

# SpinWorks Documentation, Version 2.2 (09/2003)

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# Introduction

## What is SpinWorks

SpinWorks has two functions: The first is to provide easy basic off-line processing of 1D NMR and 2D 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 UNIX Xsim program.

SpinWorks 2.2 is the third release of first SpinWorks version to contain 2D processing. Full support is included for Bruker (XwinNMR/UXNMR) and Varian (Unix VNMR) data formats. Included  $F_1$  detection modes include States, TPPI, States-TPPI, Single Detection (QF), and echo-antiecho. There have also been some improvements and bug fixes in the 1D and simulation routines, and these should be at least as stable as those in version 1.3.

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 only 1 data set at a time. However, most new computers have sufficient memory to run two or three copies of SpinWorks simultaneously. This can be very useful when examining the rows and columns of a 2D data set.

### Things that don't yet work or need improvement:

1. 2D display in contour mode is still a bit slow, especially on older computers and at very low contour levels. If you find the contouring too slow, it is best to stick to image mode except on expanded regions of the spectrum.
2. As always, the manual and help files aren't quite up to the level of the software. I like writing code a lot better than writing help files. In particular, the dialog pictures in this manual may not be quite up to date with those in the latest release.
3. In 2D mode, the program 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. Future versions may store imaginary data on disk after transform and phasing. 2D SpinWorks is currently about 20% faster than XwinNMR on the same computer. 2D matrices up to 2k by 1k shouldn't create problems on a machine with 64 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 on my 256 MB machine. Considerable additional memory is used transiently during transform and display contouring.

### Sample 2D transform timings (with image mode display) on a 1.7 GHz 256 MB P4 under Win2000:

- 4k by 512 States HSQC with phasing applied in  $F_1$ : 6 sec.

- Comparison: XwinNMR 2.6: 7 sec.
- Comparison: VNMRJ, Sun Blade 150: 14 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 3 to 4 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 (2D Processing and Display dialog). You may have to click several times if the level was much too low.

### **Computer Requirements**

SpinWorks requires a 486 or higher processor (Pentium recommended) running Windows 95, 98, NT 4.0 or Windows 2000 Pro (NT 4.0 or Win 2000 recommended). Windows ME and XP are probably O.K., but are untested. Installation currently requires about 5 Mbytes of disk space exclusive of NMR data. 32 Mbytes or more of RAM are recommended, depending on NMR data set and simulation sizes. SVGA 800 x 600 or better display required (1024 x 768 or better recommended). For 2D processing a Pentium class processor with 64 Mbytes of memory is the practical minimum. For 2D you should also have your display set to at least 16 bit colour, otherwise the image and contour levels will be strange. I have received reports (but have not confirmed) that SpinWorks will run under Linux with the WINE package and on a Mac with SoftWindows.

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 also serves as the middle mouse button.

### **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\_2.2.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. The installation procedure will add the program to the Start menu and it is an easy matter to make a shortcut on the desktop. Please read the license agreement and agree with the conditions before proceeding.

### **Registration**

Although SpinWorks is currently being distributed as Freeware, and there are no license keys or

“dongles” required, I do, however, request that a SpinWorks user register their use by email to:

[kirk\\_marat@umanitoba.ca](mailto:kirk_marat@umanitoba.ca) (**Please note changed address**)

Please include: an email contact, the machines and OSs that you are running it on (e.g. Pentium 266 Win 98), 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.

## 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, Woody Connover, Mike Coplien, Klaus Eichele, Mike Englehardt, Phil Hultin, Vera Mainz, Virginia Miner, Gareth Morris, Rudy Nunlist, Georgy Salnikov, Rich Shoemaker and David Vanraes. My apologies to anyone I may have missed.

## Obligatory Disclaimer

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.

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## Online Help

Standard Windows type context-sensitive help is available. Most of the help files are extracted from this manual, but may not be complete. In version 2.2, this manual is available from the help menu as a pdf file.

## What's New in Release 2.2

1. The most important new feature in Version 2.2 is that the program automatically saves and loads processed data. (Varian VNMR and Bruker XwinNMR/UXNMR data only) This can save considerable time when working with 2D data sets. When you select an *fid* or *ser* file, SpinWorks first looks in the data folder for SpinWorks processed data. If found, these processed data (spectra) will be loaded and displayed. Size and reference information are also read, and will take precedence over the size and reference information stored with the raw data by XwinNMR or VNMR. If you do not want SpinWorks saving and loading processed data, this feature can be disabled by unchecking the appropriate boxes in the **Set Preferences...** dialog of the **Options** menu. Note that SpinWorks *never* modifies the parameters or data of the original data set.

2. 2D Spectra can be plotted in a square format with parameters at the right side of the spectrum. A grid can be included on a 2D plot.
3. The integral and peak pick listings now display an absolute intensity as well as a relative intensity. This can be used to compare intensities between spectra.
4. Drift correction (offset and slope) baseline correction has been added to 2D processing. This is essentially polynomial baseline correction of order 1, and can be useful when the usual polynomial baseline correction can't find enough baseline points for a good fit.
5. First-point correction parameters have been added to 2D processing.
6. The tracking cursor (or 2D cross-hair cursor) can be toggled on and off by double-clicking in the data window.
7. A link (button) to the 2D parameter editor has been added to the 2D processing and display dialog.
8. A link (button) to the 1D parameter editor has been added to the 1D processing dialog.
9. A command line option has been added. Many of the menubar and toolbar commands are now duplicated with keyboard commands. Many of the commands actually have several aliases, and often duplicate the equivalent function in XwinNMR or VNMR. Although a command line may seem like "creeping featurism" in these days of menus and dialogs, a command processor is a first step towards a macro processor.
10. A Gaussian mask function has been added to the HOGWASH routine.
11. HOGWASH enhancement has been added to the  $F_1$  dimension of 2D spectra.
12. Negative 2D contours can be printed in a different colour, if desired.
13. The  $F_1$  linear prediction routine is more stable.
14. The pdf version of this manual can be displayed from the **Help** menu. This requires that a copy of the Adobe Acrobat reader be installed on the computer.
15. Integration regions can be saved to a disk file.

## **Use of the Mouse, and General Display Manipulation**

While the cursor is in the data window, the mouse 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.


The *right* mouse button (on both two- and three-button mice) 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). (This is somewhat counter to standard Windows behavior, but is consistent with Xsim and I couldn't figure out a better way to do it.)

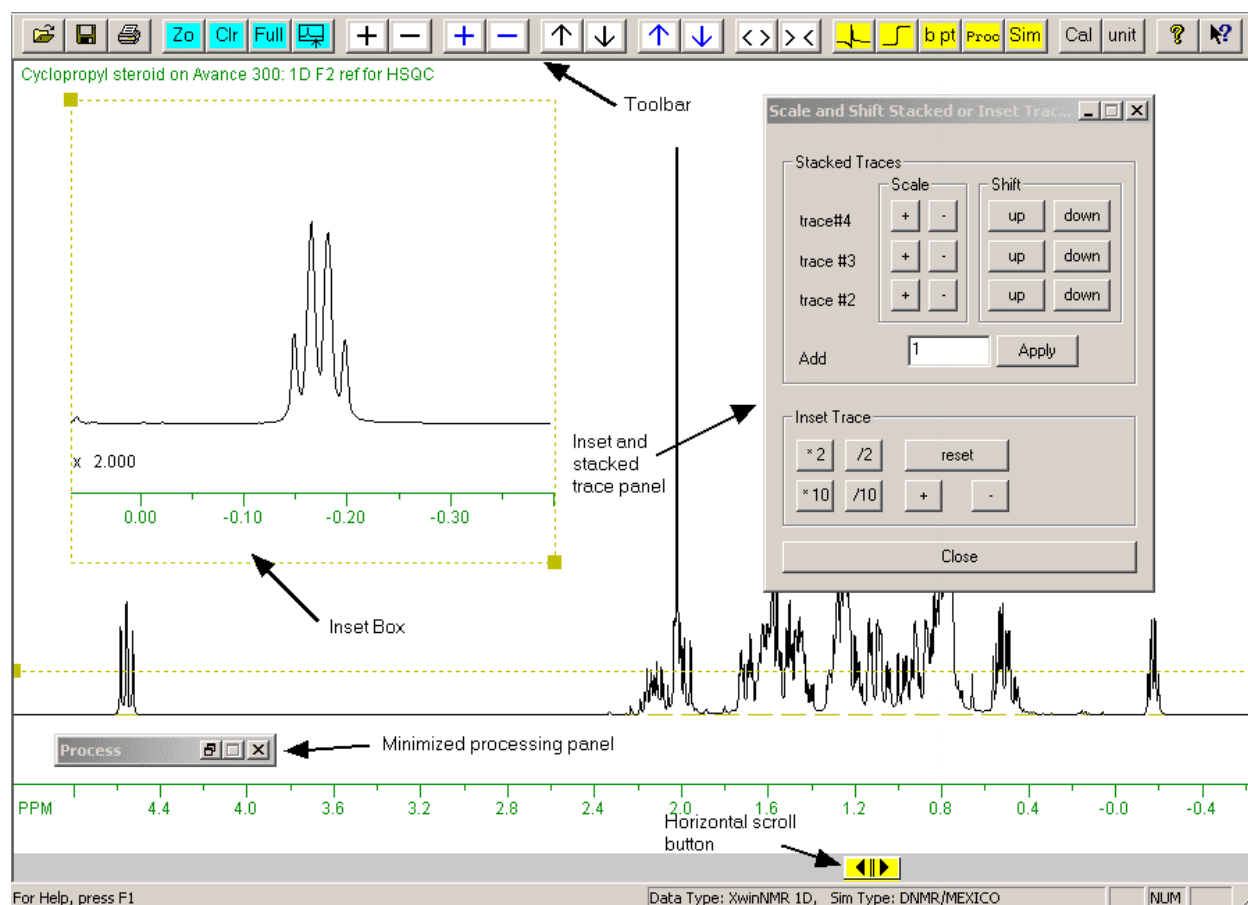
In 2D mode the *right* mouse button selects rows or columns of the 2D matrix (e.g. for phasing).



Spectral regions can be zoomed by defining two reference points and clicking the **zoom** button on the toolbar or the middle button of a three-button mouse. Spectra can also be expanded and contracted with the buttons labeled **< >** and **> <**. A tracking vertical cursor line can be turned on/off with the appropriate selection in the **Options** menu. The scrollbar at the bottom of the data window can be used to pan through an expanded spectrum. 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 toolbar **+** and **-** buttons. The black buttons are for the experimental spectrum and the blue buttons are for the simulated spectrum. Spectra may also be scaled in smaller steps with the keyboard up and down arrow keys. The toolbar up and down arrow keys are used to shift 1D spectra vertically.

It is possible to expand a region of a 1D spectrum and display it in a separate inset box. Simply define the region with the cursors and click on the  button on the toolbar. The cursor-defined region will be displayed in a re-sizable box and a dialog box for scaling the inset will be displayed as well. The inset trace can be turned off and back on at any time with the **Show Inset Box** selection in the **View** menu. 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 on the inset just above the axis. 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.



## 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

**Left and Right arrows** Move the simulated spectrum transition cursor. Holding the **control** key down at the same time moves the cursor in steps of 5 transitions


**Up and Down arrows** Adjust the vertical scaling of the experimental spectrum. Holding the **control** 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.

- “t” key** Toggles the tracking cursor (2D cross-hair pointer) on or off. This is an alternative to using the **Options** menu.
- “r” key** Refreshes the display. Occasionally, the tracking cursor or the 2D cross-hair cursor can leave behind marks on the screen if it has been covered by a dialog. To remove these traces simply hit the “r” key.
- “a” key** 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.
- “d” key** Deletes any assignment for the currently pointed to transition.

## Toolbar

The toolbar is displayed across the top of the application window, below the menu bar. To hide or display the Toolbar, choose **Toolbar** from the **View** menu. The toolbar is “dockable”, meaning that it can be moved to any convenient location. The toolbar provides quick mouse access to many tools used in SpinWorks:

- Open an existing NMR data set. SpinWorks displays a dialog for selecting data sets.
- Print the current NMR spectrum.
- The blue buttons labeled **Zo** **Clr** and **Full** Zoom the spectrum between red two cursor lines, Clear the cursors and return to the full spectral width, respectively. Note that if you have defined a region with two cursors lines, you can use the middle mouse button (if you have one) to zoom in place of the blue **Zo** button.
- The blue  button transfers a cursor-defined region to a re-sizable inset box. This box may be re-sized with the handles located at the upper left and lower right corners. The vertical scaling of the inset is independent of the scaling of the main spectrum display. Not available in 2D.
- The + and – 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, these buttons adjust the scaling of any displayed rows or columns.

