

ADVANCED DATA PROCESSING

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Tranferring Files via FTP

1. From your networked computer, open an FTP program.
2. FTP to the host: echo.chem.umn.edu and login with your NMR account and password.

Making Backups via Zip disk

The NMR lab periodically backs up data on CD-ROMs for your convenience (**[Files]** – **[Oldfids]**). If you would like your own personal backups, you can do this via the computer called “spectrum”.

1. Insert a PC-formatted 100MB zip disk into the zip drive.
2. Select **[Unix Shell]**.
3. In the shell window, change to the directory from which you want to copy, e.g.:
 - > **cd /data/6-lettercode** (for current data)
 - > **cd /v3data/6-lettercode** (VAC-300 files)
 - > **cd /v2data/6-lettercode** (VAC-200 files)
 - > **cd /old/b8/6-lettercode** (for archived data)
 - > **cd /cdrom/cdrom0/6-letter code** (for data on the CD-ROM drive)
- 4a. Copy files to the zip drive:
 - > **cp -r filename /rmdisk/zip0** (for a single file)
 - > **cp -r * /rmdisk/zip0** (for all files)
- 4b. For data archived on the CD-ROMs, you will have to type:
 - > **tar cf - filename | (cd /rmdisk/zip0; tar xf -)**
5. To eject your zip disk, type: **eject zip0**

Printing Insets

1. Plot the full spectrum manually: **pl pscale pirn ppa** (Do NOT type 'page'!)
2. Put the cursors around the region you would like printed as an inset and type **inset**.
3. Use the mouse buttons to move the spectrum to the area where you would like the inset to be printed.
The left mouse button moves the inset region back and forth on the screen.
The middle mouse button adjusts intensity.
The right mouse button adjusts the width of the spectrum.
To adjust the vertical position of the spectrum, use the **vp** command.
(You can also do all of this manually by adjusting the following values: sc, wc, vp)
4. Plot the inset and send it all to the plotter: **pl pscale pirn page**
5. To get back to the original spectrum, type **vp=12 f full**

Arrays: Creating, Processing, Displaying, and Plotting

CREATING ARRAYS

Several spectra can be combined into one dataset of arrayed spectra. This is very useful for comparing spectra collected under the same conditions, e.g., to compare peak heights over a series of spectra.

1. Load each FID into a separate experiment, leaving exp5 empty. **Do NOT process your data.**
(If you do not have enough experiments, create them by typing **cexp(#)** where # is the experiment number you want to add.)
2. Join experiment one: **jexp1** and type **clradd**. This deletes exp5.
3. Type **add('new')** in experiment 1.
Join all the other experiments: **jexp2** and type **add('new')** etc. until all fids have been added to experiment 5.
4. Join experiment five: **jexp5**
5. Type **setvalue('arraydim',#, 'processed')** where # is the total number of fids that you have added.

PROCESSING AND DISPLAYING ARRAYED DATA

The data in exp5 will now behave as an arrayed experiment. Save it if you wish.

wft will process all the fids

wft(1) will process just the first fid, **wft(2)** will process the second, etc.

ds(1) will display the first fid, **ds(2)** will display the second, etc

dssa will display all fids stacked vertically

dssh will display fids in a line horizontally

PLOTTING ARRAYED DATA

To plot the entire array as displayed on the screen, type: **pl('all') pscale page**

To plot individual spectra, display the spectrum with the **ds(#)** command, then **pl pscale page**

Comparison of Spectra

METHOD 1

If you would like to compare the same regions of two spectra and want the vertical scales and ppm ranges to be identical, you can create saved regions.

