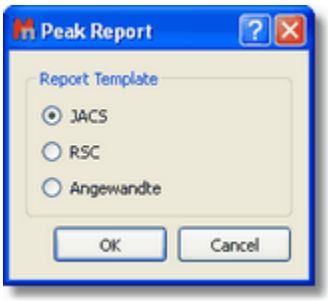
To delete an undesired peak, click on the **'Delete'** icon  of the Peak List, or select 'Delete Manually' on the Peak Picking scroll bar icon



By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* and also as a **'Peak List'** by clicking on the **'Report Special'** icon 

. The

user will be able to select the 'Peak Report Template' between JACS, RSC or Angewandte.



The user will be able to copy the **'Peak List'** report to the clipboard by selecting 'Copy Special' on the 'Report Special' scroll bar menu.

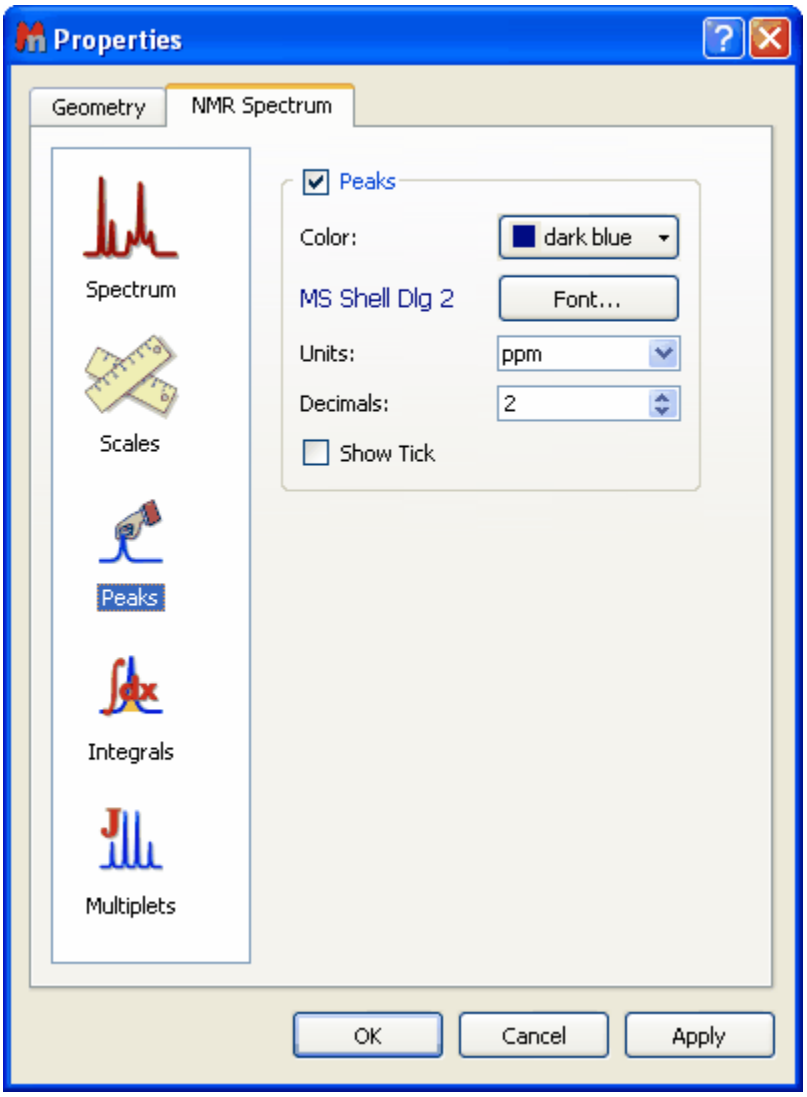
See also:

Peak Picking Properties

9.2.1 Peak Picking Properties

The user can modify the properties of the **Peak Picking** by following the menu 'Edit/Properties/Peak Picking' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Peak Picking* from the *pop-up* menu.

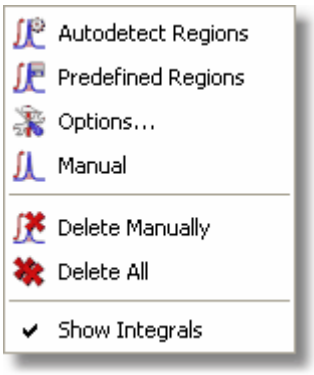
Then, the user can display or hide the peaks (or the Tick) by checking or unchecking the 'Peaks' (or the 'Show Tick') box. The user can also choose the color, the font and the units (ppm or Hz) of the 'peak picking label'; as well as the number of decimal figures to be shown on screen.



9.3 Integration

Spectral integration can provide invaluable quantitative information about a molecule as the integral of a spectrum indicates the number of nuclei which contribute to a given line (or set of lines).

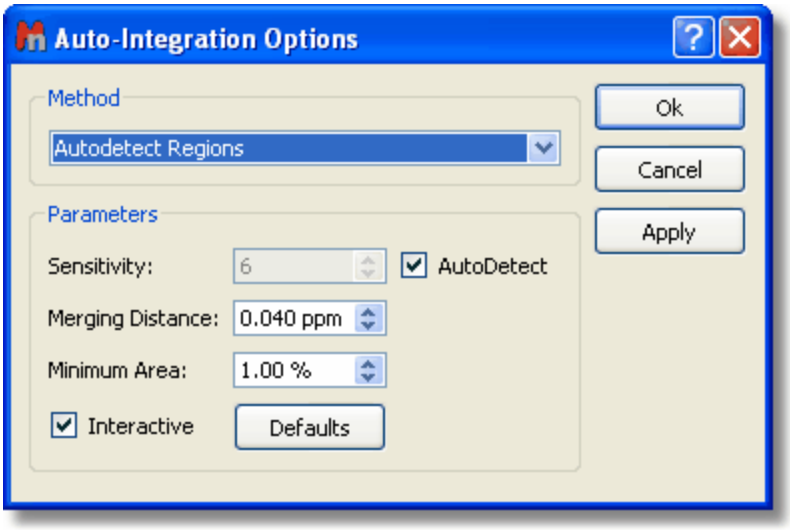
Mnova implements a series of integration algorithms for both 1D and 2D data sets. These algorithms can be accessed via the menu item 'Analysis/Integration' or via the scroll menu immediately to the right of the **'Integration'** icon  on the toolbar. This scroll menu will allow you to use Automatic, Predefined or Manual Integration:



Autodetect Integration

1D spectra

The program automatically determines the regions containing signals. The algorithm uses the following **options**:

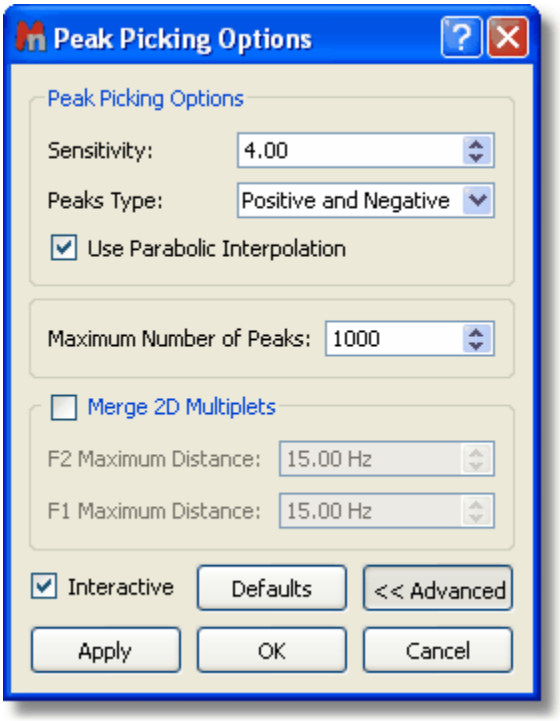


- **Sensitivity**: This value controls how small peaks can be detected. It is a kind of smoothing parameter so that large values will filter out noisy signals. Peaks of small intensity will also be discarded in the automatic integration. Reduce this value if some small signals of interest are not detected.
- **Merging distance**: This parameter is used to control the separation of integrals. Integral regions separated by less than this value will be merged into a single one
- **Minimum Area**: Integrals with a value smaller than a given percentage of the largest integral will be discarded

2D spectra

Automatic integration of 2D spectra is based on the 2D peak picking algorithm. If no peak picking has been applied when the automatic integration command is issued, **Mnova** will automatically apply a 2D peak picking on the background and then, for every peak detected, it will find the corresponding volumes.
Thus, the options that control the 2D peak picking procedure also apply to the 2D automatic integration algorithm.

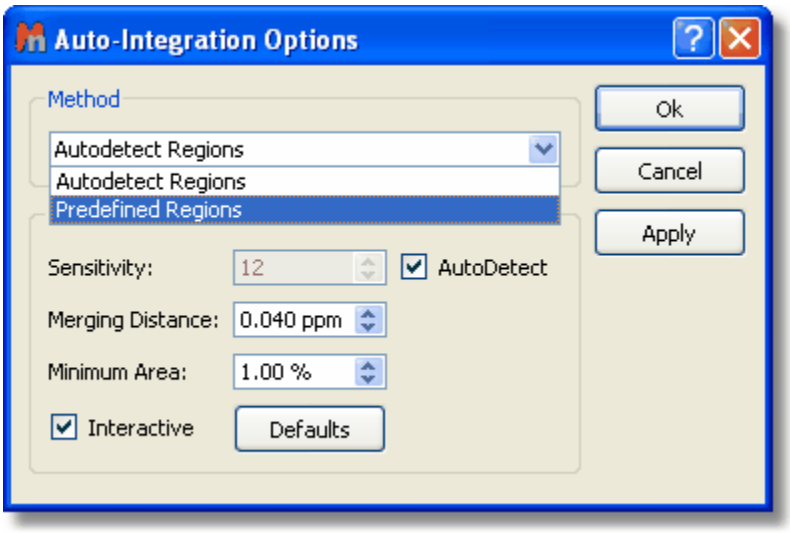
A series of Automatic Peak Picking Options can be controlled on the 'Peak Picking Options' dialog box, which can be accessed via the Peak Picking scroll menu (see the 'Peak Picking' section of this guide). The user can choose the noise factor (the higher the factor the less peaks will be picked), the peak type, whether to use parabolic interpolation, merging options for 2D multiplets, maximum number of peaks, etc. All these options can be evaluated interactively, so that their results can be viewed on screen in **real time**, by making sure the **'Interactive'** box is selected.



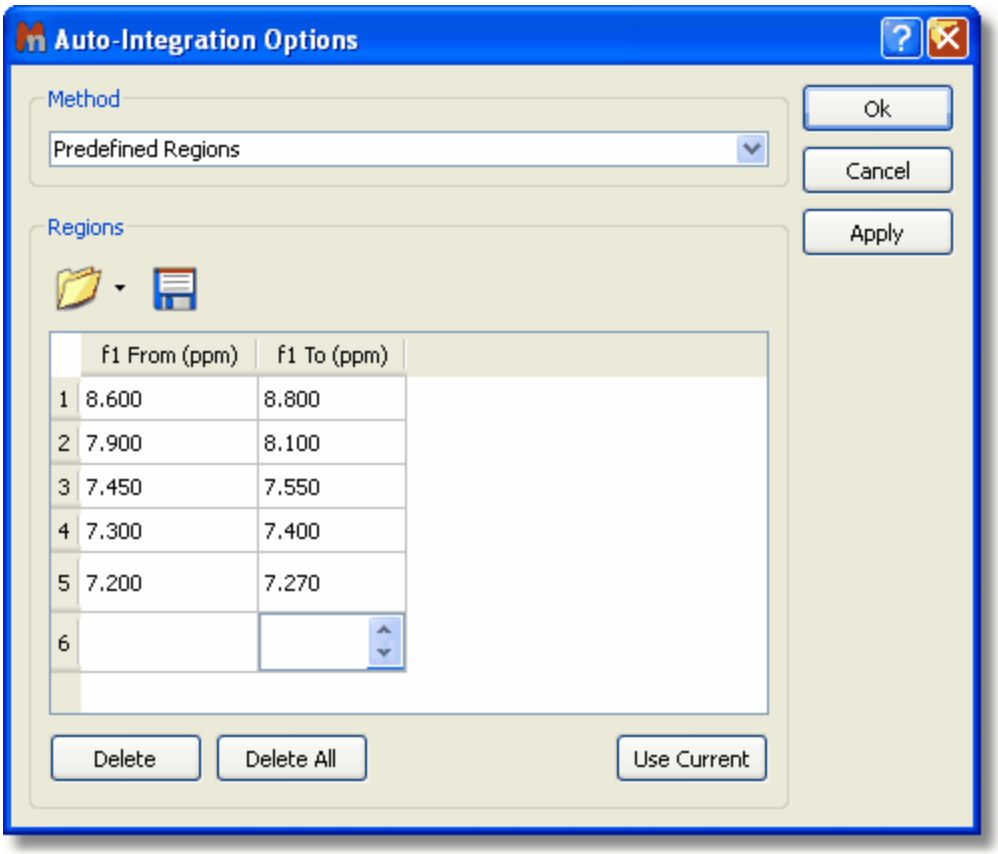
Predefined Regions Integration

The user will be able to predefine the integral ranges to automate the integral analysis. Just go through the following procedure:

1. Open the 'Integral Option' dialog box (by clicking on 'Options' in the 'Integration' scroll down menu) and select the Method: **'Predefined Regions'** as is shown in the picture below:



2. This will display a new dialog box, where the user will be able to select the desired ranges to apply the integration:

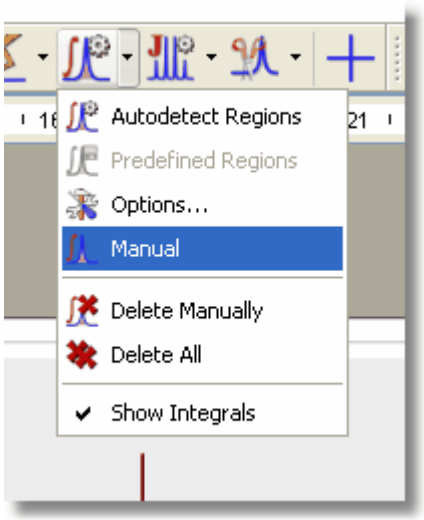


Clicking on OK, will apply the integrations to the current spectrum and keep the regions to apply further integrations to additional spectra by just clicking on 'Predefined Regions' in the Integral scroll down menu:

The user will be able to save these regions for later uses, by clicking on the 'Save Integral Regions' icon  which will be loaded by clicking on the 'Load Integral Regions' icon  . It is also possible to save the integral regions by following the menu 'File/Save as' and selecting 'MestReNova Integral Regions'.

Manual Integration

First, select integration mode by clicking on the Manual integration command as depicted below:



.

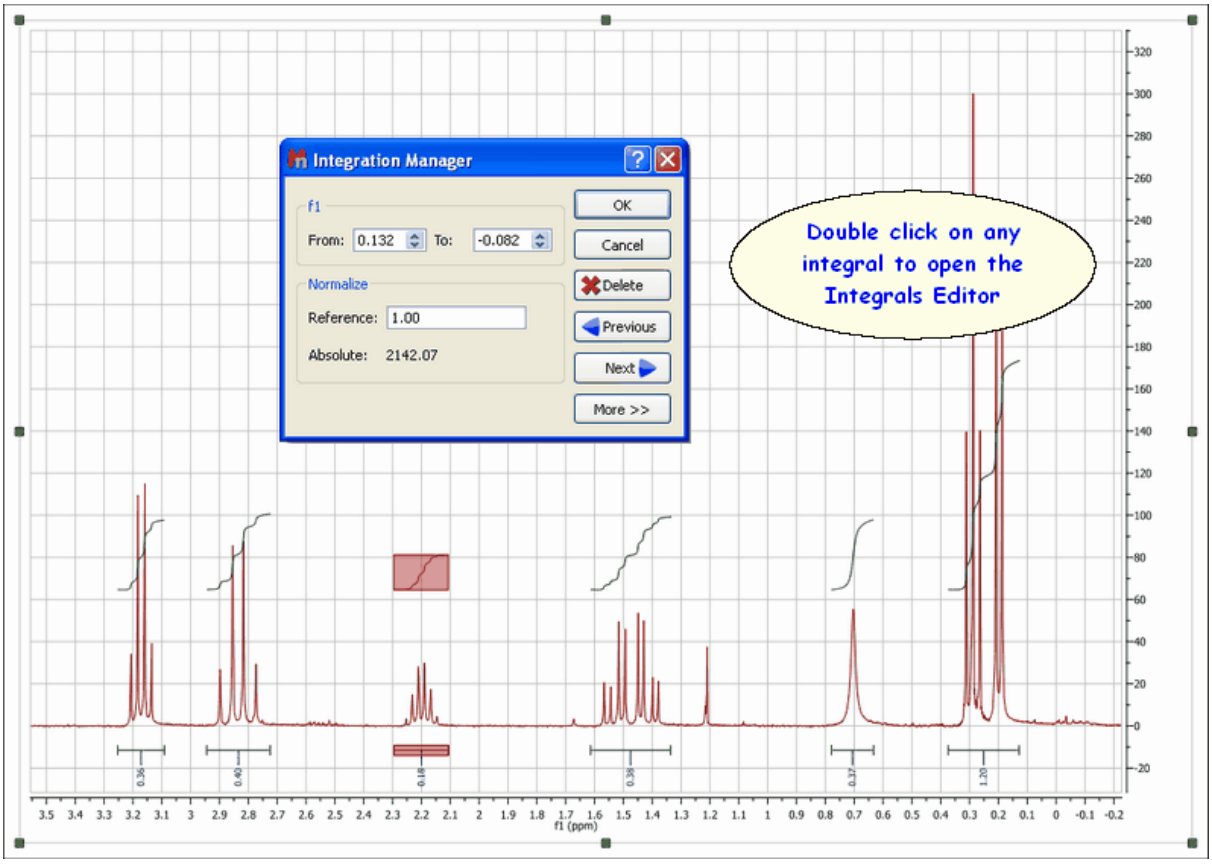
- Next, click and drag with the mouse to select the area to be integrated
- The first integral will be normalized to 1.0. All following integrals will be referenced relative to the first integral.

It is possible to save the integrals list as a 'text file' by following the menu 'File/Save as' and selecting 'MestReNova Integrals (*.txt)'

Integral Settings:

Setting integrals with Mnova is very easy and intuitive. All the integral curves are mouse-sensitive and they respond to usual mouse operations. If you want to move up or down all the integrals, just click and drag (with the left mouse button) over anyone of the integrals (notice that hovering the mouse over the integral will highlight it in red). If you keep pressed the SHIFT key at the same time, the integral curves will be resized.

The integral editor will be displayed by double clicking on any integral curve, as you can see in the figure above:



Likewise, if you press the right mouse button on an integral, you will get a pop-up menu with the following options:

Edit Integral: displays the Integration Manager dialog box.
Delete Integral: deletes the current integral.
Delete All: deletes all integrals.

The user can resize the limits of the integral by using the arrow buttons  or entering the desired value on the corresponding edit box:

Bear in mind that you are able to navigate over the integrals by using the **'Previous or Next'** icons.

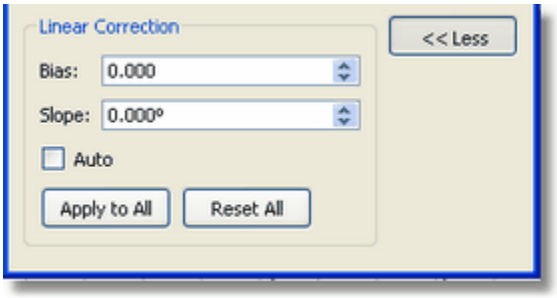
If you want to resize the height of the integral curve, just hover the mouse over one of the integral curves and hold down SHIFT, click on left mouse button and drag up or down (to increase or decrease the height).

If you want to normalize the integrals, just overwrite the desired value on the 'Reference edit box' (on the Integrals Editor') and press OK, all integrals will update with reference to the chosen one.

The user can also delete an integral one by one by clicking on the 'Delete' button: 

If you want to apply an integral correction or rather, a linear baseline correction just on the integral region, just click on 'More >>' button, 

this will display the 'Linear Correction' dialog box, where you will able to adjust the *Bias* and *Slope* parameters.



Use the controls for *Bias* and *Slope* correction as follows:

- Adjust the *Bias* parameter until the initial (left) part of the integral is flat.
- Adjust the *Slope* parameter to flatten the top (right) part of the integral curve.

The *Auto* box computes the *Bias* and *Slope* values automatically.
The button *Apply to All* is used to correct all the integrals simultaneously.

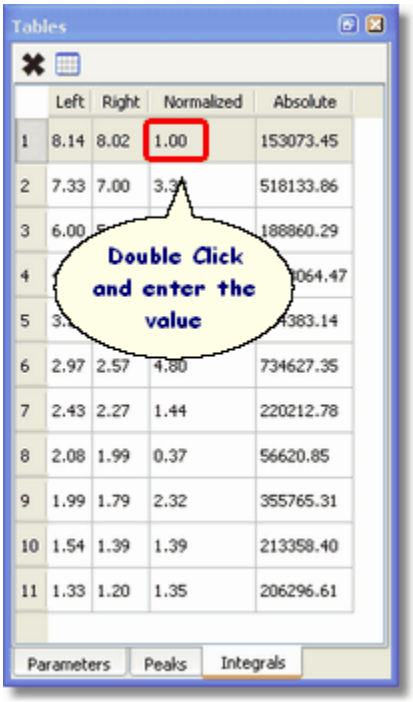
We strongly recommend to correct the baseline prior integration using any of the baseline correction algorithms.

Integral Table:

The Integrals Table (by following the menu: 'View/Tables and then select: Integrals') includes information about the integral range (in ppm) and the normalized and absolute values of each integral.

In this table the user can delete a desired integral by clicking on the **'Delete'** icon  . The user can also normalize the integrals by double clicking on the normalized value of the integral which the user would like to set (in the red square on the capture below). Once it is highlighted, the user has to overwrite the desired value, press return, and all integrals will update with reference to the chosen one.

This method is an alternative way to normalize integrals. The user can obtain the same result by double clicking directly on the integral curve on the spectrum, as we have explained a few lines above.



See also:

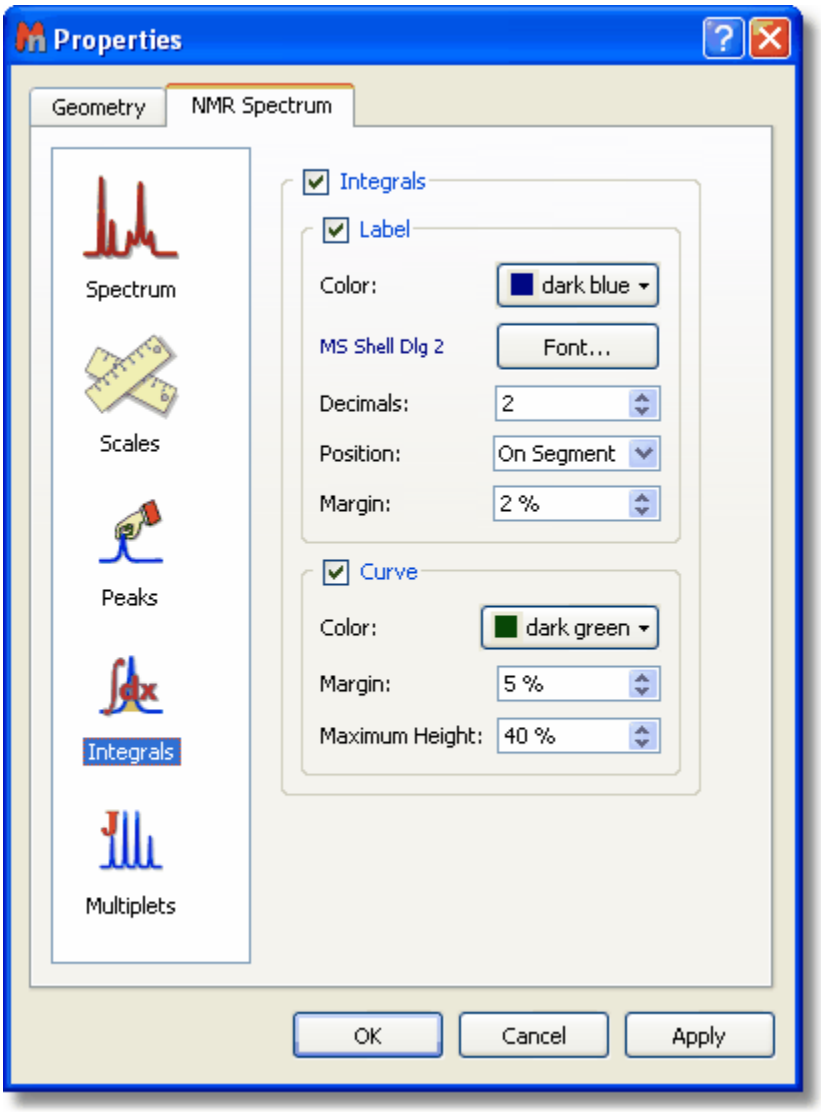
Integral Properties

9.3.1 Integral Properties

The user can modify the properties of the integrals by following the menu 'Edit/Properties/Integrals' or by double clicking the left mouse (or pressing the right mouse) button on the spectrum display and selecting *Properties/Integrals* from the *pop-up* menu.

The options are different in 1D and 2D-NMR:

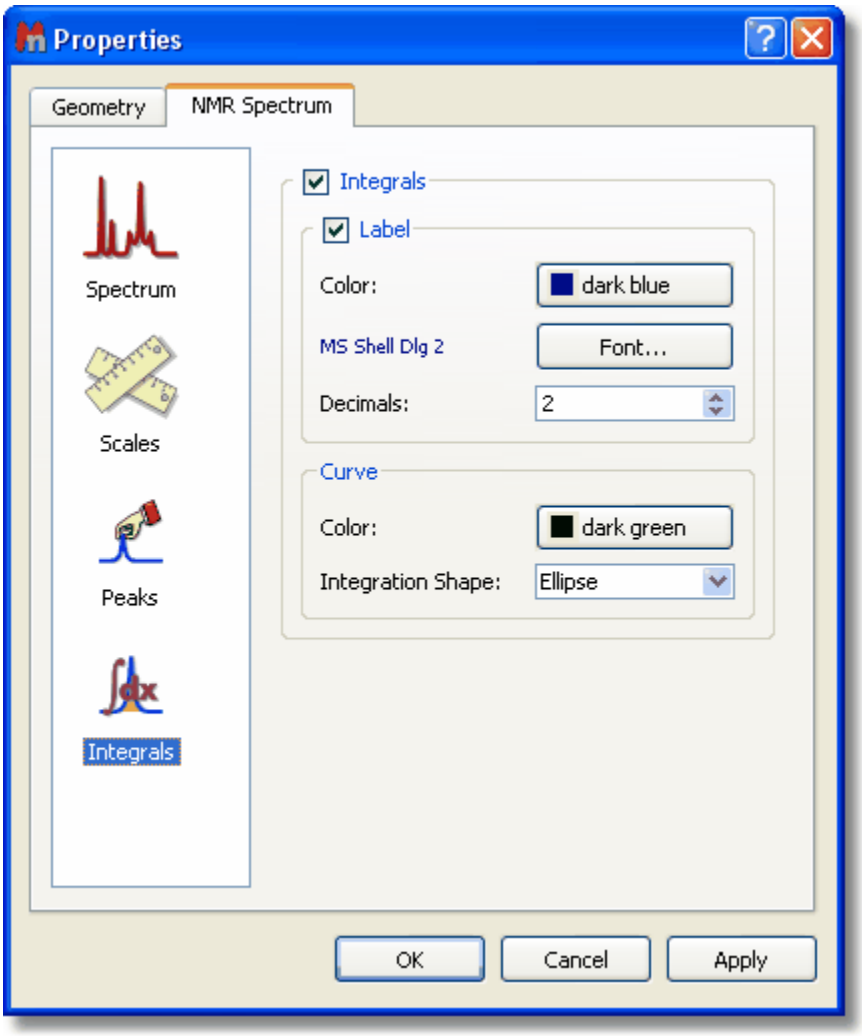
1D Spectra



Integrals: Check/Uncheck this option if you want to display/hide the integrals (labels + curves)
- **Label**: Check/Uncheck this option if you want to display/hide the integral labels
- **Color**: Select the font color
- **Font**: Select the font type
- **Decimals**: Select the number of decimal figures to be shown on screen
- **Position**: Use this option to show the integral label on the curve or on the integral segment

Curve: Check/Uncheck this option to show/hide the integral curves
- **Color**: Select the curve color
- **Margin**: distance (in % with respect to the spectrum item height) from the top
- **Maximum height** of the integral curve.

2D Spectra



Integrals: Check/Uncheck this option if you want to display/hide the integrals (labels + curves)
- **Label**: Check/Uncheck this option if you want to display/hide the integral labels
- **Color**: Select the integral label color
- **Font**: Select the font type
- **Decimals**: Select the number of decimal figures to be shown on screen

Curve: Check/Uncheck this option to show/hide the integral curves
- **Color**: Select the integral color
- **Integral shape:** Select the integral shape (rectangular or elliptic) to be used for 2D integration

9.4 Multiplets analysis

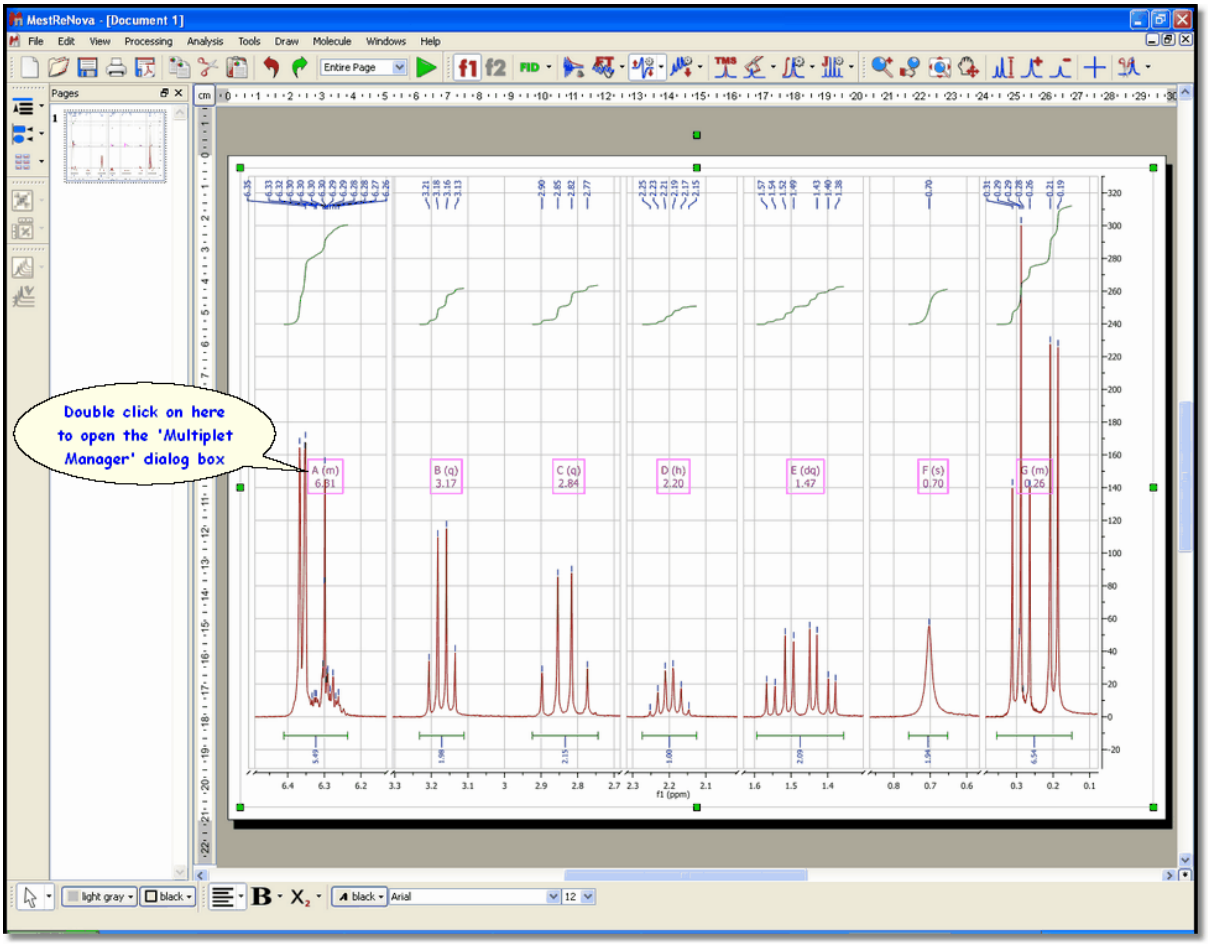
Multiplet Analysis (Not available in Mnova Lite):

Mnova incorporates an intuitive and powerful multiplet analyzer. The user will carry out an automatic multiplet analysis by clicking on its respective icon, placed on the analysis toolbar menu  , or by following the menu 'Analysis/Multiplets Analysis/Automatic'. Bear in mind that when you select an automatic multiplet analysis, Mnova will also apply automatic peak picking and integration, so you may need to set the sensitivity in both cases (or even the merging distance of the integrals) to obtain good results.

If you select a 'Manual Multiplet Analysis', please check also the sensitivity used for peak picking, or apply a 'Manual Peak Picking' to obtain a good result. You can also select the peaks by using the ' Peak by Peak' feature. Note that, by default, Mnova will find the peak top by using parabolic interpolation. To pick a peak at an arbitrary position (e.g. not at a local extreme or a shoulder), hold down <Shift> and click.

The user can also apply a manual integration followed by an automatic multiplet analysis.

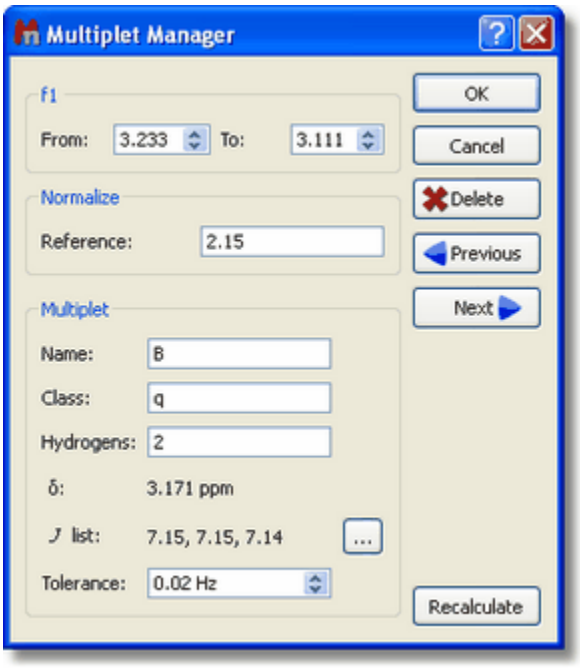
In the capture below, you will see the result of applying an automatic multiplet analysis:



The user will also be able to set the multiplet parameters by double clicking on the magenta square containing the multiplet information. This will display the 'Multiplet Manager' dialog box, where you will be able to modify the integral limits as well as to normalize its value. In this dialog box, you will also be able to change the multiplet parameters (name, class, number of hydrogens and the coupling constant tolerance limit). The number of hydrogens will be rounded (up or down) by default with respect to the integral value. Bear in mind that, if you change the integral value, the number of hydrogens of the multiplet will be altered, but this will not happen in the opposite way, that is, changes in the number of hydrogens of the multiplet will not affect the integral value.

As you see in the capture below, you can also delete a multiplet by clicking on the 'Delete' button 

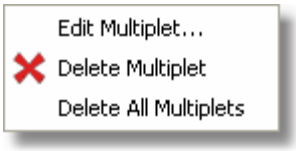
or navigate through multiplets by using the 'Previous' or 'Next' buttons.



The 'Recalculate' button is used to obtain the new result after having made changes in the spectrum (as a 'refresh' button), or to recover the initial values (useful if you made changes in the 'Multiplet Manager' edit box but you do not remember them).

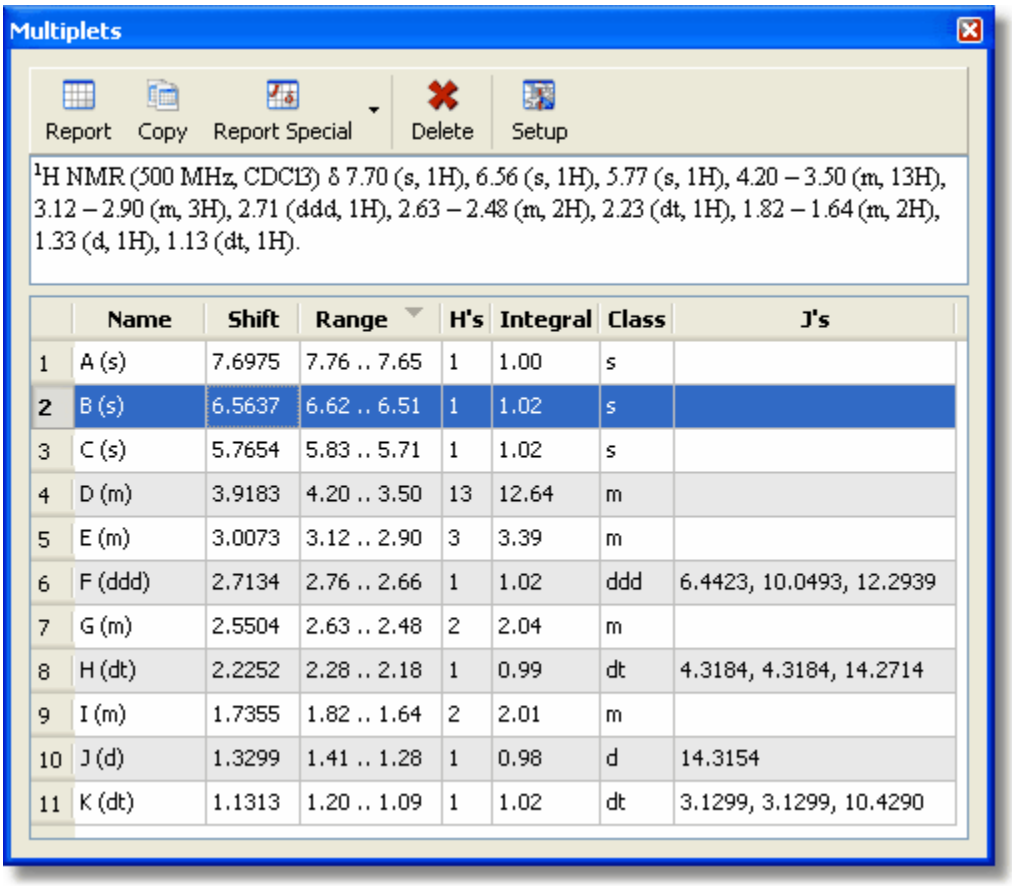
The user will also be able to add or delete a coupling constant by clicking on  . After that, the 'J´s Editor' dialog box will be displayed:

Likewise, if you press the right mouse button on a multiplet box, you will get a pop-up menu with the following options:



Edit Multiplet: displays the Multiplet Manager dialog box.
Delete Multiplet: deletes the current multiplet.
Delete All Multiplets: deletes all multiplets.

The user will be able to obtain the 'Multiplets' table by following the menu 'View/Tables'. The 'Multiplets' table includes information about the name (by default Mnova will have named the multiplets in alphabetical order, but the user is able to change this order in the 'Multiplet Manager'), the chemical shift (ppm), the range (ppm), number of hydrogens, integral value, type of multiplet and coupling constant values (Hz).

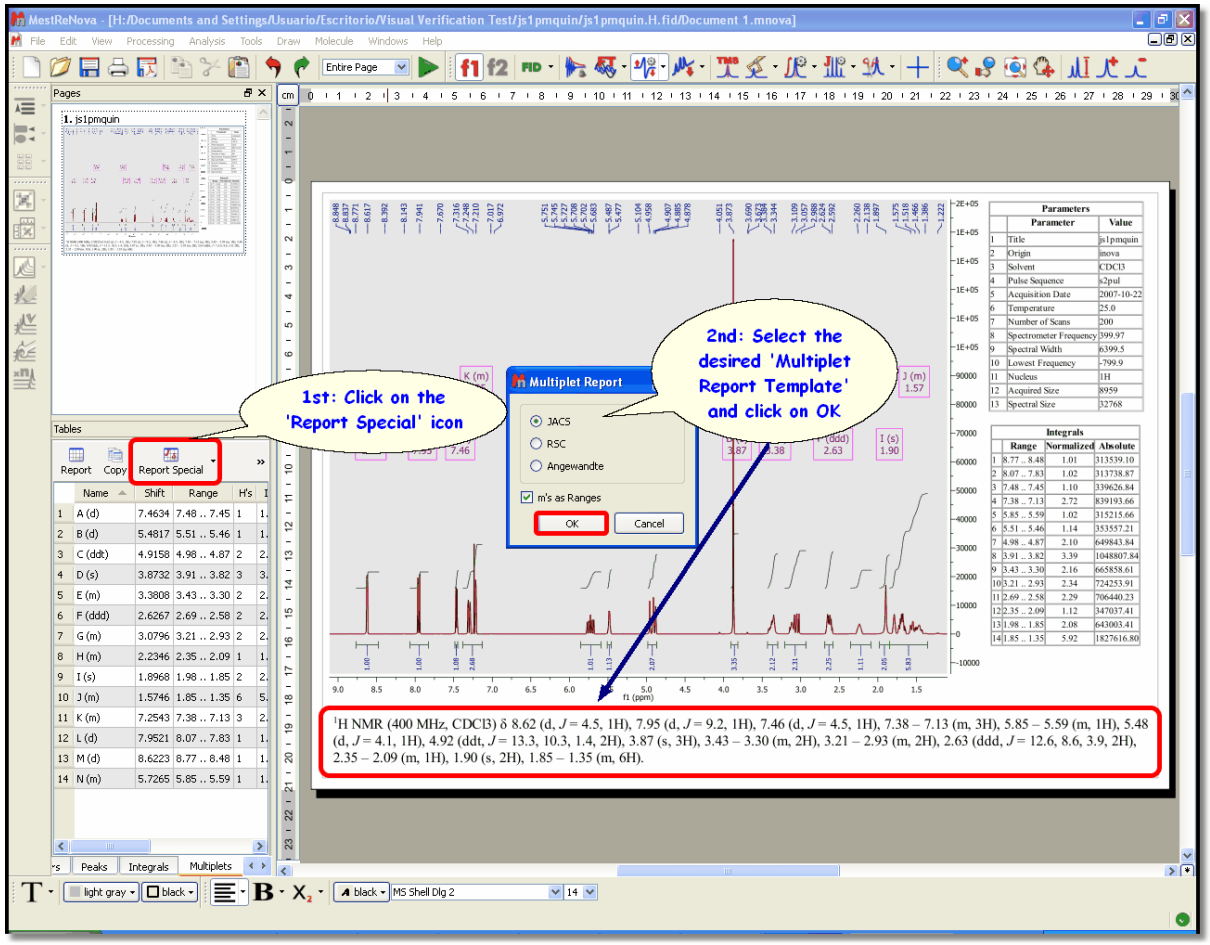


	Name	Shift	Range	H's	Integral	Class	J's
1	A (s)	7.6975	7.76 .. 7.65	1	1.00	s	
2	B (s)	6.5637	6.62 .. 6.51	1	1.02	s	
3	C (s)	5.7654	5.83 .. 5.71	1	1.02	s	
4	D (m)	3.9183	4.20 .. 3.50	13	12.64	m	
5	E (m)	3.0073	3.12 .. 2.90	3	3.39	m	
6	F (ddd)	2.7134	2.76 .. 2.66	1	1.02	ddd	6.4423, 10.0493, 12.2939
7	G (m)	2.5504	2.63 .. 2.48	2	2.04	m	
8	H (dt)	2.2252	2.28 .. 2.18	1	0.99	dt	4.3184, 4.3184, 14.2714
9	I (m)	1.7355	1.82 .. 1.64	2	2.01	m	
10	J (d)	1.3299	1.41 .. 1.28	1	0.98	d	14.3154
11	K (dt)	1.1313	1.20 .. 1.09	1	1.02	dt	3.1299, 3.1299, 10.4290

The user can paste the 'Multiplets' table on the spectrum by clicking on the **'Report'** icon  and also can delete a multiplet from this table by using the **'Delete'** icon  when the undesired multiplet is highlighted.

Finally, the user will be able to obtain the multiplet report list by clicking on the **'Report Special'** icon 

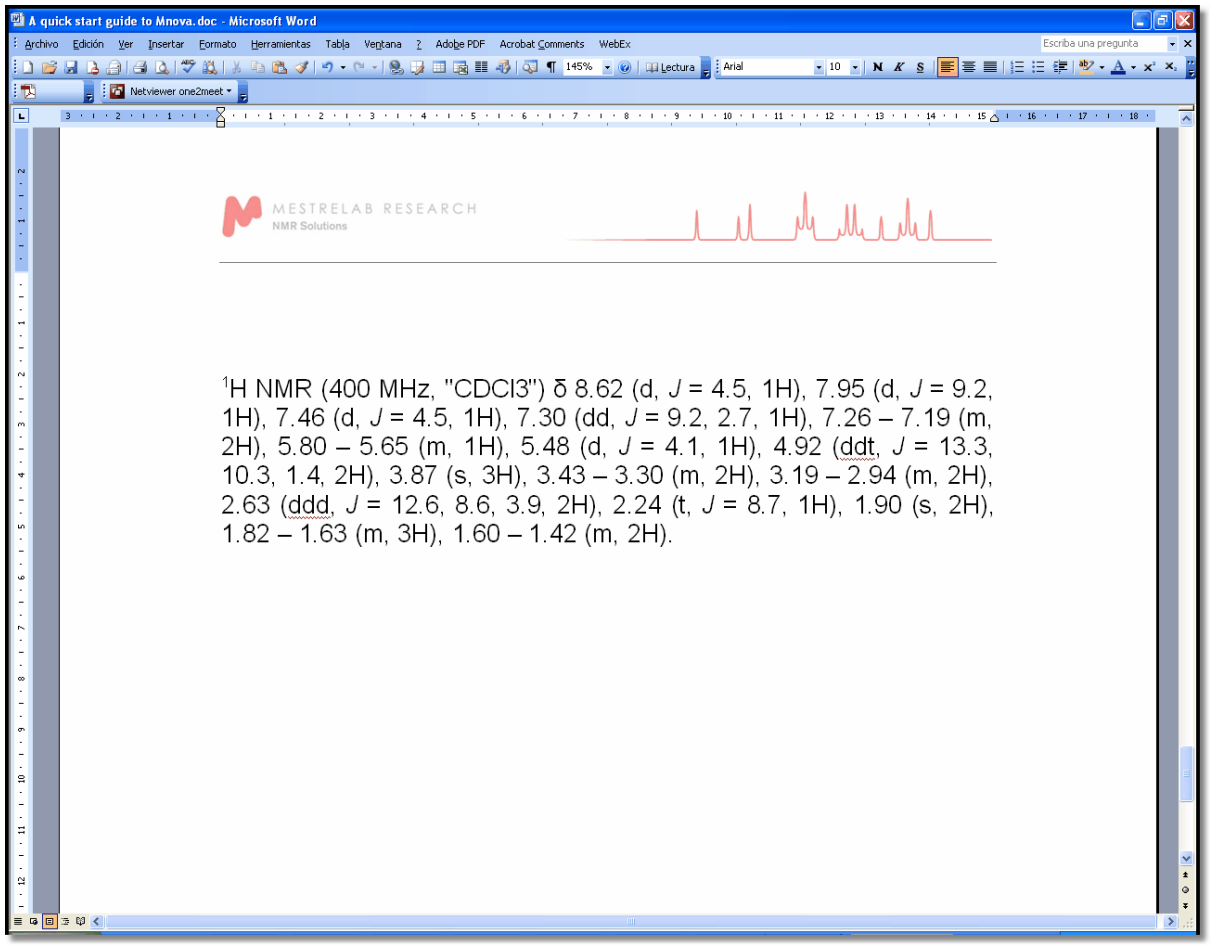
. This will display the 'Multiplet Report' dialog box, which will allow the user to select the desired template (JACS, Royal Society of Chemistry and Angewandte).



After that, you will obtain in your spectrum the multiplet report as it is shown in the picture above.

The user will be able to copy the multiplet report to the clipboard by selecting 'Copy Special' on the 'Report Special' scroll bar menu:

And then paste the multiplet report on another document just by using Ctrl+V or Cmd+V.



The user will be able to change the multiplet report templates; by following the menu 'File/Edit Script' and opening the corresponding script file ('MultipletReporter.qs' in this case):

```
// This function defines JACS-like multiplet report.
// To customize report, edit this class or implement another one.
function JACSMultipletReporter()
{
        MultipletReporter.call(this);

        this.onlyElementName=false;
// Define font size and font family
        this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";
```

```
        this.
nucleusTemplate="%1";
// Report header. %1 will be
replaced with nucleusString,
%2  with frequency, %3 with
solvent
        this.header  =  "%1
NMR (%2 MHz, %3) &delta;
";

// Multiplet templates. %1 -
delta, %2 - category, %3 -
nH
        this.reportRange  =
true;  // set to true to get
multiplet range instead of
delta.
        this.
withoutJsTemplate  =  " %1
(%2,  %3H)";  //  multiplet
template without J's
        this.withJsTemplate
= " %1 (%2, %4, %3H)"; //
 multiplet template with J's
        this.rangeTemplate
= "%1 &ndash; %2";

// J's list template. %1 - list of
J's
        this.jListTemplate  =
"<i>J</i> = %1";

        this.jPrecision = 1; //
J's precision
        this.deltaPrecision =
 2; // delta precision
        this.mSeparator = ",
"; // multiplet separator
        this.jSeparator = ", "
; // J's separator

        this.start = this.font;
        this.end = ".</font>";
}

JACSMultipletReporter.
prototype        =        new
MultipletReporter();
JACSMultipletReporter.
prototype.toString        =
function()      {      return
"JACSMultipletReporter()"; }

(...)
```

The user will be able to change the multiplet report template to obtain the desired multiplet report; for

example:

this.onlyElementName=false; changing false with true, we will obtain only the element name without the atomic mass (For example: H, C instead of ^{1}H, ^{13}C).