- The toolbar up and down arrow buttons shift the spectra up and down the screen. In 2D mode these arrows select which of any extracted rows or columns is highlighted or “active”.
- The < > and >< buttons expand and contract the spectra horizontally. Usually, it is easier to use the cursors and the ZO button, but some routines (e.g. integration) preempt the cursor for their own purposes. Not available in 2D mode.
- The yellow button with a picture of an out of phase peak starts the interactive phasing routine. (1D and 2D)
- The yellow button with the picture of an integral trace starts the integration routine. Not yet available in 2D.
- The yellow button labeled **b pt** starts the baseline point routine. Not available in 2D mode.
- The yellow **Proc** button is used to start a processing dialog that can be a convenient alternative to the commands in the **Processing** menu. (1D and 2D)
- The yellow **Sim** button is used to start a simulation dialog that can be a convenient alternative to the commands in the **Simulation** menu. The **Proc** and **Sim** pop-ups help prevent “mouse wrist” ☺. Not available for 2D.
- The **Unit** button toggles the axis units between Hz. and PPM.
- The **Cal** 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.

## Getting Started: Basic 1D NMR Processing

### 1. Setting the Data Format

SpinWorks needs to know the format of any data before it can be processed. The current default format is UXNMR, which is appropriate for data from a Bruker AMX, ARX, ASX or Avance series spectrometer. In order to process data from other spectrometers, use the **Data Format...** selection in the **Options** menu. The correct format for a Bruker AM or AC spectrometer is DISNMR. Use the **Varian** setting for Varian Unity, Unity+ and Inova 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 current data format is displayed in the status bar at the bottom of the screen. The **Set Preferences...** dialog in the **Options** menu can be used to change the default data format. JEOL data support is limited to the "JEOL generic" format. Current JEOL spectrometers do not use this format, but can convert their data into this format. TECMAG support is for NTNMR. Neither the JEOL nor the TECMAG formats have been extensively tested.

1D Data can also be read and saved in JCAMP DX format.

If there is sufficient interest, I *may* add support for Bruker WinNMR and Varian Gemini files

Note that 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. For JEOL data, both the .bin and .hdr files must be present in the same 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 or similar.

## 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" menu is presented 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.

**Bug:** If a data set is selected with the program set to the wrong data type (e.g. trying to read DISNMR data with the program set to UXNMR/XwinNMR) an error message will be displayed. However, you will not be able to select the *same* data set after changing to the correct data format unless you either:

1. Select a different data set first or
2. Exit and re-start the program. Hopefully, this will be fixed shortly.

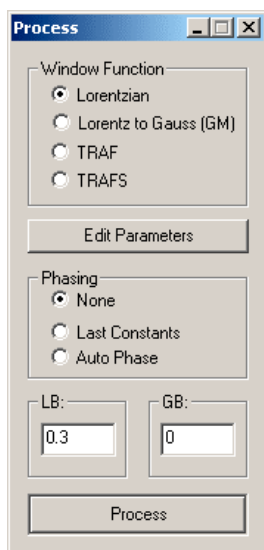
**Data on CD:** SpinWorks occasionally writes scratch files or other information, usually in the same folder as the experimental data. This creates a problem for data on CDs where you cannot usually (except for CD-RW) write data. Therefore, data on CD *must* be transferred to the hard disk before processing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

### 3. Processing Data

Data processing involves application of a window function (Exponential Multiplication) followed by Fourier transformation. These two processes are combined in the **em+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, but a reasonable parameter is set by default. After processing, a spectrum will be displayed on the screen.

Resolution enhancement may be applied by setting the LB parameter to a negative value, with the negative of the natural line width being a good starting point. The GB 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 GB to  $0.33$ . Then process the fid with **gm+ft+pk**. Some experimentation may be required. If you are re-processing the same data that you have just processed and phased, then commands with a **+pk** appended will phase the data with the last phase constants known to SpinWorks. Note that resolution enhancement may alter the relative integrals of the signals.

As an alternative to using the menu bar commands for processing, a processing dialog box can be started from the **Proc** button on the toolbar. The minimize (iconize) button can be used to keep this



panel handy for use at any time.

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.

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 linewidths of the peaks in the absence of any apodization, but some experimentation may be required. 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).

HOGWASH resolution enhancement is left for a later section.

#### **4. Linear Prediction**

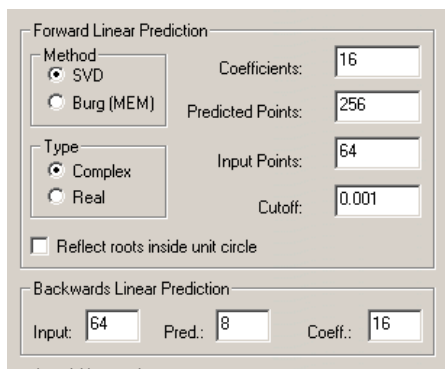
Backward linear prediction is useful for the removal of artifacts (baseline roll) resulting from probe ringdown, acoustic ringing and wideline probe background. In this procedure the first few points of the FID are discarded and replaced with a backward extrapolation of the remaining data. Forward linear 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.

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 4 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 then performed using the **LP Process 1D (Backward)** selection in the **Processing** menu. The FID can then be processed normally with any of the usual 1D processing functions in the **Processing** menu or the pop-up **Process Dialog**. If the selection of LP parameters was inappropriate make the necessary changes, re-apply the LP, and re-process. Note that backward LP in SpinWorks uses the SVD algorithm.

For forward LP, the SVD method is generally preferred to the Burg method. The “reflect roots inside unit circle” checkbox applies only to the Burg method, and should generally remain unchecked. The cutoff parameter specifies a significance level for the coefficients. The default value is usually good, but may be raised to speed calculation.<sup>†</sup>

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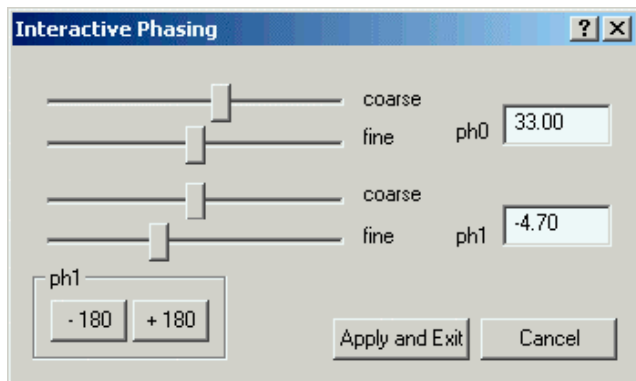
<sup>†</sup> For a good discussion of linear prediction see: NMR Data Processing by J.C. Hoch and A. S. Stern, Wiley-Liss, NY (1996) (ISBN 0-471-03900-4).



For the use of forward LP in two-dimensional spectra, please see the 2D section of this manual.

## 5. Phasing Data

After processing, the resulting spectrum must be phased. The phasing mode is entered by clicking on the **phase** button on the toolbar. A phase dialog box will be displayed and the zero-order reference point will be marked with a vertical green line. 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. Version 2.0 added 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 enter apply phase constants, use the **Edit Processing Parameters** or **Edit 2D Processing Parameters** dialogs (Edit menu).



## 6. Baseline Correction

For accurate integration it will be necessary to apply a baseline correction to the data. Click the yellow **BPTs** button in the toolbar. Then use the cursor to define at least 6 baseline points in the spectrum. Click the pop-up **Return** button to exit the baseline points mode. Then select **Automatic 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. For spectra that have well defined regions with peaks and baseline, there is now a fully automatic baseline correction function available in the **Processing** menu.

## 7. Integration

To integrate the spectrum select the yellow **integrate** button from the toolbar. 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 start and end 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, 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 **Delete All** button of the integration dialog. In SpinWorks 2.2, 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.



## 8. Peak Picking

Peak frequencies are picked with the **Pick Peaks** and **Append to List** selection in the **PeakPick** menu. The minimum intensity for peak picking is shown with a dashed line across the screen. The height of this line can be set by dragging it with the small box at the left end of the line. 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 **PeakPick** menu. Specific peaks can be deleted with the **Clear Peaks in Region** selection.

A Notepad list of frequencies may be generated with the **List** selection of the **PeakPick** menu. This list may then be printed or saved to disk if desired. The **Peak Pick (On Plot) 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 **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 **Cal** toolbar button. A dialog will then ask for the calibration frequency. The value is entered in Hz. or PPM, depending on the currently used axis unit.

## 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. In order to change to portrait orientation, select **Edit Plot Options and Parameters...** in the **Edit** menu and uncheck the box labeled **Force Landscape Orientation**. The plot orientation can then be set to either portrait or landscape via the **Print 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.

A plot title may be entered or edited with the **Edit Plot Title...** selection in the **Edit** menu. For Uxnmr/XwinNMR data the existing plot title as defined on the spectrometer will be read, and can be edited if desired.

Note that some printer drivers are broken with respect to the rotated font used for peak frequencies and integral values. The Win 95 drivers for the HP LJ6 seem especially bad (Win NT 4.0 and Windows 2000 seem OK). All print drivers seem to show the peak and integral labels slightly displaced in **Print Preview** although they may be correct on the actual printed page. The errant drivers rotate the peaks the opposite direction in **Print Preview** and on the printed page, with the integral labels overlapping the axis and the peak labels overlapping the label lines. If you find that this is the case, use the **Set Preferences...** dialog in the **Options** menu to select **Alternate Peak Rotation**.

## 10. Printing to a MetaFile

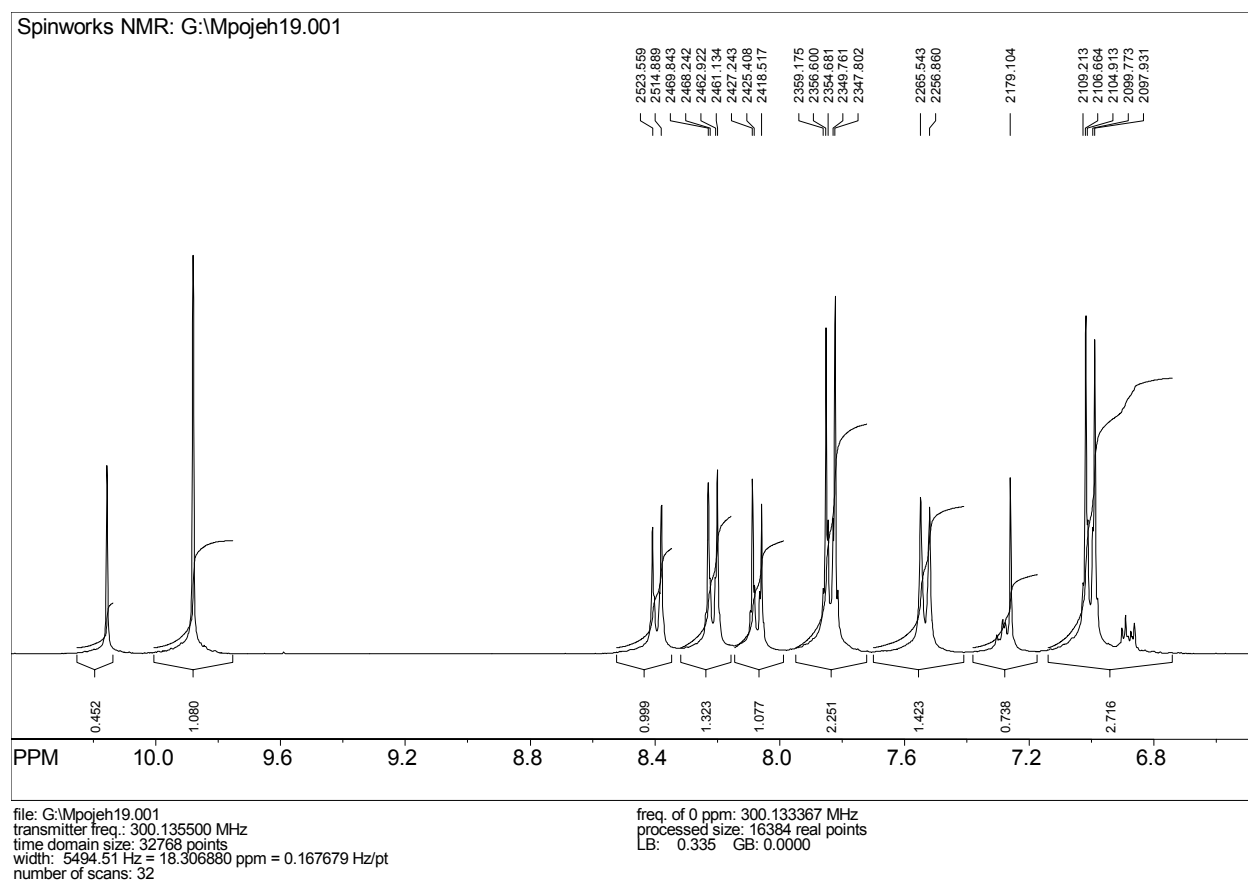
The **Copy to MetaFile...** command in the **Edit** menu can be used to produce a copy of the spectrum as it is set up to print as a Windows Enhanced MetaFile (\*.emf). This file can then be imported into Word (e.g. with **Insert: Picture: From File**), PowerPoint, etc. This command opens up a file save 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. One possible problem is that the scaling of the

SpinWorks graphics are often too big for a Word or PowerPoint document (due to the difference in units used by the different programs). The solution is (in Word or PowerPoint) to reduce the zoom factor (the list box on the toolbar with things like “75%” on it) to about 25 %. Then use the resize handles on the imported SpinWorks graphics to size the spectra appropriate to the document. The zoom factor can then be restored to whatever value you normally use.

