

# SpinWorks Documentation, Version 4.0.5 (2014/06/04)

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## Introduction

SpinWorks has two functions: The first is to provide easy basic off-line processing of 1D, 2D and 3D NMR data on personal computers. SpinWorks other function is the simulation and iterative analysis of complex second order spectra including dynamic NMR problems and certain solid-state NMR problems, in a manner similar to our old UNIX Xsim program.

SpinWorks 3.0 was the first release of SpinWorks intended to run under the Microsoft .NET environment. Full support is included for Bruker (XwinNMR/UXNMR), Varian (Unix VNMR/VNMRJ) and Anasazi (CDFF type 2) data formats. JEOL and JCAMP-DX formats are supported for 1D only. JEOL 2D and Tecmag formats will hopefully be added in the future. Included indirect detection modes include States, TPPI, States-TPPI, Single Detection (QF), and echo-antiecho.

***Bruker DISNMR and DISMSL format data are no longer supported.***

While the program is to the point where it should (I hope) be useful, there will, no doubt, be bugs and there are things that don't yet work. The aim of the program is to make a program easy enough for undergrads to process magnitude COSY spectra (for example) with a single mouse click, and yet still be flexible enough for research use.

SpinWorks currently handles up to 7 data sets at a time.

### **Things that don't yet work, or need improvement in SpinWorks 4:**

1. The .NET version is a bit of a memory hog. This is because all of the data remains memory resident. This does, however, greatly speed up the processing. 2D Imaginary quadrants can be cleared from memory after transform and phasing. 2D matrices up to 2k by 1k shouldn't create problems on a machine with 256 MB of ram. You may want to watch how many other apps you have running, however. A 4k by 1k TPPI HSQC uses up about 32 MB of memory. Considerable additional memory is used transiently during transform and display contouring.
2. Not enough OS/printer/printer-driver combinations have been tested. In particular, I would like to hear from anyone having printing problems with Linux.
3. 2D MQMAS shearing transforms still need more testing with Bruker data.
4. Linear prediction is a bit slow compared to earlier versions. However, the LP routines are now .NET clean and should run under other .NET environments (e.g. Mono).
5. For Varian data, the referencing values imported with the dataset *may* be off by one data point. This is not normally noticeable for data with a large number of points (usually 1D), but may be annoying on smaller (usually 2D) datasets. Using the SpinWorks **Calibrate** function will correct this problem.
6. For normal processing, SpinWorks doesn't care if there are blanks embedded in the path to the data. For example, if you put your NMR data in the "My Documents" folder (with the blank between "My" and "Documents") SpinWorks won't care. However, the UNIX-derived dynamic NMR simulation routines (DNMR3 and MEXICO) won't tolerate blanks in a file path (because UNIX doesn't like blanks in a file path). So, if you are running DNMR or MEXICO simulations with experimental data present, the data must be in a folder with no

blanks in the entire path. For example, create a folder like C:\nmrdata (or whatever), put your data there, and everything will be fine. ***Note that some vendors' software now allows blanks in file names, but I think practice should be discouraged.***

7. The autophase routine really sucks! Does anyone have a good algorithm that they want to share?
8. The Linux version still needs a lot of work. Or, I might say, the mono run-time needs a lot of work. On many printers, the Linux version can't seem to print in landscape mode. There has been a report that the "Copy to Inset Box" feature doesn't work under mono.
9. The simulation modules still need to be compiled for Linux.

## Fonts

In this manual, Arial is used for menu items and other text that you see on the screen. ***Bold italics means: listen up, this is important!***

## Timings

Sample 2D transform timings (with image mode display) on a 1.86 GHz CoreDuo 2 GB (Dell Precision 390) under WinXP:

- 4k by 512 States HSQC with phasing applied in F<sub>1</sub>: 6 sec.
- 1k by 1k States TOCSY with phasing applied in F<sub>1</sub>: ca 2 sec.
- Contour display of 1k by 1k TOCSY (Strychnine): ca 2 to 3 sec.

***What does the message "Display buffer exceeded, raise the floor!" message mean?***

It means that the lowest display level (contour or image) is too low. Simply click the Floor + button (button panel). You may have to click several times if the level was much too low.

## Computer Requirements

SpinWorks requires a Pentium class processor running Windows 2000 Pro, Windows XP, Windows Vista, Windows 7 or Windows 8. Installation currently requires about 12 Mbytes of disk space exclusive of NMR data. 512 Mbytes or more of RAM are recommended, depending on NMR data set and simulation sizes. Of course, more memory is required for 2D and 3D!

SVGA 1024 x 768 or better display required (1280 x 1024 or better recommended).

***Release 2.0 (or higher) of the .NET runtime environment is required.*** This can be downloaded from Microsoft and is also included on the SpinWorks ftp site. Release 4 or higher is recommended.

For 2D you should also have your display set to at least 16 bit colour, otherwise the image and contour level colours will be strange.

A three-button mouse is ideal, but SpinWorks will work just fine with a two-button mouse. Note that on "Wheel Mice" the mouse wheel can serve as the middle mouse button. The mouse wheel can also be used for vertical scaling of 1D spectra or the "projection" traces of 2D spectra.

## 64 Bit Operating Systems

SpinWorks seems to work just fine under Windows 8, 64 bit Windows 7 and 64 bit Vista. However, the default folder is C:\Program Files (X86)\SpinWorks\_4. In order to use the simulation modules you must update the External Module Path entry (Options menu, Set Start-Up Options... selection) to reflect this change.

It turns out that there is a bug in .NET for Windows 7 64 bit (maybe Vista 64 bit?, maybe Windows 8?) in that the Print Dialog box doesn't work. A workaround has been to use the print button in the Print Preview dialog. Starting in 3.1.8 beta 4, an option has been added to use an alternate Print Dialog. This can be selected with the Set Start-up Options... dialog in the Options menu.

## Mono

I am *hoping* that the program will run under other releases of .NET, such as the open source mono project under LINUX or MacOS X. I am currently testing SpinWorks running under MONO 3.0.6 on OpenSUSE 13.1. A slightly modified version of SpinWorks also seems to compile just fine with MonoDevelop 3.0.6 under OpenSUSE13.1. Testing so-far has been encouraging, but there are still a few minor snags to be straightened out. The external simulation modules have not yet been compiled for Linux.

SpinWorks seems to run just fine under "Parallels" or "Boot Camp" on Macs and "Wine" on Linux.

## Download

The program is currently available by anonymous ftp from <ftp://davinci.chem.umanitoba.ca> in the pub/marat/SpinWorks/ directory. The current release is SpinWorks\_405.zip. The documentation, available as a pdf file, is available for separate download, but is also included in the zip distribution.

## Installation

Run the setup.exe program in the unzipped folder and follow the instructions. In most cases, it is safe to use the default settings. Please read the license agreement and agree with the conditions before proceeding. The installation procedure will add the program to the Start menu and make a shortcut on the desktop.

## Windows Command Line Start-Up

SpinWorks can be started from the Windows command line and given the full path to start-up data set as a command line argument. For example, a command line entry like:

```
SpinWorks.exe C:\nmrdata\cyclosporin\1\fid
```

will start SpinWorks on and load the fid for experiment 1 on the cyclosporine data set. If the path to the data contains any blanks, it will be necessary to enclose the path in quotation marks.

E.g. SpinWorks.exe "C:\my nmrdata\cyclosporin\1\fid".

If the “autoload” feature is turned on and data set contains processed data, this will be displayed. This feature can be very useful if one wants another program or web browser to display an NMR data set by launching SpinWorks.

## Registry Entries

SpinWorks 4 uses a different set of registry entries than earlier (release 2 and 3) SpinWorks versions. This means that you will probably want to go to the **Options** menu and use the **Set Preferences...** dialog to configure your start-up preferences. Usually, this will mean just setting your start-up data path and a few desired defaults... a few seconds at most. Display colours and the recent files list are also stored in the registry.

## Redistribution

You are free to redistribute this software to others, provided that:

- You don't charge anything for it.
- You don't modify it.
- You distribute it in its entirety.

## Registration

Although SpinWorks is currently being distributed as freeware, and there are no license keys required. I do, however, request that a SpinWorks user register their use by email to:

[kirk.marat@ad.umanitoba.ca](mailto:kirk.marat@ad.umanitoba.ca)

Please include: an email contact, the machines and OSs that you are running it on (e.g. i7 3.4 GHz, Win 7 Pro), and a note of any problems etc. that you had with the download and installation. I intend (someday) to maintain an email list so that I can inform users of bugs, updates, patches, etc. If you *don't* want to be on this list, please let me know.

More detailed comments, questions, bug reports, etc. can be also directed to the above address, and are *always* (!) appreciated.

## Acknowledgements

Many people have helped with the design and testing of SpinWorks and the older UNIX Xsim program, providing numerous bug corrections, suggestions and test data. I would especially like to thank: Alex Bain, Klaus Bergander, Alan Brisdon, Woody Connover, Mike Coplien, Witold Danikiewicz, Klaus Eichele, Mike Englehardt, Tom Ferris, Pascal Fricke, Deby Harris, Phil Hultin, Anne Kaintz, Christian Landvogt, Vera Mainz, Virginia Miner, Gareth Morris, Rudy Nunlist, G. Dan Pantos, Sebastian Pfeifer, Georgy Salnikov, David Sandquist, Georg Schitter, Nils Schloerer, Rich Shoemaker, Martin Suetterlin, Harold Toms, Jian-Xin Wang and David Vanraes.

My apologies to anyone I may have missed.



## Obligatory Disclaimer (Lawyer Talk)

*SpinWorks is being distributed as “freeware” in the hope that it will be useful. However:*

*The author disclaims all warranties with regard to this software, including all implied warranties of merchantability and fitness. In no event, shall the author or the University of Manitoba be liable for any special, indirect or consequential damages, or any damages whatsoever resulting from loss of use, data or profits, whether in an action of contract, negligence or other tortuous action, arising out of or in connection with the use or performance of this software.*

*All trademarks mentioned (e.g. XwinNMR, TopSpin, VNMR(J), Windows, etc.) are property of their respective owners.*

## Referencing SpinWorks

Please check the “Instructions to Authors” for whatever journal you are writing for, but it should be something like:

SpinWorks 4.0.5, Copyright © 2014  
Kirk Marat  
University of Manitoba

If you make use of the simulation modules (DNMR3, MEXICO, NUMMRIT or SIMPSON) you must, of course, ***reference the original authors work as well***. These references are given in the corresponding section of this manual

## Online Help

PDF and Word format manuals are available. The standard windows help has been phased-out, and may be replaced with an XML or HTML based help system. The manual can also be viewed directly from SpinWorks in PDF format (requires Adobe Acrobat reader) or in Word format (requires a word processor).

## Error Logging

SpinWorks logs exceptions and other error conditions into a log file (SW\_error.log) in the folder defined as the scratch folder (usually C:\Temp). This is the first place to look if there is a problem with the program. Additional more detailed program tracing information can be generated by selecting Extended Debug Logging in the Options menu.

## FTP Clients

Bruker and Varian data sets are directory (folder) structures containing a mixture of binary data and ASCII parameters and text. FTP transfer of binary data with the ftp client set to ASCII will result in a corrupt data set. Some FTP clients (notably FileZilla) have an “auto” setting ***which unfortunately doesn’t detect the binary data properly***. For these clients set the transfer type to binary (or bin) and everything should be fine. In FileZilla this is the transfer type setting in the Transfer menu. Transferring the ASCII parameters in binary mode creates no problems.

## New (or different) in version 3 relative to version 2.5.5

1. New, streamlined, more colourful user interface. (I got tired of the Microsoft battleship gray.)
2. Requires .NET 2.0 or higher.
3. More options and flexibility for stacked and inset plots.
4. More options for colours, font sizes, etc.
5. Provisions for automatic processing and peak picking of Varian “Arrayed” experiments.
6. Several selections have been moved from one menu to another. For example, “Peaks and Match” has been moved from Options to View.
7. The keyboard shortcuts for recalling regions of interest have been changed to: <Alt>1, <Alt>2 and <Alt>3.
8. Added FID math toolbox.
9. Added support for JCAMP-DX FIDs produced by XwinNMR/TopSpin.
10. Added support for JCAMP-DX Spectra produced by Tecmag NTNMR.
11. Added support for solid state simulations (spectra and FIDs) produced by the SIMPSON program.
12. Uses Bruker “FnMODE” parameter (if defined) for F1 quad detection setting. This parameter was added with TopSpin (possibly XwinNMR3.5 as well). If FnMODE is not defined then the MC2 parameter is used. This is consistent with the way TopSpin handles the data.
13. Added manual 1D and 2D peak picking using right mouse button.
14. Removed “double-click” toggle of the data area cursor. The View menu or the keyboard “t” shortcut can be used instead.
15. Added support for 2D and 3D processed data produced by NMRPIPE.
16. Added peak deconvolution (band fitting).
17. Added 1D and 2D Hilbert transforms.
18. Added manual picking of individual peaks.
19. Fixed “blank-free” file path restriction for NUMMRIT simulations.
20. Versions 3.1.1 to 3.1.5 add numerous small enhancements and bug corrections. Many of the bug corrections correct problems resulting from many parts of the world using a “,” as the decimal separator rather than “.” As used in North America. ***Note, however, that all values entered into dialog boxes in SpinWorks must use the North American “.” Decimal separator.***
21. Plot sized and options (sizes and objects) are saved with the processed data (3.1.3). A default option for A4 paper size has also been added (3.1.3).
22. Added command line start-up with a specific data set (3.1.4).

- 23. Added error logging and tracing (3.1.5).
- 24. Increased number of workspaces to 7 (3.1.5).
- 25. Added keyboard shortcuts “z” for zoom, “f” for full spectrum, and “l” for last expansion (3.1.5).
- 26. Added Return (Enter) key as a shortcut to exit baseline peak pick minimum routines (3.1.5).
- 27. Added log spacing for 2D contours, and made this the default. Linear spacing can be selected in the View menu (3.1.5).

### **Added in 3.1.6**

- 28. Added support for JEOL 1D data (JEOL 2D is still under development).
- 29. Added cubic spline baseline correction.
- 30. Added “sum” mode to the projection display and calculation. Previous projection calculation was “skyline” only.
- 31. For band fitting x,y points files of the individual peaks as well as the synthetic spectrum can be produced.
- 32. Added summing a stacked trace to the experimental.

### **Added in 3.1.7**

- 33. Added support for Anasazi format data (1D and 2D, FIDs and spectra).
- 34. Many small (and not so small) bug fixes.
- 35. The code is hopefully more “Linux friendly.”
- 36. Improved JCAMP-DX format handling. Packed, Squeeze and Diff/Dup compression modes are supported.
- 37. A Dual 2D display mode has been added.
- 38. Improved processing for Bruker “arrayed” (T<sub>1</sub>, diffusion, etc.) type experiments that are recorded as a pseudo-2D experiment in a ser file.

### **Added in 3.1.8**

- 39. The fully automatic baseline correction routine has been greatly improved. The “Whittaker smoother” routine is used.
- 40. The FID display has been improved, and the current window function is displayed with the FID.
- 41. Font Size Options added. Standard menu and button panel fonts slightly bolder and (hopefully) more readable.
- 42. Alternate print dialog box (Set Start-up Options...) for Windows 7 64 bit.
- 43. Added data “binning”.

## New (or different) in version 4.0.5 relative to version 3.1.8

1. 3D Processing (Bruker and Varian/Agilent data only).
2. Workspace buttons as an alternative to the Workspace drop-down (drop-down may be deleted in future versions).
3. An option for coarse vs. fine contour level spacing (View menu). The coarse setting is the default, and speeds up the 2D display.
4. Integration slope and bias added. Slight change in the way a current integral is defined.
5. Increased default Whittaker Lambda value for fully automatic baseline correction.
6. Peak Pick menu now includes an option for setting the number of digits to the right of the decimal.
7. Referencing dialog includes buttons for setting the reference to common solvent values ( $^1\text{H}$  and  $^{13}\text{C}$ ).
8. Fixed several bugs in the JCAMP-DX file reading routines.
9. Option for setting number of digits for integral and peak display.
10. Integrals and peaks are automatically read in when loading processed data.
11. The increase and decrease Bruker experiment number buttons will now skip gaps in the experiment number list.
12. You can automatically process and stack display a series of Bruker experiment numbers (e.g. for a 1D NOE or ROE series or time study).
13. Scaling of metafiles has been changed internally as a work-around to a bug in MS Word's pdf creation procedure. These changes should be transparent to the user.
14. Start-up defaults are suitable for 64 bit operating systems (32 bit still fully supported).

## Use of the Mouse, and General Display Manipulation

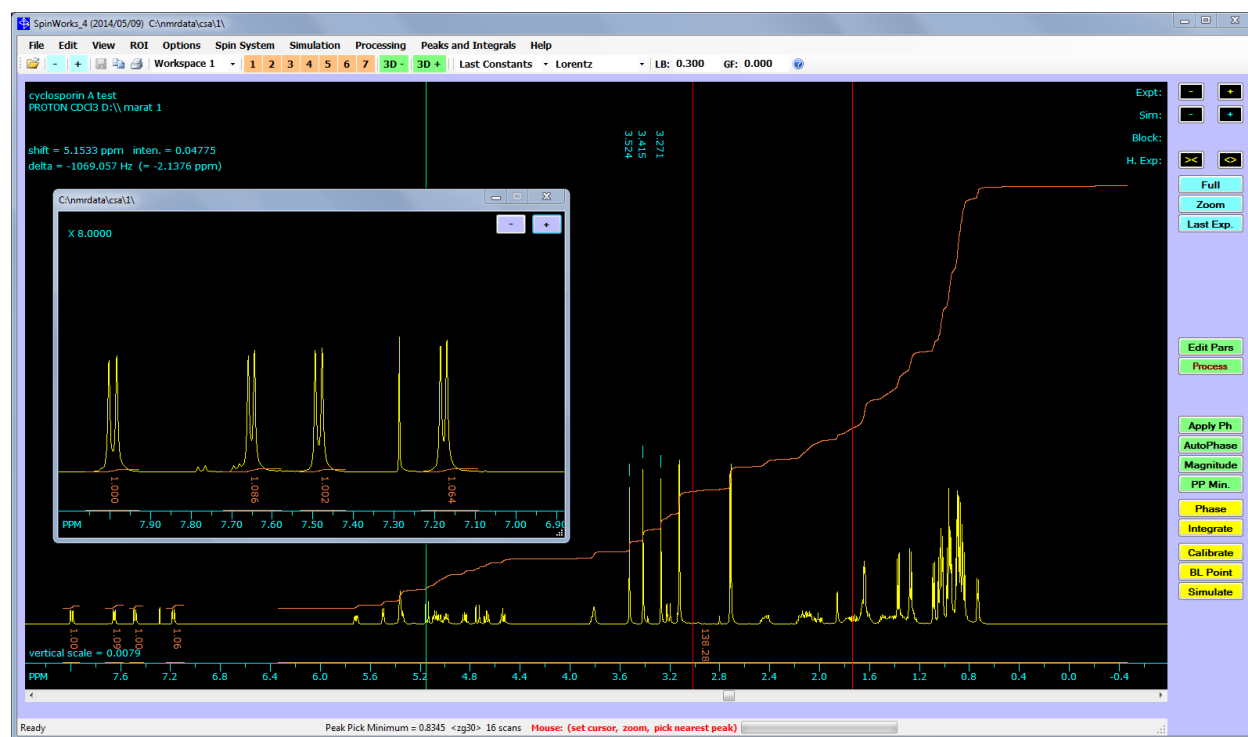
While the cursor is in the data window, a left mouse click can be used to mark one or two red reference lines on the spectrum. A third click will remove the lines. The frequency and frequency difference of these lines is displayed on the screen and can be used to estimate shifts and couplings, etc. These lines are also used by several routines that require defined reference points. I am considering adding a “drag to zoom” feature as an alternative.

The function of the **right** mouse button (on both two- and three-button mice) in 1D depends on the presence of simulated data. If there is an experimental spectrum present but *no* simulated spectrum, the right mouse button can be used for manual peak picking. The program will use the current peak picking parameters (minimum intensity and noise threshold) to try to find a peak within a reasonable distance from the current cursor location. If no peak is found (or interpolation is turned off), the raw cursor location is used as the peak location. These manually picked peaks are appended to the current peak list, so that a mixture of normally picked peaks and manually picked peaks can be created. This can be very useful if you want a peak list to include peaks that are difficult (broad and/or noisy) for the automatic peak picking algorithm to detect.

If there *is* a simulated spectrum as well as an experimental spectrum, the right mouse button is used to assign calculated transitions to observed lines, but only when the pointer is actually within the data window (the part of the screen displaying the spectra).

In 2D mode the **right** mouse button selects rows or columns of the 2D matrix (e.g. for phasing), or can be used for interactive 2D peak labeling/picking.

Display colours can be set with the Display Colours... dialog in the Edit menu.



**Figure 1.** Typical 1D display

Spectral regions can be zoomed by defining two reference points and clicking the **Zoom** button on the right hand button panel, or the middle button of a three-button mouse. Spectra can also be zoomed with the Z keyboard shortcut.

Spectra can also be expanded and contracted with the buttons labeled .

A tracking vertical cursor line can be turned on/off with the appropriate selection in the View menu or with the “t” keyboard shortcut.

The scrollbar at the bottom of the data window can be used to pan through an expanded spectrum.

The spectrum baseline or zero can be moved by “dragging” it with the cursor. This works for both experimental and simulated data.

A number of specific display limits or plot scales (e.g. 9 to -0.5 ppm or 10 Hz/cm) are available through the View menu. The toolbar Full button will always show the full recorded spectral width.

1D Spectra are scaled vertically with the button panel **+** and **–** buttons. The yellow buttons are for the experimental spectrum and the bluish buttons are for the simulated spectrum. Spectra may also be scaled in smaller steps with the keyboard up and down arrow keys. The spectra can be shifted vertically by dragging the blue zero intensity reference line.

It is possible to expand regions of a 1D spectrum and display it in inset boxes. Simply define the region with the cursors and select **Copy to Inset Box** on the **View** menu. The cursor-defined region will be displayed in a re-sizable box. Likewise, the button panel controlling the scaling of the inset trace can be closed and then re-opened at any time with the **Scale/Shift Stacked or Inset Traces...** selection in the **View** menu. Note that the vertical scaling of the inset is completely independent of the regular spectrum scaling, but the relative scaling of the two is always shown in the upper left corner of the inset. The relative scaling of the inset can be set to be the same as the regular spectrum by using the reset button on the **Scale and Shift Stacked or Inset Traces** dialog box.

Up to three regions of interest (regions that you might want to look at again) may be defined for each spectrum. You can define these regions with the **Define User Specified Limits...** dialog from the **View** menu, and the saved region can be displayed with the **ROI** menu. You can also use **<ctrl>1**, **<ctrl>2** or **<ctrl>3** to define the current screen view as a region of interest. The regions can then be recalled with the **ROI** menu or the **<Alt>1**, **<Alt>2**, or **<Alt>3** keys. These regions of interest are saved with processed data saved by SpinWorks (when the auto save feature is on) and recalled when the data is retrieved. These regions are *not* saved with data in JCAMP-DX format.

In 1D mode, SpinWorks can display the experimental spectrum, a simulated spectrum, and a series of stacked traces. It is important to note that there are slight difference between the experimental trace, the simulated trace, and the stacked traces.

1. The size, spectral width and offset of the *simulated trace* are equivalent to the experimental spectrum. In essence, the spectral characteristics of the simulated trace are tied to the experimental spectrum.
2. The size, spectral width and offset of the *stacked traces* are independent of the experimental data. Each stacked trace is an independent object, and its spectral parameters are copied from the source spectrum when the stacked trace is created. This allows one to compare spectra that may have been recorded under different conditions.

## Keyboard Arrow Keys

The keyboard left and right arrow keys move the simulated spectrum transition cursor from peak to peak. Holding the **<ctrl>** key down while pressing the arrow keys moves the cursor in steps of 5. This is very useful for moving the cursor rapidly on a spectrum with many degenerate or near degenerate transitions.


The keyboard up and down arrow keys adjust the vertical scale of the experimental spectrum. The scaling steps are smaller than those of the toolbar **+** and **–** buttons. Holding the **<ctrl>** key down while pressing the arrow keys scales both the experimental and calculated spectra.

## Keyboard Function Summary

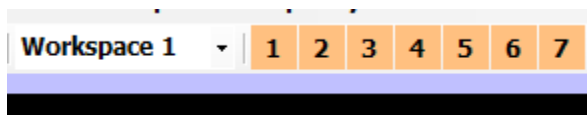
<b>Left and Right arrows</b>	Move the simulated spectrum transition cursor. Holding the <ctrl> key down at the same time moves the cursor in steps of 5 transitions
<b>Up and Down arrows</b>	Adjust the vertical scaling of the experimental spectrum. Holding the <ctrl> key down at the same time adjusts the scaling of both the experimental and simulated spectra. If you have a "Wheel Mouse", you can use the mouse wheel to adjust the vertical scaling of 1D spectra. If the pointer is in the bottom half of the data window the scale of the experimental spectrum will be adjusted. If the pointer is in the upper half of the screen the scaling of the simulated spectrum will be adjusted.
<b>"t" key</b>	Toggles the tracking cursor (2D cross-hair pointer) on or off. This is an alternative to using the Options menu. Double clicking in the data area will also do this.
<b>"a" key</b>	Assigns the transition pointed to by the transition cursor to the nearest observed line. This function uses the peak picking algorithm, so is influenced by the peak picking threshold and noise discrimination factor.
<b>"d" key</b>	Deletes any assignment for the currently pointed to transition.
<b>"z" key</b>	Zooms the region between the cursors.
<b>"f" key</b>	Displays the full spectrum.
<b>"l" key</b>	Displays the last expansion.
<b>&lt;Ctrl&gt;1, &lt;Ctrl&gt;2, &lt;Ctrl&gt;3</b>	Defines the currently display as a region of interest that can be recalled later. You can also define these regions from the View menu.
<b>&lt;Alt&gt;1, &lt;Alt&gt;2, &lt;Alt&gt;3</b>	Recall a previously defined region of interest. The ROI menu from the menu bar can also be used.

## Toolbar

The toolbar is displayed across the top of the application window, below the menu bar. The toolbar provides quick mouse access to several SpinWorks functions:

-  Open an existing NMR data set. SpinWorks displays a dialog for selecting data sets.





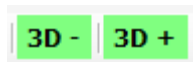
- 

Selects the current workspace. There are 7 available workspaces. Starting in SpinWorks 4 there are a series of workspace selection buttons to the right of the Workspace pull-down.



- 

Decreases or increases the experiment number for Bruker data.



- 

Decreases or increments the current 3D plane number.



- 

Print the current NMR spectrum. Various plot options can be set with the Plot Options and Parameters... dialog available in the Edit menu.



- 

Save the processed spectrum in JCAMP-DX format in the current data folder. The file will be named: "spectrum.dx". In order to save the file with a particular name, use the **Save As...** selection in the **File** menu. This button is most useful for saving 1D reference spectra to plot along the axes of 2D spectra. A copy of the processed data is also saved in SpinWorks own format as well.



- 

Saves a copy of the current output as a Windows enhanced metafile (emf) in the Windows clipboard. These files can be used to include the spectrum in documents, etc.




- 

Define the pre-transform window (weighting) and post-transform phasing to be applied with a 1D Fourier transform.





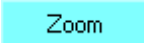




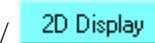
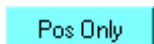
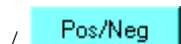

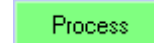
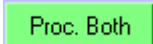
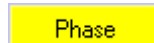

## Button Panel



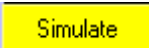
The button Panel at the right of the data window can be used for display manipulation and processing. The number and identity of these buttons will change with the current state of the program. The buttons are grouped and colour coded according to function. Cyan (blue-green) is for display functions, green in for processing and yellow starts post-processing dialogs and routines such as interactive phasing.

- The  buttons scale the spectra vertically. The colour of the symbol on the button matches the colour of the spectrum. In 2D mode, these buttons control the scaling of any displayed rows or columns. If you have a "Wheel Mouse" you can use



the mouse wheel to adjust the vertical scaling of 1D spectra. If the pointer is in the bottom half of the data window, the scale of the experimental spectrum will be adjusted. If the pointer is in the upper half of the screen the scaling of the simulated spectrum will be adjusted. In 2D mode, the buttons with the **Scale:** label to the left adjust the scaling of any displayed rows or columns.

- The   buttons are used in 2D mode to highlight a particular overlaid row or column. In an arrayed experiment (Varian) selects the index of the array.
- The   buttons contract and expand the spectra horizontally. Usually, it is easier to use the cursors and the  button, but some routines (e.g. integration) pre-empt the cursor for their own purposes. Not available in 2D mode.
- The ,  and  buttons Zoom the spectrum between red two cursor lines, return to the full spectral width, or return to the previous expansion, respectively. Note that if you have defined a region with two cursors lines, you can use the middle mouse button (if you have one) or the Z keyboard shortcut to zoom in place of the blue Zoom button. The F keyboard shortcut will display the full spectrum while the L keyboard shortcut will display the last expanded region.
- The  /  button switches between 1D and 2D display mode for 2D data. The label on the button described the action of the button, and not the current display state of the program. i.e. If you are displaying 2D data in contour or image mode, the button will be labeled 1D Display in order to switch to 1D mode for displaying individual rows.
- The  /  button switches the 2D contour and image displays between displaying only positive intensities (e.g. for magnitude mode spectra) and displaying both positive and negative intensities (e.g. for phase sensitive data).
- The  button starts the processing parameter dialog (1D and 2D).
- The  (1D) button is used to process the data with the current processing parameters. The pre-transform window (weighting) function and the post-transform phasing can be set with the tool-bar drop-down menus. In 2D mode, the  button will process both dimensions according to the current processing parameters.
- The  button starts the interactive phasing routine. (1D and 2D)
- The  button starts the integration routine. (1D and 2D).

- The  button calibrates the spectrum. To calibrate a spectrum: define a point with a cursor line, click the Cal button, and then enter the correct value in Hz. or PPM (depending on the current axis units). In 2D mode, you will be asked for the calibration in *both* dimensions.
- The  button starts the baseline point routine. Not available in 2D mode. After all baseline points have been defined click the button (now labeled return) again or hit the **Enter** key to exit the baseline routine.
- The  button starts the pop-up simulation panel.

# Getting Started: Basic 1D NMR Processing

## 1. Setting the Data Format (not normally necessary)

SpinWorks 4 can identify the most common formats (VNMR, XwinNMR/UXNMR/TopSpin, JCAMP-DX, JEOL 1D and Anasazi) automatically. In order to process data in other supported formats or processed data from NMRPIPE, use the Data Format... selection in the Options menu. The Varian setting is for Varian Unity, Unity+, Mercury, Inova, VNMRS and MR400 spectrometers. VXR/S data will probably also work with this setting, but this hasn't been tested. VXR, Gemini and XL data formats are not supported. The Bruker setting is appropriate for data from a Bruker AMX, ARX, ASX or Avance series spectrometer. ***DISNMR / DISMSL data are no longer supported.*** JEOL data support is currently limited to the "jdf" format, ***ID only.***

1D FIDs in JCAMP-DX format written by XwinNMR or TopSpin can be read and should be automatically recognized by the program. 1D Processed data can also be read and saved in JCAMP DX format.

Part of the design philosophy of SpinWorks is that it uses the data in the format provided by the spectrometer. Any and all format conversions are internal to the program and are essentially transparent to the user. This also means that the ***complete*** data set must be transferred to your PC! For UXNMR/XwinNMR data, the entire data tree from the experiment name down should be transferred. This is important, since SpinWorks gets referencing and processing information from the *proc* and *procs* files, and expects to find them in the same relative place that they were on the spectrometer. For Varian data, SpinWorks expects to find the fid, procpa and text files under the *experiment\_name.fid* directory. The easiest way to transfer entire directories with contents to your PC is with one of the smart ftp programs such as WS\_FTP95, "FileZilla" or similar. ***Note that the file transfer mode must be set to binary.*** This seems to be a particular issue with FileZilla.

**Tip for JEOL and Anasazi data:** Bruker, Varian and NMRPipe data sets are actually folders contain a number of data and parameter files. SpinWorks writes processed data, simulation files etc. into this folder. JEOL and Anasazi data can be problem in that the data sets are single files, whereas many features of SpinWorks assume that a data set is a folder. An easy work around it to create a folder for each JEOL data set. For example, if you have a data set called brucine\_1D1H.jdf just place the file into a folder called brucine\_1D1H. All SpinWorks created files will then be placed into the folder with the raw data set.

## 2. Selecting the Data

Data selection is done with Open... selection in the File menu. You will then be presented with a standard Windows file selection menu. Navigate through directory system and select your data. For Bruker AMX and Avance data, navigate from the experiment name directory to the experiment number directory and select the file named "fid". A "recent files" selection is available at the bottom of the File menu and can be used as a shortcut for data selection. If you have selected a valid data set then it will be displayed on the screen after closing the File menu. JEOL files will have a ".jdf" extension while Anasazi files will have a variable (or no) extension. Anasazi data can be either time domain (fids) or processed spectra.

**Tip:** The Default Data Path setting in the Set Start-Up Options... dialog in the Options menu

is a convenient way to short cut selection of your data.

**Data on CD:** SpinWorks occasionally writes scratch files, processed data or other information, usually in the same folder as the experimental data. This creates a problem for data on CDs (or any other write-protected file system) where you cannot usually (except for CD-RW) write data. Therefore, data on CD *must* be transferred to the hard disk before processing with SpinWorks. ***This also means that you must have write permission in the folder where the data are located.***

### 3. Processing Data

1D data processing involves application of a window function (Exponential Multiplication) followed by Fourier transformation. These two processes are combined in the **window+ft** selection in the **Processing** menu. The other selections are for more advanced processing. The line broadening parameter for the exponential multiplication can be set in the **Processing Parameters...** selection of the **Edit** menu or the **Edit Pars** button on the button panel, but a reasonable parameter is set by default. After processing, a spectrum will be displayed on the screen.

The **Process** button on the right hand button panel will process the spectrum with the window function and phasing options selected by the drop-downs on the menu bar. Note that it is *not* necessary to re-read a data set to reprocess it. Once a data set has been selected *once*, (e.g. *via* the **File** menu) then one can just issue processing commands - the program will automatically re-read the raw data from disk each time.

Sometimes, there is a DC bias in the FID, which can cause a zero frequency spike in the spectrum. This can be removed by checking the **FID bias correction** in the **1D Processing Parameters...** dialog. FID bias correction is not the default setting for two reasons: Low frequency signals near the centre of the spectrum (usually water) can cause problems with the algorithm; Modern spectrometers have very little DC bias in their receivers. Systems with digital receivers (Bruker Avance, Varian VNMRs) should have *no* DC bias in the signal.

However, for spectra with a low number of scans, especially if the number of scans is not a multiple of the phase cycle, bias correction might be required on older systems.

