

# **NUTS Cheat Sheet**

by CG Fry, TC Stringfellow, and M Ivancic: updated 2013.01.25

The NUTS on-line Help is excellent, but extensive; it does not provide a shortened reference guide. This *Cheat Sheet* is presented as a brief outline of common NUTS usage. See the on-line Help, and NUTS help section at [www.acornnmr.com](http://www.acornnmr.com) for more complete tutorials and help.

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**0a. A Common Command Sequence for 1H 1D Processing**

- GA**      **File → Open/Import**      ;works better (more consistently) than IM
- BC**      ; time-domain baseline correction
- LB**      ; check line broadening, =0.3 is common for 1H spectra
- EM**      ; exponential line broadening; removes sinc wiggle artifacts
- ZF**      ; zero-fill; performing a 2<sup>nd</sup> ZF may improve the appearance of the data
- FT**      ; Fourier transform
- ZO**      ; or *double-click*; *left-drag* across region, then *right-click* to Zoom  
    <RET> ; to exit zoom
- left-hold—P*      ; set phasing pivot point; choose peak at either side of spectrum
- PH**      ; phase, *left-mouse* for 0-order at pivot point, *right-mouse* for 1<sup>st</sup>-order  
    <RET> ; to exit PH routine
- left-hold—R*      ; on reference (e.g., TMS) peak
- FB**      ; flatten baseline routine
- L**      ; after correcting red strips computes least-squares fit  
    <RET> ; applies baseline correction and exits FB
- ID**      ; integrate routine  
    <RET> ; exit integrate routine
- ^I**      ; display integrals at Base level
- left-hold—M*      ; set minimum height (for peak picks)
- PP**      ; auto-select peak picks
- ^P**      ; display peak picks at Base level
- DP**      ; enter Define Peaks routine
- H**      ; change peak labels to Hz  
    <RET> ; exit DP routine
- IS**      ; inset regions
- NO**      ; notes
- SB**      ; save spectrum
- PL**      ; print spectrum
- DT**      ; generate data tables, useful for generating publication listings

**0b. A Common Command Sequence for 13C 1D Processing**

- GA**      **File → Open/Import**      ;works better (more consistently) than IM
- BC**      ; time-domain baseline correction
- LB**      ; check line broadening, =1 or 2 Hz are common for 13C spectra
- EM**      ; exponential line broadening; removes sinc wiggle artifacts
- ZF**      ; zero-fill; performing a 2<sup>nd</sup> ZF may improve the appearance of the data
- FT**      ; Fourier transform
- ZO**      ; or *double-click*; *left-drag* across region, then *right-click* to Zoom
- left-drag*      ; across one region of peaks
- 1**      ; to set 0-order phase region
- left-drag*      ; across a 2<sup>nd</sup> region of peaks
- 2**      ; to set 1-order phase region
- <RET> ; to exit zoom
- PE**      ; phase 0-order using *left-mouse*, phase 1st-order using *right-mouse*
- <RET> ; to exit PE routine
- left-hold—R*      ; on reference (e.g., CDC13) peak
- left-hold—M*      ; set minimum height (for peak picks)
- PP**      ; auto-select peak picks
- ^P**      ; display peak picks at Base level
- IS**      ; inset regions
- NO**      ; notes
- SB**      ; save spectrum
- PL**      ; print spectrum
- DT**      ; generate data tables, useful for generating publication listings

Listed below are common commands that can be run with a mnemonic, shown bold as in **FT**, and the same menu command, found by mouse clicking as in **Process**→**Transform**→**Fourier Transform**; the mnemonic, if one exists, is listed in the menus.

### 1. Import Data

**GA**      **File**→**Open**      ;we strongly recommend the use of **GA** for all opens *and* imports; **IM** fails when the path gets too large; the nuts.ini file may need modification to import bruker data correctly.