You can also copy SpinWorks spectra to programs like Word and PowerPoint with the Windows Clipboard. The **Paste Special...** function seems to work best, allowing the Spectrum to **Float over Text**. This method seems to incorporate spectra at the correct size, at least for Word.

SpinWorks 1.3 had difficulties using the Windows XP clipboard. This has been corrected as of SpinWorks 2.0.

Metafiles are created with a resolution that is greater than screen resolution (the default is 2 times screen resolution) but with less resolution than that on printed spectra. To increase the resolution of metafiles you can use the **MetaFile Resolution** parameter in the **Plot Options and Parameters...** dialog (**Edit** menu). Using higher resolution will use more disk space for the metafiles and any documents that incorporate them.



**Figure 1** Student spectrum with poor shim imported into Word

## 2D Processing

### Introduction to 2D Processing

SpinWorks now has the capability to process 2D data sets from UNIX and NT based Bruker (A?X and Avance) and UNIX based Varian (Inova, Unity, Mercury, Gemini) spectrometers. Provision has been made for Hypercomplex (States, States-TPPI, echo-antiecho), TPPI and Magnitude mode data. Echo-antiecho Varian data did not work properly prior to release 2.0 PL6.

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

In the **Options** menu set the **Data Format** according to the brand of spectrometer that you are using (Bruker or Varian) and also set the 2D selection. The default data format can be changed in 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 tries to get 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. Separate panes are available for the detection ( $F_1$ ) dimension and the evolution ( $F_2$ ) 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** This is the value (in MHz) used as the calibration reference point in the spectrum. 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** ( $F_1$  only) 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.
- **Reverse** ( $F_1$  only) Check this box if you find that the  $F_1$  direction needs to be reversed. This is pulse sequence and spectrometer model dependent.
- **First Point Correction** Applies a multiplier to the first point of the FID. The default values of 0.5 is good for the vast majority of data sets. Experiment with the values if you need to remove some baseline offset in the transformed spectra. An alternative is to apply drift correction (2D Processing menu) after processing.
- **Window Function** This is the pre-transform apodization function applied to the data. Choices are: none, Lorentz, Gauss (actually Lorentz to Gauss or the Bruker "GM" function), Sine, Sine squared, TRAF and TRAFS. 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 Lorentz, Gauss, TRAF and TRAFS functions.
- **Linear Prediction** Specifies the parameters used for linear prediction of the data set. This is typically used in  $F_1$  only, when the number of time domain data points is small. You can specify the number of points to be predicted, the number of coefficients (Maximum entropy poles), the number of input points used for prediction, and whether you want forward or reverse prediction. In version 2.0, 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. Typically, you can usually double the number of time domain data points in  $F_1$ , 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.

There is also an alternate forward LP routine that can be used by setting the LP type to **Forward (old)**. This routine can sometimes provide better resolution than the "standard" forward LP routine, but it also has a tendency to "blow up" on noisy data. There are two algorithms that can be used for linear prediction. The first is the singular value decomposition (SVD) method, and the second is the Burg or maximum entropy (MEM) method. SVD almost always works better, but you can experiment to see which works best for your data. The Burg and SVD settings apply only to the "old" LP algorithm.

**Note:** In *most* cases simply selecting **Forward** and leaving all of the other parameters at their

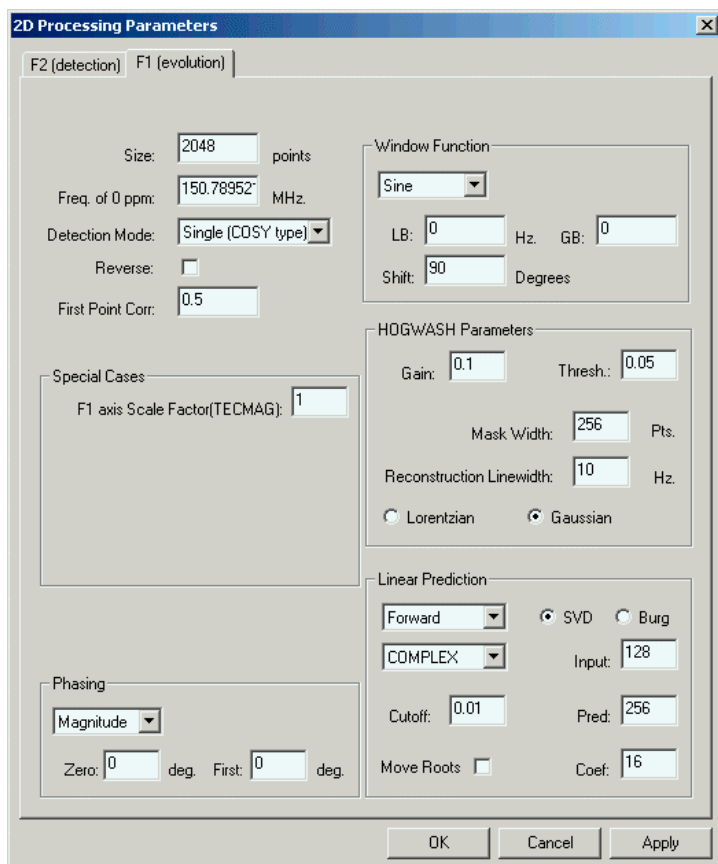
default values will provide the best results.

**Note:** Linear prediction works well then the 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 dimension) it is *always* better to increase the resolution by increasing the acquisition time (narrowing SW or increasing TD) 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.

**Ignore** the Move Roots Into Unit Circle checkbox.

- **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  $F_1$  dimension for magnitude COSY and HMBC) and **power**. Note that applying magnitude or power spectrum processing to the detection dimension ( $F_2$ ) would be pretty silly.
- **HOGWASH** parameters. These are described separately in the HOGWASH section of this manual.

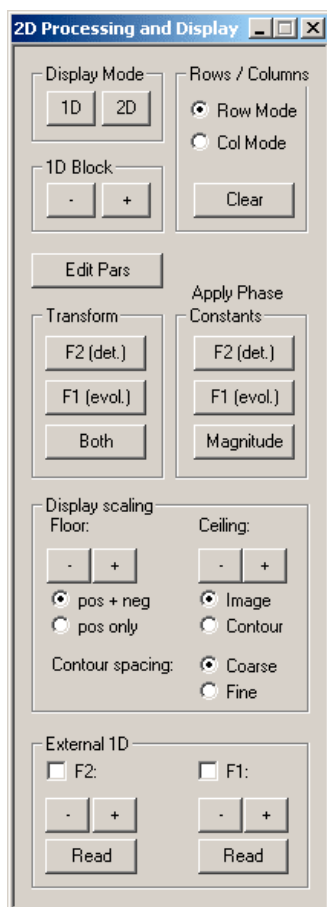


## Processing 2D Data

### Transform

Once you have selected your 2D data and have set the processing parameters, you will need to process it. In 2D mode the yellow **Proc** button on the toolbar will start the “stay-up” 2D processing and display dialog box. This box can be minimized at any time if desired. Although you can process the  $F_2$  and  $F_1$  dimensions separately, it is usually easiest to click in the **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.)

Some less frequently used 2D processing commands are 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 in the 2D Processing and Display dialog. 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 **ceiling** buttons. Note that **-** means moving the floor level down, or closer to the noise. Therefor click the **-** button to see smaller peaks. Similarly, moving the ceiling 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 ceiling so that the highest peaks are displayed in the most intense colours. For spectra with very large dynamic range, lowering the ceiling even further will give more contours on the smaller peaks at the expense of the more intense peaks.

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

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 **Zo** button on the toolbar. The cross-hair lines can be erased by clicking the left mouse button a third time or with the blue **Clr** button on the toolbar.

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 **column mode** in the **2D Processing and Display** dialog box 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 2.1), columns cannot be transferred to the 1D display mode. However, columns can be saved as JCAMP-DX files (see below).

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 (toolbar **+** and **-** buttons). The currently active trace can be changed with the toolbar up and down arrows.

**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 **Options** menu. You can use the keyboard “t” key as a shortcut to toggle the cross-hairs on or off. Occasionally, if the cross-hairs have been overlapped by a pop-up dialog, there will be stray pieces of cross-hairs left on the screen when the dialog is moved or closed. You can clear these cursors by refreshing the screen with the keyboard “r” key.

## Phasing

There are several ways in which the phase sensitive data may be phased.

- 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  $F_2$  (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.
  - Select either the row mode or column mode in the 2D Processing and Display dialog (use the yellow PROC toolbar button if this dialog is not already active).
  - 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 + or – buttons on the toolbar. The highlighted trace only will be changed. Which trace is highlighted can be changed with the toolbar up and down arrows.
  - 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.
  - 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.
- Now phase the other dimension, if required. Note that most Varian pulse sequences are written so that the  $F_1$  phase correction is zero (or very close to zero). In such cases no  $F_1$  phase correction is usually required. However, due to differences in algorithms, some data that have zero  $F_1$  phase correction in VNMR may require exactly a 90 degree correction in SpinWorks.

### 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 third order polynomial is normally used, but this can be increased with the Edit Processing Parameters dialog. If the baseline is offset or sloped, but not very curved, It is probably 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.

### F2 and F1 Reference Spectra

Processed 1D reference spectra can be displayed and printed along the  $F_2$  and  $F_1$  dimensions.

- Process the 1D data normally and save the processed spectra as JCAMP-DX files (File menu).

- After processing your 2D data, use the External F2 and F1 buttons in the 2D Processing and Display panel to Read in the JCAMP-DX files as "projections."
- The vertical scaling can be adjusted with the + and - buttons, and the individual traces can be turned on or off with the appropriate check box.
- Note that the 1D reference spectra do *not* have to have been recorded with the same SW as the 2D spectrum. They do, however, have to be recorded at the same field.

## 2D Processing Tutorial

A number of 2D data sets are available for download. They are in the file *sample\_2D\_data.zip*, and contain data acquired on both Varian and Bruker spectrometers, with a number of detection modes.