The function: **this**.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">"; will define the font size and the font family of the multiplet report.

The line: **this**.header = "%1 NMR (%2 MHz, %3) δ "; is used to print the header of the report, where %1 will be the nucleus (H or C), %2 the frequency of the spectrometer (in MHz), and %3 the solvent, followed by a delta symbol (δ). For example: "^{1}H NMR (500 MHz, CDCl3) δ".

The sentence: **this**.reportRange = true is used to obtain the multiplet range instead of the chemical shift.

The functions: **this**.withoutJsTemplate = " %1 (%2, %3H)" and **this**.withJsTemplate = " %1 (%2, %4, %3H)" are used to customize the appearance of the multiplet report by changing the positions of %1, %2, %3H, or %4 (where, %1 means: chemical shift; %2 means: type of multiplet (s, d, t, etc); %3H means: number of hydrogens and %4 means the coupling constant value). As you can see, the first line shows a multiplet without coupling constants, (while the last line shows a multiplet with coupling constants).

So, if you need to obtain something like this (japanese format):

1H NMR (300 MHz, Solvent) δ ppm 6.43-6.22 (1 H, m), 3.17 (1 H, q, J = 7.15 Hz) etc…

You should modify both lines, as you can see below:

```
this.withoutJsTemplate = %1 (%3H, %2)";
this.withJsTemplate = " %1 (%3H, %2, %4)";
```

If you prefer to obtain something like this:

1H NMR (300 MHz, Solvent) δ ppm 1.23 (d, *J* = 1.2 Hz, 3 H), etc…

Just replace the original lines with:

```
this.withoutJsTemplate = %1 (%2, %3H)";
this.withJsTemplate = " %1 (%2, %4, %3H)";
```

To obtain the coupling constant symbol in normal instead of *'italic'*, just modify the script by removing the italic format (J). If you prefer to obtain it in "**bold**" just type:

```
this.jListTemplate =  "<b>J</b> = %1";
```

The following paragraph will be used to customize the appearance of the coupling constants list:

```
sion= 1; //J'sprecision this.deltaPrecision= 2; //deltapre
```

```
cision this.mSeparator = "','";

//multiplet separator

this.jSe
```

```
parator="','";//J'sseparator
```

The first line is used for the precision of the coupling constants values, the second will be used for the precision of the chemical shift and the remaining two lines will print the separation between the multiplets and the coupling constants values.

If you need to obtain the coupling constants in descending order, replace 'true' with 'false' in the following line:

```
var jList= new JList(mu
```

```
l
t
i
p
l
e
t
.
j
L
i
s
t
(
)
)
;
j
L
i
s
t
.
s
o
r
t
(
t
r
u
e
)
;
```

If you want to obtain the multiplet chemical shifts in ascending order, just replace the 'false' with 'true' in the script:

```
var multiplets = new Multiplets (spectrum.multiplets()); // get multiplets from spectrum
jList.sort(true);
```

To obtain the multiplet range in ascending order, just replace the rangeMin with rangeMax in the below line of the script:

```
shiftStr = this.rangeTemplate.argDec(multiplet.rangeMax, 0, 'f', this.deltaPrecision).argDec(multiplet.rangeMin, 0, 'f', this.deltaPrecision);
```

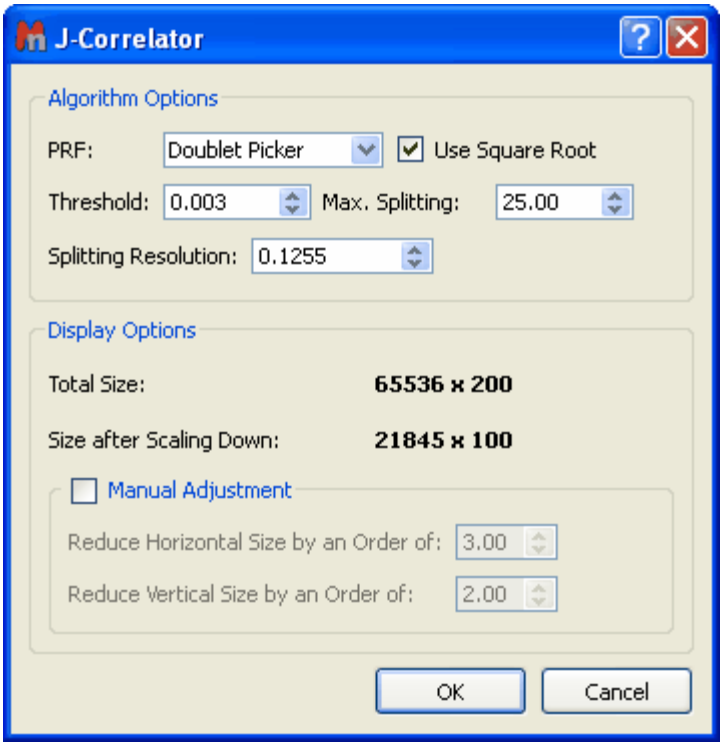
9.5 J-Correlator

J-Correlator (Not available in Mnova Lite): is a new method for detecting spin connetivities in ^{1}H NMR spectra. The main goal of this method is to convert a 1D spectrum into a 2D-like plot in which it is easy to correlate multiplets containing the same **splitting**. Its main purpose is to rule out couplings between

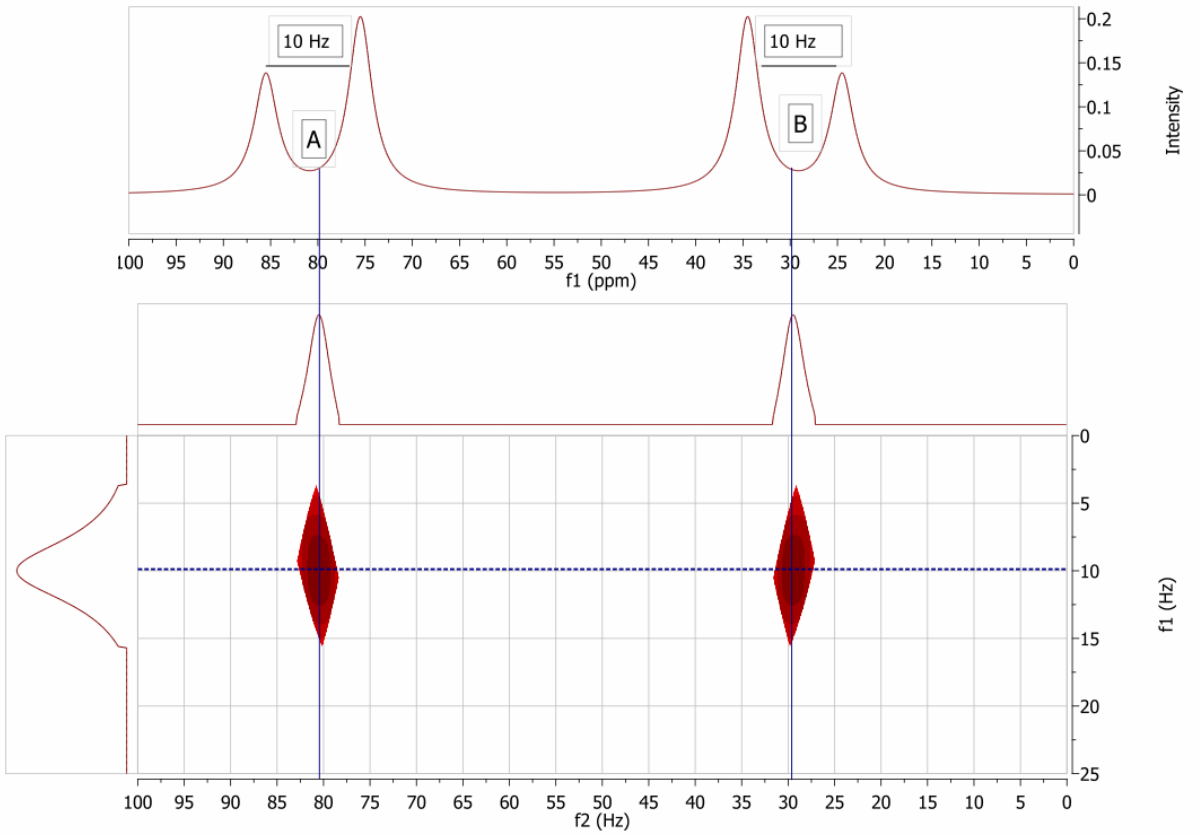
multiplets. It is suitable for human inspection.

The basic idea is to generate a 2D plot from a 1H NMR spectrum in such a way that the horizontal dimension conveys the chemical shifts whereas the vertical one represents the separation between peaks in the spectrum (***splittings*** from now on). Basically, it is a special kind of 2D 1H - D experiment (where D = splitting).

Just load your ^{1}H NMR spectrum and follow the menu 'Tools/J Correlator' to display the J Correlation dialog box to select the desired algorithm and display options:



As usual, a picture is worth a thousand words:



In this figure we have simulated 1D spectrum with two coupled spins, A = 80 Hz and B = 30 Hz. JAB = 10 Hz.

In the new 2D plot (JCorr from now on) we can see two cross peaks at the positions of A and B respectively and at the same d-trace (vertical dimension or splitting dimension).

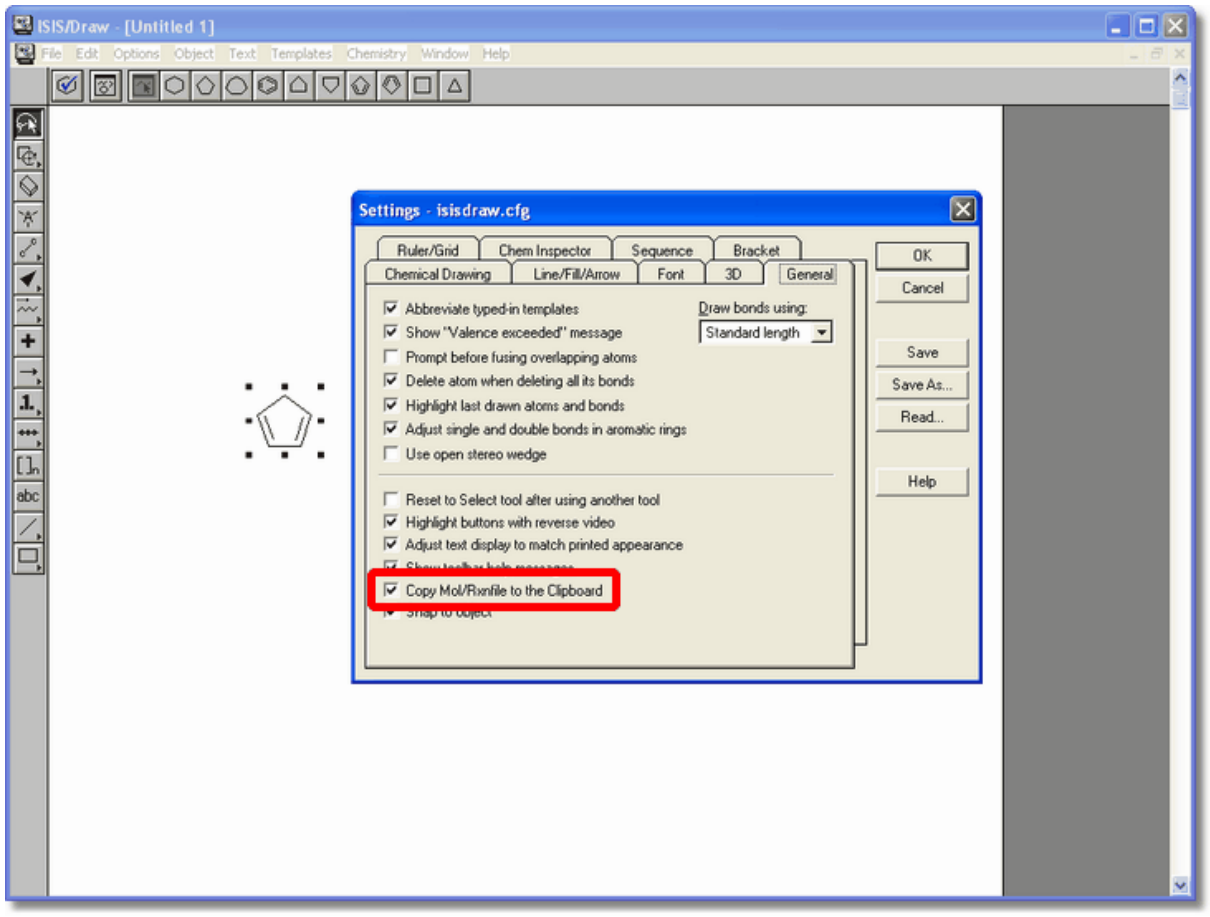
It's very important to remember that what we see in the JCor plot are just splittings, not Coupling Constants, even though in some cases these splittings can coincide with coupling constants.

9.6 Molecular Structure

Mnova allows the user to import Molecular Structures, and to interact with them.

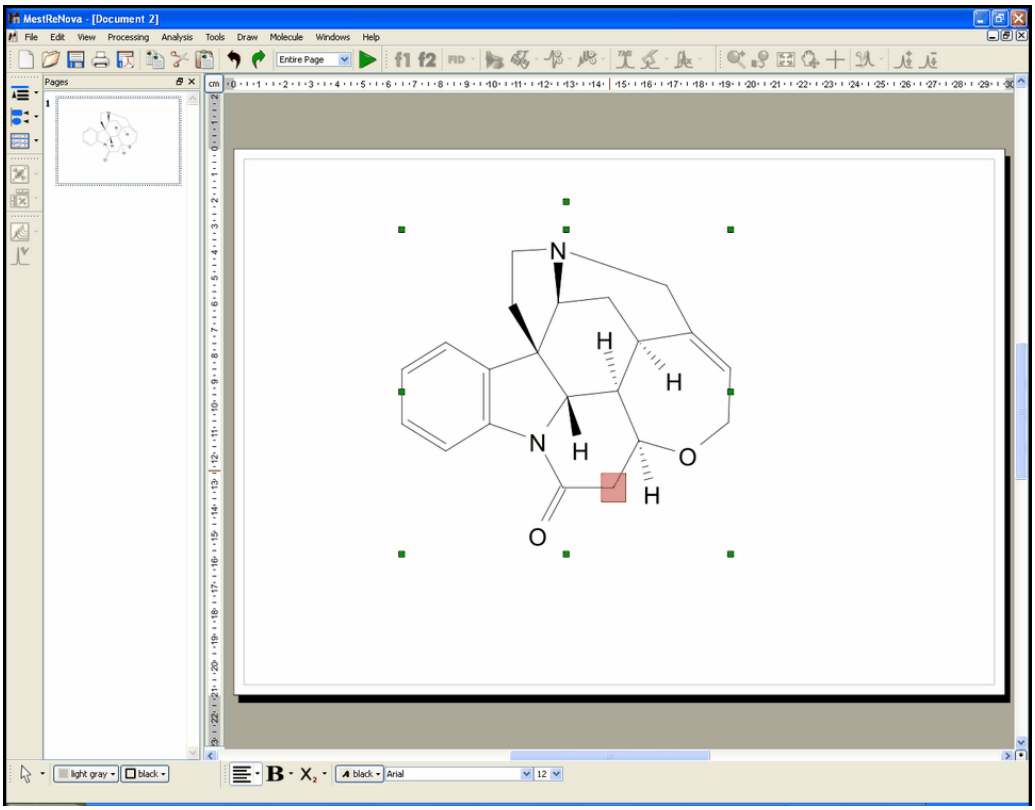
Currently, molecular structures can be imported as a **.mol** file by simply using the Open command exactly in the same way as this would be applied to a spectrum. Support to 'copy & Paste' or 'drag & drop' operations are currently limited to **ACD ChemSketch,** but extension to **ChemDraw** and **IsisDraw** will be included in the oncoming release version. Bear in mind, that you are actually able to copy&paste molecular structures from **ChemDraw** and **IsisDraw** but not drag&drop them.

If you are using IsisDraw, please keep in mind that you will need to select the capability to copy your molecules as mol files in the 'Options/General Settings' menu.

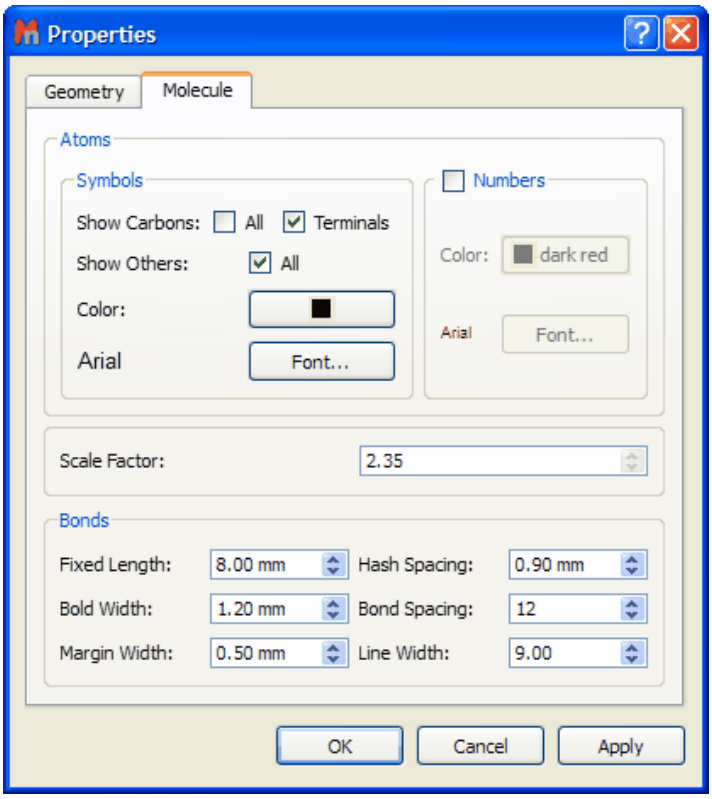


The molecular structure can be handled (resized, moved, aligned, copied, pasted, etc) exactly in the same way as all other Mnova objects. Molecular structures can be imported in a separate page or into a page already occupied by another object, such as for example its corresponding spectrum.

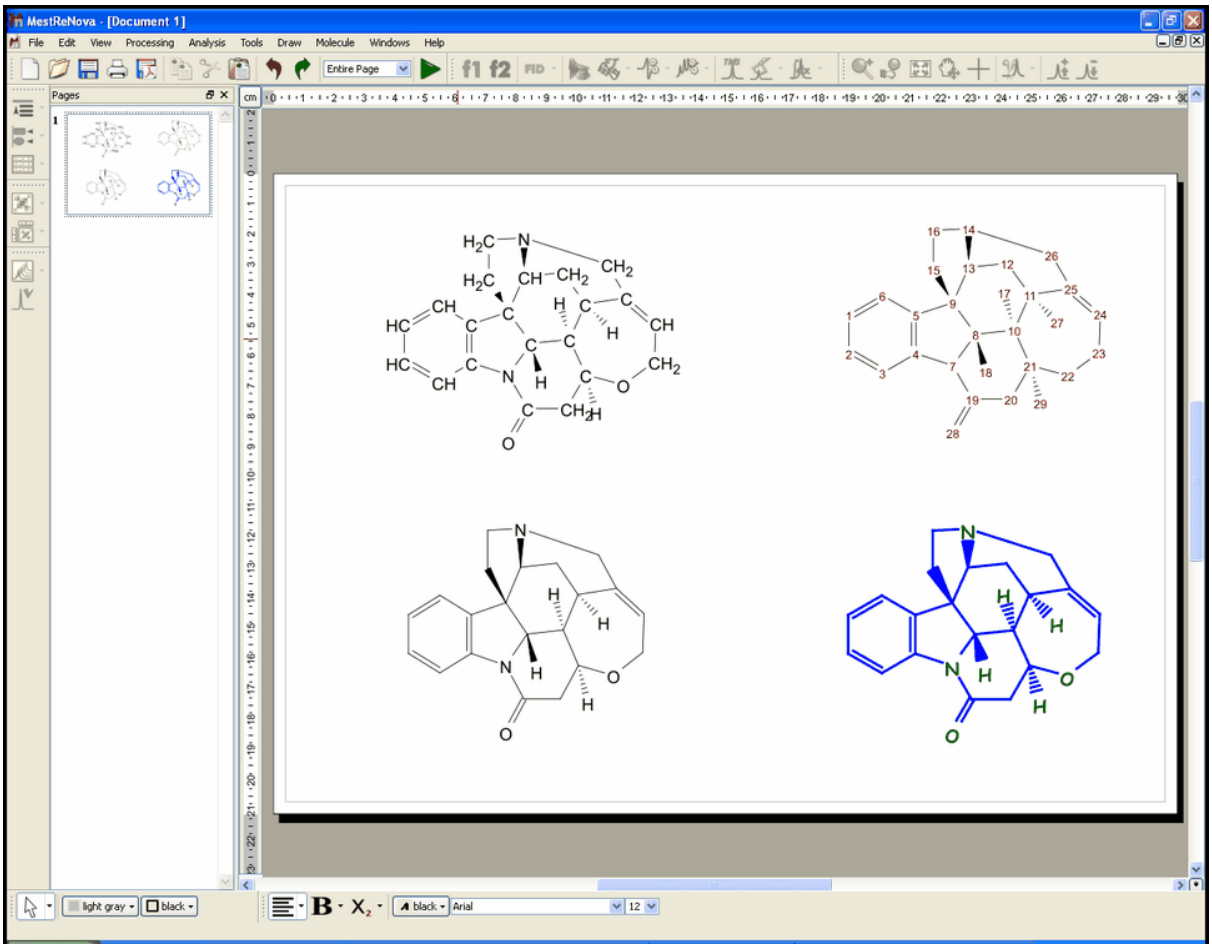
The first thing you will notice when interacting with molecular structures, is the fact that atoms will become highlighted in a red transparent box when the mouse hovers over them. Clicking on an specific atom will make it the active atom and highlight in a red box with pink background, as can be seen in the figure below. This atom selection will become essential when interacting with the molecule, as will be explained in the following chapters.



Mnova will allow you to customize the appearance of the molecule, by using the object 'Properties' dialog box. This box can be accessed by double clicking on the molecule, or by right clicking and then scrolling down to 'Properties'.



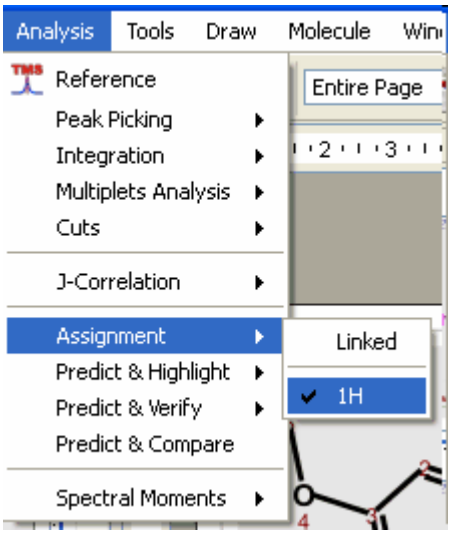
The 'Properties' dialog box will allow you to customize the size of the molecule, its angle with respect to the paper, and all representation properties, such as whether or not to show explicit carbon and hydrogen atoms, whether to show atom numbers, the length and spacing of bonds, etc. It is therefore possible to achieve many different representations of the same molecule, as the figure below shows.



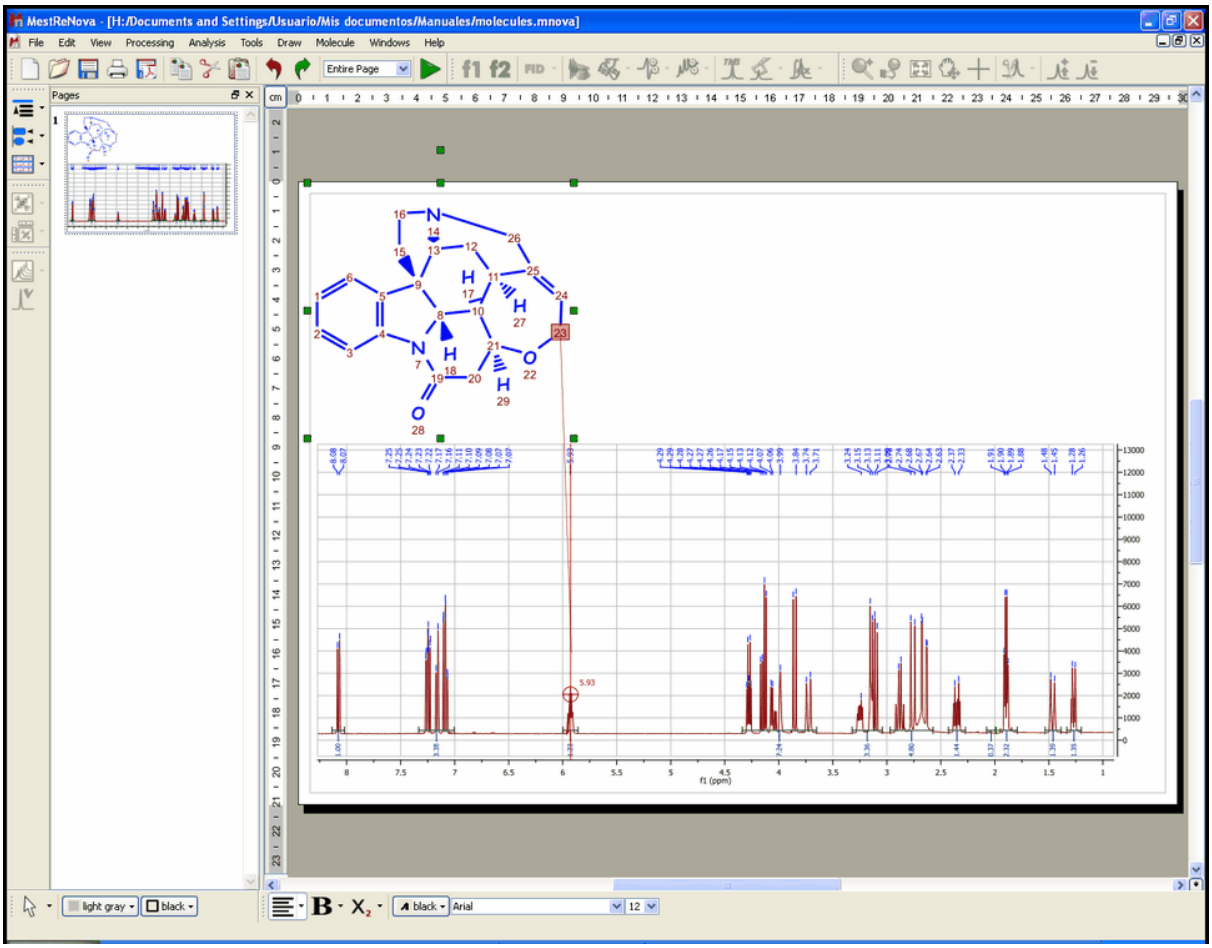
9.6.1 Molecular Structure Assignment

It is now possible within Mnova to carry out atom to peak assignments, both to 1D and 2D spectra **(Please note that this feature is not completely finished in this version and that it will be greatly enhanced for the oncoming release version)**

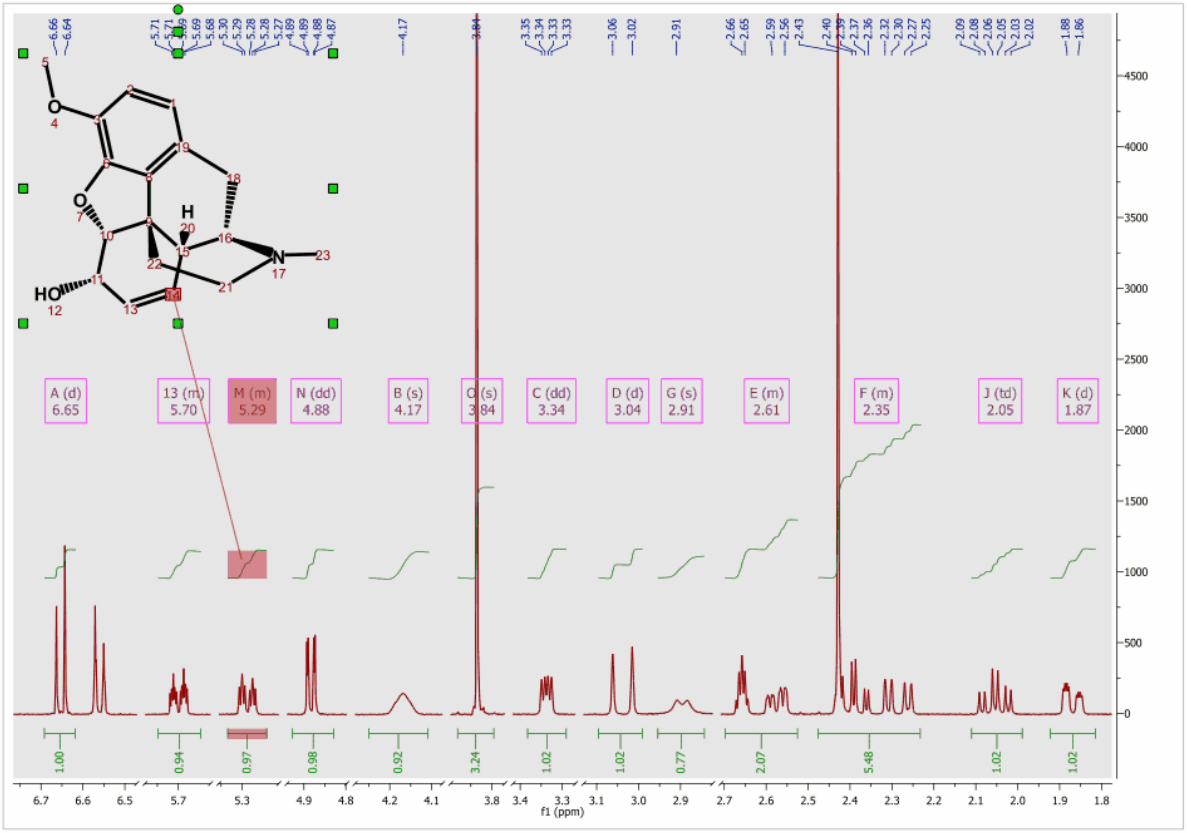
Mnova provides a very simple interface to assign your molecule. Just highlight the molecule and follow the menu 'Analysis/Assignment' to select the corresponding assignment.



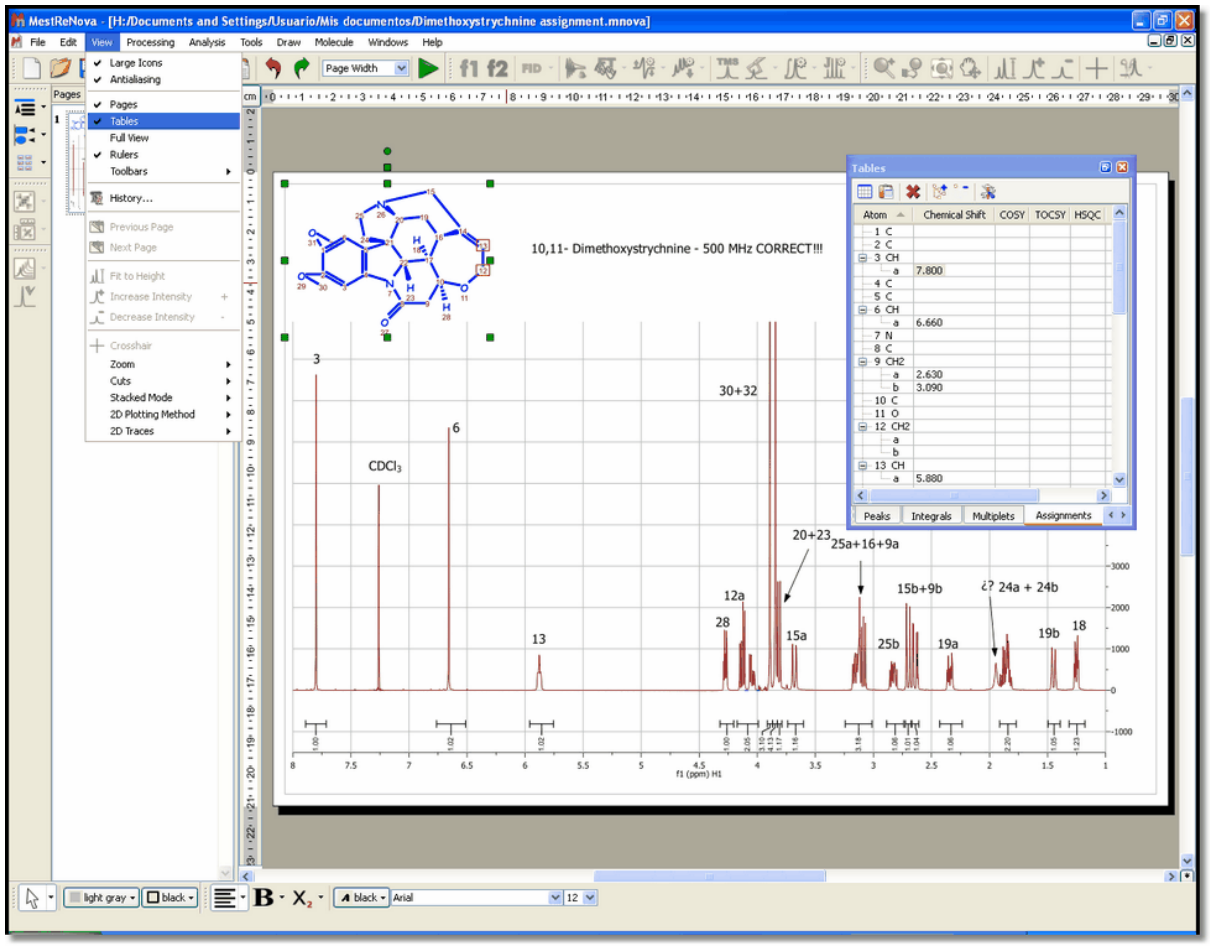
Finally, left click on the desired atom, so that it becomes highlighted in a red box and drag the mouse without releasing it to your desired peak and click again on it. This peak will now be assigned to the atom. The user can also assign a multiplet, by clicking, dragging and releasing the mouse over the desired multiplet (or even over the integral curve or the multiplet box). Once the assignment has been made, hovering the mouse over the atom will highlight the corresponding peak in the spectrum, and hovering the mouse over the peak will highlight the corresponding atom on the molecular structure.



It is also possible to assign a multiplet by dragging the mouse to the '**multiplet box**' (when assigning to a multiplet, its name is replaced with the atom number) or the '**integral curve**', as you can see in the picture below:



Once you have finished with the assignation you can visualize the assignment list by following the menu 'View/Tables/Assignments' (with the spectrum highlighted) where you will be able to edit any entry by double clicking on it.



A few notes on Molecular Structure Assignment

- ✓ The current release version of Mnova allows visualization or exportation of assignments in a table or reporting format. However, this functionality will be available in the oncoming release version.
- ✓ We plan for the oncoming release of Mnova to also implement the functionality to carry assignments through different data sets in different pages (thus, for example, it would be possible to assign a 2D spectrum and for those assignments to be applied automatically to the corresponding 1D spectra).

See also:

How to carry out Assignments?

9.7 NMR Prediction

NMR Prediction: The handling of molecular structures within **Mnova** also allows the user to carry out **spectral predictions**. Although the process described here could be applied to predictions by means of any commercially available or in-house prediction package, currently **Mnova** will carry out predictions by using the prediction application **NMRPredict**, by **Modgraph Consultants Ltd**.

A few notes on NMR Prediction

- ✓ Our intention is for **Mnova** to support predictions with a range of prediction packages as wide as possible.
- ✓ Currently, predictions can be carried out by using **NMRPredict**, the prediction software by Modgraph Consultants Ltd. An evaluation version of NMRPredict can be downloaded from http://www.modgraph.co.uk
- ✓ **NMRPredict** comes into two different flavours:

  NMRPredict Desktop
  NMRPredict server-based

- ✓ Customers who have their own in-house prediction package are welcome to contact us to discuss a similar, seamless integration of their own prediction package. Please write to us at support@mestrec.com.
- ✓ Please note that NMRPredict is a different software package to Mnova and therefore, in order to carry out predictions via this package, a license of NMRPredict must be held separately, once the NMRPredict evaluation has expired.

Mnova incorporates a fast simulation mode of ^{1}H and ^{13}C-NMR spectra, called **NMRPredict Desktop.** This application does not need to connect itself to a server for the spectra prediction. Besides, it makes uses of the fastest prediction algorithms (**Neural Network, Charge** and **Increments**) implemented into **NMRPredict**.

9.7.1 Modgraph NMR Predict Desktop

Mnova integrates a fast simulation module of ^{1}H and ^{13}C-NMR spectra, called **Modgraph NMRPredict Desktop**.

NMRPredict Desktop is faster than **NMRPredict server-based**, because it does not need to log in to a server to predict a spectrum. Besides, it makes use of the fastest prediction algorithms (**Neural Network, Charge**, **Increments** and **Best**) implemented into **NMRPredict**. Other very significant advantages are that the software can be used without an internet connection, there are no access issues with firewalls, and no risk to confidential data being sent over the internet.

NMRPredict Desktop uses a Neural Network system for the prediction of ^{13}C-NMR spectra, and the NMR tables (**increments** methodology) of Professor Ernö Pretsch (ETH, Zürich) for a fast simulation mode of ^{1}H-NMR spectra, as well as the **CHARGE** program which offers ^{1}H-NMR prediction based upon partial atomic charges and steric interactions.

CHARGE offers the **first** quantitative prediction of the proton chemical shifts of a variety of organic compounds. It works by first generating 3D conformers from a 2D structure through a choice of *force fields* and then predicting proton spectra for all the conformers. The program works by looking at functional groups which have been parametrised by Professor Abraham.

To read more about the scientific theory behind CHARGE, please visit the following link:

http://www.modgraph.co.uk/product_nmr_proton.htm

The **CHARGE** and the **Increment** algorithms included in **NMRPredict Desktop** are the same as used in the Server-based version to predict ^{1}H-NMR spectra, whereas for the prediction of ^{13}C-NMR spectra; **NMRPredict Desktop** uses a **Neural Network** system, but not the HOSE database methodology implemented additionally in the server-based application.

The **Best** algorithm is the combined approach between the Increments and the Conformers algorithm that is capable of producing significantly improved proton NMR predictions for the data set upon which it has been tested down to below the 0.2 ppm target error.

You will find further information about this feature in:

http://www.modgraph.co.uk/best_proton_press_release.htm

Please bear in mind that we are not giving any guarantees that the “Best” will always give prediction accuracy as low as 0.18 ppm. Statistically this is what we got against the 1.1 million shift values in the 90,000 data we used. This may or may not be the case with user data.

The **Neural Network algorithm** is much more general and error tolerant than the HOSE code approach (based on a reference spectra database) and is much more accurate at predicting shifts not found in the database.

The Neural Network prediction method in **NMRPredict Desktop** was developed during the mid 1990s in the group of Professor Robien at the University of Vienna. It was developed by V Purtuc and gave a very broad application range to cover general organic chemistry.

The basic design principles as defined in 1995 were:

- to be general enough and be able to handle problems from basic organic chemistry to complex natural products.
- to be able to handle "unusual" organic chemistry like organometallics.
- to be solvent specific during prediction.
- to be able to use stereochemical information not restricted by ring size, in the same way as could be done within the HOSE code technology.
- to make sure that only **inter**polation and not **extra**polation occured when making predictions.

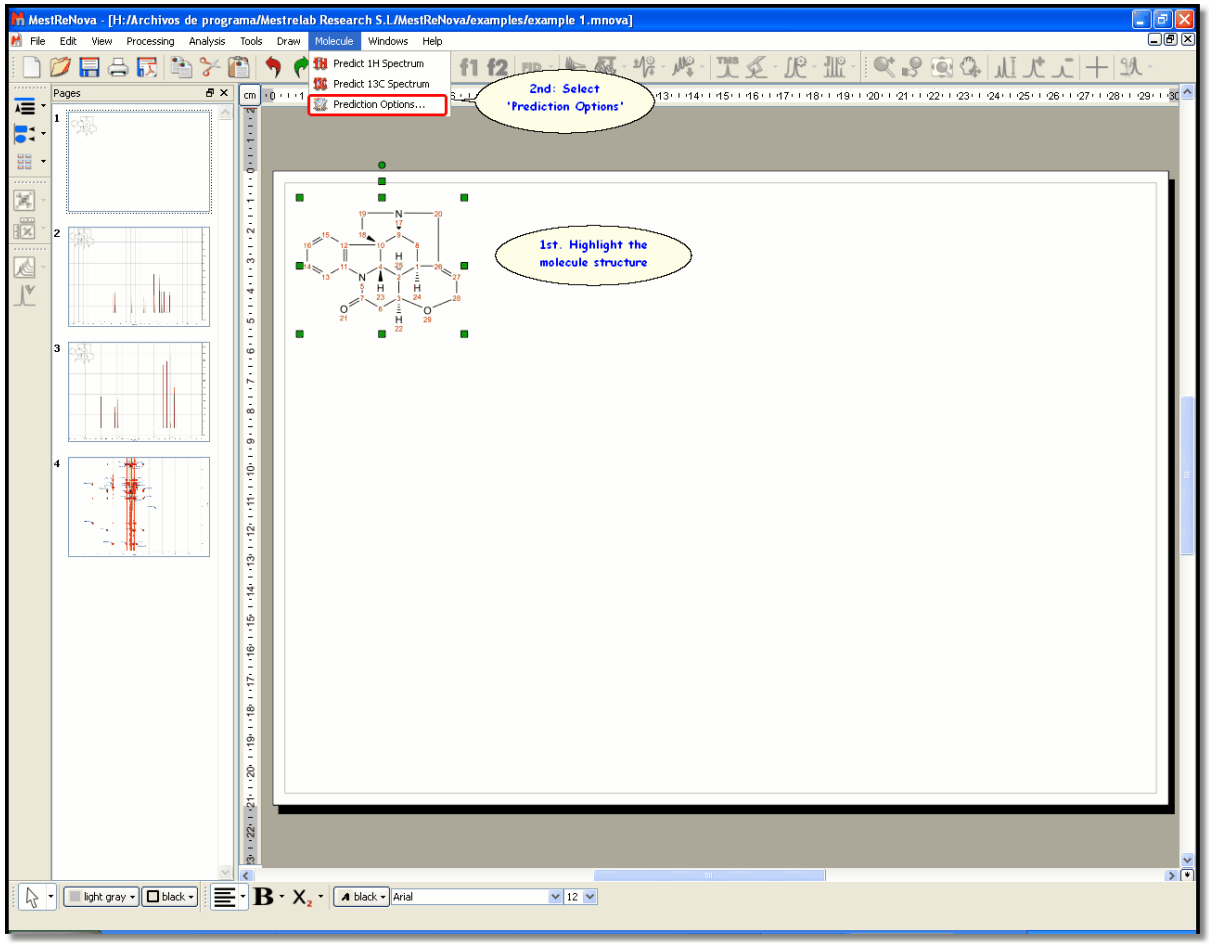
The Neural Network implemented into **NMRPredict Desktop** has been thoroughly tested and proved to be both reliable and accurate. The 4,000,000 assigned chemical shift values of the available 345,000 reference spectra can be predicted with an average deviation between experimental versus

calculated of below 2.00 ppm. This includes compounds which are not well handled by traditional prediction programs, such as ferrocenes and chromium complexes.

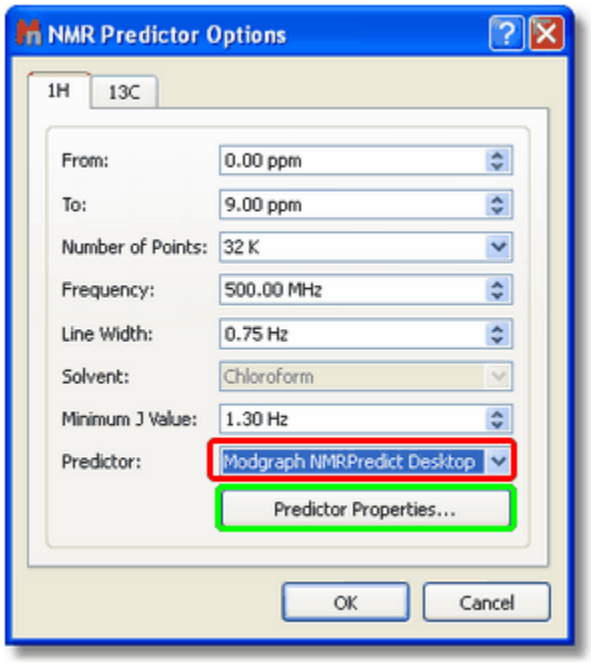
Carrying out of predictions in Mnova with **NMRPredict Desktop** is very easy and intuitive. With a molecular structure highlighted in the active page of Mnova, just go to the 'Molecule' menu and select 'Prediction Options'.

ATTENTION

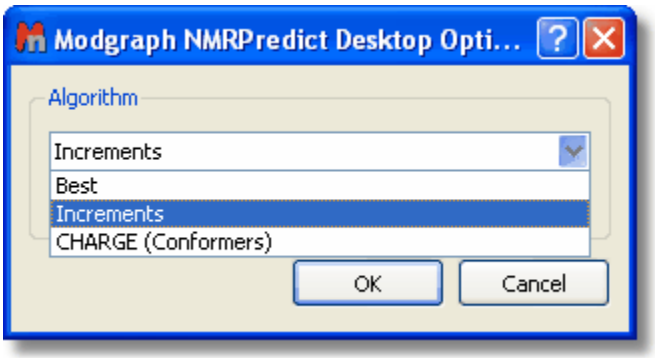
Bear in mind that, in terms of stereochemistry, drawing a structure as a chair or as a boat means nothing to the .mol file and the computer. In those cases the user must draw all the corresponding bonds to show the stereochemistry of the molecule, i.e., you must explicitly draw up and down bonds using wedges.

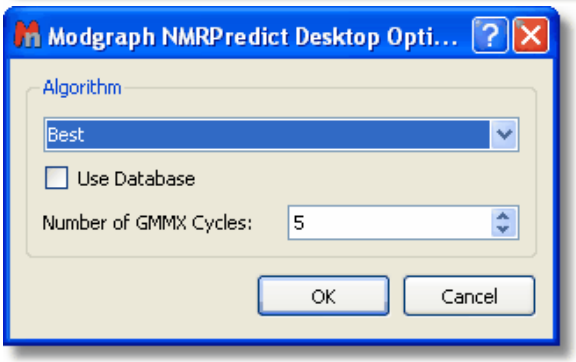


This will open the 'NMR Predictor Options' dialog box where you have to select 'Modgraph NMRPredict Desktop' in the Predictor edit box (in the red square in the capture below).