A pronounced baseline offset in the *spectrum* can usually be corrected by adjustment of the **First Point Correction** parameter (1D or 2D processing parameter dialogs.). Typical values will be in the 0.4 to 0.6 range (the theoretical value is 0.5). Note that any baseline offset in the spectrum as well as reasonable amounts of baseline curvature can also be removed after processing with the baseline correction features in the **Processing** menu.

Resolution enhancement may be applied by setting the window type to **Lorentz to Gauss**. The **LB** parameter should be set to a negative value, with the negative of the natural line width being a good starting point. The **GF** parameter should be set to the relative fraction of the FID where the signal decays into the noise. For example, if the spectrum has peaks with a natural linewidth of 1 Hz, and the FID is gone 1/3 of the way into the acquisition time, then set **LB** to -1.0 and **GF** to 0.33. Set the Window function to **Lorentz to Gauss** and **Process** the data. Some experimentation may be required. Note that resolution enhancement may alter the relative integrals of the signals.

The **TRAF** function provides resolution enhancement as described by Traficante and Ziessow in *J. Magn. Reson.* **66**, 182 (1986). This function uses the **LB** parameter to calculate  $T_2$ . Optimum

performance is usually achieved when LB is set to the linewidth of the peaks in the absence of any apodization, but some experimentation may be required. Usually, a smaller value of LB will be needed for an acceptable signal to noise ratio. For routine organic samples in CDCl<sub>3</sub>, a value of 0.5 to 1.0 Hz seems to work well. A larger value will provide more enhancement, but a poorer signal to noise ratio.

The TRAFS function is a modification of the function which optimizes both the resolution and signal to noise ratio, as described by Traficante and Nemeth in *J. Magn. Reson.* **71**, 237 (1987).

The Gaussian selection in the processing panel applies a pure Gaussian window function to the time domain data. This function uses the LB parameter (*not* GF). In this case, the LB parameter specifies the width of 1  $\sigma$  in the normal distribution, and is slightly closer to the baseline than half-height.

HOGWASH resolution enhancement is left for a later section.

## 4. Linear Prediction

There are two situations where backward linear prediction may be useful for 1D spectra.

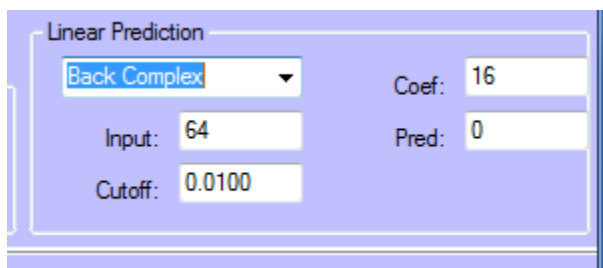
The first is where the first few points are corrupted by probe ringdown, baseline background, acoustic ringing, etc. In this procedure, the first few points of the FID are discarded and replaced with a backward extrapolation of the remaining data. After selecting a data set, the parameters for backward LP may be set in the Edit Processing Parameters... dialog of the Edit menu. The number of points to predict will typically be 2 to 16, and should be an even number. The number of coefficients will typically be 16 to 32, while the number of input points used for prediction must be greater than or equal to twice the number of coefficients. Generally, the more input points the better. However, specifying too many will result in noisy regions of the FID being used in the calculation of the coefficients. 64 Input points are suitable for many cases. The linear prediction itself is applied as part of the next transform. The cutoff parameter specifies a significance level for the coefficients. The default value is usually good, but may be raised to speed calculation. The FID can then be processed normally with any of the usual 1D processing functions in the Processing menu or the Process button. If the selection of LP parameters was inappropriate, make the necessary changes and re-process. Note that backward LP in SpinWorks always uses the SVD algorithm.

The second situation occurs with spectra recorded with very wide spectral widths, typically in solid state NMR. Here, the first data point is sampled after many dwell times, although the Fourier transform assumes that the first point is at zero time. For example, with a spectral width of 2.5 MHz, the dwell time (time per data point) is 200 ns, while the “DE” value on our Avance III 500 is about 8  $\mu$ s. This means that the first data point (which should be at time zero) is sampled about 40 dwell periods too late. This creates a huge ( $\sim 6000^\circ$ ) first order phase correction which then results in baseline “issues”. The trick here is to right shift (that’s correct, *right shift*) the data so that the first sampled data point occurs at the proper time in the fid. In a normal right shift, the points at beginning of the fid are filled in with zeros while the points at the end of the FID are lost. However, the zero points at the beginning of the fid can also be replaced by back-predicted points based on the remainder of the fid. In SpinWorks, this is accomplished as follows:

1. For Bruker dsp filtered data (essentially all recent Bruker instruments), you *must* first convert the data from “digital” to “analogue” by using the “convdta” command in TopSpin or XwinNMR. This must be done with the Bruker software, as there is currently no equivalent function in SpinWorks.
2. For Bruker data, divide the DE value by the dwell time ( $DW = 1 / (2 * SW)$ ). In the example above,  $8 \mu s / 200 ns = 40$  points. If the number is not even, round down to the next *even whole number*. This is, in theory, the number of points to right shift. In practice, a number slightly less than this may be required due to the finite length of the pulse and propagation delays in the instrument. For the above values, we found that 38 points rather than 40 points was optimum. Enter the *negative* of this number as the **Left Shift Points** value in the processing parameter dialog. In SpinWorks, a negative left shift is a right shift.
3. Varian/Agilent data can be treated on the same way. In one example data set with a SW of 5 MHz ( $DW = 100 ns$ ) and an AD value of  $4 \mu s$  ( $RD = 0$ ) it was found that the optimum shift was 40 points ( $4 \mu s / 100 ns$ ). Note that the AD and RD parameters exist only within the “SolidsPack” library.
4. Set the linear prediction type (drop box) to **Back Complex**, and the **Pred:** value to the same number used for the right shift (*positive* this time!).
5. Set the **Coef:** value to a fairly large number, 256 would be a typical value. Values much larger than this make the calculation fairly slow.
6. Set the **Input:** value to a fairly large value but, of course, no larger the TD value. Currently, SpinWorks limits this value to 1024. If  $T_2$  is very short, you may be able to get away with a fairly small value.
7. Process the data. The optimum right shift value is the one that gives the smallest first order phase correction.
8. Adjust the **Coef:** and **Input:** values, if necessary, for optimum baseline.

Thanks to Martine Monette at Bruker Canada for pointing this method out to me!

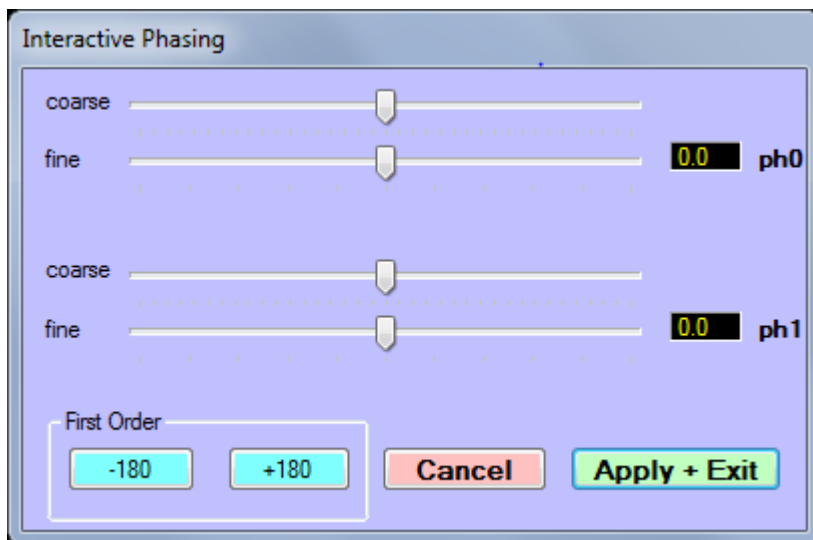
Forward linear prediction has very little application in 1D NMR, but is used extensively in the indirect dimensions of multi-dimensional NMR experiments. 1D Forward LP is included in the 1D routines of SpinWorks for instructional purposes, and for the rare time when the acquisition time must be cut short. For the use of forward LP in two-dimensional spectra, please see the 2D section of this manual.



**Figure 2.** LP parameters

## 5. Phasing Data

After processing, the resulting spectrum usually must be phased. The phasing mode is entered by clicking on the **Phase** button on the button panel.



**Figure 3.** Interactive Phasing Dialog

A phase dialog box will be displayed, and the zero-order reference point will be marked by an orange bar on the axis. Adjust the zero order phase sliders to give a properly phased upright peak at the reference mark and adjust the first order sliders to phase the peaks that are furthest from the reference line. Should a large first order correction be required, there are buttons to adjust ph1 in 180° steps. This should rarely be necessary in 1D spectroscopy, but may be useful in the F<sub>1</sub> dimension of some 2D experiments. If necessary, the spectrum may be zoomed or scrolled. When you are satisfied with the phase of the spectrum, click on the Apply and Exit button. Note that you can't edit the values displayed in the ph0 and ph1 boxes – they simply display the values determined by the sliders. If you want to manually enter phase constants, use the Edit Processing Parameters dialog (Edit menu) or the Edit Pars button.



Some spectra in JCAMP-DX format do not include the imaginary part of the data. However, these



data can still be phased by performing a Hilbert transform (Processing menu) on the data.

## 6. Baseline Correction

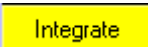
For accurate integration or for other reasons, it may be necessary to apply a baseline correction to the data. For most spectra you can simply use the Fully Automatic Baseline Correction function in the Processing menu. There are some adjustable parameters that can be found in the Edit Processing Parameters dialog, but the default values are good for most cases. The algorithm used (Whittaker smoother<sup>1</sup>) has a habit of treating very broad peaks as baseline, and large groups of closely clustered peaks may be overcorrected a tiny bit, with a slight negative baseline offset. These problems can often be corrected by **increasing** the filter Lambda value (Processing Parameter dialog). If you want to treat broad peaks as baseline (e.g. to remove probe background) then **decrease** the filter Lambda value. You may need to reduce the lambda value by a factor of 1000 or more for the removal of broad peaks.

If you need or want to define your own baseline points, click the  button on the button panel. Then use the cursor to define at least 6 baseline points in the spectrum. Click the  button to exit the BL Point mode. Then, select Baseline Correction (Least Squares) in the processing menu. A third order polynomial correction will then be applied to the data. If necessary, the default correction parameters can be changed in the Processing Parameters... dialog in the Edit menu.

The polynomial is defined such that increasing curvature is to the right or low frequency (high field) end of the spectrum. If your data has significant baseline curvature at the left end of the spectrum, simply reverse the spectrum, correct the baseline, and then reverse the spectrum to its correct orientation.

A cubic spline baseline correction is also available with the Baseline Correction (Spline) selection.

## 7. Integration

To integrate the spectrum, select the  button from the button panel. A pop-up integration dialog box will appear on the screen. The integration regions can then be selected by using the cursor to define the starting and ending points of the integral. An integral can be deleted by selecting it with a single cursor and clicking the Delete Current button in the integration dialog. The integration can be calibrated by selecting an integral with the cursor (current integral trace changes to red), entering a calibration value into the box in the integration dialog (default is 1.0), and clicking on the integration dialog Calibrate button. The integration traces can be scaled up and down independently of the spectrum scaling by using the appropriate buttons in the integration dialog. Clicking Close in the integration dialog will remove the dialog and integral traces from the screen, but integration regions are retained until cleared with the Delete All button or the program is closed. Integration regions are retained when reading a new data set. This can be useful when it is necessary to integrate a series of spectra over the same region. If this is not desired simply use the

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<sup>1</sup> P. H. C. Eilers, *Anal. Chem.* **75**, 3631 (2003); J. C. Cobas *et al.*, *J. Magn. Reson.* **183**, 145 (2006).



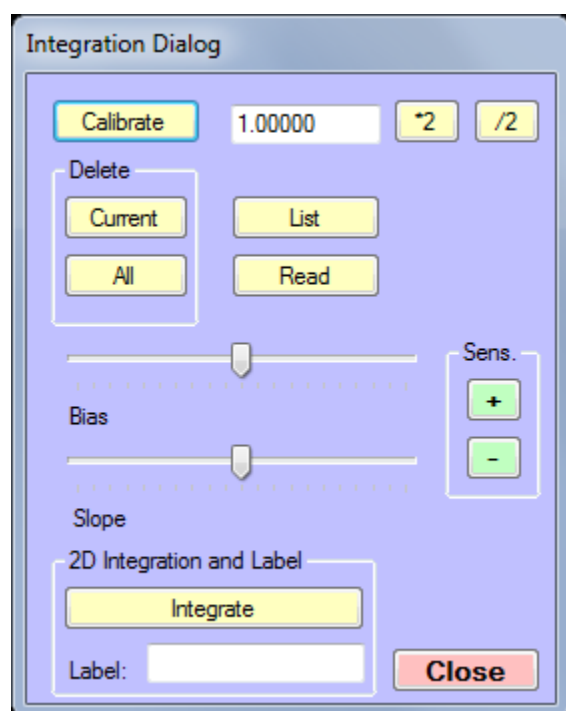
Delete All button of the integration dialog. The integration regions are saved in a file with the data on disk, and can be read in if you wish to re-process the data with the same integration regions.

It is possible to adjust the slope and bias of individual integrals, using the sliders on the integration dialog. First select the integral with a left mouse click - the integral colour will become red to indicate that this is the current integral. Then adjust the slope and bias with the sliders. The sensitivity of the sliders can be adjusted with the + and - sensitivity buttons. The integral can then be unselected by left-clicking or by selecting another integral. The integration slope and bias values are saved in the integration regions file.

However, adjusting the slope and bias on an integral can create a form of “confirmation bias” where you are adjusting the integral to the number you want, not what it actually is. Properly phased spectra with good baselines rarely need any adjustment on the integrals. Slope and bias adjustments should **not** normally be used to get “flat” integrals on overlapping peaks. Band deconvolution is much better for this.

SpinWorks 3.1 added the ability to integrate 2D spectra. After selecting a region of the 2D file to integrate, it is necessary to click the Integrate button to add the region to the integrals list. It is also possible to attach a label to the region by filling in the Label field before integrating the region. 2D integrals can be calibrated and deleted in the same fashion as 1D integrals.

The integrals can be listed (in Notepad or whatever other program is associated with a .txt file) with the List button or an equivalent selection in the Peaks and Integrals menu.



**Figure 4.** Integration dialog (1D and 2D)

## 8. Peak Picking

Peak frequencies are picked with the **Pick Peaks and Append to List** selection in the **Peaks and Integrals** menu. The minimum intensity for peak picking can be set by clicking on the **PP Minimum** button on the button panel. The desired minimum can be set by clicking in the data window. A line will then be drawn at the selected height. When the desired height has been selected, click the **Return** button on the button panel. If desired, the peak picking minimum intensity can also be set in the **Processing Parameters...** dialog in the **Edit** menu. This dialog can also be used to set the noise discrimination threshold for peak picking. Each peak pick appends to the current peak list. This enables one to pick certain regions of the spectrum and omit others. The list may be cleared with the **Clear Peak List** selection of the **Peaks and Integrals** menu. Specific peaks can be deleted with the **Clear Peaks in Region** selection.

The number of digits displayed to the right of the decimal can be set from a dialog in the **Peaks and Integrals** menu.

A text file list of frequencies may be generated with the **List** selection of the **Peaks and Integrals** menu. This list may then be printed or saved to disk if desired. The **Units** selection is used to choose between Hz. and PPM (default) for the peak display and print.

The **Interpolation** selection is used to set whether the raw cursor frequency is used for the calibration and assignment routines or whether an interpolated peak frequency is used. The default is **ON**.

The **Sign** sub-menu can be used to select whether peaks of both positive and negative intensity, only positive peaks, or only negative peaks should be displayed. The default is for both intensities to be displayed.

It is possible to manually add peaks to the peak list by clicking near a peak with the right mouse button. If a peak matching the peak pick parameters (minimum intensity and noise threshold) is found near the cursor frequency, it is added to the peak list. If no peak is found near the cursor frequency, the raw cursor frequency and intensity is added to the list. This can be very useful if you want to add broad and/or noisy peaks to the peak list.

The **Calibrate** selection is used to reference the spectrum. Select the reference peak with the cursor (a red line will be displayed) and then use the **Calibrate** selection (or the **Calibrate** button on the button panel). A dialog will then ask for the calibration frequency. The value is entered in Hz. or PPM, depending on the currently used axis unit.

For some applications (e.g. metabolomics) it is often convenient to divide the spectrum into a series of equally spaced bins, and then report the integrated, average or maximum value in that bin. This can be accomplished with the **Bin Data** selection in the **Peaks and Integrals** menu. The binned values will be listed, and can be edited or saved to a named file if desired. The binning parameters (start and stop points, bin width, binning method) can be set in the **Edit Binning Parameters...** dialog available from the **Edit** menu. The choices of binning methods are: integral (sum over points in the bin), mean value of the bin, and the maximum value in the bin.

## 9. Printing

Spectra are printed essentially as they are seen on the screen. Integrals will be printed if defined. Peak frequencies will also be printed if peak picking has been done and **Peaks and Match** in the **Options** menu is turned on (default). Printing defaults to landscape orientation. The plot orientation can then be set to either portrait or landscape via the **Page Setup...** dialog in the **File** menu. This selection remains until changed or the program is exited. Select **Print Preview...** in the **File** menu to confirm that the spectrum will print as desired. The **Print** selection or the toolbar **Print** button is then used to print the spectrum.

The **Edit Plot Options and Parameters...** selection in the **Edit** menu can be used to customize the plot. You can select objects to be printed, colours, font sizes, etc.

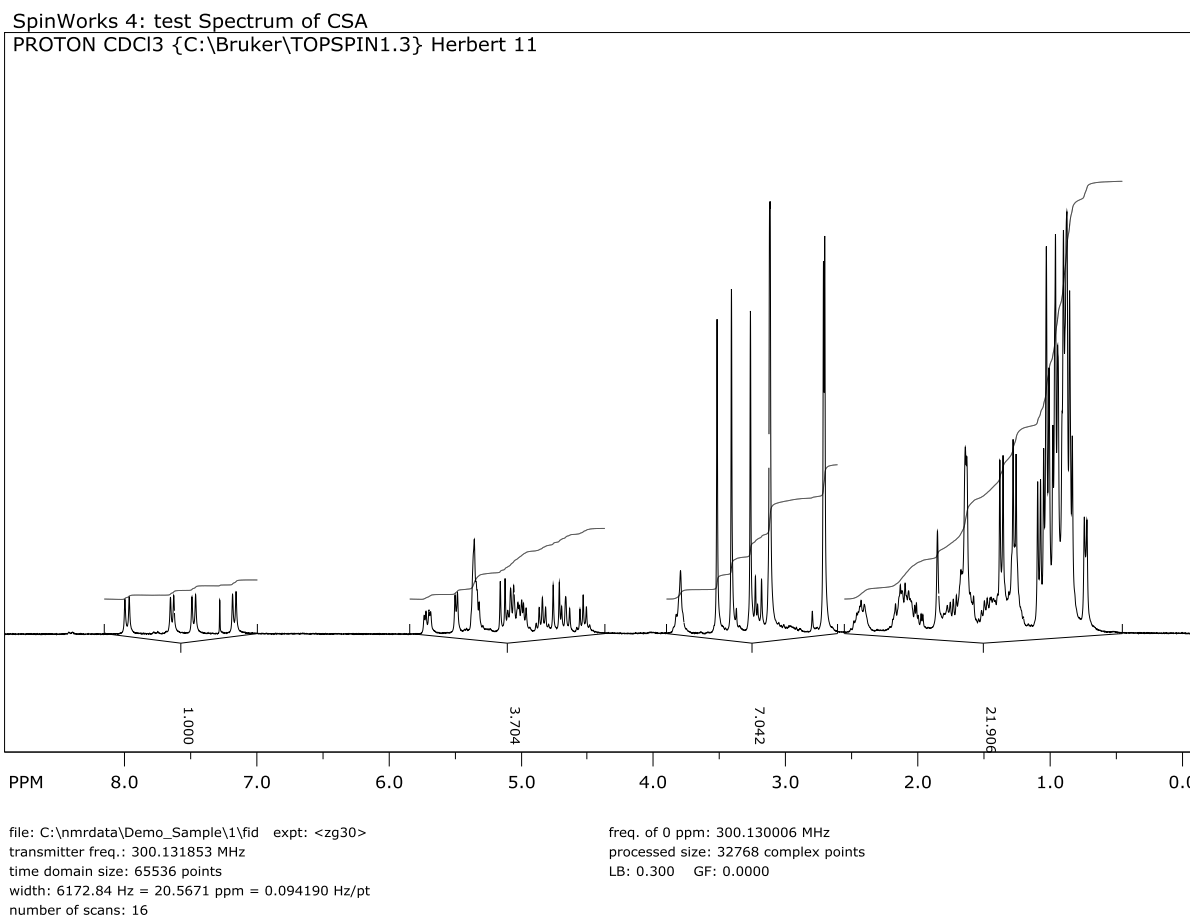
A plot title may be entered or edited with the **Edit Plot Title...** selection in the **Edit** menu. The existing plot title as defined on the spectrometer will be read, and can be edited if desired. This edited title is saved with any processed data.

## 10. Printing to a MetaFile

The **Copy to MetaFile...** command in the **Edit** menu can be used to produce a copy of the spectrum as it is set up to print as a Windows Enhanced MetaFile (\*.emf). This file can then be imported into Word (e.g. with **Insert: Picture: From File**), PowerPoint, etc. This command opens up a file save dialog, allowing you to save the file to any desired folder. The .emf extension will be automatically added to the file name. The various objects to be included in the metafile can be set with the **Edit Plot Options...** selection in the **Edit** menu.

Note that there are two options for saving to the Clipboard. The first saves in a new .NET Clipboard format, which is unfortunately not understood by many other applications. The second uses the older Win32 Clipboard format, which is fine for applications like Word and PowerPoint.

Saving to the Win32 clipboard is not allowed in the Mono/Linux version. The .NET clipboard may be supported under Linux, and printing to a Metafile works as expected in Linux.



**Figure 5.** Spectrum imported into Word.

## JEOL Data

Support for JEOL data is currently limited to 1D data in the so-called “jdf” format. This format can be identified by the “.jdf” file extension. It is recommended that a separate folder be used for each single file data set. For example, if you have a data set called brucine\_1D1H.jdf, then put it in a folder called brucine\_1D1H. All of the ancillary files created by SpinWorks (processed data, integrals, simulation files, etc.) will then remain associated with the data. (This is automatic with Bruker and Varian as these data sets are folders to begin with.)

Recent JEOL and Tecmag spectrometers seem to be using digital filters similar to those used by Bruker. That is, there is no compensation for filter group delay. These data are characterized by a “build-up” period at the beginning of the FID and spectra with extreme first order phase requirements. SpinWorks should calculate the appropriate group delay and phase correction

from parameters in the JEOL data set.

However, if this does not work, here are two ways to process these data:

1. In the interactive phasing mode click the **ph1: +180** button repeatedly and take note of the oscillation in the baseline and the phase of the peaks. You should see the frequency of the oscillation decrease and at some point the baseline will be flat and the peaks should all have nearly the same phase. Then adjust the zero and first order phases until the spectrum is phased properly.
2. The FID can also be left-shifted to compensate for the filter delay. The left shift can be set in the 1D processing parameter dialog. With the sample data that I have, a 20 point left shift was optimum.

**Note that the two methods do not produce identical results**, although both methods are acceptable. For Bruker Avance data, SpinWorks understands the characteristics of the digital filters and applies the necessary phase shift (as in method 1 above) automatically at the time of transform. SpinWorks does **not** left shift Avance data, unless you want a left shift for some other reason. Note that Bruker digital data must be converted to “analog” format (CONVDTA) in TopSpin) before left or right shifting in either SpinWorks or TopSpin.

It is also possible to specify the group delay for a non-standard digital filter by specifying Custom DSP as the F2 Detection Mode in the Edit Processing Parameter Dialog. The value for Group Delay can then be entered manually. You can use this for JEOL and TECMAG data, or for older Bruker data with a non-standard DSP firmware version. Recent Bruker data sets specify the group delay for all available digital filters, and SpinWorks automatically uses this value. Most JEOL data seems to require either a group delay of 19.96, although some may require a group delay of 15. Note that sharper filters have higher group delays. Bruker digital filters are **extremely** sharp, with group delays on the order of 70 points.

You can also correct for filter group delay with a circular left shift, although this procedure is **mathematically identical** to applying a first order phase shift. Note that circular left shifting **does not** remove the “smilies” (or “frownies”) sometimes seen with this type of data, as has been claimed by others.

There has been some discussion in various “blogs” of alternate methods of filter group delay correction, referring to these as “time correction” of the filter delay or “enhanced” correction.<sup>2</sup> While these methods do eliminate the “smilies”, they produce an overall baseline that is in fact much worse than that resulting from a simple first order phase correction. With newer Bruker systems, using the “BASEOPT” digital filter results in a spectrum with a very flat baseline with virtually no DC offset, no “smilies”, and no first order phase correction other than that resulting from the group delay correction ( $360^\circ \times \text{GRPDLY}$ ).

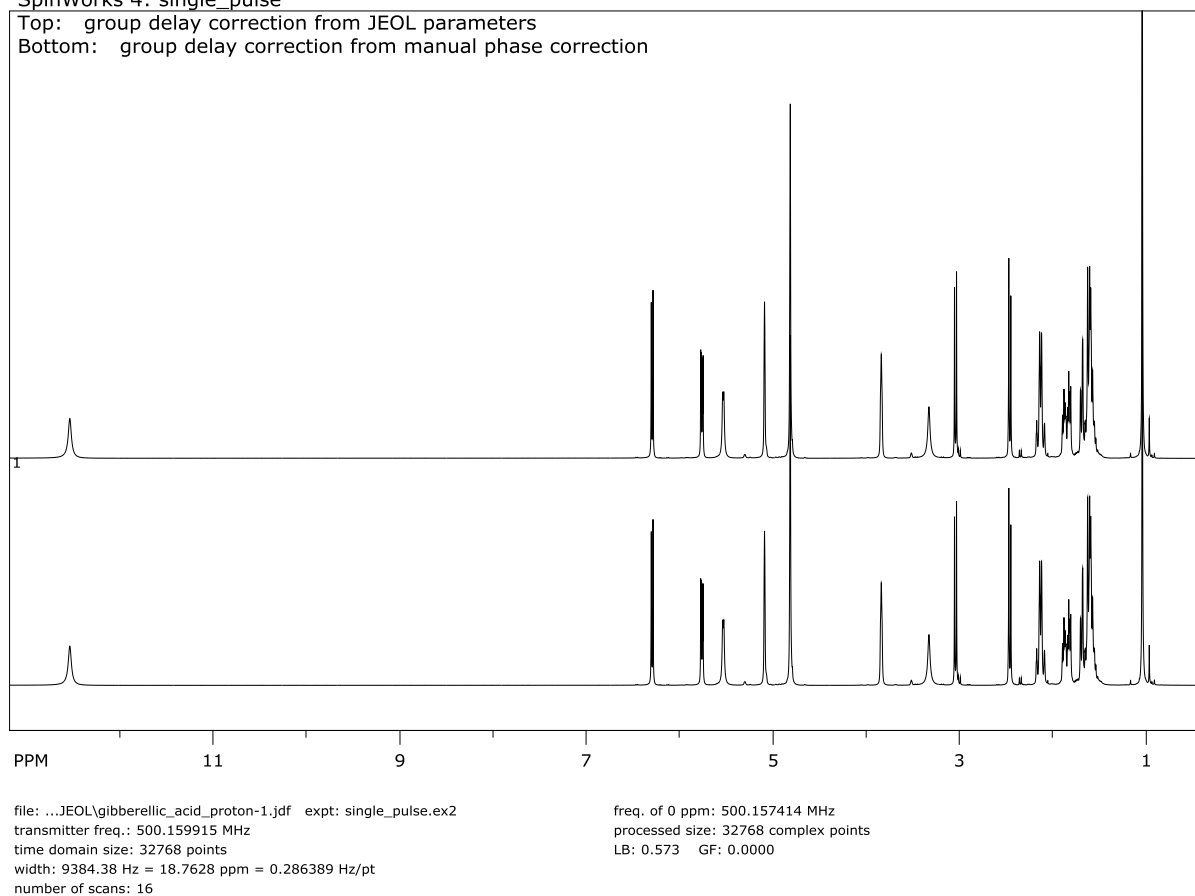
---

<sup>2</sup> Examples would include Bruker’s “CONVDTA” command and mNova’s “enhanced” group delay correction.

SpinWorks 4: single\_pulse

Top: group delay correction from JEOL parameters

Bottom: group delay correction from manual phase correction

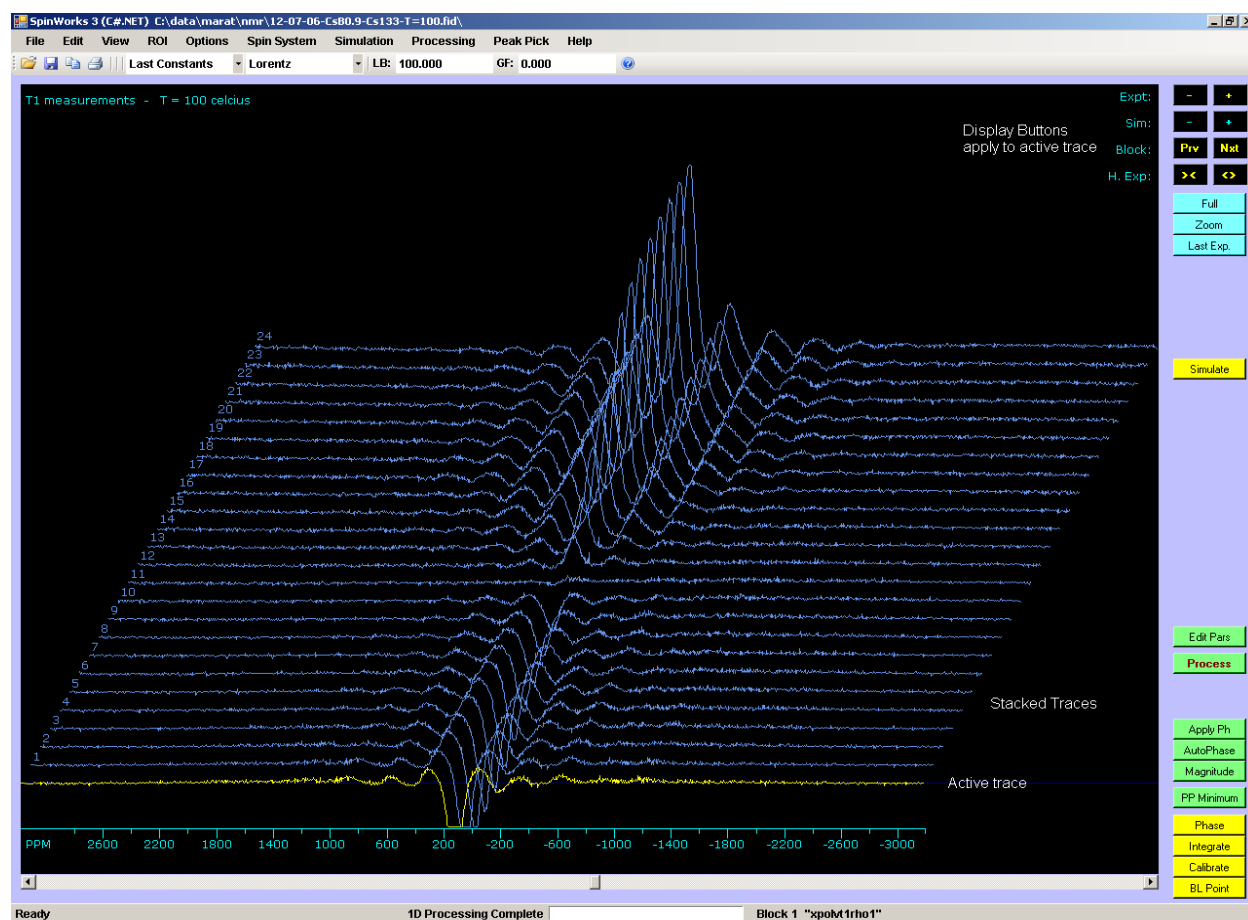


**Figure 6.** Digital filter group delay correction for JEOL data

## Processing Arrayed Data

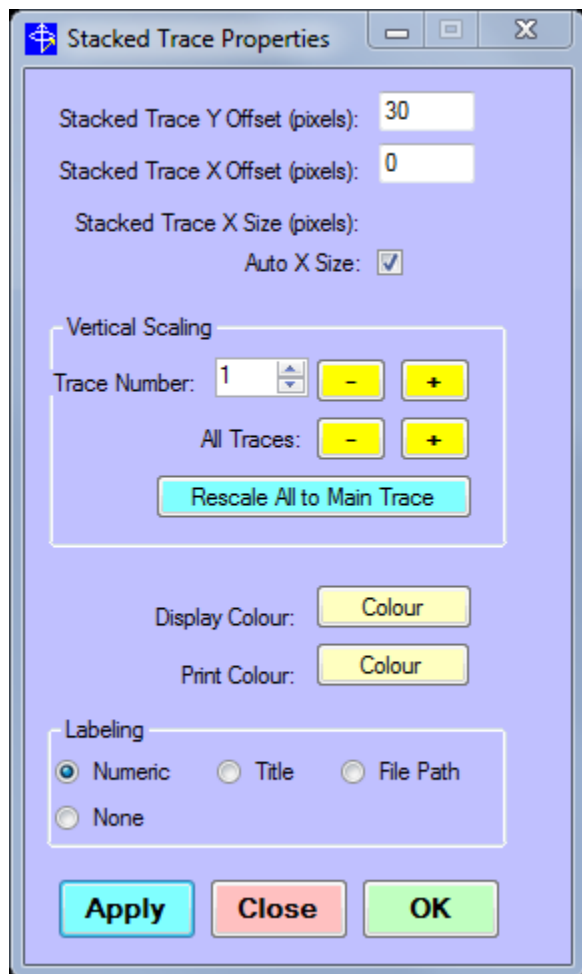
Varian and Anasazi spectrometers can array parameters and produce an FID file which is actually a collection of FID blocks. An example would be a  $T_1$  experiment where each  $\tau$  value would be a different block. To process arrayed experiments, select the fid file as you would with any other experiment. If SpinWorks replies: “This appears to be 2D data, Switch to 2D mode?” click on **No**. The first FID will be displayed, and you can process the data normally. Each subsequent block can be automatically processed and displayed by clicking on the Prv and Nxt buttons on the button panel. If the Phasing parameter (toolbar) is set to Last Constants, each block will be phased using the current phase constants.

You also process and display an entire arrayed experiment with the Process Array selection in the Processing menu. Each block will be processed and displayed in stacked display. The first block will be the first stacked trace (in blue) while the active trace (in yellow) can be any of the arrayed traces. You can select which of the stacked traces is the active trace with the Prv and Nxt buttons. *The cursor frequency and intensity, and the vertical scaling, refer to the “active” trace.* Display of active trace can be turned off by the appropriate selection in the View menu. Use the Edit Plot Options and Parameters... dialog (Edit menu) to turn off the active trace in the plot.