### Magnitude (COSY type) Data

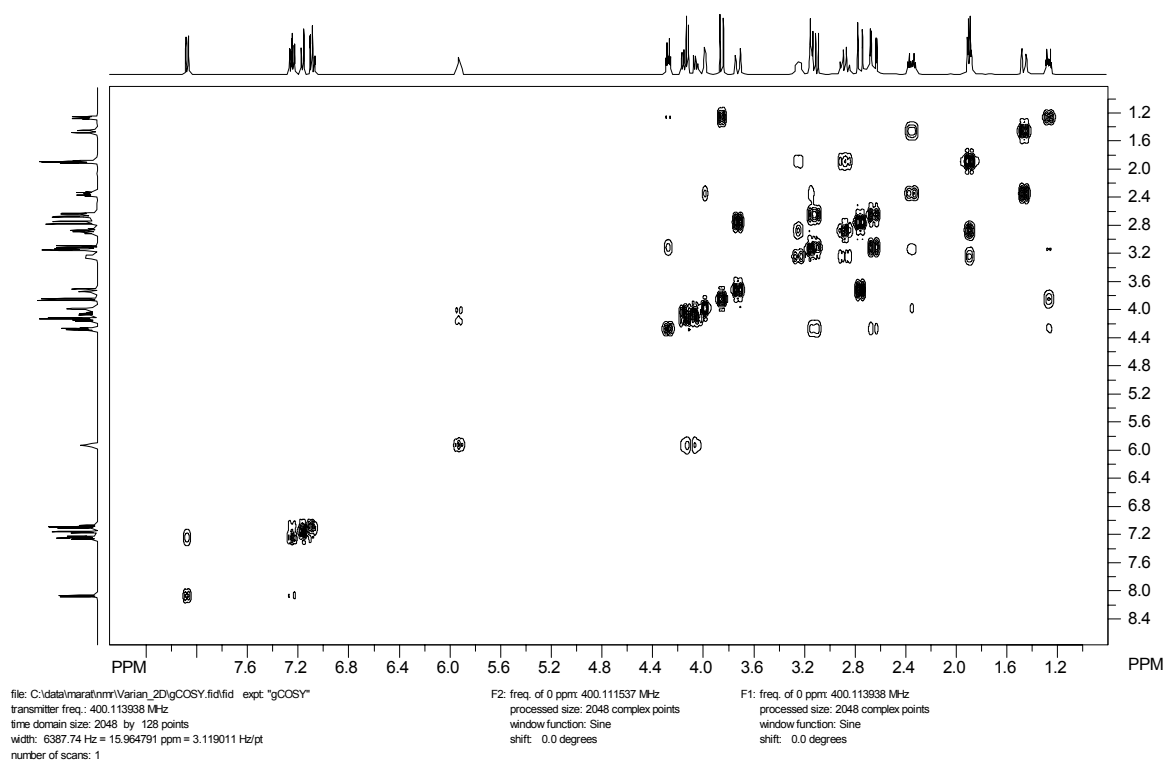
These data sets are usually processed with unshifted sine window functions in F<sub>1</sub> and F<sub>2</sub>, magnitude phase correction in F<sub>1</sub> (only!) and "single" type detection mode.

1. Set the data format according to the spectrometer type that you most commonly use, either Bruker UXNMR/XwinNMR or Varian VNMR and set the data type to 2D.
2. 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.
3. 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 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. 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. 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.
4. Close the processing parameter dialog and start the 2D Processing and Display dialog by clicking the yellow Proc button on the toolbar. Click the Both button in the Processing group. 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 the contour radio button. 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 positive only ratio button to display the positive contours in a more complete set of colours. The floor

(lowest contour) and ceiling (highest contour) can be changed with the appropriate buttons.

5. 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 blue **Zo** button on the toolbar or by clicking the *middle* mouse button. Clicking the left mouse button in the data window a third time will erase the cursors.
6. 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. Leave the algorithm set to **SVD**. 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.
7. **Print** and **Print Preview** in the **File** menu work as expected.
8. If you wish to apply baseline correction or symmetrize the data, these functions are available in the **Processing** menu.

SpinWorks 2.0: strychnine



**Figure 2** Magnitude gCOSY spectrum of Strychnine recorded on an Inova 400. SVD linear prediction has been applied in  $F_1$ , but the data have not been symmetrized.

## Phase Sensitive (HSQC type) Spectra

There are several ways in which phase sensitive 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. However, we commonly use an echo-antiecho HSQC. The comments included with each Bruker pulse sequence 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).

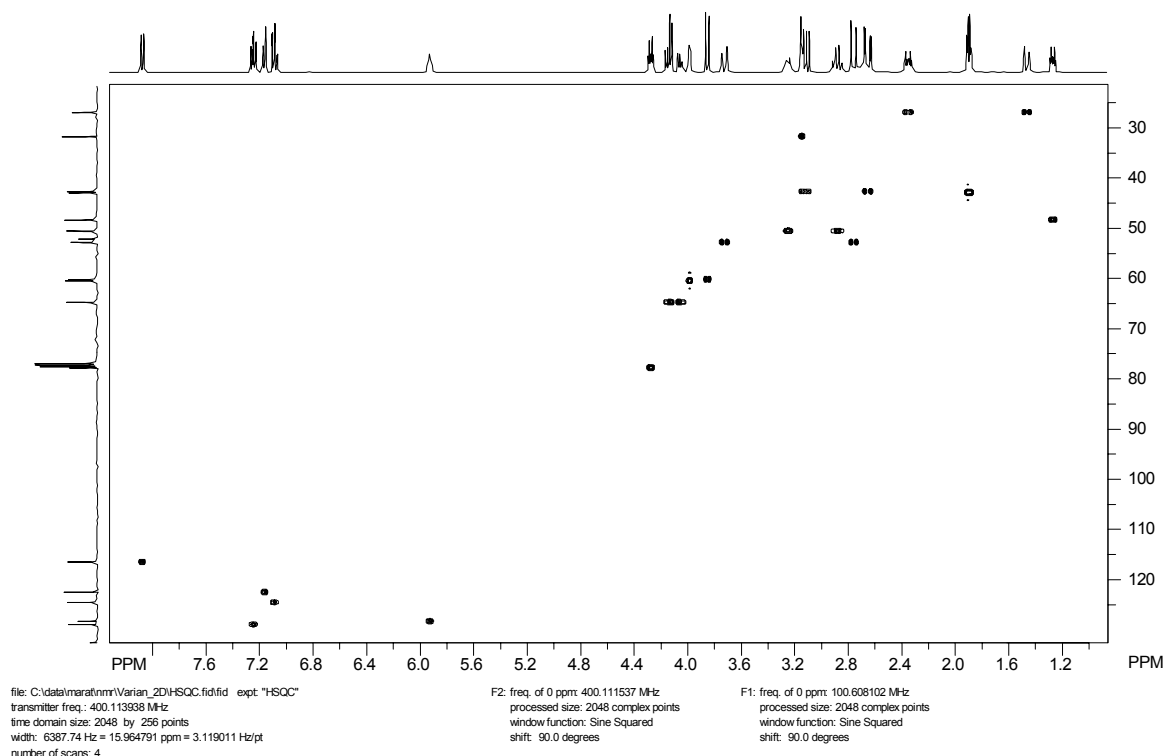
It is also possible to adjust the timings in a pulse sequence so that there is no required  $F_1$  phase correction. This approach is seen in most Varian data.

For Varian data, SpinWorks guesses the  $F_1$  detection mode from the name of the pulse sequence. Most phase sensitive Varian pulse sequences 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. Set the data format according to the spectrometer type that you most commonly use, either Bruker UXNMR/XwinNMR or Varian VNMR and set the data type to 2D.
2. 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.
3. 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 a 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 be necessary to

optimize the Windowing parameters for optimum signal to noise ratio.

4. Close the **Edit 2D Processing Parameters...** dialog and click the toolbar **Proc** button to start the **2D Processing and Display** dialog. Click the **Both** button in the **Processing** group to process the data.
5. 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 provided that they contain peaks near both ends of the spectrum. The vertical scaling of the displayed 1D traces can be adjusted with the black **+** and **-** buttons on the toolbar.
6. Use the left mouse button to select a peak to be used as the pivot point.
7. Click the toolbar **Phase** button (the yellow one with the picture of the out of phase peak). 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.
8. In the **2D Processing and Display** dialog, switch to **Col Mode** in the **Rows/Columns** group and do any required  $F_1$  phase correction.
9. Use the **Clear** button in the **Rows/Columns** group to remove any displayed rows or columns and print any desired regions of the spectrum.
9. 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. Leave the algorithm set to **SVD**. 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.
10. Print the spectrum, if desired.



**Figure 3** HSQC spectrum of Strychnine recorded on an Inova 400. SVD linear prediction has been applied in F<sub>1</sub>.

## 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++.

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\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.

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). Note that you must **ONLY** edit the numerical part of these entries. Do NOT change the coupling labels such as J(1,2) J(A,B) The program needs these labels to identify the couplings.
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, even though 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 Ortho-dichlorobenzene Spectrum**

1. Start SpinWorks, and make sure that it is in DISNMR mode (**Options** menu)
2. Read the ODCB spectrum (**File: Open...**). This is the file odcbs.001 usually located in C:\Program Files\SpinWorks\odcb. The ODCB spectrum should now be displayed on the screen.
3. Read in a starting spin system with by loading the odcb\_ni file (**File: Read Spin System File...**). You can examine the starting parameters with the shift and scalar coupling editors if you desire (**Spin System** menu). Particularly note how explicit 2 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<sub>(AB)</sub> and that
4. Select **Edit Simulation Parameters...** in the **Simulation** menu. Set the **Display Linewidth (Hz)** setting to 0.06 (Yes, really).
5. 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.
6. The left and right keyboard arrow keys will move a transition cursor across the simulated



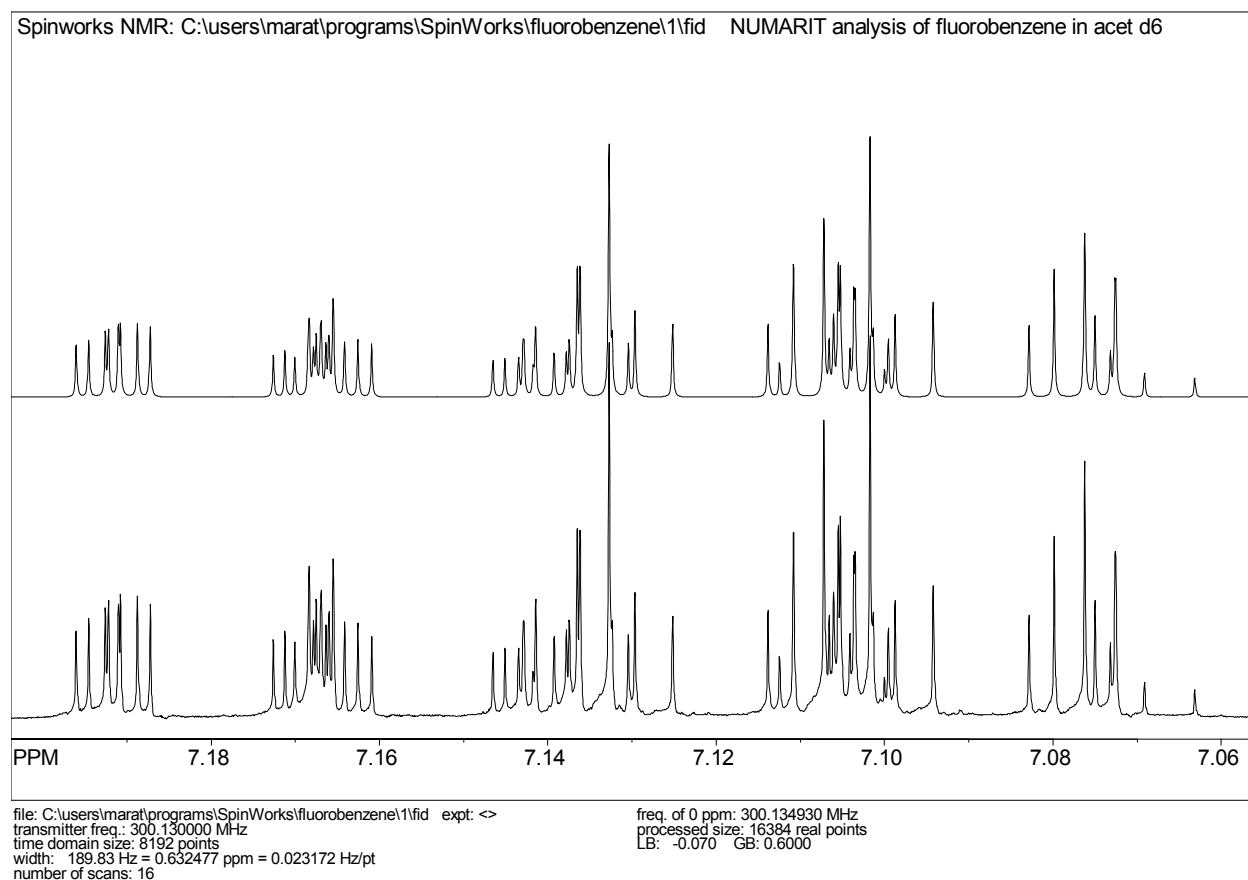
spectrum. Assign lines to these transitions by pointing to the corresponding experimental peak and clicking with the right mouse button. The peak picking routine is used to find the peak closest to the cursor position. If no peak is found within a reasonable distance of the cursor the transition is assigned 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 misassigned transition can be deleted with the keyboard "d" key.

7. 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 Simulation menu select Edit Simulation Parameters... . Check the Optimize, Autoignore and Autoassign boxes.
8. 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.
9. If the simulation was satisfactory, load the new parameters (Spin System: 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 0.005 Hz at this point.
10. 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.