*example for Varian and Avance data:*

[click into a foldername (Varian) or an exp# (Avance) and double click on fid ]

[for Avance, you can go down into pdata and a pexp# and click on **1r** — in this case you don't need the following as you are importing the processed spectrum]

**BC**    **Process**→**Baseline Correct**      ; removes 0Hz/center glitch  
**LB**                                           ; set up an appropriate line-broadening parameter  
**EM**                                           ; apply exponential multiplication apodization  
**FT**                                           ; Fourier transform the data

We have run into two bugs with importing (**IM**) data that occur somewhat regularly with NUTS. If you get an error message when trying to import data, try the following:

- Always use **GA** instead of **IM**. When the directory structure gets deep, **IM** fails whereas **GA** always seems work ok.
- On occasion, a Bruker AC dataset will not import properly; in these cases, NUTS seems to not to be able to figure out the data type properly. The following solves this problem:

```
2F
im -type BRUKERASPECT↵
2N↵
```

### 2. Apply Multipliers

**LB**    **Process**→**Conditions**→set **LB**  
Set value for multiplication (0.1 to 0.3 Hz for <sup>1</sup>H, -0.5 to 3 for <sup>13</sup>C)  
**EM**    **Process**→**Window Function**→**Exponential Mult.**

### 3. Transform

**BC**    **Process**→**Baseline Correct** (a DC-offset correction of the FID)  
**ZF**    **Process**→**Zero Fill**  
**FT**    **Process**→**Transforms**→**Fourier Transform**

#### 4. Zoom & Display

- a) *double-left-click* to enter Zoom Mode, or type **ZO**
  - b) *left-drag* to select region to zoom (Alternatively, typing **F** in Zoom Mode provides a menu in which to specify horizontal and vertical display limits.)
  - c) *right-click* to expand the specified region
  - d) *return* will exit Zoom Mode
  - e) Outside of Zoom Mode, *double-right-click* to toggle between full view and zoomed view (mode stays at top level); **Ctrl-E** shows expanded view, **Ctrl-F** shows full view. **DC** (at Base Level) sets the vertical display position via a slider bar in the upper left portion of the screen.
- Ctrl-A** toggles the horizontal axis display units between ppm, Hz, points and none.

#### 5. Phasing

The following recommended methods both work quite well. **PH** is a bit easier to learn and works nicely. **PE**, although a bit more complicated to set up, allows very accurate setting of the phase. Keep in mind also that scaling up the spectra (using the right-hand slide bar, or the **>** **<** and **↑** **↓** keys on the keyboard) usually helps with phasing by allowing better observation of the baseline.

##### Recommended method #1 – PH:

At the Base Level:

- i) – *left-click-and-hold* mouse button on a large upfield or downfield peak
  - press **P** to set toggle point while still holding the mouse button down
- ii) **PH Process→Phasing...→PHasing by mouse**
- iii) 0-order correction: *left-drag* up-down/left-right and fix phase about toggle point
- iv) 1<sup>st</sup>-order correction: *right-drag* up-down/left-right

##### Recommended method #2 – PE:

Enter Zoom Mode by *double-left-clicking*, then

- i) – *left-drag* over a downfield region (called region #1) that has well-resolved peaks
  - DO NOT right click to zoom to this region
  - after releasing the *left-drag*, enter **1** on the keyboard to define this as region #1
- ii) – *left-drag* over an upfield region (called region #2) that has well-resolved peaks
  - DO NOT right click to zoom to this region
  - after releasing the *left-drag*, enter **2** on the keyboard to define this as region #2
- iii) – press return to exit Zoom Mode
  - *left-click-and-hold* mouse button on one downfield peak (one in region #1)
  - press **P** to set toggle point while still holding the mouse button down
- iii) **PE Process→Phasing...→Phasing Expanded**
- iii) – region #1 is automatically displayed
  - perform a 0-order correction by *left-drag* up-down/left-right
  - region #2 is displayed automatically by *right-dragging* to perform 1<sup>st</sup>-order phase corrections; *left-drag* will automatically toggle back to region #1
- iv) press Enter to exit PE mode

Other possibilities for phasing:

- QP** Process→Phasing...→Quick Phase (will do both, but is slow)  
**QA** no mouse/menu equivalent; sets only 0-order phasing  
**AP** Process→Phasing...→Auto Phasing (not recommended)

**6. Referencing**

- i) *left-click-and-hold* mouse button on desired peak
- ii) press **R** to open reference windows while holding mouse button down
- iii) note various options in reference window such as *find maximum* and *interpolate*

Note: As of June, 2012 the following results in an incorrect X-nucleus chemical shift!!! The X-nucleus chemical shift is approximately 5-6ppm off. We are working to correct this issue, in the meantime please use MestreNova's software for Unified Scale referencing.