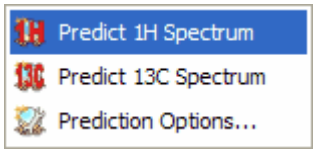


In this dialog box, the user is able to select the chemical shift limits of the spectral window, as well as other parameters, such as number of points, frequency, Line Width, solvent and minimum coupling constant value). If you are going to predict a ^{1}H-NMR spectrum, you can also select the prediction algorithm (Increments or Charge) by clicking on the 'Predictor Properties' button (in the green square in the capture above). After that, the **'NMRPredict Desktop Options'** dialog box will be displayed and you will be able to select the desired prediction algorithm (Increments, Charge or Best). If you use the Increments methodology, you will have the option to use or not use the Database, whereas if you use the CHARGE or the Best algorithm you can set the 'number of GMMX cycles', as you can see in the picture below:

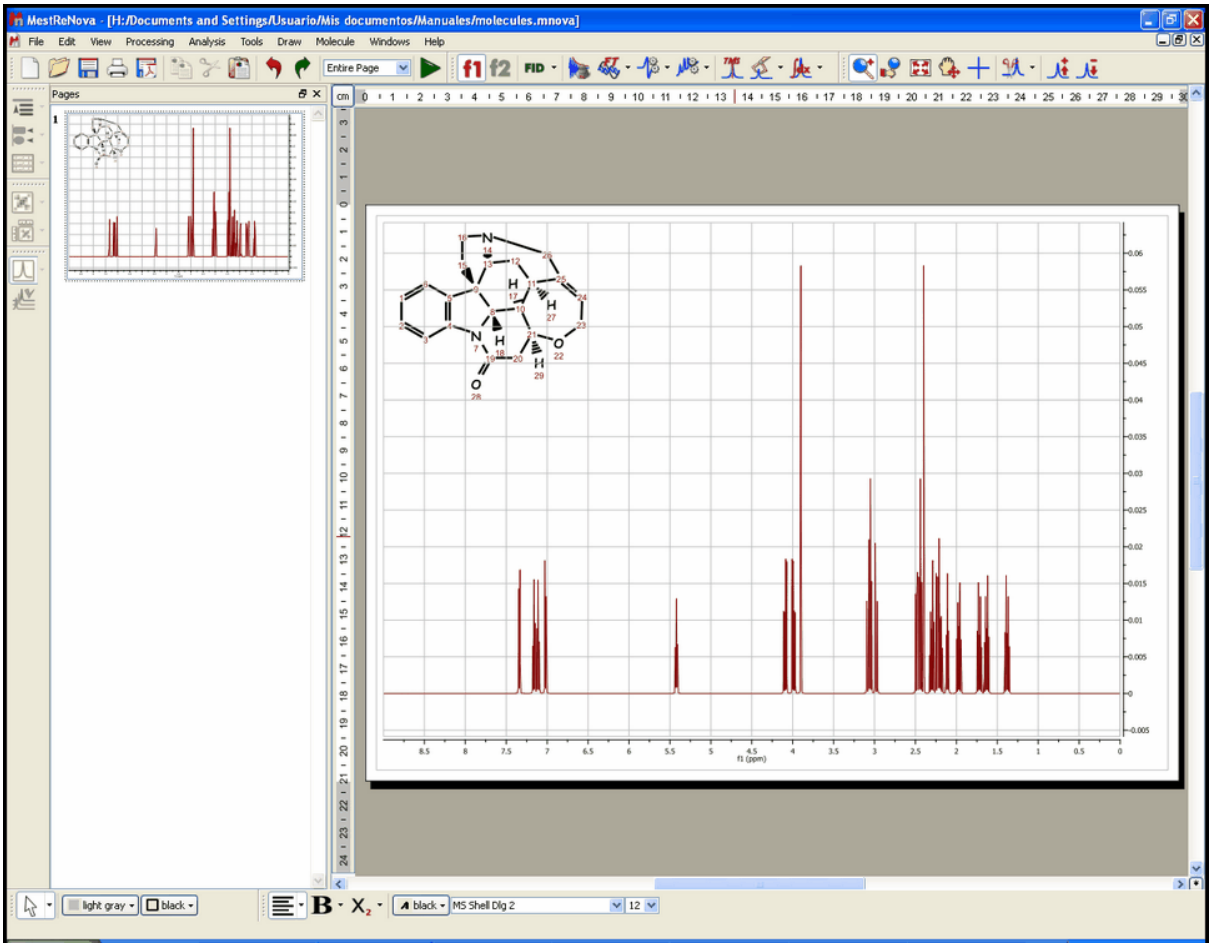




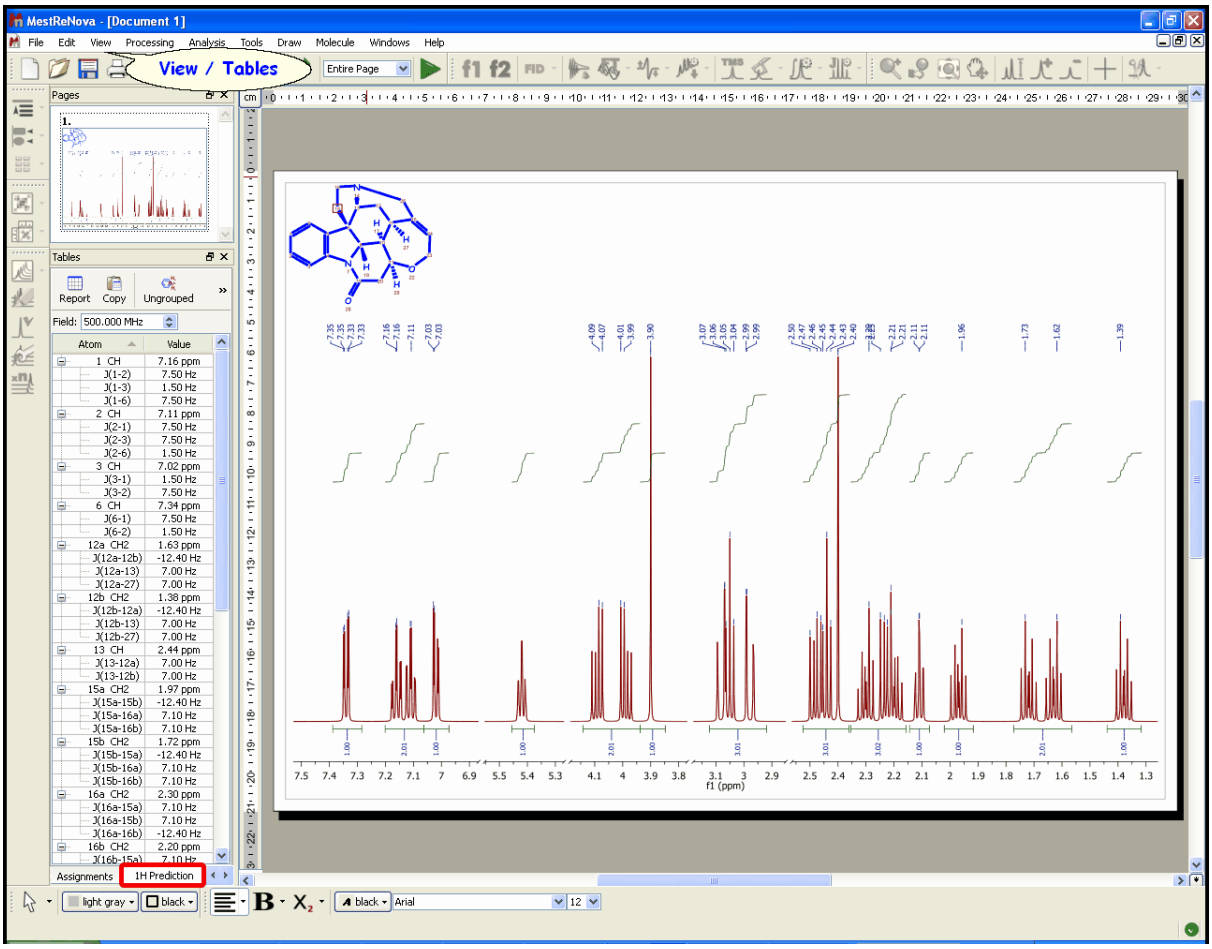
Click 'OK' and back again to the 'Molecule' menu to select 'Predict 1H Spectrum':



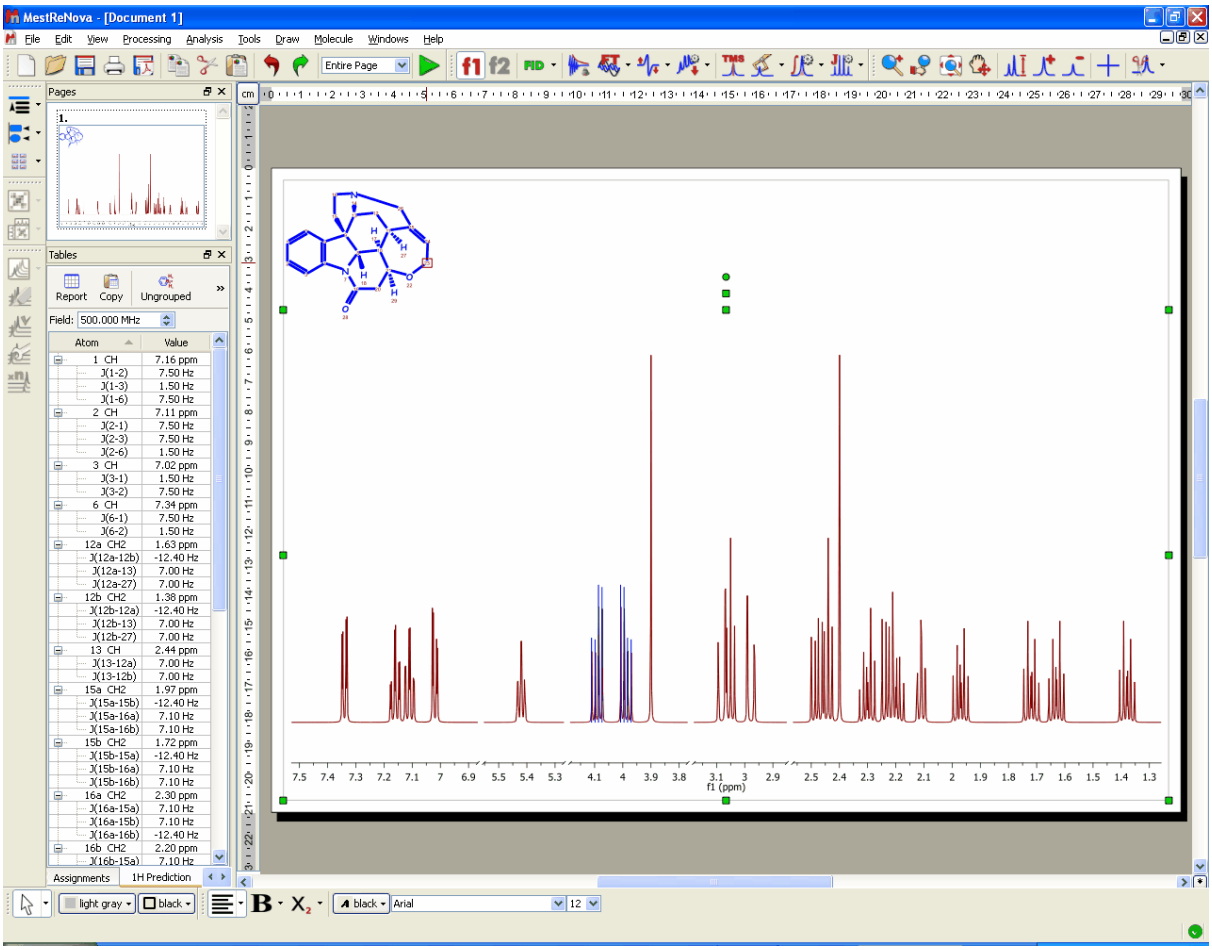
After that, you will obtain the desired predicted ^{1}H-NMR spectrum:



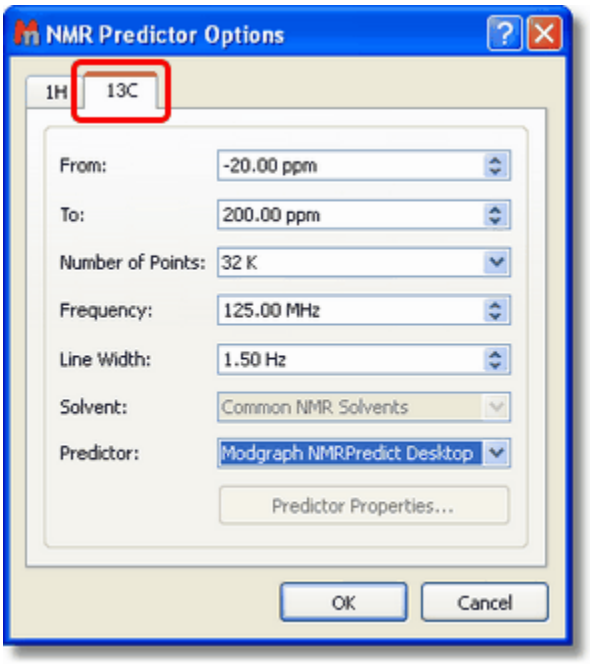
This predicted spectrum can be analyzed as a real one (e.g. it can be integrated, etc). The user can also visualize and edit the chemical shift and coupling constants values from the tables of the predicted spectrum by following the menu 'View/Table'.



Finally, to know the "predicted assignment", just hover the mouse over the desired atom, (so that it becomes highlighted in a red box) and the corresponding signal in the spectrum will automatically highlight in blue (increasing slightly its intensity); or vice versa (hovering the mouse over the peak will highlight the corresponding atom on the molecular structure).



If you need the ^{13}C-NMR predicted spectrum, follow the same instructions but selecting the options for '13C' (be sure that you select the correct tab for 13C in the 'NMR Predictor Box' dialog, red square in the figure below). As you can see in the 'NMR Predictor Option' box below, you can select the limits, the number of points, the frequency and the line width of the spectrum.



See also:
How to simulate a spectrum with NMRPredict Desktop?

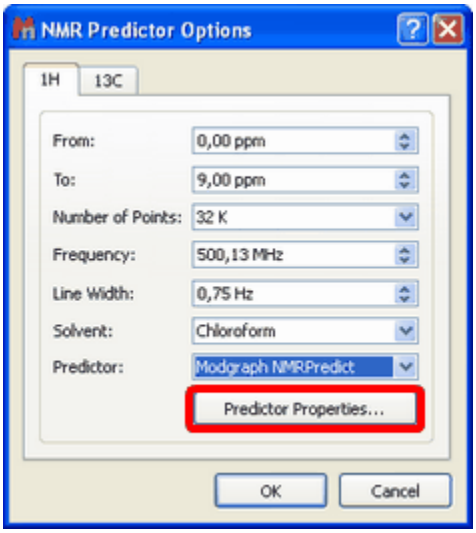
9.7.2 NMRPredict Server-Based

NMR Prediction: The handling of molecular structures within Mnova also allows the user to carry out **spectral predictions**. Although the process described here could be applied to predictions by means of any commercially available or in-house prediction package, currently Mnova will carry out predictions by using the prediction application **NMRPredict**, by Modgraph Consultants Ltd. Please note that this is a separate plugin which must be licensed in addition to Mnova.

A few notes on NMR Prediction Server-based

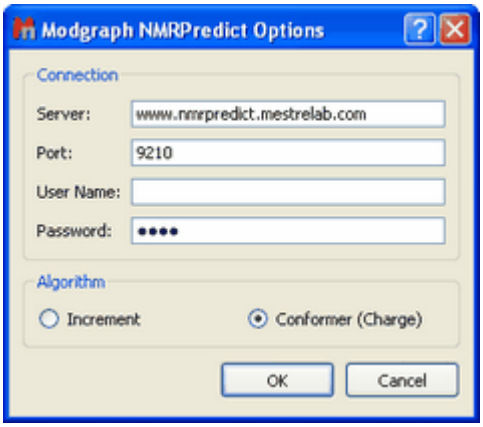
- ✓ As opposed **to NMRPredict Desktop, NMRPredict server-based** is a **client-server** application, so to carry out the predictions you will have to log in to the server via a dialog box shown by Mnova. Log in details will be provided to you when downloading **NMRPredict**. This login will only have to be carried out the first time you run a prediction with Mnova. After that, the software should remember your login details and do the log in in the background.
- ✓ Because it is a client-server application and the data are being sent over the internet to the server, industrial testers may wish to have an in-house installation of NMRPredict for security reasons. If this is the case, just write to us with your request at nmrpredict@mestrec.com.
- ✓ Customers who have their own in-house prediction package are welcome to contact us to discuss a similar, seamless integration of their own prediction package. Please write to us at support@mestrec.com.
- ✓ Please note that NMRPredict is a different software package to Mnova and therefore, in order to carry out predictions via this package, a license of NMRPredict must be held separately, once the NMRPredict evaluation has expired.

Carrying out of predictions in Mnova is very simple. With a molecular structure open in the active page of Mnova, just go to the 'Molecule' menu and select Prediction Options; then a dialog box will open.



This open dialog allows the user to set a series of prediction parameters before carrying out the prediction: limits, number of points, frequency of the spectrometer, line width, solvent and the Predictor methodology. To carry out a server-based NMR prediction the user has to select 'Modgraph NMRPredict Server' in the 'Predictor' edit box.

Clicking on the 'Predictor Properties' (red square in the capture above), the user will be able to select the algorithm for the ^{1}H-NMR prediction (Increment or Charge):



The Increment algorithm (based in Ernö Pretsch´s NMR tables) is the same as NMRPredict Desktop uses to predict NMR spectra

The CHARGE program offers ^{1}H-NMR prediction based upon partial atomic charges and steric interactions. This program offers the **first** quantitative prediction of the proton chemical shifts of a variety of organic compounds.

The program works by first generating 3D conformers from a 2D structure through a choice of *force fields* and then predicting proton spectra for all the conformers. The program works by looking at functional groups which have parametrised by Professor Abraham.

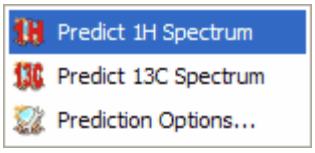
To read more about the CHARGE theory, please visit the following link:

http://www.modgraph.co.uk/product_nmr_proton.htm

Please remember that:

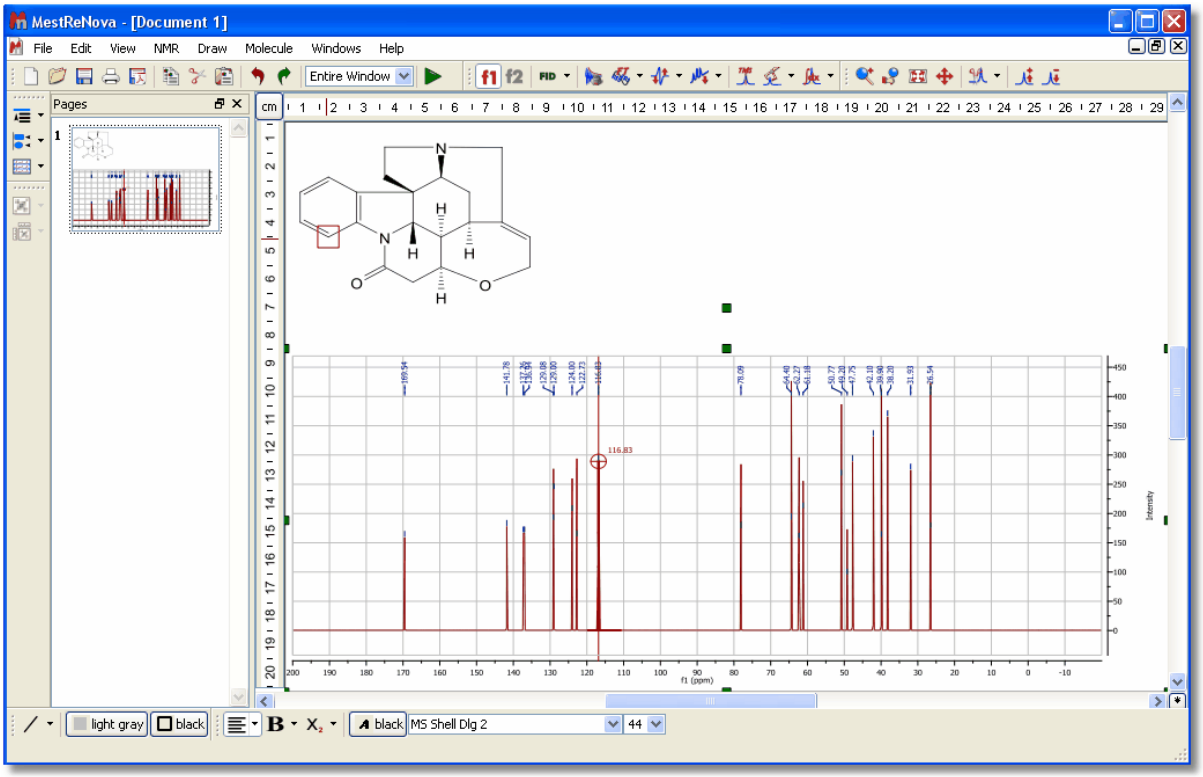
NMRPredict is a **client-server** application, so to carry out the predictions you will have to log in to the server via a dialog box shown by Mnova. Log in details will be provided to you when downloading **NMRPredict**. This login will only have to be carried out the first time you run a prediction with Mnova. After that, the software should remember your login details and do the log in in the background.

Finally, just go to the 'Molecule' menu and select 'Predict 1H Spectrum' or 'Predict 13C Spectrum'.



This will send a request to the server, which will run a prediction and return a set of values which Mnova will display as a spectrum on the screen. You will immediately notice that this will be an assigned spectrum, and therefore hovering of the mouse over the atoms will highlight the

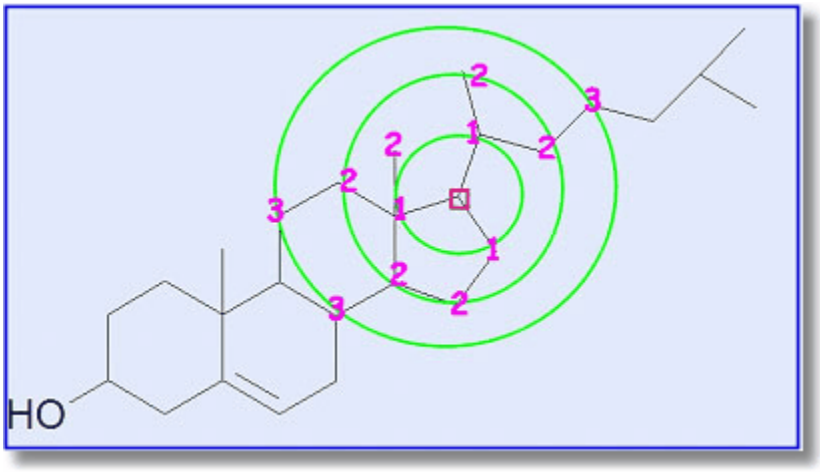
corresponding peak on the spectrum and viceversa.



A few notes on the synthetization of NMR 1H spectra

✓ It is important to note that the simulation carried out by Mnova when displaying the predicted values as a spectrum **is not first order**. The software is carrying out the **full quantum-mechanics calculations** to arrive at the displayed spectrum. This is very computationally intensive and results in such simulation module having a limitation, for 1H spectra, which prevents it from simulating spin systems larger than 13 spins. However, we have developed a new algorithm to overcome this problem.

The prediction of the 13C-NMR is based on the HOSE code approach. The **HOSE** (**H**ierarchical **O** rganisation of **S**pherical **E**nvironments) code approach starts at the carbon atom whose shift is to be predicted, looks one bond away from the carbon and tries to find this same environment in its database. If it is successful it moves two bonds away and tries again and so on until it either comes across something not represented in the database or it reaches the boundary of the molecule.



The HOSE code approach works very well for query structures which are well represented in the reference collection. If atoms can be predicted to three spheres or more (NMRPredict goes to a maximum of five spheres) the prediction can be considered to be very reliable.

However, if the query structure is not well represented in the database and the atom can only be predicted to one or two spheres the prediction cannot be relied upon at all. Also the HOSE code approach exactly reproduces the contents of the reference database – including every error within the reference database.

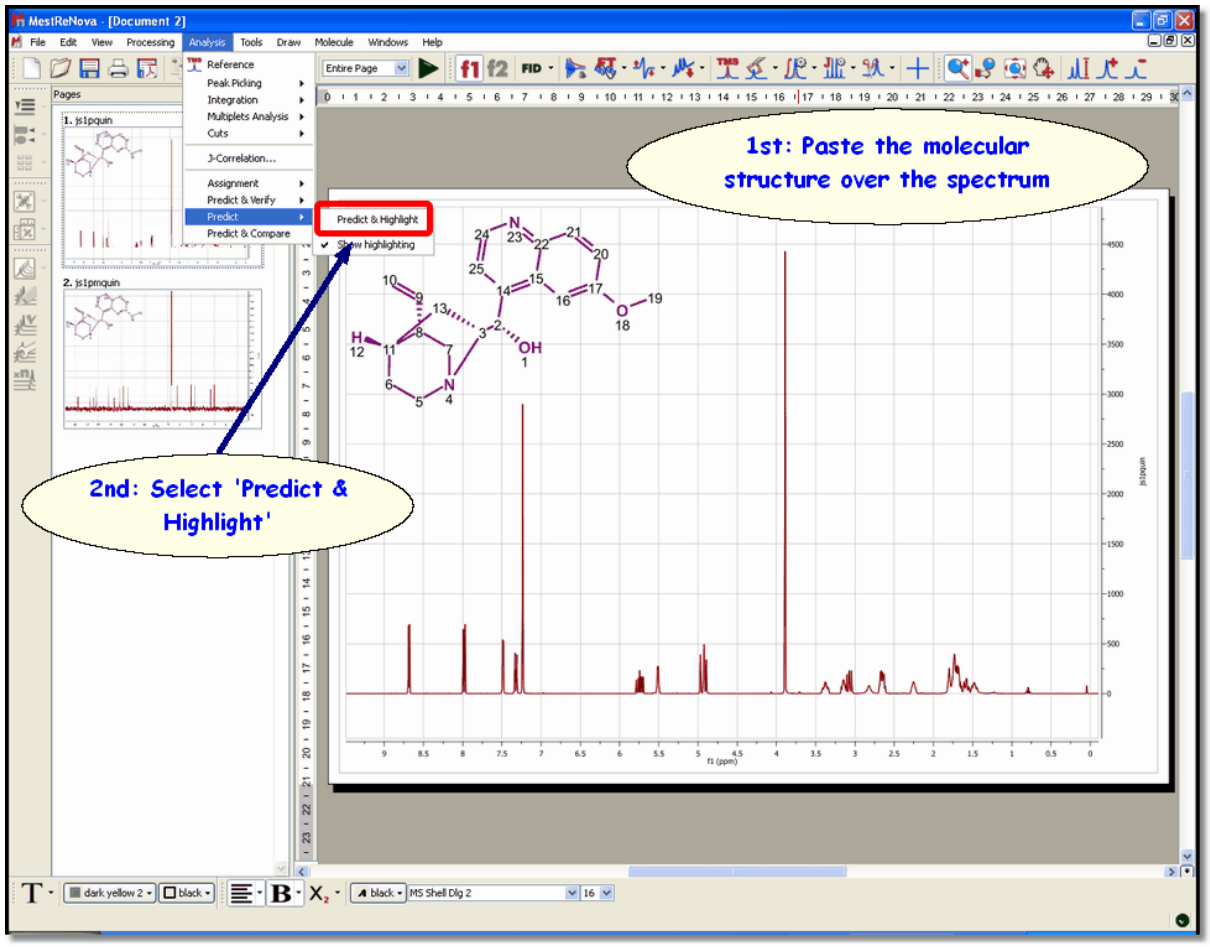
If atoms can only be predicted to one or two spheres the only solution traditionally was to add your own similar data and use that for prediction. This is now possible with NMRPredict by using NMRPredict DBA. However, NMRPredict also uses a unique Neural Network algorithm, which is much more general and error tolerant than the HOSE code approach and is much more accurate at predicting atoms it has not seen in its database.

9.7.3 Predict & Highlight

Predict & Highlight:

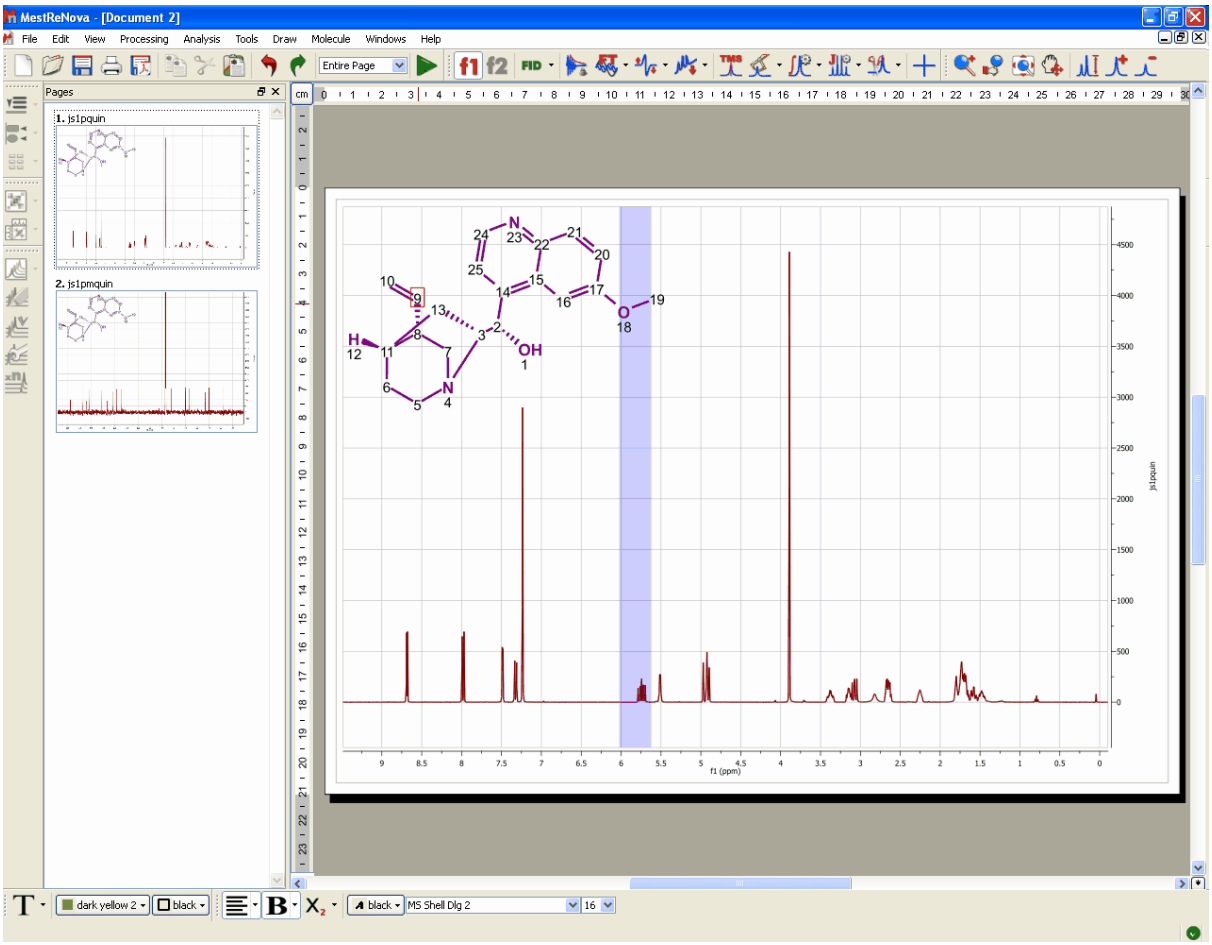
This feature will calculate in the background a simulation of the spectrum of the molecular structure present in the spectral window, highlighting the expected chemical shifts when the user hover the mouse over a proton or a carbon. This tool will be very useful to help the user in the process of assigning of the 1D NMR spectra.

The user will be able to use this tool with ^{1}H and ^{13}C NMR spectra just by pasting the corresponding molecular structure (quinine, in this example) over the spectrum and following the menu 'Analysis/ Predict/Predict & Highlight', as is shown in the picture below:

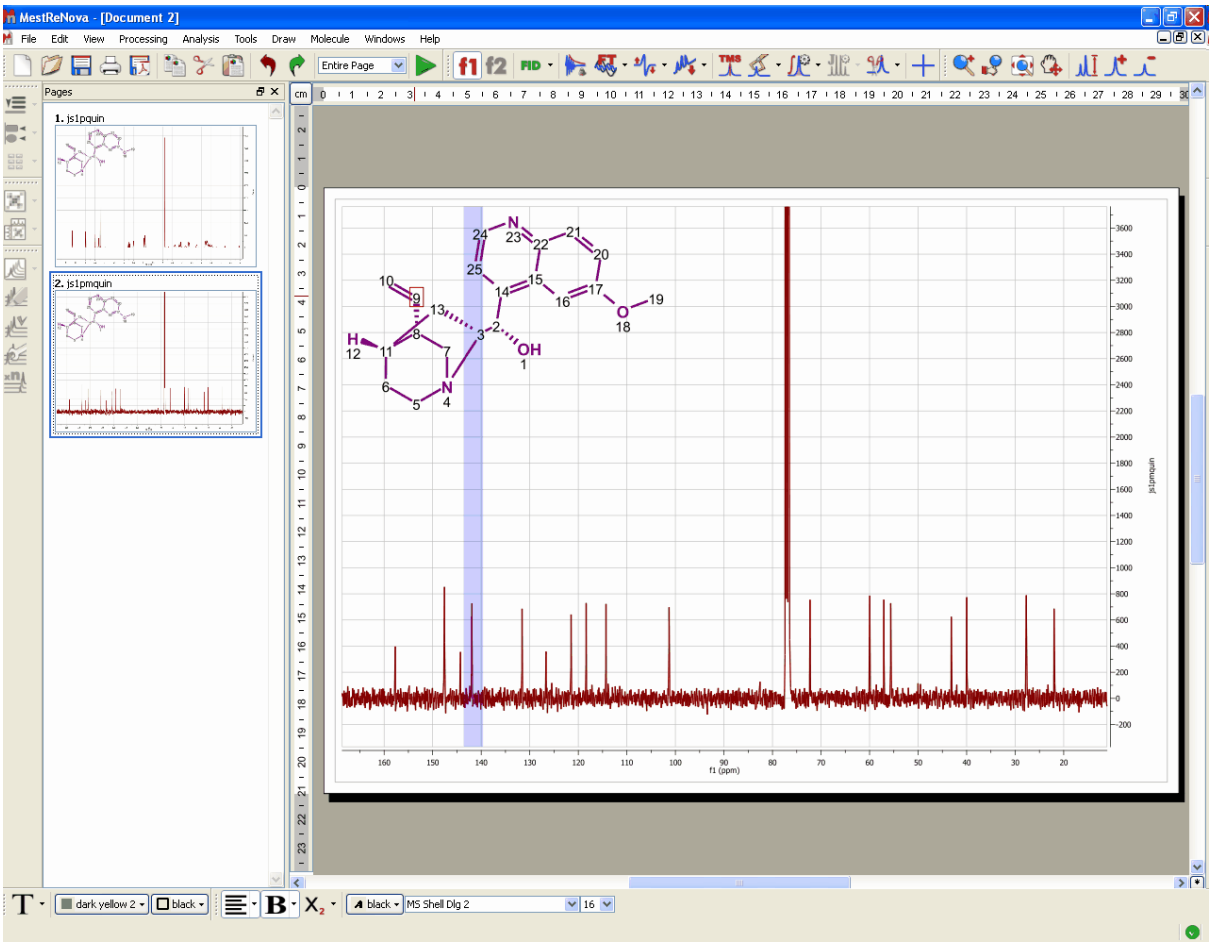


The algorithm of the simulation carried out in the background will be the selected in the 'Molecule/ Prediction Options' menu. The user will be able to select the '**Increments**' or the '**Charge**' algorithm in the ^{1}H simulation and the '**Neural Network'** system (by using **NMRPredict Desktop)** or the **HOSE** database methodology (implemented in the server-based application).

After having applied this feature, the user will be able to notice that hovering the mouse over the atom will highlight the corresponding simulated area in the spectrum. So the user will know that this signal will correspond with the proton of the carbon number 9 in the quinine.



The user will be able to apply the same feature with the ^{13}C NMR spectra, as is shown in the picture below:

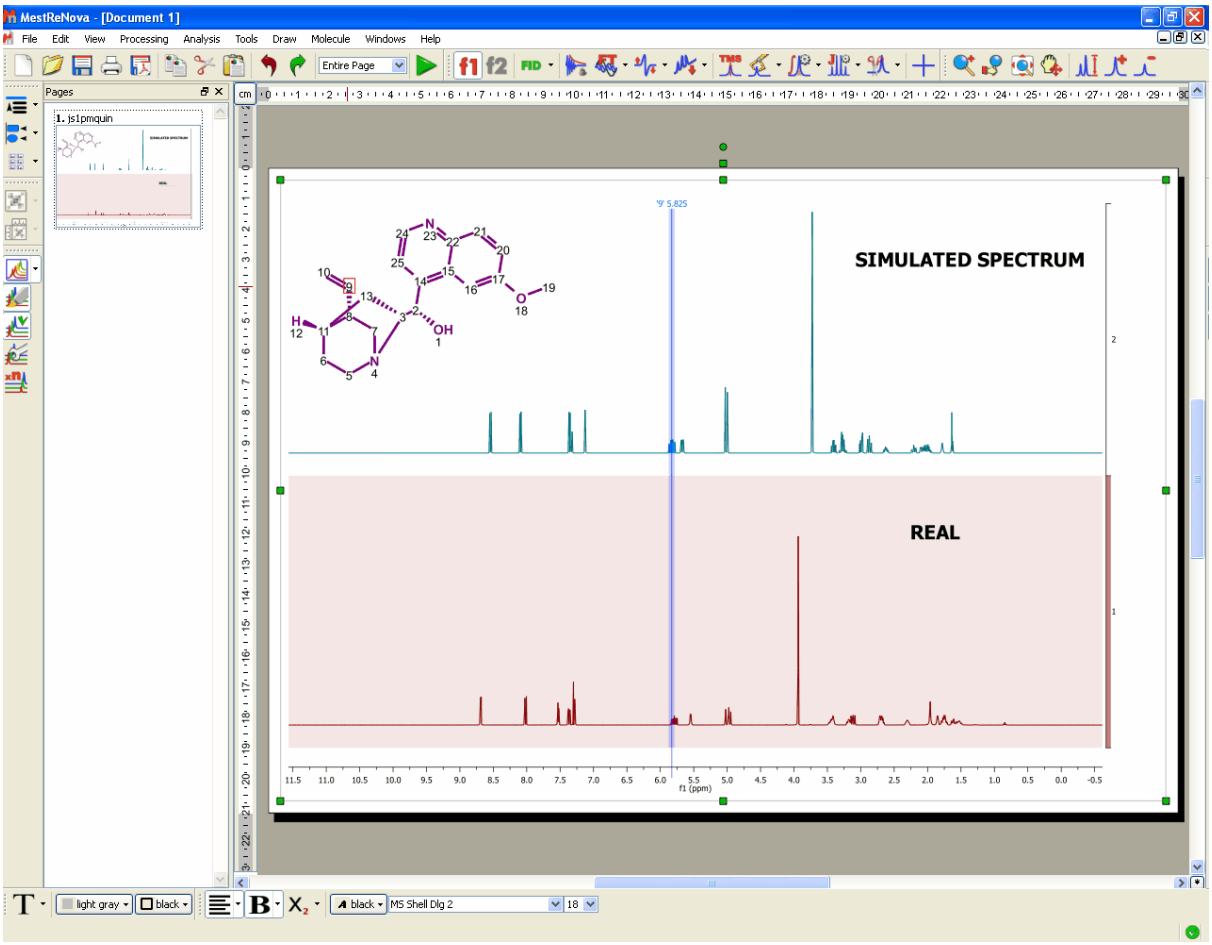


9.7.4 Predict & Compare

Predict & Compare:

This feature will display in stacked mode, a simulation of the spectrum of the molecular structure present in the spectral window, highlighting the expected chemical shifts when the user hovers the mouse over a proton or a carbon. This tool can be used of assistance in the process of assigning ^{1}H and ^{13}C NMR spectra.

The user will be able to use this tool with ^{1}H and ^{13}C NMR spectra just by pasting the corresponding molecular structure (quinine, in this example) over the spectrum and following the menu 'Analysis/ Predict/Predict & Compare', as is shown in the picture below:

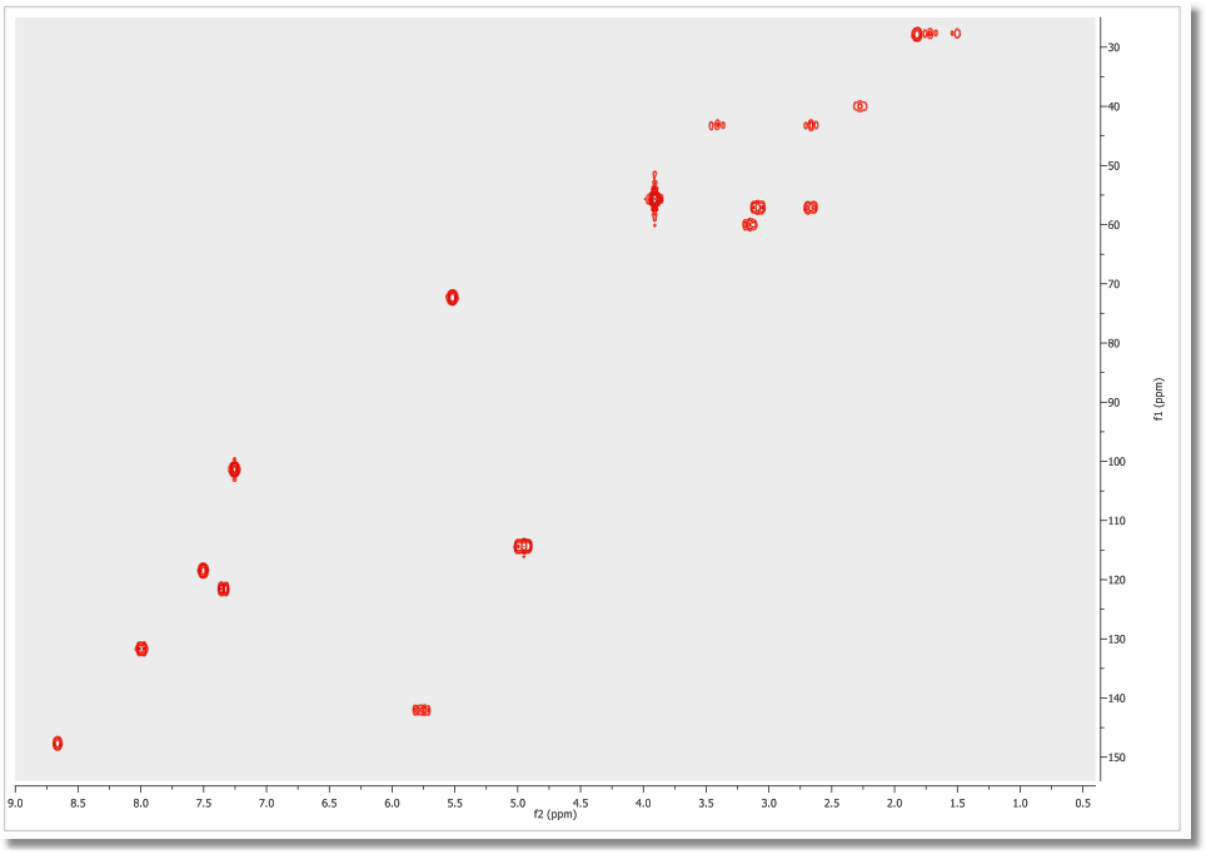


9.7.5 Predict & Verify

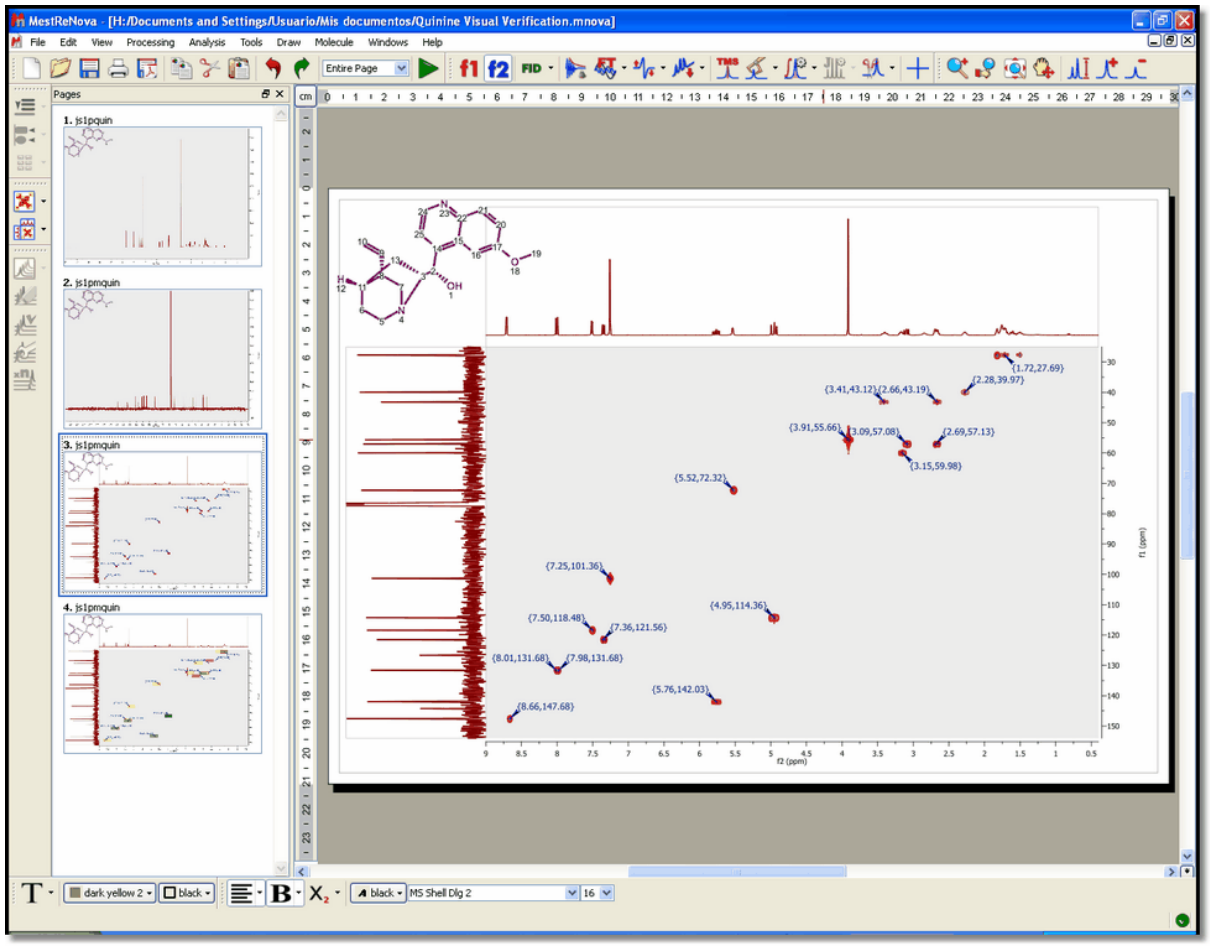
This chapter describes a new feature to validate molecular structures, which is based on the concurrent analysis of two simulated 1D (^{1}H and ^{13}C) NMR spectra and a real 2D NMR one-bond correlation spectrum, such as HMQC or HSQC (experiments which correlate the chemical shift of the proton with the chemical shift of the directly bonded carbon).

The procedure to apply a **Visual Verification** with Mnova is very easy, just load your HSQC or HMQC spectrum, and follow the menu: 'Analysis/Predict & verify/HSQC' with the corresponding molecular structure pasted on the spectral window as is shown in the pictures below:

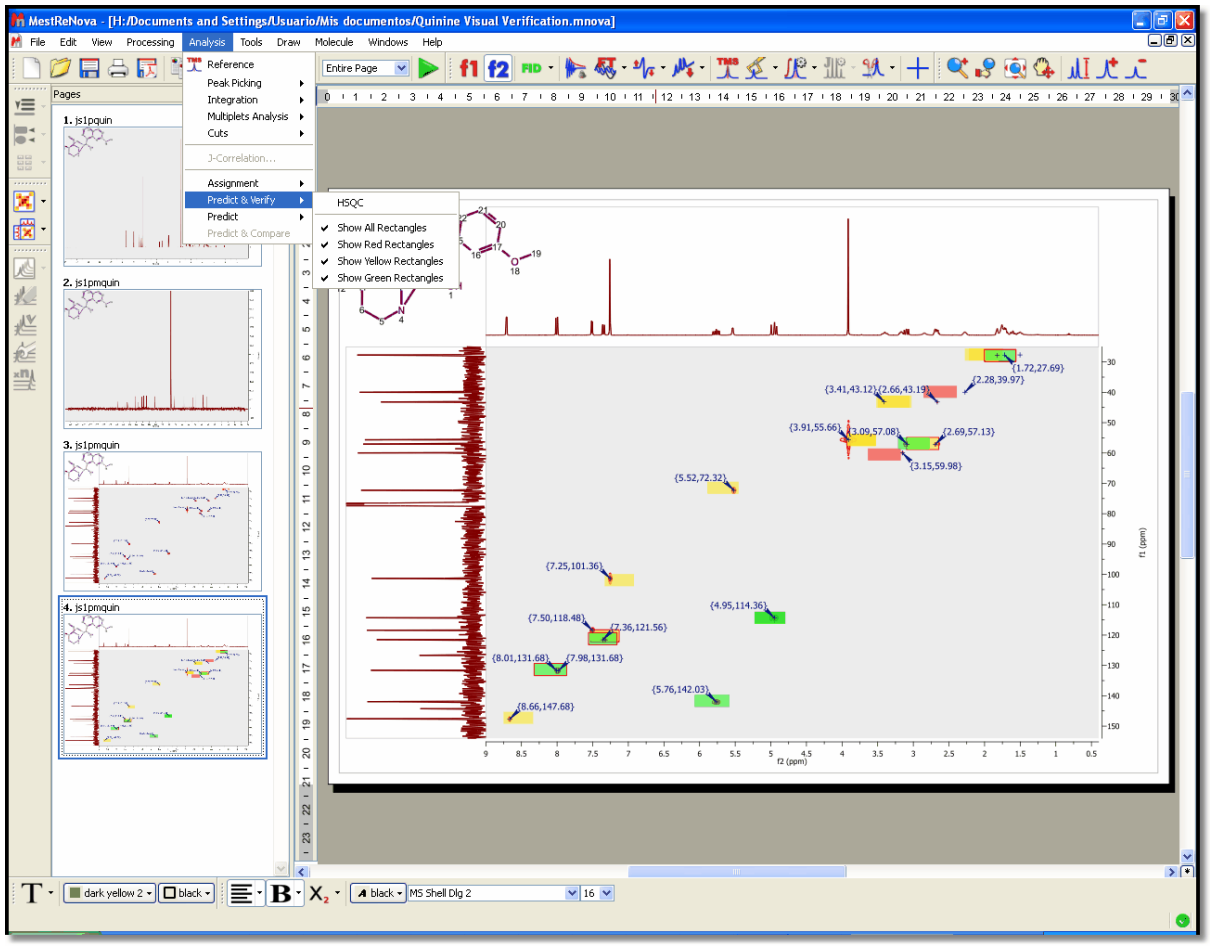
1st: Load the HSQC or the HMQC spectrum in Mnova:



2nd: Paste (or load) the corresponding molecular structure. Bear in mind that molecular structures can be imported as a **.mol** file by simply using the 'Open' command exactly in the same way as this would be applied to a spectrum. Keep in mind that you are also able to copy & paste structures from **ChemDraw, ChemSketch** and **IsisDraw**.