1. Call up each spectrum in different experiments (eg. exp 1 and 2) and process them as desired.
2. In experiment 1, type **s1** to save the region (that's a one, not an L). You can save up to 9 regions. The **s** command saves a copy of all the current display parameters. The saved parameters are data-independent, i.e., the fid is not being saved.
3. Type **md(1,2)** to move those display parameters (all the **s** files you created) from experiment 1 into experiment 2.
4. Go to experiment 2 (**jexp2**) and type **fr1** or **r1** to recall the data region. **fr1** does a full recall. **r1** will not recall phase, drift correction, integrals, or reference parameters. It does recall chart info (wc, sc, wp, sp, etc), horizontal and vertical offsets, vertical scaling, and ai/nm mode.
5. Type **ds** (or **dcon1** if 2D) to display the region.

METHOD 2

This method only keeps the chemical shift range constant.

In each spectrum, set sp and wp to the desired chemical shifts, e.g., **sp=0p wp=10p**

sp is the start of the chart in ppm

wp is the width of the chart in ppm

PLOTTING SPECTRA AS STACKED PLOTS ON THE SAME PAGE

For a set of three spectra in exps 1-3, after each has been set to the same regions using the methods above, join each experiment and plot the spectrum without typing "page" until the final spectrum.

Ex: For the bottom spectrum,

jexp1

type **ds** and adjust the height with the middle mouse button bearing in mind that 3 spectra need to fit on the same page

pl pscale

For the middle spectrum,

jexp2

type **ds** and adjust the height with the middle mouse button

vp=70 pl

For the top spectrum,

jexp3

type **ds** and adjust the height with the middle mouse button

vp=140 pl page

Experiment with **vp** (vertical position) and **vs** (vertical scale) to adjust the spacing between spectra. For 4 spectra, try vp's of 45, 90, and 135.

Creating Postscript Files

Spectra, both 1D and 2D, can be saved as postscript files. This is useful when you need to make figures for a paper and would like to edit the file with labels, colors, etc.

1. Log in to any workstation in the NMR lab.
2. Choose a PS-AR plotter (for landscape plots) using the **[Change Plotter]** button.
3. Process your spectrum. Display it the way you want it for the figure.
4. Make sure you are in your data directory. **[Main Menu] - [File] - [Data]** or by typing:
cd('/data/6-lettercode')
5. Type in all the commands necessary for plotting the spectrum, but give page a filename, e.g.:
For 1D spectra: **pl pscale pirn ppa ppf('top') page('filename.ps')**
For 2D spectra: **pcon(10,1.2) pap page('filename.ps')**
6. Transfer your files via zip or FTP.

Creating ASCII Files

In some cases, you may want to save your spectra as an ASCII file. This is a file consisting of 2 columns, the first is the ppm value and the second is the intensity.

1. Log in to any workstation in the NMR lab.
2. Recall your FID.
3. Change the zerofill number (**fn**) to something more reasonable, e.g. **fn=16000**. This method creates a new entry for each datapoint; this is the reason for reducing the fn number.
4. Reprocess (**wft**) and make sure you do not lose too much resolution.
5. Make sure you are in your data directory. **[Main Menu] - [File] - [Data]** or by typing:
cd('/data/6-lettercode')
6. Type **writespec('filename')** in the command line. *Use a different filename than that of your original fid.*
Otherwise you will not be able to read the original fid later on.
7. Transfer your files via zip or FTP.

Solvent Subtraction Filtering

Sometimes it is necessary to remove a large solvent peak that was not suppressed sufficiently during acquisition. This can be done post-acquisition using digital filtering.

There are two types of algorithms for digital filtering:

lfs (low-frequency suppression) in which a low-pass filter is applied to the FID. This filter attenuates all signals that lie outside the filter, leaving only the on-resonance solvent signal. This filtered FID is then subtracted from the original FID to remove the solvent peak contribution.

zfs (zero-frequency suppression) in which the FID is also low-pass filtered, but then fitted with a polynomial (specified by the parameter *ssorder*). This is then subtracted from the original FID. This gives a FID in which the signal *that is exactly on-resonance* has been removed. (Use this when you have moved the tof to the solvent signal.)