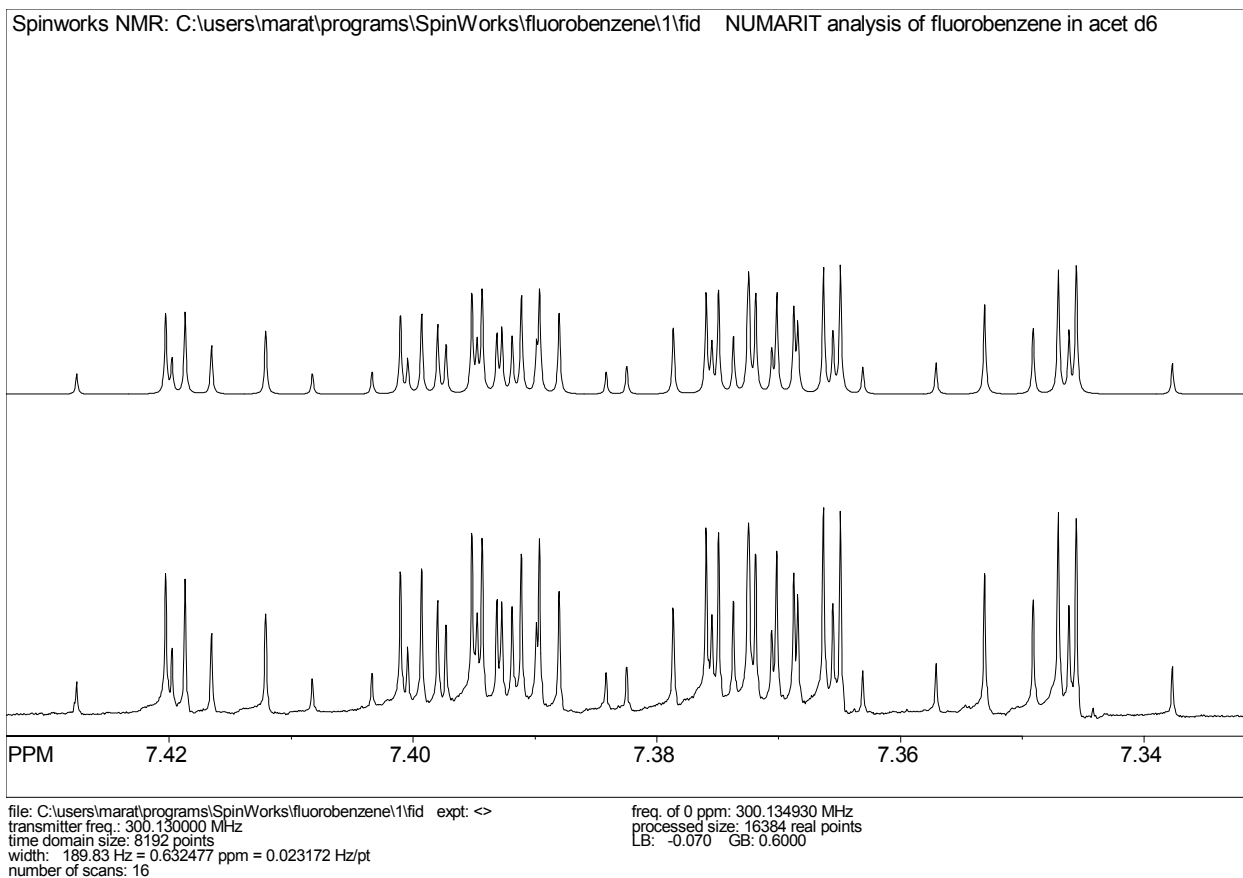
## Analysis of the Fluorobenzene Spectrum

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. This has no effect on spectra recorded at "normal" resolution. Note that the spectrum shown below is an older one recorded on an AM300.
2. Read in the spin system file pfb.ss (File: Read Spin System File...). 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 (Simulation 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 (**Spin System** 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 missassigned 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 4** 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 5** 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 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 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 or Xsim. 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 File 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 MASKSIZE data points (4000 points as supplied). The larger the range the more slowly the program will execute.

## 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**

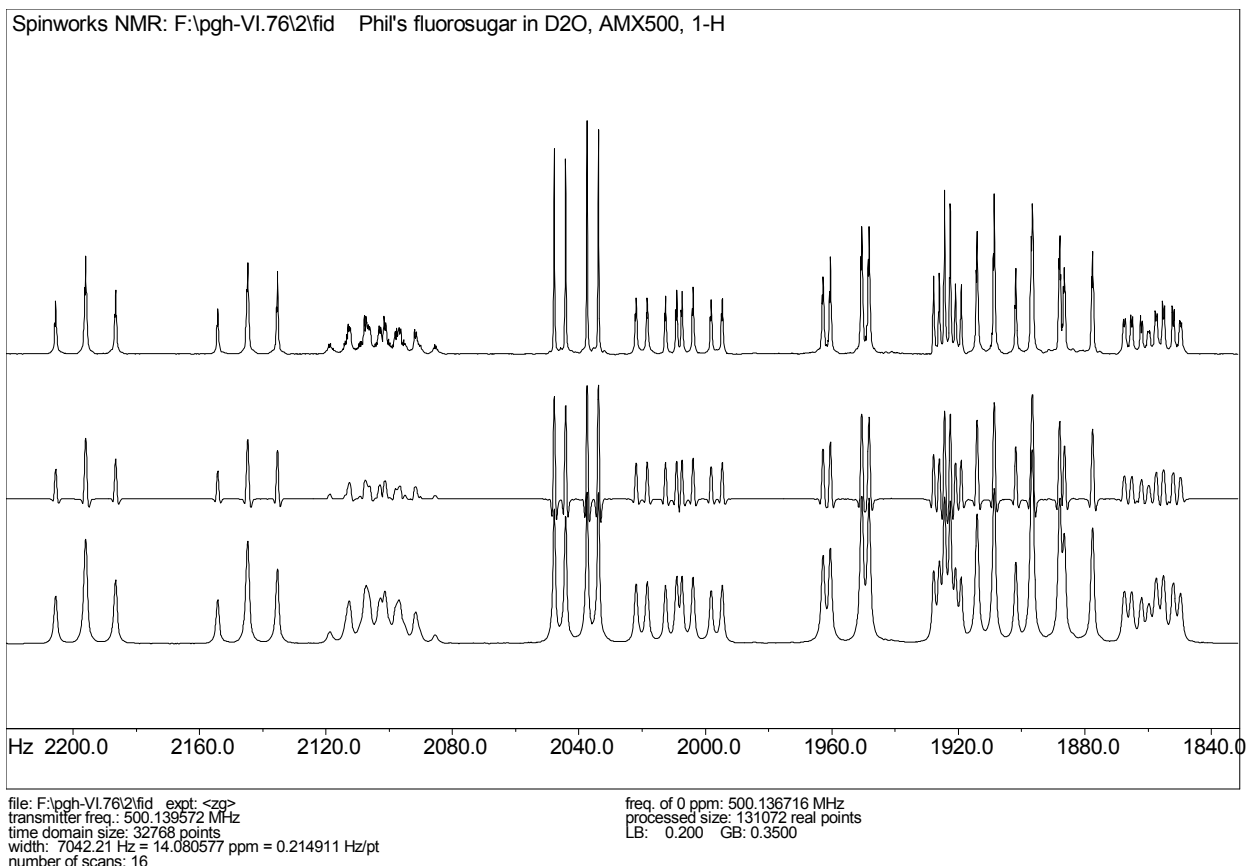
The enhancement is started from **Processing** menu. *The enhancement is only applied to the displayed region of the spectrum.* If other regions of the spectrum are to be enhanced, then they can be done serially, avoiding the need to apply the enhancement to baseline or uninteresting portions of the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

### **Saving Enhanced Spectra**

Enhanced processed spectra can be saved in JCAMP-DX format with the appropriate command in the **File** menu.

### **Speed**

Typical calculation times are from a few seconds to a few minutes, depending on the problem and the computer used.



**Figure 6** HOGWASH processing of a fluorosugar spectrum, 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 required about 20 seconds on a 100 MHz Pentium under Windows NT 4.0.

## 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>‡</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<sup>†</sup> but not often appreciated

<sup>‡</sup> Not to be confused with Maximum Entropy Reconstruction.

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

problems and artifacts. The Burg algorithm, in particular, is prone to problems such line splitting (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 be 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)).

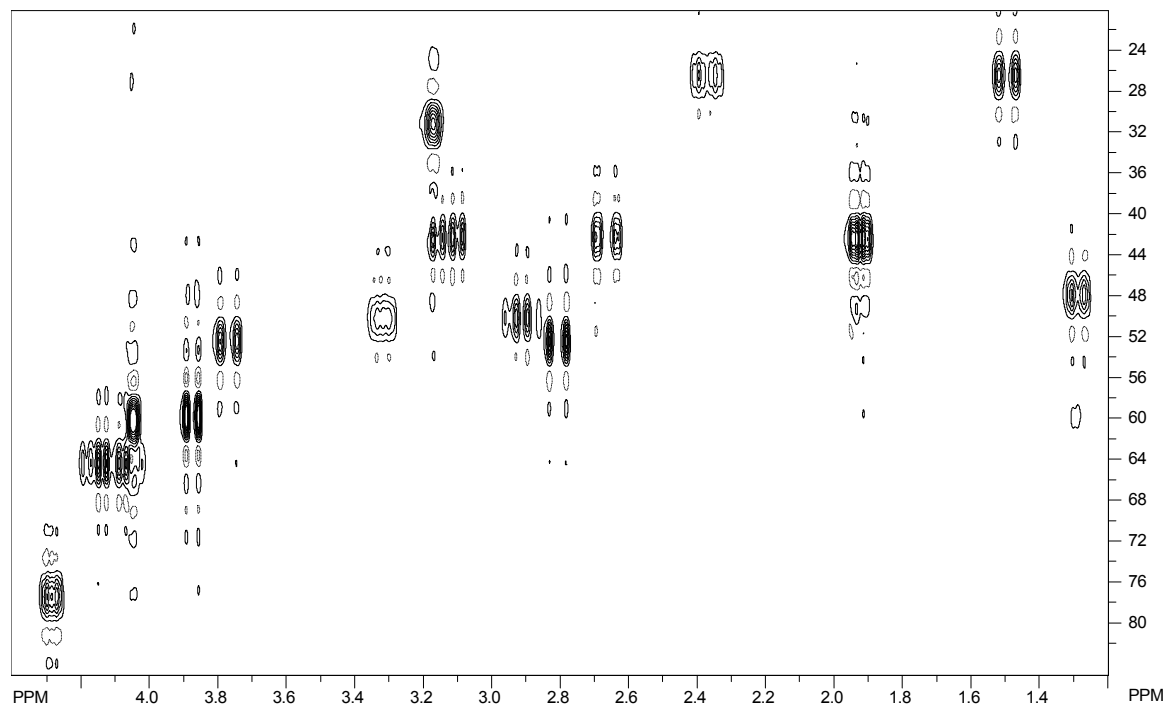
## 2D HOGWASH Parameter Selection

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.