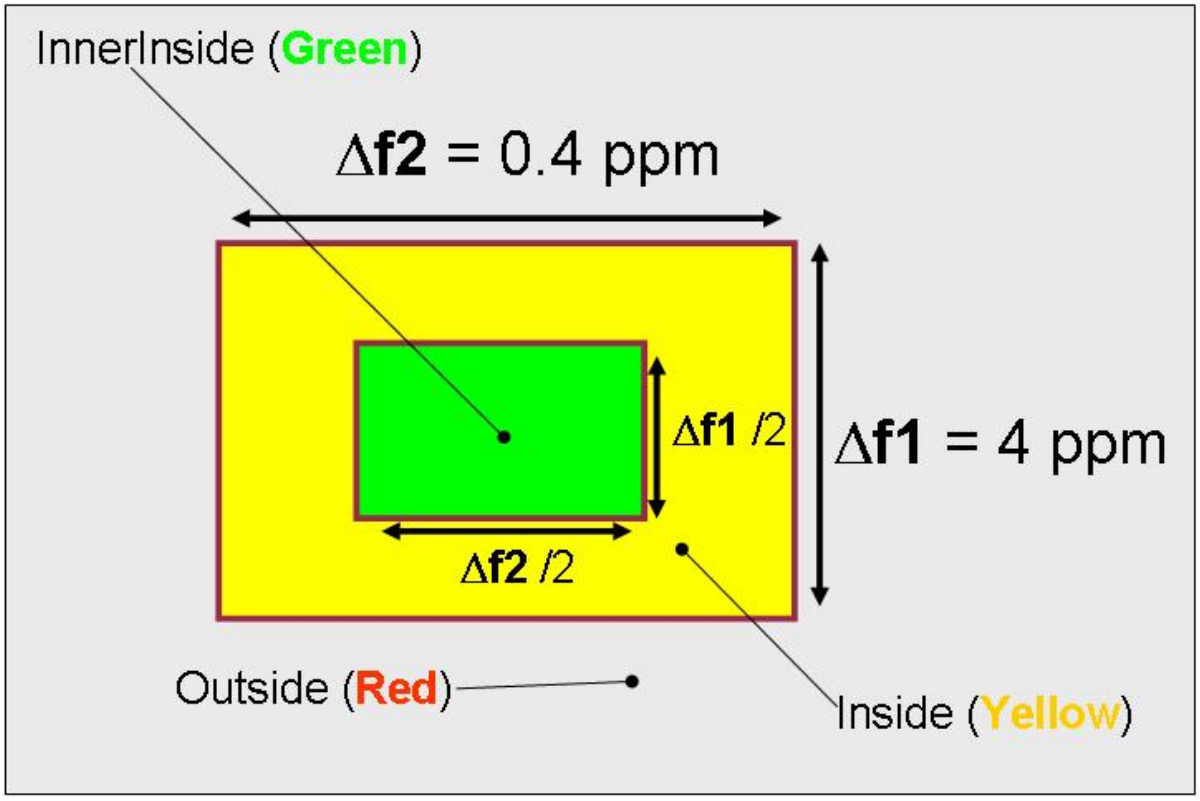


3rd: Follow the menu: 'Analysis/Predict & Verify/HSQC' to obtain the corresponding analysis:



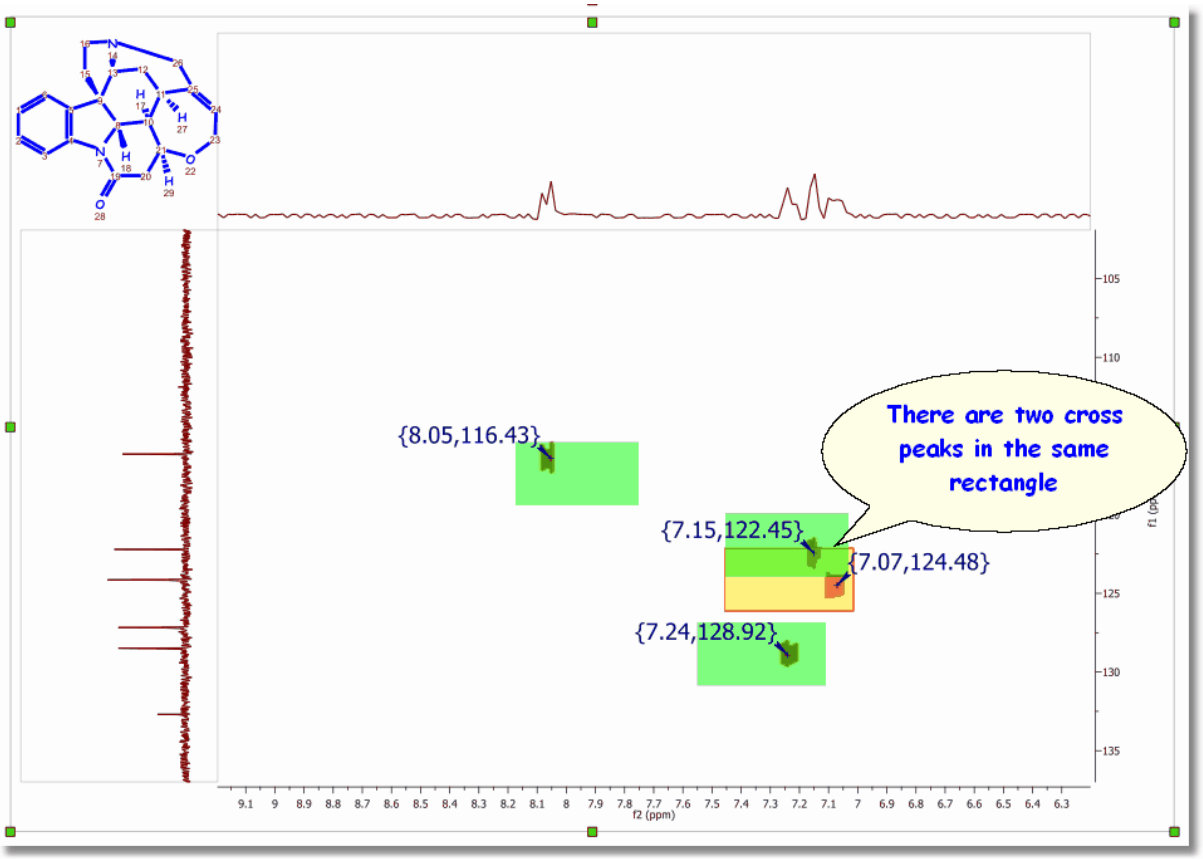
As you can see in the picture above, green, yellow and red rectangles will be obtained in the analysis.

A green rectangle will appear when the simulated value fits with the experimental one (the experimental value falls within a rectangular window, drawn around the simulated value, with dimensions of 0.2 ppm along the proton dimension and 2 ppm along the carbon dimension). A yellow rectangle will be displayed when an experimental peak falls within a window which is between 0.2 and 0.4 ppm along the proton dimension and 2 and 4 ppm along the carbon dimension. Finally, the red rectangles are displayed when no experimental peak is found within the latter rectangle, with the simulated value at its center and dimensions of 0.4 ppm along the proton dimension and 4 ppm along the carbon dimension.

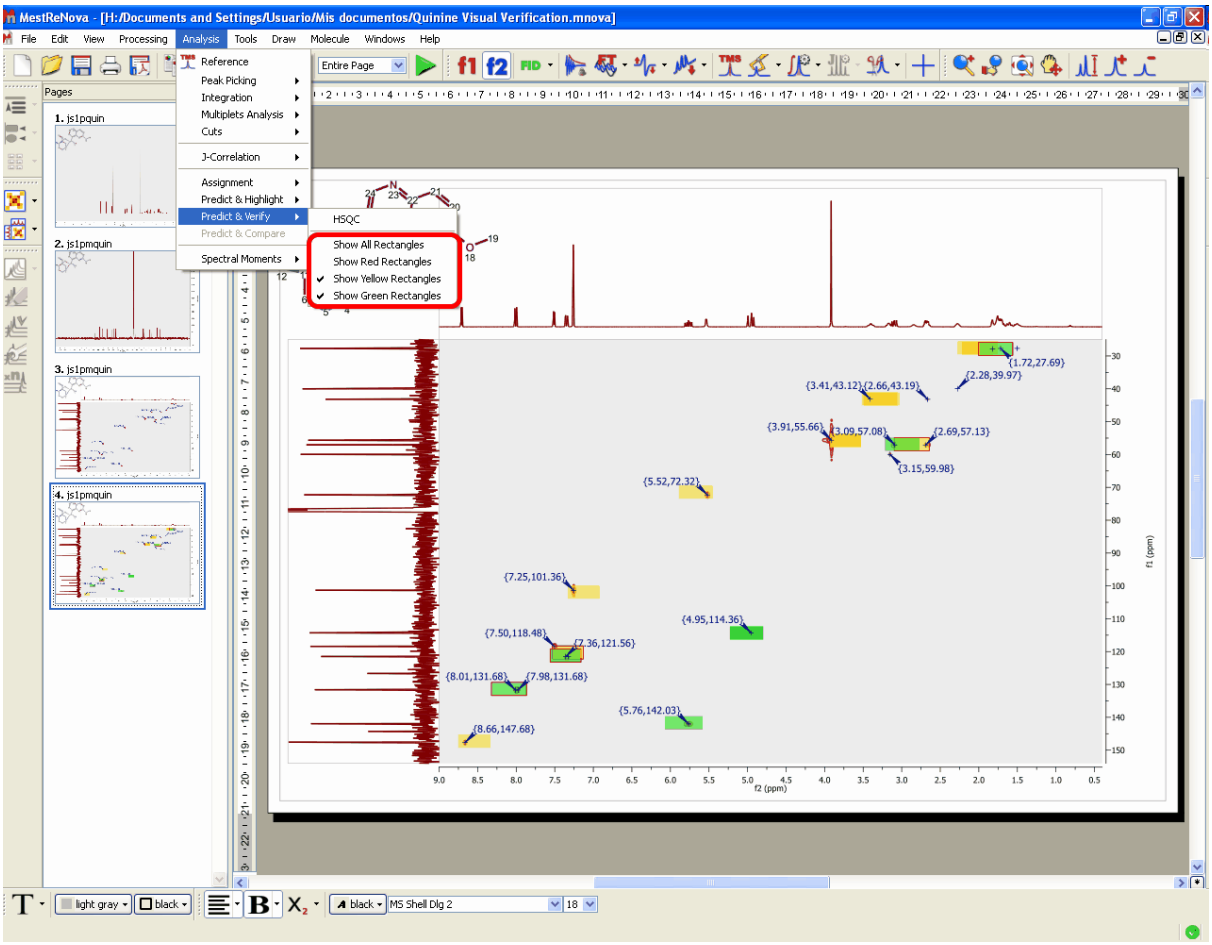


For example, if we have a real cross peak in the HSQC spectrum at (1.00, 10.0) ppm and a simulated value of (1.05, 10.2) ppm, then we shall obtain a green rectangle, because there is a experimental peak within the rectangle with a predicted peak at its center and sides of 0.2 ppm along the proton and 2 ppm along the carbon dimensions, which we will call 'inner inside'. If we would have a simulated value of (1.25, 11.0) ppm, then we would get a yellow rectangle drawn around the simulated value, as the experimental peak values would fall outside the 'inner inside' but within the area, drawn around the predicted peak, with sides of 0.4 ppm along the proton dimension. In this case the carbon simulation would be fine, but we would get a yellow rectangle due to the deviation in the proton prediction. If the simulated value for this cross peak was (1.41, 15.0) ppm, then we would obtain a red rectangle.

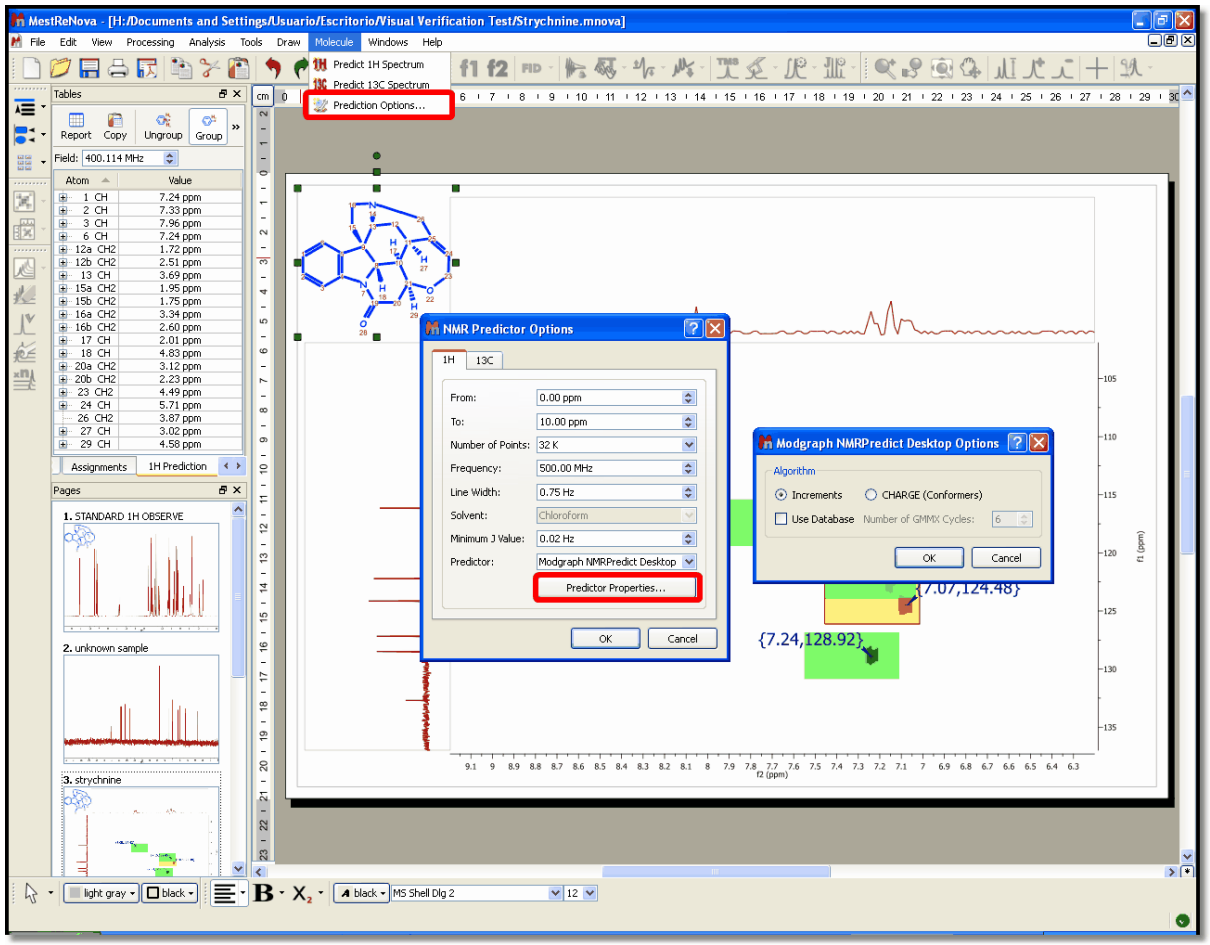
Finally, we shall get a rectangle with a red border when more than one experimental cross peak falls within the green or yellow rectangles drawn around a predicted peak, as is shown in the picture below:



The user also has the ability to display any combination of rectangles, such as only red rectangles or green and yellow rectangles, for example. These options are available from the 'Analysis/Predict & Verify' menu.

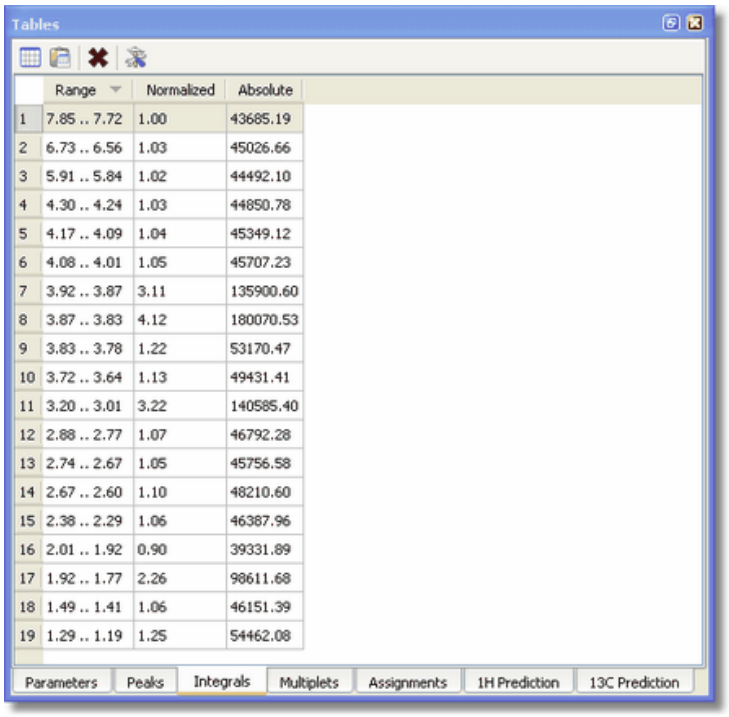


Bear in mind that the results reported by visual verification will depend on the prediction algorithm used in the spectral simulation. The user will be able to change this algorithm by following the menu: ‘ Molecule/Prediction Options/Predictor Properties’.



9.8 Tables

Mnova groups all spectra relevant information into the so-called **Tables window**, available from the ***View*** menu.



From this set of tabbed pages, the user can find information, about:

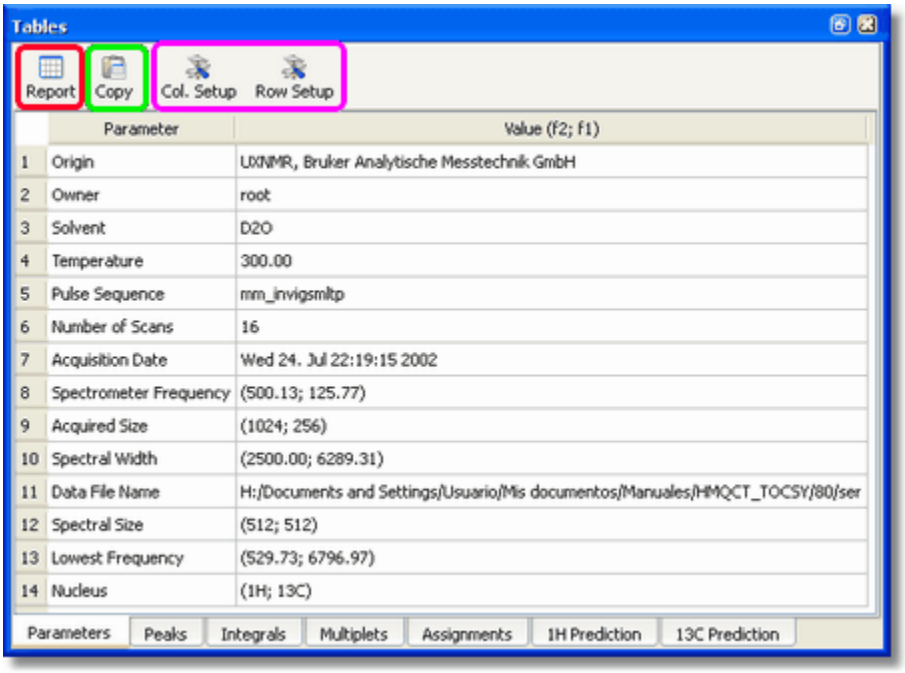
- Spectral Parameters
- Peaks List
- Integrals List
- Multiplets
- Assignments
- ^{1}H Prediction
- ^{13}C Prediction

Spectral Parameters: This table shows information about:

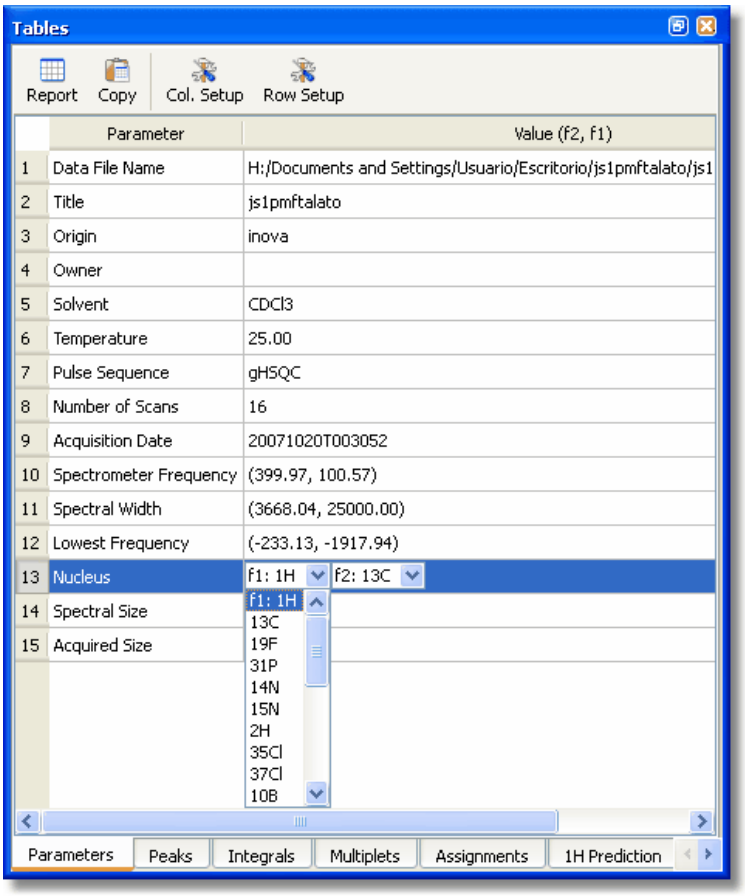
- Experiment Title
- Data File Name
- Origin of the Experiment (type of spectrometer used)
- The Owner
- Solvent Used ($CDCl_3$ in the example below)
- The Frequency of the Spectrometer Used to Carry Out the Experiment
- Number of Acquired Data Points
- Spectral Width
- Spectrum Size After FT
- Lowest Frequency (in Hz) Used for Referencing Purposes, etc.

Please note that the current version of Mnova includes the ability to add more parameters into this list and customize the way it is displayed on the screen. **See also:** Parameters Table

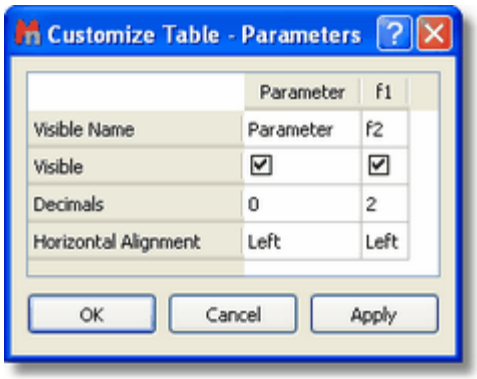
The user can directly paste the spectral parameters table on the spectrum by clicking on the **'Report'** icon (red square in the capture below). You can also export the table to another document by clicking on the **'Copy to Clipboard'** icon  (green square in the screenshot below).



The user will be able to modify some of the parameters (Spectrometer frequency, Spectral Width, Lowest Frequency, Nucleus...) by just double clicking on the value as is shown in the picture below:

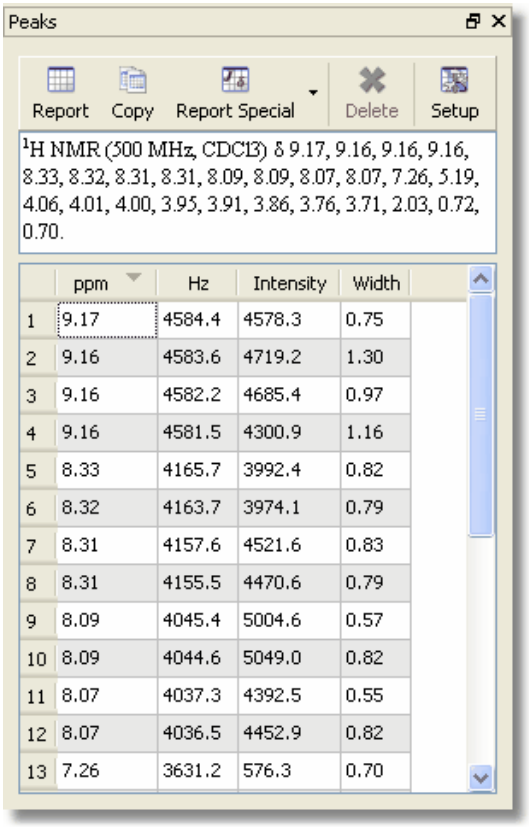


You can customize the table, by clicking on the **'Column or Row Setup'** icon  . The 'Customize Table' dialog box will be displayed and will allow you to change the name of the columns, hide them, change the number of decimals as well as the horizontal alignment.



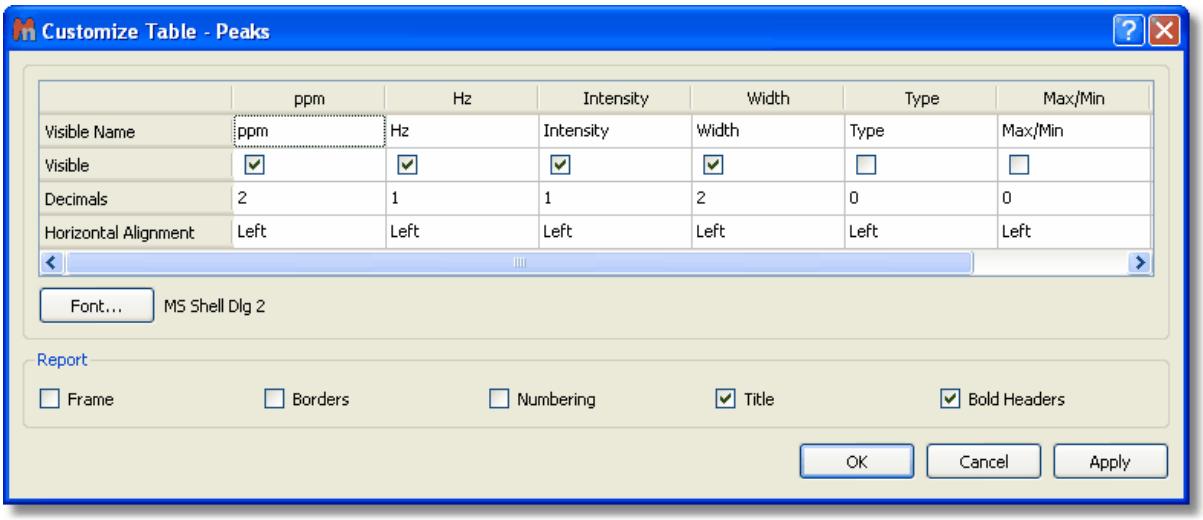
See also:
Parameters Table

Peak List: By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* by following the menu 'View/Tables/Peaks'.



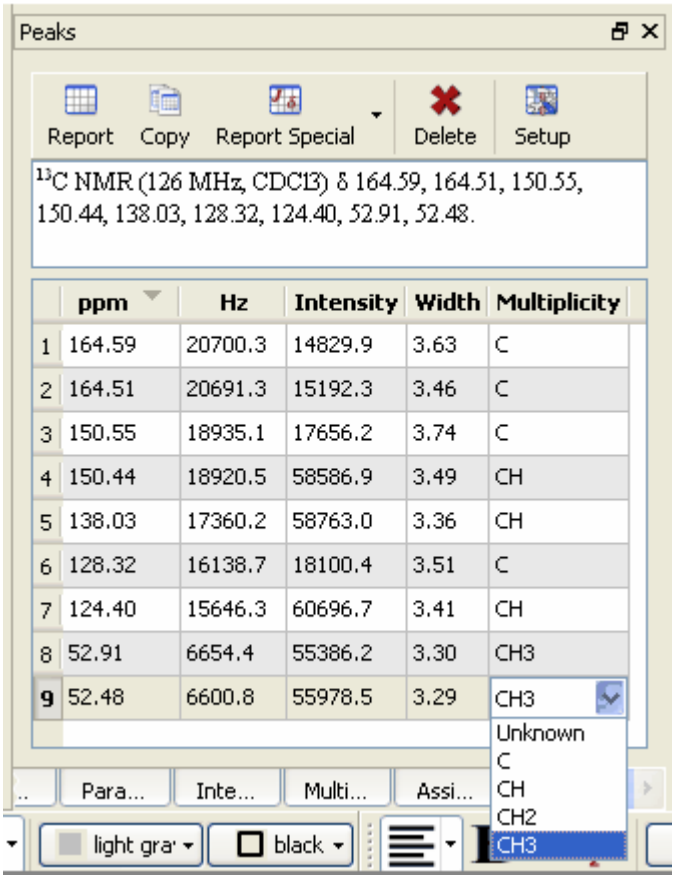
	ppm	Hz	Intensity	Width
1	9.17	4584.4	4578.3	0.75
2	9.16	4583.6	4719.2	1.30
3	9.16	4582.2	4685.4	0.97
4	9.16	4581.5	4300.9	1.16
5	8.33	4165.7	3992.4	0.82
6	8.32	4163.7	3974.1	0.79
7	8.31	4157.6	4521.6	0.83
8	8.31	4155.5	4470.6	0.79
9	8.09	4045.4	5004.6	0.57
10	8.09	4044.6	5049.0	0.82
11	8.07	4037.3	4392.5	0.55
12	8.07	4036.5	4452.9	0.82
13	7.26	3631.2	576.3	0.70

This table displays the list of peaks, including the following information: chemical shift in ppm and Hz, intensity (height) and width. The user can report the Peak table directly on the spectrum by clicking on the **'Report'** icon  . It is also possible to customize the appearance of this report (an also of the table), by clicking on the 'Setup' button  to display the 'Customize Table-Peaks' dialog box:

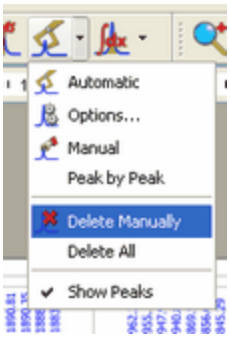


This dialog box will allow the user to change the name of the columns, hide them, change the number of decimals as well as the horizontal alignment. It is also possible to change the Font Family and also to customize the appearance of the table report (Frame, Borders, Numbering, Title and Bold Headers).

The user will also be able to select the multiplicity of the carbon signals, just by double clicking in the corresponding cell, as you can see in the picture below:

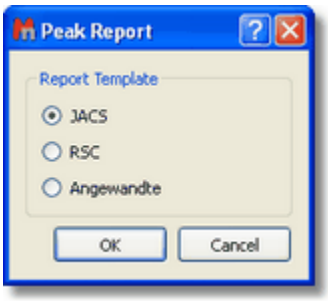


To delete an undesired peak, click on the **'Delete'** icon  of the Peak List, or select 'Delete Manually' on the Peak Picking scroll bar icon



By default, peak frequencies are listed directly on the spectrum but they can also be accessed via the *Peak Picking table* and also as a **'Peak List'** by clicking on the **'Report Special'** icon 

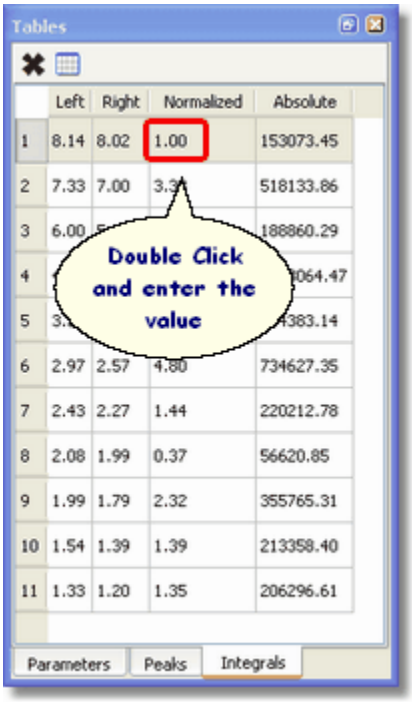
. The user will be able to select the 'Peak Report Template' between JACS, RSC or Angewandte.



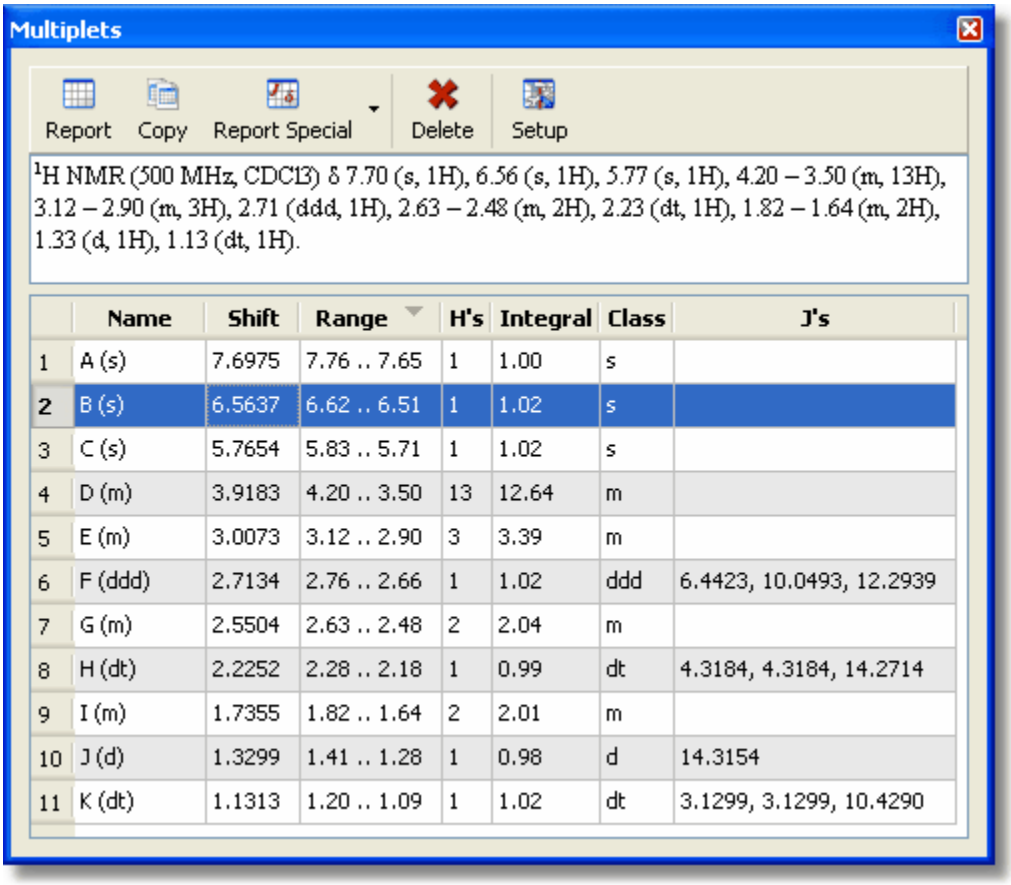
Clicking on the 'Setup' icon

Integral List: The Integrals Table includes information about the integral range (in ppm) and the normalized and absolute values of each integral. In this table the user can normalize the integrals by double clicking on the normalized value of the integral which the user would like to set (in the red square on the capture below). Once it is highlighted, the user has to overwrite the desired value, press return, and all integrals will update with reference to the chosen one. You will obtain the same effect by double clicking directly on the integral label on the spectrum.

The user can also delete an integral from this table by clicking on the **'Delete'** icon  when the undesired integral is highlighted. To paste the integral list on the spectrum, just click on the **'Report'** icon  .



Multiplets: The multiplet table includes information about the name (by default Mnova named the multiplets following the alphabetical order, but the user is able to change it in the multiplet manager), the chemical shift (ppm), the range (ppm), number of hydrogens, integral value, type of multiplet and coupling constant values (Hz).

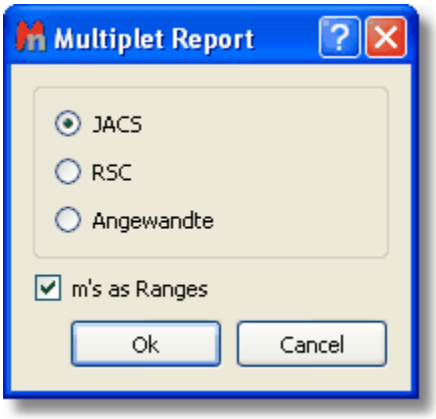


	Name	Shift	Range	H's	Integral	Class	J's
1	A (s)	7.6975	7.76 .. 7.65	1	1.00	s	
2	B (s)	6.5637	6.62 .. 6.51	1	1.02	s	
3	C (s)	5.7654	5.83 .. 5.71	1	1.02	s	
4	D (m)	3.9183	4.20 .. 3.50	13	12.64	m	
5	E (m)	3.0073	3.12 .. 2.90	3	3.39	m	
6	F (ddd)	2.7134	2.76 .. 2.66	1	1.02	ddd	6.4423, 10.0493, 12.2939
7	G (m)	2.5504	2.63 .. 2.48	2	2.04	m	
8	H (dt)	2.2252	2.28 .. 2.18	1	0.99	dt	4.3184, 4.3184, 14.2714
9	I (m)	1.7355	1.82 .. 1.64	2	2.01	m	
10	J (d)	1.3299	1.41 .. 1.28	1	0.98	d	14.3154
11	K (dt)	1.1313	1.20 .. 1.09	1	1.02	dt	3.1299, 3.1299, 10.4290

The user can paste the multiplet table on the spectrum by clicking on the **'Report'** icon and also can delete a multiplet from this table by using the **'Delete'** icon when the undesired multiplet is highlighted. You can also customize this table by clicking on .

Finally, the user will be able to obtain the multiplet assignment list by clicking on the **'Report Special'** icon 

. This will display the 'Multiplet Report' dialog box, which allow the user to select the desired template (JACS, Royal Society of Chemistry and Angewandte).



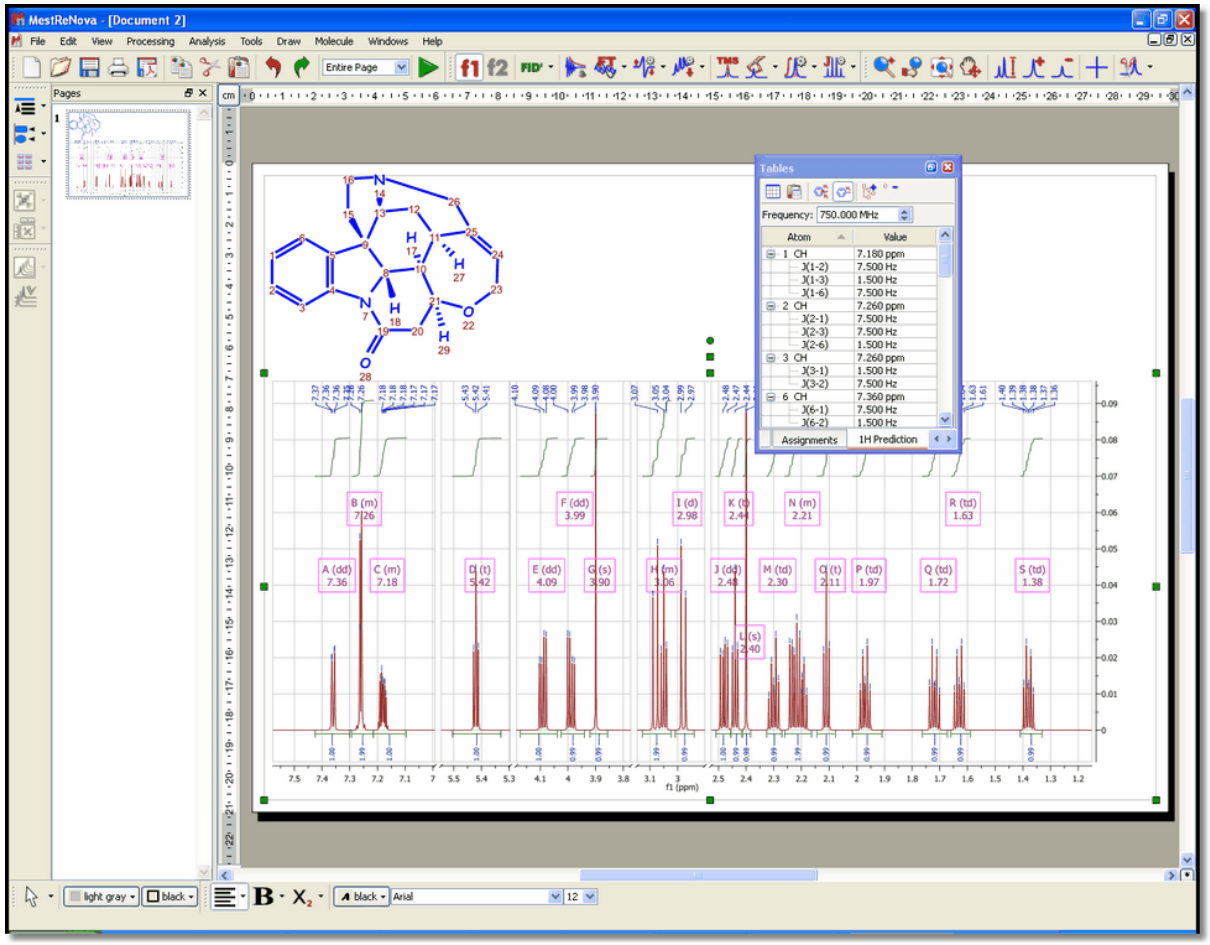
Unchecking the 'Multiplets as Ranges' box, will print the multiplet as a chemical shift value which corresponds with the middle point of the range.

After that you will obtain in your spectrum the assignment list, like this one: 1H NMR (300 MHz, CDCl3) δ 6.30 (m, 5H), 3.17 (q, *J* = 7.1, 2H), 2.84 (q, *J* = 12.9, 2H), 2.20 (m, 1H), 1.47 (ddd, *J* = 6.4, 15.2, 21.1, 2H), 0.70 (s, 2H), 0.27 (m, 6H).

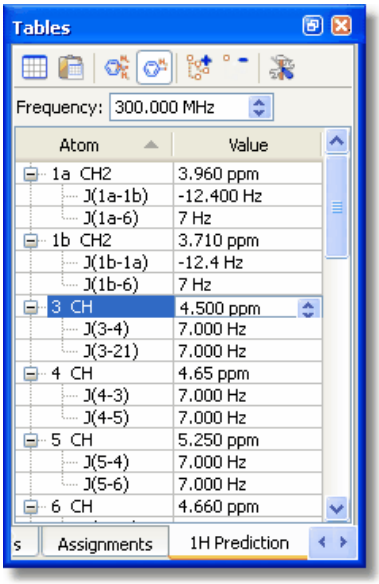
The user will be able to copy the multiplet report to the clipboard by selecting 'Copy Special' on the 'Report Special' scroll bar menu.

The user will able to customize the format of this special report by selecting 'Setup' and then modifying the corresponding script. See also 'Multiplet analysis' and Scripts.

1H Prediction: In this table, the user can visualize the ^{1}H assignments of the predicted shifts (in NMR Predict mode). As you can see in the picture below, the carbon atoms are labeled to make easy the proton assignment. The user can also select the frequency of the desired predicted spectrum (on the frequency edit box) as well as do (or not) groups magnetically equivalents (by clicking on the suitable icons: ) and also expand or collapse the predicted values by clicking on the corresponding buttons 

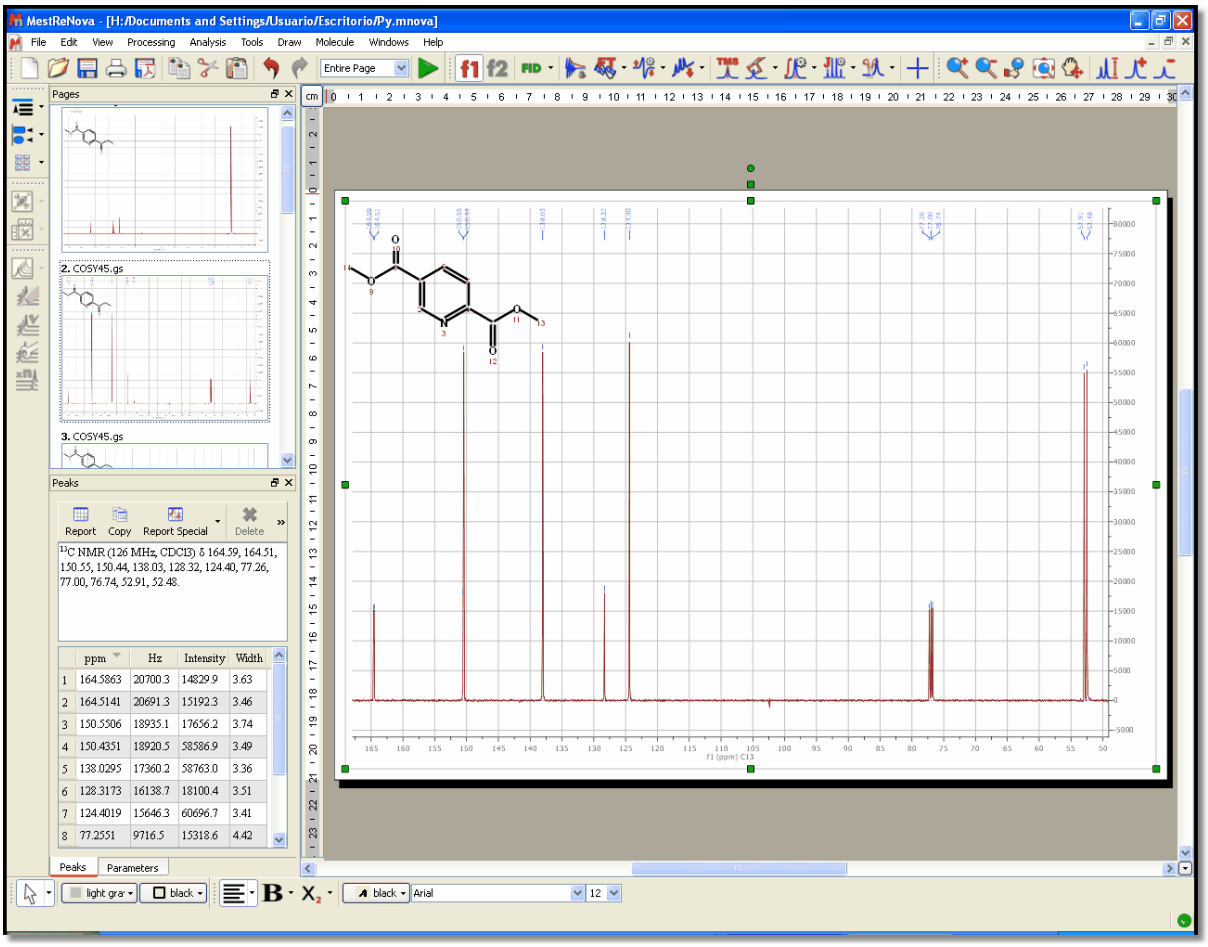


You can also modify the chemical shift and coupling constant values directly from this tables, just by double clicking on the corresponding box and entering the desired value.

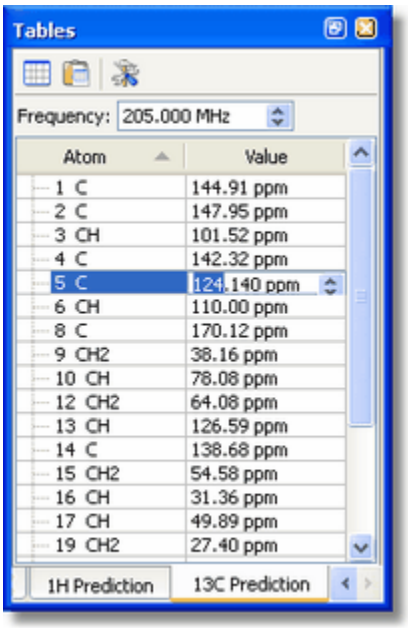


Please bear in mind that you can also put the table on the 'Page Navigator' just by clicking on the table

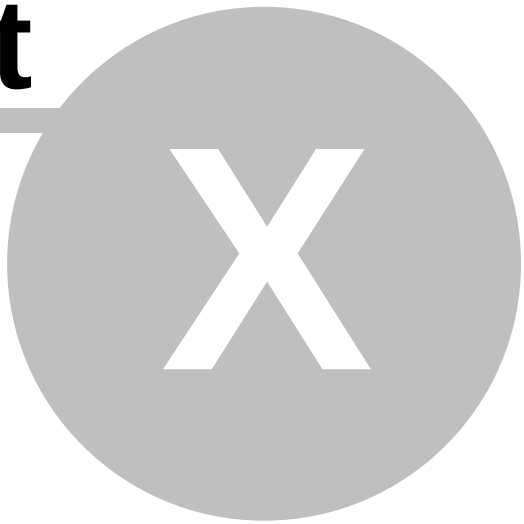
and dragging it to the 'Page Navigator', as you can see in the picture below:



^{13}C Prediction: In this table, the user can visualize the ^{13}C assignments of the predicted shifts (in NMR Predict mode). You can also select the frequency of the desired predicted spectrum (on the frequency edit box). The user can paste the ^{13}C Prediction table directly on the spectrum by clicking on the **'Report'** icon or copy it to the clipboard by clicking on the corresponding icon. You can also customize this table by clicking on .