**Figure 7.** *Stacked trace display*

Properties such as scaling, offset and colours for the stacked traces can be adjusted with the Stacked Traces Properties dialog, available from the View menu. The Rescale All to Main Trace button resets the scaling of all of the stacked traces to be equal to that of the experimental (main) trace.



**Figure 8.** *Stacked trace dialog*

## Bruker arrayed (2D) data processing

$T_1$  and similar experiments from Bruker spectrometers are recorded as pseudo-2D experiments in a “ser” file. These experiments can be processed with the following steps:

1. Select the ser file just as you would for any other 2D file.
2. Use the Edit Pars button (button panel) to check the processing parameters. In particular, check that the window function in  $F_2$  is reasonable (Lorentz with appropriate LB parameter) and the size in  $F_1$  is bigger than the number of individual experiments or slices **but not greatly larger**. In many cases you can use the minimum size of 64. The chief problem here



is that if you inherit an excessively large F1 size from the data set, the resulting processed data will be huge.

3. Process the data with the **Process Array (Bruker)** selection in the **Processing** menu.
4. The data will then be displayed in a stacked trace fashion. The bottom (yellow) trace is called the “active trace” and all 1D display manipulation is performed on this trace. Above this is a stack (blue) of all the traces. You can make any of the stacked traces to be the active trace by using the **Prv** and **Nxt** buttons on the right hand button panel. The trace number of the active trace will be displayed in the upper left hand corner of the screen. Display of the active trace can be turned off, if desired, by unchecking the **Show Spectrum** setting in the **View** menu. Use the **Edit Plot Options and Parameters...** dialog (**Edit** menu) to turn off the active trace in the plot.
5. The offset, scaling, colour, etc. of the stacked traces can be adjusted with the **Define Stacked Trace Properties...** dialog, available from the **View** menu.
6. You have three choices for baseline correction of arrayed data. If you pick baseline points in the active trace, you can do either a polynomial or a spline correction of the entire array from the **Processing** menu (**Least Squares Baseline Correct Array** or **Spline Baseline Correct Array**). ***Remember to click the red **Return** button to exit the baseline points mode before doing the actual baseline correction.*** For certain spectra, you can also use the **Auto Baseline Correct Array** selection to baseline correct the data without defining the points. The fully automatic correction works well for spectra with sharp well-defined peaks (e.g.  $^{13}\text{C}$  spectra), and often does not do well with digitally filtered data that have a sharp curvature at each end of the spectrum.
7. You can peak pick an array of spectra by placing a single cursor on top of a peak in the active trace and then using the **Peak Pick Stacked Array** selection in the **Peak Pick** menu. If you use two cursors to define the left and right edges of a peak then this function will provide the area (integral) under the peak. This can be useful for noisy spectra or broad peaks.
8. The active trace can be integrated as any other 1D spectrum. If you change the active trace with the **Prv** or **Nxt** buttons the integrals will reflect the new active trace. After you have defined the integral regions in the active trace you can obtain a list of the relative integrals in all of the traces with the **Integrate Stacked Array** selection in the **Processing** menu. These values are uncalibrated (raw) integral values. The integrated values are listed in two formats: The first ordered by trace number and the second ordered by peak number.

You can also process a consecutive series of Bruker “experiment numbers” in a similar fashion with the **Process Series of ExNo (Bruker)** selection. An example would be a series of 1D NOE experiments. You can then transfer these spectra to the stack with the **Copy Series of Bruker Experiment Numbers to Stack** selection in the **View** menu.

## Deconvolution or Band Fitting

Sometimes, it is desirable to fit or “deconvolute” overlapping peaks, in order to determine position, width and intensity. SpinWorks has this feature included in the **Simulation** menu. Note that spectra to be deconvoluted should be well digitized and should have good baselines.

Use the following steps to deconvolute a set of overlapping peaks:

1. Define a set of starting values. It is usually easiest to define the starting shifts by generating a peak list with the manual peak picking by right clicking on the location of each suspected peak. You can also use the regular peak pick command, but most peaks that you would want to deconvolute don't peak pick very well.
2. Transfer this peak list to a deconvolution list with the **Copy Pick Peaks to Deconvolution List** selection in the **Simulation** menu. This will generate and display a synthetic spectrum based on the shifts and intensities of the peak list. You can edit these values or add other peaks with the **Edit Deconvolution Peak Parameters...** selection in the **Simulation** menu. At this point you will want to enter estimates for the peak widths as the initial list will have default values only. Each parameter has a check box
3. Start the **Interactive Deconvolution Panel...** from the **Simulation** menu. In this panel, individual peak parameters can be adjusted with the **+** and **-** buttons, and can be optimized with the **Optimize** buttons. The current RMS deviation between the simulated and calculated spectra is shown at the bottom of the dialog. The **Peak number** box selects which peak to optimize.
4. A global optimization of selected parameters can be performed with the **Optimize** button at the bottom of the dialog. The actual parameters to be adjusted can be selected with check boxes in the **Edit Deconvolution Peak Parameters...** dialog. Several rounds of optimization may be required for an optimum fit. The fit is optimum when the RMS deviation is no longer changing significantly. Note that the percentage of Gaussian character in the peaks cannot currently be globally optimized (it correlates very highly with the other parameters, baseline offset and phasing).
5. The **List** button will provide a tabular output of the peak information and the **Stack** button can be used to copy the individual peaks to a stacked plot.
6. The **Rescale Display** button sets the absolute scale of the simulated spectrum to be equal to that of the experimental spectrum.
7. If desired, you can drag the vertical position of either the simulated or experimental spectrum to overlay the spectra. Simply hold the left mouse button down on the zero position of either spectrum and drag it to the desired position.
8. The **Stack** button can be used to display the individual peaks as stacked traces. The properties of the stacked traces can change with the **Define Stacked Trace Properties...** dialog in the **View** menu.
9. You can use the **Copy Simulated to Stack** selection in the **View** menu to calculate the final simulated spectrum into the stack. This makes for a neater display than displaying the simulated spectrum along with the stack. You can then turn off display or printing of the simulated spectrum.
10. The **Dump** button can be used to dump the simulated spectrum as well as any stacked traces to X,Y data files. These files will be located in the same folder as the fid file.

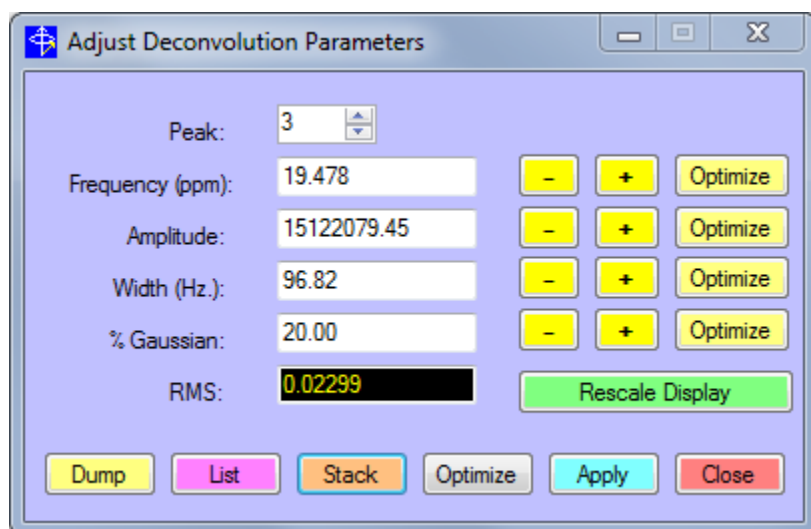
Enter or Edit Peaks for Deconvolution

	Frequency (ppm)		Amplitude		Width		% Gaussian	
1	22.640	<input checked="" type="checkbox"/>	13574180.97	<input checked="" type="checkbox"/>	101.59	<input checked="" type="checkbox"/>	20.00	<input type="checkbox"/>
2	21.519	<input checked="" type="checkbox"/>	15830361.68	<input checked="" type="checkbox"/>	110.68	<input checked="" type="checkbox"/>	20.00	<input type="checkbox"/>
3	19.478	<input checked="" type="checkbox"/>	15122079.45	<input checked="" type="checkbox"/>	96.82	<input checked="" type="checkbox"/>	20.00	<input type="checkbox"/>
4		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
5		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
6		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
7		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
8		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
9		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
10		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
11		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
12		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
13		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
14		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
15		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
16		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
17		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
18		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
19		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
20		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>

Cancel

OK

**Figure 9.** This dialog can be used to enter or edit peaks for deconvolution.

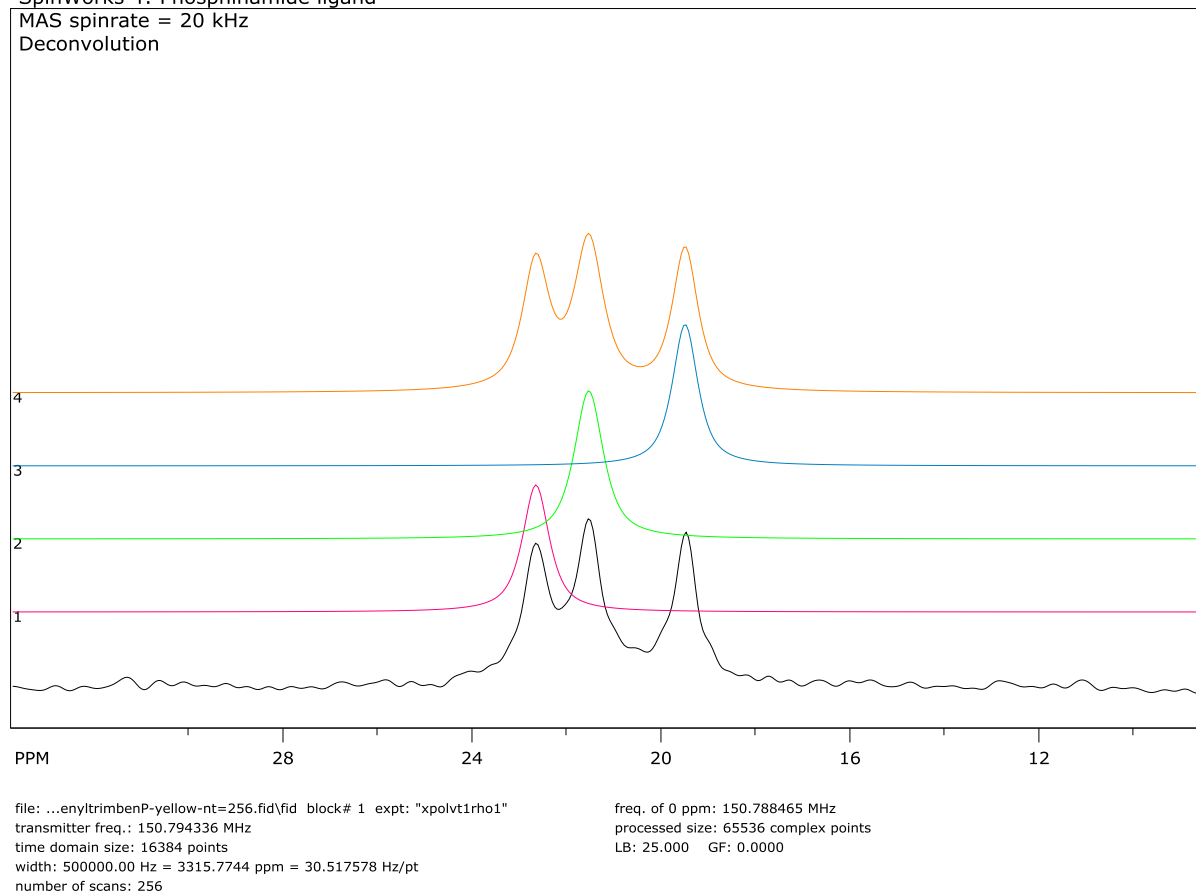


**Figure 10.** Dialog that can be used to interactively adjust the deconvolution parameters.

SpinWorks 4: Phosphinamide ligand

MAS spinrate = 20 kHz

Deconvolution



**Figure 11.** Deconvolution of Phosphinamide ligand ( $^{13}\text{C}$  MAS)

## 2D Processing

### Introduction to 2D Processing

SpinWorks has the capability to process 2D data sets from UNIX/Linux and Windows based Bruker (A<sup>+</sup>X and Avance), UNIX/Linux based Varian/Agilent (VNMRs, Inova, Unity, Mercury, Gemini), and Anasazi spectrometers. JEOL 2D is not yet supported. Provision has been made for Hypercomplex (States, States-TPPI, echo-antiecho), TPPI and Magnitude mode data. Every attempt has been made to make the 2D mode of SpinWorks as consistent as possible with the 1D mode. SpinWorks also tries to set processing parameters and modes that are appropriate for the data, resulting in very easy processing. However, inappropriate parameters (e.g. referencing, detection mode, etc.) may be inherited from the original data. In these rare cases, the parameters can be overwritten in SpinWorks.

### Selecting the 2D Data Set

SpinWorks should automatically detect Varian, Bruker and Anasazi data formats, and can distinguish between 1D and 2D data. However, should this not work for some reason, you can use the **Options** menu set the **Data Format** according to the brand of spectrometer that you are using and also set the **2D** selection.

In order to read processed data from the NMRPIPE program, select **NMRPipe Processed Data** from the **Data Format** sub-menu of the **Options** menu.

In the **File** menu, use the **Open...** command or the recent files list to select the appropriate raw spectral data. Select the **ser** file for Bruker data and the **fid** file for Varian data. If you use the **Open** command, you will have to navigate through the directory tree to the correct file. Bruker data sets have the structure: `.../exname/ex#/ser` while Varian data sets have the structure: `.../exname.fid/fid`. When you are transferring data make sure that you transfer the entire data set, as SpinWorks requires information from the various parameter sets that are stored with the raw FID data (the `procpars`, `acqus`, `procs`, `proc`, `title`, etc. files).

You can use the **Set Preferences** selection in the **Options** menu to set the default data directory for the **File Open...** dialog.

After the data set has been opened, the first FID (first  $t_1$  value) will be displayed on the screen.

### Setting the 2D Processing Parameters

SpinWorks gets most of the necessary processing parameters from the parameter files of the data set. If you want to override these, or to simply check on the parameters, you can use the **2D Processing Parameters...** selection in the **Edit** menu or the corresponding green **Edit Pars** button on the button panel. Separate panes are available for the detection ( $F_2$ ) dimension and the evolution ( $F_1$ ) dimension. The following parameters can be edited:

#### Size

The number of complex data points in each dimension. This value must be a power of 2, and will be rounded up to the nearest power of 2 if it isn't.

## Frequency of 0 ppm

The value (in MHz) used as the calibration reference point in the spectrum and is equivalent to the Bruker SF parameter. If the data were properly calibrated on the spectrometer, then this value should not need to be changed. Referencing can also be accomplished (more conveniently) with the **Cal** button on the toolbar.

## Detection Mode

For  $F_1$ , this checkbox specifies whether the data were recorded in single (magnitude or Bruker QF mode) TPPI, States, States-TPPI or echo-antiecho, mode. SpinWorks can normally determine this from the data set parameters. The sequential setting is not currently used. Note that the Varian gHSQC sequence uses echo-antiecho, and sequences that the Varian documentation specifies as States-TPPI, processes in SpinWorks as States. The necessary correction to convert the data to States format has been applied at the spectrometer. For Varian data, the  $F_1$  detection mode is determined from the “flcoef” and “phase” parameters.

The Varian gHMBCAD experiment requires echo-anti-echo.

The  $F_2$  detection mode is can be one of **Simultaneous**, **Sequential** or **DSP**, and is normally determined correctly from the acquisition parameters.

## Reverse

Check this box if you find that the  $F_1$  direction needs to be reversed. This is pulse sequence and spectrometer model dependent. SpinWorks should set this correctly for *most* data sets. An exception seems to be magnitude mode data (e.g. COSY and HMBC) from Bruker AMX spectrometers. Data from Avance series spectrometers should be O.K. Using the  $F_3$  (a.k.a. Y or decoupler B) channel for HSQC type experiments on an AMX spectrometer may also result in a reversed  $F_1$  dimension.

Reversing  $F_2$  should never be necessary.

## First Point Correction

Applies a multiplier to the first point of the FID. The default value of 0.5 is good for the vast majority of data sets. Experiment with the value if you need to remove some baseline offset in the transformed spectra. An alternative is to apply drift correction (2D Processing sub-menu) after processing.

## Bias Corr.

Determines whether a DC offset or bias correction should be applied to the FID. The default value is **OFF**, and it should only be turned on if there is a noticeable zero frequency “spike” at the centre of the spectrum.

## Window Function

This is the pre-transform apodization function applied to the data. Choices are: none, Lorentz, Lorentz to Gauss (the Bruker “GM” function), Sine, Sine Squared, TRAF, TRAFS and Gaussian. Details of the TRAF and TRAFS functions can be found in the 1D processing section. The shift parameter is the shift (in degrees) for the Sine and Sine

squared function, while the LB and GB parameters are used by the Lorenz, Lorentz to Gauss, TRAF, TRAFS and Gaussian functions. Note that the Lorentz to Gauss selection uses the LB and GB parameters, while the Gaussian Selection uses LB only.

For Bruker data, the window function and parameters specified on the spectrometer will be used. For Varian data, SpinWorks currently selects window function parameters based on the detection mode and pulse sequence name (if a standard Varian pulse sequence was used). The reason for this is that the window function definition and parameters used in SpinWorks differ somewhat than those used by VNMR. Future versions will hopefully select the window function and parameters based on the values set in the spectral parameters.

## Linear Prediction

Specifies the parameters used for linear prediction of the data set. This is typically only used in the indirect dimensions where the number of time domain data points is small. You can specify the number of points to be predicted, the number of coefficients, the number of input points used for prediction, and whether you want forward or reverse prediction. Only *forward* prediction is supported in  $F_1$ , while only *backwards* prediction is supported in  $F_2$ . These parameters may require some experimentation, but the default values are usually satisfactory. You can usually double the number of time domain data points in the indirect dimensions, and the number of coefficients will typically be 8 to 16. The number of Input Points can usually be set to the one half the number of time domain points in  $T_1$ , which is the default value. If you get what appears to be streaking in  $F_1$ , you can increase the number of input points and/or the number of coefficients. The Cutoff value can be left at the default value for most cases, but raising it can speed up the LP procedure.

The newest LP addition is the Zhu-Bax “forward-backward” algorithm (*J. Magn. Reson.* **100**, 202 (1992)). This routine seems better than the others, and can extrapolate the data further than the others. For example, extrapolation from 128 to 512 points is possible for data with a good signal to noise ratio. It is not recommended to raise the number of coefficients above 16, and the input points parameter is not used for this algorithm (the whole data set is used). Future releases of SpinWorks may have this as the standard or default LP algorithm.

**Note:** In *most* cases simply selecting Forward or Zhu-Bax and leaving all of the other parameters at their default values will provide the best results.

**Note:** Linear prediction works well when an indirect dimension has been abbreviated in order to save experiment time. *Applying linear prediction to a data set that has already decayed to the noise will not provide any improvement in resolution, and will likely result in poorer sensitivity.*

For  $F_2$  (the detection or acquisition dimension) it is *always* better to increase the resolution by increasing the acquisition time (narrowing SW or increasing TD or NP) rather than by relying on linear prediction. This does not increase the experiment time, as the increase in acquisition time can be compensated by a reduction in the relaxation delay. The only time when this may not be appropriate is when there is severe decoupler



heating during acquisition.

## Phasing

Specifies the type of phase correction to be applied to the data during the transform. Possible values are: **none** (you will phase the data interactively later) **constants** (the specified zero and first order corrections will be applied to the data during transform), **magnitude** (appropriate for the F<sub>1</sub> dimension for magnitude COSY and HMBC) and **power**. Note that applying magnitude or power spectrum processing to the detection dimension (F<sub>2</sub>) would be pretty silly.

## HOGWASH (F<sub>1</sub> only) parameters.

These are described separately in the HOGWASH section of this manual.

## Solvent Filter (F<sub>2</sub> only)

This provides a high-pass filter for removing a strong solvent signal (e.g. H<sub>2</sub>O) from the centre of the spectrum. **Gauss** or **Sine** usually work best, and the number of points determines the selectivity of the filter. A larger number of points creates a narrower filter, with the default value giving good results for typical proton 2D spectra recorded in water. However, you should experiment to achieve the best result. Note that solvent filtering is purely cosmetic and does nothing to actually improve the dynamic range or baseline of the original data.

## F1 Ref Freq (F<sub>1</sub> only)

Specifies the frequency to be used for Hz to ppm conversion in the F<sub>1</sub> dimension. Buttons are available for the Observe transmitter, Decoupler or Decoupler 2. The frequency can also be entered manually. These buttons are most often used to correct bad “refsource1” or “refsource2” parameters in Varian data sets.

**Edit Processing Parameters**

F2 (Detection) X    F1 (Evolution) Y

Size: 1024

Freq. of 0 PPM: 500.1300000 MHz.

First Point Corr.: 0.5000

FID Bias Correction: ☐

Reverse: ☒

Detection Mode: DSP

Group Delay:

Aquisition Seq. (3D):

Left Shift Points: 0

Circular Left Shift Points: 0

Peak Pick Minimum: 0.100

Peak Pick Sensitivity: 0.020

Baseline Poly. Degree: 5

Phasing

Constants

Zero: -111.14    First: 2.21

Window Function

Sine Squared

LB: 1.000 Hz.    GF: 0.0000

Sine Shift: 90.0 deg.

Solvent Filter

No Filter    Points: 19

Note: A larger number of points creates a narrower filter

Special Cases

Virtual Spectrometer Freq.: 500.132028 MHz.

Automatic Baseline

Filter Width: 2    Lambda: 10000000

Threshold: 1.500

Linear Prediction

LP Off

Coef: 16

Input: 64    Pred: 0

Cutoff: 0.0100

Cancel    OK

**Figure 12.**  $F_2$  (and 1D) processing parameter page

**Edit Processing Parameters**

**F2 (Detection) X** **F1 (Evolution) Y**

Size: 1024

Freq. of 0 PPM: 125.7577882 MHz.

First Point Corr.: 0.5000

FID Bias Correction: ☐

Reverse: ☐

Detection Mode: Echo-Antiecho

**Special Cases**

F1 Reference Frequency: 125.76716817 MHz.

F1 Spectral Width: 20831.9800 Hz.

Observe Decoupler Decoupler2

**Window Function**

Sine Squared

LB: 0.300 Hz. GF: 0.1000

Sine Shift: 90.0 deg.

**HOGWASH Parameters**

Gain: 0.10000 Thresh: 0.05000

Mask Width: 256 Pts.

Recon. Linewidth: 10.000 Hz.

Recon. Lineshape: Gaussian

**Automatic Baseline**

Filter Width: 2 Lambda: 10000000

Threshold: 1.500

**Linear Prediction**

LP Off

Coef: 16

Input: 128 Pred: 256

Cutoff: 0.0100

**Phasing**

None

Zero: 0.00 First: 0.00

Cancel OK

*Figure 13. 2D  $F_1$  processing parameter page*

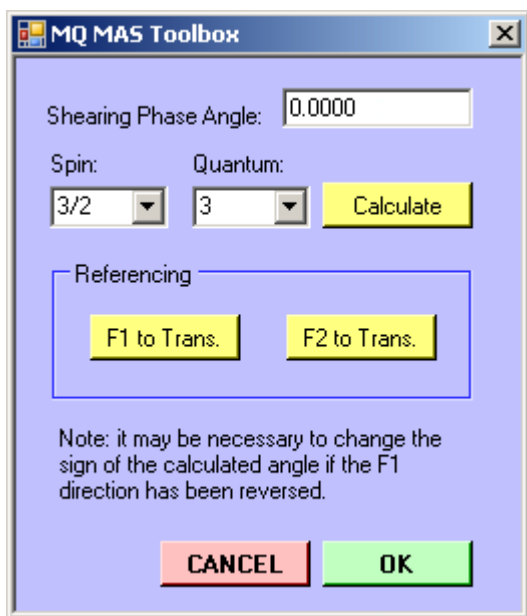
## Processing 2D Data

### Transform

Once you have selected your 2D data and have set the processing parameters, you will need to process it. In 2D mode the green **Process** buttons on the button panel will process the data as specified by the current processing parameters. Although you can process the  $F_2$  and  $F_1$  dimensions separately, it is usually easiest to click in the **Proc. Both** button to do a complete

transform of the data. For 2D data sets that do not require phasing (e.g. COSY, HMBC and HETCOR), clicking the **Both** button may be the only processing that the data set requires. This will be true if appropriate window functions and other processing parameters were set up correctly at the time of acquisition. (For experiments run with IconNMR or with the standard parameter sets, this will always be so.)

2D MQ MAS spectra require a special type of transform called a “shearing transform,” in which a  $T_1$  dependent first order phase correction is applied to each row of the spectrum after the first ( $T_2$ ) transform. The phase constant or “shearing angle” depends on the spin of the nucleus and the quantum selection of the experiment. The angle can be calculated with the MQ MAS Toolbox... dialog available in the 2D Processing sub-menu of the Processing menu. A particular angle can also be entered, if desired.



**Figure 14.** MQMAS shearing transform dialog

The referencing buttons adjust the referencing so that each of the dimensions can be referenced to the transmitter, if desired. The **Calculate** button is used to calculate the required shearing angle after the **Spin** and **Quantum** values have been set.

The shearing transform for MQMAS data has only been tested on Varian data. Testing with Bruker data is still required.

Note that the scaling of the  $F_1$  axis retains the scaling of the original unsheared data. There are several conventions for the scaling and referencing of MQMAS data, and there does not seem to be a consensus as to which one is best. Consult:

Y. Millot and P. P. Man, *Solid State Nuclear Magnetic Resonance*, **21** 21-43 (2002) and other solid state NMR literature for a detailed discussion.

Some other less frequently used 2D processing commands are also found in the 2D Processing sub menu of the Processing menu.

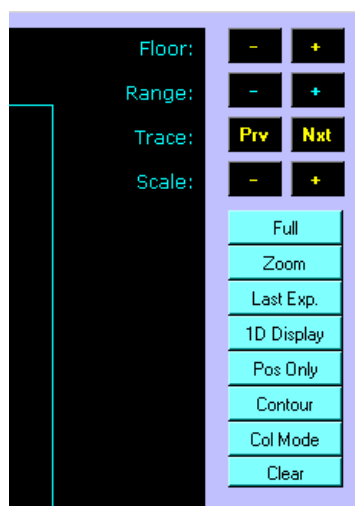
## 2D Display

The default mode for the 2D display is “image” mode where both the positive and negative intensities will be displayed. For magnitude data and phased phase sensitive data, a **Pos Only** mode can be selected from the Button Panel. It is also possible to display the data in a “contour” mode and to select coarse or fine contour spacing. The lowest displayed contour and the highest displayed contour can be adjusted with the **floor** and **range** buttons. Note that **-** means moving the **floor** level down, or closer to the noise. Therefore click the **-** button to see smaller peaks. Similarly, moving the **range** up or down will control the number of levels displayed. As a starting point, set the floor just above the noise (or artifact) level and adjust the **range** so that the highest peaks are displayed in the most intense colours. For spectra with very large dynamic range, lowering the **range** even further will give more contours on the smaller peaks at the expense of the more intense peaks. The normal contour level spacing is logarithmic, but may be changed to linear spacing with the appropriate selection in the **View** menu.

Note that buttons that toggle between modes display what the **action** of the button will be, not the current state. For example, if the button is labeled **Contour**, clicking it will switch *from* image mode *to* contour mode.

In image mode, all levels below the “floor” will be black, while all levels above the range or ceiling will be displayed in the colour of the highest level.

The “image” display mode is considerably faster than the contour mode. Printing is always done in contour mode, irrespective of the display setting.



**Figure 15.** 2D display buttons

Clicking the left mouse button with the cursor in the data area will cause a set of red cross-hair lines to be drawn. Clicking again will cause a second set of lines to be drawn, and will thus define a region. This region can be zoomed by clicking the middle mouse button (if available) or the blue **Zoom** button. The cross-hair lines can be erased by clicking the left mouse button a third time.

Individual rows or columns of the 2D matrix can be selected and displayed with clicks of the right mouse button. The selection of **Row Mode** or **Col Mode** determines which will be displayed. Switching to **1D Display** mode will transfer the last highlighted row to the 1D display. You can examine and plot individual 2D rows in this manner, using all of the 1D tools of SpinWorks. You can select which of the rows is highlighted (in yellow) by selecting it with the toolbar up and down arrows. Currently (version 3.0), columns cannot be transferred to the 1D display mode. However, columns can be saved as JCAMP-DX files (see below), and then read as 1D spectra.

Rows or columns selected with the right mouse button can be saved as JCAMP\_DX files by using the **Save Displayed Columns** or **Save Displayed Rows** selections in the **File** menu. These spectra will be saved with names like row482.dx in the folder containing the folder containing the current raw data. The titles in these files will have the chemical shift of the extracted row or column attached. These rows and columns can then be read by SpinWorks in 1D mode, *but be sure to set the data type to JCAMP-DX first.*

When extracted rows or columns are displayed on the screen, one will be highlighted in yellow, while the others will be displayed in blue-green. The highlighted trace is one to which vertical amplitude scaling will be applied (**Scale: +** and **-** buttons). The currently active trace can be changed with the button panel **Prv** and **Nxt** buttons.

**Hint:** If you have sufficient memory in your computer, you can start a *second* (or third) copy of SpinWorks for examination and printing of the extracted rows and columns. This allows you to keep the 2D data in the first copy.

A cross-hairs cursor can be displayed on the screen by using the **tracking cursor** selection in the **View** menu. You can use the keyboard “t” key as a shortcut to toggle the cross-hairs on or off. “Double clicking” will also toggle the cross-hair cursor. Spectra can be displayed and printed with the F<sub>1</sub> axis horizontal by using the **Rotate 2D** selection in the **View** menu. Note that interactive phasing requires that F<sub>2</sub> axis be horizontal. Spectra can be rotated to the desired format after phasing, of course.

## Phasing

2D data may be phased in several ways:

- Phase constants set in the **2D Processing Parameters** dialog (**Edit** menu) can be applied at the time of transform. If the data have been previously phased with XwinNMR or VNMR, the phase constants read in with the data should work in SpinWorks.
- Phase constants set in the **2D Processing Parameters** dialog (**Edit** menu) can be applied after the transform. This is accomplished with the **F2 (det.)** **F1 (evol.)** or **Magnitude** buttons in the **Phasing** section of the **2D Processing** dialog.
- The first increment FID can be transformed and phased to give the F<sub>2</sub> (acquisition

dimension) phase constants. Note that this method will not work for experiments that have zero intensity in the first increment. For example, some phase sensitive COSY experiments.

- The 2D spectra can also be phased interactively. The method is essentially the same as used in 1D mode. Note that the spectra must be in the normal (to me) “F<sub>2</sub> horizontal” mode for interactive phasing. Use the View menu to turn the Rotate 2D option off if necessary. Spectra can be rotated to the “F<sub>1</sub> horizontal” mode after phasing, if desired. To phase spectra interactively:
  1. Select either the row mode or column mode in from the button panel.
  2. Select rows or columns that contain peaks in diverse regions of the spectrum by clicking on the peak with the right mouse button. The extracted row will be displayed on the screen. The amplitude of the extracted traces can be changed with the **Scale: +** or **–** buttons on the button panel. The highlighted trace only will be changed. Which trace is highlighted can be changed with the **Prv** and **Nxt** buttons.
  3. Select the peak to be used as the pivot point (the point where the first order phase is always zero) by marking it with the left mouse button.
  4. Use the phase button on the toolbar to start the interactive phasing routine. Adjust the zero order phase (ph0) on the selected pivot peak (it should be marked with a green line) and adjust the first order phase (ph1) on the other peaks. Note that in column mode, positive is to the right. When satisfied with the phase, click on the **Apply** and **Exit** button.
  5. Now phase the other dimension, if required.

Note that most Varian pulse sequences are written so that the F<sub>1</sub> phase correction is zero (or very close to zero). In such cases no F<sub>1</sub> phase correction is usually required. However, due to differences in processing algorithms, *some* (usually echo-antiecho) data that have zero F<sub>1</sub> phase correction in VNMR may require exactly a 90 degree correction in SpinWorks. SpinWorks can recognize some of these sequences and set the phasing constant appropriately.

Many Bruker experiments calculate the F<sub>1</sub> phasing based on the delays and pulse lengths in the sequence. These phase constants will work in SpinWorks with the F<sub>1</sub> phasing set to **Constants**.

For NMRPIPE data the imaginary parts of the spectra are not normally stored with the data. In this case, use the F<sub>2</sub> and F<sub>1</sub> Hilbert transform function available in the **2D** sub-menu of the **Processing** menu.

### Baseline Correction

2D spectra in organic solvents rarely require baseline correction. However, if baseline correction is required, it can be applied to either dimension with the **Baseline Correct F2** or **Baseline Correct F1** selections of the **2D Processing** sub-menu of the **Processing** menu. A Whittaker smoother algorithm is used. The default parameters are usually good, but can be adjusted in the processing parameter dialog if necessary.

If the baseline is offset or sloped, but not very curved, it may be better to use “drift correction” instead of baseline correction. This corrects any slope and bias in the baseline, and will be

familiar to VNMR users. The drift correction commands are also in the 2D Processing sub-menu of the Processing menu.

Note that the first point correction value (2D Processing Parameters dialog) will also affect the DC offset of the spectrum.

### **F<sub>2</sub> and F<sub>1</sub> Reference Spectra**

Processed 1D reference spectra can be displayed and printed along the F<sub>2</sub> and F<sub>1</sub> dimensions.

- Process the 1D data normally and save the processed spectra as JCAMP-DX files (File menu).
- After processing your 2D data, use the Read F1 Trace... or Read F2 Trace... buttons in the File menu to read in the JCAMP-DX files as "projections."
- The vertical scaling can be adjusted by clicking near the 1D projection and using the Scale: + and - buttons, and the individual traces can be turned on or off with the drop-down menus on the tool-bar. The F<sub>1</sub> and F<sub>2</sub> reference spectra can also be scaled by placing the cursor near the appropriate 1D spectrum and using the mouse wheel (if you have one).
- Note that the 1D reference spectra do *not* have to have been recorded with the same SW as the 2D spectrum. They should normally be recorded at the same field. However, there is now a Convert Field function (Processing menu) which can be used if you want to use a 1D reference spectrum recorded at a different field.

### **Projections**

As an alternative to displaying external 1D spectra along the edges of a 2D spectrum, it is also possible to display projections of the 2D data. This can be very useful for a dimension where a 1D reference spectrum cannot be recorded. An example would be the <sup>13</sup>C dimension of HSQC, HMQC and HMBC type experiments. For these experiments, data can often be obtained for samples that are too dilute for conventional <sup>13</sup>C spectroscopy. The projections can be calculated and displayed with the F1 and F2 trace drop-down boxes on the toolbar. The projections can be scaled by placing the cursor near the appropriate 1D spectrum and using the mouse wheel (if you have one), or by clicking near the projections and using the Scale: + and - buttons on the button panel. The projections can also be calculated with the appropriate selection in the Processing Menu (2D sub-menu).