The Unified Scale can be used within NUTS to properly reference 1D X-nucleus spectra based on a referenced  $^1H$  spectrum. The procedure in brief is:

1. Acquire  $^1H$  and X-nucleus data from the compound during the same session (same lock, same shims). Save as the raw data on the spectrometer for importation into NUTS.
2. Work the data up as normal in NUTS, and properly reference the  $^1H$  spectrum. Save both as NUTS spectra.
3. With the  $^1H$  spectrum properly referenced and open in NUTS, run the appropriate macro using **RU**. The facility provides the following macro:

```
absref_13C.mac
absref_19F.mac
absref_31P.mac
```

All are simple modifications of the example described in AcornNMR's on-line documentation. Macros for other nuclei can easily be made.

4. Open the X-nucleus (NUTS-format) spectrum as requested by the macro. This spectrum will now be properly referenced.
5. When publishing data based on the Unified Scale, use a statement similar to the following:

*The X-nucleus spectra were referenced to TMS in the  $^1H$  spectra, using the Unified Scale [citation: R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, R. Goodfellow, and P. Granger, "NMR Nomenclature. Nuclear Spin Properties and Conventions for Chemical Shifts (IUPAC Recommendations 2001)", Pure and Applied Chem 73, 1795-1818 (2001)].*

**7. Baseline Correction**Simple Baseline Corrections

- BC** Process→Baseline Correct uses end points of the complete data set for DC and tilt corrections
- BF** Process→Baseline Flatten uses only end points of the zoomed region (whether zoomed or not!) for DC and tilt corrections; applies DC to all data

Sophisticated Baseline Corrections

- FB Process→Fit Baseline** 7<sup>th</sup> order polynomial fit to baseline
- *left-click* toggles region on/off for inclusion in fit
  - **L** (recommended) linear-least squares fit of baseline regions
  - **C** simplex (slower) fit of baseline regions
  - **RM** at top level brings up **Process→Conditions...** window to change RM
  - **P** draws the fitted polynomial
  - **X** quits FB routine without applying baseline correction
  - **Q** quits simplex optimization
  - **Enter** applies the correction and exits

**8. Integration**Manual Integration

- i) **ID Process→Integrate Display**, or  
**I** in Zoom Mode (equivalent to ID; no mouse path)
- ii) – Baseline correction (use FB [preferred] or BF) should be done prior to starting integrations, and/or from within **ID**:
  - **B** 0-order and tilt correction on integral
    - *left-drag* 1<sup>st</sup> to correct 0-order at left side of integral
    - *right-drag* 2<sup>nd</sup> to correct tilt (baseline to the right side of screen)
  - **F** toggles between sub-integral and full integral
- iii) – *left-click* (1<sup>st</sup> time) to start a sub-integral region
  - *left-click* (2<sup>nd</sup> time) to define the beginning of a sub-integral region
  - *left-click* (3<sup>rd</sup> time) to define the end of a sub-integral region
  - **L** deletes the last defined sub-integral region
  - **C** deletes all sub-integral regions defined
  - *left-click* then **D** deletes the sub-integral region under the mouse cursor
  - **Q** aborts cursor and sub-integral definition process
- iv) – *left-click* then **V** opens a sub-integral amount window for the region under the mouse cursor, e.g., to specify 1 proton (set the amount = 0 to remove labels)
  - **M** toggles the integral number position from top of integral line to top of screen
  - *left-click* then **1**, **2**, or **3** shifts the position of the sub-integral label for the sub-integral region under the mouse cursor
  - **Z** displays a 2<sup>nd</sup> left-side scroll bar to position starting point of all sub-integral regions
- v) – **T** send integrals to a clipboard inside the integral routine (equivalently, use **IL** at the Base Level); **CB** or **Ctrl-B** displays the clipboard contents
  - **Ctrl-I** at the top level toggles integral display on/off (equivalently, use **AN** or **AF**)