1. Recall the fid and process it.

2. Type **addpar('ss')**

This creates the following parameters:

ssfilter specifies the full bandwidth of the low-pass filter; default is 100 Hz.

sslsfrq specifies the location of the center of the solvent-suppressed region. Setting this to a non-zero value shifts the solvent-suppressed region by *sslsfrq* Hz.

ssntaps specifies the number of "taps" (coefficients for the digital filter). Default is 121, but the value can range from 1 to $np/4$.

ssorder specifies the order of the polynomial used to fit the FID

3. Decide which algorithm (*lfs* or *zfs*) is appropriate for your dataset and enter the correct parameters according to the following guidelines:

For *lfs*,

ssfilter is set to a value other than "not used"

ssorder='n'

To set *sslsfrq*:

Set **axis='h'**

Put the cursor in the middle of the spectrum (i.e., find the tof) by typing: **cr=sw/2-rfl+rfp**

Put the right cursor on top of the peak to be subtracted. (If the peak is to the left of the tof cursor, use the left cursor on the peak to be subtracted and the right cursor on the tof.)

Repeat this step if you have a second peak you would like to subtract.

Set **sslsfrq=delta** (Use **-delta** if peak is to the left of tof; use an array if you are subtracting 2 peaks.

ssntaps--default value is suitable

For *zfs*,

ssfilter is set to a value other than "not used." Default is 100 Hz.

sslsfrq='n' (suppresses a region centered around the transmitter frequency (tof).

ssntaps--use values between 3 and 21

ssorder-- set to a value from 1 to 20.

4. Type **wft** to perform the subtraction.

Spin Simulation

You can simulate a spectrum of up to **EIGHT** closely coupled, non-equivalent spins (ABCDEFGH) using the vnmr software. Equivalent spins can be treated by magnetic equivalence factoring (e.g., A3B2CD3). Nuclei are treated as different types if there is at least one spare letter in the alphabet between their groups (e.g. ABD and ABX). Frequencies, intensities, energy levels, and transitions can be listed and simulated spectra can be displayed and plotted. Parameters can be iteratively adjusted to approach an experimental spectrum.

Example of an AX2Y system using menu buttons:

1. Load an FID and transform it.
2. Expand the multiplet you wish to simulate.
3. Adjust the threshold so that all peaks are picked.
4. Enter **dll** to display a line listing (will be used later).
5. Select **[Main Menu] - [Analyze] - [Simulation]**
You are now in the Spin Simulation Main Menu.
6. Select **[Spin System]** - choose the spin system (use **[other]** to get more options)
The example used here is an AX2Y system.
7. Select **[Set Params]**. The spectrum will reappear.
8. Click the left button on the center of the multiplet and enter **A=cr**
This sets the chemical shift of spin A to the position of the cursor
9. Click the left button on the center of the left-most line of the multiplet, and the right button in the center of the second left-most line, enter **JAY=delta**
This sets the coupling constant to match the difference frequency.
10. Click the right button on the center of the third line and enter **JAX=delta**.
11. Select **[Main Menu] - [Analyze] - [Simulation] - [Show Params] - [Simulate]**
The simulated spectrum will appear.
12. To continue iteratively, type **iterate?**
The status window will display: `iterate='A, JAX, JAY'`
13. Select **[assign] - [auto assign]**
The assign macro is executed, assigning the lines from the dll listing to the lines from the previous simulation.
14. Select **[iterate]**
15. Select **[list]**
This listing contains the values of the A, JAX, and JAY parameters that give the best iterated fit to the experimental spectrum.

If you want to simulate a spin system from scratch, load a H1 dataset at the same field strength for which you would like to simulate. This will ensure that your coupling constants are correct.

1. Click on **[Main Menu] - [Analyze] - [Simulation]**
You are now in the Spin Simulation Main Menu.
2. Click on **[Spin System]** - choose the spin system (use [other] to get more options)
3. Click on **[Set Params]** The spectrum will reappear.
4. Enter chemical shifts for each peaks in Hertz: **A=#### B=#### X=####** etc.
5. Enter the coupling constants in Hertz. **JAB=#### JAX=#### JBX=####** etc.
6. Click on **[Main Menu] - [Analyze] - [Simulation] - [Show Params] - [Simulate]**
The simulated spectrum will appear.