Part



10 Tutorials

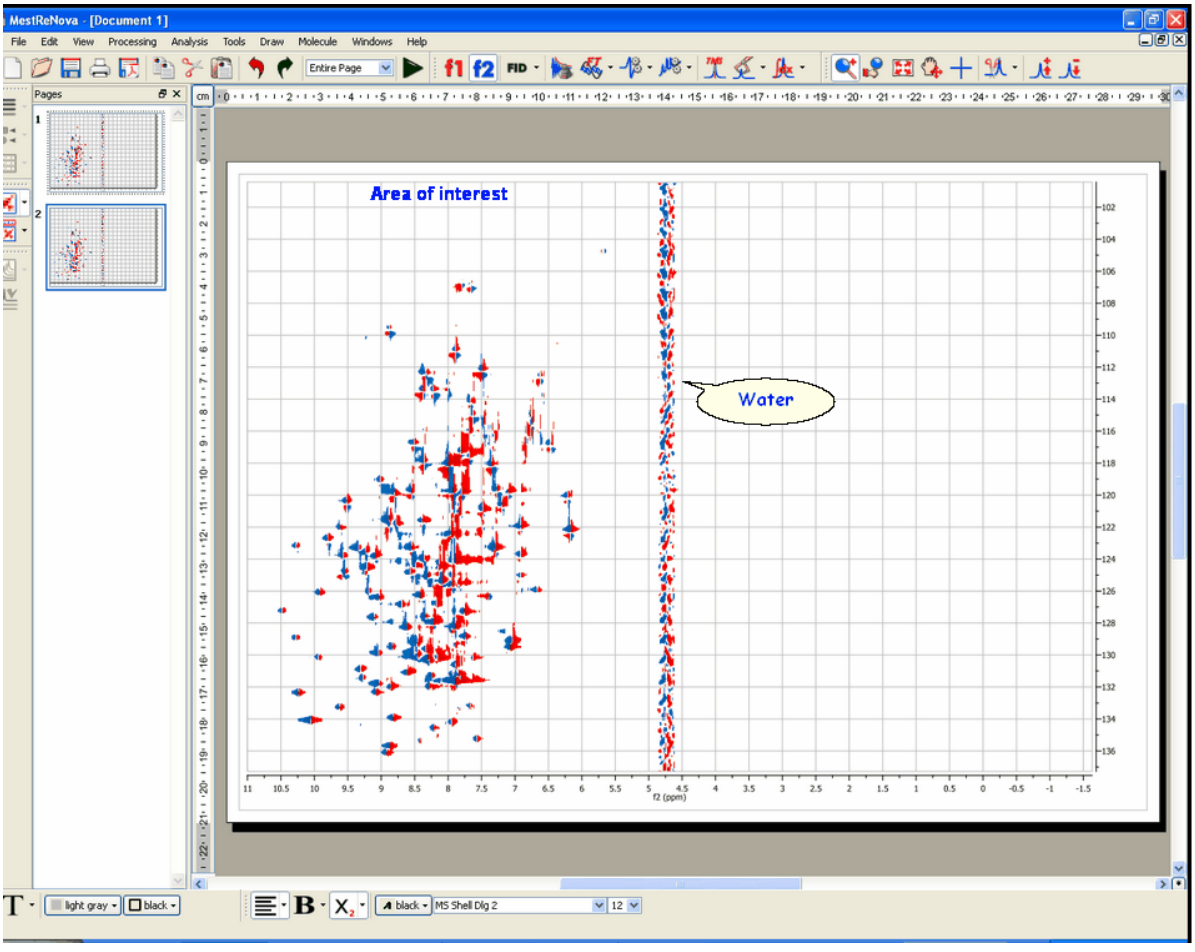
The goal of the present chapter is to give you a fast introduction to the basic analysis tools which are commonly used to solve structural problems. Not everything is explained in detail; the rest of the manual provides a more comprehensive description of all topics covered here.

We are going to start with a tutorial about the 'Manual Phase Correction in 2D' and in the following days we will add more tutorials as soon as we have them ready.

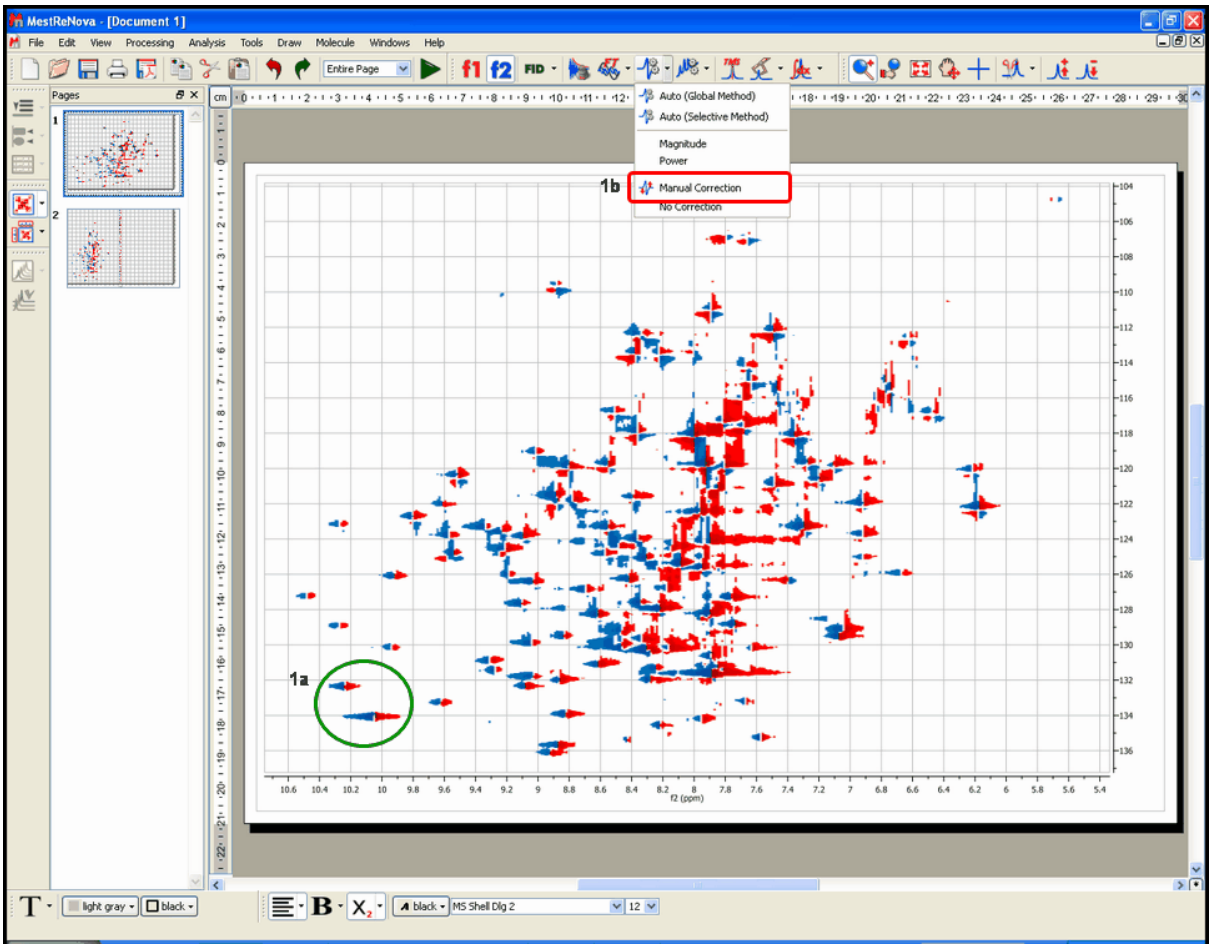
10.1 Manual Phase Correction in 2D-NMR

In this tutorial, we will explain how to phase the spectrum in real time, directly on the 2D spectrum.

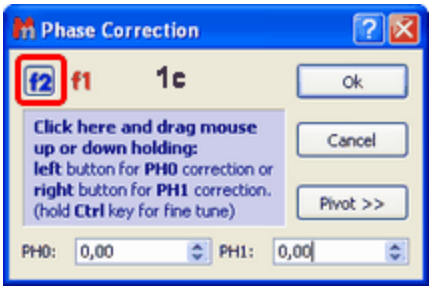
We will show how to correct the phase of a gNHSQC experiment (see figure below).



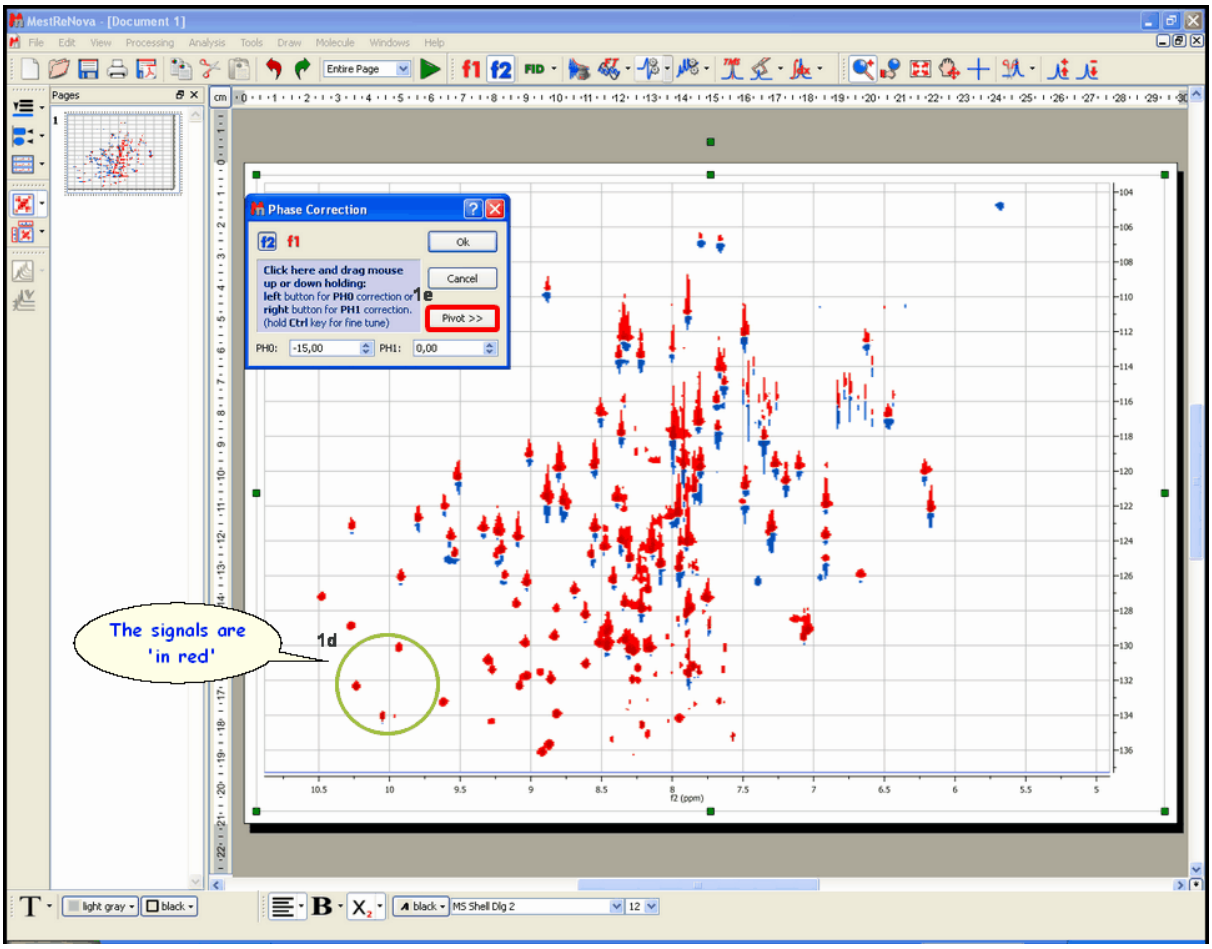
Once you have opened the spectrum; *'zoom in'* the area of interest (in this case, we only need to worry about the left half of the spectrum):



1. Then, take a look at the signal, or the group of signals (**1a**), which appear in a corner of the spectrum (for example, the signals highlighted with a green circle in the capture above); select 'manual correction' (**1b**) from the drop down menu on the 'Phase Correction' toolbar button and adjust the zero order (PH0) phase parameter along f2 (by dragging the mouse up or down over the blue rectangle in the 'Phase Correction dialog box' whilst holding the left mouse button (**1c**)).

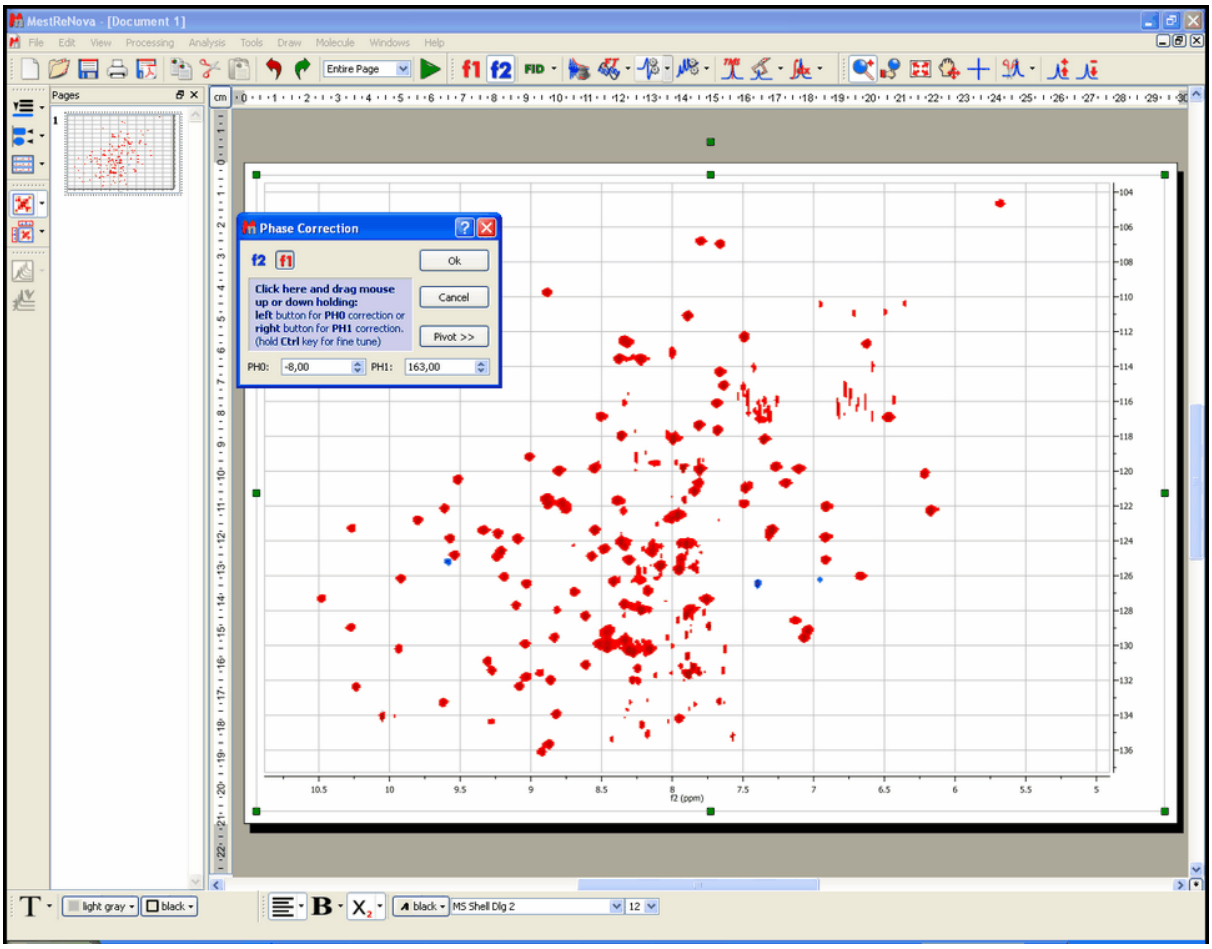


The goal (**1d**) will be to make all the **signals of the area of interest** (highlighted with the green circle in the picture above) positive (note that in this example, positive signals are red whereas negative are blue). Remember that we are adjusting the phase along f2, so do not worry if the phase of the peaks along the indirect (vertical) dimension (f1) is not correct.



Next, if some peaks on the right side of the area of the spectrum shown on screen require phase correction (remember, still along f2), you can place the pivot point (**1e**) over the signal we have selected as reference (in the green circle) and carry out the first order phase correction PH1 (by dragging the mouse up or down over the blue rectangle in the 'Phase Correction dialog box' whilst holding the right mouse button). In this particular case, the spectrum does not require first order correction.

2. Finally, if necessary (as in this case), you can proceed in the same manner to correct the phase along **f1.** Just click on the f1 button on the dialog box and then proceed as explained along f2, by starting with a zero order phase correction and following with a first order phase correction (remember: along **f1**):



Bear in mind that this procedure is exactly the same as with 1D-NMR spectra, but it is necessary to correct the phase along 2 dimensions.

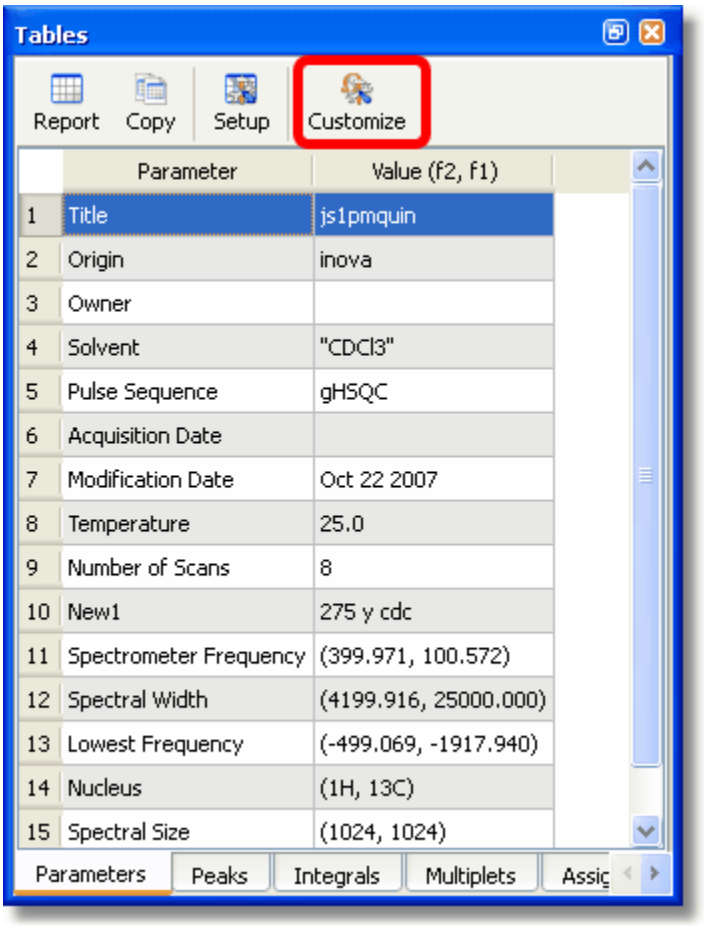
Note that, in our specific example, the resulting spectrum has 3 negative signals, but this is something that a phase correction is not able to correct.

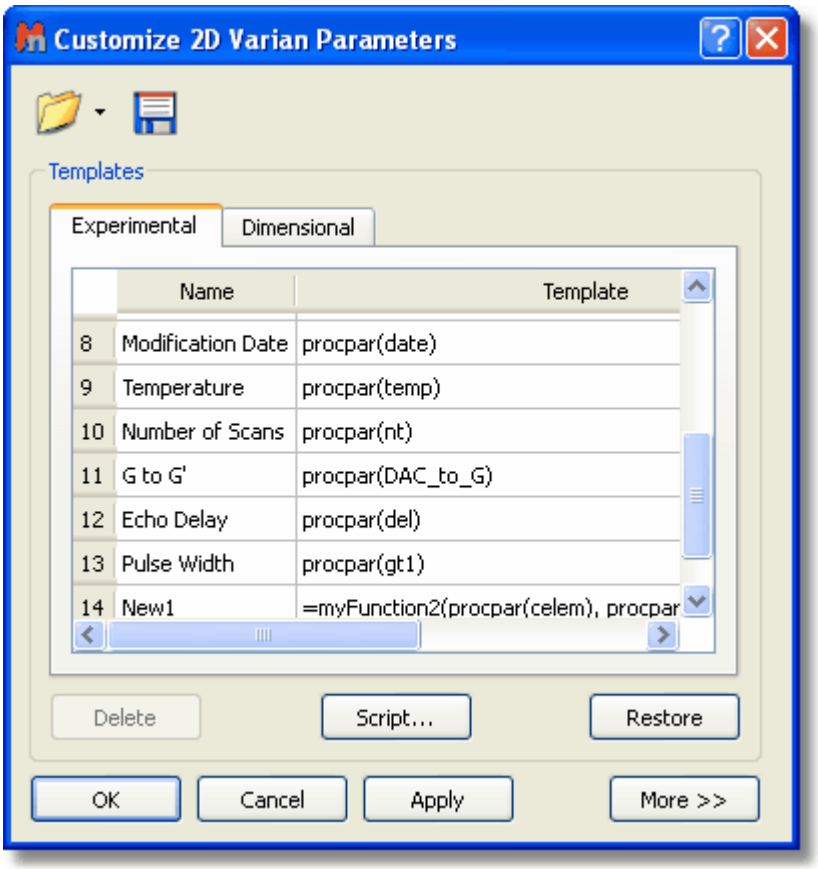
10.2 Importing Spectrum Parameters

The 'Importing Spectrum Parameters' feature will allow Mnova users to include any variable into the Parameters Table from the raw data file. In addition, the users will be able to create 'Parameters Templates' in order to obtain the same parameters in all of their processed spectra. Before this feature was implemented, only standard spectrum parameters could be included in the corresponding table.

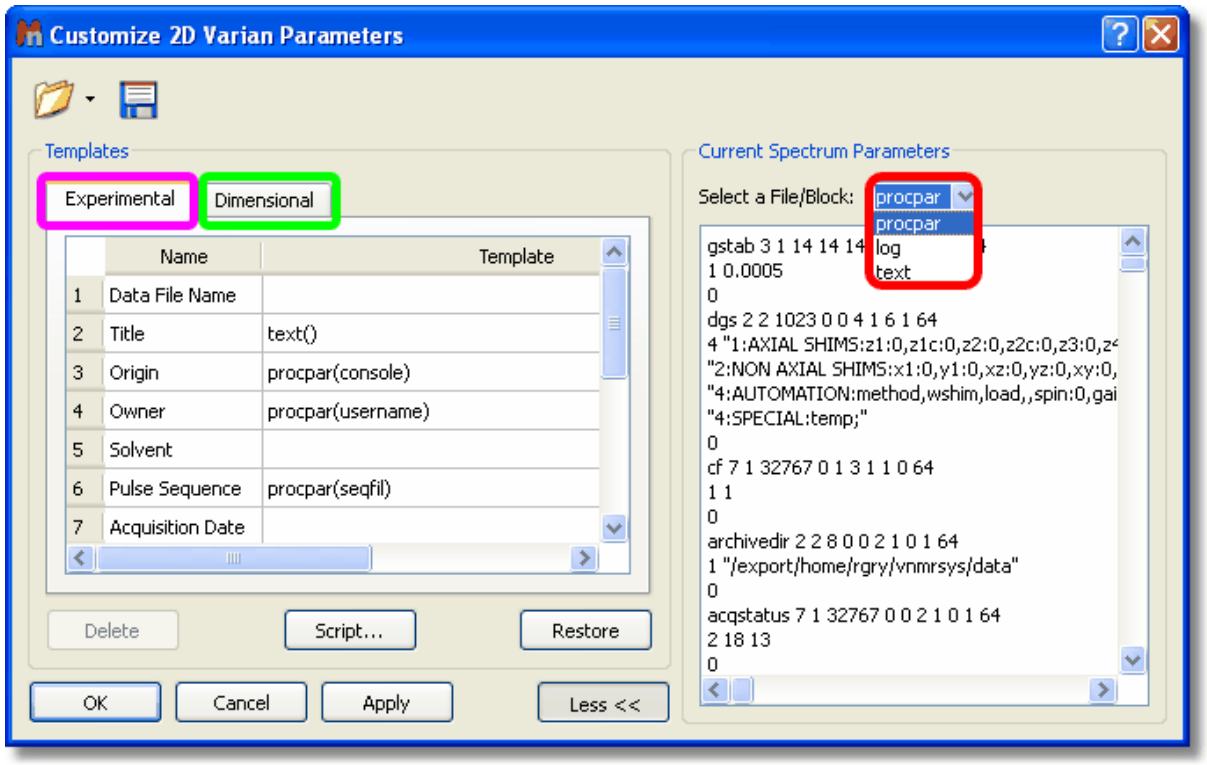
The user will easily be able to add, create, remove or modify any spectrum parameter or even to create templates in order to obtain the same parameters in any processed spectra.

Customization of the parameters table in Mnova is very intuitive, just follow the menu: View/Tables/Parameters and click on the ‘Customize’ icon to display the 'Customize Parameters' dialog box.



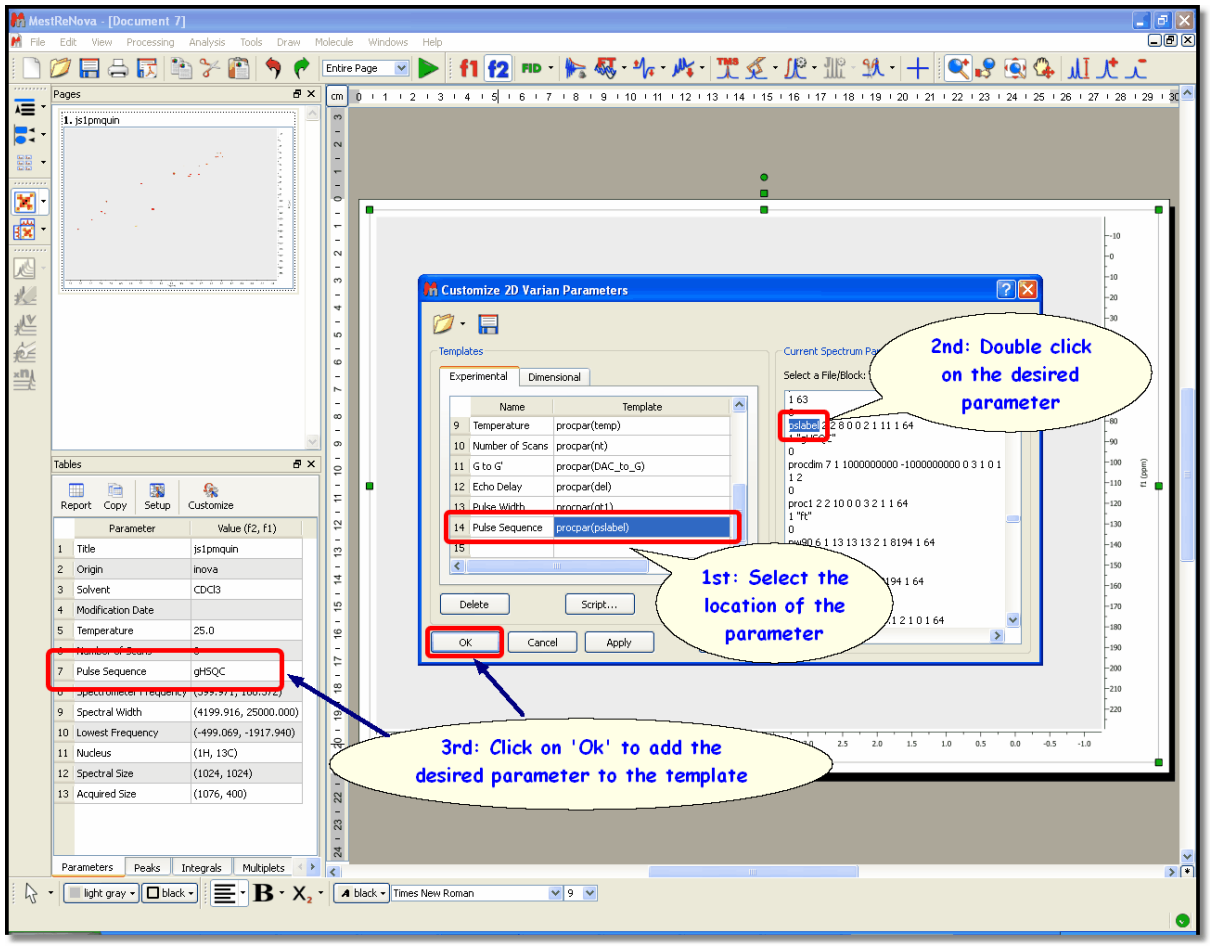


Clicking on the 'More>>' button will display the 'Current Spectrum Parameters' window, as you can see in the picture below. The user will be able to obtain any parameter from any raw data file (procs, acqus, title, etc...) by selecting the desired file on the 'File/Block' box (red square in the picture below):

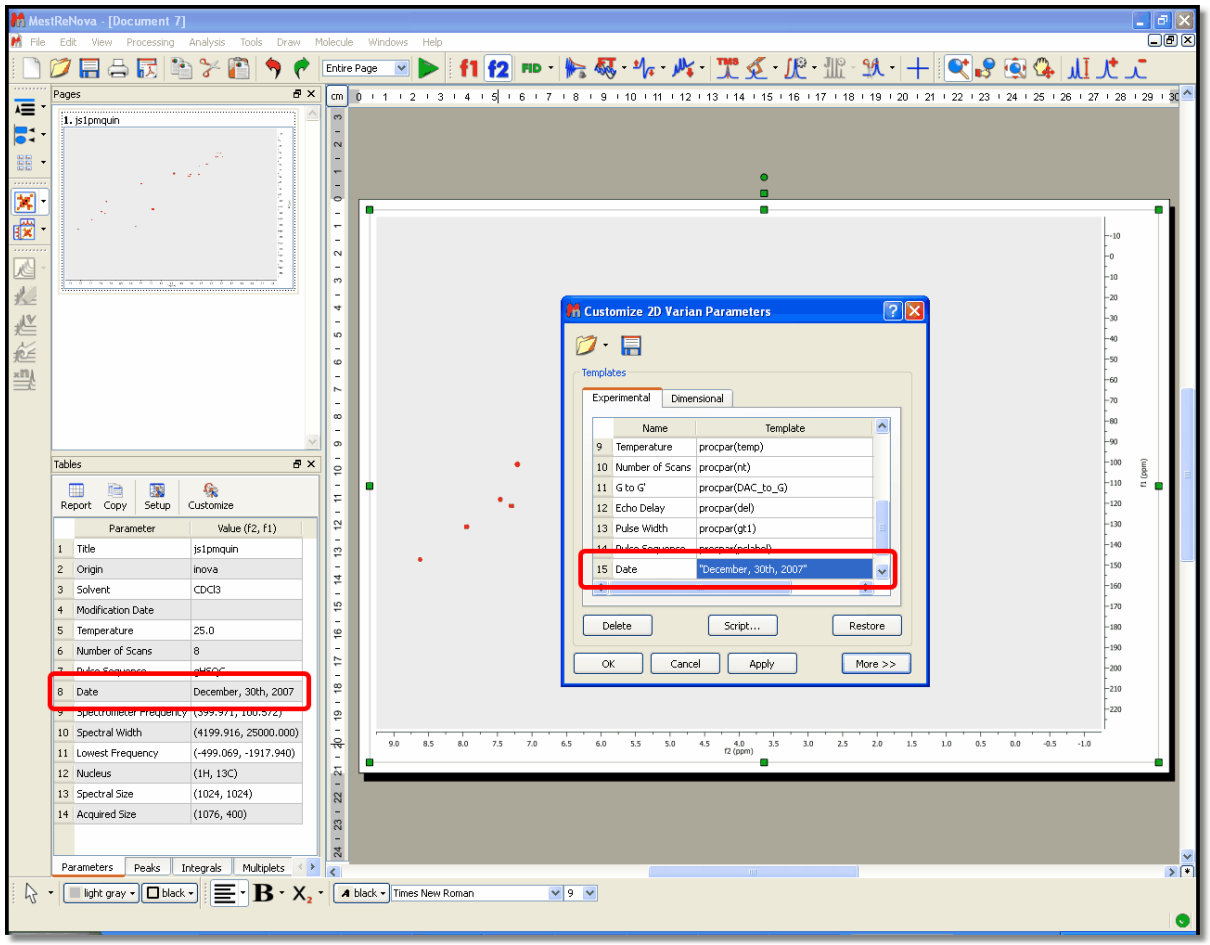


In the 'Customize Parameters' dialog box, the user will see an ‘Experimental Parameters Template’ (pink square in the picture above) which will be common for 1D and 2D (with information about: Title, Origin, Owner, Solvent, Temperature, etc.) and the ‘Dimensional Parameters Template' (green square in the picture above) with information about the 'Spectrometer Frequency', "Acquired Size', 'Spectral Width', etc.). Both sections have blank rows in their last position to allow for addition of further parameters.

You can easily add any existing parameter from the raw data. Start by selecting the location of the parameter in the Experimental or Dimensional Parameters Template by clicking on the relevant cell of the 'Template' column on the table. Once this has been selected, double click on the desired parameter (from the 'Current Spectrum Parameters' box). This will add the chosen parameter to the template.

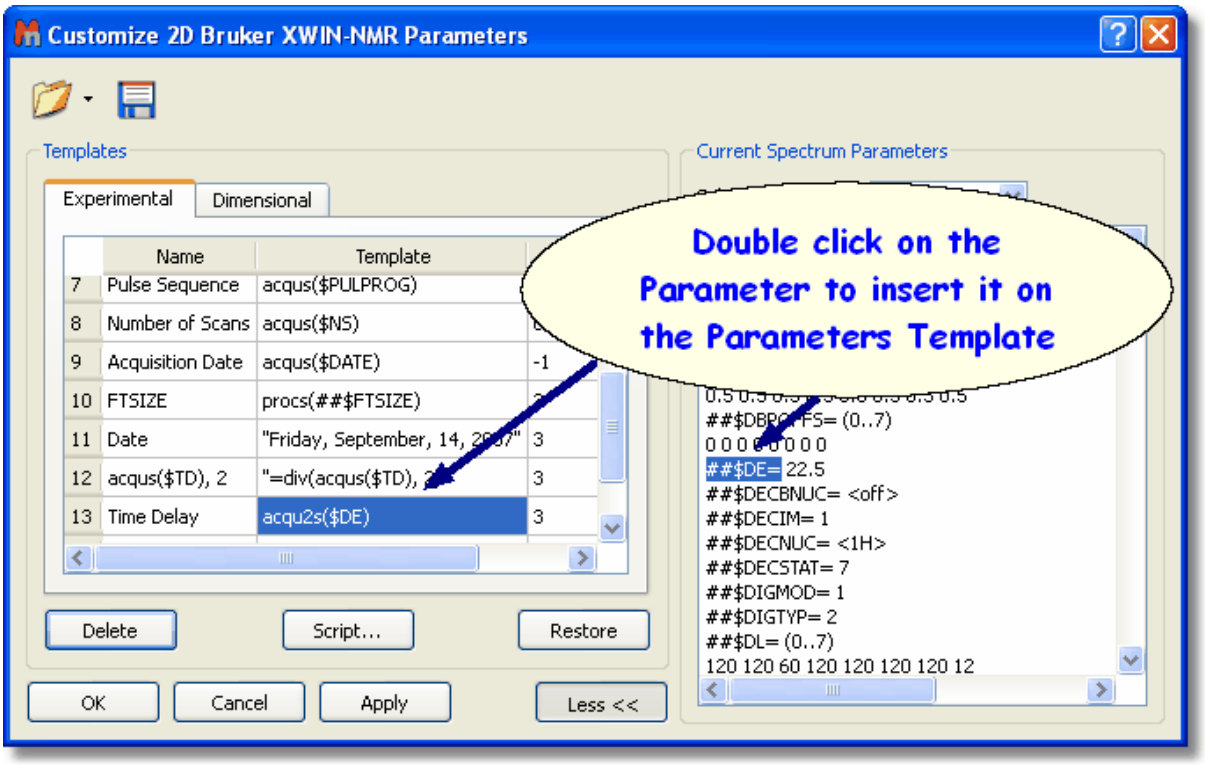


The user will also be able to add his/her own parameters by double clicking on the table and typing the corresponding parameter name (between "quotation marks") and value. In the picture below, we have added manually the text "December, 30th, 2007" in the field: 'Original Name' and 'Date' as the 'Visible Name'

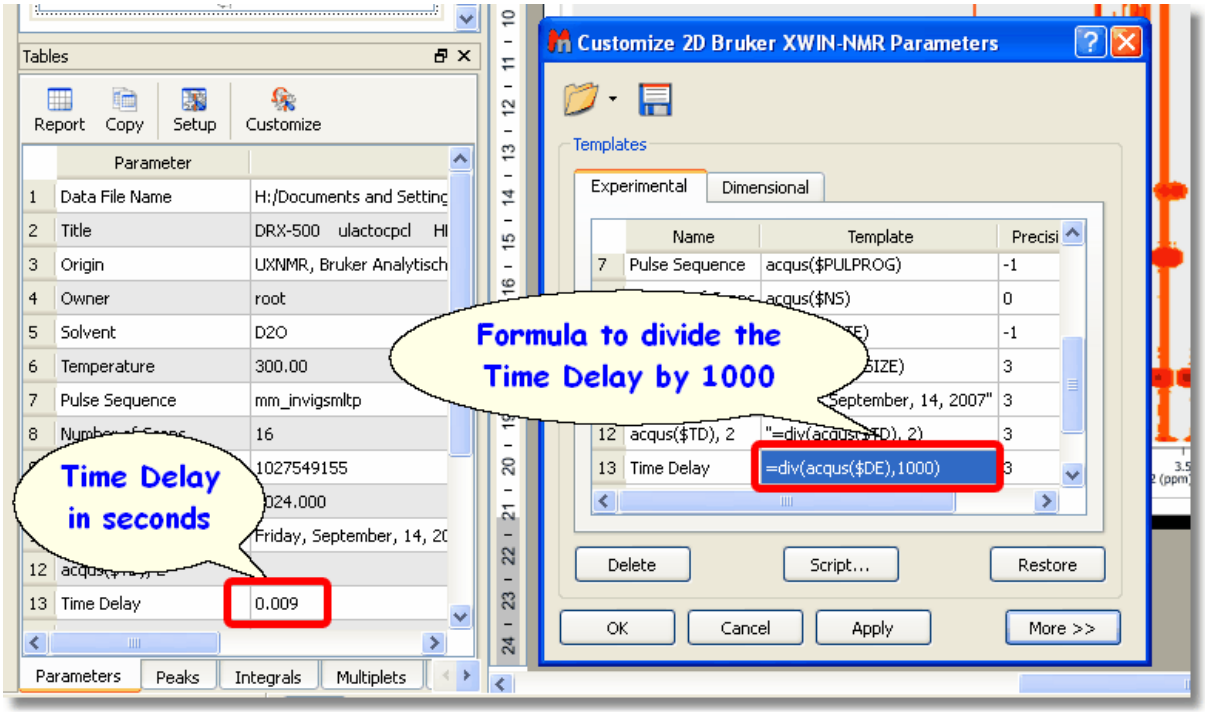


The user will also be able to introduce formulas (available in the script) by typing an *equal* symbol followed by the desired formula (numbers or parameters), for example: =sum(a, b), or =div(acqus($TD), 2). Please note that formulas are case sensitive and should be lower case.

The following example shows the procedure to show the 'Time Delay' in seconds. Firstly, we need to double click on the desired parameter in the 'Current Spectrum Parameters' list to add it to the ' Parameters Template':



By default the Time Delay is showed in milliseconds, so we need to divide the value by 1000 to obtain it in seconds. So, the user will need to type '=div(acqus($DE),1000)' in the 'Parameters Template' to get the division. In this way the user will obtain the 'Time Delay' value in seconds in the Parameters Table, as you can see in the picture below:

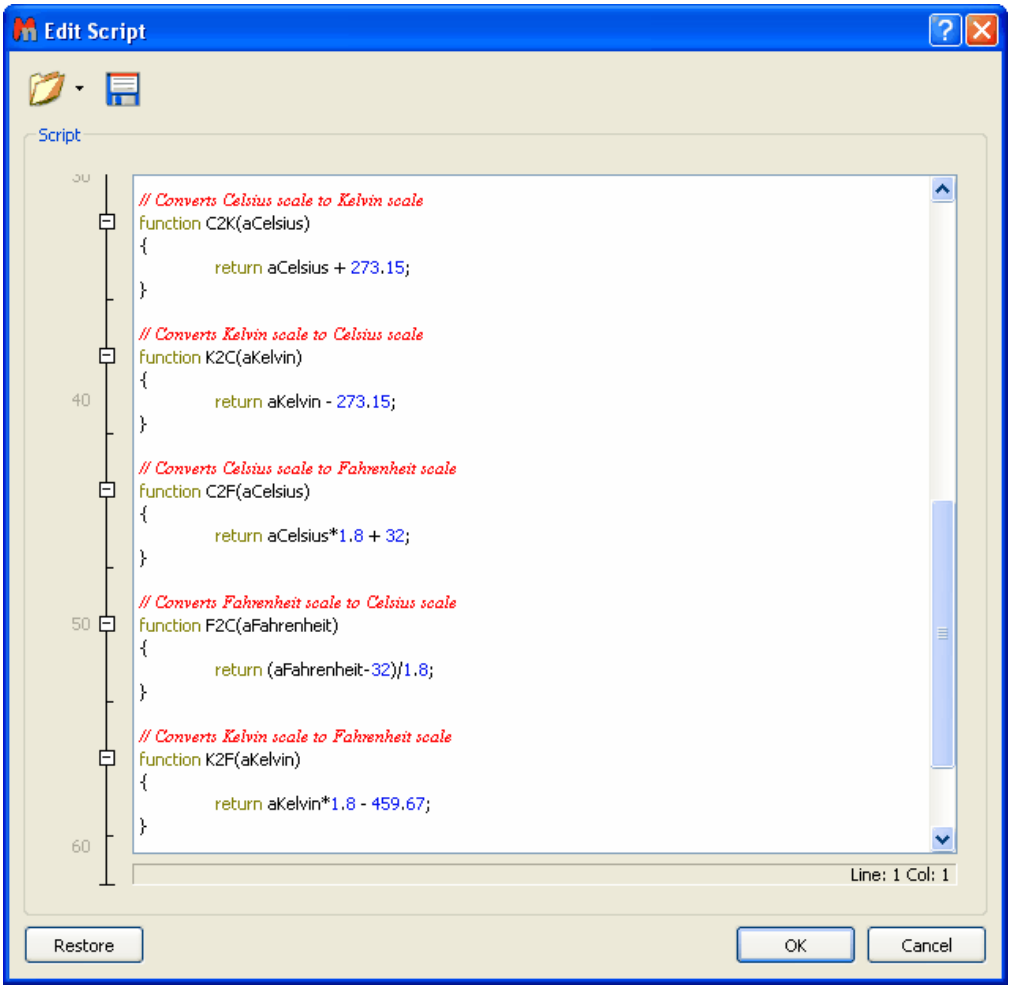


The parameters will be hidden in the table display if the user removes the corresponding tick mark on the 'Visible' Column. The user can also delete any parameters from the table by highlighting each of them and clicking on the 'Delete' button.

The 'Restore' button is used to recover the default template. The user will be able to save his/her own template by clicking on the 'Save Template' icon  . To load a template, just click on the 'Open Template' button  . Clicking on 'OK' will save the template in the registry and Mnova will remember it for futures uses. The 'Apply' button is used to view the effect of any changes directly on the parameters table without needing to close the 'Customize Parameters' dialog box.

The user will be able to edit the 'Parameters' script by clicking on the 'Script' button 

. This will display the script with some functions (to sum, divide, convert Celsius to Kelvin or to Fahrenheit, etc.).



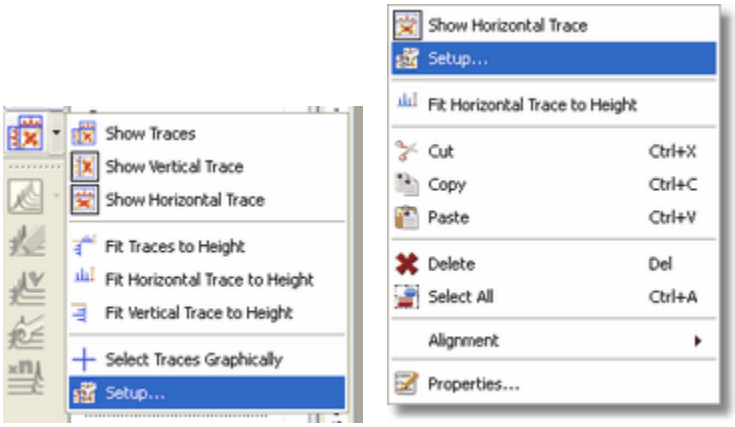
See also:

Scripts

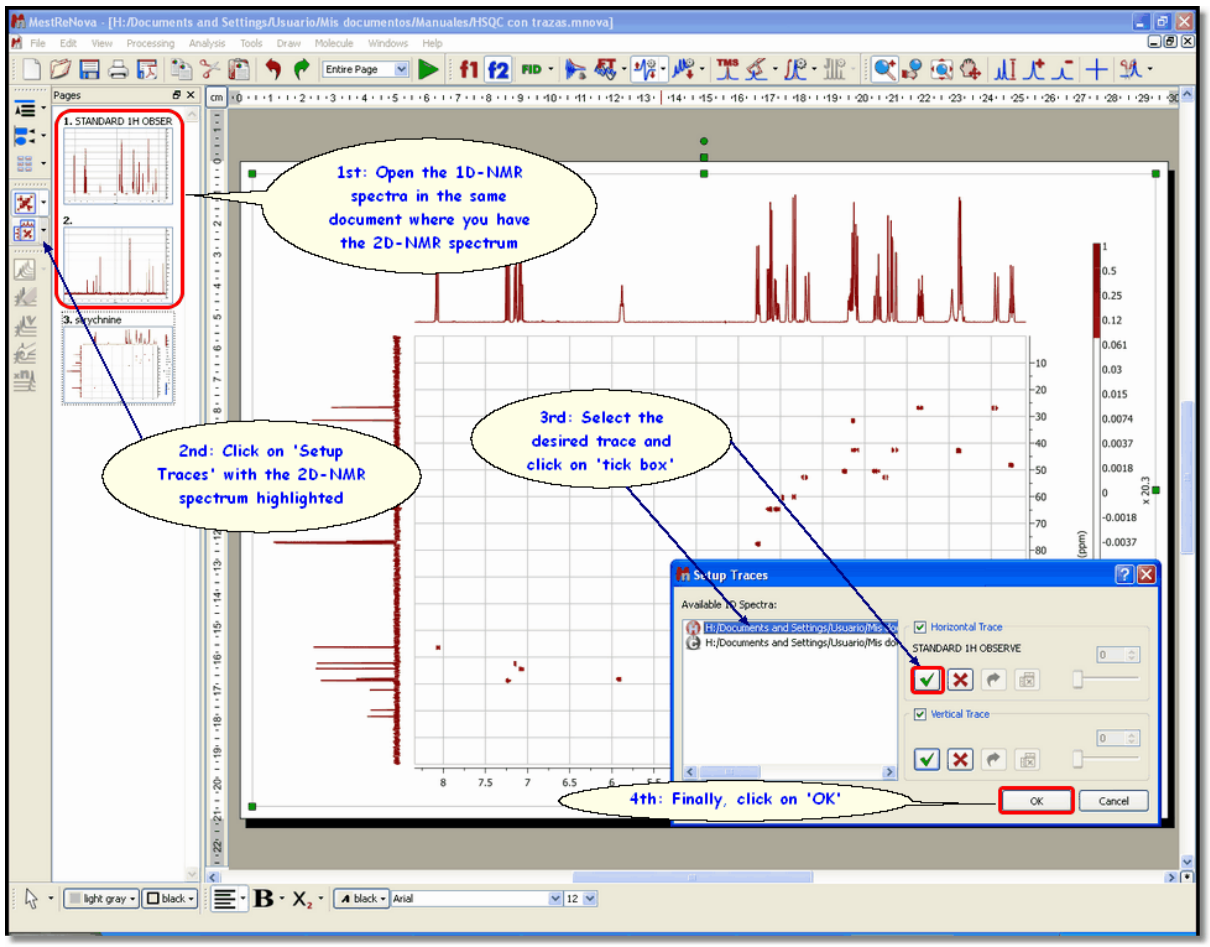
10.3 Show Traces in 2D-NMR

The user can show the traces of the 2D-NMR spectrum, just by clicking on the **'Show Traces'** icon  (or by using the contextual menu by right clicking over the spectral window). On the same icon, you will find a scroll bar menu to setup the vertical and horizontal traces of a bidimensional spectrum. The user can increase or decrease the intensity of the traces simply by scrolling the mouse wheel, after hovering the mouse over the desired trace. To move the trace, just hover the mouse over the top-right areas of the slices (the arrow cursor will change to a vertical panning cursor) and then click & drag your mouse in the desired direction. You will obtain the same result by holding the 'Ctrl' key while scrolling the mouse wheel over the trace.

You can choose any currently opened 1D-NMR spectrum as a trace by clicking on 'Setup' on the **'S how Traces'** scroll bar menu (or by using the traces contextual menu by right clicking over one of the traces).

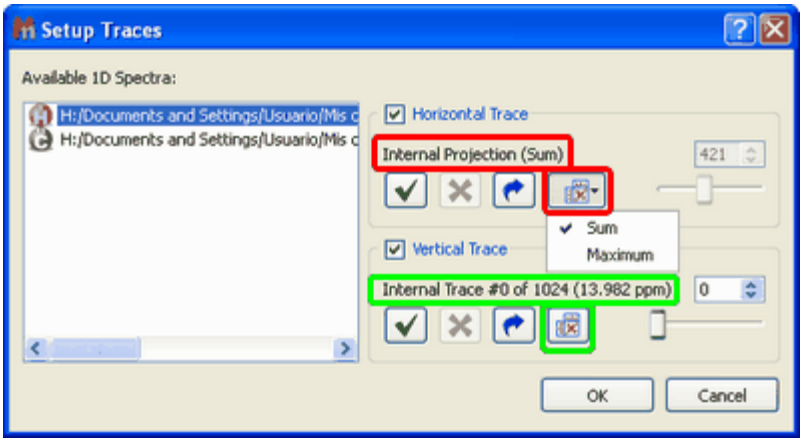


and next selecting the desired 1D-NMR spectrum for the horizontal and then for the vertical trace.