Automatic Integration

- AI** at the Base Level performs automatic integrations; **ID** then allows editing of regions (baseline correction is recommended before running AI)

## 9. Peak Picking and Annotations

### Automatic Peak Picking

**PP** displays peak pick lines above selected peaks

**MH** minimum height (in percent) for the PP command

*left-hold* of mouse cursor, plus **M**, sets the minimum height for the PP command

**RM** sets the multiples of RMS noise that a peak must decrease from its maximum before selecting another peak (try a smaller value if not seeing small couplings; try bigger if getting lots of peaks on a broad line)

**CB** or **Ctrl-B** displays clipboard (PP automatically sends a peak list to the clipboard)

### Manual Peak Picking with Annotations

**DP** enters the Define Peaks routine

- *left-click* to define a new peak
- *right-click* on peak label to edit the label (positioning is definitely *not* WYSIWYG, so define a peak close to the desired horizontal position)
- *left-drag* on label to position
- **FH** or **FV** at the Base Level to set font parameters for horizontal or vertical peak labels
- **A** Automatically pick peaks (Minimum Height must be set before entering DP)
- **C** Clear (delete) all chosen peaks
- **G** Get peak file. Recalls previously saved peak list and displays the corresponding peaks, deleting any peaks which had been selected.
- **H** Toggles peak labels from ppm to Hz
- **I** Label peaks with text label, rather than chemical shift (necessary for annotations)
- **K** Delete the peak closest to the cursor location
- **S** Save peak file.
- **T** Copy peak list to the clipboard
- **#** Re-order the peak list, from highest to lowest chemical shift

**Ctrl-P View→ShowPeakLabels** at the Base Level to display peak labels

### Single Text Annotation

Only one clipboard text can be used in NUTS. This includes peak pick lists, integral lists, or a single annotation. To get more annotations, use **NO** (preferred) or **DP** as described above.

- a) Take text from any Windows program (e.g., Notepad) and Copy it into the clipboard
- b) **CB View→Show Clipboard Text** (or use **Ctrl-B**)
  - *left-hold* plus **C** moves the upper left corner of the clipboard contents to the cursor location
  - **FC Edit→Set Fonts→Clipboard Font** changes the text font parameters
  - this text cannot be resized, whereas the **DP** text labels can
- c) Graphics-based text (e.g., from MS Draw or Paintbrush) can be imported into NUTS via the **MO** command (see the next discussion on inset or section plots).



## 10. Section (Inset) Plots

### Manual Sections

- a) Zoom to define the spectral region desired, then return to the Base Level.
- b) **IS A** **Tools**→**Inset Plots**→**Add Zoomed Region** adds the current view
- c) *left-drag* inside to move the object, or on a corner to resize
- d) *right-click* inside the object to edit parameters, e.g., to set Hz/cm, printer fudge factor (1.0084 is correct for HP printers), or axis units (ppm, Hz, points or none)
- e) **Enter** to exit IS routine

## 11. Dual Display and Add/Subtract

Perform the following general sequence to compare two spectra:

- a) **IM** – read in FID, process as normal (or **GA** to open an existing spectrum)  
**AL** – to put that spectrum in buffer  
**IM** – read in 2<sup>nd</sup> FID and process (or **GA** to open another existing spectrum)
- b) **DD** – shows both spectra (toggles Dual Display mode on/off)
  - i) **PL** plots as displayed
- c) **AS** – enters the Add/Subtract subroutine
  - i) **D** displays the current spectrum and the difference spectrum
  - ii) **B** displays both the current spectrum and the buffer spectrum (“undoes” **B**)
  - iii) +/- adds/subtracts the buffer spectrum to/from the current one and updates the current spectrum with the result

## 12. Stack Plotting using BU mode (Buffers subroutine)

In general, BU mode is recommended for making up stack plots containing relatively few 1D spectra (3 to perhaps 10 or 12), as BU mode is more intuitive and simpler to work in. The more general SP mode works best when large numbers of spectra need to be stack plotted.