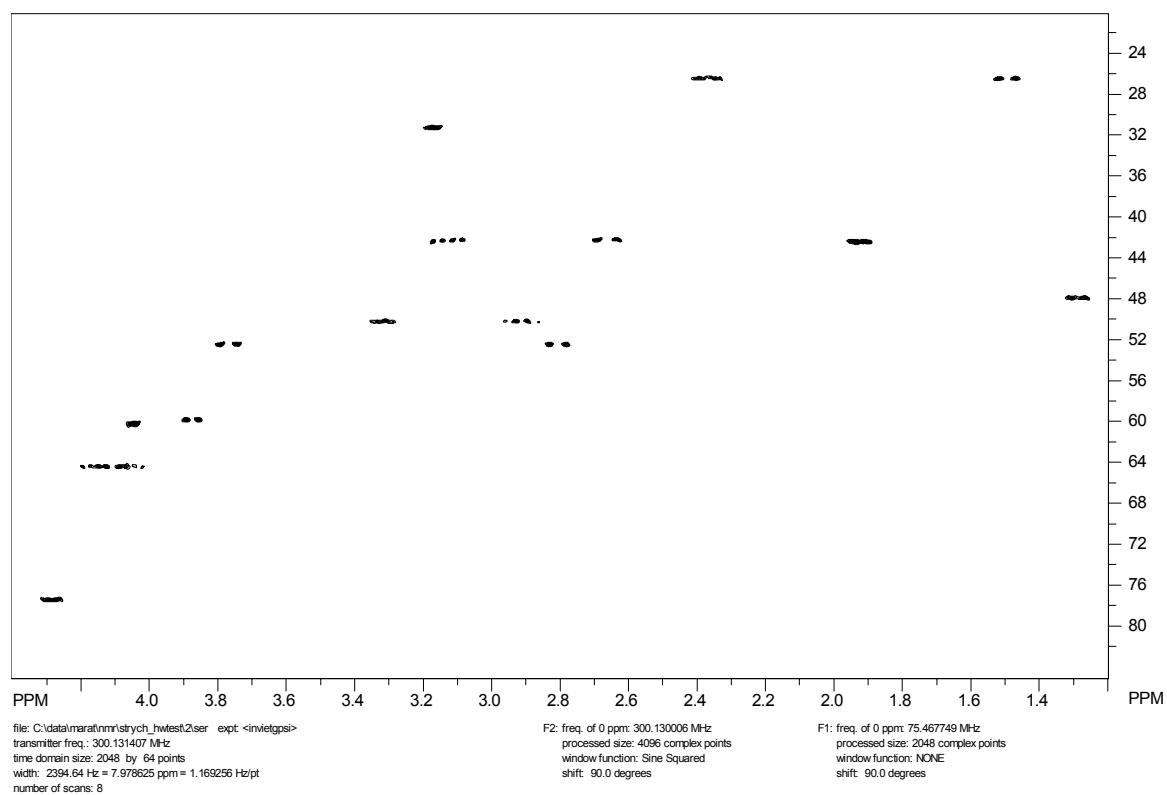
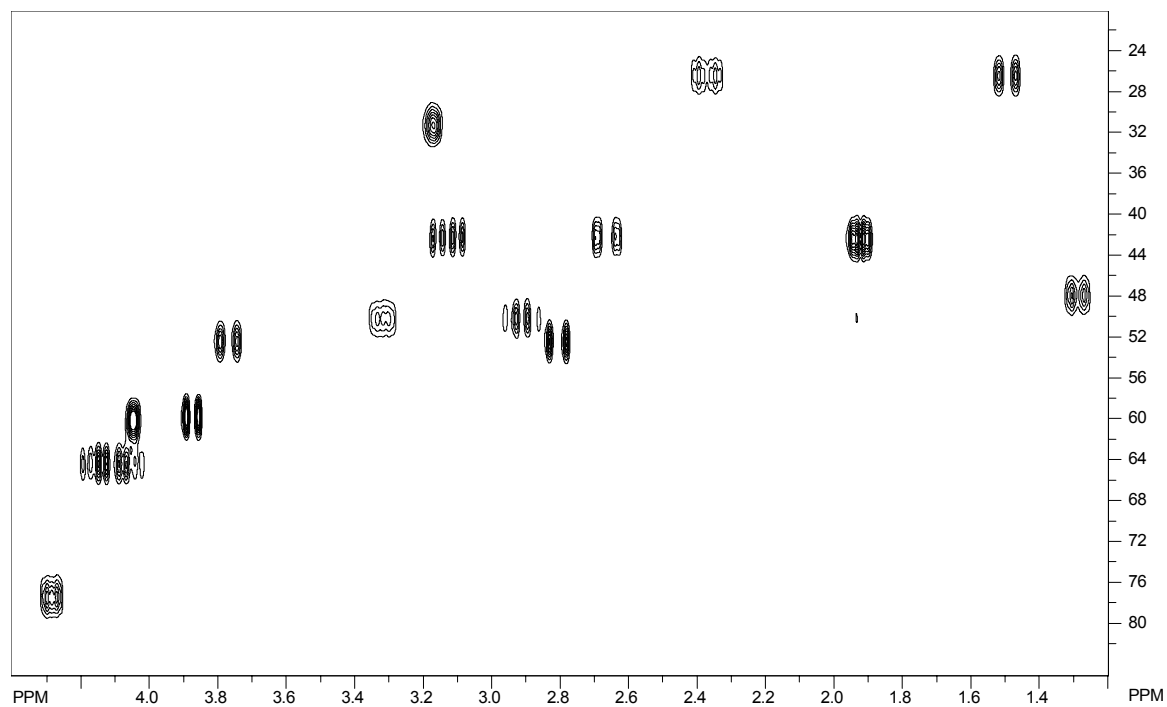
## Using the Technique

- 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.
- Phase the spectrum carefully.
- 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.
- 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.

- In the 2D Processing sub-menu of the Processing menu, select HOGWASH F1. The processing can be applied serially to other regions of the spectrum, if desired.
- If selection of HOGWASH parameters was not appropriate, it will be necessary to re-process the data (use the Both button in the 2D Processing and Display dialog).

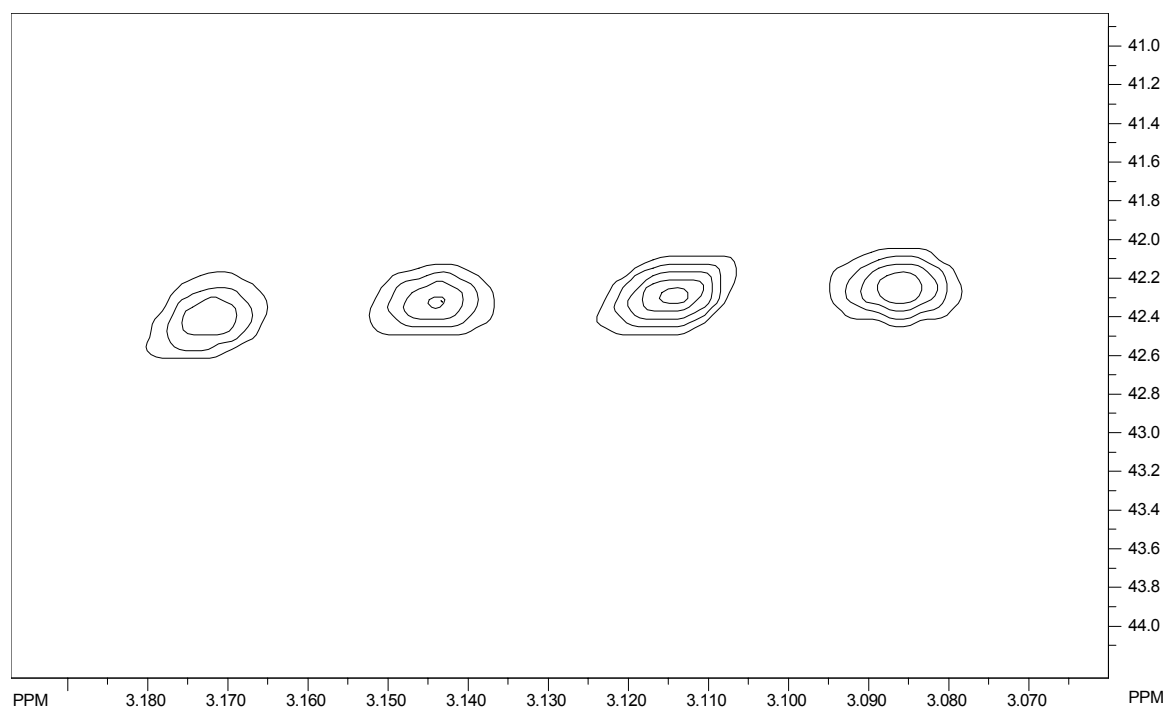




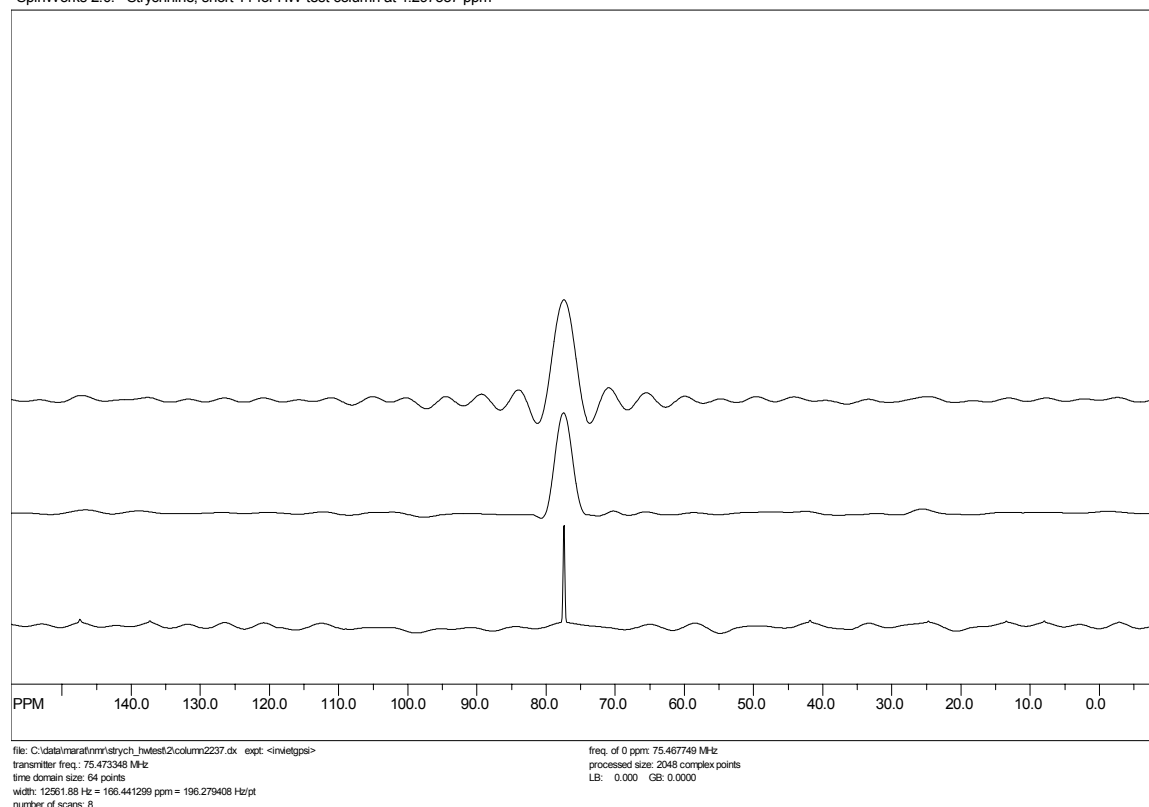


**Figure 7** The high field portion of an HSQC spectrum. 64 Time domain increments were recorded in  $F_1$ .

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 8** 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 9**  $F_1$  columns extracted from an HSQC spectrum. Top: no window function; Middle: Linear prediction followed 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 G. Binch 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.

## 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>

## General Notes on DNMR3 and MEXICO

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\SpinWorks, the default SpinWorks installation folder. If you installed SpinWorks in a different location, 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 a XwinNMR 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, MestRC) 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).

A simulation pop-up is available from the toolbar.

With this panel you can run any of the simulation routines. The rate constant for DNMR3 and MEXICO simulation can be varied, and transition assignments for NUMMRIT can be auto-assigned or deleted. The  $\chi^2$  and RMS deviation between the experimental and simulated spectra for DNMR3 and MEXICO simulations can be examined with the Chi Squared button. The Assign, Delete and Chi Squared functions apply only to the *displayed* region of the spectrum.

The Find Cursor button is used in NUMMRIT calculations to jump the transition cursor into the currently displayed region.



The following is a summary of the simulation panel 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 <b>Edit DNMR/MEXICO Parameters...</b> dialog in the <b>Spin System</b> menu.
Edit Group	These three buttons are used to edit the simulation parameters, chemical shifts of scalar couplings. These start edit dialogs identical to those that can be started from the <b>Spin System</b> 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.
Find Cursor	Moves the transition cursor from wherever it is into the displayed region of the spectrum. This eliminates the older practice of leaning on the arrow key to accomplish this task.
Chi Squared	Displays the relative fit between the simulated and experimental spectrum over the displayed region. Primarily used for DNMR3 and MEXICO calculations.