When the projections are generated, copies are also saved in the current data folder as JCAMP-DX files. These have the names f1\_proj.dx and f2\_proj.dx, and can be read by SpinWorks in 1D (JCAMP-DX) mode. They can then be peak picked, printed, etc. just as for normal 1D spectra.

### **F<sub>2</sub> and F<sub>1</sub> Traces (Scanning)**

Interactive scanning of F<sub>2</sub> and F<sub>1</sub> traces can be displayed by clicking the F2 Scan and/or the F1 Scan check boxes. The cross-hair cursor will also be turned on, in order to make it easier to see where the trace is being extracted.

### **F<sub>1</sub> Referencing**

In a perfect world, spectrometer manufacturers would provide parameter sets for all of the common 2D sequences with correct referencing information in F<sub>1</sub>. Failing this, the facility staff



should set up the referencing information. Alas, the real world seems to be quite different. Bruker data recorded with standard parameter sets, especially if recorded with ICONNMR, seem to be reasonably good. Varian data sets seem to be much poorer, especially for indirectly detected heteronuclear correlation experiments (HSQC, HMBC, etc.) Homegrown parameter sets may or may not have correct referencing information.

A problem that often occurs with Varian data is an incorrect “refsource1” parameter. This specifies the channel that is used for Hz to ppm conversion in evolution dimension. For experiments like HSQC, gHSQC, gHMBC, etc. this parameter needs to be set to “dfrq”, but quite often is set to “sfrq”. Likewise, some BioPack sequences such as gNhmqc have “refsource1” set to “sfrq” whereas in this case it should usually be “dfrq2”. To correct problems with F<sub>1</sub> channel selection, the F1(evolution) panel of 2D Processing Parameters dialog has buttons to set the channel used for Hz to ppm conversion in F<sub>1</sub>. In the Special Cases group box, there are buttons to set the F<sub>1</sub> channel to the observer transmitter, the decoupler or the second decoupler. The frequency can also be entered manually into the edit box, but this should rarely, if ever, be required.

Once it is certain that F<sub>1</sub> is using the correct frequency for Hz to ppm calculations, it may be necessary to set the actual reference position. With properly edited parameter sets (on the spectrometer end) this value is usually pretty good, but is rarely perfect due to differences in concentration, temperature, solvent, etc. There are several ways that calibration of the indirect (F<sub>1</sub>) dimension can be accomplished:

1. If the shift of a peak is known (usually from a 1D spectrum) then the yellow **Calibrate** button (button panel) can be used to set a peak to a known shift value. Simply select the desired peak with the cursor, click the **Calibrate** button and then enter the correct shifts in the dialog box.
2. If the absolute frequency of 0 ppm is known, it can be entered into the appropriate box in the F<sub>1</sub> page of the 2D Processing Parameters dialog. This value can usually be seen on 1D plots produced by SpinWorks, XwinNMR or TopSpin (SF parameter). If this value is not available for your particular sample, (often because you haven't run a carbon spectrum) then the value from another sample in the same solvent at the same temperature will be very close.
3. An automatic referencing of F<sub>1</sub> for many solvents can be accomplished using the **Auto Reference F1** sub menu in the Processing menu. Select the F<sub>1</sub> nucleus and solvent appropriate to your data. This procedure assumes that the F<sub>2</sub> dimension is proton, and has been correctly referenced. The reference values used are valid for dilute solutions at 25°C. The homonuclear option will set the referencing in F<sub>1</sub> to be the same as that in F<sub>2</sub>.

Note that even if the referencing in the 2D spectrum is identical to that in the 1D spectrum, there may not be perfect alignment between the two in some experiments. There are two reasons for this. In the F<sub>2</sub> dimension of an HSQC or HMBC type experiment (or F<sub>1</sub> of a HETCOR or COLOC experiment), the observed signals are due to the <sup>13</sup>C isotopomer, while what is observed in the 1D proton experiment is the <sup>12</sup>C isotopomer. There is an isotope shift between the two, which becomes more noticeable at higher fields. The second reason for a misregistration of peaks between 1D and 2D experiments is due to the sample heating which may occur from X nucleus decoupling. This effect is most noticeable in solvents where the lock shift is temperature dependent, usually D<sub>2</sub>O. Adequate cooling air and newer low power (adiabatic) decoupling

sequences can reduce this effect.

*Remember, though, that with decent parameter sets on the spectrometer, manual adjustment of the  $F_1$  referencing should only be required in special circumstances. E.g. different temperature or pH, odd solvent, etc.*

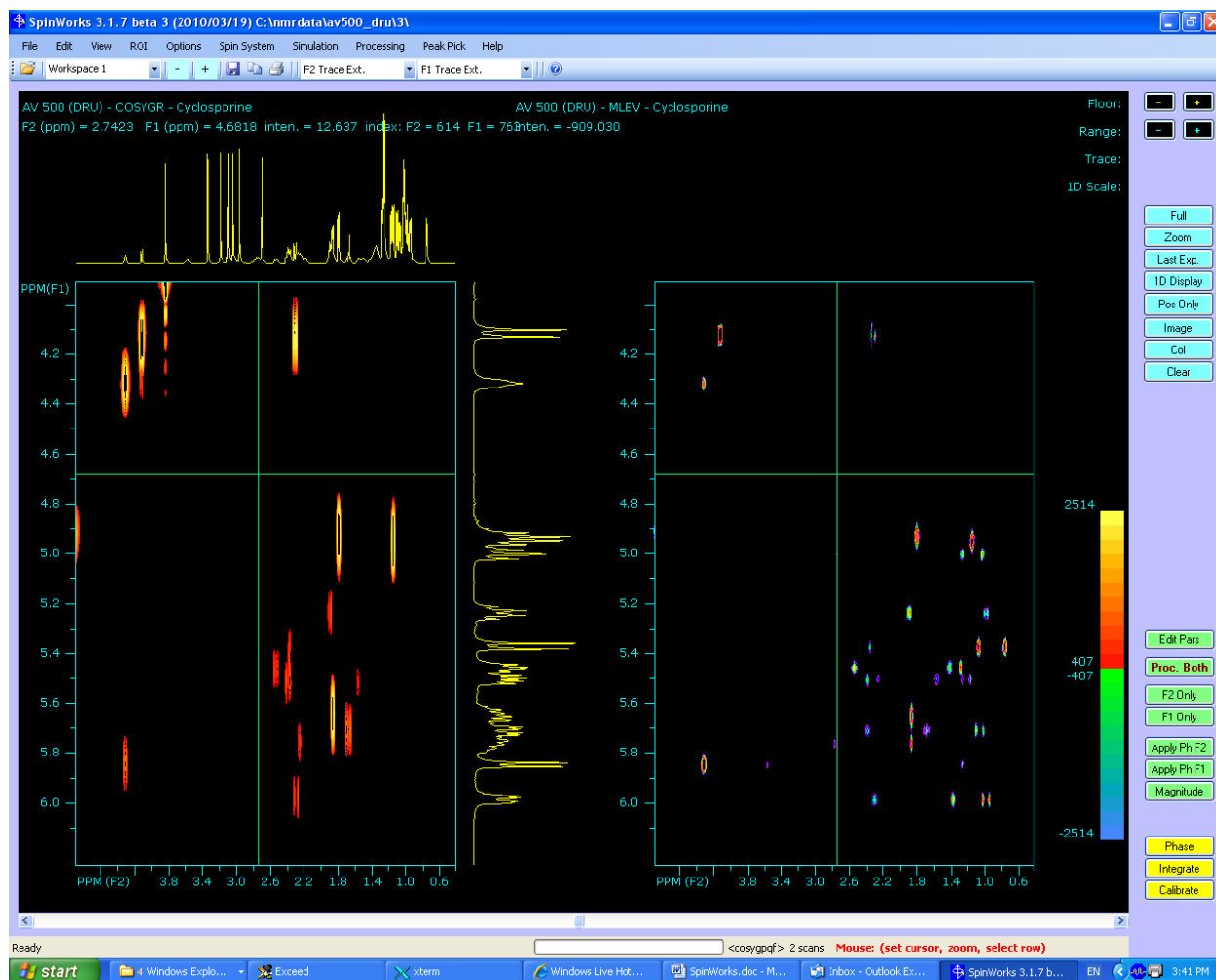
## 2D Integration

2D Spectra can be integrated with the same dialog used for 1D integration. However, the method used to define the integrals, though, is slightly different. After defining a region to be integrated with two cross-hair cursors, it is necessary to click on the **Integrate** button to generate the integral. You can also define a label for the integral by entering text into the **Label** box *before* clicking the integrate button.

## Dual 2D Display

It is possible to display two 2D spectra in a side-by-side dual display fashion. The left hand spectrum corresponds to the current workspace, and the right hand spectrum is a processed 2D “imported” from another workspace. The two data sets do not have to have identical spectral widths or sizes, but at least part of the chemical shift ranges of the two data sets must overlap. All display manipulation and processing commands work on the current workspace. That is, the left hand data set. The display limits of the right hand data set will be automatically adjusted to match the current workspace values. The following method can be used to display two data sets:

1. Select a workspace and then select and process a 2D data set in the normal fashion.
2. Move to another workspace. Select and process another 2D data set. Set an appropriate contour threshold, colour mapping, etc.
3. Return to the original workspace of step 1.
4. In the View menu use the **Dual Display of Workspace** sub-menu to choose the workspace with the second data set. The frequency limits of this second data set will match those of the first. If you zoom or expand in the first data set the display limits of the second data set will be adjusted to match.
5. If you need to adjust the contouring levels in the second data set you must switch to the second data set, make the necessary adjustments, return to the first data set, and then use the **Dual 2D Display of Workspace** sub-menu again.

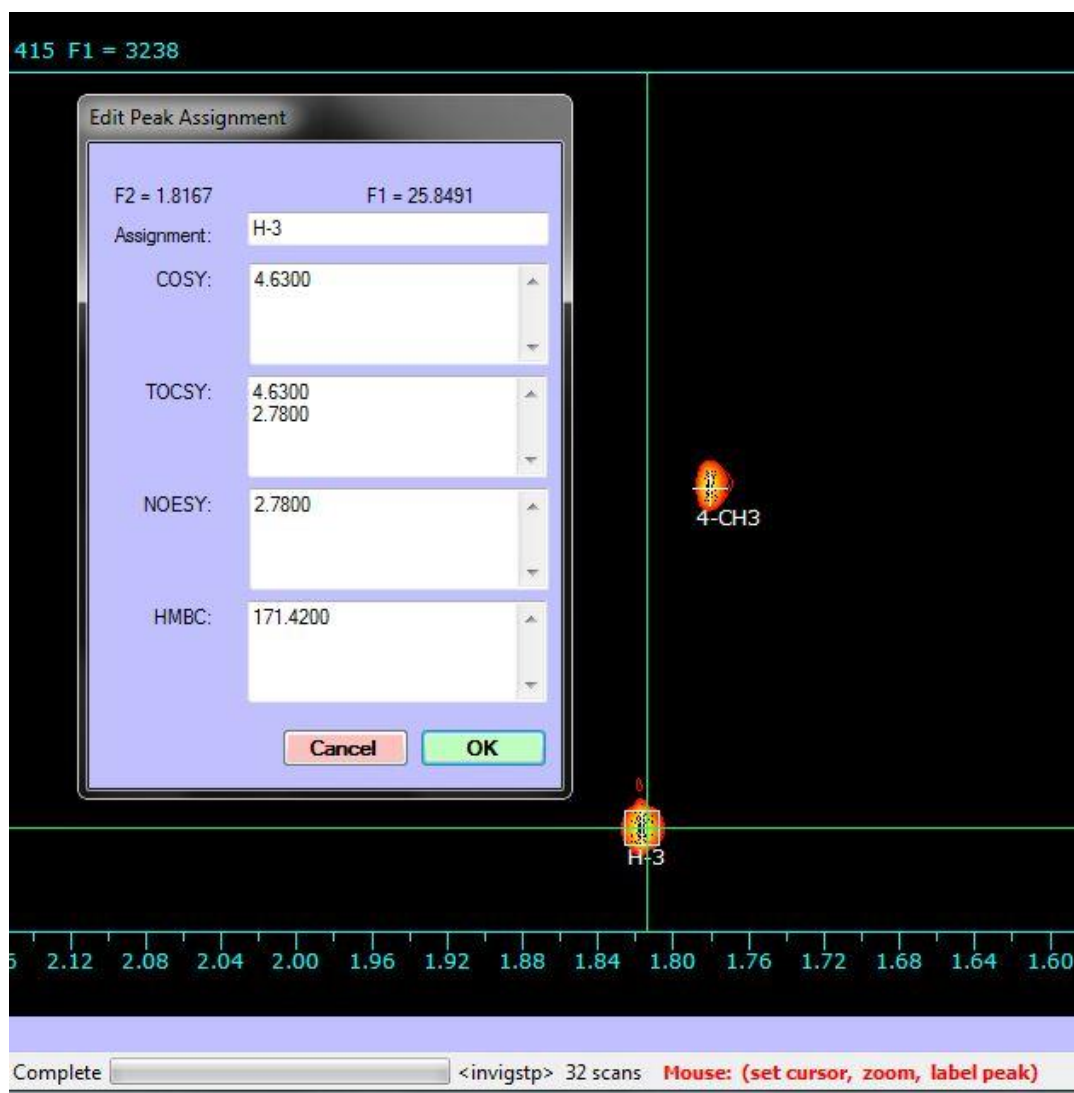


**Figure 16.** Dual 2D display of Cyclosporine A with the COSY spectrum on the left and the TOCSY spectrum on the right.

## 2D Peak Assignment Tool (under construction)

When solving a structure, it is often useful to use the HSQC or HMQC spectrum as a “worksheet” and use combined COSY, TOCSY, NOESY and HMBC in conjunction with the HSQC data in order to piece together the carbon framework of the molecule. SpinWorks has a notebook tool that allows you to keep track of these peak assignments. Although this tool would typically be used with a CH correlation (HSQC, HMQC or HETCOR) spectrum it could, in principle, be used with any 2D spectrum. The peak assignment tool is used as follows:

1. Click on the Row/Col/Label button (button panel) in order to get to the peak labeling mode.
2. The **right** mouse button can now be used to label cross-peaks. Information on COSY, TOCSY, NOESY and HMBC correlations to this peak can then be entered or edited.
3. The compiled assignments can be listed with the List function in the Peak Pick menu.



**Figure 17.** 2D Label and assignment tool.

## 2D Processing Tutorial


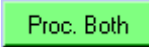

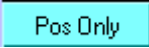

A number of 2D data sets are available for download, and contain data acquired on both Varian and Bruker spectrometers, with a number of detection modes.

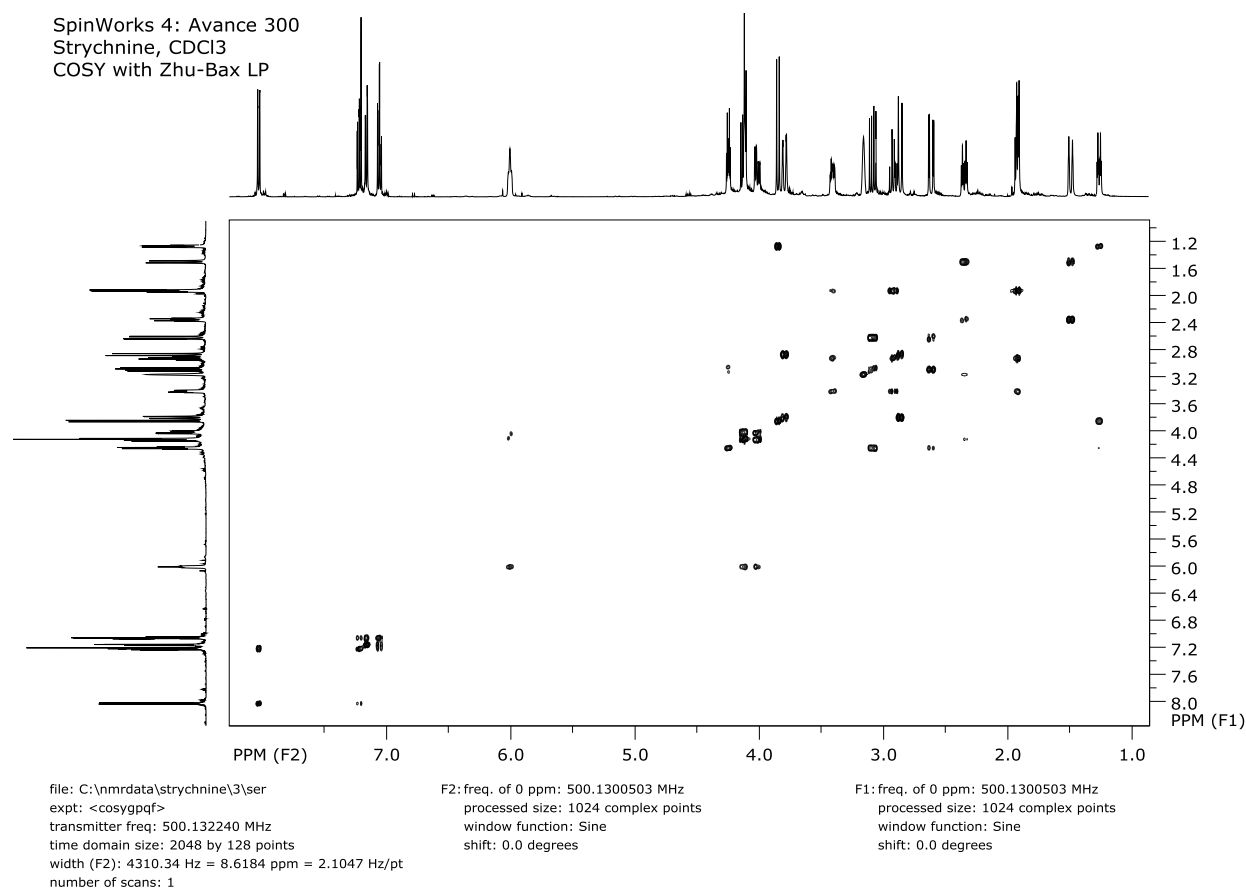
### Magnitude (COSY and HMBC type) Data

These data sets are usually processed with upshifted sine window functions in  $F_1$  and  $F_2$ , magnitude phase correction in  $F_1$  (only!) and "single" type detection mode. It should not be necessary to specify whether the data are Varian, Bruker or Anasazi as the program can identify the data format automatically. It should also automatically determine whether the data are 1D or 2D. For Varian data, a confirmation question will be asked before switching to 2D mode.

1. Use the File Open command to navigate through the supplied sample data and select either the ...gCOSY.fid/fid Varian file or the ...cosy\_qf/3/ser Bruker file. After selecting the file,

the first block (FID) of the 2D data set will be displayed. If these data sets are not available, any Bruker or Varian COSY, gCOSY or HMBC type data set could be used. Note that Varian gHMBCAD experiment, however, requires echo anti-echo as the F1 detection mode.

2. Use the Edit 2D Processing Parameters... selection in the Edit menu or  on the button panel to examine the 2D processing parameters. With the supplied sample data, these will all be correct, and are typical of what would be used for a magnitude COSY spectrum. Note that for Bruker data the processing parameters are read from the data set. If the data set was set up correctly on the spectrometer, it should process on SpinWorks without any adjustment of the processing parameters. For Varian data, the default processing parameters are based on the name of the pulse sequence, the "flcoef" parameter and the "phase" parameter. If SpinWorks recognizes the pulse sequence, it should default to reasonable parameters. If SpinWorks doesn't recognize the pulse sequence, then you may have to set some processing parameters yourself. Note that the COSY type parameters are also suitable for HMBC spectra. However, the window functions should be changed to sine or sine squared with a 45 to 90 degree shift.
3. Process the data in both dimensions by clicking the  on the button panel. The status of the transform will be displayed at the bottom of the screen, and when finished an image mode 2D spectrum will be displayed on the screen. This can be changed to contour mode by selecting  on the button panel. Note that the current contouring algorithm in SpinWorks can be a bit slow on some machines. Since this is magnitude mode data, mapping negative levels doesn't make any sense. Therefore, select the  button to display the positive contours in a more complete set of colours. The floor (lowest contour) and range (highest contour) can be changed with the appropriate buttons.
4. Clicking the left mouse button in the data window will mark the point with a set of red cross-hair cursors. Clicking the left mouse button in the data window a second time will define a region that can be zoomed with the  button or by clicking the *middle* mouse button. Clicking the left mouse button in the data window a third time will erase the cursors.
5. Use the Edit 2D Processing Parameters... selection in the Edit menu to set F<sub>1</sub> linear prediction to Zhu-Bax, the number of coefficients to 16 and the number of predicted points to 512. Exit the dialog by clicking the O.K. button. In the 2D Processing and Display dialog, click the Both button to re-process the data. You should see a significant improvement in the resolution in the F<sub>1</sub> dimension.
6. Print and Print Preview in the File menu work as expected.
7. If you wish to apply baseline correction or symmetrize the data, these functions are available in the Processing menu.



**Figure 18** COSY spectrum on an Avance 300 processed with Zhu-Bax linear prediction.

## Phase Sensitive (HSQC type) Spectra

There are several ways in which phase sensitive 2D data can be recorded. The data can be purely hypercomplex (two FIDs recorded 90° out of phase, but with the same  $t_1$  value - often known as the States method), TPPI ( $t_1$  and phase incremented at the same time), a hybrid called States-TPPI, or the echo-antiecho method, which applies to certain types of gradient experiments. SpinWorks must know how the data were recorded in order to process it correctly.

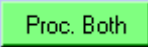






For Bruker data, this information is obtained from the processing parameters in the data set. Data recorded with standard Bruker parameter sets (e.g. in IconNMR) will *almost* always have correct processing parameters. Parameter sets created by users may, however, be incorrect. Most phase sensitive Bruker pulse sequences use TPPI, although we commonly use an echo-antiecho HSQC. The comments included with each Bruker pulse sequence usually indicate the detection mode used.

Note that the  $F_1$  phasing can be calculated, and does not change unless you change the  $F_1$  spectral width. On the supplied Bruker data, the  $F_1$  phase constants (as calculated by XwinNMR or set up in the parameter set) should do a good job of phasing  $F_1$  if you tell SpinWorks to apply phasing during the transform. (Edit 2D Processing Parameters... →  $F_1$  (evolution) → Phasing →

Constants).

It is also possible to adjust the timings in a pulse sequence so that there is no required  $F_1$  phase correction. This approach is seen in most Varian data, although a little phasing in  $F_1$  can be useful.

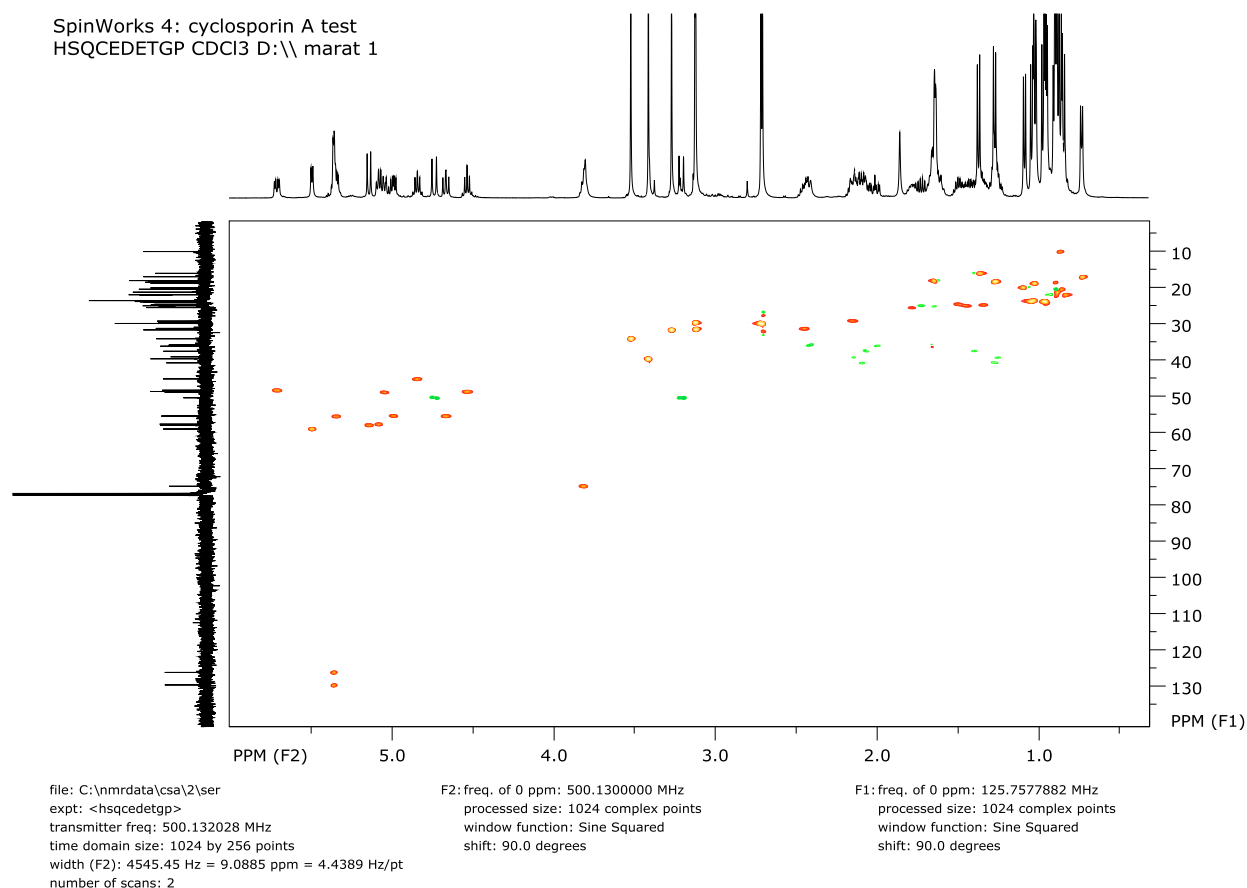
For Varian data, SpinWorks guesses the  $F_1$  detection mode from the name of the pulse sequence. If the pulse sequence name is not recognized by SpinWorks, SpinWorks then uses the “flcoef” and “phase” parameters. If the “flcoef” parameter is null or not recognized, SpinWorks will assume either COSY type or States type data, depending on the “phase” parameter. Most phase sensitive Varian pulse sequences are either hypercomplex (States) detection (e.g. HSQC, TOCSY, NOESY) or echo-antiecho (e.g. gHSQC). Some sequences in BioPack or ProteinPack are written as States-TPPI, but the data seem to be converted to pure hypercomplex (States) when the data are stored to disk. These data sets can be processed exactly like States data.

1. Use the File Open command to navigate through the supplied sample data and select either the ...HSQC.fid/fid Varian file or the ...hsqc\_av300/2/ser Bruker file. After selecting the file, the first block (fid) of the 2D data set will be displayed. If these data sets are not available, any Bruker or Varian HSQC, gHSQC or HMQC type data set should work.
2. Use the Edit 2D Processing Parameters... selection in the Edit menu to examine the 2D processing parameters. With the supplied sample data, these will all be correct, and are typical of what would be used for an HSQC spectrum. Note that for Bruker data, the processing parameters are read from the data set. If the data set was set up correctly on the spectrometer, it should process on SpinWorks without any adjustment of the processing parameters. For Varian data, the default processing parameters are based on the name of the pulse sequence. If SpinWorks recognizes the pulse sequence name, it should default to reasonable parameters. If SpinWorks doesn't recognize the pulse sequence, then you will have to set the processing parameters yourself. With some data sets, it may also be necessary to optimize the Windowing parameters for optimum signal to noise ratio.
3. Close the Edit 2D Processing Parameters... dialog and click  on the button bar to process the data in both dimensions.
4. After the processing is finished and the spectrum is displayed, select rows containing peaks with the right mouse button. Usually, two or three rows are sufficient, if they contain peaks near both ends of the spectrum. The vertical scaling of the displayed 1D traces can be adjusted with the Scale:   buttons and the   buttons.
5. Use the left mouse button to select a peak to be used as the pivot point.
6. Click the  button. Adjust the phase of 1D traces. When satisfied, click on the Apply and Exit button. The 2D spectrum with the applied  $F_2$  phase correction will now be displayed.
7. In the button panel, switch to column mode with  and do any required  $F_1$  phase correction.
8. Use the Clear button in the Rows/Columns group to remove any displayed rows or columns



and print any desired regions of the spectrum.

8. Use the **Edit 2D Processing Parameters...** selection in the **Edit** menu to set  $F_1$  linear prediction to forward, the number of coefficients to 16 and the number of predicted points to 256. Exit the dialog by clicking the **O.K.** button. In the **2D Processing and Display** dialog, click the **Both** button to re-process the data. You should see a significant improvement in the resolution in the  $F_1$  dimension.
9. Print the spectrum, if desired.



**Figure 19.** Edited phase sensitive HSQC spectrum of cyclosporine A recorded on an Avance III 500. Zhu-Bax LP has been applied in  $F_1$ .

## 3D Processing

SpinWorks 4 includes 3D Processing for Bruker and Varian/Agilent data. The data are processed and displayed as a series of 2D planes, and most of the information on 2D processing is relevant to 3D processing.



## Definitions:

1.  $F_3$  is the direct or acquisition dimension, and is also referred to as the X dimension. Currently, SpinWorks always displays this dimension along the horizontal axis.
2.  $F_2$  is an indirect dimension, also referred to as the Y dimension. For Bruker data, this is the dimension defined by the parameter set acqu2s and the delay d10. For Varian/Agilent data this will be known as the “second indirect” dimension.
3.  $F_1$  is an indirect dimension, also referred to as the Z dimension. For Bruker data this is the dimension defined by the parameter set acqu3s and the delay d0. For Varian/Agilent data this will be known as the “first indirect” dimension.

## Selecting the Data

Currently, SpinWorks cannot automatically discriminate 3D vs. 2D data, so it is necessary to set the data format to 3D *before* selecting the data. Use the Data Format selection in the Options menu. The data set can then be selected (ser or fid file). The raw data (fids) from the first block will be displayed.

## Processing 3D Data

3D data are processed as a series of 2D planes, followed by processing all of the individual columns of the third dimension. You can process the XY planes followed by the Z dimension, or the XZ planes followed by the Y dimension. Which order you do it in usually doesn't matter, with the following exception: *If one of the indirect dimensions is “echo-antiecho” (sometimes called “Rance-Kay”), that dimension must be processed first.* You can also process the first plane of either the XY or XZ dimensions. This is useful for determining phase angles and weighting parameters. All of the 3D processing commands are available in the 3D sub-menu of the Processing menu.

## Displaying the Data

The processed data are displayed as a series of 2D planes. You can display either XY planes or XZ planes. Display of YZ planes is not currently available. You can step through the planes with the 3D- and 3D+ buttons on the toolbar. The chemical shift of the currently displayed orthogonal dimension (e.g. Z if you are displaying XY planes) is shown on the status bar at the bottom of the screen.

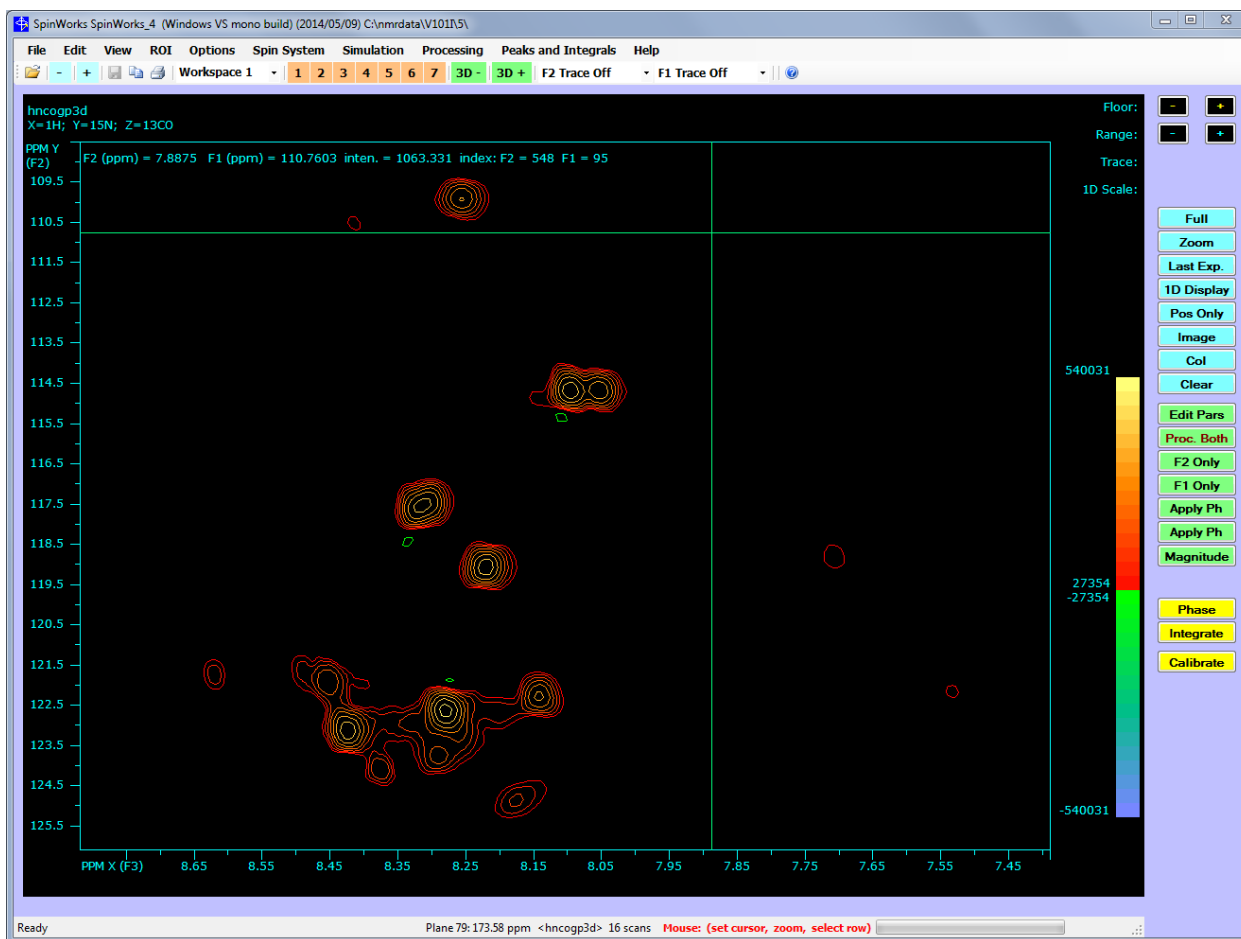
## Steps in Processing a 3D Data Set

It is assumed that you have some experience processing 1D and 2D data with SpinWorks.