**BU** will put you into ‘Buffers Control’ mode.

- a) **A** adds the current worked-up spectrum to the buffer,
- b) exit BU with ↵
- c) **IM** to import a new fid, or  
**GA** to open another spectrum,
- d) then **BU** to enter buffers mode (after fid is processed), and
- e) another **A** to add this one to the buffer.

The buffers are numbered in the order in which they are added. Additional spectra can be added in this manner, c) thru e).

- Select a buffer by simply rolling the cursor over it; the selected spectrum will blink.

- **E** will bring up an edit dialog box for the current buffer, for changing the color of the spectrum, and adding a comment line that will be positioned on the spectrum.
  - *left-click* over the baseline and then dragging to reposition the spectrum.
  - **<** and **>** arrows decrease/increase the intensity of the selected buffer.
  - **D** deletes the current buffer
  - **C** clears all the buffers
- f) View -> 'Display buffers outside of BU' will leave spectra display after exiting BU mode with ↵ (where all normal commands, such as notes, are available)
- g) **S** saves a NUTS file with the buffer spectra/info included (or use **File** -> **Save file** within Buffers mode). *Note that a normal Save file outside of BU mode will NOT save the buffers spectra or information that goes with it!!*

### 13. Stack Plotting using SP (pseudo 2D) mode

- a) Files must have the same prefix, and the suffix must be serially incremented from 001 (e.g., kinetics.001, kinetics.002, kinetics.003, ..., kinetics.006).
- b) The following commands and links are straightforward on a computer where you have complete privileges. The facility PCs, however, do not allow delete privileges in the spectrometer folders; e.g., you cannot delete files in N:\phoenix\. Use the following folders for all **Save As** requests (for all **SB** and **SC** commands):

**P:\**            or            **C:\temp**

- c) **IM** – read in 1<sup>st</sup> FID (or spectrum); process normally (e.g., EM, FT, phase)

For <sup>13</sup>C plus DEPT spectra, it is easier to ignore the next link command, and process each dataset manually, followed by an **SB** to save each dataset into NUTS format.

**LI** – run a link (e.g., **IM BC EM FT PS SB IN**) to process a series of FIDs into NUTS-format spectra  
or a link (**IM SB IN**) to write a series of NUTS-format files from spectra

- d) **LI** – run a link (**GA SC IN**) to turn NUTS-format spectra into a NUTS 2D file  
(Steps c and d can be combined if desired: **IM BC EM FT PS SC IN**)

**GA**– read in the newly created NUTS 2D file

- i) **VW** with **N** or **B** will run through spectra quickly
- ii) **SS** sets the vertical scale according to the tallest peak in the 2D data set

**SP** – initiates the start stack-plot routine

- i) **O** sets the x- and y-axis offsets
- ii) **A** changes the vertical amplitude
- iii) **W** toggles the whitewash feature
- iv) **P** plots the stacked plot

- e) To fully enable stacked plotting of expanded sections, first use **ZO**, then **F** to access the **Zoom Offset Information** menu. Set **Vertical Dimension**, **Start of Zoom Slice** and **End of Zoom Slice** appropriately to display those spectra desired.
- f) Scaling one spectrum independently of the others is done in a fairly primitive manner in NUTS. Using a version of 2D NUTS is easiest:
  - i) if using 2D NUTS, enter the arrayed mode using: **AR**
  - ii) use the view **VW** command, and go to the slice you want to scale  
type  $\downarrow$  to exit with this slice being shown on the display
  - iii) type **2f** to exit two-letter command mode
  - iv) enter **multiply 5** or **divide 2** to multiply(divide) the spectrum by 5(2)
  - v) type **2n** to re-enter two-letter command mode
  - vi) if using 1D NUTS, you must resave the spectrum using **S2** ; use the same psuedo-2D NUTS filename
  - vii) use **SP** to view the stack plot