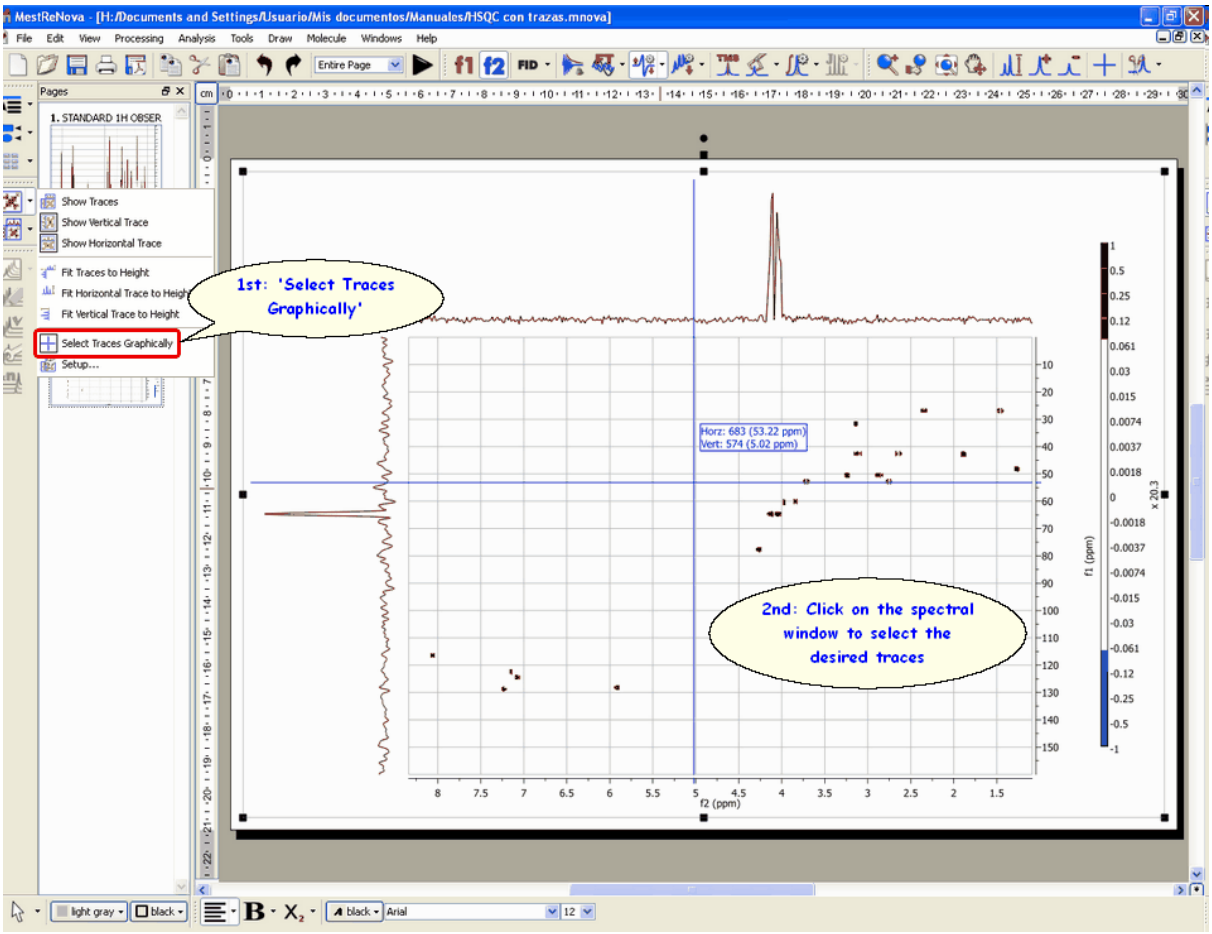


The user will be able to remove the external trace by clicking on the **'Delete'** icon  .

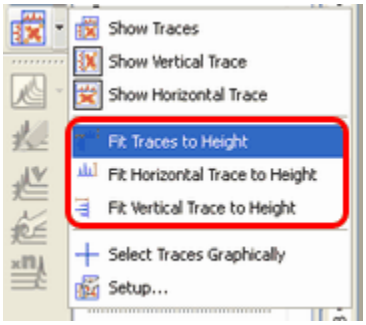
It is now possible to show the **'Internal Projections'** in 2D spectra as either a calculation of the sum of all traces across a given dimension (Sum) or by computing the maximum of every trace (Maximum), just by clicking on the 'Projections icon'  . Click again on this icon  to show the **'Internal Traces'**.



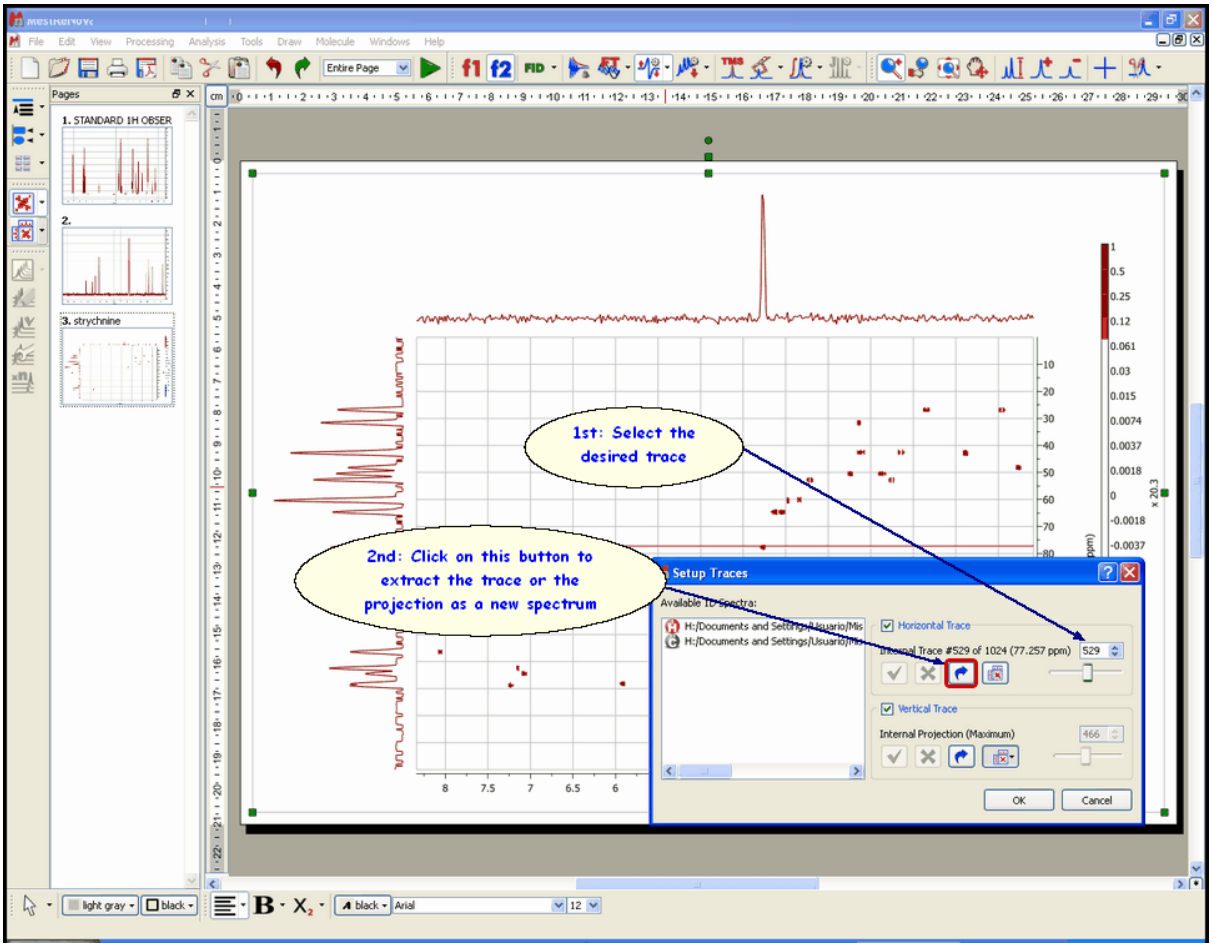
The user can also select the traces directly from the 2D-NMR spectrum by using the **'Select Traces Graphically'** tool in the 'Show Traces' scroll bar menu.



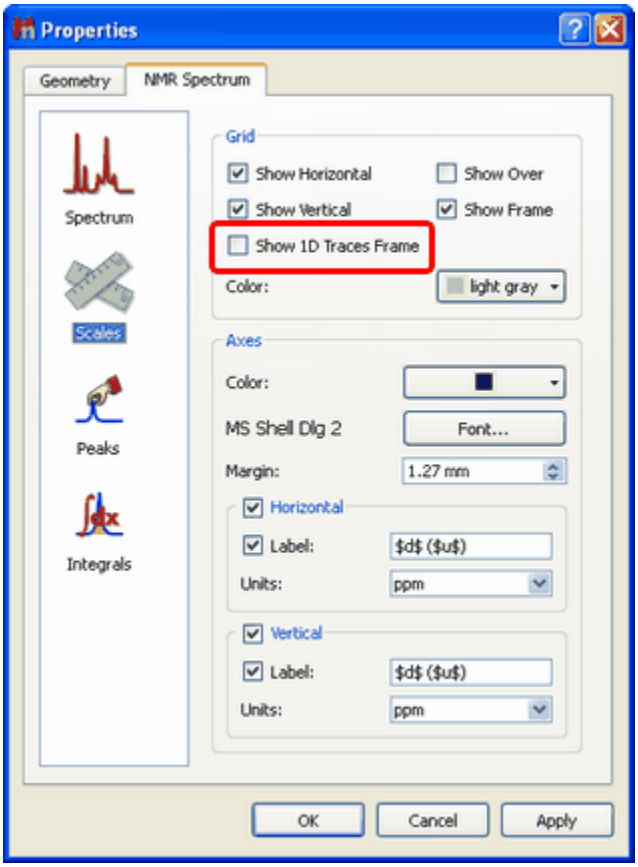
The user will be able to **'Fit Traces to Height'** (the vertical, the horizontal or both), just by following the menu 'View/2D Traces' or by using the **'Show Traces'** scroll bar menu on the left vertical toolbar:



The user can also extract a current trace (or projection) as a new spectrum by clicking on the button  in the 'Setup traces' dialog box. In the figure below, you can see how to extract the vertical trace (# 517).



The user will be able to add or remove the 1D traces frame in the 'Scales Properties' dialog box (displayed by double clicking on the 2D-NMR spectral window):



10.4 How to simulate a spectrum with NMRPredict Desktop?

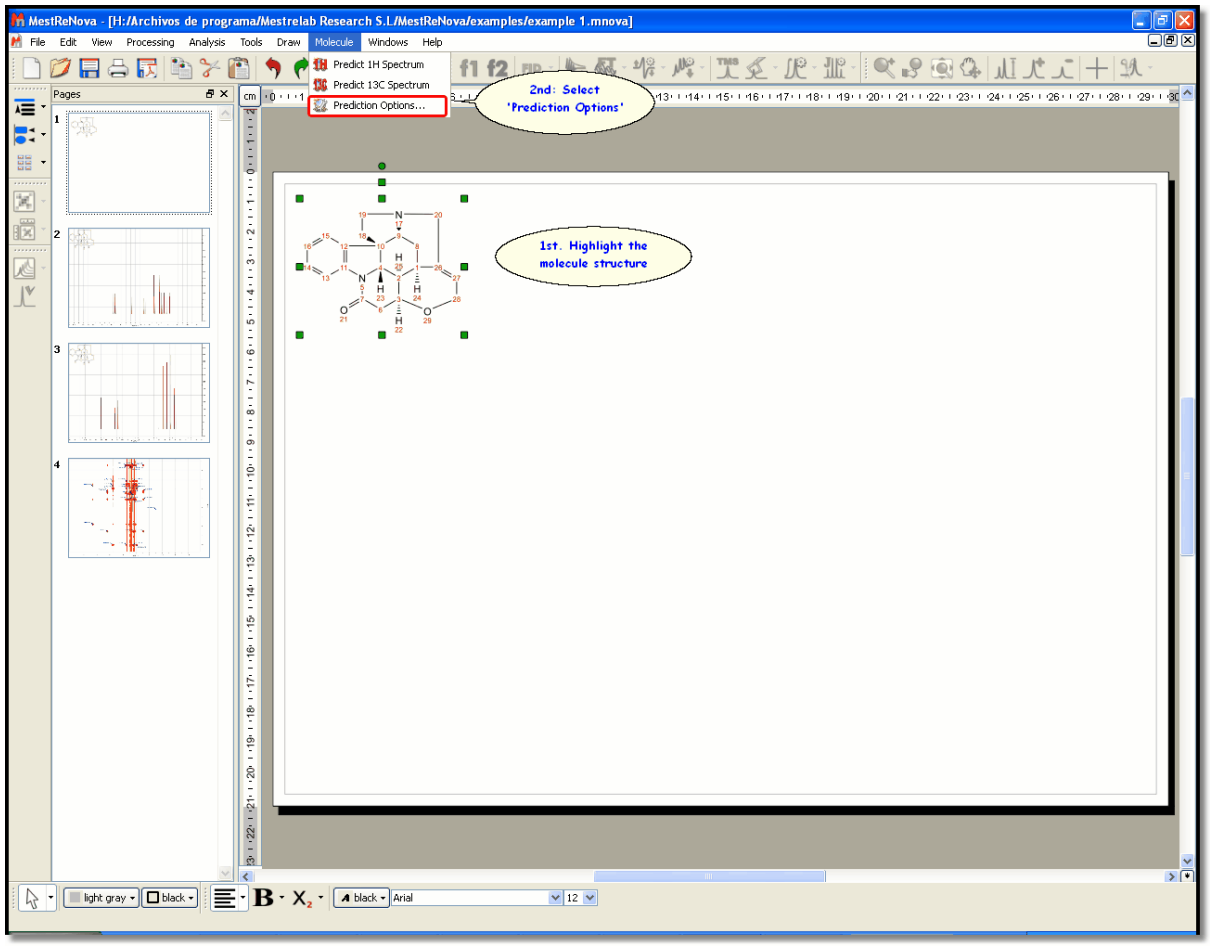
Carrying out of predictions in Mnova with the **NMRPredict Desktop** plugin is very easy and intuitive.

1. Just, import a molecular structure. Currently, molecular structures can be imported as a **.mol** file by simply using the Open command exactly in the same way as this would be applied to a spectrum. Bear in mind, that you are actually able to copy&paste **.cdx** structures from **ChemDraw** and **.mol** from **IsisDraw** but not drag&drop them. Support to 'drag & drop' operations are currently limited to **ChemSketch.**

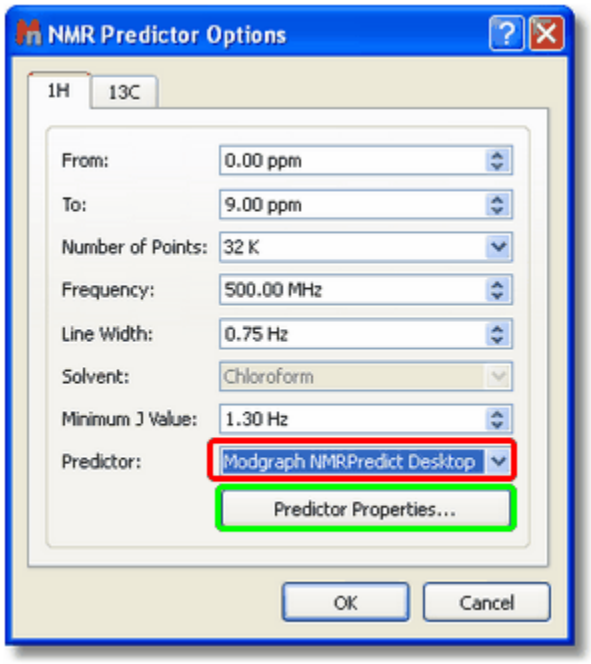
ATTENTION

Bear in mind that, in terms of stereochemistry, drawing a structure as a chair or as a boat means nothing to the .mol file and the computer. In those cases the user must draw all the corresponding bonds to show the stereochemistry of the molecule, i.e., you must explicitly draw up and down bonds using wedges.

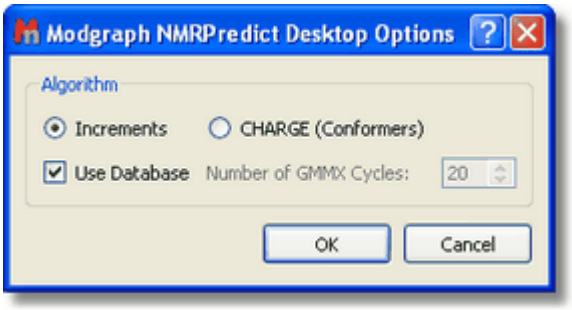
2. Highlight the molecule structure in the active page of Mnova and select 'Prediction Options' in the 'Molecule' menu .



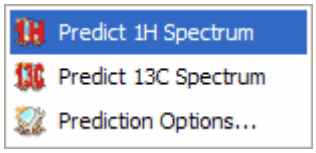
3. This will open the 'NMR Predictor Options' dialog box where you will have to select 'Modgraph NMRPredict Desktop' in the Predictor edit box (red square in the figure below).



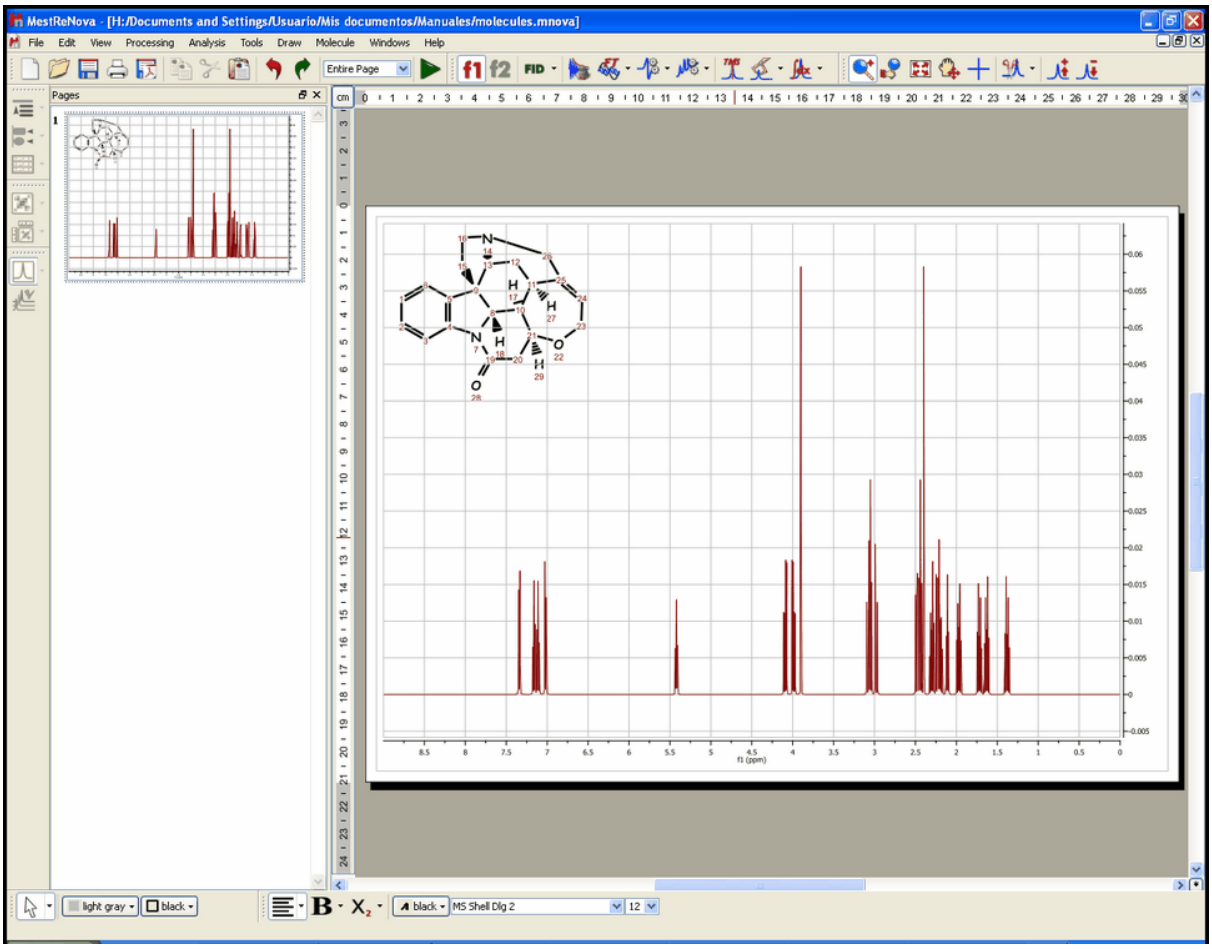
4. In this dialog box, the user is able to select the chemical shift limits of the spectral window, as well as other parameters, such as number of points, frequency, Line Width, solvent and minimum coupling constant value). If you are going to predict a ^{1}H-NMR spectrum, you can also select the prediction algorithm (Increments or Charge) by clicking on the 'Predictor Properties' button (in the green square in the figure above). After that, the **'NMRPredict Desktop Options'** dialog box will be displayed and you will be able to select the desired prediction algorithm (Increments or Charge). If you use the Increments methodology, you will have the option to use or not use the Database, whereas if you use the CHARGE algorithm you can set the 'number of GMMX cycles', as you can see in the picture below:



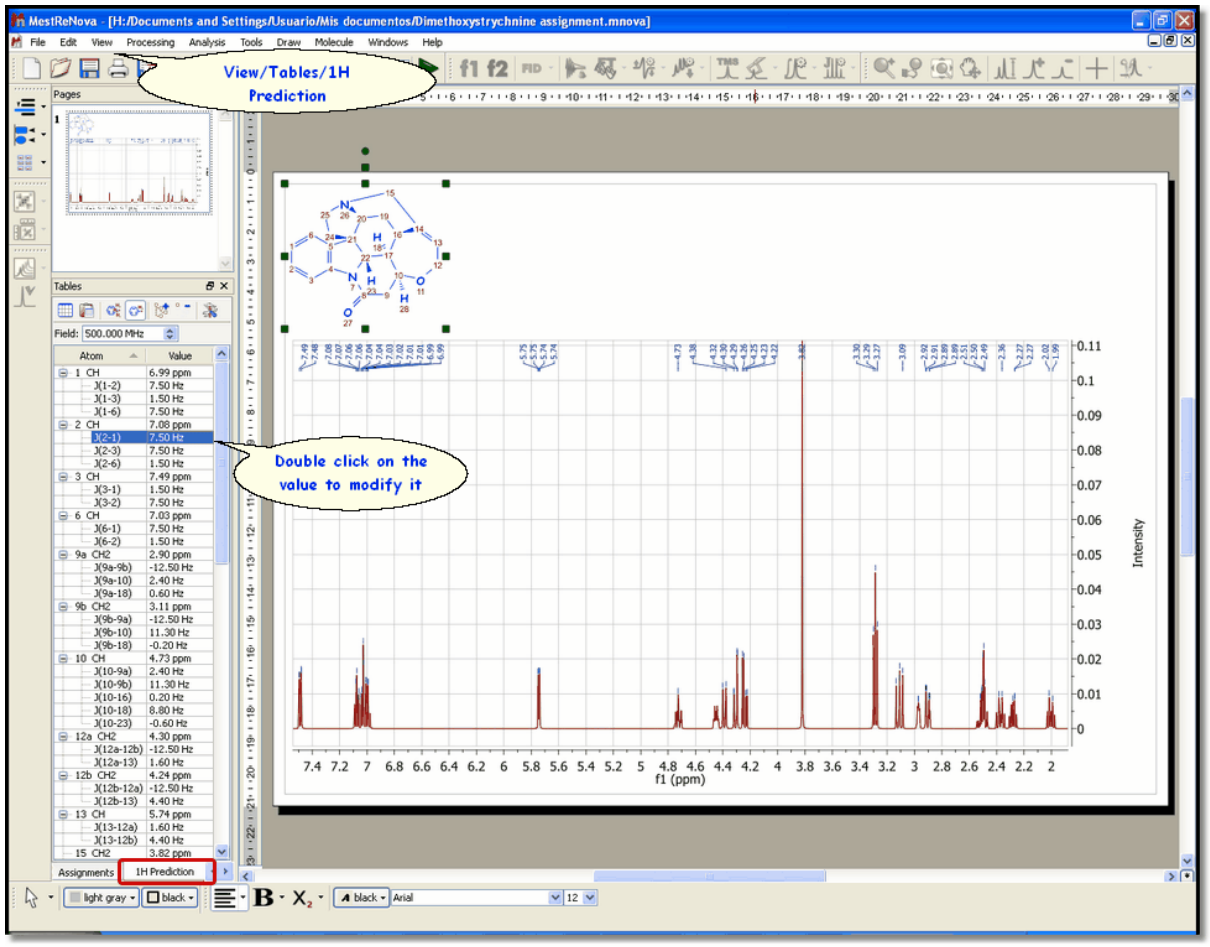
5. Click 'OK' and back again to the 'Molecule' menu to select 'Predict 1H Spectrum':



6. After that, you will obtain the desired predicted ^{1}H-NMR spectrum. This predicted spectrum can be analyzed as a real one (e.g. it can be integrated, peak picking, etc).

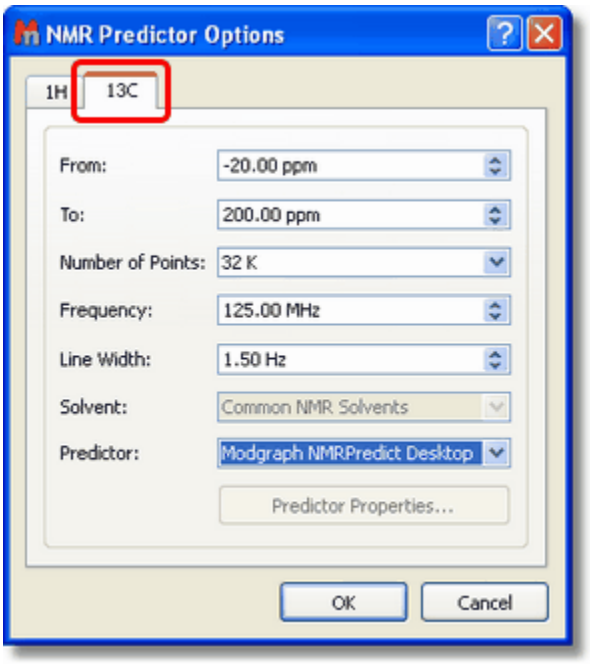


You can also modify the chemical shift and the coupling constant values from the table of the predicted spectrum. Just, highlighted the spectrum, follow the menu 'View/Table/1HPrediction' and then double click on the value that you want to edit. The changes made on the table will take effect automatically in the simulated spectrum.

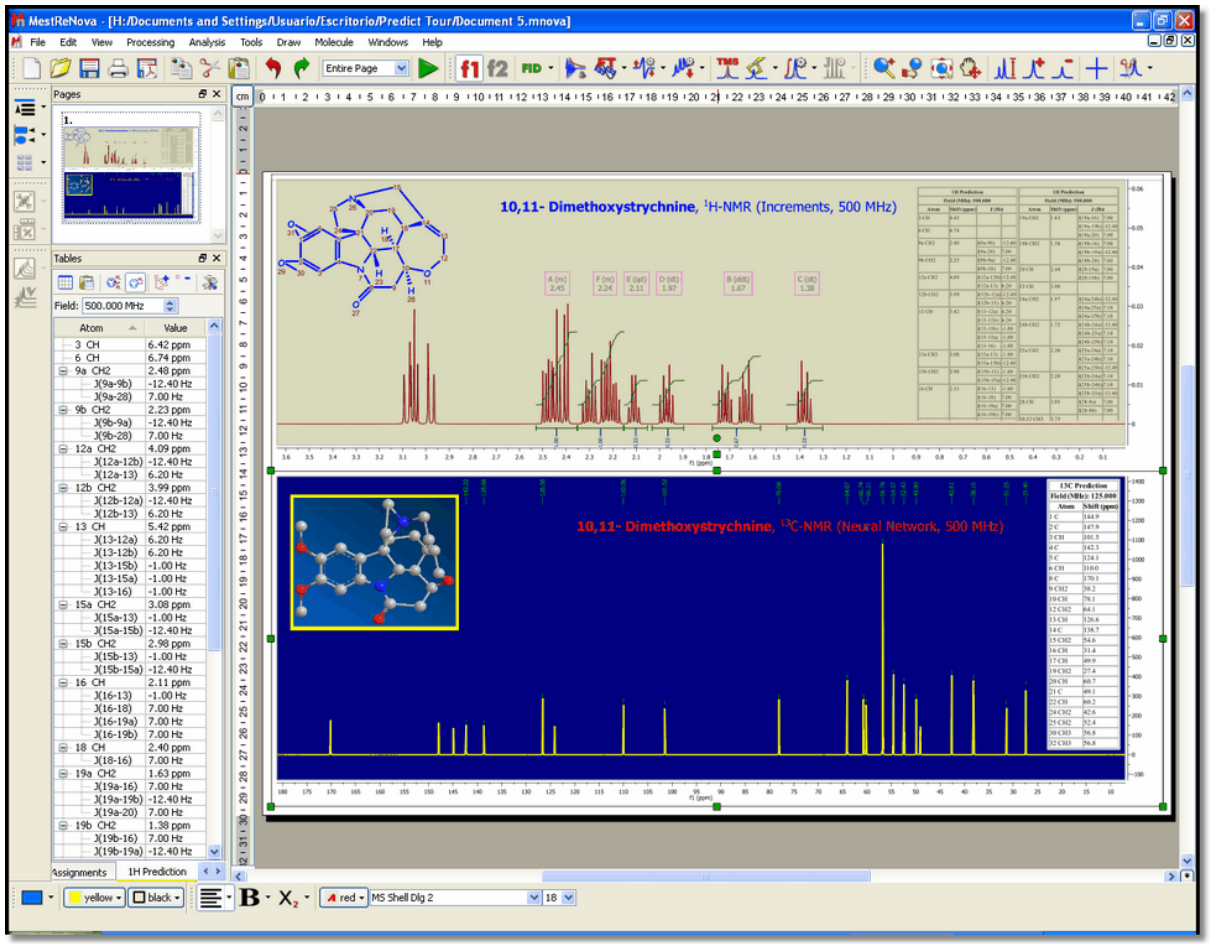


Finally, if you want to know the "predicted assignment", just hover the mouse over the desired atom, (so that it becomes highlighted in a red box) and the corresponding signal in the spectrum will automatically highlight in blue (increasing slightly its intensity); or vice versa (hovering the mouse over the peak will highlight the corresponding atom on the molecular structure).

If you need the ^{13}C-NMR predicted spectrum, follow the same procedure but selecting the options for '13C' (be sure that you select the correct tab for 13C in the 'NMR Predictor Box' dialog, red square in the figure below). As you can see in the 'NMR Predictor Option' dialog box below, you can select the limits, the number of points, the frequency and the line width of the spectrum.



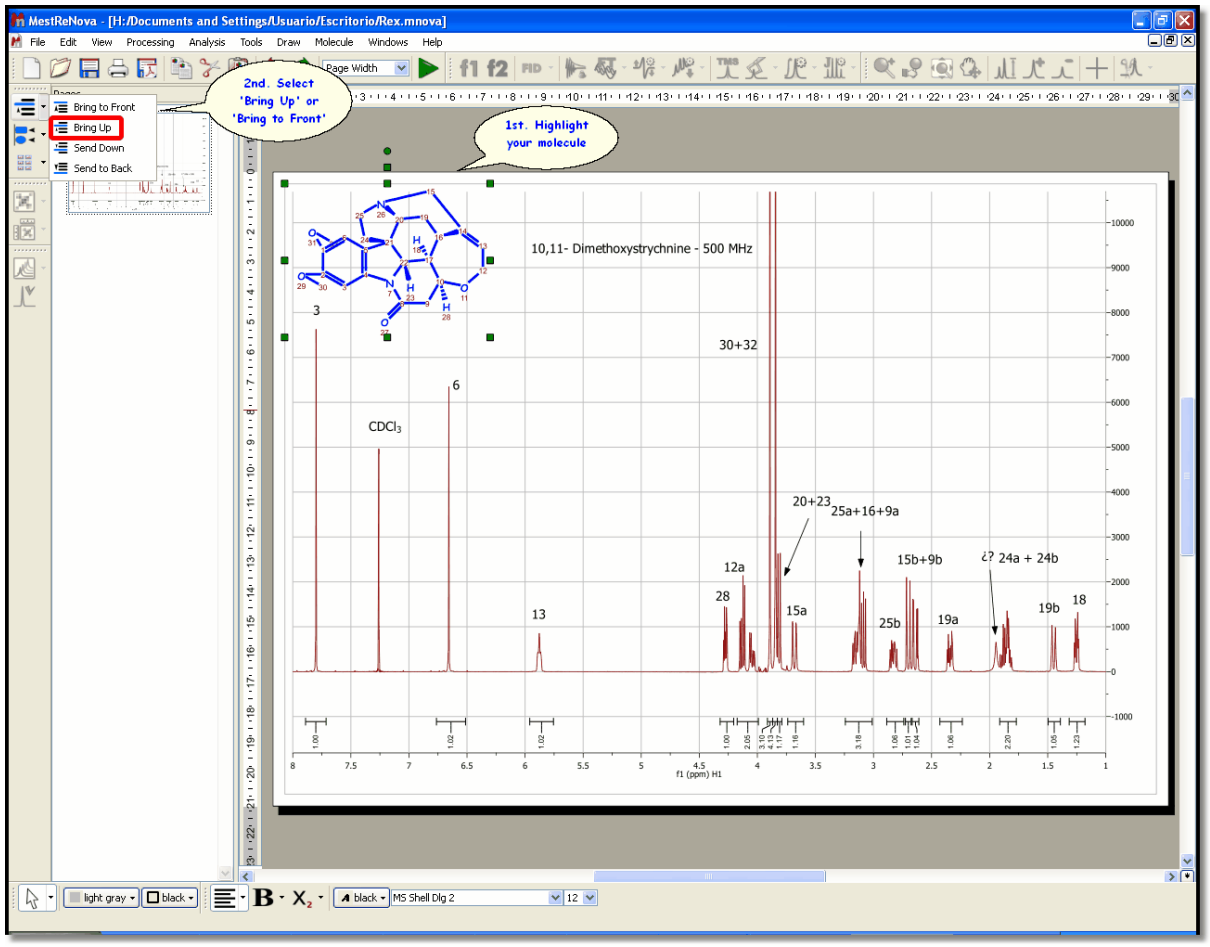
Of course you will be able to change the appearance of your simulated spectra as you prefer. Here is a sample:



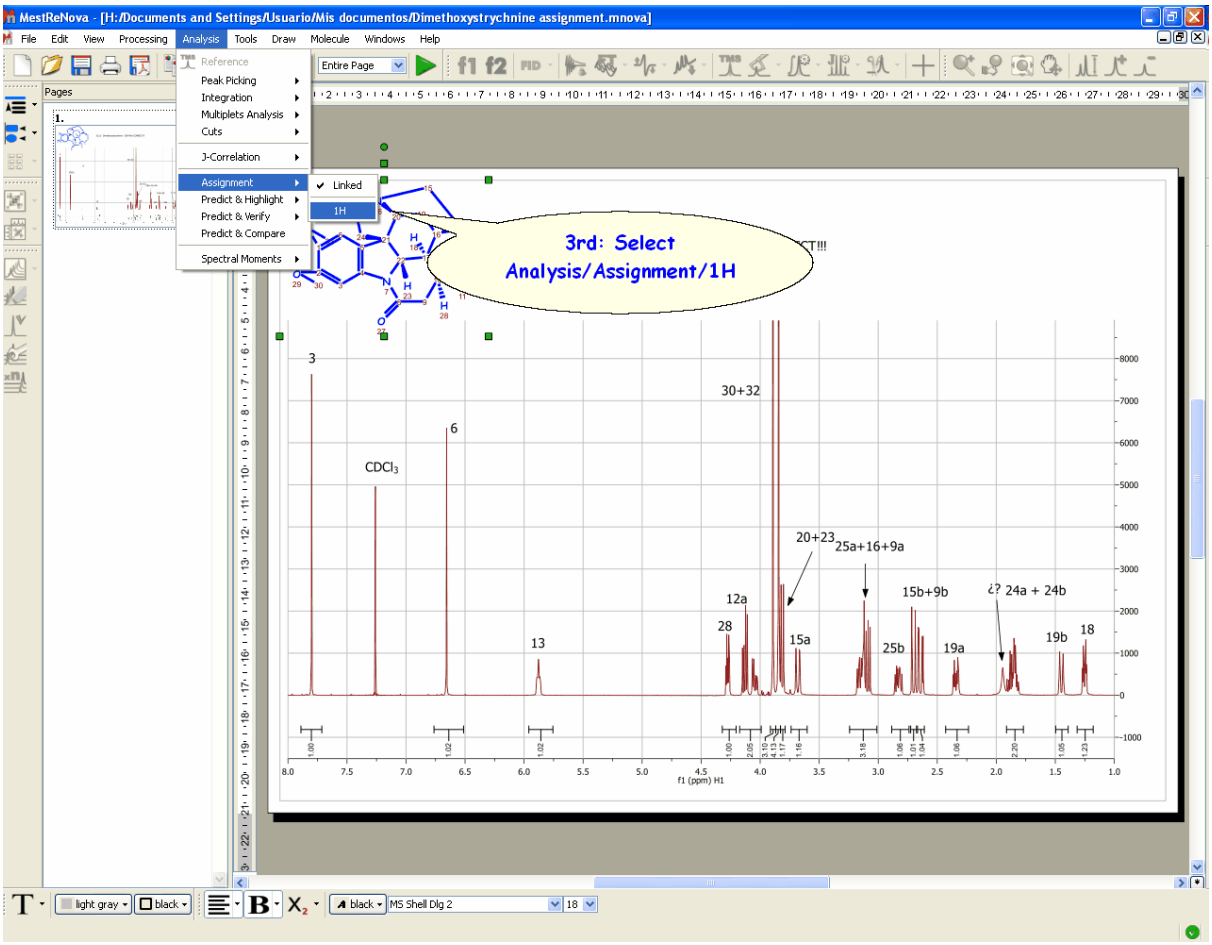
10.5 How to carry out assignments?

It is now possible within Mnova to carry out atom to peak assignments, both to 1D and 2D spectra **(Please note that this feature is not completely finished in this version and that it will be greatly enhanced in the oncoming release versions)**

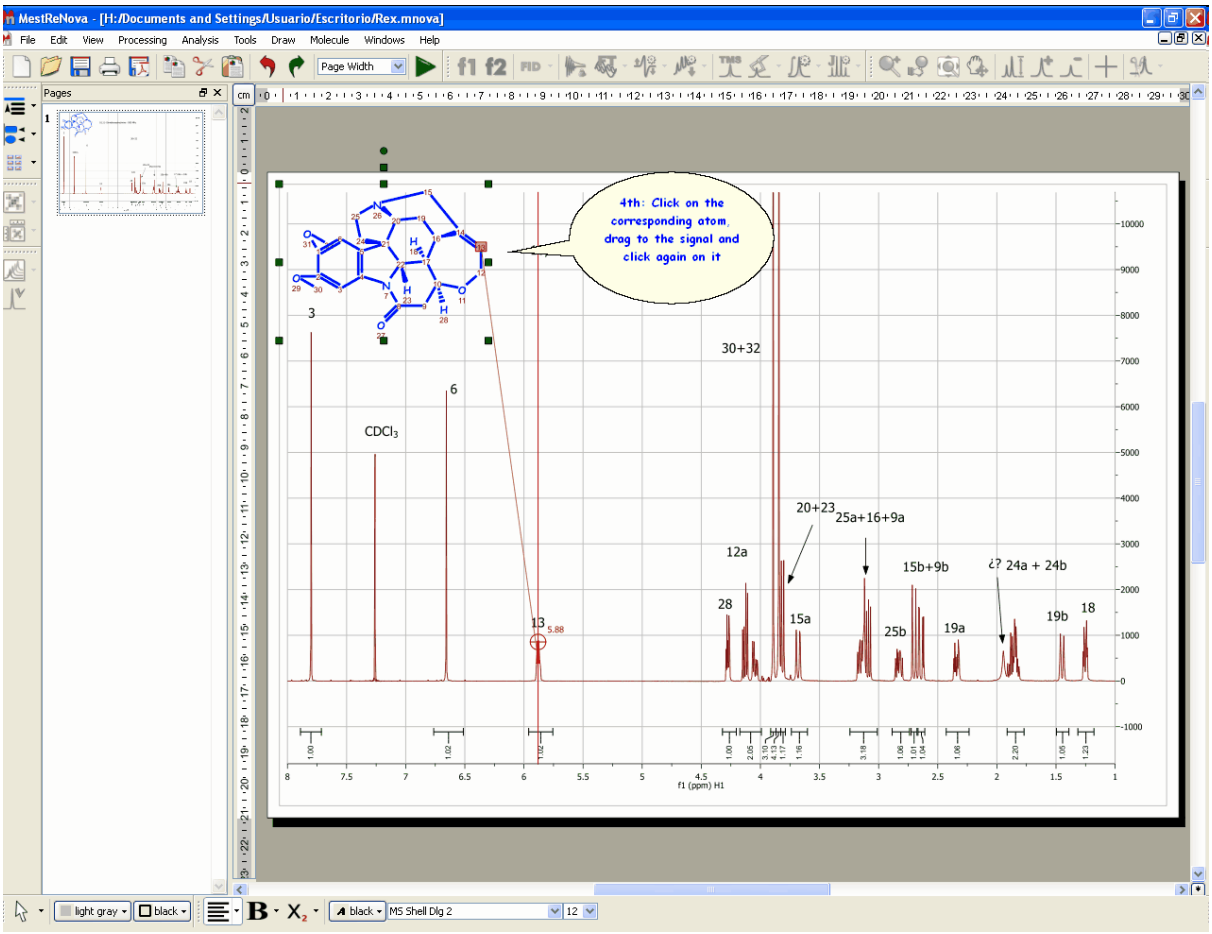
Mnova provides a very simple interface to assign your molecule. Just highlight the molecule and be sure that it is over your spectrum, (if the molecule is under the spectrum, click on 'Bring Up').



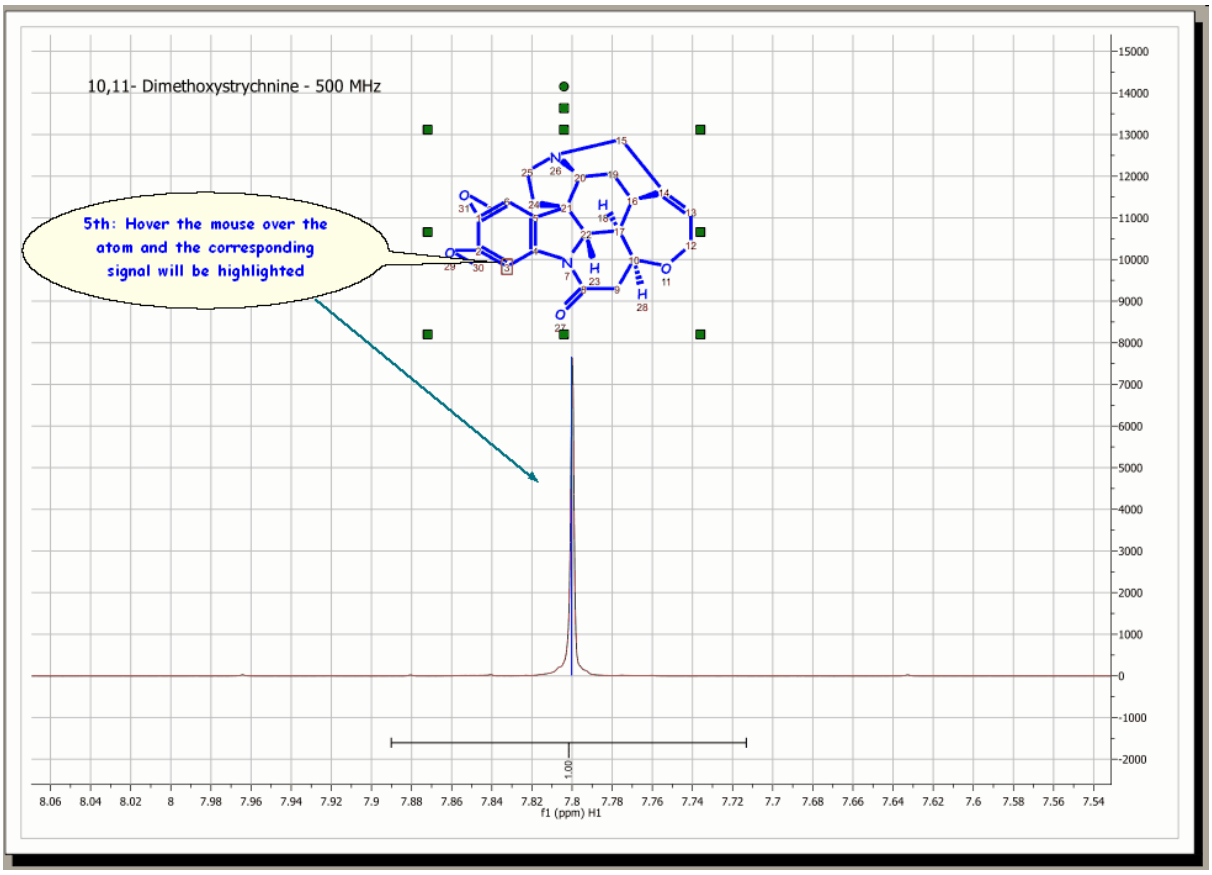
After that, please follow the menu 'Analysis/Assignment' to select the corresponding assignment. The option '**Linked**' will keep the assignment connected to the molecular structure (useful for example, to copy the assignments of the molecular structure to another page, in order to compare a real spectrum with a simulated one).



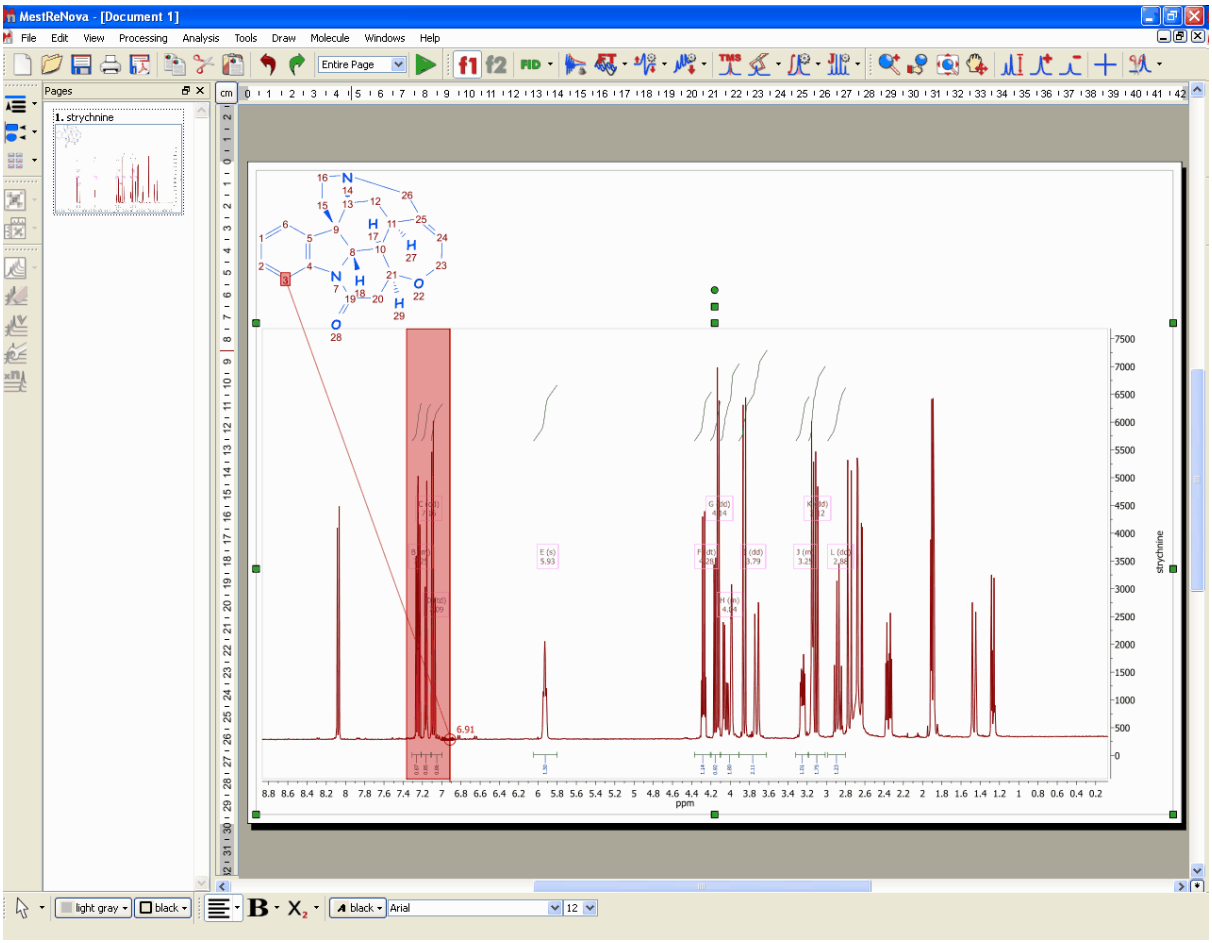
Finally, left click on the desired atom, so that it becomes highlighted in a red box and then release the mouse and drag it to your desired peak. Once your desired peak is highlighted on the spectrum, click on it to assign it.