1. Switch SpinWorks to 3D mode and set the data type (Varian or Bruker).
2. Select the 3D data set. You must select the ser file (Bruker) or the fid file (Varian/Agilent) the entire series of fids is read into memory, so this may take a few seconds.
3. Use either the button panel or the Edit menu to display the parameter editor. Check that the size parameters are reasonable for a 3D data set. Also check that the frequencies in the indirect dimensions are correct. These should be specified in the processing parameters for the data set, but can be wrong if the data were not processed with the

spectrometer vendor's software. Varian are especially bad in this regard, Bruker data sets seem to be much better.

4. Varian/Agilent indirect dimensions that use States detection should have the **reverse** checkbox checked. For Varian/Agilent indirect dimensions that use Echo-antiecho detection, leave the **reverse** check box unchecked, but make sure that the zero order phasing is set to  $-90^\circ$  in this dimension and that the **Phasing** mode is set to **Constants**.
5. Process either the first XY or the first XZ plane (Processing Menu: 3D). For data that are States or States-TPPI in both of the indirect dimensions, it really doesn't matter which dimension you do first. ***For data that are echo-antiecho (Rance-Kay), then you must select the plane containing the echo-anti-echo indirect dimension.*** For example, for Bruker and Varian HNCO experiments, this would be the X-Y ( $F_3$ - $F_2$ ) dimension.
6. After you have processed the first plane, phase the data as you would for any 2D data set. Normally, only X ( $F_3$ ) will require phasing. Use the parameter editor to set the **Phasing** mode in X ( $F_3$ ) to **Constants**. If you have made any phase adjustments to the indirect dimension, also set the **Phasing** mode in this dimension to **Constants**. Note that Varian (**only!**) echo-antiecho data should already have the **Phasing** mode in this dimension set to **Constants**, and should already have a zero order phase of  $-90^\circ$ . For many NH detected experiments, what you should see at this point will look like a normal NH HSQC spectrum.
7. You can process the first plane again if desired, just to be sure that the phasing is correct.
8. Now process all the planes, either XY or XZ as appropriate (Processing Menu: 3D: Process XY Planes or Process XZ Planes). This may take several minutes.
9. After all of the planes have been processed, you can step through the individual planes with the 3D- and 3D+ buttons (Toolbar). What you should see is a series of 2D spectra, where the amplitude of the cross-peaks varies with the plane number (evolution time in the third dimension). The phase in the processed dimension (XY or XZ, as the case may be) should not generally change as a function of the evolution time in the unprocessed dimension.
10. It is now necessary to process the third dimension. This will be the Z dimension if you have processed the XY planes, and the Y dimension if you have processed the XZ planes. This is done through the 3D sub-menu of the **Processing** menu. Processing the third dimension normally takes less a few minutes or less.
11. You can now step through the individual planes using the blue 3D- and 3D+ buttons on the toolbar. Since all three dimensions have been processed, you have the choice of either XY or XZ planes available in the **View** menu.
12. During 3D processing, SpinWorks discards unneeded imaginary quadrants. If necessary, in order to touch up the phase after transform, you must regenerate the imaginary quadrant using a Hilbert transform. You can do a Hilbert transform of the direct or current indirect dimension of the current plane with the appropriate selection in the 3D Processing sub-menu.



**Figure 20.** NH plane of an HNCQ experiment (AVIII 500) Processed with SpinWorks. Zhu-Bax LP has been applied in the Y and Z dimensions.

## NMRPIPE 2D and 3D Processed Data

SpinWorks can read 1D, 2D and 3D processed data from the NMRPIPE program. SpinWorks makes the following assumption about the data:

1. The processing scripts for 2D and 3D use the `-di` option in the phasing command to discard the imaginary parts of the data. For NMR Pipe 1D data you can eliminate the `-di` option, and the imaginary data will be available for phasing.
2. File names for 1D data, the filename has to have the `.ft` extension, while for 2D data the filename has to have the `.ft2` extension, and for 3D data the filename has to have the `.ft3` extension.
3. File names for 3D files have the format `*###.ft3`, where `*` is any text, `###` is the 3 digit plane number and the `.ft3` suffix (extension) identifies it as 3D data. For example `test014.ft3` would be the 14<sup>th</sup> plane (in the Z direction) of the 3D data set. The last line in the processing script would therefore look something like:

```
| pipe2xyz -out ft/test%03d.ft3 -z
```

Note that most NMRPIPE scripts either use the above conventions or can easily be modified to do so.

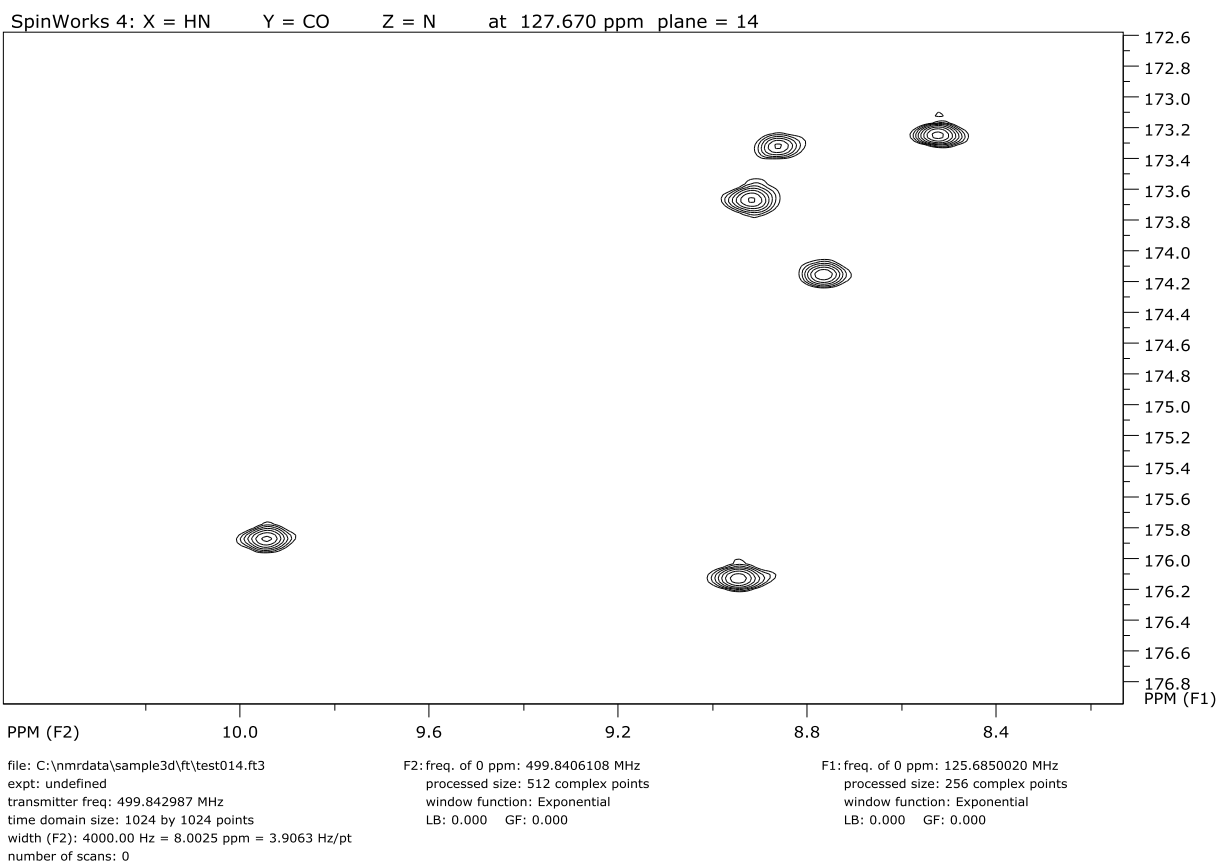
## **2D Data**

Use the Data Format sub-menu on the Options menu to set the data format to NMRPIPE. Then select the 2D processed data (usually a file with the “.ft2” extension) with the **Open** command (**File** menu or toolbar). The data can then be displayed and printed like any other 2D data. In order to phase the data, you must Hilbert transform the data first (Processing menu) first.

## **3D Data**

NMRPIPE stores 3D processed data as a series of 2D planes. Each 2D plane contains the X and Y dimensions, and the series of 2D planes is the Z dimension. After setting the data format to NMRPIPE, each of these 2D planes can be read, displayed and printed as for any other 2D data. There are two important differences with 3D data:

1. NMRPIPE produces data files with extensions .ft1, .ft2 and ft3. Only the .ft3 files (processed in all three dimensions) are valid 3D files for SpinWorks.
2. If the file name contains a 3 digit plane number (e.g. test013.ft3) it is possible to step through the individual planes with the blue **+** and **-** buttons on the toolbar.



**Figure 21.** X-Y Plane of a 3D HNCO experiment processed with NMRPipe. The Z dimension is the  $^{15}\text{N}$  Chemical shift.

## FID Math

For some experiments it may be necessary to perform non-standard mathematical operations on an FID before transform. The FID Math Toolbox (available from the Processing menu) can be used to accomplish this. More operations can be added if requested.

### Notes:

1. SpinWorks *never* (!) alters the original acquisition data. Therefore, The FID Math Toolbox will *not* allow you to save FID files with the names “fid” or “ser”. Names such as “fid1” or “ser3” are fine. For 2D files that will be read in for further processing by SpinWorks, the output names “ser1” “ser2” or “ser3” are strongly recommended. SpinWorks will recognize these names and switch modes properly.
2. Some of the operations in the toolbox operate on memory resident files, and others operate on files in the current experiment folder. Operations on files require that the files be in the correct format. The files must have the same byte order of Intel processors, and must not have any prepended file or block headers. The only native (raw spectrometer) files currently in the correct order would be FID files from Bruker spectrometers with

X32 or PC computers. However, operations exist to convert files to this format, and back into Varian format, if necessary.

3. Because of differences in the way SpinWorks processes 1D vs. 2D data, some of these operations operate slightly differently on 1D or 2D data.

For 2D data, after a file is selected (e.g. **File Open**) the acquisition data are read into memory and all processing uses this memory resident data as the input. This means that if an FID Math operation produces undesirable results, you then have to re-select the data (**File Open** or recent files list) to restore the original acquisition data to memory.

For 1D data, all processing uses the FID file on disk. That means that any FID Math operation must save the file to disk and that you must select this file to process it.

### **Operations available:**

#### **Split Hypercomplex 2D Data into Separate Files**

This operation will split the current memory resident “fid” or “ser” data into two files representing the two quadrature “channels” in  $F_1$ . The default file names are “cos” and “sin” but other names can be entered, if desired.

#### **Phase Non-Hypercomplex 2D File (From Split)**

This operation will apply a zero-order phase correction to each increment in a 2D file. A time ( $t_1$ ) dependent phase correction can be applied by using a non-zero value for the increment (incr:). The increment is the amount by which the phase is incremented for each  $t_1$  value. Note that a time dependent zero order phase shift is equivalent to a frequency shift. The file from the split (typically “sin” or “cos”) overwritten with the phase shifted data. The original fid (raw data) file is not altered.

#### **Phase Current 1D or 2D FID and Save in File**

A zero order phase correction is applied to the current memory resident 1D or 2D FID data. If the data is a hypercomplex 2D data set, the phase correction is applied to both the real and imaginary parts of  $t_1$ . A time ( $t_1$ ) dependent phase correction can be applied by using a non-zero value for the increment (incr:). The phase corrected FID file is retained in memory (if 2D), and can be written to an output file (1D or 2D) if desired.

#### **Save Current FID in Correct Format**

Saves the current fid data to disk in the correct format for the FID Math functions. The file will have the byte order of the Intel processor and will have no file or block header information.

#### **Comb. FIDs in $t_1$**

This function can be used to reassemble two split FID files into a hypercomplex file for processing. The multiplier values can be used to scale the individual FIDs, but these values will typically be 1 or -1.

#### **Add/Subt. FIDs**

Produces a linear combination of two 1D or 2D FID files. The files can be hypercomplex

or split. Setting the multipliers to 1 will add the files, will setting one of the multipliers to -1 will produce a difference file.

## Multiply FIDs

Not yet implemented.

**Figure 22.** *FID math dialog*

## Trivial FID Math Example

1. Select a 2D experiment that has been recorded with States or States-TPPI detection in F<sub>1</sub>.
2. Use the **Split Hypercomplex...** function to split the 2D data sets into 2 separate files, called “cos” and “sin”. These represent the real and imaginary parts in T<sub>1</sub>.
3. In the **Phase Non-Hypercomplex 2D file...** box give “sin” as the file name and use a value of 180 for the ph0 value. Click on the **phase FID** button.
4. In the Bottom Row (under **Comb. FIDs in t1**) use “cos” and “sin” as the file names, leave the multipliers at 1.0, and leave the output file name as “ser1”. Click on the **Comb. FIDs in t1** button.
5. Use **File Open** to select the “ser1” file. Process the data. Notice that the F<sub>1</sub> direction is reversed? This is one way of reversing the F<sub>1</sub> direction of a 2D data set. In SpinWorks, it is easier to select the reverse checkbox in the F<sub>1</sub> panel of the **Edit Processing Parameters** dialog.

6. Try the above with slightly different values for  $\text{ph0}$ . Notice the appearance of  $F_1$  quad images now that the two quadrature channels in  $T_1$  are not orthogonal?

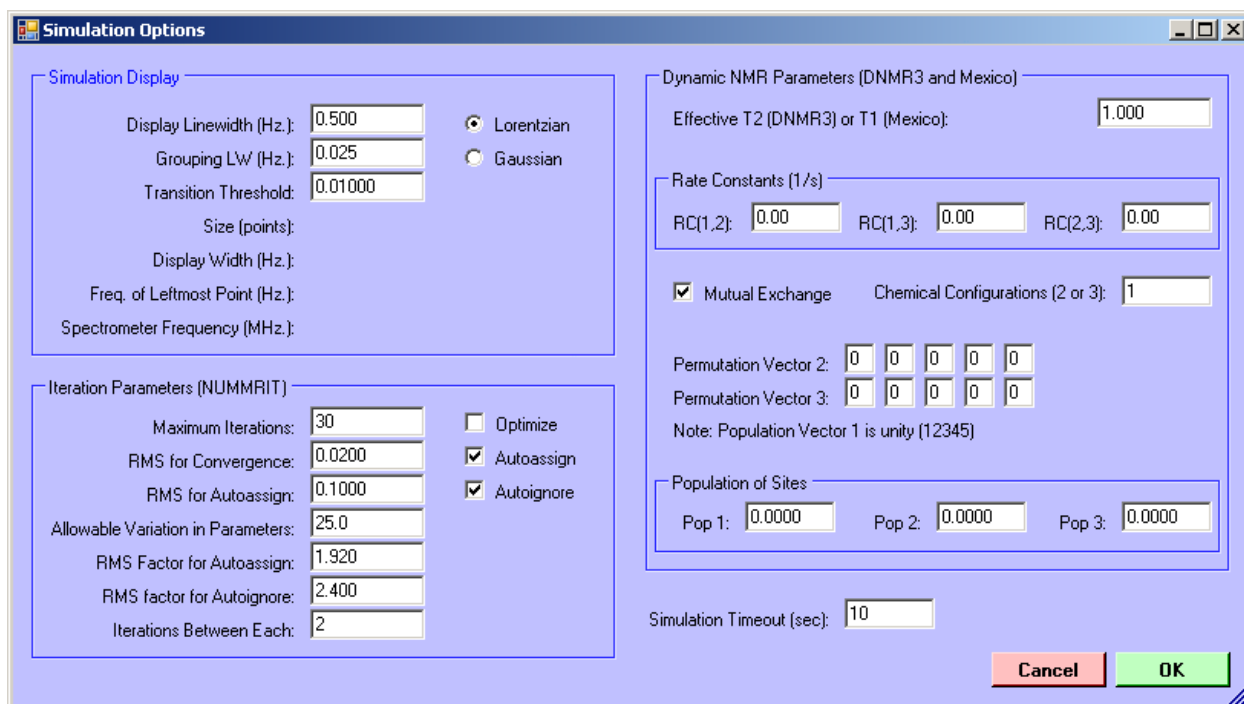


## Simulation Tutorial

SpinWorks uses the NUMARIT algorithm as described in: J. S. Martin and A. R. Quirt, *J. Magn. Reson.* **5**, 318 (1971), and modified by Rudy Sebastian and others at the University of Manitoba (who re-named it NUMMRIT). However, none of the original FORTRAN code is used in SpinWorks. SpinWorks uses a completely original implementation of the NUMARIT algorithm in C++.

There are four dialogs available from the **Spin System** menu that can be used to specify simulation parameters.

- In the **Edit Chemical Shifts...** dialog you define the nuclei or groups of nuclei that define the spin system. You must specify:
  1. The number of nuclei in each group. For example, a single nucleus would be entered as 1 and a methyl group would be 3. A symmetric pair (AA') would be entered as 2\*1.
  2. A symbolic label for each group. Letter, numbers, underscores and dashes can be used. Terms like "Hpara" or "CH3" are fine.
  3. A species identifier. Identifying different groups as different species will cause them to be treated as "weakly coupled". Different nuclei should always be treated as different species, but you can identify different groups of the same nucleus as different species if the chemical shift between the groups is much larger than any coupling between them.
  4. The spin of the nucleus. Spin ½ is default.
  5. The chemical shift in Hz.
  6. A check-box that defines whether a particular chemical shift should be optimized during an iterative calculation.
- The **Edit Scalar (J) Couplings...** dialog is used to enter conventional J values in Hz.
- The **Edit Dipolar (D) Couplings...** dialog is used to enter dipolar couplings in Hz. Note that with dipolar couplings, couplings within an equivalent group are observed.
- The **Edit Simulation Options and DNMR Parameters...** dialog is used to enter all of the simulation parameters that are not actually part of the Spin System. The DNMR parameters are described in more detail in the DNMR section of this manual.



**Figure 23.** *Simulation options dialog with DNMR parameters*

A small library of sample spin systems is included with the program. In a standard installation, these will be located in: C:\Program Files\SpinWorks\_4\SpinSystems.

## ABX Spectrum

1. Start SpinWorks, but do not select any experimental data.
2. In the Spin System menu select Edit Chemical Shifts... You will then be presented with a dialog for entering chemical shift and other information describing the spin system. For Group 1, enter 1 for the number of spins, A for the label, proton for the species and 1000 for the chemical shift. For Group 2, enter 1 for the number of spins, B for the label, proton for the species and 1010 for the chemical shift. For group 3, enter 1 for the number of spins, X for the label, proton for the species and 3000 for the chemical shift. Leave the Spin values at ½.
3. In the Spin System menu select Edit Scalar (J) Couplings... Enter -12 for J(A,B), 2 for J(A,X) and 10 for J(B,X).
4. In the Simulation menu select Run NUMMRIT Simulation. In a few seconds, a simulated spectrum will appear on the screen. Zoom in on the X multiplet at *ca* 6 ppm. Use the cursors to measure the multiplet spacings. Note that they do not relate all that well to the coupling constants! This is a second-order or virtual coupling effect, and is much more common than one might think.
5. Change the J(A,X) coupling to 0. Use Run NUMMRIT Simulation in the Simulation menu again. Note that the X multiplet is still a doublet of doublets, although it is coupled to only *one* of the other protons. Closer examination of the multiplet shows that there are small

satellite peaks (more properly called combination lines) displaced about 16 Hz. from the centre of the multiplet. These lines are an important clue that the spectrum is second order, but are easily lost in baseline noise.

6. Set the B chemical shift to be identical to the A chemical shift (**Edit Chemical Shifts...**) and re-simulate the spectrum (**Run Simulation**). The X multiplet looks like a triplet (with small combination lines) despite the fact that it is only coupled to 1 other proton.

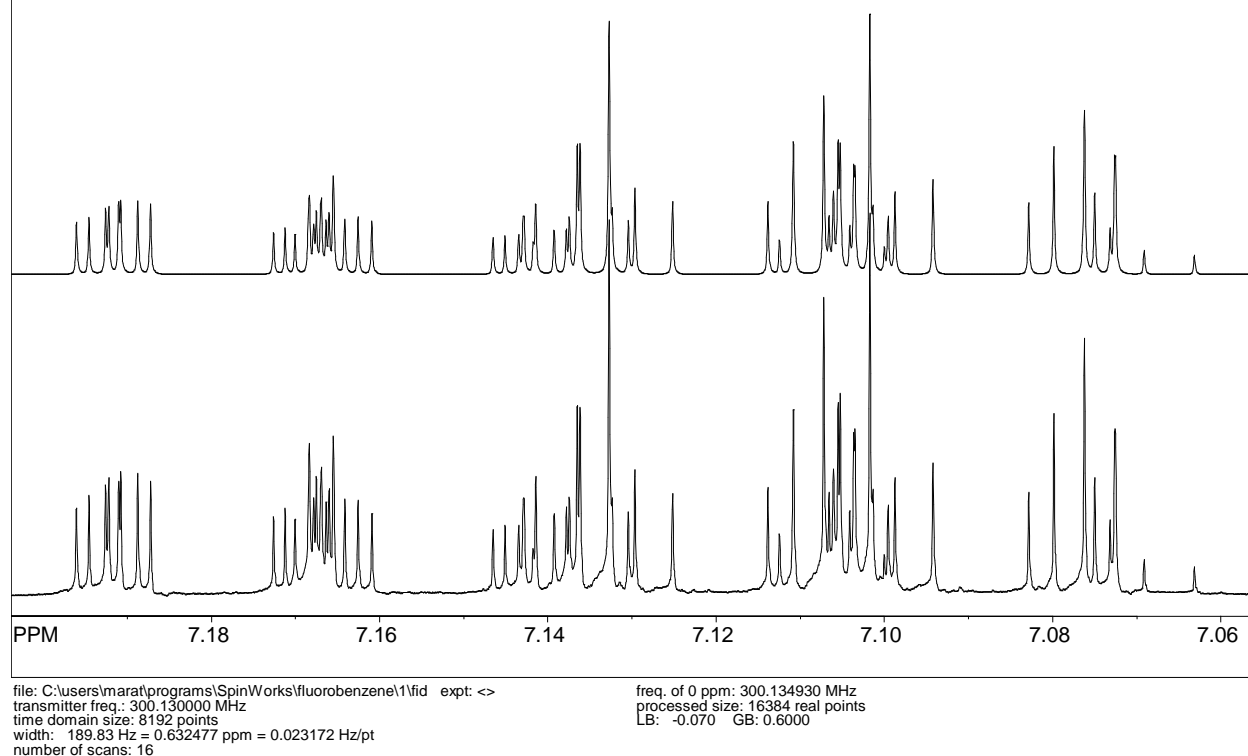
## Analysis of the Orthodichlorobenzene Spectrum

1. Locate the sample ODCB data. This data set normally resides in C:\Program Files\SpinWorks\_4\BrukerData.
2. Read the ODCB data set (**File: Open...**). This is the file odc\_b\2\fid located in the data directory as described in step 1.
3. Process the spectrum with no window function.
4. Read in a starting spin system with by loading the odc\_b\_ni file (**File: Read Spin System File...**). This file can be found in the same folder as the fid file. You can examine the starting parameters with the shift and scalar coupling editors if you desire (**Spin System** menu). Particularly note how explicit two fold symmetry is described with the 2\*1 entries in the spins field. Also note that in a symmetric spin system like AA'BB',  $J_{(A'B')}$  will automatically be taken to be equal to  $J_{(AB)}$  and that  $J_{(A'B)}$  will be taken to be equal to  $J_{(AB')}$ .
5. Select **Edit Simulation Options and DNMR Parameters...** in the **Spin System** menu. Set the **Display Linewidth (Hz)** setting to 0.05 (Yes, really).
6. Run the simulation (**Simulation: Run NUMMRIT Simulation**). A simulated spectrum somewhat like the experimental will be displayed above experimental spectrum. The vertical offset and scaling of the calculated spectrum can be adjusted with the blue +, -, up-arrow and down-arrow buttons on the toolbar.
7. The left and right keyboard arrow keys will move a transition cursor across the simulated spectrum. Assign lines to these transitions by pointing to the corresponding experimental peak and clicking with the right mouse button. The peak picking routine is used to find the peak closest to the cursor position. If no peak is found within a reasonable distance of the cursor the transition is assigned to the raw cursor frequency. When a transition is assigned, a red line will be drawn between the transition and its corresponding peak. Continue assigning all of the lines in the spectrum. A wrongly assigned transition can be deleted with the keyboard "d" key.
8. In the **Spin System** menu, use the **Edit Chemical Shifts...** and **Edit Scalar (J) Couplings...** dialogs to check the iterate boxes for both chemical shifts and all four couplings. In the **Spin System** menu select **Edit Simulation Options and DNMR Parameters...** Check the **Optimize**, **Autoignore** and **Autoassign** boxes.
9. Run the simulation (**Simulation: Run NUMMRIT Simulation**). The new spectrum that will be displayed should be a very close match to the experimental. If not, check the simulation output (**Simulation: List Simulation Output**) for possible clues as to what might be wrong.

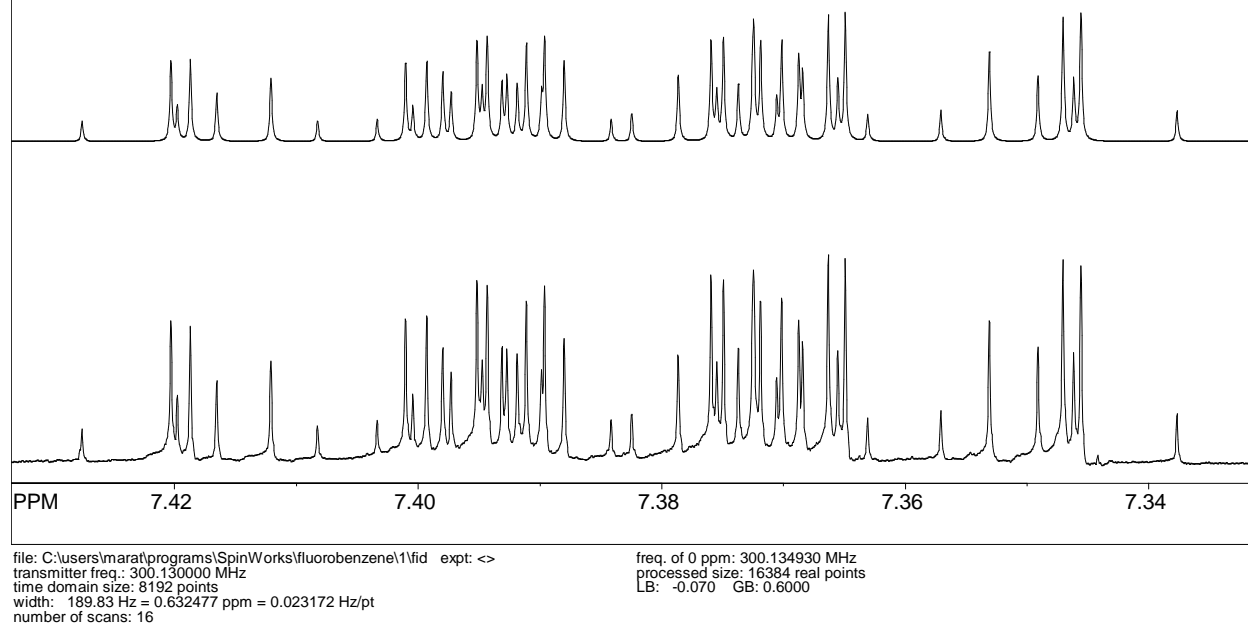
10. If the simulation was satisfactory, load the new parameters (Simulation: Load Optimized Parameters) into the spin system editor, and re-run the simulation. Examine the simulation output (Simulation: List Simulation Output). The RMS deviation between the experimental and calculated spectra should be less than 0.01 Hz at this point.
11. Load these final optimized parameters (Spin System: Load Optimized Parameters) and save the new spin system to disk (File: Save Spin System As...) and likewise save the assigned transitions (File: Save Assigned Transitions As...) for future use.

## Analysis of the Fluorobenzene Spectrum

1. The fluorobenzene spectrum supplied with SpinWorks is in XwinNMR format. Using File: Open..., select the fid file of experiment number 1, and process with FT. Phase the spectrum. This spectrum was recorded on an Avance 300 using gradients and a selective pulse to reduce the effective sample length and thus improve resolution (pulse sequence available on request). Slight baseline distortions are observed due to the lock hold circuitry releasing immediately before acquisition and diffusion within the sample. This has absolutely no effect on spectra recorded at "normal" resolution. Note that the spectrum shown below is an older one of the same sample recorded on an AM300. Note that this sample data set will have to be moved to a path with no embedded blanks, just as described for the orthodichlorobenzene sample above.
2. Read in the spin system file pfb.ss (File: Read Spin System File...). This file will normally reside in the same folder as the raw data (fid) for the fluorobenzene data. Set the simulation display linewidth to 0.06 Hz (Simulation: Edit Simulation Parameters...). Yes, we really can shim our 300 that well ☺.
3. Run a non-optimized simulation.
4. Assign as many transitions as possible. Note that this is a more difficult analysis and that not all of the transitions can be assigned at this point.
5. Check that all proton shifts and all couplings are selected for optimization (Spin System menu), and Set the simulation to run with optimization, autoassign and autodelete (Spin System menu).
6. Run the simulation. The fit at this point should be fair, but not great. If it looks like a reasonable improvement then load the optimized parameters (Simulation menu), assign more of the transitions, and re-assign any that were obvious mistakes. If the fit did not improve, it is probable that you did not assign enough lines or misassigned too many. Repeat steps 3 and 4.
7. Run the simulation. The fit should be quite good at this point. Continue assigning lines and loading optimized parameters until the fit no longer improves. The RMS at this point will typically be 0.006 Hz or better.
8. Print the spectra.



**Figure 24.** Analysis of the fluorobenzene spectrum: high field portion. This is the final fit after two rounds of iteration. The RMS deviation between experimental and calculated peak positions is 0.004 Hz. The Simulated spectrum has a calculated linewidth of 0.06 Hz.



**Figure 25.** Low field portion of the fluorobenzene analysis

## HOGWASH Resolution Enhancement

This is an implementation of Ray Freeman's HOGWASH (*Second-Hand Experiment for the Elucidation of Partially resolved Data using an Iterative Procedure*) algorithm incorporated into the Xsim/SpinWorks simulation package. The original papers (*J. Magn. Reson.* **76**, 476 (1988); **56**, 463 (1984); **56**, 294 (1984)) should be consulted for details of how the technique works.

The current implementation will process data with a real data size of up to 512k points (2 Mbytes). This is not an array size limit but is included to force a degree of reasonableness.

### General Comments

Be reasonable. Resolution enhancement (especially non-linear processing) is not the solution to all resolution problems. Enhancement factors of up to *ca* 5 are possible with careful application of the program to good data. Users are strongly encouraged to read the homily on page 492 of the paper by Davies *et al.* (*J. Magn. Reson.* **76**, 476 (1988)).

The choice of a synthetic vs. an observed mask (reference) peak depends on the nature of the resolution problem. In cases where the peaks can reasonably be assumed to be Lorentzian (i.e., relaxation is the problem) then a synthetic Lorentzian mask is appropriate, although the synthetic mask peak may be either Lorentzian or Gaussian. If field inhomogeneity is limiting the resolution, an observed mask (reference) peak is essential. This must be a sharp single line of reasonable intensity in a clear region of the spectrum. Note that TMS and other silylated reference compounds are usually poor choices because of the  $^{29}\text{Si}$  satellite peaks. Both the spectral region being enhanced and the mask peak must be well phased and baseline corrected if necessary. The compound chosen for the mask peak should be reasonably well  $T_2$  matched to the peaks to be enhanced, especially if a component of natural line width is to be removed from the spectrum. If the mask peak has a considerably greater  $T_2$  than the compound under investigation, then only a small component of natural line width will be removed from the spectrum. Any instrumental contribution will still be removed, however. If the mask peak has a considerably shorter  $T_2$  than the compound under investigation, severe baseline distortion will result. The frequency domain data (spectrum) must be *very* well digitized. This may involve zero-filling the FID to 4 or even 8 times the original time domain size. The spectrum should also have a reasonable signal to noise ratio and spectra with extremely high dynamic range should be avoided.

### Using the Technique

The spectrum to be enhanced should be processed normally using SpinWorks. Pay particular attention to phasing and baseline. If the acquisition time is considerably longer than the effective  $T_2$ , some EM is appropriate to optimize the signal to noise ratio without excessively broadening the peaks. If an observed mask peak is being used, record its frequency or shift.

Before using the enhancement package, there are a number of parameters that must be set. These can be set in the Edit HOGWASH Parameters...dialog of the Edit menu:

## Mask Peak Information

When using a synthetic mask peak some degree of trial and error is required. It is best to start with a fairly narrow mask and increase it until the desired degree of enhancement is obtained. Severe baseline distortion will result from the use of a too-wide mask peak. For an observed mask peak, enter the position of the peak and the range over which the mask should be defined. This range should be at least 10 times the line width of the mask peak but is limited to 4000 points. The larger the range, the more slowly the program will execute.

You can define the position of the mask peak by defining it with a single cursor before starting the HOGWASH parameter dialog.

## Loop Gain and Threshold

Typical values for the loop-gain ( $\gamma$ ) are less than 0.03, with 0.01 usually giving good results. Smaller values are better but slower. The termination threshold is defined as a fraction of the tallest peak in the region being enhanced. The threshold value depends on the dynamic range of the spectrum, and should be above the noise level - otherwise the program will attempt to synthesize the baseline noise. A threshold value just above the noise level is usually appropriate. The default value of 0.01 works well in most cases provided that the signal to noise ratio is good.

## The Reconstruction Line Width

This is another variable that often requires some experimentation. It must be several times the digital resolution, however. A value 3 to 5 times narrower than the mask peak half-height width seems to give good results. Too high a value will result in insufficient enhancement. Too low a value may introduce false structure into the spectrum.

## Starting the Calculation

The enhancement is started from the **Processing** menu. *The enhancement is only applied to the displayed region of the spectrum.* If other regions of the spectrum are to be enhanced, then they can be done serially, avoiding the need to apply the enhancement to baseline or uninteresting portions of the spectrum. If the procedure, for whatever reason, messes-up the spectrum, it will be necessary to re-process the FID. (e.g. with the **Process** button or with the **Processing** menu).