Deconvolution

Complex patterns of multiplets can be deconvoluted into their component parts for integration purposes.

The following parameters are available for each line:

Frequency (in Hz)

Intensity

Linewidth (in Hz) at half-height of line

Gaussian fraction of line (0.0 for completely Lorentzian line; 1.0 for completely Gaussian line)

All parameters can be fit simultaneously or selected parameters can be removed from the fit. A linear baseline correction is always added to the fit to avoid errors from baseline effects.

The following text files are used:

fitspec.inpar--contains starting parameters

fitspec.indata--contains point-by-point intensity of the spectrum

fitspec.outpar--contains final parameters after a fit

mark1d.out--contains results of a **mark** operation

For best results, use $fn = 2 * np$

For complex problems, use **usemark** to set the best possible guesses (see below).

Example using the menu buttons:

1. Load an FID.
2. Enter **fn=65000**
3. Enter **lb=1.0**
4. Transform the fid: **wft**
5. Expand on the region of interest so that it fills the screen.
6. Type **ai** for absolute intensity mode.
7. Choose a threshold value that picks all the peaks of interest and type **dpf** to check this.

If all peaks of interest are not picked, you will need to use the **[Mark]** function. Put the cursor on each peak you are interested in and select **[Mark]**. This will create a file called *mark1d.out* in your *vnmr/sys/exp#* directory. For each deconvolution you plan to do, this file must be zeroed such that there are no entries in it as the **[Mark]** command appends to the file: **mark('reset')**

8. Click on the buttons: **[Main Menu] - [Analyze] - [Deconvolution]**
9. Click on **[Use Line List]** or **[Use Mark]** depending on the outcome of step 7.
This creates a line list and file containing the starting point for the deconvolution and automatically measures the linewidth of the tallest line on the screen and uses that as the starting linewidth for the calculation.
10. Click on **[Fit]**
This performs the deconvolution. When finished, the calculated spectrum is displayed and the numerical results appear in the text window.
11. To plot the results, click on **[Plot]**
This plots the original spectrum, the calculated spectrum, and each of the component lines, along with the numerical results. At the end of this operation, the original spectrum is again displayed.
12. To return to the calculated spectrum, click on **[Main Menu] - [Analyze] - [Deconvolution] - [Show Fit]**
13. To view the original and calculated spectra simultaneously, click on **[Add/Sub]**
14. Select **[sub]** from the menu to view the difference between the two.

More details:

slw--starting linewidth. Set automatically using the menu buttons or can be bypassed using the **usemark** macro with 2 cursor input. Typical value is 1.

fitspec performs deconvolution by fitting experimental data to Lorentzian and/or Gaussian lineshapes. This macro uses the file *fitspec.inpar* (containing information on frequency, intensity, linewidth, Gaussian fraction) which must be created before the calculation. Any number followed by an asterick(*) is held fixed during the calculation.

fitspec creates the file *fitspec.indata*, a text representation of the spectral data. After the calculation, the results are contained in the file *fitspec.outpar*.

The **setgauss** macro modifies the output of the last deconvolution (*fitspec.outpar*) and makes it the input for subsequent analysis (*fitspec.inpar*), after first modifying the Gaussian fraction:

To allow this fraction to vary, use the format **setgauss(fraction)** where fraction is the Gaussian fraction of the lineshape, from 0 to 1, e.g., **setgauss(0.4)**

To fix the fraction, use the format **setgauss('fraction*')**, e.g., **setgauss('1.0*')**

Display and Plotting Deconvolution:

The results are written into the file *fitspec.outpar*. The macro **showfit** converts the data into a useful format for analysis and printing.

The command **dsp('fitspec.outpar')** displays the theoretical spectrum. The macro **plfit** produces a complete output plot with the observed spectrum, the full calculated spectrum, each individual component, as well as the numerical results of the analysis (or use the **[Plot]** button in the Deconvolution menu).