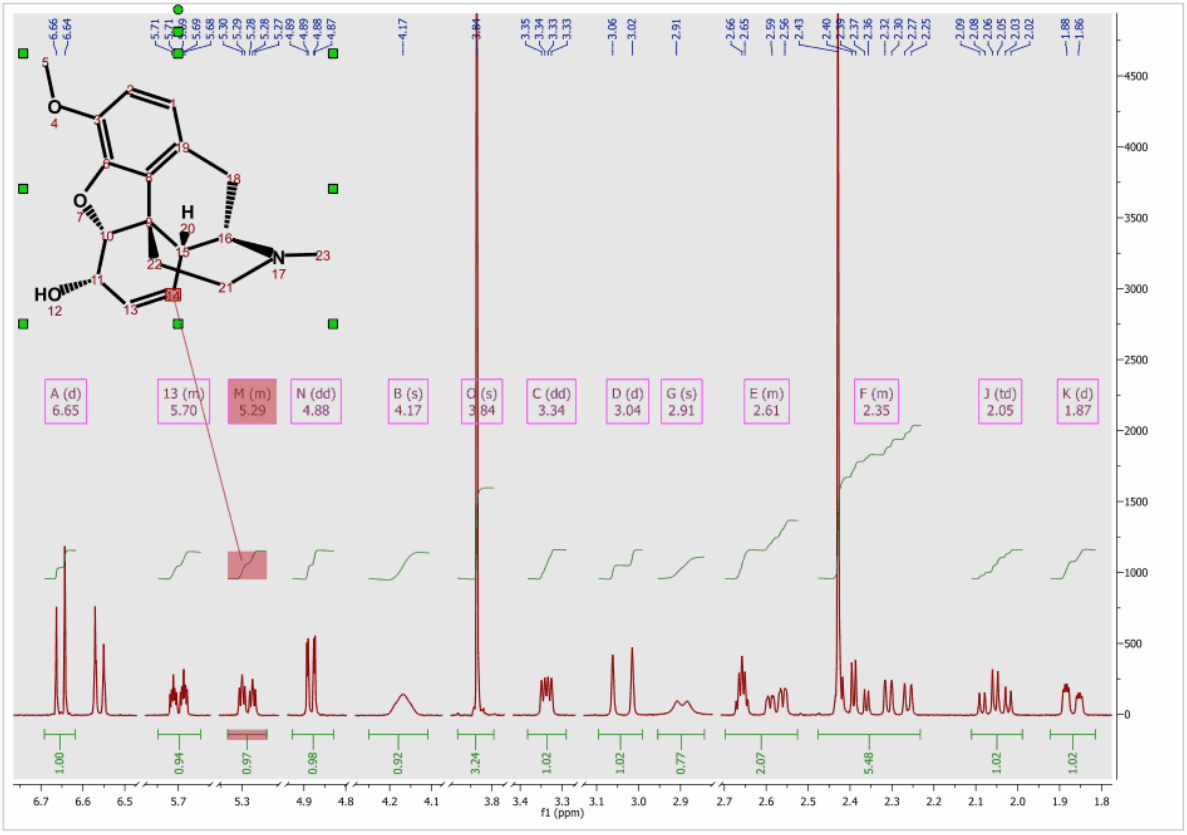
This peak will now be assigned to the atom. Once the assignment has been made, hovering the mouse over the atom will highlight the corresponding peak in the spectrum, and hovering the mouse over the peak will highlight the corresponding atom on the molecular structure.



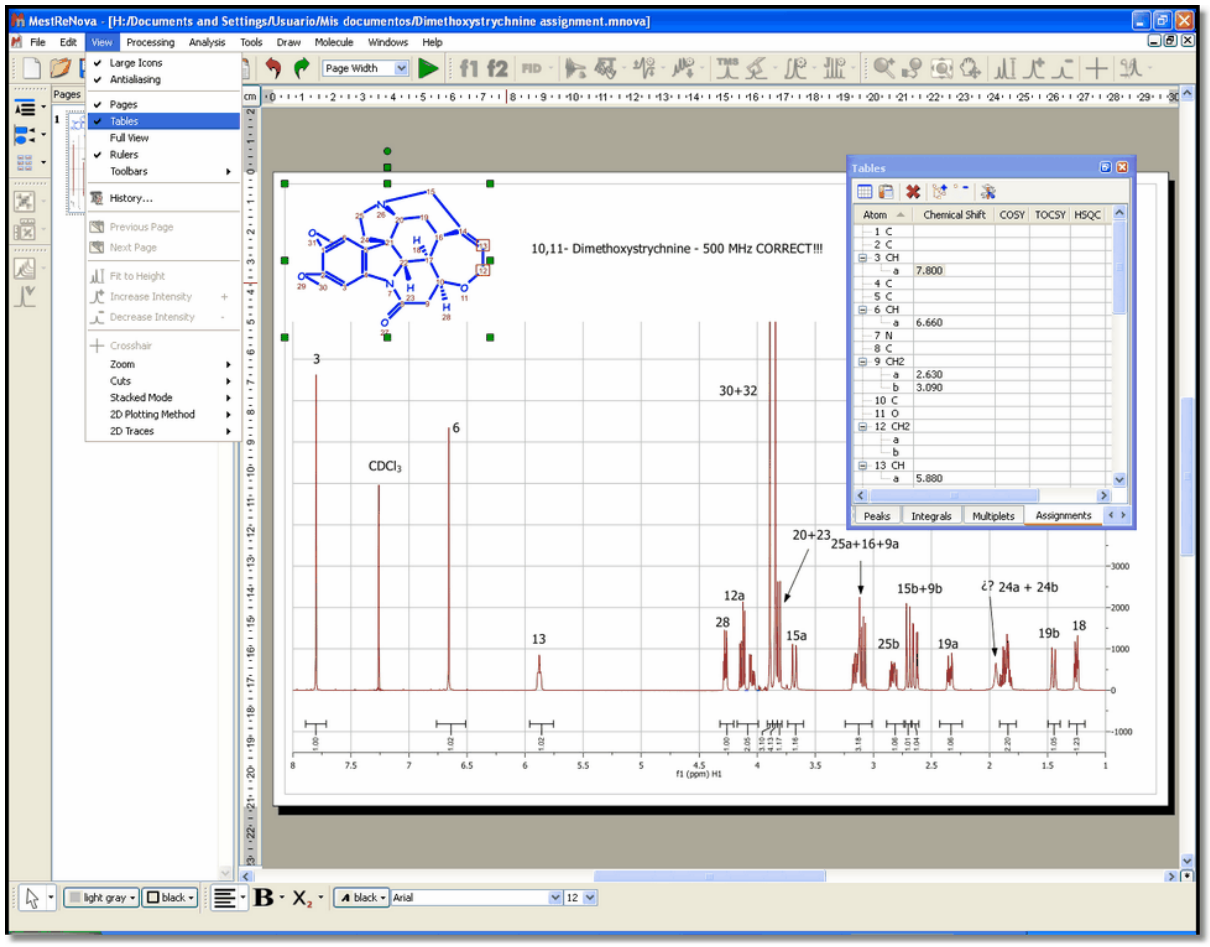
The user can also assign a multiplet, by clicking, dragging and releasing the mouse over the desired multiplet.



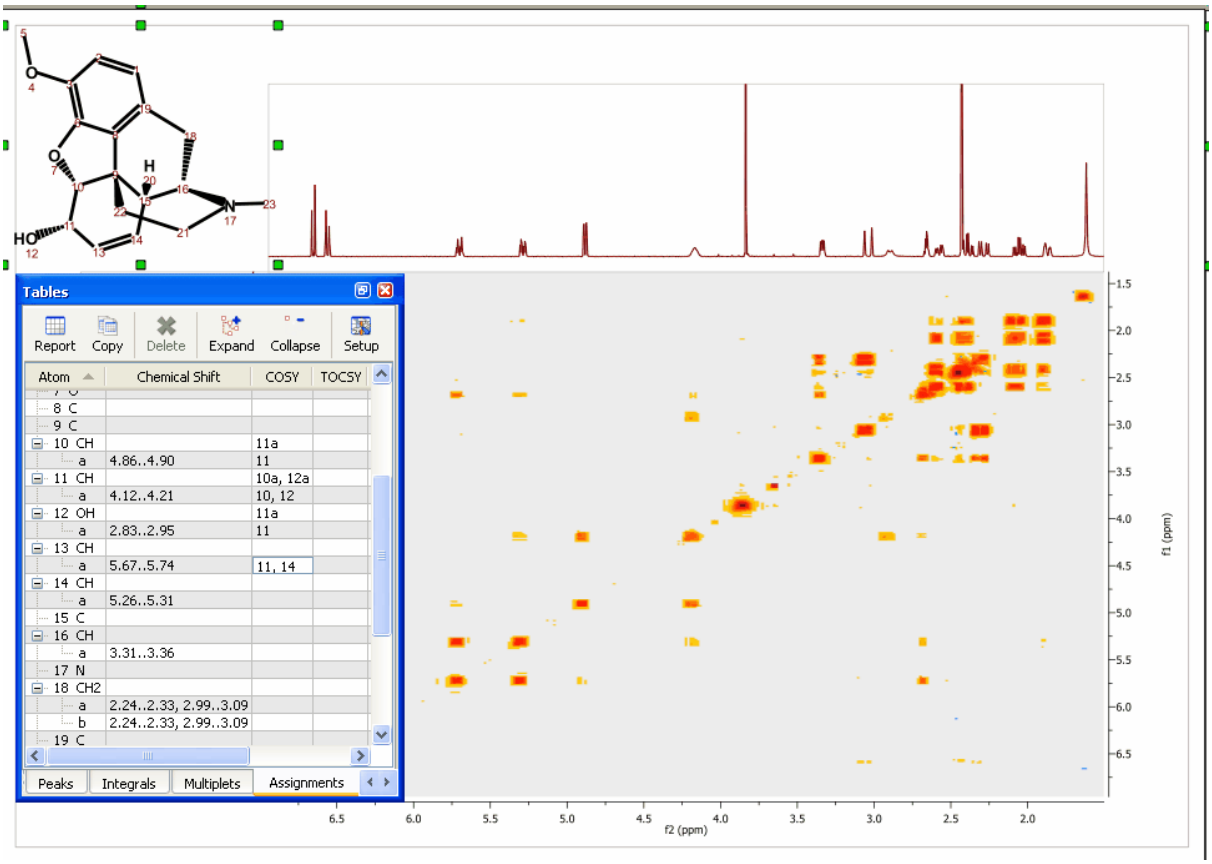
It is also possible to assign a multiplet by dragging the mouse to the '**multiplet box**' (when assigning to a multiplet, its name is replaced with the atom number) or the '**integral curve**', as you can see in the picture below:



Once you have finished with the assignment, you can visualize the assignment list by following the menu 'View/Tables/Assignments' (with the spectrum highlighted) where you will also be able to edit any entry by double clicking on it and overwriting the new value. Bear in mind that you are also able to carry out the peak assignment directly from this table by typing the corresponding chemical shift in the edit box.



Currently, it is only possible to carry out assignments to 2D spectra by typing the corresponding number of the atom in the 'Assignments Table' :



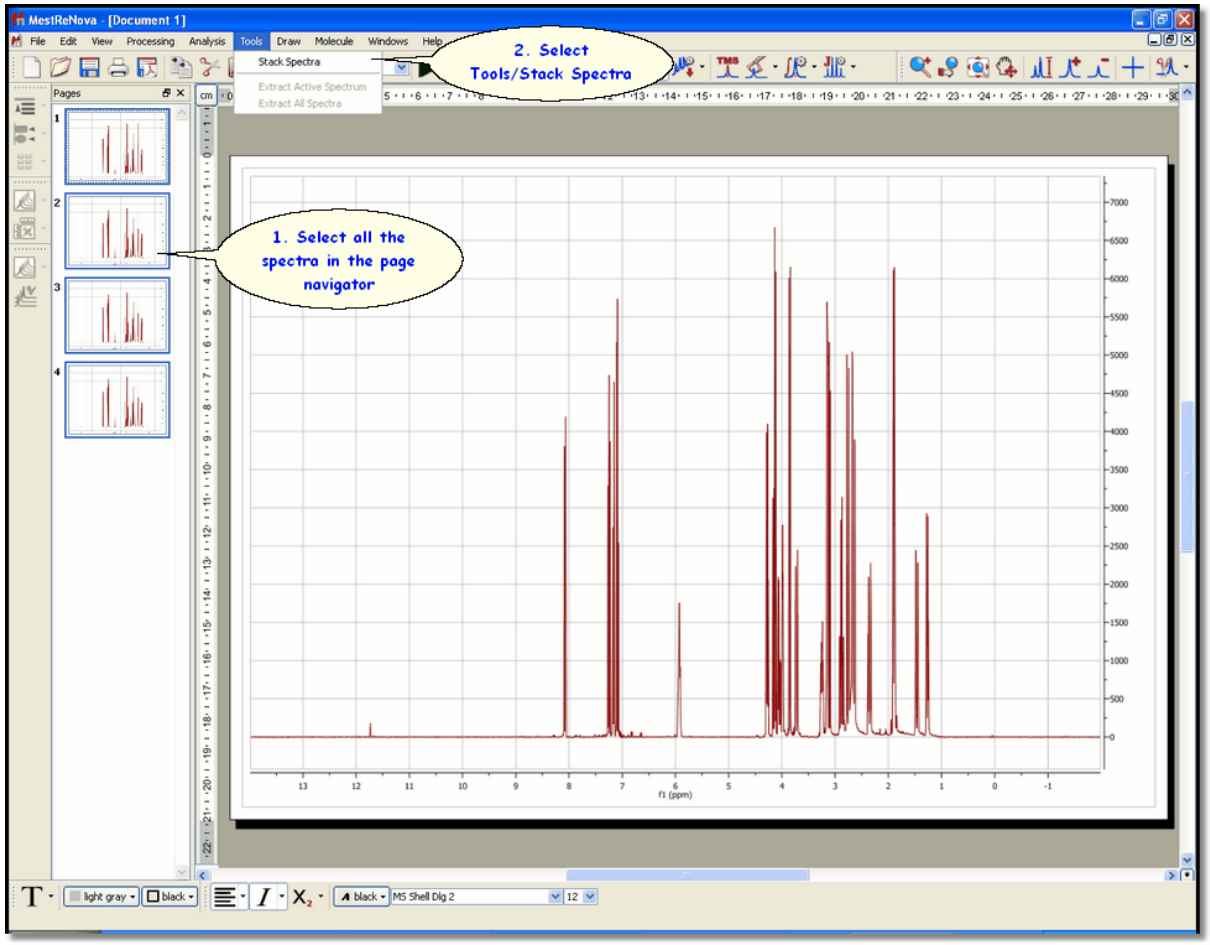
A few notes on Molecular Structure Assignment

- ✓ The current release version of Mnova allows visualization or exporting of assignments in a table. The reporting functionality will be available in the forthcoming versions of Mnova.
- ✓ We plan for the oncoming releases of Mnova to also implement the functionality to carry assignments through different data sets in different pages (thus, for example, it would be possible to assign a 2D spectrum and for those assignments to be applied automatically to the corresponding 1D spectra).

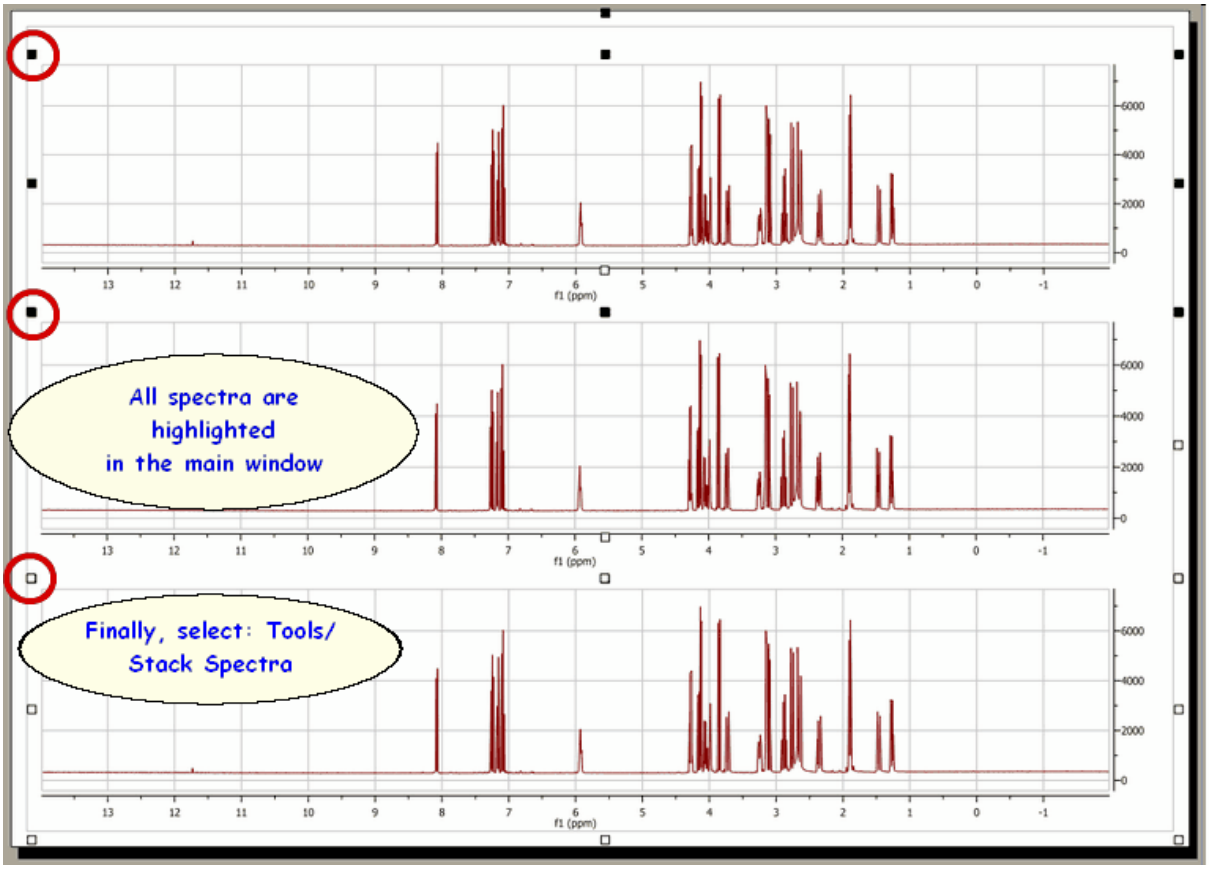
10.6 Stacked Spectra

Many types of experiments require obtaining a series of FIDs, related to each other through the variation of one or more parameters (temperature, concentration, solvent...). The user, for example, may need to run several spectra at different temperatures: 270K, 300K, 330K, 360K and 390K. Instead of acquiring five different sets of data, the user is able to create an array in which the temp parameter is given for successively different values.

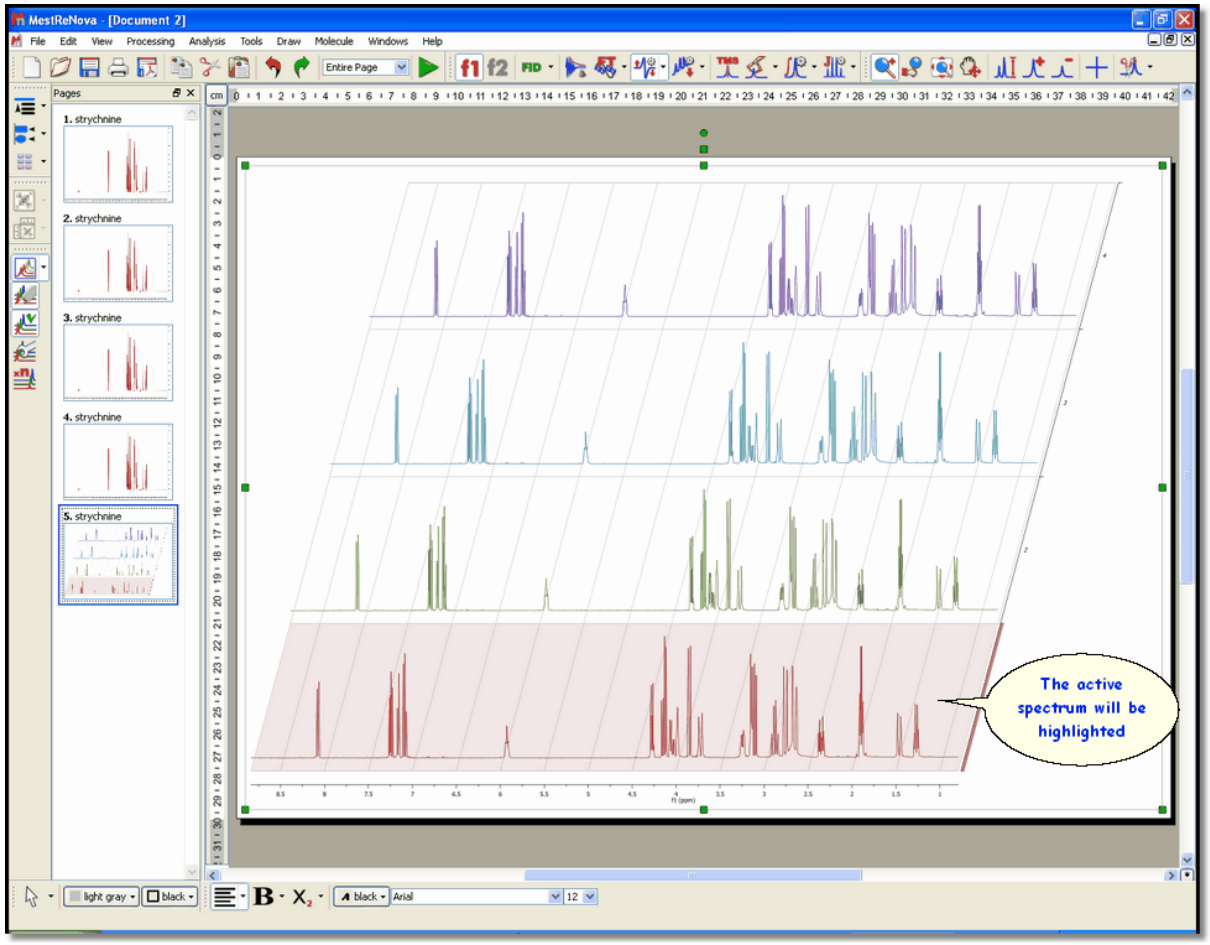
Creating a stacked (or pseudo 2D) spectrum with Mnova is very easy. Just select the desired spectra in the page navigator (by holding down 'CTRL key' or 'Cmd' while clicking on each spectrum) and then issue the command 'Tools/Stack Spectra':



The user can also stack spectra by selecting the desired spectra in the main window (be sure that all desired spectra are highlighted). If you want to highlight all the spectra on the main window, just follow the menu 'Edit/Select All' (or use the shortcut: Ctrl+A, or Cmd+A in Mac).



Once you select 'Tools/Stack Spectra'; an arrayed spectrum will be displayed:

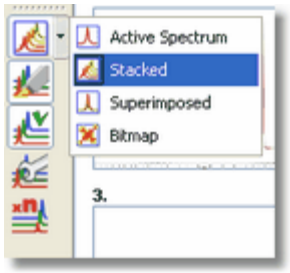


A few tips on Stacked Spectra

- You will be able to change the stacked angle to zero (15º by default) in the properties dialog box (by double clicking on the spectral window).

- You can normalize the spectra (if they have different intensities) by using the command: 'Processing/Normalize'.

The user can select the stacked NMR representation mode by clicking on the scroll menu belonging to the **'Stacked NMR'** icon :



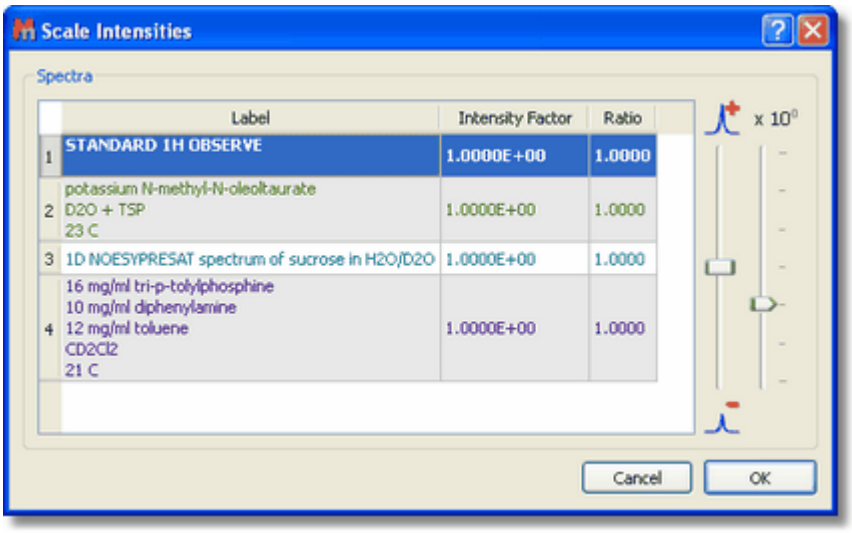
In this scroll bar menu you will also find:

'Active Spectrum': to see only the highlighted spectrum.
'Stacked': to see all the spectra in arrayed mode.
'Superimposed': to see all the spectra overlayed (one over another).
'Bitmap': too see all spectra in a bitmap mode.

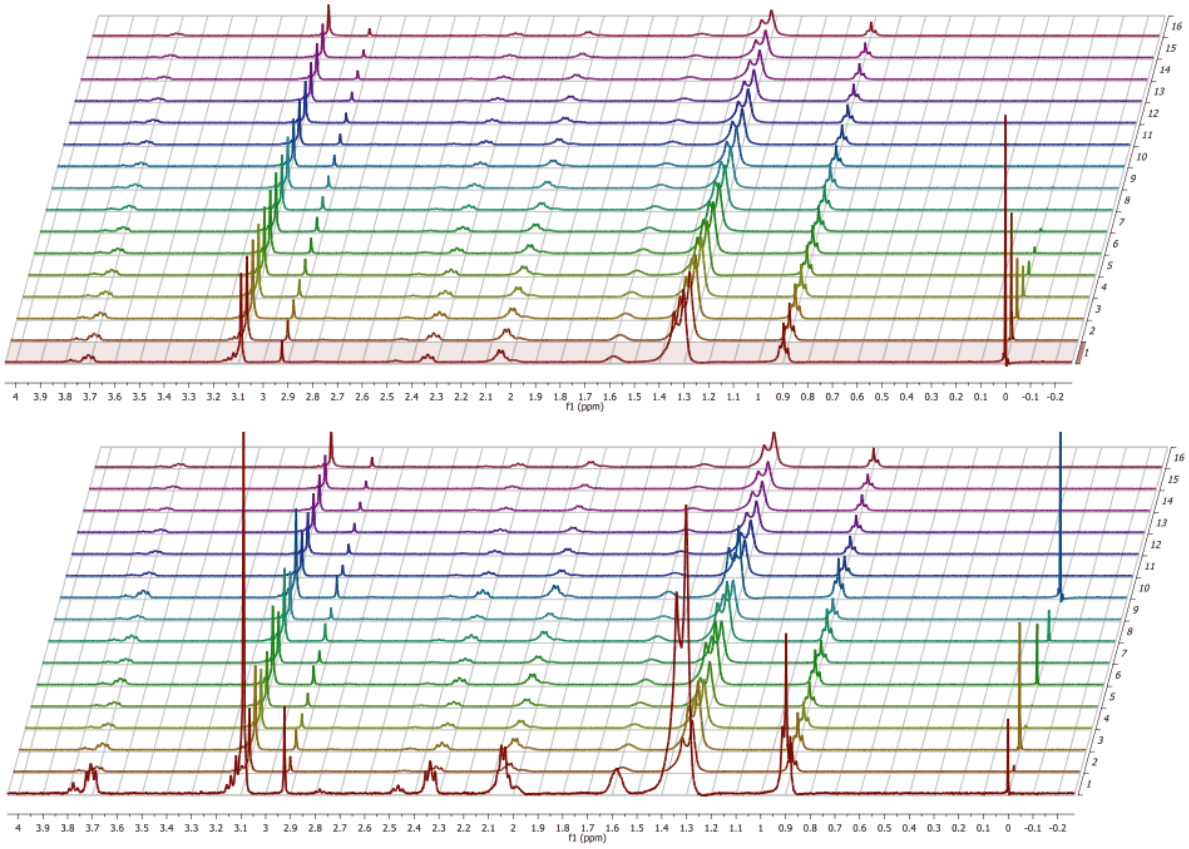
The user can also apply changes only to one slice instead of to all of them together (which is the default behaviour) by simply clicking on the corresponding **'Active Spectrum'** icon, which will turn to to indicate that the processing is only being carried out on the active spectrum. To navigate through the traces, press and hold down **SHIFT** and use the up or down arrow key (or use the mouse scroll wheel). The active trace will be highlighted by default. If you do not wish to highlight it, just click on the **'Active Spectrum Highlighting'** icon .
The user can easily sort the traces of the arrayed spectrum by clicking on the **'Setup Stacked Spectra'** icon to display the dialog box below:

Once there, click on the spectrum for which you want to change the location and then use the right side buttons (top, up, down, bottom, invert).

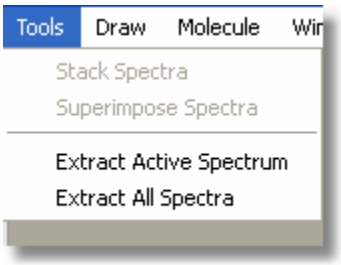
The user can also change the intensity of the traces in the 'Scale Intensities' dialog box, displayed by clicking on the 'Scale Stacked' icon . Use the right side vertical bars to increase or decrease the intensity of the selected trace.



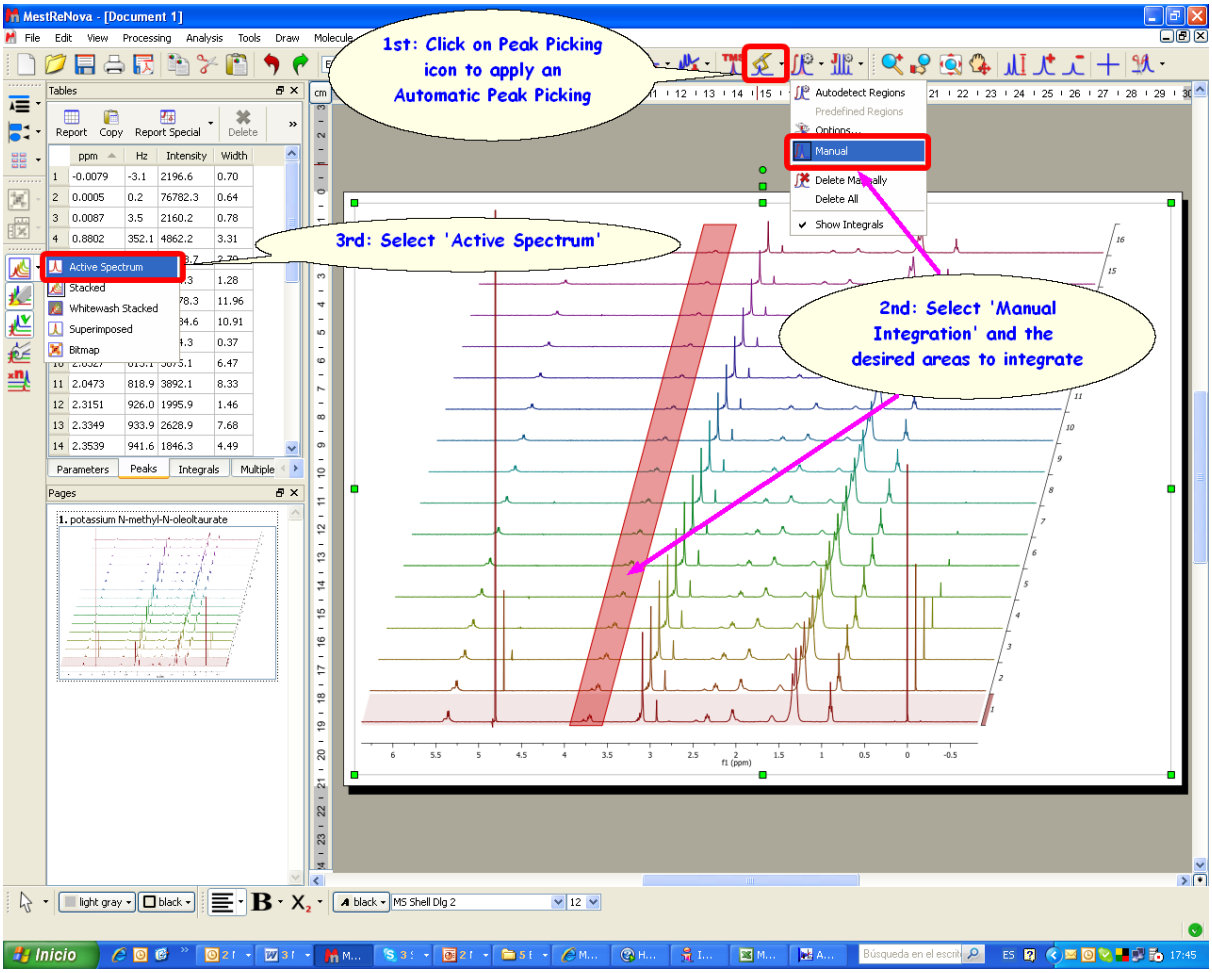
This picture shows the result of a rearrangement of the traces, of a change of their intensity one by one, as well as of the removal of the highlighting of the active trace, for example prior to reporting.



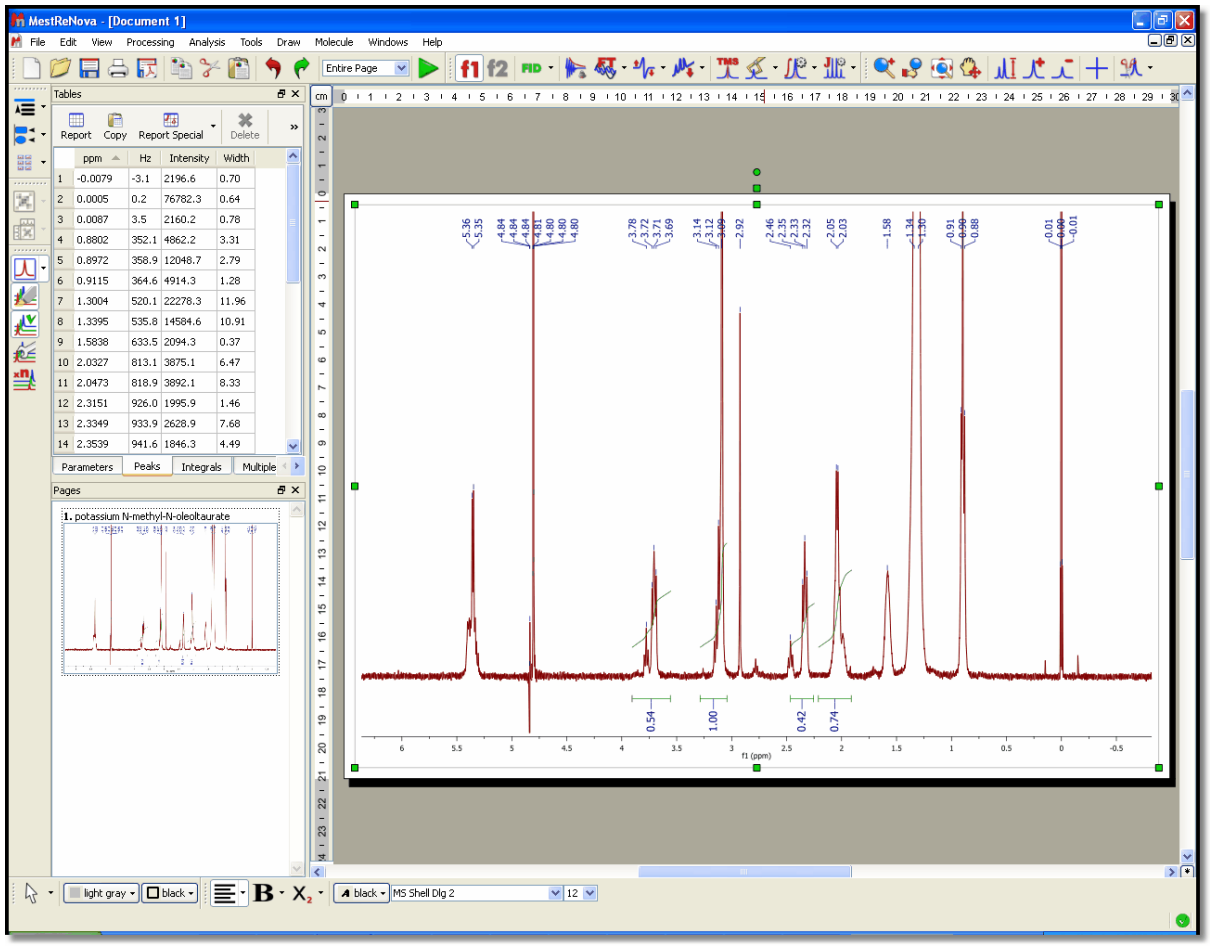
The user is also able to extract the 'Active Spectrum' or 'All Spectra' together by following the 'Tools menu':



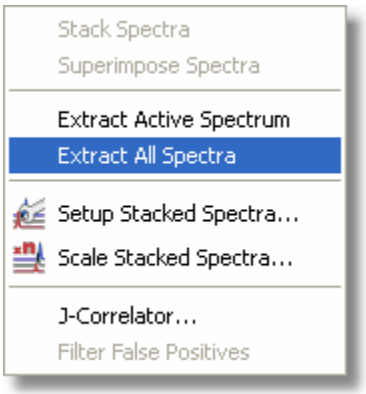
It is very easy to apply a peak picking or an integration analysis to a stacked spectra, just by clicking on the corresponding icon (or selecting the desired analysis function). In the picture below, we have applied an automatic peak picking and a manual integration of several areas of interest:



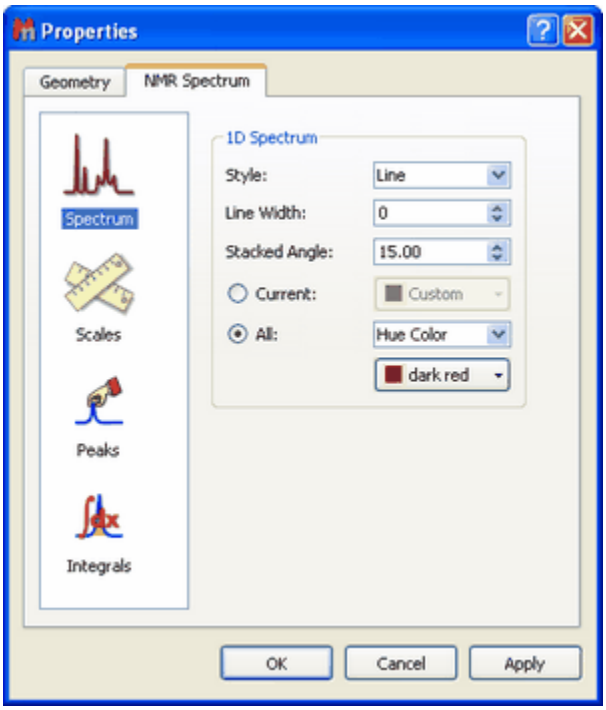
Then, just click on 'Activate Spectrum' in the vertical toolbar to obtain only the highlighted spectrum and you will be able to see the integral and also the peak picking. To navigate through the traces, press and hold down **SHIFT** and use the up or down arrow key (or use the mouse scroll wheel):



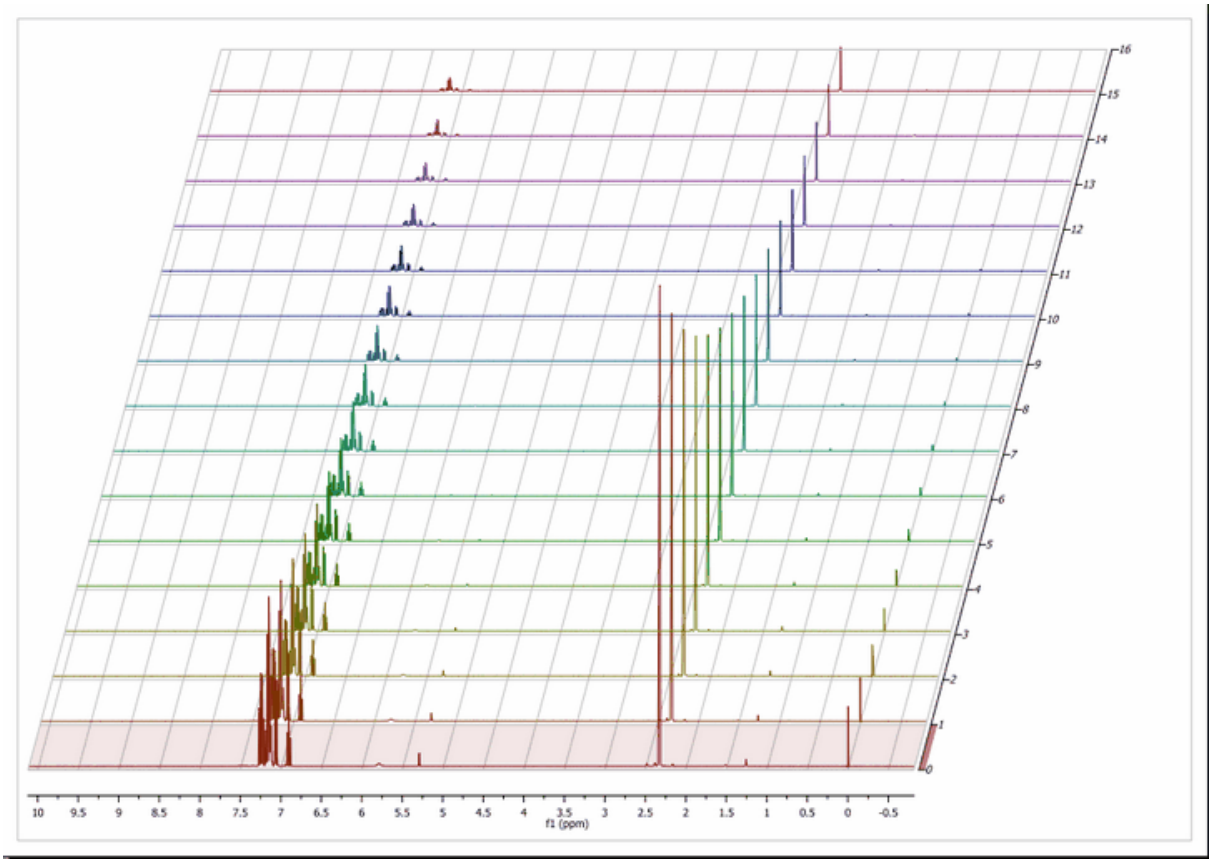
The user can 'Extract the Active Spectrum' or 'All Spectra' by selecting the corresponding command in the 'Tools' menu.



The user can change the stacked spectra properties by double clicking on the spectrum window; this will display the Properties dialog box, where the user can modify the Style (lines, crosses or circles), the Line Width, the Stacking Angle and the color of the current slice as well as that of the spectrum (single color, gradient or hue color).

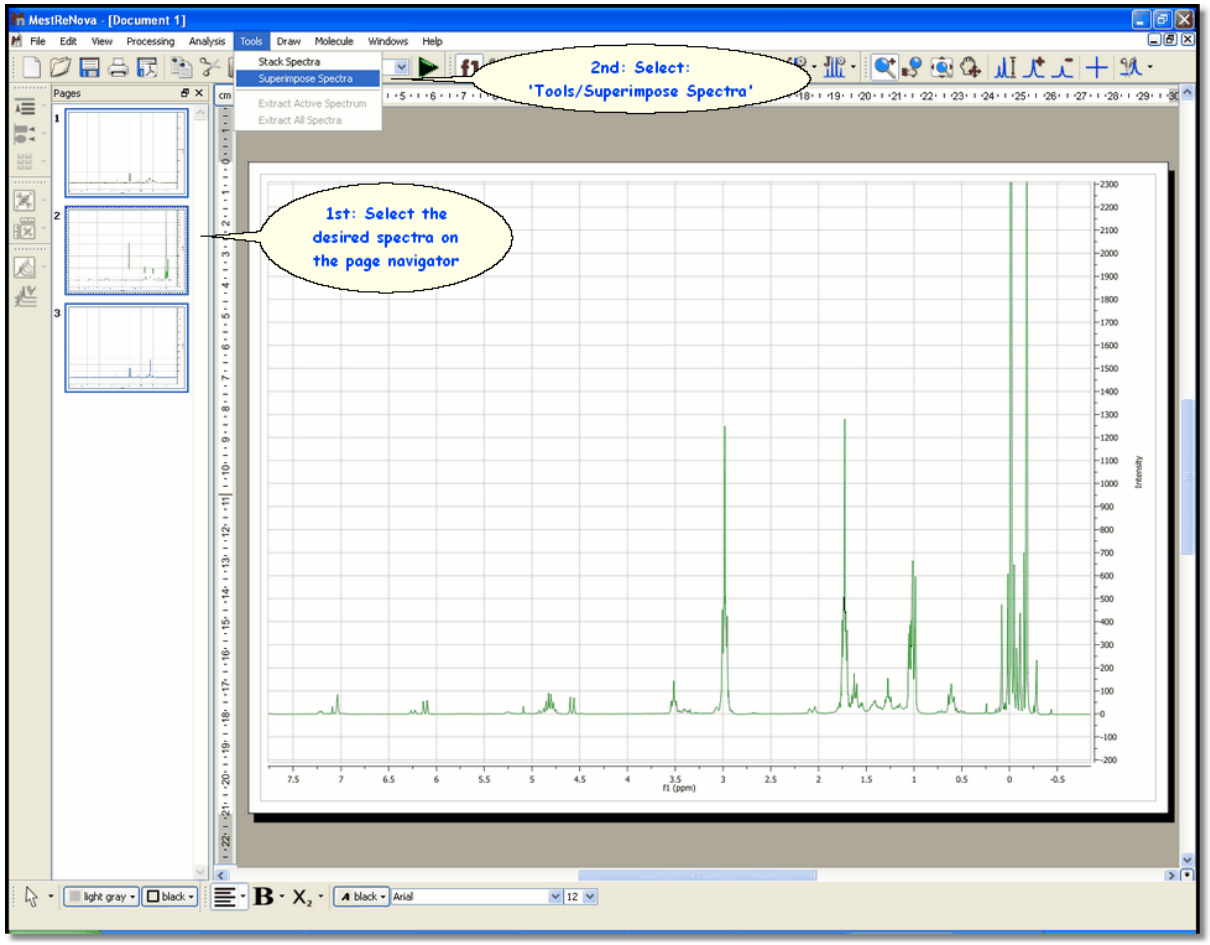


Additionally, Mnova can detect spectra which have been acquired in the so-called arrayed-mode (or pseudo 2D), typically used in relaxation, kinetics or diffusion experiments. For all these cases, Mnova will only process the acquired dimension (t2). Basically, Mnova will process only the rows of the data matrix and, by default, will display the spectrum as a stacked plot (see below).