## Saving Enhanced Spectra

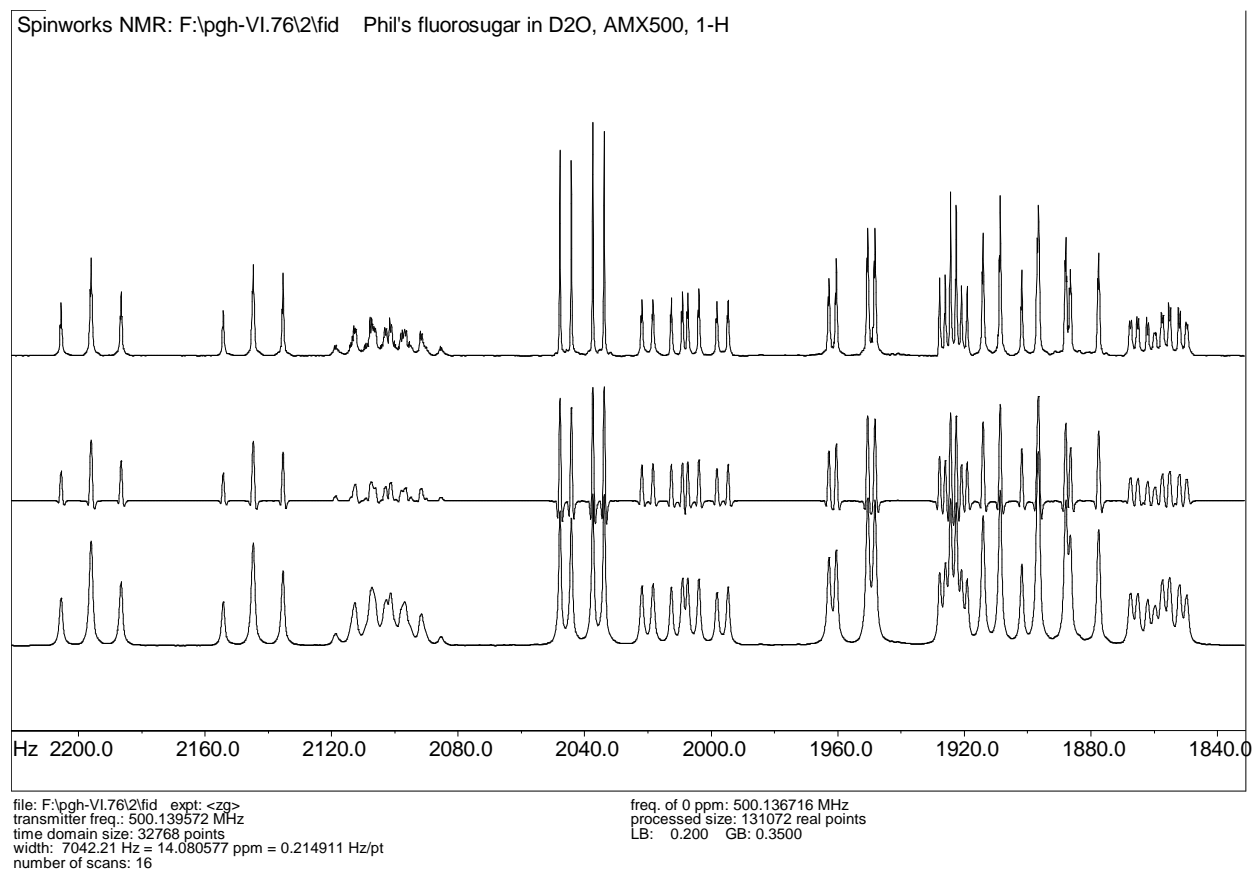
Enhanced processed spectra can be saved in JCAMP-DX or SpinWorks native format with the appropriate commands in the **File** menu. Enhanced spectra are also saved in SpinWorks format if the auto save feature is turned on (**Set Preferences...** selection in the **Options** menu)

## Speed

Typical calculation times are from a few seconds to a few minutes, depending on the problem and the computer used. The figure below shows HOGWASH processing of a fluorosugar spectrum with: loop gain = 0.02, threshold = 0.01, reconstruction linewidth = 0.2 Hz., mask = N-Acetyl peak (1036.5 Hz), mask width = 20 Hz, zero-filling to 128 k., Top: HOGWASH



enhancement, middle: GM enhancement, bottom: normal processing (LB = 0.2). This calculation requires only a few seconds on a recent computer.



**Figure 26.** HOGWASH processing of a fluorosugar spectrum. See text for details.

## Application of HOGWASH Processing to the Indirect Dimension ( $F_1$ ) of 2D Spectra

Multidimensional spectra often suffer truncation artifacts (“sinc wiggles”) resulting from short acquisition times in the indirect dimensions. The obvious solution of lengthening these acquisition times often results in an unacceptable increase in experiment time, and can decrease the signal to noise ratio. Apodization, of course, can reduce or eliminate the artifacts at the expense of resolution. Most NMR processing software includes linear prediction routines to extend the time domain data. Linear prediction uses a set of coefficients derived from singular value decomposition (SVD) or maximum entropy methods<sup>3</sup> (the so-called Burg algorithm) to extend the data in the truncated dimensions. However, “there is no such thing as a free lunch”, and linear prediction can create a number of well documented but not often appreciated problems and artifacts.<sup>4</sup> The Burg algorithm, in particular, is prone to problems such as line splitting

<sup>3</sup> Not to be confused with maximum entropy reconstruction.

<sup>4</sup> See, for example, A. S. Stern, K. Li, and J. C. Hoch, *J. Amer. Chem. Soc.* **124**, 1982 (2002).

(introducing false multiplet structure) and frequency shifting.

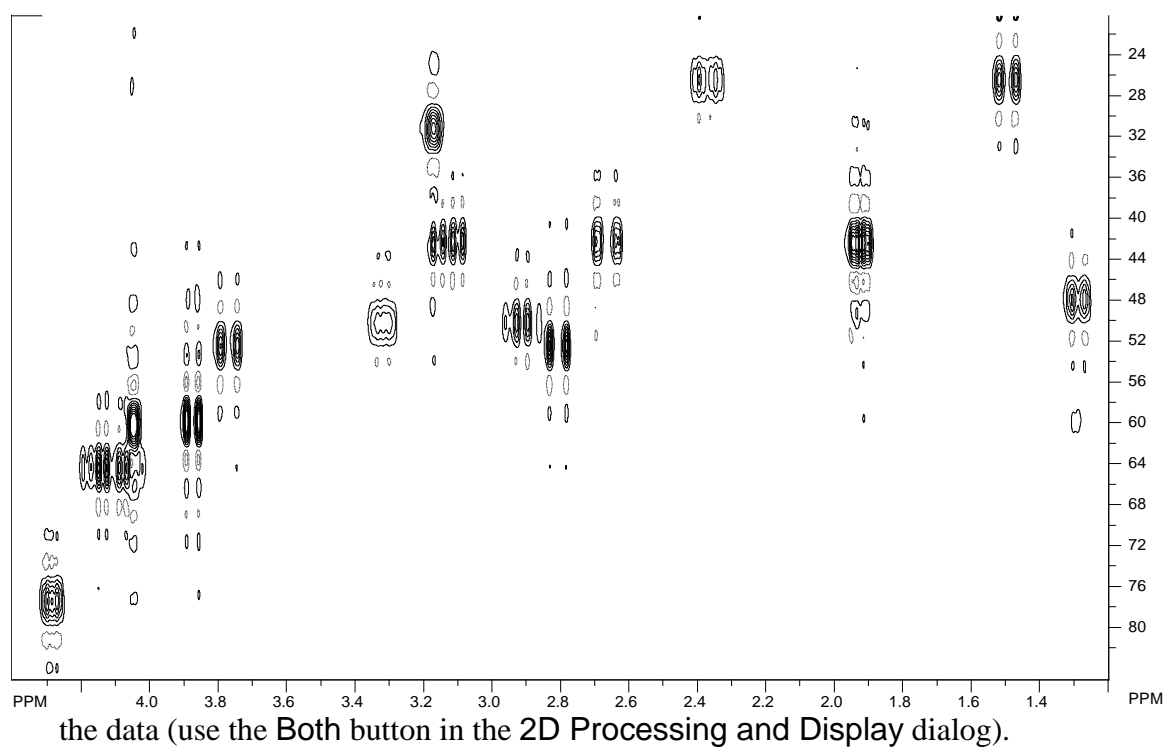
HOGWASH is modeled on a procedure used to solve an exactly analogous in radio astronomy. The finite aperture of the radio telescope relative to the wavelength creates artifacts similar to the truncation artifacts seen in multi-dimensional NMR. In both cases, the instrument lineshape function can be calculated or measured, and can readily be deconvoluted out of the spectrum in a step-wise procedure. For two-dimensional NMR, the instrument lineshape function can easily be calculated as the Fourier transform of a step function running from 0... $t_1$ , as field inhomogeneity usually has little effect for short values of  $t_1$ . Any additional apodization can be applied to this function, if desired. This application of HOGWASH to the removal of truncation artifacts was first proposed by Keeler (*J. Magn. Reson.* **56**, 463 (1984)) but, until now, the method has not been incorporated into readily available software packages. Note that other lineshape problems, such as “phase twist”, can be treated with the same method (Shaka *et al.* *J. Magn. Reson.*, **56**, 294, (1984)).

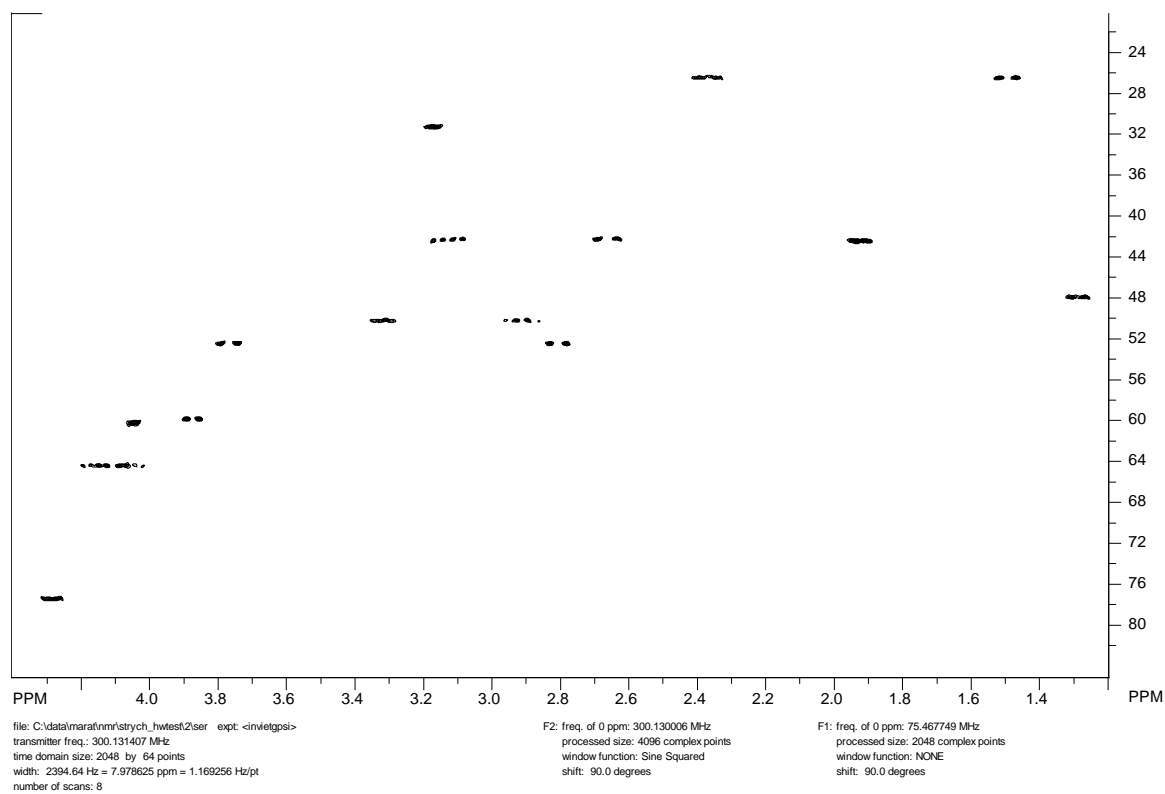
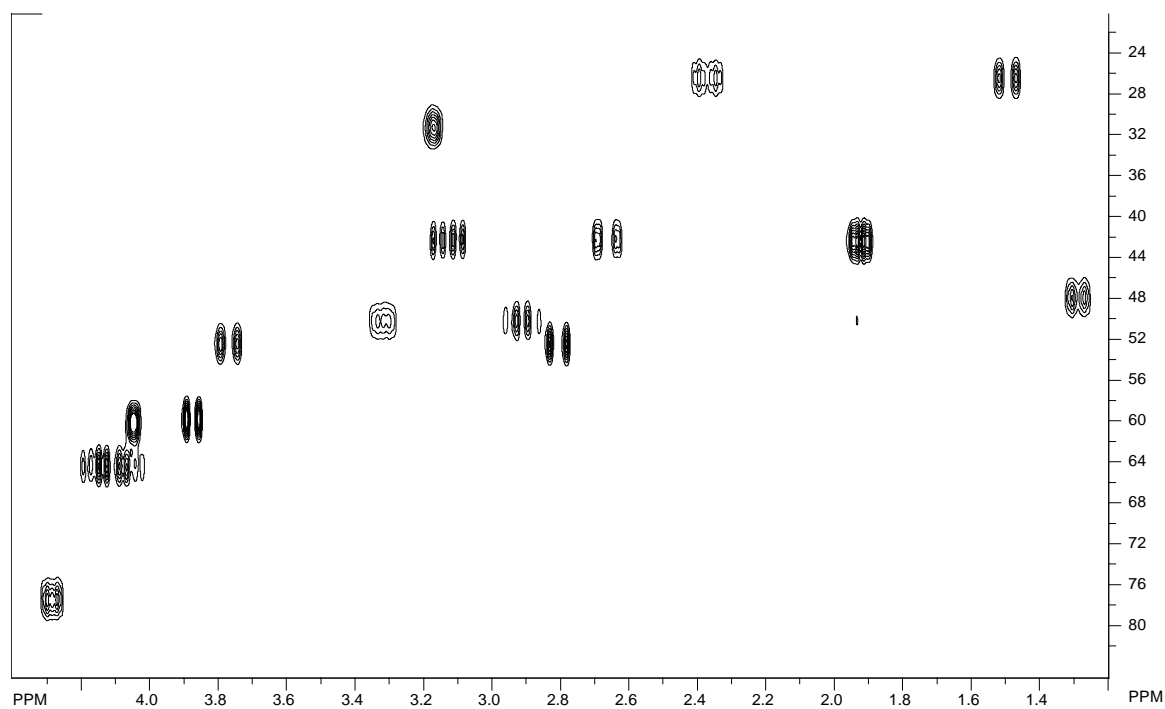
## 2D HOGWASH Parameter Selection

The optimum parameters for applying HOGWASH to 2D truncation problems are somewhat different than when using the procedure for general 1D resolution enhancement. The loop gain and termination threshold can be considerably higher. The peak width used for reconstruction must be larger, on the order of the digital resolution in  $F_1$ . The default parameters are appropriate for many situations. These parameters enable the 2D version of HOGWASH to run considerably faster than the 1D version. For 2D, the default reconstruction lineshape is Gaussian. This is primarily a cosmetic choice, as it seems to give nicer 2D contour plots than Lorentzian.

## Using the Technique

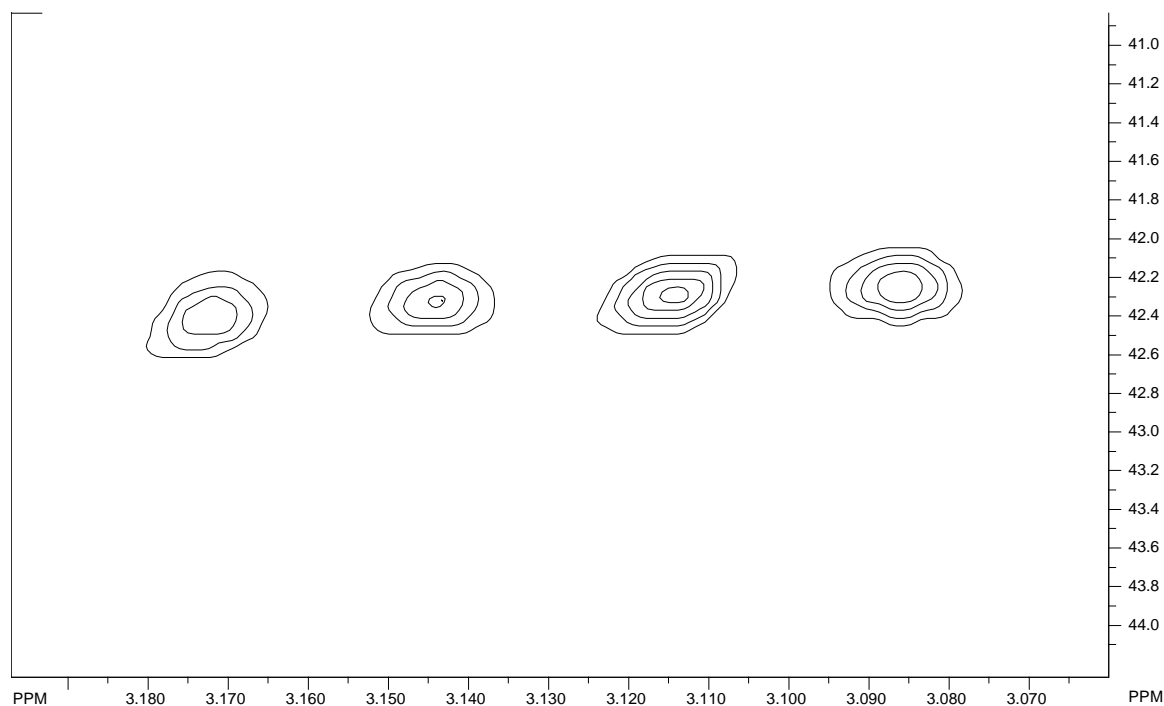
1. Process the 2D spectrum with normal window functions and the phasing mode set to none. (Edit menu, Edit 2D Processing Parameters...) Use considerable zero filling in  $F_1$ . If the number of time domain points in  $F_1$  was 256, zero filling to 1K would be reasonable.
2. Phase the spectrum carefully.
3. In the Edit 2D Processing Parameters... dialog, set the phasing mode in both dimensions to constants, and set the  $F_1$  window function to none. In the  $F_1$  HOGWASH Parameters, group set the Mask Width parameter to one half of the  $F_1$  size parameter. The Reconstruction linewidth should be set to approximately the digital resolution (Hz/point) in the  $F_1$  dimension. This may be 10 Hz to 20 Hz for an experiment like HSQC, but may be 1 Hz to 5 Hz for a homonuclear correlation experiment such as COSY.
4. Process the spectrum in both dimensions. You should see a phased 2D spectrum with truncation artifacts in  $F_1$ . Select the region to apply the HOGWASH processing. This can be the entire spectrum, if desired. Note that HOGWASH is applied to the entire  $F_1$  width, but only to the  $F_2$  region displayed on the screen.
5. In the 2D Processing sub-menu of the Processing menu, select HOGWASH  $F_1$ . The processing can be applied serially to other regions of the spectrum, if desired.
6. If selection of HOGWASH parameters was not appropriate, it will be necessary to re-process



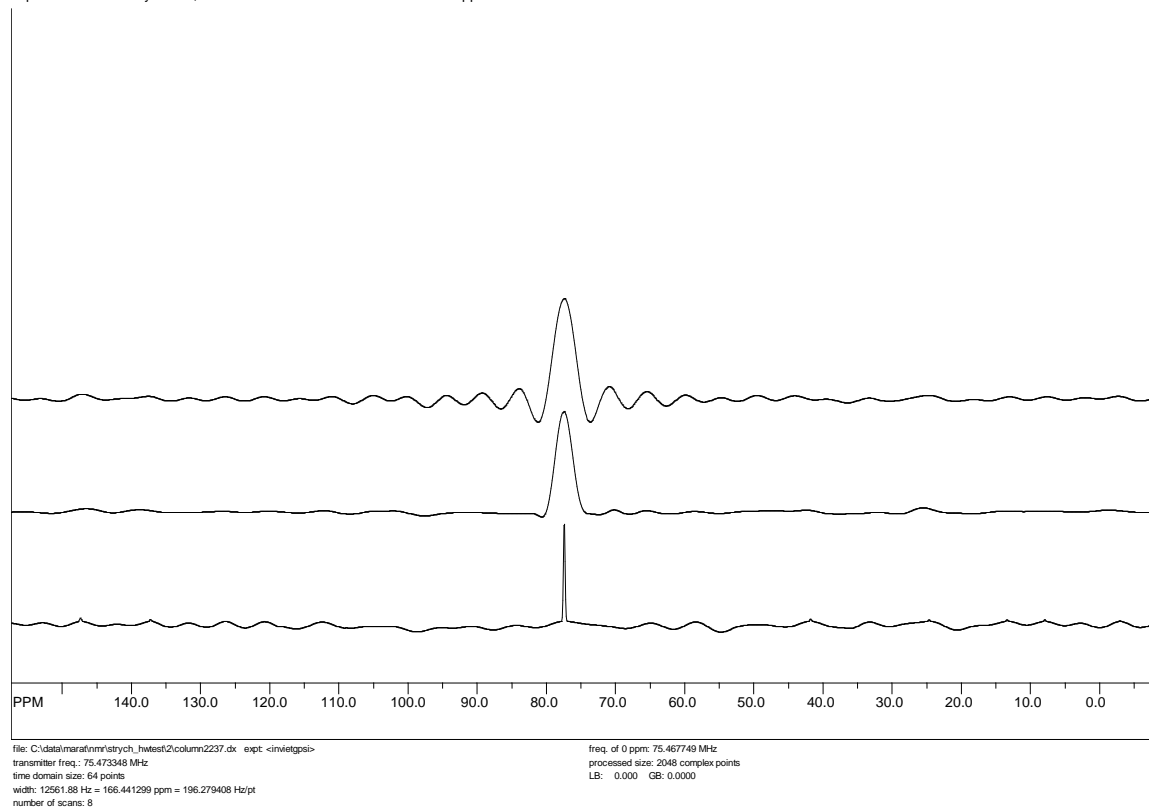


**Figure 27.** HOGWASH processed HSQC spectrum of strychnine

1. (Top) Processing with no window function in  $F_1$ . Severe truncation artifacts are observed in  $F_1$ .
2. (Middle) Processing with 8 coefficient SVD linear prediction to 128 points in  $F_1$ .
3. (Bottom) HOGWASH Processing. The parameters were: loop gain = 0.10, termination threshold = 0.05, mask width = 512 points, reconstruction linewidth 15 Hz (Gaussian). This calculation required approximately three seconds on a 1.7 GHz Pentium 4 under Windows 2000.



**Figure 28.** HOGWASH processing (expanded plot) showing resolution in  $F_1$  of approximately 15 Hz. Only 64 increments were recorded in  $F_1$  over a total width of 12,000 Hz, for a raw digital resolution of about 190 Hz.



**Figure 29.**  $F_1$  columns extracted from an HSQC spectrum

*Top: no window function*

*Middle: Linear prediction followed by sine squared apodization*

*Bottom: HOGWASH processing*

*Note that linear prediction has minimal effect on the resolution, but allows a less severe apodization by extending the effective acquisition time. The “sinc wiggles” that remain in the outer edges of the HOGWASH spectrum result from using a mask width that was too narrow (512 points).*

## Dynamic NMR Simulation

SpinWorks can interface to two external programs for the simulation of exchanged broadened NMR spectra. The first is the venerable old DNMR3 program of D. Stephenson, G. Binsch and D. Kleier, while the second is the much newer MEXICO of Alex Bain. The following summarizes the features and drawbacks of the two programs:

### DNMR3

- Handles fairly large spin systems, including groups of equivalent spins (e.g. methyl groups.) It can also use symmetry (e.g. AA') factoring, but the SpinWorks user interface does not include this. I doubt that symmetry factoring would be that useful for most dynamic NMR problems – anyone care to prove me wrong? The limit is 5 individual spins, or more if you have equivalence.
- The program can handle both mutual (e.g.  $AB \leftrightarrow BA$ ) exchange and non-mutual (e.g.  $AB \leftrightarrow CD$ ) type exchanges.
- Up to three chemical configurations can be included. For example,  $AB \leftrightarrow CD \leftrightarrow EF$ .
- The program can occasionally suffer from numerical instability.
- Reference: D. S. Stephenson and G. Binsch, *J. Magn. Reson.* **30**, 625 (1978).

### MEXICO

- Handles very large spin systems, but does not include symmetry or equivalence factoring. It can include weak coupling (X approximation) though. Since memory in this program is dynamically allocated, the maximum size of the spin system is unknown. With the SpinWorks interface, the practical limit is 5 individual spins. Bigger systems can be handled by manually editing the mechanism file.
- The program can handle both mutual and non-mutual exchange.
- The program is currently limited to two chemical configurations.
- The program is much faster and considerably more stable than DNMR3.
- The treatment of relaxation is more accurate than that in DNMR3.
- The complete MEXICO manual is available from Alex Bain at: <http://www.chemistry.mcmaster.ca/faculty/bain>
- References:
  1. A. D. Bain and G. J. Duns, *Can. J. Chem.* **74**, 819, (1996).
  2. A. D. Bain, D. M. Rex and R. N. Smith, *Magn. Reson. Chem.* **39**, 122 (2001).

### General Notes on DNMR3 and MEXICO

**The DNMR parameters for SpinWorks 3 are now in the Simulation Options and DNMR Parameters... dialog of the Spin System menu.**

These programs run as external modules (.exe files) to SpinWorks. SpinWorks expects to find these modules (dnmr3.exe and mexico.exe) in C:\Program Files (X86)\SpinWorks\_4, the default SpinWorks installation folder. If you installed SpinWorks in a different location (or if a 32 bit version of Windows installed it in a different location, e.g. C:\Program Files\SpinWorks\_4), then you must configure this in the **Set Preferences...** selection in the **Options** menu. Information is passed back and forth between these modules and SpinWorks *via* files, which are normally generated in C:\Temp. For MEXICO, this scratch folder can be changed in the **Set Preferences...** selection in the **Options** menu. DNMR3, however currently requires the presence of the C:\Temp folder to operate. All current Windows versions should have this folder. If you don't, then simply create it. The following files are generated:

- **Dnmr.mch** This is the input file for dnmr3, and is generated by the SpinWorks spin system editors.
- **Mexico.mch** This is the input (mechanism) file for MEXICO, and is generated by the SpinWorks spin system editors.
- **Dnmr.out.** This is the output spectrum from DNMR3, and is read by SpinWorks to create the displayed simulated spectrum. This is an ASCII file and X,Y frequency-intensity pairs.
- **1r** This is the digital spectrum produced by MEXICO. It has exactly the same format as the file of the same name in Bruker's XwinNMR. The byte order is that of the INTEL processor so, if you import this file into an XwinNMR or TopSpin data, set the appropriate parameter in the procs file must be correct. SpinWorks reads this file in order to display the simulated spectrum.

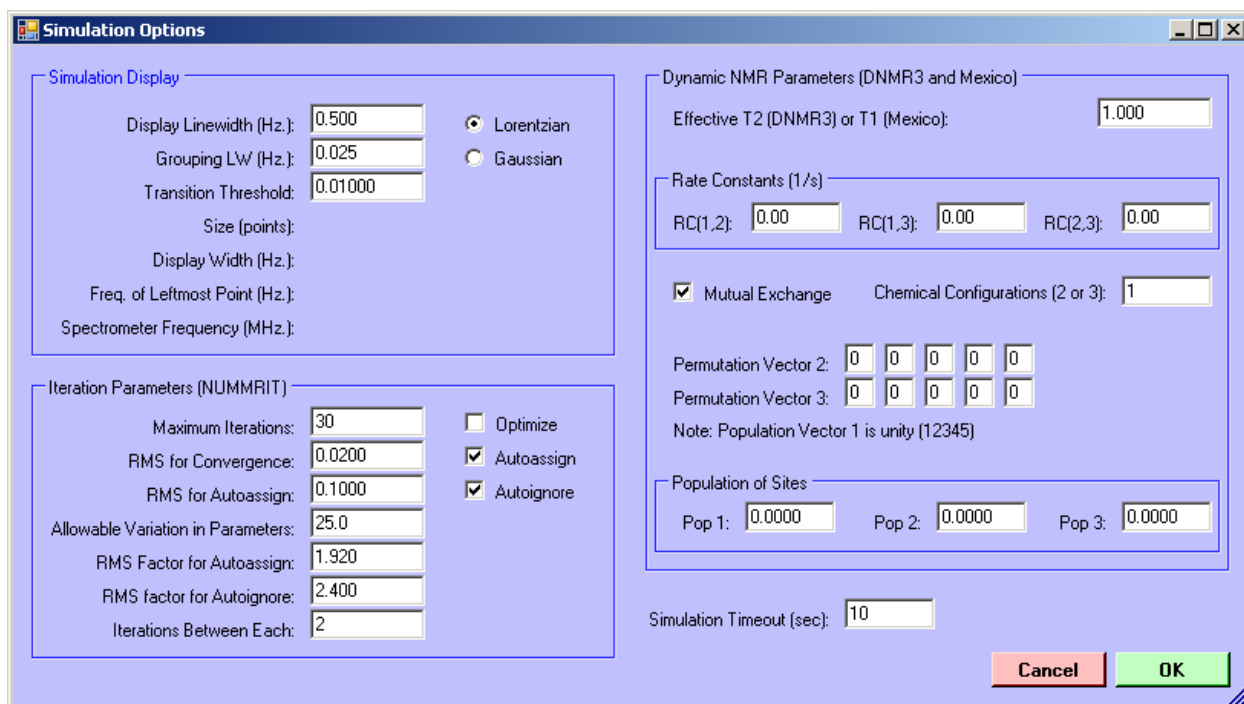
Note that the versions of DNMR3 and MEXICO supplied with SpinWorks have been modified from the originals in order to work with SpinWorks. The original MEXICO (for SGI IRIX, and designed to work in conjunction with Bruker's XwinNMR, or, I hate to admit, mNova is available from Alex Bain at: [bain@mcmaster.ca](mailto:bain@mcmaster.ca). The original DNMR3 program is available from numerous places on the web.

Note that all valid DNMR/MEXICO parameter sets are also valid for NUMMRIT calculations, and that any NUMMRIT parameter set that would be suitable can be turned into a DNMR/MEXICO parameter set by adding the appropriate DNMR parameters (Spin System menu).

If you are doing DNMR3 or MEXICO calculations with experimental data present, then the entire path to the experimental data can contain no blanks. This is a result of the UNIX style programming practices used in DNMR3 and MEXICO. Therefore, do not place your data in a folder like "C:\my nmr data", but instead use something like "C:\my\_nmr\_data" instead. Putting NMR data in the "My documents" folder creates similar problems.

The **Edit Simulation Options and DNMR Parameters...** (Spin System menu) dialog is used to specify many of the dynamic NMR parameters.





**Figure 30.** *Simulation options menu*

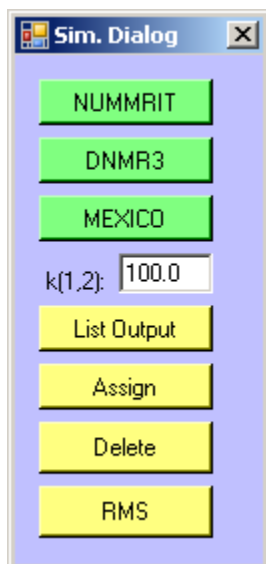
Despite what some people might tell you, Windows *is* a true multi-threaded multi-tasking operating system. In SpinWorks, simulations are run as a separate “thread”, and the program polls the simulation thread to see if it has finished. If a simulation hasn’t finished after a certain number of seconds, a timeout will occur. The default for this time period is 10 seconds, but can be by changing the **Simulation Timeout (sec)** value in the **Simulation Options** dialog.

Simulations can be started from the **Simulation** menu or with a pop-up dialog that can be started with the yellow **Simulate** button on the button panel. The following is a summary of the simulation dialog functions:

- NUMMRIT** Starts a NUMMRIT simulation using the existing spin system definition. Since all DNMR3 and Mexico spin systems are also valid NUMMRIT spin systems, this button will also work in DNMR3/MEXICO mode
- DNMR3** Starts a DNMR3 simulation. SpinWorks must be in DNMR/MEXICO mode and a valid DNMR3 spin system must exist.
- MEXICO** Starts a MEXICO simulation. SpinWorks must be in DNMR/MEXICO mode and a valid MEXICO spin system must exist.
- K(1,2)** Sets the rate constant for a two site DNMR3 or MEXICO simulation. For systems with more than 2 sites use the **Edit Simulation Options and DNMR Parameters...** dialog in the **Spin System** menu.
- Assign** This button is used to autoassign peaks in the displayed region of the spectrum. This would be used in an iterative NUMMRIT analysis. The fit must be

reasonably good for this feature to work, so a few rounds of manual assignment and iteration may be required first.

Delete	Deletes assignments in the displayed region.
RMS	Displays the relative RMS deviation and $\chi^2$ fit between the simulated and experimental spectrum over the displayed region. Primarily used for DNMR3 and MEXICO calculations.



**Figure 31.** *Simulation panel*

## Treatment of Relaxation

DNMR3 treats relaxation in a very rudimentary fashion. An effective  $T_2$  determines the linewidth of each spectral line in the absence of chemical exchange or at the extreme fast exchange limit. MEXICO's treatment of relaxation is far more sophisticated. A separate  $T_1$  value can be entered for each nucleus.  $T_2$  is considered to be equal to  $T_1$ .  $T_1$ , however, also affects the linewidth of any coupled nuclei, and this is properly handled in MEXICO. An example of this is the self decoupling of  $^{14}\text{N}$  from  $^1\text{H}$ . With SpinWork's DNMR/MEXICO interface, a single  $T_1$  is entered, and is considered to be equal for all nuclei. This should be adequate for most applications. If you find it necessary to enter separate  $T_1$ s, it is a simple matter to manually edit the MEXICO mechanism file. (Spin System menu).

## Example Files

A number of sample spin system files suitable for DNMR calculations are included in the DNMR folder of the standard installation (C:\Program Files\SpinWorks\_4\DNMR). These have the additional necessary DNMR parameters. These example files currently include:

- dnmr\_test3.ss AB to BA mutual exchange. This file is suitable for both DNMR3 and MEXICO.

- dnmr\_test4.ss AB to CD non-mutual exchange. This file is suitable for both DNMR3 and MEXICO.
- dnmr\_test5.ss  $A_2B_2$  to  $B_2A_2$  mutual exchange. This file is suitable for DNMR3 but cannot be used for MEXICO because there is more than one spin in a group. MEXICO could probably handle this system as ABCD to CDAB with  $\delta A = \delta B$  and  $\delta C = \delta D$ ;  $J(A,B) = J(C,D) = 0$ , and  $J(A,C) = J(B,C) = J(A,D) = J(B,D)$ . The permutation vector for the exchange would be 3412. Give this a try, if you like.
- dnmr\_test6.ss AB to CD to EF three site non mutual exchange. This file is suitable for DNMR3 only as MEXICO is currently restricted to two sites.
- dnmr\_test7.ss ABC to BAC mutual exchange. Suitable for both DNMR3 and MEXICO.
- dnmr\_test8.ss ABC to BAC to CAB three site mutual exchange. DNMR3 only.