#### 14. Deconvolution Fitting

- a) Before beginning, perform **BF** or **FB** to correct the baseline.
- b) Zoom to display the region to be fitted, then exit zoom. (*Do not include too much baseline or too many points; set the number of points  $\leq \sim 1000$  total.*)
- c) **LF Tools**→**Line Fit** enters the line-fit routine
- d) *left-click* on each peak; a red line with estimates of peak's parameter appears  
or **P Fit**→**Peak Pick** for automatic selection of peaks  
or *left-click* below axis for automatic peak max selection (recommended)
  - *right-click* down curve to adjust width (works on current/red peak only)
  - **I Fit**→**Information On Line** opens dialog box about estimates
  - *left-drag* across Relative Area and replace with 1; Apply will give simpler comparison of estimated areas
- e) **A Fit**→**Optimize All**
  - **Q** will quit the optimization in progress

#### 15. Relaxation Analysis

[see NUTS help]

**16. Data Tables (DT) and Publishing NMR Data (Word/PowerPoint)**
**For  $^{13}\text{C}$  spectra and publication listings:**

1. Open and process the  $^{13}\text{C}$  spectrum as normal.
2. Perform a peak pick:
  - *left-click-n-hold* the mouse cursor at a level just below the lowest peak of interest
  - press **m**
  - remove or add peaks using the **DP** (define peaks) utility
3. Import the peak pick data into a NUTS data table:
  - **2f** ; turns off 2-letter command mode (**2n** turns it back on)
  - **dt getpeaks** ; creates a data table and pastes in peak pick data
  - **dt ppmdecimals 1** ; if want only 1 decimal place for  $\delta$  (then redo getpeaks)
4. Edit multiplicity and/or assignment information in the 3<sup>rd</sup> column:
  - *right-click* in a cell and type in the information
  - Most often, only multiplicities are provided with the chemical shifts in  $^{13}\text{C}$  listings, and are entered in the 3<sup>rd</sup> column. The 4<sup>th</sup> column can be used to document assignments.
5. Save the data table (which is NOT saved with the NUTS spectrum with **sa** or **sb**):
  - **dt export** ; use **dt import** to read back in later
6. Create a publication listing:
  - **dt cacs** ; please provide feedback about the format; it may change (or have other formats added, e.g., **dt corg**) in the future
  - paste into Word and make final corrections
7. Type **2n** to re-enter two-letter command mode.

**For  $^1\text{H}$  spectra and publication listings:**

1. Open and process the  $^1\text{H}$  spectrum as normal.
2. Peak picks can be used for  $^1\text{H}$  spectra, but the method discussed below is usually more convenient. If you decide to use peak picks, make certain it is in Hz for computing the  $J$ -couplings.
3. Open a data table in NUTS:
  - **2f** ; turns off 2-letter command mode (**2n** turns it back on)
  - **dt** ; creates a blank data table and pastes in peak pick data
  - **dt ppmdecimals 3** ; will set 3 decimal places for  $\delta$  values
4. Use NUTS to directly enter each multiplet's  $\delta$  and  $J$ -coupling values:
 

$\delta$  (chemical shift) values

  - zoom (double-left-click, drag, right-click) so the multiplet is easily seen on-screen
  - *left-click* into a PPM cell
  - *left-click-n-hold* with the cursor centered on the multiplet
  - press **s** ; the cursor position ( $\delta$  value) will be entered into the table

Multiplicity, # protons, Assignment: *right-click* on the cell and type in

J-couplings

- *left-click* into the J cell
- *left-click-n-hold* on one peak of the splitting, *right-click-n-hold* on the other peak
- press **d** ; the J-coupling will be entered in the table
- repeat for each J-coupling in the multiplet

Repeat the procedure above for each multiplet in the spectrum.

- Add rows by *right-click* on any cell, and the ADD ROWS button in the panel.
5. Save the data table (which is NOT saved with the NUTS spectrum with **sa** or **sb**):
    - **dt export** ; use **dt import** to read back in later
  6. Create a publication listing:
    - **dt hacs** ; please provide feedback about the format; it may change (or have other formats added, e.g., **dt horg**) in the future
    - paste into Word and make final corrections
  7. Type **2n** to re-enter two-letter command mode.