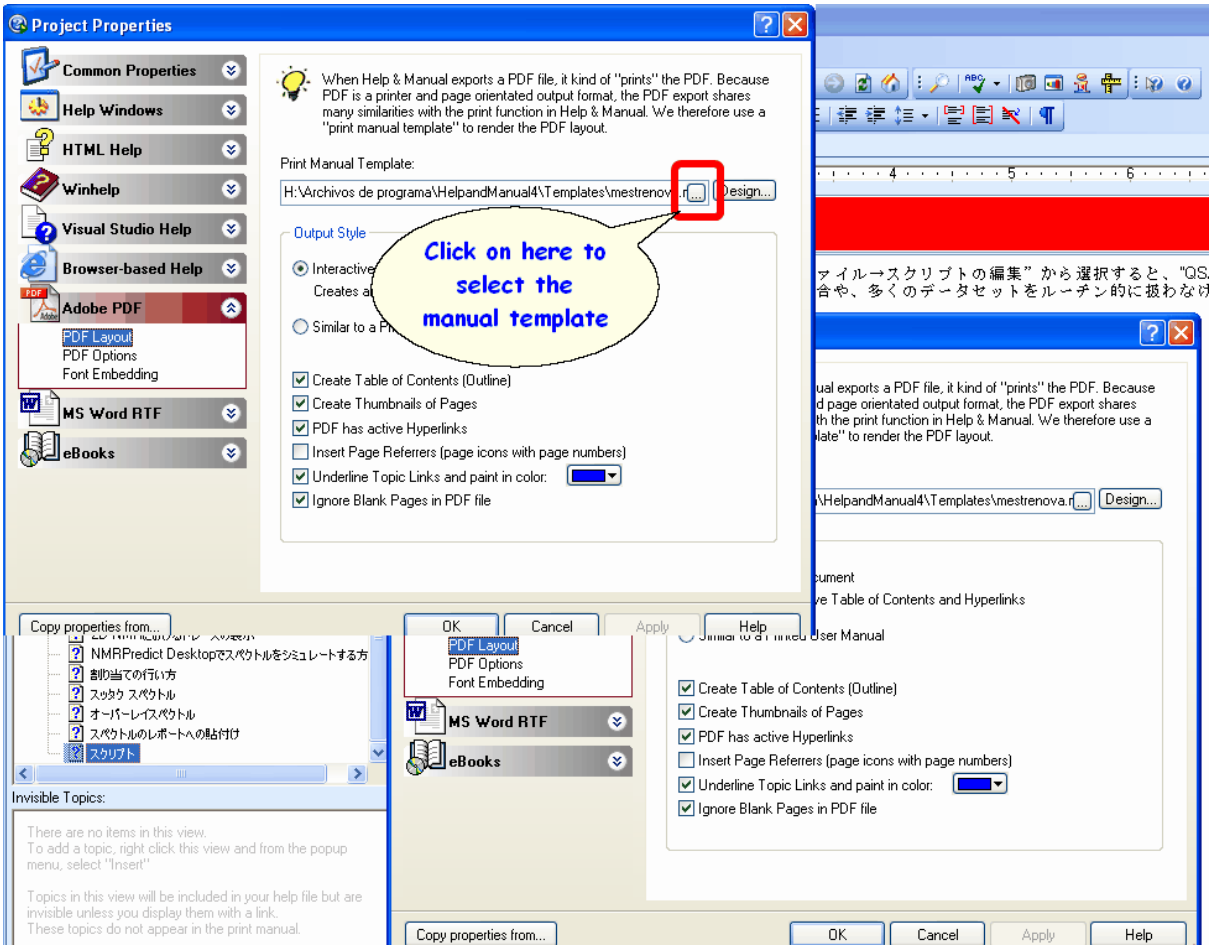


10.7 Superimposed Spectra

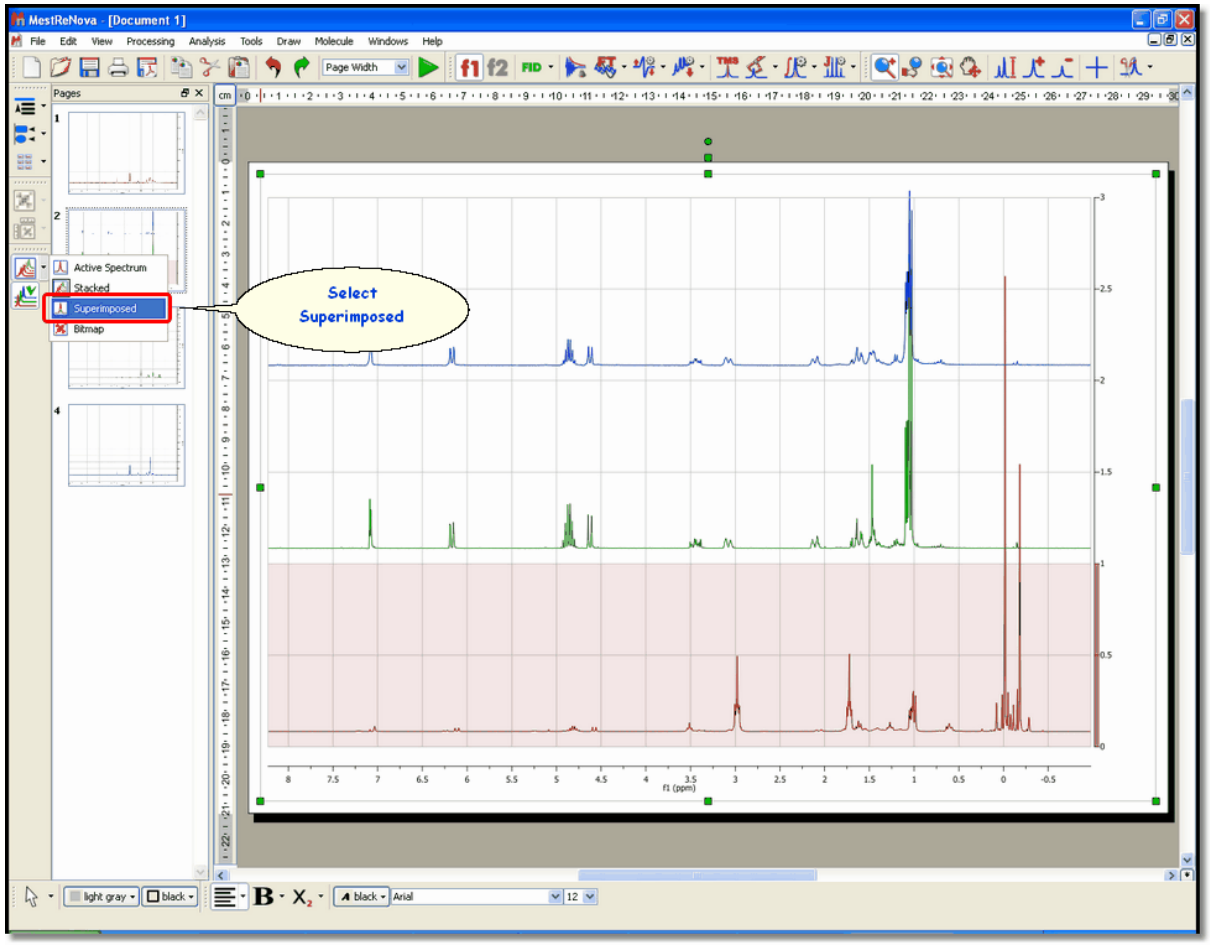
It is very easy to **Superimpose Spectra** with Mnova. Just select the desired spectra on the page navigator (by holding down 'CTRL key' while clicking on each spectrum) and then issue the command 'Tools/Superimpose spectra'.



Finally, you will obtain the superimposed spectra (one spectrum on top of the others):



You will also be able to obtain the superimposed spectrum from a stacked one by selecting **'Superimposed'** on the stacking mode (vertical-left toolbar) or by following the menu 'View/Stacked Mode/Superimposed':



If you have several spectra on the same page (to obtain it, just paste the desired spectra to one page, by using CTRL+C, to copy, and CTRL+V, to paste), you can also superimpose them by following the menu 'Edit/Alignment/Align Left (or by using other alternatives in the vertical-left toolbar) after having selected all of them (by CTRL+A, or Edit/Select All').

10.8 Pasting Spectra into Reports

When the user copies a spectrum, he is copying a graphic. A graphic is any kind of image displayed by a computer. There are two kinds of graphics: vector graphics and raster graphics. A vector graphic (metafile) is a graphic drawn as lines, polygons and text while a raster graphic is drawn as an array of colored dots (bitmap).

A ***metafile*** is a list of commands that can be played back to draw a graphic. Typically, a metafile is made up of commands to draw objects such as lines, polygons and text and commands to control the style of these objects. So, internally, a metafile is an array of variable-length structures called metafile records. The first records in the metafile specify general information such as the resolution of the device on which the picture was created, the dimensions of the picture, and so on. The remaining records, which constitute the bulk of any metafile, correspond to the graphics device interface (GDI) functions required to draw the picture. These records are stored in the metafile after a special metafile device context is created. This metafile device context is then used for all drawing operations required to create the picture. When the system processes a GDI function associated with a metafile, it

converts the function into the appropriate data and stores this data in a record appended to the metafile.

NOTE: Some people equate metafiles with vector graphics. In most cases this is correct but, strictly speaking, a metafile can contain any mix of vector and raster graphics. For example, a metafile could contain just one command to display a bitmap. This is the approach used in Mnova, which by default pastes the spectrum as a metafile which contains a bitmap in order to reduce the file size (without too great a loss in resolution). Mnova also allows the user to use (only in Windows) the graphical format known as ***Enhanced Windows Metafiles*** in order to keep the maximum graphical resolution possible (at the expense of an increase in the file size). This will usually be the preferred way to copy NMR spectra.

Enhanced metafiles provide true device independence. You can think of the picture stored in an enhanced metafile as a “snapshot” of the video display taken at a particular moment. This “snapshot” maintains its dimensions no matter where it appears—on a printer, a plotter, the desktop, or in the client area of any application.

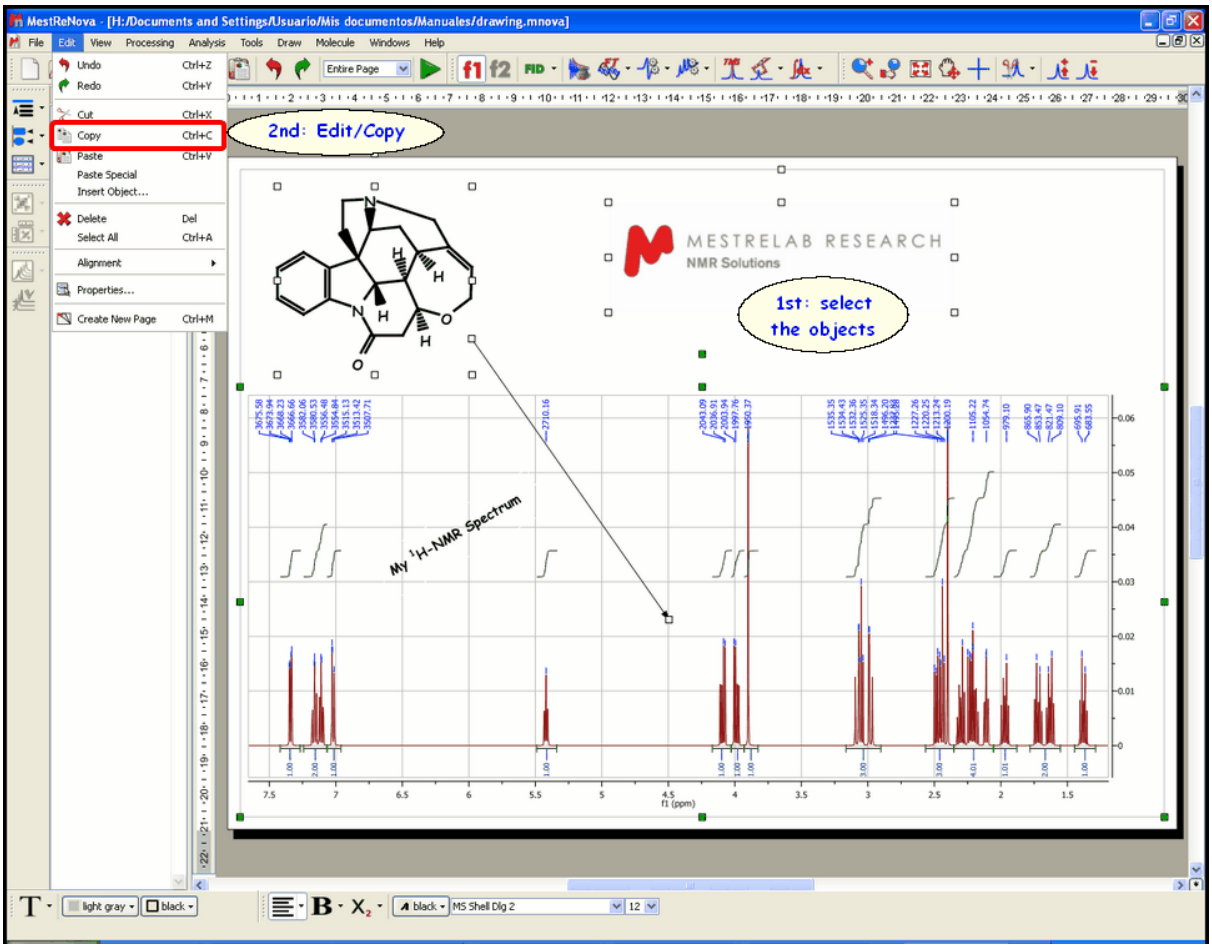
You can use enhanced metafiles to store a picture created by using the GDI functions (including new path and transformation functions). Because the enhanced metafile format is standardized, pictures which are stored in this format can be copied from one application to another; and, because the pictures are truly device independent, they are guaranteed to maintain their shape and proportions on any output device. In addition, the user is able to work with the copied spectrum in the document just by double clicking on it (Mnova will automatically open due to the Object Linking and Embedding technique; OLE).

A ***bitmap*** is another name for a raster graphic. In Windows, these are typically saved in a BMP file. However, other common bitmap formats include PC Paintbrush (PCX), Tagged-Image File Format (TIFF), Graphics Interchange Format (GIF) and JPEG.

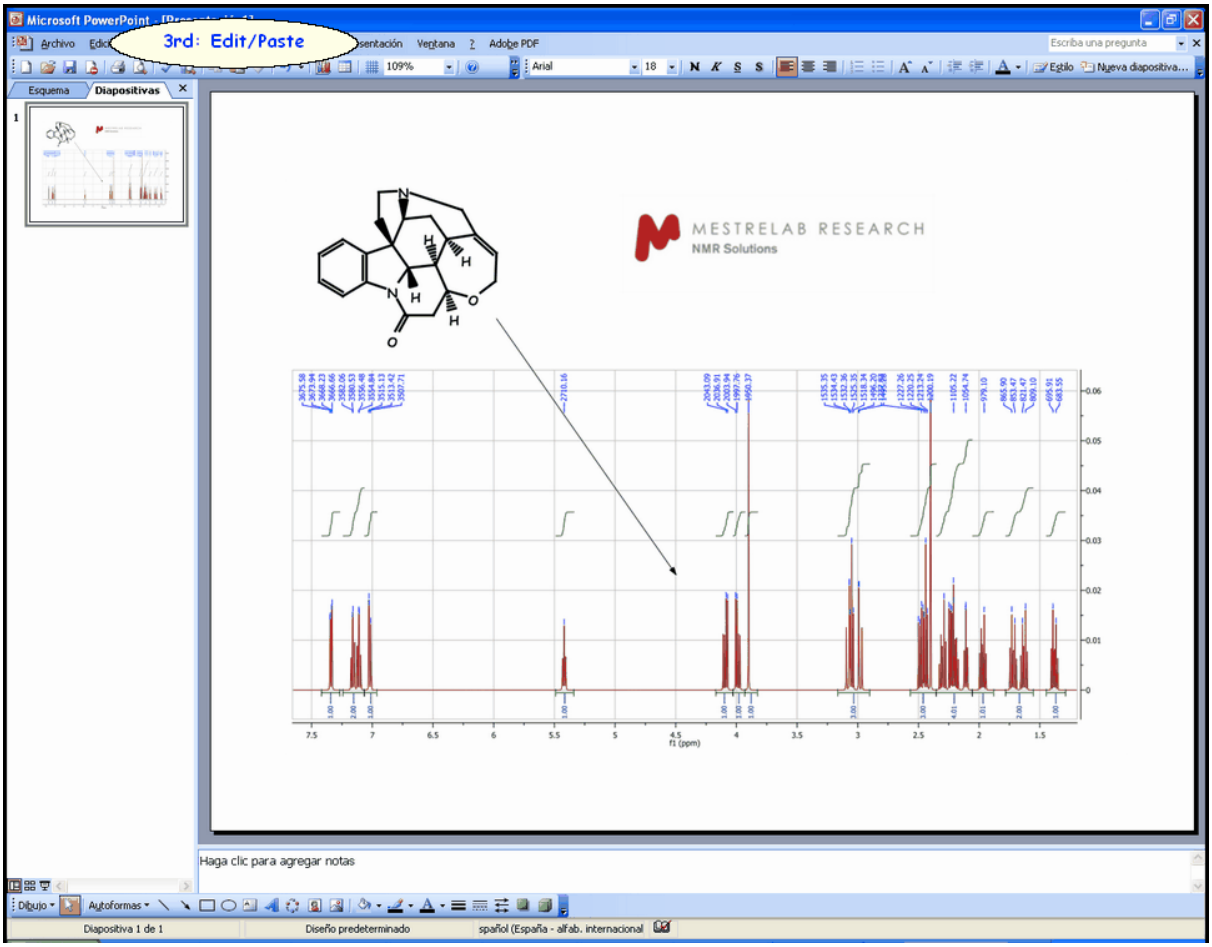
In general, vector graphics take longer to display but can be scaled to any size with no degradation, whilst raster graphics are faster to display but image quality suffers when they are scaled up or down.

How to paste a spectrum in a Report?

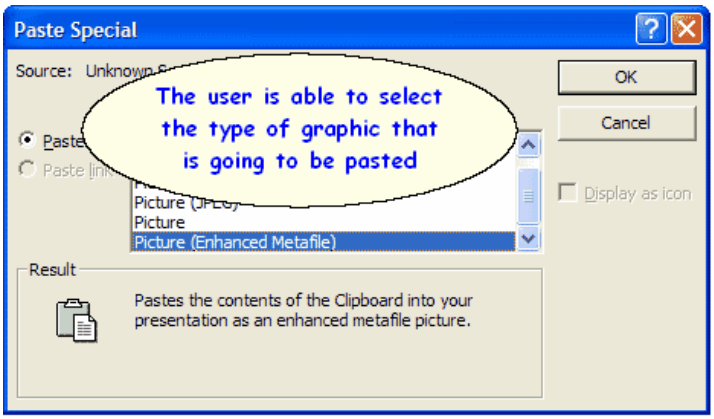
Mnova provides the ability to copy&paste spectra into other applications (e.g. MS Office apps) via the clipboard. Just be sure that you have highlighted the desired objects (Edit/Select All, if you want to select all the objects) and then follow the menu 'Edit/Copy' or alternatively use the shortcut 'Ctrl+C' (or 'Cmd+C' in Mac).



Next, open the document (PowerPoint in this case) where you want to paste the spectrum and follow the menu Edit/Paste or alternatively use the shortcut, Ctrl+V (or Cmd+V in Mac).

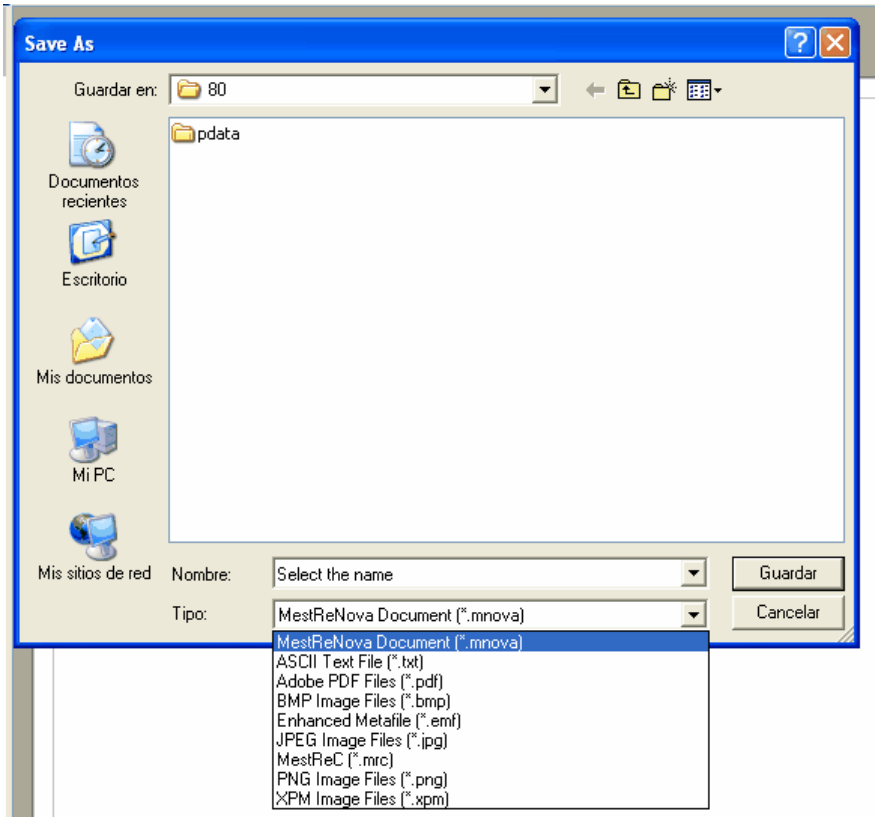


Mnova will, by default, paste the object as a metafile which contains just one command to display a bitmap. However, the user can change this default by following the menu 'Edit/Paste Special' before pasting (in the corresponding document; PowerPoint in this case). It will open the 'Paste Special' dialog box where the user will be able to select between Mnova Document (by default), Enhanced Windows Metafile, Bitmap, etc.



The user can also save the spectrum by following the menu 'File/Save as' and then 'paste it' in the corresponding document by selecting 'Insert/Picture'. Mnova allows the user to save the data in Mnova (*.Mnova) or MestRe-C (*.mrc) formats as well as in ASCII text (*.txt), adobe PDF or several kinds of

image files, such as bitmap (bmp), enhanced metafile (emf), JPEG, PNG, PostScript, SVG, TIFF, XBM, XPM.



'ASCII Text File' (*.txt): It is essentially raw text just like the words you are reading now. Each byte in the file contains one character which conforms to the standard ASCII code. This kind of text file becomes a common denominator between applications which do not import each other's formats. If both applications can import and export ASCII files, you can transfer your files between them. Almost all word processors import and export ASCII files as well as many database and spreadsheet programs.

'Adobe Text File' (*.pdf): Portable Document Format (PDF), is an open file format used for representing two-dimensional documents in a device independent and resolution independent fixed-layout document format. Each PDF file encapsulates a complete description of a 2D document which includes the text, fonts, images, and 2D vector graphics which compose the document.

'JPEG Image File' (*.jpg): JPEG stands for Joint Photographic Experts Group; it is a lossy compression algorithm which has been conceived to reduce the file size of natural, photograph-like true-colour images as much as possible without affecting the quality of the image as perceived by the human sensory engine.

'PNG Image File' (*.png): PNG (Portable Network Graphics) is another bitmapped image format which employs lossless data compression.

XPM Image Files (*.xpm): XPM stores image data in the form of ASCII text formatted as a Standard C character string array. This type of format allows XPM files to be edited easily with any text editor, to have comments inserted at any point within the file, to be included as data in C and C++ programs, and to be easily transmitted via electronic mail.

10.9 Scripts

Scripts: selecting this option, via the menu 'File/Edit Script', a window will open. From here, you can create a new or edit an existing script in QtScript (the language used in Mnova). Qt Script is based on the ECMAScript scripting language and the originating technology for this ECMA Standard is JavaScript. There are several books about JavaScript, so in this chapter we will show you some of our contribution to this programming language, used in the implementation of Mnova .

EMCAScript is an object-oriented programming language for performing computations and manipulating computational objects within a host environment. ECMAScript as defined here is not intended to be computationally self-sufficient; indeed, there are no provisions in this specification for input of external data or output of computed results. Instead, it is expected that the computational environment of an ECMAScript program will provide not only the objects and other facilities described in this specification but also certain environment-specific *host* objects, whose description and behavior are beyond the scope of this specification except to indicate that they may provide certain properties that can be accessed and certain functions that can be called from an ECMAScript program.

A ***scripting language*** is a programming language that is used to manipulate, customize, and automate the facilities of an existing system. In such systems, useful functionality is already available through a user interface, and the scripting language is a mechanism for exposing that functionality to program control. In this way, the existing system is said to provide a host environment of objects and facilities which completes the capabilities of the scripting language. A scripting language is intended for use by both professional and non-professional programmers, and therefore there may be a number of informalities and built into the language.

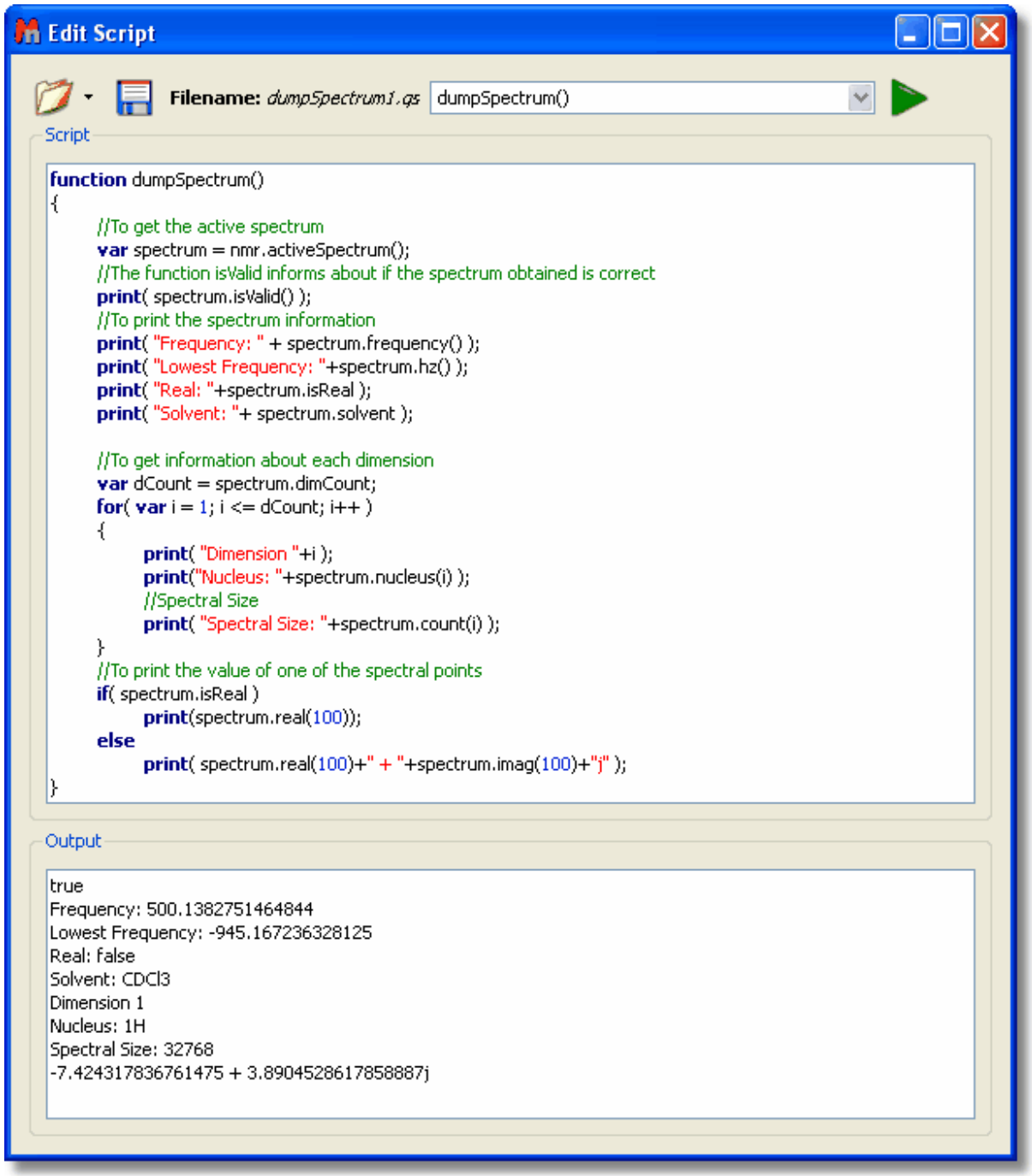
Let´s start with a very simple script. Run Mnova, follow the menu: 'File/Edit Script' and copy the following script:

```
function dumpSpectrum()
{
        //To get the active spectrum
        var spectrum = nmr.activeSpectrum();
        //The function isValid informs about if the spectrum obtained is correct
        print( spectrum.isValid() );
        //To print the spectrum information
        print( "Frequency: " + spectrum.frequency() );
        print(
```

```
"Lowest Frequency: " +spectrum.hz() );
        print( "Real: " +spectrum.isReal );
        print( "Solvent: "+spectrum.solvent );

        //To get information about each dimension
        var dCount = spectrum.dimCount;
        for( var i = 1; i <= dCount; i++ )
        {

        print( "Dimension "+i );

        print( "Nucleus: " +spectrum.nucleus(i) );


//Spectral Size

        print( "Spectral Size: " +spectrum.count(i) );
        }
        //To print the value of one of the spectral points
        if( spectrum.isReal )

        print (spectrum.real(100));
        else
```

```
        print(
spectrum.real(
100)+"    +    "
+spectrum.ima
g(100)+"j" );
}
```

This script is used to obtain information about the spectrum. Typing " dumpSpectrum() " in the combobox, will show the spectral information in the output, as you can see in the picture below:



The code that you see in the picture above uses the variable "**var** spectrum = nmr.activeSpectrum();" to obtain the active spectrum in the window. The line **print**(spectrum.isValid()); tell us if the spectrum is or not valid. The following 4 lines in the script will print the spectrum information (frequency, lowest frequency, real or complex and the solvent used in the experiment).

The variable **var** dCount = spectrum.dimCount; and the loop **for**(**var** i = 1; i <= dCount; i++) will show the number of the dimensions of the spectrum (in this case as you can see in the output of the picture above, the spectrum is a 1H-NMR, so the dimension is 1). The commands **print**(spectrum.nucleus(i)); and **print**("Spectral Size: " + spectrum.count(i)); will show the nucleus and the spectral size (1H and 32768 points, in this case).
Finally, the last lines are used to display the value (including the real and the imaginary part) of one of the spectral points (in this case, the selected point was the 100, but you can introduce any number from 1 to 32768).

Of course, the user will be able to modify the script to obtain any other desired parameters in the output and to save this changes by clicking on the 'Save' button  .

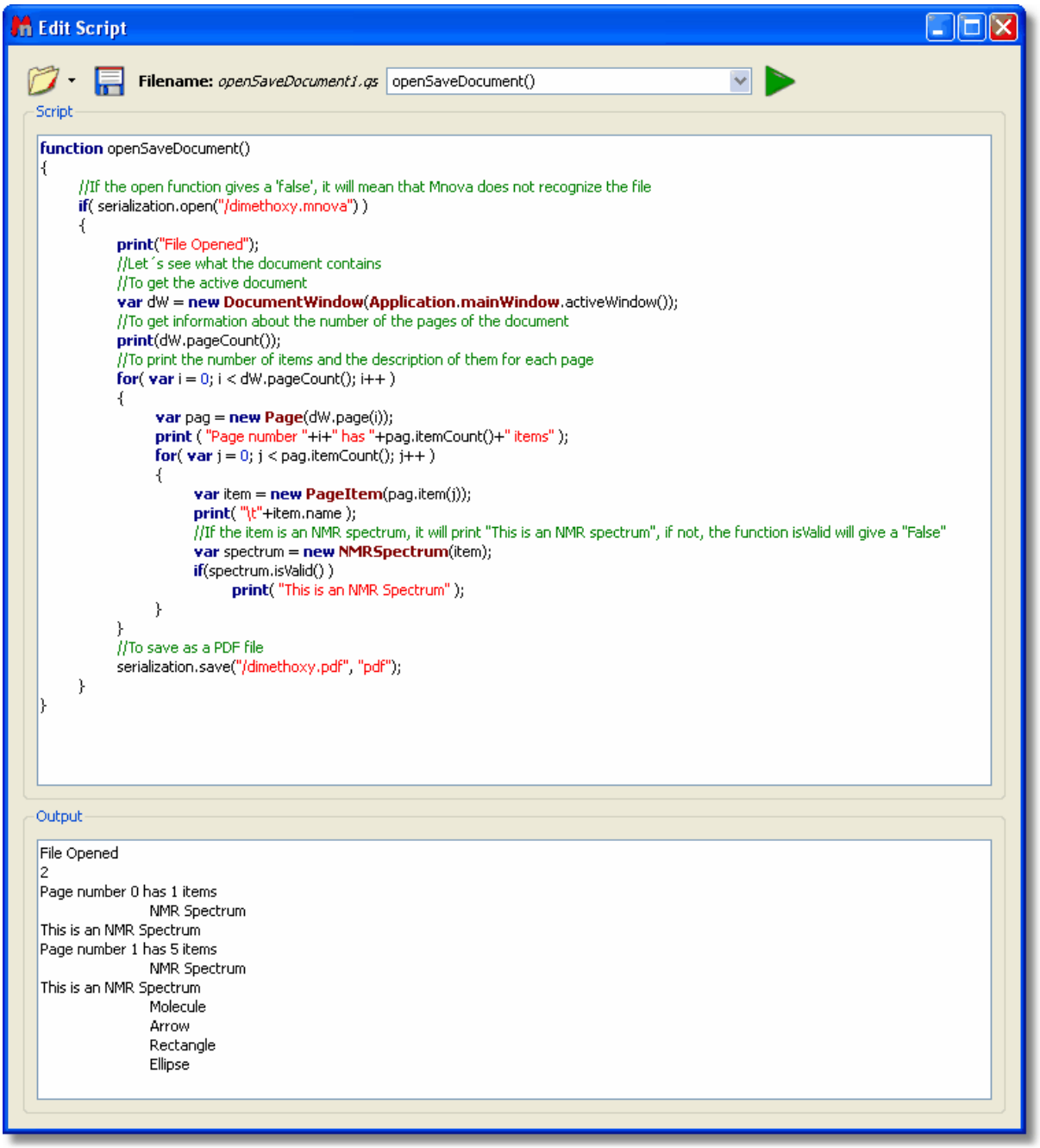
You will find below a script used to open a .mnova file named 'Dimethoxy' and save it as a PDF file:

```
function openSaveDocument()
{
	//If the open function gives a 'false', it will mean that Mnova does not recognize the file
	if( serialization.open("/dimethoxy.mnova") )
	{
		print("File Opened");
		//Let´s see what the document contains
		//To get the active document
		var dW = new DocumentWindow(Application.mainWindow.activeWindow());
		//To get information about the number of the pages of the document
		print(dW.pageCount());
		//To print the number of items and the description of them for each page
		for( var i = 0; i < dW.pageCount(); i++ )
		{
			var pag = new Page(dW.page(i));
			print ( "Page number "+i+" has "+pag.itemCount()+" items" );
			for( var j = 0; j < pag.itemCount(); j++ )
			{
				var item = new PageItem(pag.item(j));
				print( "\t"+item.name );
				//If the item is an NMR spectrum, it will print "This is an NMR spectrum", if not, the function isValid will give a "False"
				var spectrum = new NMRSpectrum(item);
				if(spectrum.isValid() )
					print( "This is an NMR Spectrum" );
			}
		}
		//To save as a PDF file
		serialization.save("/dimethoxy.pdf", "pdf");
	}
```

```
}
```

To run this script, just copy it to the 'Edit Script' dialog box (or load it by using the open icon ) and type "openSaveDocument()" in the combobox. Please bear in mind that, you will need to write the corresponding path of the saved Mnova file in the line 4 of the script (in this case the dimethoxy.mnova file was saved in C:\ ).
As you can see in the script, if Mnova does not recognize the spectrum, you will obtain a 'False' message in the output due to the statement:
`if( serialization.open("/dimethoxy.mnova") )`

The line `print("File Opened");` will give the corresponding message in the output, as you can see in the picture below:



The object 'Application' of the variable **var** dW = new **DocumentWindow**(**Application.mainWindow**.activeWindow()); gives us the 'Active Window' of the 'Main Window', that is to say, the document which we can see in the screen. The function **print**(dW.pageCount()); is used to know the number of the pages of the document.

The following lines of the script are used to obtain information about the items included on the document. For example the function pageCount is used to know the number of pages of the document. The variable **var** pag = **new Page**(dW.page(i)); will analyze page by page to find the information. The line **print** ("Page number "+i+" has "+pag.itemCount()+" items"); will print the corresponding phrase in the output, following the number of the page (+i+) and the number of the

items included in the page `for( var j = 0; j < pag.itemCount(); j++ )`. The variable `var item = new PageItem(pag.item(j));` will inform about the type of the item, and the function `print( "\t"+item.name );` will print the name of the item. In this example (as we can see in the output of the picture below), the document has 2 pages; the page 0 has 1 item, which is an NMR spectrum and the page 1, has 5 items (NMR Spectrum, molecule, arrow, rectangle and ellipse).

The variable `var spectrum = new NMRSpectrum(item);` will rebuild the active spectrum, and if the item is an NMR spectrum, it will print "This is an NMR spectrum", if not, the function isValid will give a "False" due to the lines: `if(spectrum.isValid() ) // print( "This is an NMR Spectrum" );`.

Finally, the plugin serialization.save("/dimethoxy.pdf", "pdf"); will save the document as a PDF file in the specified location of the hard-disk (in this case, the PDF will be saved in C:\)

Let´s take a look at a very important part of the 'Multiplet Reporter' script:

```
// This function defines JACS-like multiplet report.
// To customize report, edit this class or implement another one.
function JACSMultipletReporter()
{
	MultipletReporter.call(this);

	this.onlyElementName=false;
// Define font size and font family
	this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";

	this.nucleusTemplate="%1";
// Report header. %1 will be replaced with nucleusString, %2 with frequency, %3 with solvent
	this.header = "%1 NMR (%2 MHz, %3) &delta; ";

// Multiplet templates. %1 - delta, %2 - category, %3 - nH
	this.reportRange = true; // set to true to get multiplet range instead of delta.
	this.withoutJsTemplate = " %1
```

```
(%2, %3H)"; // multiplet template without J's
        this.withJsTemplate = " %1 (%2, %4, %3H)"; // multiplet template with J's
        this.rangeTemplate = "%1 &ndash; %2";

// J's list template. %1 - list of J's
        this.jListTemplate = "<i>J</i> = %1";

        this.jPrecision = 1; // J's precision
        this.deltaPrecision = 2; // delta precision
        this.mSeparator = ", "; // multiplet separator
        this.jSeparator = ", "; // J's separator

        this.start = this.font;
        this.end = ".</font>";
}

JACSMultipletReporter.prototype = new MultipletReporter();
JACSMultipletReporter.prototype.toString = function() { return "JACSMultipletReporter()"; }

(...)
```

The user will be able to change the multiplet report template to obtain the desired multiplet report; for example:

`this.onlyElementName=false;` changing false with true, we will obtain only the element name without the atomic mass (For example: H, C instead of ^{1}H, ^{13}C).

The function: `this.font = "<font style=\"font-size: 10pt; font-family: Times New Roman\">";` will define the font size and the font family of the multiplet report.

The line: `this.header = "%1 NMR (%2 MHz, %3) &delta; ";` is used to print the header of the report, where %1 will be the nucleus (H or C), %2 the frequency of the spectrometer (in MHz), and %3 the solvent, followed by a delta symbol (δ). For example: "^{1}H NMR (500 MHz, CDCl3) δ".

The sentence: `this.reportRange = true` is used to obtain the multiplet range instead of the chemical shift.

The functions: `this.withoutJsTemplate = " %1 (%2, %3H)"` and `this.withJsTemplate = " %1 (%2, %4, %3H)"` are used to customize the appearance of the multiplet report by changing the positions of %1, %2, %3H, or %4 (where, %1 means: chemical shift; %2 means: type of multiplet (s, d, t, etc); %3H means: number of hydrogens and %4 means the coupling constant value). As you can see, the first

line shows a multiplet without coupling constants, (while the last line shows a multiplet with coupling constants).

So, if you need to obtain something like this (japanese format):

1H NMR (300 MHz, Solvent) δ ppm 6.43-6.22 (1 H, m), 3.17 (1 H, q, J = 7.15 Hz) etc…

You should modify both lines, as you can see below:

```
this.withoutJsTemplate = %1 (%3H, %2)";
this.withJsTemplate = " %1 (%3H, %2, %4)";
```

If you prefer to obtain something like this:

1H NMR (300 MHz, Solvent) δ ppm 1.23 (d, *J* = 1.2 Hz, 3 H), etc…

Just replace the original lines with:

```
this.withoutJsTemplate = %1 (%2, %3H)";
this.withJsTemplate = " %1 (%2, %4, %3H)";
```

To obtain the coupling constant symbol in normal instead of *'italic'*, just modify the script by removing the italic format (J). If you prefer to obtain it in "**bold**" just type:

```
this.jListTemplate =  "<b>J</b> = %1";
```

The following paragraph will be used to customize the appearance of the coupling constants list:

```
this.jPrecision=1;//J's precisi
```

```
sion
this.deltaPrecision=2;
//deltaprecision
this.mSeparat
```

```
or
=
"
,
"
;
//multipletseparator
this.jSeparator='ss
```

```
separator
```

The first line is used for the precision of the coupling constants values, the second will be used for the precision of the chemical shift and the remaining two lines will print the separation between the multiplets and the coupling constants values.

If you need to obtain the coupling constants in descending order, replace 'true' with 'false' in the following line:

```
var jList = new JList(multiplet.jList());
j
```

```
List.sort(true);
```

If you want to obtain the multiplet chemical shifts in ascending order, just replace the 'false' with 'true' in the script:

```
var multiplets = new Multiplets (spectrum.multiplets()); // get multiplets from spectrum
jList.sort(true);
```

To obtain the multiplet range in ascending order, just replace the rangeMin with rangeMax (and vice versa) in the below line of the script:

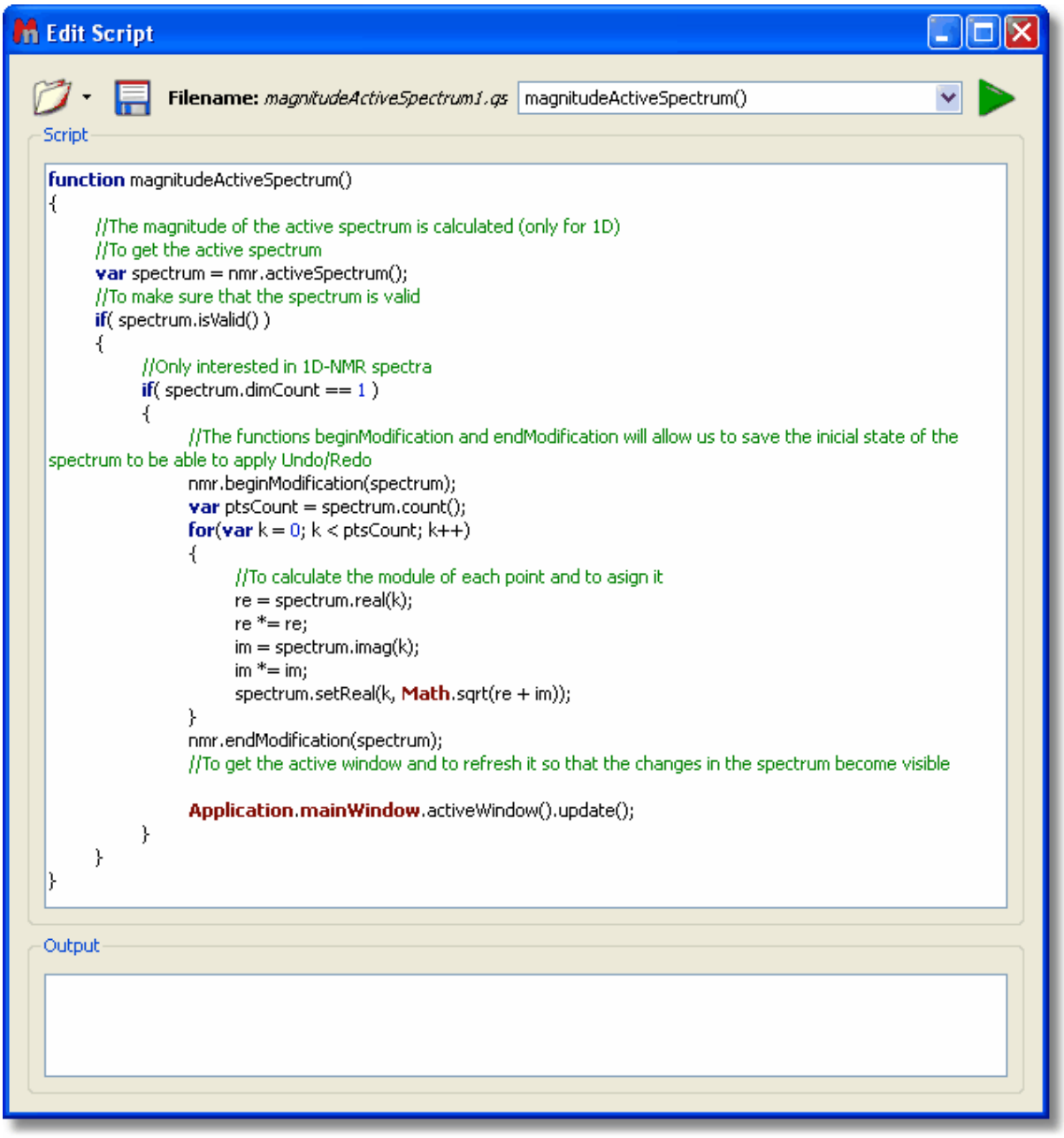
```
shiftStr = this.rangeTemplate.argDec(multiplet.rangeMax, 0, 'f', this.deltaPrecision).argDec(multiplet.rangeMin, 0, 'f', this.deltaPrecision);
```

Let´s see another script; in this case this script is used to obtain the 1D-NMR spectra in magnitude. Just open a 1D-NMR spectrum in Mnova, load this script by following the menu 'File/Edit Script', type "magnitudeActiveSpectrum()" in the edit box and click on the green triangle to run the Script. You will obtain automatically your 1D-NMR spectrum in magnitude.

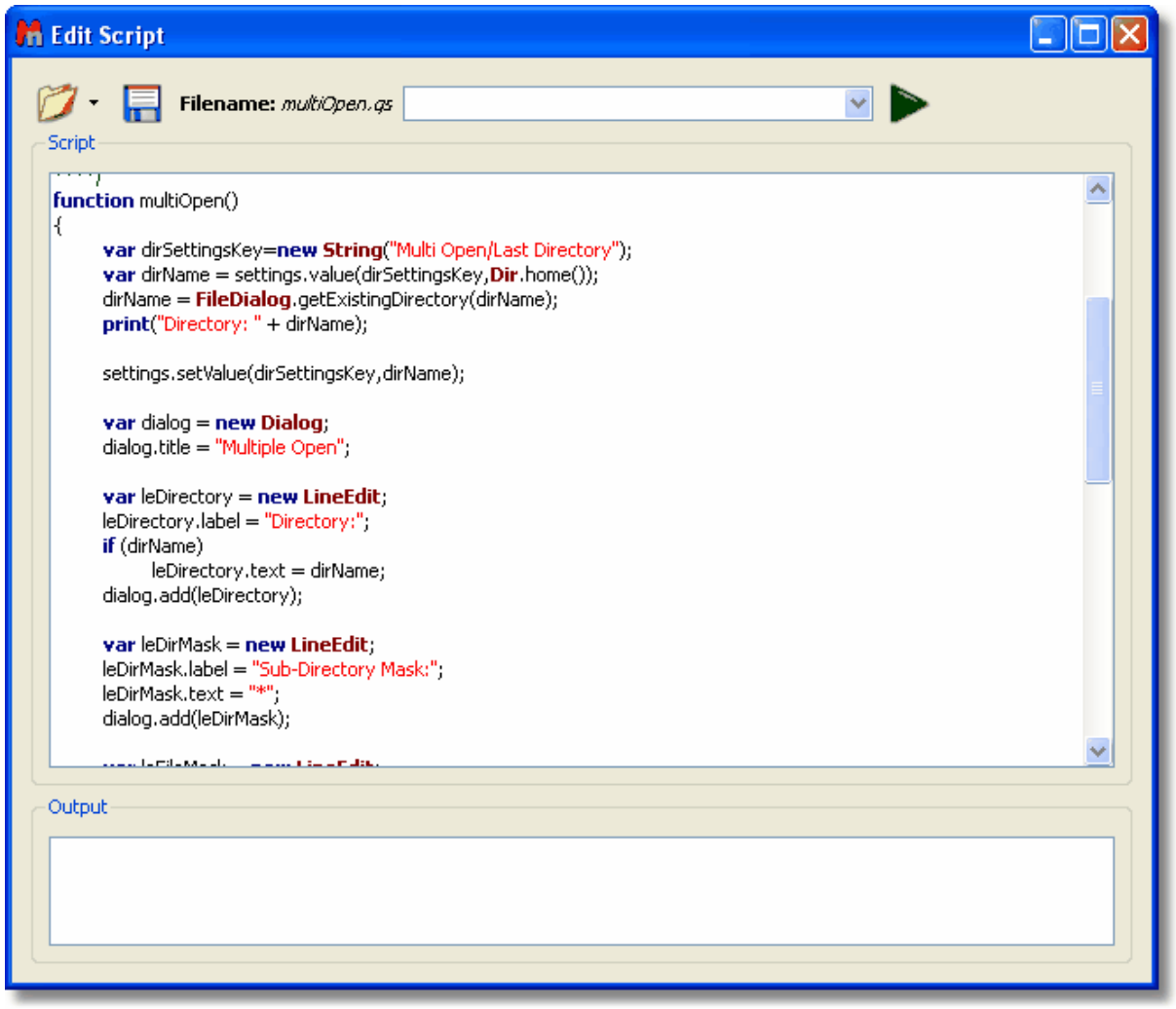
Please bear in mind that it is also possible to run scripts from the command line, just by typing the path where the Mnova .exe file and the script are located and -sf "name of the script function": **"mestrenovaPathname" "scriptPathname" -sf "scriptFunctionToRun"**

For example:

"C:\Program Files\Mestrelab Research S.L\MestReNova\MestReNova.exe" "C:\Program Files\Mestrelab Research S.L\MestReNova\scripts\magnitudeActiveSpectrum.qs" -sf "magnitudeActiveSpectrum"

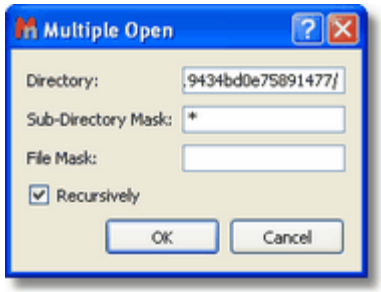


Mnova incorporates a script named 'MultiOpen'. This tool is very useful if, for example, you always process your spectra in the same way, or when you have to routinely handle a large number of datasets.



A practical example:

'*multiopen script*' allows to open simultaneously a collection of several spectra. Make sure that all spectra are located in the same folder. After that, select 'Run Script...' on the 'File Menu' or click on the **'Run Script'** icon  on the toolbar and then click 'Ok' after finding the corresponding script (in this case, the script is called 'multiOpen'). Then, select the directory where the spectra are located and a window like the one below will open:



Finally, write the 'File Mask' name (you can use wildcards, so this could be, for example, '*', '???', fid or

ser), tick 'Recursively' and click 'OK'. All selected spectra will open simultaneously.

The user can also use the 'Full Processing' command to automate the processing of both 1D & 2D NMR data sets. (You can find a full description of this tool further on in the Automated_Processing chapter.)

'Recent Scripts': dragging the mouse to this option you will be able to select recently used scripts.

Thank you!

‘Thank you for reading this Manual, and for purchasing this release version of MNova. We will be very keen to read your feedback on the application, to hear about any bugs you may find and to also listen to any additional ideas or suggestions you may have.

Please remember that you can send all those, and any queries about the software, or requests for help, to:

support@mestrec.com

Keep checking our web site (www.mestrelab.com) for additional information on our range of software packages, and for news on our company.’