## 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\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 effecting 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 DNMR Parameters...** 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 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 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

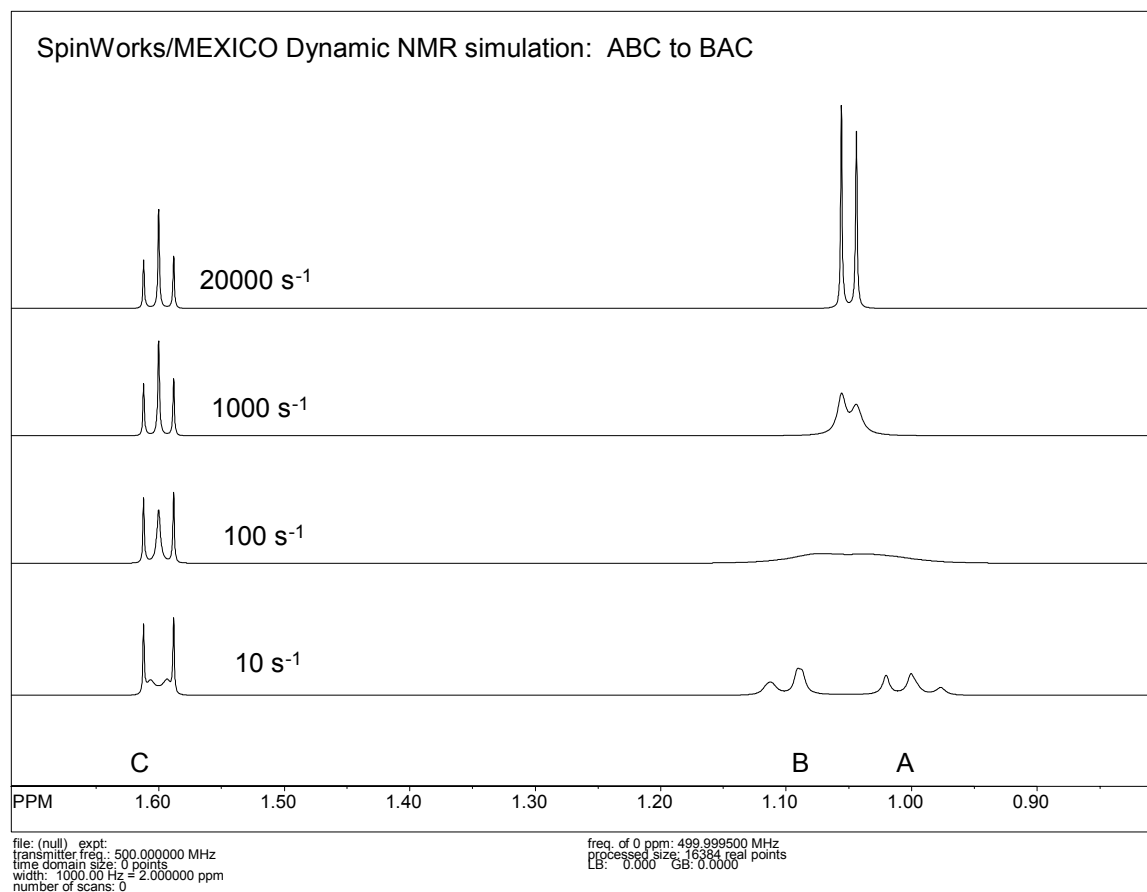


the Yellow Sim button on the toolbar.

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 Parameters... dialog in the Simulation 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 calculation to stacked trace with the Copy Simulated to # commands 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. 2.460 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 T1 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.

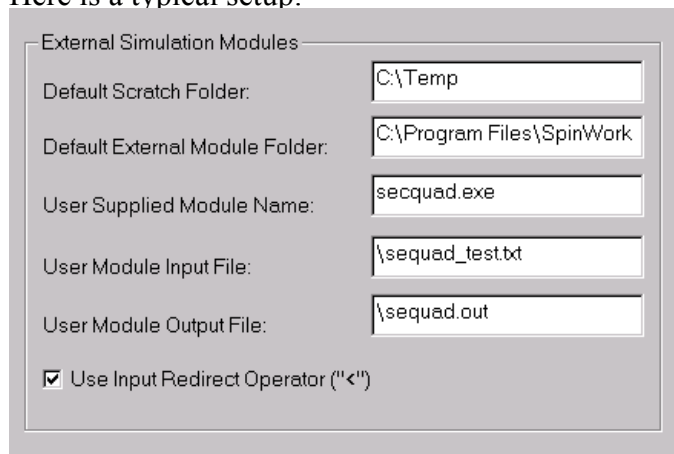
## **Running User Supplied External Modules**

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: secquad.exe

User Module Input File: \sequad\_test.txt

User Module Output File: \sequad.out

☒ Use Input Redirect Operator ("<")

In this case, the program executable is called “secquad.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 will prompt you for the following options:
  1. **File Name** Type or select the filename you want to open. This box lists files with the extension you select in the List Files of Type box. The file that you select depends on the data format. For UXNMR/XwinNMR and Varian data select the “fid” file of the appropriate experiment directory. For DISNMR and DISMSL data select the name of the data set file (e.g. MYDATA.001). For JCAMP DX spectra select the data file, usually with the .dx extension. This extension is not, however, required.
  2. **Files of Type** Select the type of file you want to open. For NMR data this should be “all files” i.e. blank or \*.\*:

3. Look in Select the directory in which SpinWorks stores the file that you want to open.
- **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 and Save as...** 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 it 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 Extracted 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*.
  - **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.
- **Print Preview** Exactly what it says.
- **Print Setup...** Sets various printer dependent parameters (which tray to use, etc.).
- The most recently used file list. The last eight NMR data sets used by SpinWorks are displayed for possible selection as the current data set.
- **Exit**

### **Edit Menu Commands**

- **Copy Metafile to Clipboard** Makes a copy of the currently displayed spectra to the system clipboard, for possible inclusion in other documents.
- **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. This method currently seems to work a bit better than the clipboard for including spectra in Word or PowerPoint.
- **1D Processing Parameters.....** Use this command to view or change the parameters used to process spectra. **Note that in future versions the peak picking parameters may be moved to a separate menu.** The processing parameters are:
  1. **Lorentzian Broadening (LB)** in Hz. The amount of broadening to be applied in an exponential multiplication of the fid. Used in the EM+FT, GM+FT commands, etc.
  2. **Gaussian Broadening. (GB)** 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 GM+FT (Processing menu) command for details.

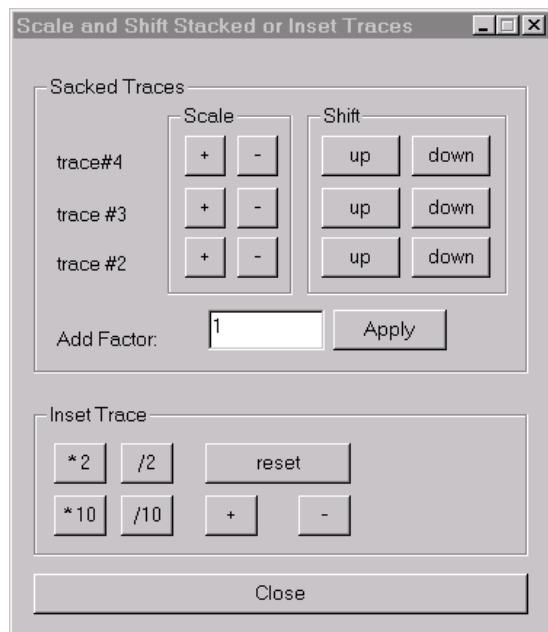
3.     **Size** The number of complex data points in the transform. The number will be rounded up to the nearest power of 2. e.g. A size value of 32000 will be rounded to 32768 and will give 32k real and 32k imaginary points after the transform. A **Size** value greater than TD/2 results in zero-filling.
  4.     **Peak Pick Minimum Intensity** The intensity threshold relative to the most intense peak in the spectrum for peak picking. Usually adjusted graphically by dragging the threshold line on the screen.
  5.     **Peak Pick Noise Threshold** provides a degree on 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.
  6.     **ABS Poly. Degree** The degree of the polynomial used in the ABS and ABSS routines. The default value is 3 and the maximum value is 6.
  7.     **Acquisition Mode** The type of quadrature detection used. 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.
- **2D Processing Parameters...** Use this command to edit the processing parameters for both dimensions of 2D experiments. The parameters are described in detail in the 2D processing section of this manual. This dialog may also be started from the "stay-up" 2D processing dialog.
  - **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.
  - **Plot Options and Parameters...** edits sizes, etc. for the plots. These should be self-explanatory. In order to change to portrait orientation uncheck the box labeled **Force Landscape Orientation**. The MetaFile Resolution parameter sets the resolution of any saved MetaFile (e.g. for incorporation into a document) relative to the screen. The default value of 2 should be suitable for most applications. Higher valued give more resolution but create much bigger MetaFiles.
  - **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.

## View Menu Commands

- **Toolbar** Use this command to display and hide the Toolbar, which includes buttons for some of the most common commands in SpinWorks, such as File Open. A check mark appears next to the menu item when the Toolbar is displayed.
- **Status Bar** Use this command to display and hide the Status Bar, which describes the action to be executed by the selected menu item or depressed toolbar button, and keyboard latch state. A check mark appears next to the menu item when the Status Bar is displayed. The current data format (e.g. DISNMR, UXNMR, etc.), status messages (e.g. FT complete) and one line help messages are also displayed.
- **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.
- **Show Simulated** Use this command to turn the display of the simulated (calculated) 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.
- **Copy Experimental to #2...#4** Use these commands to make a copy of the processed experimental spectrum into one of three alternate traces. This makes it possible to compare a series of spectra such as a temperature run or DEPT sequence on the screen. Zooming, scrolling and vertical scaling commands affect all of the traces. If it is necessary to adjust the vertical scaling of an individual trace, use the Scale Stacked Traces... command of the **View** menu. The Subtract Trace 2 from Experimental command in the **Processing** menu can be used to produce a difference spectrum.
- **Copy Simulated to #2...#4** Similar to the above, but copies the simulated spectrum to one of three alternate traces. These alternate traces can contain a mixture of simulated and experimental spectra.
- **Add Simulated to #2** 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 trace 2 by adding the current simulated spectrum to the spectrum in trace 2. 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.
- **Scale/Shift Stacked or Inset Traces...** This command starts a modeless dialog box which allows one to adjust the vertical scale of the individual stacked traces, if necessary. Each click of a **+** button increases the vertical scaling by 10% while the **-** button decreases the scaling by 10%. You can also shift the individual traces up and down the screen if desired. The **Add Factor** value is the coefficient used for adding a simulated spectrum to trace 2. The default value is 1. After changing this value you must click on the **Apply** button for the change to have effect. The **Inset Trace** grouping is used to adjust the vertical scaling of any



inset trace. The **+** and **-** buttons adjust the scaling in small steps, while the reset button can be used to return the scaling to a value identical to the experimental spectrum.



- **Delete Trace #2...4** Use these commands to delete any stacked traces. The current experimental spectrum is not affected.

## Option 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. The default mode is NUMMRIT.
- **Data Format (sub menu)** This menu selection sets the format of the current data. Currently supported data formats are: Bruker UXNMR/XwinNMR, Bruker DISNMR, Bruker DISMSL and Varian UNITY/INOVA. JEOL generic data is supported, but not thoroughly tested. 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 NTNMR data is supported, but hasn't been thoroughly tested. 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. **Note that you must select the data format (spectrometer type and 1D vs. 2D) before reading the data.**
- **Peaks and Match** These options turn ON and OFF the display of picked peak frequencies and match lines between experimental and simulated spectra. 1D mode only.

- **Tracking Cursor** This option selection turns ON and OFF a tracking vertical reference line. This can be very useful for measuring frequencies, frequency differences, etc. The default value is OFF.
- **Set Preferences...** A number of start-up preferences can be set with this dialog:

- **Default Data Folder** Sets the initial data folder (directory) for the File Open command. e.g. F: or D:\data\marat\nmr.
- **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 will 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 before selecting the data set.

- **Data Format** Sets the default data format at start-up.
- **Simulation Mode** Sets the default simulation mode (currently NUMMRIT or DNMR/MEXICO). The default is NUMMRIT.
- **Alternate Peak Rotation** Overcomes a bug in some printer drivers (not normally necessary).
- **Default Scratch Folder** Sets the scratch directory (folder) used by some of the calculations. The default is C:\Temp is should be acceptable for most users.
- **Default External Module Folder** Sets where the program expects to find external simulation modules such as DNMR3 and MEXICO. The default is C:\Program Files\SpinWorks.
- **User Supplied Module Name** The executable file name (e.g. secquad.exe) of a user supplied simulation routine.
- **User Module Input File** The name of any input file required by the external module. Set to **none** if your program requires no input name to be specified. (Either it doesn't need one or the name is hard-coded into the program.) The default is **none**. Note that the leading “\” character is required.
- **User Module Output File** The name of the output file generated by your external program. This is a text file and will consist of frequency-intensity pairs, as described in the separate section on using external modules. The leading “\” character is required.
- **Use Input Redirect Operator “<”** This check box determines whether the input file (if any) has its input redirected from standard input (UNIX style) or as a specific command-line argument.

## 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, and species identifier for a group. The standard spin systems provided in the simulation tutorial give good examples of the required format.

- **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. Only the numeric parts of the fields should be edited.