## Parameters Required for DNMR Simulation

The spin system is described in the same manner as it would be for a high resolution (NUMMRIT) simulation. There are, however, a couple of differences, primarily affecting the chemical shift editor. For MEXICO, the number of spins in each group is limited to one. For DNMR3, this can be 1, 2, or 3. The species identifier is treated quite differently. Rather than specifying a different nuclear species (e.g.  $^{13}\text{C}$  vs.  $^1\text{H}$ ), the species identifier specifies a different chemical species for cases of non-mutual exchange (e.g.  $AB \leftrightarrow CD$ ). For mutual exchange all nuclei must be the same species (MEXICO can handle weak coupling, but it must be entered differently). For the  $AB \leftrightarrow CD$  case, the first two nuclei would be entered as species 1, while the second two would be entered as species 2. The actual symbols used to describe the species don't matter, as long as they are different. Once correctly described in the chemical shift editor, the coupling constant editor will recognize all valid coupling constants. For instance, for the  $AB \leftrightarrow CD$  case, the coupling constant editor will have entries for  $J(A,B)$  and  $J(C,D)$ , but not for  $J(A,C)$  etc. In effect, you are entering two separate spin systems. In addition to these differences in defining the spin system, there are a number of DNMR specific parameters that must be entered into the Edit Simulation Options... dialog of the Spin System menu.

T2 (for DNMR3) or T1 (for MEXICO)

For DNMR3, this parameter defines the linewidth in the absence of exchange.  $T_2 = 1/(\pi\Delta\nu_{1/2})$ . For MEXICO, this is the actual (or more likely estimated)  $T_1$  value of the nuclei.  $T_2$  is assumed to be equal to  $T_1$  and both effect the linewidth in the absence of chemical exchange. The current parameter editor sets the  $T_1$  of all nuclei to be equal, but you can override this with a manual edit of MEXICO's mechanism file. Future versions will allow you to enter separate  $T_1$  values for each nucleus.

Mutual Exchange (checkbox)

This parameter defines whether the exchange is mutual, or not.

Rate Constants

For mutual exchange only a single rate ( $K(1,2)$ ) can be defined. For non-mutual exchange, the forward rate for the exchange between site should be entered.

### Number of Species

Can be set to either 2 or 3. The current MEXICO release can only handle 2 species cases.

### Permutation Vectors

This string of integers defines the identity of the nuclei after exchange. The first permutation vector is taken to be the unity permutation 1,2,...,n where n is the number of nuclei. DNMR requires these vectors only for mutual exchange, one vector for two site exchange and two vectors for three site exchange. For an  $AB \leftrightarrow BA$  mutual exchange, the permutation vector would be 2,1. MEXICO requires a permutation vector for non-mutual exchange as well, but can usually be set to the unity permutation. Please see the MEXICO manual for complete details. *Note that SpinWorks and DNMR3 numbers the nuclei starting with 1, and the permutation vectors are internally converted into MEXICO's 0 based (C type) counting.*

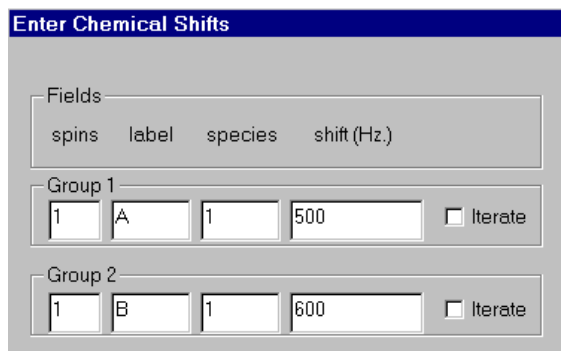
### Populations

These are the populations of the sites for cases on non-mutual exchange.

## DNMR Tutorial

### $AB \leftrightarrow BA$ Mutual Exchange

1. Start up SpinWorks with no experimental data. Use the Options menu to set the simulation mode to DNMR3/MEXICO.
2. Under the Spin System menu choose Edit Chemical Shifts... Enter two nuclei as follows:



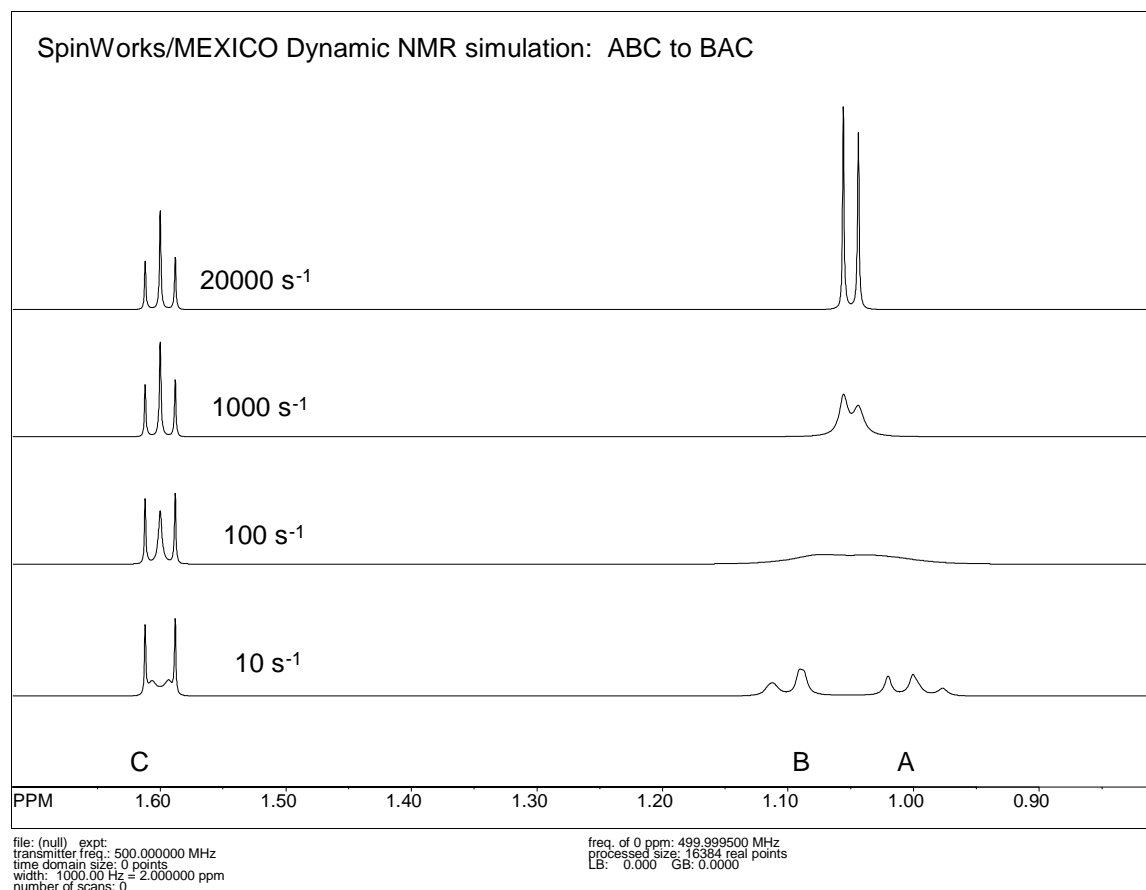
Enter Chemical Shifts			
spins	label	species	shift (Hz.)
Group 1			
1	A	1	500
<input type="checkbox"/> Iterate			
Group 2			
1	B	1	600
<input type="checkbox"/> Iterate			

3. Also under the Spin System menu, choose Edit Scalar (J) Couplings... Set  $J(A,B)$  to 12 Hz. Remember only to change the numeric part of the coupling constant field.
4. Use the Edit Simulation Options and DNMR Parameters... dialog in the Spin System menu to set the following DNMR parameters:  $K(1,2) = 5$ ; Permutation Vector (2) = 2 1 and  $T2 = 1$  (the default). Make sure that the mutual box is checked, and that the number of chemical configurations (sites) is set to 2. Since this is mutual exchange, the population values do not need to be set.

5. Use the Run DNMR simulation command in the Simulation menu to run the simulation. A black command interpreter window will appear briefly, and a simulated spectrum should appear. You can also run DNMR from the Simulation pop-up panel that can be started from the Yellow Simulate button on the button panel.
6. Run the same calculation with MEXICO by using the Run MEXICO simulation command (Simulation menu). If you see a spectrum here and in step 5, then both DNMR3 and MEXICO are installed properly and are working. Use List Simulation Output command (Simulation menu) to view the text output (e.g. for possible errors) from either of these programs.
7. The default spectral width (5000 Hz) is a bit big for this simulation, so let's reduce it. Use the Edit Simulation Options and DNMR Parameters... dialog in the Spin System menu to reduce the Display Width to 1000 Hz and the Offset to 1000 Hz. Recalculate the spectrum with either DNMR3 or MEXICO.
8. Experiment with the effect of changing the rate constant on the spectrum. You can save any of your calculations to a stacked trace with the Copy Simulated to Stack command in the View menu.
9. Print the spectrum, if desired.

### **ABC ↔ BAC Mutual Exchange**

1. Using the spin system editors, Set up an ABC spin system with  $\delta A = 500$  Hz,  $\delta B = 550$  Hz and  $\delta C = 800$  Hz. Set  $J(A,B) = 12$  Hz,  $J(A,C) = 10$  Hz and  $J(B,C) = 2$  Hz. Using the DNMR parameter editor (Spin System menu) set Permutation Vector 2 to 2 1 3 and the rate constant to  $5 \text{ s}^{-1}$ . Chemical Configurations (2 or 3) should be set to 2. This is one of the standard DNMR spin systems distributed with SpinWorks, so it can also be set up by reading in the spin system from disk. Use the Read Spin System... selection in the File menu. The file is dnmr\_test7.ss and should be located in C:\Program Files\SpinWorks\dnmr.
2. Run the simulation with both MEXICO and DNMR3. Experiment with different values of the rate constant. The spectra should look something like the following:



3. Notice that the two outer lines of the C multiplet remain sharp. Using simple  $\alpha\beta$  type spin basis functions, explain why this is so. (CHEM 4600 students take note ☺)
4. Switch the simulation mode to NUMMRIT (Options menu). Re-run the simulation with Run NUMMRIT Simulation (Simulation menu). Notice how easy it is to switch back and forth between the two calculation modes. For a strongly second order exchanging system, a good technique is to analyze the slow exchange spectrum with NUMMRIT first to obtain accurate spectral parameters. It is then an easy matter to switch to DNMR or MEXICO to simulate the exchanging spectra.

### ABC: Effect of $T_1$ Relaxation (MEXICO)

While not exactly Dynamic NMR, the following is an interesting demonstration.

1. Set up the ABC spin system exactly as in the previous example (dnmr\_test7.ss). Set the rate constant to 0, but the other dnmr parameters should be the same.
2. Simulate the spectrum using MEXICO. A nice, non-exchange broadened spectrum should be displayed.
3. Select the View or Edit MEXICO Mechanism File command in the Spin System menu. Find the relaxation rates line for the first site, and set the relaxation rate (not the exchange rate) for nucleus "C" (the third number on the line) to 10000.0. You do not need to change the rate of the second site as this is mutual exchange. (Read Alex's MEXICO manual, if you

need help understanding the format of the mechanism file).

4. Re-simulate the spectrum with MEXICO. What do you see? Why?

### **AB to CD Non Mutual Exchange**

This is a simulation of two different spin systems in equilibrium.

1. Read in the spin system file `dnmr_test4.ss` (it should be in `C:\Program Files\SpinWorks\dnmr`).
2. Using the spin system editors (in the **Spin System** menu) examine how the shifts and couplings are entered for a system of this type. Especially note how the species designator is used for the chemical configuration (site) and how the coupling editor only shows couplings within a chemical species.
3. Use the DNMR parameter editor to examine the `dnmr` parameters.
4. Simulate the spectrum with DNMR3 and MEXICO. Experiment with different values of the rate constant and populations.

## Reading SIMPSON Data

SIMPSON is a very powerful program for the simulation of many types of solid-state NMR experiments. SIMPSON can generate both time domain data (FIDs) and frequency domain data (spectra). For a complete description of SIMPSON please see: M. Bak, J. T. Rasmussen and N. C. Nielsen, *J. Magn. Reson.* **147**, 296-330 (2000). The program, examples, and a PDF version of the *J. Magn. Reson.* Paper can be downloaded from: <http://nmr.imsb.au.dk>

SIMPSON is available for Windows, Linux and several flavours of UNIX.

SpinWorks can read both SIMPSON spectra and FIDs, but the way in which the data are treated is slightly different depending on whether the data are time or frequency domain, and whether there are experimental data present. It is also important to note that there is a slight difference between the simulated trace, and the stacked traces.

1. The size, spectral width and offset of the *simulated trace* are equivalent to the experimental spectrum. In essence, the spectral characteristics of the simulated trace are tied to the experimental spectrum.
2. The size, spectral width and offset of the *stacked traces* are independent of the experimental data. Each stacked trace is an independent object, and its spectral parameters are copied from the source spectrum when the stacked trace is created. The principal purpose of this is to compare spectra that have been recorded under different conditions.

## Spectra with Experimental Data Present

If SpinWorks has a valid experimental spectrum, SIMPSON spectra can be read with the **Read Simpson Spectrum** selection in the **File** menu. The number of data points in the simulated spectrum **must** match the size of the experimental spectrum, and the spectral width of the simulated spectrum **must** match that of the experimental spectrum. The simulated data are read into the simulated trace.

## Spectra with no Experimental Data Present

In this case, the size and spectral width are read from the SIMPSON .spe file. The offset is set so that the frequencies run from SW at the left end to 0 at the right end. The spectra are read into the simulated trace, but can be copied into the experimental trace (or a stacked trace) if desired. If the simulated spectrum is copied into the experimental trace, it is then possible to re-reference the data, peak-pick, integrate, etc.

## SIMPSON FIDs

***FIDs produced by SIMPSON are treated as experimental data.*** It is just as if the data were produced by a virtual spectrometer. The spectral width and number of data points are determined from the data, and the virtual spectrometer frequency (used for Hz to PPM conversions) can be set with the **SpinWorks Edit Processing Parameters** dialog in the **Special Cases** group box. These FIDs can be processed, phased, etc., exactly like experimental data.

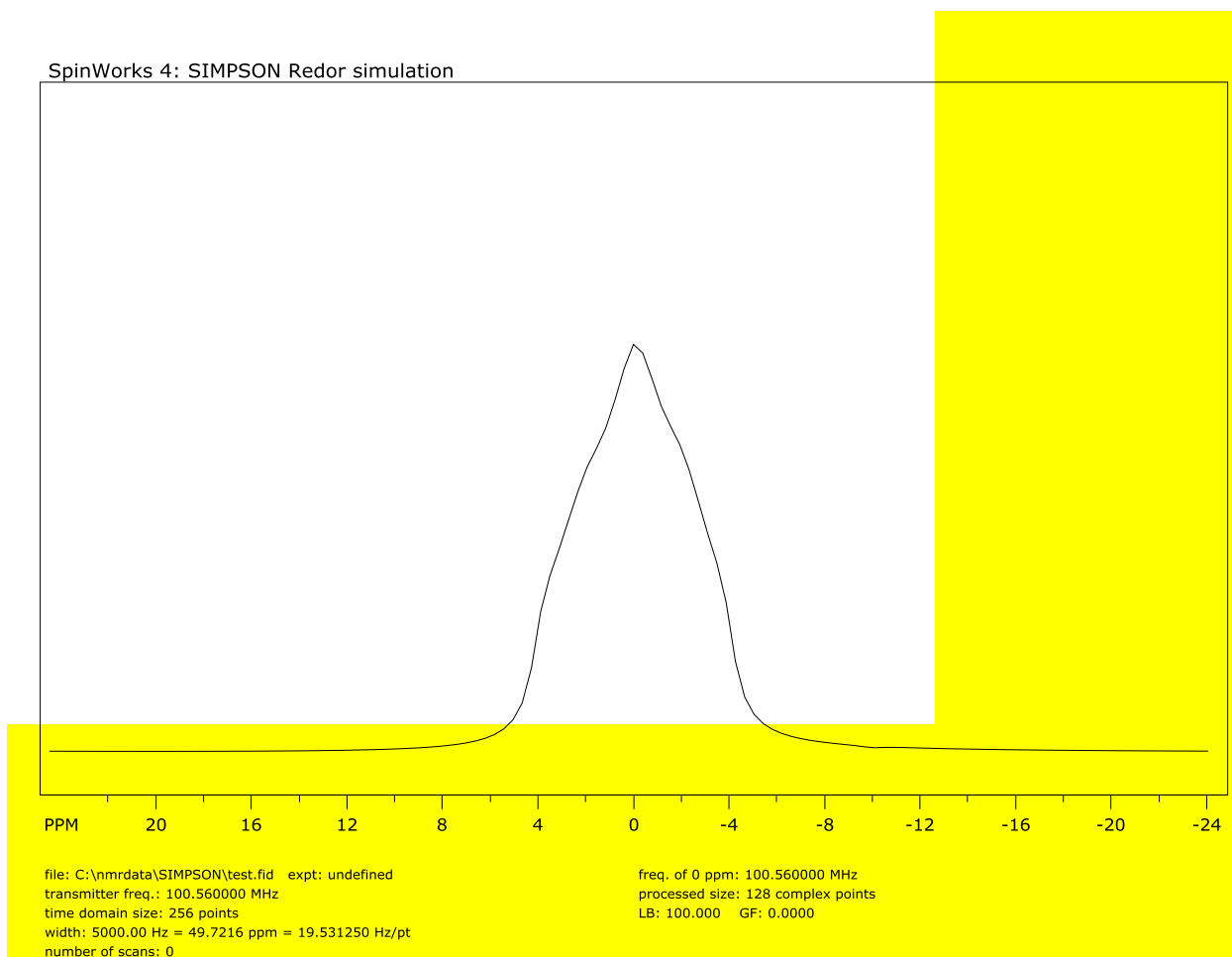


## SIMPSON Examples

### REDOR Example

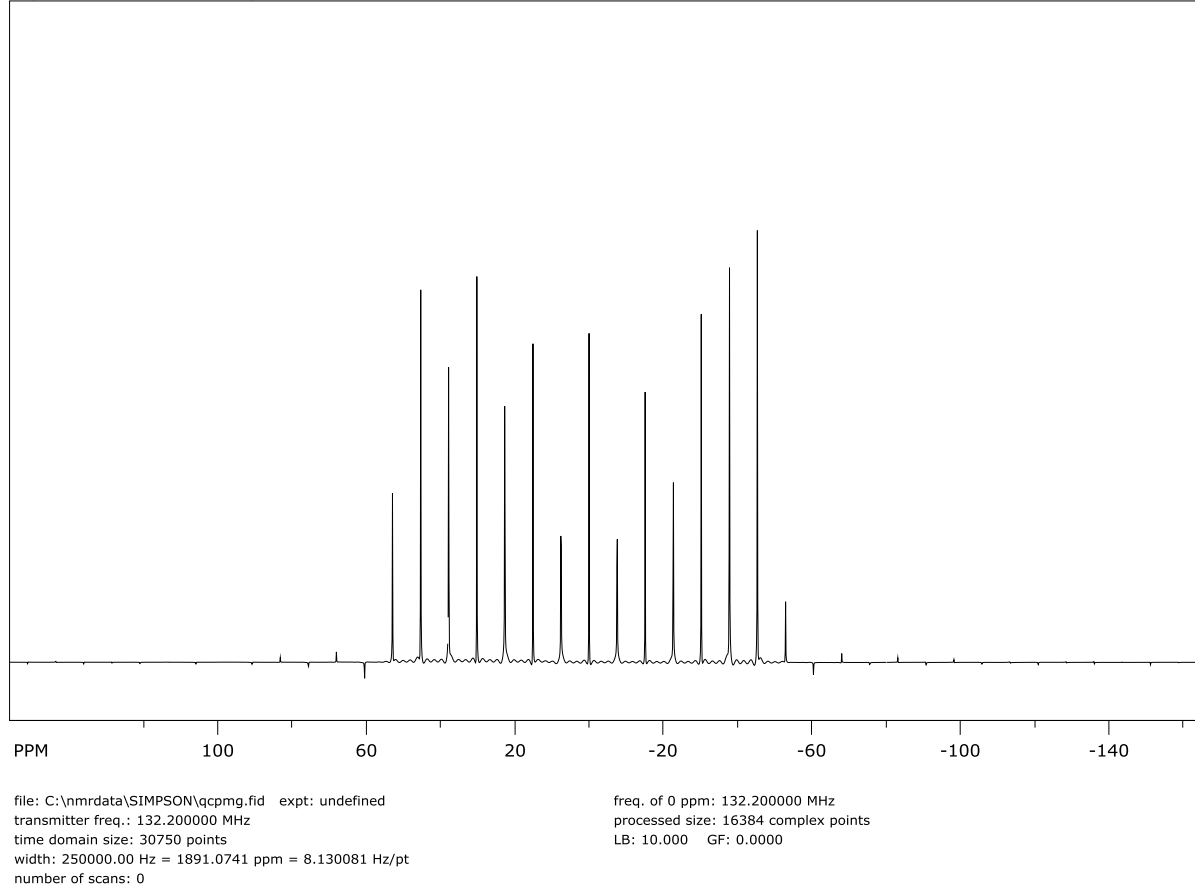
This is the REDOR example from the *J. Magn. Reson.* paper. It should be included with the Windows installation of SIMPSON (perhaps as the test.in file), but is also included with SpinWorks. It is assumed that you know how to install and run SIMPSON on your particular system.

1. Run SIMPSON to simulate the REDOR spectrum. On a Windows system this can be done with the command line: `simpson.exe redor.in`. This will produce two output files: `redor.fid` and `redor.spe`.
2. Start SpinWorks. Start the processing parameter dialog, and in the F<sub>2</sub> panel set the Virtual Spectrometer Frequency to 100.56 MHz. This is a <sup>13</sup>C experiment on a spectrometer with a proton frequency of 400 MHz. This number is only used for PPM calculations.
3. From the File menu select Read SIMPSON Spectrum..., and in the dialog select the `redor.spe` file. The spectrum should look like that in Figure 25 below.
4. From the File menu select Read SIMPSON Fid..., and in the dialog select the `redor.fid` file.
5. Set 100 Hz of line broadening and Lorentz windowing. Process the spectrum. The resulting spectrum should essentially be identical to that in the `redor.spe` file.



**Figure 32.** *SIMPSON REDOR simulation*

SpinWorks 4: QCPMG spectrum simulated with SIMPSON



**Figure 33.** *SIMPSON QCPMG simulation*

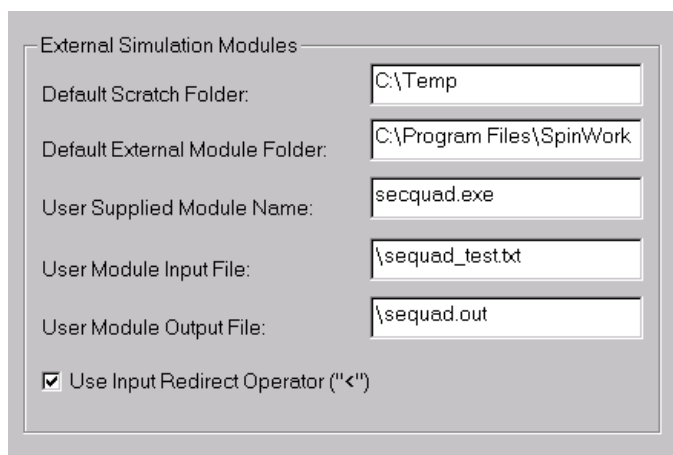
## Running User Supplied External Modules

*Not yet available for SpinWorks 4*

A new feature in SpinWorks as of version 1.3 is the ability to use SpinWorks as an interface to a user supplied external simulation module. These modules can be written in any language (C, C++, FORTRAN, Java, etc.), provided that certain conventions are observed. This feature has not been thoroughly tested. I would enjoy hearing from anyone that has a particular simulation program that they would like to use with SpinWorks.

1. The program *must* produce its output (digital spectrum) as an ASCII (text) table of frequency/intensity (x,y) pairs. The location of this file *must* be in SpinWork's scratch folder. This is set with the **Set Preferences...** selection in the **Options** menu. The name of this file must be entered into: **User Module Output File**, and note that a leading backslash is necessary.
2. If your external program requires an input file (spin system parameters or whatever) the name is entered into: **User Module Input File**, again with a leading backslash. If your external program does not require an input file name (perhaps because it has been hard coded into the program ☺) then enter **none**. Note that SpinWorks produces a number of spin system files that, depending on exactly what sort of calculation that you are doing, you may be able to use as your input file. These are called "spin\_system", "dnmr.mch" and "mexico.mch". If none of these are suitable, then it is necessary to edit your own input file by starting an editor (e.g. Notepad or WordPad) session and saving your input file before each simulation. You can also select whether your program requires its input file as a command line argument or with redirection from "standard input", UNIX style.
3. The number of data points (size), the spectral width and the frequency of the left-most point in SpinWorks (**Simulation** menu -> **Edit Simulation Parameters...**) must match that produced by the external program.

Here is a typical setup:



External Simulation Modules	
Default Scratch Folder:	C:\Temp
Default External Module Folder:	C:\Program Files\SpinWork
User Supplied Module Name:	sequad.exe
User Module Input File:	\sequad_test.txt
User Module Output File:	\sequad.out
<input checked="" type="checkbox"/> Use Input Redirect Operator ("<")	

In this case, the program executable is called "sequad.exe" and is located in C:\Program Files\Spinworks. The program produces an output file called "sequad.out" located in

C:\Temp. The program uses as input a manually edited text file called “sequad\_test.txt” and uses the Redirect operator “<” as part of the input line.

If you put SpinWorks into simulation debug mode (Simulation menu) you can see that the actual command line generated by SpinWorks is:

C:\Program Files\SpinWorks\secquad.exe < C:\Temp\sequad\_test.txt > simout.txt.

## Summary of Menu Bar Commands

### File Menu Commands

#### Open...

Specifies which NMR data set to open. The standard Windows File Open dialog.

#### Read F1 Trace... and Read F2 Trace...

Selects JCAMP-DX files to be used as 1D reference spectra (“projections”) in 2D mode. E.g. for an HSQC experiment you would select a proton spectrum for F<sub>2</sub> and a regular <sup>13</sup>C spectrum or DEPT 135 spectrum for F<sub>1</sub>.

#### Select Block (Varian)...

Varian “fid” files can actually contain more than one FID. This will be the case for arrayed parameters or experiments such as DEPT. Use this command to select which of the FIDs should be read.

#### Save (JCAMP\_DX) and Save as (JCAMP\_DX)...

These functions save the processed spectrum in JCAMP DX format. To read JCAMP DX spectra with SpinWorks set the appropriate data type with the Options menu. SpinWorks can also read JCAMP DX spectra create by XwinNMR and XwinNMR can convert JCAMP DX spectra created by SpinWorks. The data sets will have a .dx file extension. Note that these functions always use JCAMP DX format irrespective of the Data Format setting. The JCAMP-DX format used here is a subset of the full definition of the format. In particular, the data are not compressed and compressed data produced by other programs cannot be read. JCAMP-DX files saved by SpinWorks can be read by XwinNMR (although XwinNMR may gripe a bit about missing parameters). JCAMP-DX files saved by XwinNMR can be read by *SpinWorks provided that they are stored in uncompressed format*. In XwinNMR, this would be Compression Mode: FIX.

#### Save Displayed Rows or Columns...

Rows or columns selected with the right mouse button can be saved as JCAMP\_DX files by using the Save Displayed Columns or Save Displayed Rows selections in the File menu. These spectra will be saved with names like row482.dx in the folder containing the folder containing the current raw data. The titles in these files will have the chemical shift of the extracted row or column attached. These rows and columns can then be read by SpinWorks in 1D mode, *but be sure to set the data type to JCAMP-DX first*.

#### Save or Read Processed Data (SpinWorks Format)

These commands save the processed data for the current data set and load any previously saved processed data for the current data set. Processed data can also be automatically saved and loaded with the autosave and autoloading functions (Options menu). Note that SpinWorks processed data are not complete stand-alone data sets, but become part of the XwinNMR or VNMR data set. Therefore, in order to read SpinWorks processed data you must select the corresponding *fid* or *ser* file first. The presence of SpinWorks

processed data in the VNMR or XwinNMR data folder does not affect the ability of VNMR or XwinNMR to process the data.

### Delete SpinWorks Processed Data

If SpinWorks has been set to automatically save processed data (Options menu), any processed data generated by SpinWorks for this data set can be deleted with this command.

### Read Spin System File...

Reads in a spin system definition (shifts, couplings, etc.) for a simulation. A standard Windows File Open dialog will prompt you for the name and location of the file. The current spin system is also always saved in a file called "spin\_system" in the current working directory, and is updated any time the spin system changes (e.g. with the commands in the Spin System menu). Files generated by the spin system editor in Xsim are compatible with SpinWorks. If you have the Simulation Mode (Options menu) set to DNMR any additional DNMR parameters stored with the file will also be loaded. If you have no experimental data loaded, a descriptive title stored with the spin system file will also be loaded. The title of any experimental data will have precedence over this title, however.

### Save Spin System As...

Saves the current spin system to a named file on disk. A standard Windows File Save dialog will prompt you for the name of the file and the folder to save it in. This command is primarily used to save a spin system for future use and reference. Files saved with this command can be read with the Read Spin System File... command. This file is in plain ASCII text and can be examined with any text editor. These files are also compatible with the Xsim program. **Hint:** If you use a consistent file extension for your spin system files (such as .ss) they will be easy to recognize, and you can use the explorer to associate your favorite editor (e.g. Notepad, Wordpad, etc.) with this file type. If you have the Simulation Mode (Options menu) set to DNMR, any DNMR parameters will be stored with the parameters. The data set title (from the experimental data set) will also be stored with the spin system file. If you have not loaded an experimental data set, you can enter a title for the spin system file with the Plot Title... selection in the Edit menu.

### Read Assigned Transitions...

Reads a file of assigned transitions into the program. The current list of assigned transitions is also stored into a file called "asn\_trans" in the current working directory (usually the folder containing the experimental data) whenever an optimizing simulation is run. Transition files produced by SpinWorks are ASCII text files compatible with Xsim

### Save Assigned Transitions As...

Saves the currently assigned transitions to a named file on disk. This command is primarily used to save the assigned transitions for future use. Files saved with this command can be read with the Read Assigned Transitions... command.

## Print...

Prints the current spectra. Use the Edit Plot Options and Parameters... command in the Edit menu to change various aspects of the output.

## Read Simpson FID...

Reads in an FID file produced by the Simpson simulation program. The Simpson options must be set to produce the FID file in ASCII.

## Read Simpson Spectrum...

Reads a spectrum file produced by the Simpson simulation program. The Simpson options must be set to produce the spectrum file in ASCII.

## Print Preview

Exactly what it says.

## Page Setup...

Sets various printer dependent parameters (which tray to use, landscape vs. portrait, etc.).

## Recent Data Sets...

The most recently used file list. The last eight NMR data sets used by SpinWorks are displayed in a dialog for possible selection as the current data set.

## Exit

# Edit Menu Commands

## Processing Parameters...

Use this command to view or change the parameters used to process spectra. The same dialog is used for both 1D and 2D spectra. For 1D data the F1 page will be labeled inactive. *Note that in future versions the peak picking parameters may be moved to a separate menu.* The processing parameters are:

### Size (drop-down)

The number of complex data points in the transform. A Size value greater than TD/2 results in zero-filling.

### Frequency of 0 ppm (MHz):

The reference frequency for the spectrum. This value can be used to reference spectra that don't have a convenient reference line. Particularly useful in solid state NMR.

### First Point Correction

The multiplier to be applied to the first point in the FID. The default value is 0.5, but may be adjusted to obtain zero baseline offset.

### Reverse

Checkbox that selects whether the spectrum should be reversed after transform. The default is off, but may be turned on automatically for the F<sub>1</sub> dimension of some 2D



experiments.  $F_2$  would only require reversal in very rare circumstances.

### Detection mode

The choices for  $F_2$  are: Sequential, Simultaneous, DSP or Custom DSP. For  $F_1$ , the choices are: COSY/HMBC type, Seq. (currently unused), TPPI, States, States-TPPI and Echo-Antiecho. SpinWorks can usually determine these correctly from the data. A discussion of the  $F_1$  detection modes can be found in the 2D processing section of this manual. For  $F_2$ , this parameter should be correctly extracted from the data parameters, but is included here just in case it isn't. It does *not* need to be changed unless there is a problem.

### Group Delay

Only available for the Custom DSP Detection mode in  $F_2$ . This is the effective delay through the digital filters expressed in dwell periods. For JEOL digital filters, this number will typically be about 20. May also be required for Bruker Data where something other than a standard digital filter has been used.

### Special Cases

$F_1$  only. Used to adjust the frequency used for Hz to PPM conversion in  $F_1$ , or other special  $F_1$  cases. (Rarely used!)

### Baseline Poly. Degree

The degree of the polynomial used in the baseline correction routines (Processing menu). The default value is 3 and the maximum value is 6. ( $F_2$  only)

### Left Shift Points

The FID will be left shifted this many points prior to Fourier transform. Zeros will be added to the end of the FID. A negative value creates a right shift with zeros being added to the beginning of the FID. For simultaneously sampled data, this is the number of complex pairs that have been shifted. ( $F_2$  only)

### Peak Pick Minimum Intensity

The intensity threshold relative to the most intense peak in the spectrum for peak picking. Usually adjusted graphically by using the PP Minimum button on the button panel. ( $F_2$  only)

### Peak Pick Noise Threshold

provides a degree of noise discrimination in peak picking. The default value is 0.5 and may have to be lowered for spectra that have broad peaks (relative to the digital resolution) or that have high degrees of zero-filling. ( $F_2$  only)

### Phasing

Specifies the type of phase correction to be applied to the spectrum after transform. Choices are: None, Constants, Magnitude, Power and Auto (1D only). Using Power or Magnitude on the  $F_2$  dimension of a 2D spectrum would probably be silly. Zero: and First: specify the zero and first order phase constants that are applied when Phasing is

set to Constants.

#### Window Function (drop-down)

Select from No Window, Lorentz, Lorentz to Gauss (GM), Sine, Sine Squared, TRAF, TRAFS and Gaussian.

#### Line Broadening (LB) in Hz.

The amount of broadening to be applied in an exponential multiplication or Gaussian multiplication of the FID.

#### Gaussian Factor (GF)

The Bruker style Gaussian broadening factor to be applied to the FID. Used in conjunction with a negative LB value to provide resolution enhancement. See the description of the Window+FT (Processing menu) command.

#### Sine Shift

The number of degrees that a Sine or Sine Squared window function is shifted. A value of 90 will place the maximum value of the function at the origin of the FID.

#### Solvent Filter

A low pass filter that can be used to remove a strong signal at the centre of the spectrum. Primarily cosmetic. Three filter functions are provided, and the default number of points is typical for proton data. (F<sub>2</sub> only)

#### Automatic Baseline

This Group Box specifies the parameters used in the fully automatic baseline correction. The default values are good for most spectra; however they may be adjusted if necessary. The Filter Width and Threshold values are used by the peak detection routine. They can be raised for very noisy spectra. The Lambda value is the smoothing width used by the “Whittaker Smoother” baseline routine. It should be raised if the baseline routine is removing broad peaks from the spectrum (treating them as baseline), or if there are closely spaced bands of peaks and the baseline is being driven negative by the smoother.

#### Linear Prediction

Specifies the type of linear prediction applied to the data before transform. Typically, only backwards LP will be applied to 1D or the F<sub>2</sub> dimension of 2D data while only forward LP (Zhu-Bax) will be applied to F<sub>1</sub>. The value of Cutoff can be raised to speed the calculation, if necessary.