**Notes on Pasting NUTS Spectra into MS Word and PowerPoint:**

A few simple modifications will enable reasonable quality pastes of NUTS spectra into MS Word or PowerPoint. From a normal session, do the following:

1. **BW** switches to black-and-white, typical for publication. **CD** will toggle back to color.
2. The font sizes usually need to be increased for publication spectra. Use the following:
  - FA** ; change the axis font (try 16 to 20 pt for Word; unchanged or 2pt larger for PowerPoint)
  - FI** ; change the integral label font
  - FV** ; change the peak pick label font
3. **Edit** → **Copy Enhanced Metafile to Clipboard**  
is usually sufficient for following pastes into Word or PowerPoint.
4. On occasion, minor adjustments of the pasted graphic need to be done (e.g, small move of an integral label). These can normally be managed by **right-clicking** on the spectrum in Word or PowerPoint and selecting **Edit Picture**.

## 17. Processing 2D Data

Use the **NUTS 2D Pro** version—with array mode, available on all the PC's within the facility (but not available for distribution)—for 2D processing. The following are *guidelines* for processing various types of 2D data; some details may depend upon your specific acquisition and processing requirements. The online help and acorn website ([www.acornnmr.com](http://www.acornnmr.com)) both provide extensive help, examples, and demonstrations of 2D processing. Note that display of 2D data via **IP** (in **2D**) is significantly faster than via **CP**; the former command is useful for optimizing the display parameters, whereas the latter is preferable prior to printing a hardcopy.

In manner completely analogous to stack plotting, the following commands and links are straightforward on a computer where you have complete privileges. The facility PCs, however, do not allow delete privileges in the spectrometer folders; e.g., you cannot delete files in N:\athena\. Use the following folder for all **Save As** requests (for all **SA**, **SB** and **SC** commands):

S:\            or            [memory key]

### A. Magnitude COSY data from the Bruker ACs

- i. **1D projections:** Process and save (**SA** or **SB**) the 1D high-resolution spectrum for projections.
- ii. **Macro processing:** There are two macros that can be run to automate the complete procedure describe in sections III-V below:
 

**RU**↓            **cosy\_sb\_zfzf.mac**            or            **cosy\_sb2\_zfzf.mac**

The first macro performs sinebell (**MS**) apodization, and the 2<sup>nd</sup> sinebell-squared (**MS MS**) apodization. These macros will allow symmetrization *only* for datasets where  $TD = 4 \times NE = 4 \times TD1$ , because the  $t_1$ -dimension is zero-filled twice in both macros. Initiate the macros with the command **RU**; performing an **IM** prior to **RU** is *\_not\_* needed, as the macro starts by asking for the 2D dataset to open.
- iii. **Manual work-up of  $t_2$ :** To work up the 2D data set manually in the directly detected domain ( $t_2 \rightarrow f_2$ ):
  - a) **GA filename.SER**            (import data set with **array mode off** at this point)
  - b) **AR**                                    (turn on the array-mode processing; this command toggles array-mode on/off, as indicated in status line)
  - c) **BC MS FT**                            (all data are operated on simultaneously)
- iv. **Manual correction of  $t_1$ -parameters:** Set the spectral parameters:
  - a) Access the **View** → **Spectral Parameters** menu.
  - b) Set the  $t_1$  ( $f_1$ ) parameters in the right-hand column equal to their respective  $t_2$  ( $f_2$ ) parameters in the left-hand column for:
 

Frequency (SF)

Sweep Width (SW)  
Offset (O1)

Use copy (CNTL-C), TAB, paste (CNTL-V), TAB 3 times to perform this task.

- c) Note the difference between the Number of Points in both dimensions. You will have to zero-fill sufficient to make the dataset square (e.g., zero-fill twice if # point in direct dimension equals 512 and # points in indirect dimension is 128).

**v. Manual work-up of  $t_1$  domain:** Work up 2D data in the indirect detected domain ( $t_1 \rightarrow f_1$ ):

- a) **TD** (Transpose the **D**ata matrix)
- b) **MS ZF ZF FT** (perform first stage of data processing)  
(zero-fill as needed to produce a square spectral matrix)
- c) **MC** (perform a **M**agnitude **C**alculation)
- d) **SR** (**S**pectral **R**eversal to form LL-to-UR diagonal)
- f) **SS** (perform **S**pectral **S**caling of the data)

The Links (**LI**) L2–L5 have been written to automate this processing for the case of two zero-fills (i.e., for TD1=NE=TD/4). L2,L3 both symmetrize, L2 performing sinebell and L3 sinebell-squared apodization. L4,L5 leave out the symmetrization step.