**only edit the number !**

- **Edit Dipolar (D) Couplings...** Dipolar coupling is now supported for NUMMRIT (but not DNMR or MEXICO) calculations.
- **Edit Quadrupolar (Q) Couplings...** Quadrupolar (Q) coupling is not yet implemented.
- **Edit Species Spin Values...** This editor sets the spin quantum number for each group of nuclei. The default is all groups spin  $\frac{1}{2}$ .
- **Edit DNMR Parameters...** This editor is used to set the necessary dynamic NMR parameters for DNMR or MEXICO simulations. See the Dynamic NMR section for details of the parameters.
- **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.

## Simulation Menu Commands

- **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 User Supplied External Simulation** This feature is under development, but it will allow users to add their own simulation modules.
- **Edit Simulation Parameters** This command starts a dialog where it is possible to edit the simulation parameters:
  - **Display Linewidth (Hz.)** This is the linewidth (Lorentzian or Gaussian) used for in the calculation of the simulated spectrum.
  - **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.
  - **Grouping linewidth (Hz.)** The linewidth used for grouping near degenerate peaks in a peak listing. This value does not normally need adjustment.
  - **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 15.
  - **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 if 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 ALWAYS be left ON unless you really know what you are doing.
- **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.
- **Autoassign Displayed 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.
- **Delete Assignments in Displayed Region** Deletes all transition assignments in the displayed region. Single transition assignments can be deleted with the keyboard “d” key.
- **Debug Mode** Turns on the simulation debug mode. If this feature is turned on, a number of log files are dumped during a simulation. These files contain useful information in the event of difficulty. The default setting is OFF. If running a user-supplied external module the names of the input file, output file and the actual command line will be displayed as pop-ups while the external module is running.

## Peak Pick Menu Commands (1D mode)

- **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.
- **List** Displays the peaks in MS Notepad for possible printing.
- **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.
- **Peak Pick (On Plot) Units..** Selects PPM or Hz as the units for the on-plot peak list and display. This is independent of the axis units.
- **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 as a Toolbar button.

## Processing Menu Commands

Note that the processing pop-up dialog (tool bar) provides a convenient alternative to many of the following menu commands.

- **ft** Performs a Fourier transform of the current fid. with no window function Uses the **Size** parameter from the 1D Processing Parameters... dialog (Edit menu). Usually, the **em+ft** command is more appropriate.
- **em+ft** Performs a Lorentzian broadening and Fourier transform of the current fid. Uses the **LB** and **Size** parameters from the 1D Processing Parameters... dialog (Edit menu). A typical value for **LB** is  $1/(\text{acquisition time})$  or equal to the natural linewidth if the peaks are broader than this value.
- **gm+ft** Performs a Bruker-style Gaussian multiplication and Fourier transform of the current fid. Uses the **LB**, **GB** and **Size** parameters from the 1D Processing Parameters... dialog (Edit menu). This function is normally used to provide resolution enhancement of the spectrum. 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.
- **em+ft+pk** Performs a Lorentzian broadening and Fourier transform of the current fid. Uses the **LB** and **Size** parameters from the 1D Processing Parameters... dialog (Edit menu). After transformation, the data are phase corrected with the last phase constants used by SpinWorks.

- **gm+ft+pk** Performs a Bruker-style Gaussian multiplication and Fourier transform of the current fid. Uses the **LB**, **GB** and **Size** parameters from the **1D Processing Parameters...** dialog (**Edit** menu). After transformation the data are phased with the last phase constants used by SpinWorks. This function is normally used to provide resolution enhancement of the spectrum. 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. Note that resolution enhancement may affect relative integrals
- **pk** Phase correct the data using the last phase constants used by SpinWorks. This command is rarely used on its own, but more often in conjunction with other processing. e.g. The **em+ft+pk** command.
- **Automatic Phase Correction** Uses a fast algorithm to automatically phase the spectrum. This feature is still under development, but works well for spectra with good baselines and good signal to noise ratio.
- **2D Processing (sub menu)** Contains assorted 2D processing commands that are not included in the **2D Processing and Display** dialog.
  - **2D Processing Dialog** Starts the pop-up **2D Processing and Display** dialog. This command is also available on the toolbar.
  - **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** Apply 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 F1 and F2** Apply a Varian 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.
  - **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 by organic chemists. The chief problem is that it can turn an obvious artifact such as  $T_1$  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 always a better if not always practical solution.
- **Automatic Baseline Correction (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 **bp** button on the toolbar. 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 pop-up **return** button to exit the baseline points routine. Finally, execute the **Automatic Baseline Correction (least squares)** command.

- **Automatic Baseline Correction (SVD)** This command is similar to **Automatic Baseline Correction (least squares)**, but uses a different (and generally inferior) algorithm for the polynomial fit. The least squares version is usually preferred.
- **Fully Automatic Baseline Correction** Applies a least squares 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.
- **Clear Points** Clears the defined baseline points.
- **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.
- **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 **em+ft** command (**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 #2** command in the **View** menu.
  3. Open one of the on-resonance NOE experiments (**File** menu) and process it with the **em+ft+pk** command (**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.
- **Reverse Spectrum** This command reverses the spectrum – high field to low field. There are several circumstances where this may be appropriate:
  1. On some older Bruker spectrometers, fluorine spectra are reversed.

☺

2. Exchange of the two quadrature channels will reverse the spectrum (This is the default state on Varian spectrometers. Therefore, Varian data are automatically reversed by SpinWorks).
3. 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 automatic baseline routine, and then reversing the spectrum back to the normal orientation.

## 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 GB) be set first. This can be most easily done with the 1D Processing Parameters dialog (Edit menu or `edp` command), or by giving one of the parameter commands listed below.

- `em` Exponential multiplication of the fid (uses LB parameter).
- `gm` Bruker type Gaussian multiplication (resolution enhancement) of the fid (uses LB and GB parameters).
- `traf` Apply TRAF function to fid (uses LB parameter).
- `trafs` Apply TRAFS function to fid (uses LB parameter).
- `lpf` Forward linear prediction of fid. Uses the LP parameters in the Processing Parameters dialog (Edit menu). Note that forward linear prediction of 1D data is rarely productive.
- `lpb` Backward linear prediction of fid. Uses the LP parameters in the Processing Parameters dialog (Edit menu).
- `ft` Fourier transform of fid.
- `ef` Exponential multiplication followed by Fourier transform.
- `efp` Exponential multiplication followed by Fourier transform and phase correction with previous phase constants.
- `gf` Gaussian multiplication followed by Fourier transform.
- `gfp` Gaussian multiplication followed by Fourier transform and phase correction with previous phase constants.
- `trafft` TRAF function followed by Fourier transform.
- `trafsft` TRAFS function followed by Fourier transform.
- `pk` Apply previous phase constants.
- `apk` Automatic phase correction.

- **abs** Automatic baseline correction using a least squares fit. Requires defined baseline points and use parameters in the **Processing Parameters** dialog (**Edit** menu).
- **fabs** Automatic baseline correction with automatic selection of the baseline points.
- **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 Editor Commands:**

- **edproc** Edit processing parameters (1D and 2D). Alias: **edp**.
- **edhw** Edit HOGWASH parameters.
- **edshifts** Edit the chemical shifts for a simulation.
- **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.
- **eddnmr** Edit DNMR/MEXICO specific parameters.
- **edmch** Edit or inspect the MEXICO mechanism file (rarely needed).

### **Options Commands:**

These commands duplicate most of the commands in the **Options** menu.

- `vnmr` Switches to Varian VNMR mode. Alias: `varian`.
- `xwinnmr` Switches to Bruker XwinNMR/UXNMR mode. Aliases: `bruker`, `uxnmr`,

### 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.
- `gb` 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`.
- `ph0` Zero order phase constant. Alias `phc0`.
- `ph1` First order phase constant. Alias `phc1`.

## SpinWorks Bug and Revision History

### Pre version 1.2

1. Failure to read Varian floating point and 16 bit integer FIDs due to problems with Sun to Intel byte swapping routine. Fixed in 99 11 08 a.
2. Files of type... drop-down blank in File Open and Save boxes, causing user confusion. Changed to show: "all files (\*.\*)" and to display the current data format in File Open box in 2000 02 18.
3. Integral region list was retained upon reading new file. This was changed to clear the integral regions as of 2000 02 18.
4. Added dipolar couplings 2000 02 18.
5. Negative couplings or shifts not optimized properly. Fixed in 2000 05 08.
6. Problems with optimizing dipolar couplings. Fixed in 2000 05 08.
7. Would not detect Bruker DSP data if `AQ_mod` was set to `qsim` rather than `DQD`, even though `DIGMOD` was set to `digital`. Editing of the processing parameters to set the DSP flag was then necessary. Fixed in 2000 05 08.

### Version 1.2

1. DNMR3 and MEXICO now included as external modules.
2. JEOL (generic) and TECMAG (NTNMR) data format now supported.

### Version 1.3

1. Saving (and reading) of 1D spectra in JCAMP-DX format now included.
2. Inset expansion box added.
3. Zooming with middle mouse button added.

4. Axis tic spacing increases for large numbers (to avoid overlap of the labels if axis values have numbers like -87200 Hz).
5. Processing pop-up dialog box (button panel) added.
6. TRAF and TRAFS apodization functions added.
7. DNMR3 re-compiled with double precision (64 bit) floating point variables.
8. A problem with printing if C:\Temp didn't exist was fixed.
9. J editor increased to include all possible couplings from a 9 group system.
10. User supplied external simulation routines are supported (read the manual carefully!)
11. Small bug with simulation parameter editor (linking of SF and SW) fixed. You can now change the SF without the program changing SW to be the same range in ppm. The SW is now constant in Hz, even if you change SF.
12. Simulation pop-up dialog box (button panel) added.
13. Fixed a bug that caused SpinWorks to crash if a MEXICO simulation failed. A proper error message is now provided.
14. RMS deviation is now displayed on the screen after an iterative NUMMRIT simulation or a DNMR/MEXICO simulation.
15. Summing of simulated spectra into trace 2 added.
16. Fixed bug with MetaFile axis and cleaned up the MetaFile handling a bit.

### **Version 2.0**

1. The scalar and dipolar coupling editors have been expanded to handle all possible couplings from 10 spins.
2. Assorted small bugs fixed.
3. Input (user) errors in the simulation routines handled more gracefully. For example, a 19 spin system will abort with an error message rather than crash SpinWorks.
4. Problems with crashes when writing metafiles to the clipboard in Win XP fixed.
5. 2D processing of Bruker and Varian data - at "alpha" test level! Expect bugs. Expect giant mutant cockroaches!
6. Added fully automatic baseline correction. For most data sets, it is now not necessary to define baseline points - the program will find them automatically.
7. Mouse wheel support for vertical scaling added.
8. Left shift processing parameter added.

### **Version 2.1**

1. The contouring routines have been improved. In 2D image mode, bi-cubic spline interpolation is used at high expansion levels. (displayed window smaller than 512 by 256 data points).
2. Extracted rows or columns of a 2D data set can be saved as JCAMP-DX data sets for viewing in 1D mode. Individual extracted rows or columns can be selected and viewed in 1D mode and can be scaled separately.
3. Assorted small bug fixes.
4. Fixed automatic detection of Varian echo-antiecho data.

5. Fixed bug with linear  $F_1$  prediction of Varian data.
6. TECMAG 2D data format included (at test level).
7. HOGWASH available in  $F_1$  of 2D data sets.
8. Linear prediction routines improved. LP (forward and back) included for 1D.

## **Version 2.2**

1. Added rudimentary command processor.
2. Added first point multiplier parameter for 2D.
3. 2D Spectra can be plotted in a square format with parameters at the right side of the spectrum. A grid can be included on a 2D plot.
4. The integral and peak pick listings now display an absolute intensity as well as a relative intensity. This can be used to compare intensities between spectra.
5. Drift correction (offset and slope) baseline correction has been added to 2D processing.
6. 2D Spectra can be plotted in a square format with parameters at the right side of the spectrum. A grid can be included on a 2D plot.
7. The tracking cursor (or 2D cross-hair cursor) can be toggled on and off by double-clicking in the data window.
8. A link (button) to the 2D parameter editor has been added to the 2D processing and display dialog.
9. A link (button) to the 1D parameter editor has been added to the 1D processing dialog.
10. Negative contours can be printed in colour, if you have a colour printer.
11. Changed LP routine and fixed a bug with magnitude mode data.
12. Fixed a bug in 2D HOGWASH that caused it to fail on magnitude mode data.
13. Improved reference parameter extraction for Varian 2D data. SpinWorks now checks the “refsource” parameter for proper conversion of Hz to ppm.

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