The F1 (Evolution) parameter panel does not include options for solvent filter, peak picking or baseline. Some default values are also different in the F<sub>1</sub> panel.

#### Plot Options and Parameters...

Edits sizes, colours, fonts, etc. for the plots. These should be self-explanatory.

#### Display Colours...

Edits the display colours for the current workspace.

## Restore Default Colours

Sets the display colours of the workspace to the default values.

## Set Stored Colour Preferences

Sets the colours of the current workspace from the Windows registry.

## Copy Metafile to Clipboard (.NET Format)

Makes a copy of the currently displayed spectra to the system clipboard, for possible inclusion in other documents. This new format is only understood by other .NET applications.

## Copy Metafile to Clipboard (Win 32 API Format)

Makes a copy of the currently displayed spectra to the system clipboard, for possible inclusion in other documents. This format is understood by most other applications, including Word and PowerPoint.

## Copy MetaFile to File...

Saves a metafile copy of the current spectra as a named file. The format is Windows Enhanced Metafile and the file has an *emf* extension to indicate this.

## HOGWASH Parameters...

Use this dialog to set and adjust various parameters used by the HOGWASH resolution enhancement command. These parameters are detailed in the separate section dealing with HOGWASH.

## Start Up Options...

Allows you to set such start-up defaults as default data path, default scratch path, autosave and autoload functions, etc. These values are saved in the Windows registry.

## Binning Parameters...

Sets the start and stop points, bin width and binning method for data binning (Peak Pick menu).

## Plot Title...

Use this command to edit the title of the current data. This information is displayed at the top of the data window and is included on the plot. The default is to use the title stored with the data. Note that currently only the first line of the title file is read and displayed.

## View Menu Commands

### Tracking Cursor

This option selection turns ON and OFF a tracking vertical reference line (or cross-hairs, for 2D). This can be very useful for measuring frequencies, frequency differences, etc. The default value is ON.

### Peaks and Match

This option toggles the display of peak pick labels and the match lines between experimental and simulated spectra. Available for 1D only.

### Rotate 2D

This selection will cause 2D spectra to be displayed and printed with the F1 axis horizontal. A check mark and the axis labeling indicated whether the spectra are rotated or not. Note that this option must be turned off for interactive 2D phasing.

### 2D Contour Spacing

This sub-menu allows you to select linear or logarithmic spacing for the 2D contour levels. The default is logarithmic.

### Set Fixed Limits and Set Fixed Scale

Use these commands to set convenient plot limits and expansion scales for routine spectroscopy. Once an expansion scale has been set (e.g. 10 Hz/cm) it is possible to scroll through the spectrum at the same scale by using the slider at the bottom of the data window.

### Define ROI...

Define up to three regions of interest. You can toggle between these regions with the ROI menu or with the 1, 2 or 3 keyboard shortcut keys. You can also define the currently displayed region of the spectrum as a region of interest with <ctrl>1, <ctrl>2 or <ctrl>3.

### Show Spectrum

Use this command to turn the display of the experimental spectrum on or off. This will affect both the display and the print. The default state is **ON**, and a check mark is used to indicate this fact.

### Show Simulated

Use this command to turn the display of the simulated (calculated) spectrum on or off. The default state is **ON**, and a check mark is used to indicate this fact. To control **printing** of the simulated spectrum use the Plot Options and Parameters... selection in the Edit menu.

### Show Integrals

Use this command to turn display of the integrals on or off. The default state is **ON**, and a check mark is used to indicate this fact. To control **printing** of the integrals use the Plot Options and Parameters... selection in the Edit menu.

### Show Zero Line

Use this command to turn display of the true zero line on or off. Can be useful for phasing or manual baseline correction.

### FID Display

Use this command to toggle between FID and spectrum display.

### 3D XY planes

Shows 3D planes in XY mode.

### 3D XZ planes

Shows 3D planes in XZ mode. YZ mode is not currently available.

### Copy to Inset Box

Copies the region of the spectrum currently defined by the cursors to a separate window. Typically used for displaying expansions. In 1D, these inset spectra will also be printed with the same expansion, size and location as seen on the screen. Printing of 2D insets is not yet supported.

### Copy Experimental to Stack

Use this commands to make a copy of the processed experimental spectrum into a stacked plot. This makes it possible to compare a series of spectra such as a temperature run or DEPT sequence on the screen. Zooming and scrolling effect all of the traces. If it is necessary to adjust the vertical scaling of an individual trace, use the **Define Stacked Trace Properties...** command of the View menu. This dialog can also be used to adjust the vertical and horizontal offset of the traces. The **Subtract Trace from Experimental** command in the Processing menu can be used to produce a difference spectrum.

### Copy Simulated to Stack

Similar to the above, but copies the simulated spectrum to the stack. These stacked traces can contain a mixture of simulated and experimental spectra.

### Add Simulated to Stack

Occasionally, it is handy to break a simulation up into a number of smaller simulations. This command allows you to build up a composite simulation in the last stacked trace by adding the current simulated spectrum to the spectrum in the trace.. Stated another way,  $\text{trace2} = \text{trace2} + (\text{factor} * \text{simulated})$ . The factor or coefficient for the simulated can be changed from the default value of 1 in the **Scale/Shift Stacked or Inset Traces...** dialog.

### Copy Series of Bruker Experiment Numbers to Stack

A series of Bruker experiments with consecutive experiment numbers (e.g. a 1D NOE series) can be copied to the stack with this command.

### “dssh” Type Stacked Trace.

Generates a horizontal stacking of a series of experiments, very much like the Varian/Agilent “dssh” command.

### Copy Simulated to Experimental

Copies the current simulated spectrum into the experimental trace, overwriting any experimental data. This allows one to use the integration and peak picking routines on simulated data, primarily for teaching purposes.

## Add Simulated to Experimental

Adds the current simulated spectrum to the experimental trace, overwriting any experimental data. This allows one to use the integration and peak picking routines on simulated data, primarily for teaching purposes.

## Copy Experimental to Simulated

Can't quite see the point to this one, but it is there.

## Delete Stacked Trace

Use these command to delete the last stacked trace. The current experimental spectrum is not affected.

## Delete Stacked Traces

Deletes all of the stacked traces.

## Define Stacked Trace Properties...

Starts a dialog which allows you to adjust the scaling, labeling, colour, offsets, etc. of the stacked traces.

## F2 Axis: PPM/Hz

Toggles the axis units of the F<sub>2</sub> axis between PPM and Hz.

## F1 Axis: PPM/Hz

Toggles the axis units of the F<sub>1</sub> axis between PPM and Hz.

## ROI Menu Commands

This menu allows you to select up to three pre-defined regions of interest, or to start a dialog where up to three regions of interest may be defined.

## Option Menu Commands

### Data Format (sub menu)

This menu selection sets the format of the current data. Currently supported data formats are: Bruker UXNMR/XwinNMR/TopSpin, and Varian UNITY/INOVA. Current JEOL spectrometers do not record their data in this format but can convert it to this format. It may be necessary to reverse (Processing menu) converted JEOL data after transform. TECMAG JCAMP DX format is used to read spectra saved by SpinWorks or JCAMP DX files from other sources (e.g. XwinNMR). The File: Save As... function always saves data in JCAMP DX format irrespective of the Data Format setting. The Data Format sub-menu is also used to switch the program from 1D to 2D modes.

### Set Start-up Options...

A number of start-up preferences can be set with this dialog:

Default Data Path

Sets the initial data folder (directory) for the File Open command. e.g. F: or D:\data\marat\nmr.

#### External Module Path

Sets where the program expects to find external simulation modules such as DNMR3 and MEXICO. The default is C:\Program Files\SpinWorks.

#### Writable Scratch Path

Sets the scratch directory (folder) used by some of the calculations. The default is C:\Temp is should be acceptable for most users. This folder must exist, and must be writable in order to perform calculations. For DNMR3 calculations the scratch path *must* be set to C:\Temp.

#### Processed Data

Two check boxes that determine whether processed data should be stored to disk and automatically loaded upon selection of a data-set. These features only apply to Varian and Bruker XwinNMR/UXNMR data. Occasionally, an inconsistently processed data set can cause the Auto Load feature to fail and SpinWorks may crash. This most often happens when the parameters say that the data were of one type (e.g. States), but this designation was wrong and the selection was overridden when the data were processed. If this happens, re-start SpinWorks and turn the Auto Load feature *off* (unchecked) before selecting the data set.

#### Display Font Size

Gives a choice of “normal”, “larger” and “largest” for the fonts used in the data window. Note that with the “larger” or “largest” settings the dialog and fonts will also be larger.

#### A4 (European) Paper Size.

Sets default plot sizes suitable for European A4 paper size.

#### Alternate Print Dialog (for Windows 7 64 bit)

Uses an alternate (Windows XP style) print dialog as a work-around for a bug in .NET for 64 bit Windows 7.

#### Start-Up Size

Sets the size (width and height) of the SpinWorks Window at start-up. Why? Because someone asked for it.

#### Extended Debug Logging

Turns on detailed logging (tracing) of SpinWorks actions. These will be written into a file called SW\_error.log in the current scratch folder (usually C:\Temp). This information can be very informative in the event of a problem with the program.

#### Clear Log File

Deletes the log file to keep it from getting too large.

## **Spin System Menu Commands**

### **Edit Chemical Shifts...**

This command starts the chemical shift editor, where it is possible to enter the number of spins, chemical shift, group label, species and spin identifiers for a group. The standard spin systems provided in the simulation tutorial give good examples of the required format. Note that if you don't provide a group label a default label will be used. This label can be overwritten by the user at any time.

### **Edit Scalar (J) Couplings...**

This command starts the coupling editor, where all J couplings relevant to the spin system can be edited. Note that the chemical shift editor must be called first.

### **Edit Dipolar (D) Couplings...**

Dipolar coupling is now supported for NUMMRIT (but not DNMR or MEXICO) calculations.

### **Edit Simulation Options and DNMR Parameters...**

This command starts a dialog where it is possible to edit the simulation parameters. This dialog is also used to set the necessary dynamic NMR parameters for DNMR or MEXICO simulations. See the Dynamic NMR section for details of the DNMR parameters.

### **Display Linewidth (Hz.)**

This is the linewidth (Lorentzian or Gaussian) used for in the calculation of the simulated spectrum. The default value is 0.5 Hz.

### **Grouping linewidth (Hz.)**

The linewidth used for grouping near degenerate peaks in a peak listing. This value does not normally need adjustment.

### **Transition Threshold**

The minimum intensity for calculated transitions to be saved. Too high a value may cause some small transitions to be missed. Very large tightly coupled spin systems generate large numbers of very weak (and usually unobservable) transitions. Too low a value may cause transition table to overflow on these systems. The default value of 0.01 is suitable for most cases.

### **Size (data points)**

The number of points used in the calculation of the simulated spectrum. The default value is 16k. In an experimental spectrum has been read the size parameter from the experimental data will be used instead.

### **Display Width (Hz)**



The width of the spectral window used to calculate the simulated spectrum. If an experimental spectrum has been read, the spectral width parameter from the experimental data will be used instead.

#### Offset (leftmost frequency in Hz)

The frequency of the left-most simulated data point in Hz. If an experimental spectrum has been read, the corresponding parameter from the experimental data will be used instead.

#### Spectrometer Frequency (MHz)

The frequency used to convert from Hz to ppm for the axis of the simulated spectrum. If an experimental spectrum has been read, the corresponding parameter from the experimental data will be used instead.

#### Maximum number of iterations

Puts a limit on the number of iterations allowed in an iterative simulation. The default value is 30.

#### RMS limit for convergence (Hz)

An iterative simulation (optimization) will stop if the RMS deviation between the experimental and simulated peak frequencies falls below this level.

#### RMS level for autoassign (Hz)

The RMS deviation must fall below this level for automatic assignment to occur. The default is 0.1 Hz and the maximum value is 10 Hz. The default is suitable for high resolution spectroscopy and can be raised to 1 to 2 Hz for more routine work.

#### Allowable variation in pars

The allowable variation in a parameter during an iteration. The default value is 25% and the minimum value is 1%. This parameter rarely requires adjustment.

#### RMS factor for autoassign

Transitions with frequency differences within this factor times the current RMS deviation will be automatically assigned. The default value is 2.7 and the minimum allowed value is 1.28. This value must be less than the RMS factor for autoignore. This parameter rarely requires adjustment.

#### RMS factor for autoignore

Transitions with absolute differences greater than this value times the current RMS deviation will be automatically ignored during an optimization. The default value is 3, and the minimum allowed value is 1.6. This parameter rarely requires adjustment.

#### Iterations between each autoassignment

The number of iterations between each autoassignment. The default value of 3 rarely requires adjustment.

#### Optimize check box

Determines whether an iterative simulation (optimization) should be performed. The default value is **OFF**. Make sure that you have selected some parameters for optimization (Spin System menu) and that you have assigned sufficient transitions before running an iterative simulation.

#### Autoassign check box

Determines if transitions should be automatically assigned during an optimization. The default value is **ON**. Note that using Autoassign too early in a difficult analysis can lead to problems.

#### Autoignore check box

Determines if transitions with excessively large deviations should be ignored (and possibly Autoassigned) during an optimization. The default value is **ON**. This parameter should generally be left **ON** unless you really know what you are doing.

#### Simulation Timeout (sec.)

Specifies the maximum time that a simulation is allowed to run. The default value of 10 sec. should be good for most simulations on most computers. For very large spin systems on really old computers you may have to increase this value.

## Simulation Menu Commands

### Simulation Mode

Use this command to set the simulation mode to either **NUMMRIT**, which is used for spin simulation, iterative analysis, and double resonance simulation, or to **DNMR/MEXICO** which is used to simulate dynamic NMR experiments, or to **Deconvolution** for band-fitting. The default mode is **NUMMRIT**.

### Run NUMMRIT Simulation

This command calculates a simulated spectrum based on the current spin system, Simulation Parameters and Simulation Mode (Options menu). After a calculation the calculated (simulated) spectrum will be displayed along with the experimental spectrum (if any).

### Run DNMR Simulation

Runs DNMR3 as an external program.

### Run MEXICO Simulation

Runs Alex Bain's MEXICO program as an external program.

### Run Deconvolution

Generates a simulated spectrum based on the current deconvolution peaks list. More commonly, this will be done with the interactive deconvolution panel.

## List Simulation Output

Starts an MS Notepad session with the textual output of the last simulation run. Also, use this command to print the simulation output.

## Load Optimized Parameters

This command loads the post-optimization parameters into the coupling and shift editors for further refinement. They can then also be saved with the **Save Spin System...** command of the **Edit** menu.

## Autoassign Peaks in Region

Automatically assigns the transitions to observed peaks in the displayed region. The peak picking threshold and sensitivity values are used for peak assignment. If no observed peak can be found within a certain distance of the transition cursor, or if the peak is below the peak picking threshold or sensitivity, no peak will be assigned. Single transitions can be assigned with the keyboard “a” key.

## Edit Deconvolution Peak Parameters...

With this dialog you can enter or edit peak parameters (frequency, intensity, width, % Gaussian) for the deconvolution peak list.

## Copy Picked Peaks to Deconvolution List

Uses the current peak pick list (generated by the peak picking commands) to generate the peak list for deconvolution. This will generate estimated values for the frequency and amplitude of each peak, with a default value for the width.

## Interactive Deconvolution Panel

This stay-up dialog box will allow you to interactively adjust and optimize the parameters of the deconvolution peaks. You can also transfer copies of the individual peaks to the stack display. To copy the simulated spectrum to the stack display, use the **Copy Simulated to Stack** function in the **View** menu.

## Delete Assigned Peaks in Region

Deletes all transition assignments in the displayed region. Single transition assignments can be deleted with the keyboard “d” key.

## Calculate RMS

Calculates and displays the current normalized RMS deviation between the simulated spectrum and the experimental spectrum. This command usually used for DNMR3 or MEXICO calculations. For deconvolution analysis, an unnormalized RMS is displayed

in the Interactive Deconvolution Panel. The RMS is calculated over the displayed region of the spectrum only.

## Processing Menu Commands

Note that the button panel Process button is more commonly used for basic processing.

### FT

Performs a Fourier transform of the current FID with no window function. Uses the **Size** parameter from the **Edit Processing Parameters...** dialog (Edit menu). Usually, the **Window + FT** command is more appropriate.

### Window + FT

Applies the specified window function followed by Fourier transform of the current FID. Uses the **LB**, **GF**, **Size** and **Window Function** parameters from the **Processing Parameters...** dialog (Edit menu). For 1D, the window parameter functions may also be set with the toolbar. For a Lorentz window function, a typical value for **LB** is  $1/(\text{acquisition time})$  or equal to the natural linewidth if the peaks are broader than this value. A Lorentz to Gauss window function will perform a Bruker-style Lorentz to Gauss resolution enhancement. Typically, set **LB** to the negative of the observed linewidth and **GB** to the fractional point in the time domain where the signal vanishes into the noise. e.g. If the acquisition time is 5 seconds and the signal disappears into the noise by 2 seconds then **GB** should be set to 0.4. These are only trial parameters, and may need to be readjusted to suit particular circumstances.

### Window + FT+ Phase

Applies the specified window function followed by Fourier transform of the current FID followed by phase correction with the last specified phase constants. This is equivalent to the Bruker “efp” command or the Varian “wft” command.

### Process Series of ExNo (Bruker)

Applies a weighted Fourier transform to all experiments in a contiguous series of experiment numbers (Bruker data). Each block will also be phased if **Last Constants** is specified as the phasing parameter. Typically used for experiments such as 1D NOE.

### Process Array (Bruker)

Applies a weighted Fourier transform to all blocks of an arrayed experiment (Bruker data) where the individual FIDs are stored in an “SER” file (a pseudo-2D experiment). Each block will also be phased if **Last Constants** is specified as the phasing parameter. After transform, the blocks will be displayed as a stacked plot. Typically used for experiments such as T1.

### Process Array (Varian and Anasazi)

Applies a weighted Fourier transform to all blocks of an arrayed experiment (Varian and Anasazi data). Each block will also be phased if **Last Constants** is specified as the phasing parameter. After transform, the blocks will be displayed as a stacked plot.

## Least Squares Baseline Correct Array

After defining a series of baseline points (Yellow BL Point button), this function will apply a least squares baseline correction to all spectra in an array. The degree of polynomial used can be adjusted in the processing parameters dialog. See the detailed description under Automatic Baseline (Least Squares) below.

## Spline Baseline Correct Array

After defining a series of baseline points (Yellow BL Point button), this function will apply a cubic spline baseline correction to all spectra in an array. See the detailed description under Automatic Baseline (Spline) below.

## Auto Baseline Correct Array

Do a fully automatic baseline correction of a stacked array.

## Integrate Stacked Array

This function will provide an integral list for all spectra in an array. You must first define the integral regions in the active trace. The absolute integrals will be listed in two formats: the first by trace number and the second by peak number. The later may be preferable if you want to paste the data into a spread sheet.

## Drift Correct

Applies a Varian style linear baseline correction to the spectrum.

## Baseline Correct (Spline)

This command applies a cubic spline correction to the displayed region of the spectrum. It is first necessary to define some baseline points. This is accomplished by clicking on the yellow BL Point button on the button panel. Then use the mouse to define at least six points in the displayed region that represent baseline. More points are better. Note that the baseline point routine averages a region 16 points wide, so don't select points close to large peaks. After selecting enough points, click on the return button (or the keyboard Enter/Return key) to exit the baseline points routine. Finally, execute the Automatic Baseline (Spline) command.

## Baseline Correct (Least Squares)

This command applies a polynomial baseline correction to the displayed region of the spectrum. The default polynomial order is 3, and may be changed in the Edit Processing Parameters... dialog (Edit menu). It is first necessary to define some baseline points. This is accomplished by clicking on the yellow BL Point button on the button panel. Then use the mouse to define at least six points in the displayed region that represent baseline. More points are better, and it is important to include points near each end of the region. Note that the baseline point routine averages a region 16 points wide, so don't select points close to large peaks. After selecting enough points, click on the return button (or the keyboard Enter/Return key) to exit the baseline points routine. Finally, execute the Automatic Baseline (Least Squares) command.

## Clear Baseline Points

Clears the defined baseline points.

### Fully Automatic Baseline Correction

Applies a spline correction to the baseline, but determines the baseline points automatically. This procedure works well for spectra with clear regions of baseline and a decent signal to noise ratio.

### Reverse 1D Spectrum

Reverses the spectrum – high field to low field. There are several circumstances where this may be appropriate:

1. Exchange of the two quadrature channels will reverse the spectrum (This is the default state on Varian and JEOL spectrometers. Therefore, Varian and JEOL data are automatically reversed by SpinWorks).
2. If a spectrum has a distinct baseline curvature at the left (low field) end of the spectrum, it is possible to straighten it by reversing the spectrum, using the least squares automatic baseline routine, and then reversing the spectrum back to the normal orientation.

### HOGWASH

Applies Ray Freeman's HOGWASH procedure to the displayed region of the spectrum. The HOGWASH parameters must first be set (Edit menu). Please see the HOGWASH section of this manual for details.

### 2D Processing (sub menu)

Contains assorted 2D processing commands that are not included in the 2D button panel:

#### Symmetrize

Applies a symmetrization algorithm to the data. The data *must* be inherently symmetric. That is, the size, spectral width and referencing must be identical in the two dimensions, as should be the case with COSY data. This routine is frowned upon by spectroscopists, but is still commonly used. The chief problem is that it can turn an obvious artifact such as T<sub>1</sub> noise or tailing into something that looks like a real cross peak. Correcting the problem at source (vibration isolation, stable instrument environment, better phase cycling, using gradients, etc.) is a better if not always practical solution.

#### Smooth

Applies a smoothing algorithm to the data. This command will improve S:N slightly and will "smooth-out" jagged contours. Usually, time domain processing (adjusting window function, zero-filling, linear prediction, etc.) is preferred.

#### Baseline F2 and Baseline F1

Applies an automatic baseline correction to the rows or columns of the 2D matrix. Rarely required when working in organic solvents, but may be useful for samples recorded in water.

## Drift Correct F2 and F2

Apply a Varian/Agilent style drift correction (first order baseline correction) to the rows or columns. This is useful when some baseline flattening is desired but polynomial baseline correction is inappropriate.

## HOGWASH (F1)

Applies Ray Freeman's HOGWASH procedure to the F<sub>1</sub> dimension displayed region of the spectrum. The HOGWASH parameters must first be set (Edit menu). Please see the HOGWASH section of this manual for details.

## Calculate F1 and F2 Skyline Projections

These commands calculate the F<sub>1</sub> or F<sub>2</sub> projections of the data, and save the projections in the current data folder as JCAMP-DX files with file names f1\_proj.dx and f2\_proj.dx. An F<sub>1</sub> projection of HSQC and/or HMBC data is particularly useful for samples that are too dilute for conventional <sup>13</sup>C spectroscopy in a reasonable amount of time. These commands are also available in the 2D Processing and Display Dialog. When activated from this dialog, the projections will also be displayed along the edges of the 2D spectrum and can be printed with the 2D spectrum as well.

## Calculate F1 and F2 Sum Projections

These commands calculate the F<sub>1</sub> or F<sub>2</sub> sum projections of the data, and save the projections in the current data folder as JCAMP-DX files with file names f1\_proj.dx and f2\_proj.dx. A sum projection will usually have poorer signal to noise ratio than a skyline projection.

## Clear Imaginary Data

This command clears 2D imaginary data for the current workspace, thus freeing some of your computer's memory. A copy of the all quadrants of the data is automatically saved on disk (The File Save command is issued) before the imaginary quadrants are cleared. Although you *can* regenerate the imaginary quadrants (for phasing) using the Hilbert transform functions, it is better to simply read back in the full processed data (File menu).

## Hilbert Transform F2 or F1

These commands re-generate the imaginary data for processed 2D data. These commands are normally used with processed 2D and 3D data from the NMRPIPE program.

## Auto Reference F1

An automatic referencing of F<sub>1</sub> for many experiments can be accomplished using the Auto Reference F1 sub menu in the Processing menu. Select the F<sub>1</sub> nucleus and solvent appropriate to your data. This procedure assumes that the F<sub>2</sub> dimension is proton, and has been correctly referenced. The reference values used are valid for dilute solutions at 25°C. A homonuclear option will set the referencing in F<sub>1</sub> to be the same as that in F<sub>2</sub>. This command makes use of the constant ratio of the reference peak in proton vs. that of



the X nucleus, for a given solvent and temperature.

### Hilbert Transform

This command will re-generate the imaginary data if it is not available. This would most likely be used with JCAMP-DX data where the imaginaries are not available. You would need the imaginary data if you wanted to phase the spectrum.

### Subtract Trace 2 from Experimental

This command can be used to create a difference spectrum by subtracting trace 2 from the current experimental spectrum. For example, to process an NOE difference experiment:

1. Process the reference (off resonance) spectrum normally with the **Process** button command (or **Processing** menu) followed by phasing. Do not apply baseline correction at this point.
2. Transfer a copy of the processed reference spectrum to trace 2 with the **Copy Experimental to Stack** command in the **View** menu.
3. Open one of the on-resonance NOE experiments (**File** menu) and process it with the **Process** button (or **Processing** menu). Do not use baseline correction.
4. Use the **Subtract Trace 2 from Experimental** command (**Processing** menu) to replace the current experimental spectrum with the difference between the two. If necessary, apply baseline correction now.
5. Peak pick, integrate, and plot the difference spectrum as desired
6. Repeat steps 3 to 5 for each of the on-resonance NOE experiments in the run.
7. Anyone with gradients will, of course, be using the GOESY experiment instead. ☺

### Add Trace 2 to Experimental

This command can be used to create the sum of two spectra by adding trace 2 to the current experimental spectrum.

### MQMAS Toolbox...

Use this dialog to calculate the required phase angle or adjust the referencing for the 2D Shearing Transform.

### 2D Shearing Transform

Applies a  $T_1$  dependent first order phase correction to the rows of a 2D data set. This transform is used to generate orthogonal MQ and MAS dimensions in a MQMAS experiment. The phase angle required depends on the spin of the nucleus and, the quantum selection of the experiment, and the spectral widths in both dimensions. The shearing angle is most conveniently calculated using the MQMAS Toolbox... Dialog (2D Processing sub-menu).

### FID Math Toolbox...



This dialog can be used for FID manipulation. Hypercomplex 2D data can be split into separate files, FIDs can be phased and combined in various ways. Please see the appropriate section of this manual for details. These are advanced features, and are not required for typical 2D processing.

### Convert Field (For 2D reference)

Occasionally, it is handy to use a 1D spectrum recorded at a different field as a reference for a 2D spectrum. For example, we often record superb HSQC spectra on our AMX500. However, we don't have a decent  $^{13}\text{C}$  observe probe for this instrument. With the field convert feature, we can use a  $^{13}\text{C}$  spectrum recorded on our INOVA 600 or Avance 300 as a reference spectrum.

The Field Convert function has a dialog which will display the current transmitter frequency, and allow you to enter the target transmitter frequency. Make sure that you use the transmitter frequency. This will be SFO1 (or SFO2 or SFO3) for Bruker data and sfrq (or dfrq or dfrq2) for Varian data. After the conversion process, save the spectrum as a JCAMP-DX file. This file can then be used as a 1D reference spectrum for 2D spectra.

Note that this procedure is most appropriate for fully decoupled spectra like  $^{13}\text{C}$ , and would not generally be useful for proton spectra.

## Peaks and Integrals Menu Commands

### Pick Peaks and Append to List

Picks the peaks in the currently displayed region and appends them to the peak list. The minimum intensity for peak picking is displayed as a dashed line across the screen and can be changed by dragging it with the small box displayed at the left end of the line. This value, along with the noise discrimination parameter can be changed manually with the 1D Processing Parameters... dialog in the Edit menu.

### Clear Peak List

Erases all of the picked peaks.

### Clear Peaks in Region

Erases only those peaks in the displayed region.

### Peak Pick or Integrate Stacked Array

Picks a peak at the cursor defined frequency in an arrayed experiment. You must use one of the Process Array command in the Processing menu first. If a frequency range is defined with two cursors than the integral over that region is listed.

### Load 1D Peaks

Loads previously picked peaks. Note that the referencing must be identical to the original spectrum for the peak labels to be accurate.

### Load 2D Peak Assignments

Loads previously assigned 2D peaks.

#### List Peaks

Displays the peaks in for possible printing. The program will use whatever program is defined for a “.txt” file – usually MS notepad or WordPad.

#### List Integrals

Displays the integrals in for possible printing. The program will use whatever program is defined for a “.txt” file – usually MS notepad or WordPad.

#### Interpolation

Determines whether parabolic peak interpolation is to be used for the calibration and peak assignment routines. If turned OFF, the raw cursor frequency will be used instead. The default value is ON.

#### Sign

Determines whether only positive peaks, negative peaks, or both positive and negative peaks should be picked.

#### Units

Selects PPM or Hz as the units for the on-plot peak list and display. This is independent of the axis units.

#### Digits to Right of Decimal (Peaks)

Sets the number of digits displayed and printed to the right of the decimal for peak picking.

#### Digits to Right of Decimal (Integrals)

Sets the number of digits displayed and printed to the right of the decimal for integrals.

#### Calibrate...

Sets a particular peak or point in the spectrum to a particular shift. Enter in the same units as the axis display. Also available on the button panel.

#### Bin data

Divides the data set into a set of “bins” and reports the integral, mean or maximum value in each bin. The binning method, bin width, and start and stop points can be set with the Edit Binning Parameters... dialog available in the Edit menu.

The peak pick minimum intensity and noise threshold can also be entered in this menu as the last two items.

## Commands Available with the Command Line

### Processing Commands:

The following commands can be used to process 1D data. Many of the commands require that the appropriate processing parameters (e.g. LB or GF) be set first. This can be most easily done with the **Processing Parameters** dialog (Edit menu or **edp** command), or by giving one of the parameter commands listed below. Many of the commands have several aliases. This is so that the commands will hopefully be familiar to both Bruker and Varian users.

- **ft**            Fourier transform of FID.
- **ef**            Window multiplication followed by Fourier transform. The window function used is specified by the Window parameter (toolbar of **Processing Parameter** dialog)
- **efp**            Window multiplication followed by Fourier transform and phase correction with previous phase constants. (Alias: **wft**) Note that commands like **gfp**, **trafft**, etc. that were available in earlier versions have been removed. To use these window functions, set the window parameter (toolbar or **Processing Parameter** dialog) and use the **efp** command.
- **process**    Process the data (1D or 2D with the window and phasing parameters defined in the processing parameters (toolbar or dialog). Alias: **proc**.
- **pk**            Apply previous phase constants. (alias: **phase**)
- **pk\_f1**        Apply previous phase constants in F1. (alias: **phase\_f1**)
- **apk**          Automatic phase correction.
- **abs**          Automatic baseline correction using a polynomial and defined points.
- **fabs**          Automatic baseline correction with automatic Whittiker smoother.
- **rev**          Reverse spectrum, low field to high field.
- **hw**          HOGWASH enhance the displayed region of the spectrum. You must define the parameters in the **HOGWASH Parameters** dialog (Edit menu or **edhw**) first. Alias: **hogwash**

### File Handling Commands

- **open**        Opens a new NMR data set.
- **re #**        Reads experiment number # for UXNMR/XwinNMR data only. For example, if you are currently working on the proton spectrum **mydata/1** and you want to switch to the carbon spectrum **mydata/4**, using the command **re 4** will switch you to the carbon data set without going through the file open dialog. Alias: **read #**.

## Simulation Commands

The following commands can be used to run the various SpinWorks simulation routines. The program must be in the correct mode (NUMMRIT or DNMR/MEXICO) before running the simulation, and you must edit the parameters first (Spin System or Simulation menus).

- **sim** Run a NUMMRIT simulation.
- **dnmr** Run DNMR simulation.
- **mexico** Run MEXICO simulation.

## Parameter Editing Commands:

- **edproc** Edit processing parameters (1D and 2D). Alias: **edp**.
- **edplot** Edit plotting parameters.
- **edshifts** Edit the chemical shifts for a simulation.
- **edtitle** Edit the plot title. Alias: **setti**.
- **edj** Edit scalar coupling constants for a NUMMRIT or DNMR simulation.
- **edd** Edit dipolar couplings for a NUMMRIT simulation.
- **edsim** Edit general simulation parameters for a simulation, including DNMR parameters.

## Parameters that Can Be Set with the Command Line:

Many SpinWorks parameters can also be entered on the command line. They are entered by typing the number followed by the value. e.g. **lb 1.2** changes the value of the **LB** parameter to 1.2 Hz.

- **lb** Exponential line broadening, in Hz.
- **gf** Gaussian broadening factor (Bruker style).
- **size** Transform size in complex points. i.e. 8182 means 8192 real points plus 8192 imaginary points. Aliases: **si**, **fn**. This value must be a power of 2, and will be rounded up to the nearest power of 2 if it isn't.
- **ph0** Zero order phase constant. Alias: **PHC0** and **phase0**.
- **ph1** First order phase constant. Alias: **PHC1** and **phase0**.
- **ph0\_f1** Zero order phase constant in  $F_1$ . Alias: **PHC0\_f1** and **phase0\_f1**.
- **ph1\_f1** First order phase constant in  $F_1$ . Alias: **PHC1\_f1** and **phase0\_f1**.
- **vscale** Sets the vertical scaling of the spectrum. The current vertical scaling is shown just above the axis at the left side of the screen. Alias: **vs**
- **floor** Adjusts the 2D contouring floor (lowest displayed level) by the given factor. e.g. **floor 1.5** will raise the floor by a factor of 1.5.
- **range** Adjusts the range of the 2D contours (the difference between the lowest and

highest levels) by the given factor.

## Format of SpinWorks Processed Data

SpinWorks writes its processed data in the same folder as the original FID file. The processed data consists of several files:

- 1D\_par, 2D\_par, 3D\_par      These are the parameter files for the processed data. They are “human readable” ASCII text files, and the parameters should be self-explanatory. Note that the **SF** parameter is the frequency (in MHz) of 0 ppm. This is equivalent to the Bruker SF parameter.
- 1D\_real, 1D\_imag      The real and imaginary parts of a 1D processed spectrum. The data are signed 32 bit integers, with the byte order of the INTEL processors.
- 2D\_rr,..., 2D\_ii      The four hypercomplex quadrants of a processed 2D spectrum. The data are signed 32 bit integers (INTEL byte order), and are stored in a row-major fashion.
- 1D\_integ, 2D\_integ      ASCII integration region files for 1D and 2D data.
- SW\_3D (folder)      The folder containing the SpinWorks processed 3D data, containing:
  - 3D\_XYt\_rr.1      etc. 3D planes processed in X and Y but time domain in Z.
  - 3D\_XZt\_rr.1      etc. 3D planes processed in X and Z but time domain in Y.
  - 3D\_XYZ\_rrr.1      etc. 3D planes processed in X, Y and Z.

Note that several SpinWorks commands (e.g. peak picking, simulation, etc.) also produce ASCII output files that are also stored in the current data directory.

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