**vi. Symmetrization:** Viewing the dataset prior to symmetrization is recommended. Use care in interpreting any small/less-intense crosspeaks in a COSY dataset; view the dataset prior to symmetrization to see whether the crosspeaks might be a “symmetrization trap” artifact (see Derome, Fig 8.32):

**SY** (SYmmetrize the data)

**vii. Display and plotting:**

- a) **2D** (enter the 2D display routine, intensity display, IP)
- b) **MH** (set the parameter for **M**inimum **H**eight)
- b) **Borders** → **Pick the \_\_\_\_\_ Spectrum** (specify the files for projections)
- c) **Edit** → **Edit Display Parameters** (set scaling for projections)
- d) **ZO (F)** (**Z**Oom in to select the region of interest)
- e) Reference the spectrum if necessary, using the normal procedure.
- f) **CP** (display the 2D data set in **C**ontour **P**lot mode)
- g) **PL** (print the spectrum)

These print files can be quite large. It is unlikely there will be printer memory problems within the facility. But if you are printing from your lab or home, you may have to reduce the printer resolution to 300dpi or smaller to get the prints to work.

**B. gCOSY/Gcosy processing (magnitude mode COSY data: works also for gcosy, COSY, and cosy) from Varian's VNMR**

- i. Open the file named *fid* inside the folder of data that is the gCOSY or gcosy, etc., you want to process.
- ii. Start the macro by typing **RU** :

brings up dir: G:\Common\NetNUTS\NUTS\mac on the facility PCs.

**iii.** Choose the macro **varian\_mag\_cosy\_sb2\_?zf.mac** .

This macro will process both dimensions using ? ZFs (zero fills) in the indirect dimension, and put NUTS into **2D** mode with the direct dimension on the x-axis (horizontal) and the indirect dimension on the y-axis (vertical).

**iv.** Use **SY** to symmetrize the data.

**v.** Use **TD** to transpose the matrix (i.e., reverse the axes, or rotate by 90°).

**vi.** Use **NO** to enter notes.

**vii.** Enter **1D** to go back to the one dimensional mode. You can save the data only in the **1D** mode (more on saving data in section F below).

**viii.** Enter **2D** or **IP** (intensity plot) to get back to the two dimensional display mode.

**ix.** Enter **CP** (contour plot) if you'd prefer the peaks to be displayed as contours, and *always* prior to plotting the data.

**C. *gHSQC processing (echo-antiecho, phase sensitive data) and HSQC/HSQCAD processing (hypercomplex data) from Varian's VNMR***

**i.** Open the file named *fid* inside the folder of data that is the gHSQC or HSQC/HSQCAD you want to process.

**ii.** Start the macro by typing **RU** .

**iii.** Choose the macro **varian\_ghsqc.mac** or **varian\_hsqc.mac**, depending on whether you chose the gradient or non-gradient version of the HSQC experiment in the setup.

This macro will process both dimensions, and leaves NUTS in the two dimensional display mode with the direct axis ( $^1\text{H}$ ) on the x-axis (horizontal) and the indirect axis ( $^{13}\text{C}$ ) on the y-axis (vertical).

**iv.** To phase the data see “Phasing 2D data” in section F below.

**v.** To save the data, follow the instructions in section F below.

**D. *gHMBC and gHMBCAD processing (magnitude mode) from Varian's VNMR***

**i.** Open the file named *fid* inside the folder of data that is the gHMBC or gHMBCAD you want to process.

**ii.** Start the macro by typing **RU** .

**iii.** Choose the macro **varian\_ghmhc.mac** or **varian\_ghmhcad.mac** depending on the pulse sequence run. ( $^1\text{H}$  will be on the x-axis,  $^{13}\text{C}$  on the y-axis)

**iv.** This data does NOT need phasing, since it is collected in magnitude mode.

**v.** To save the data, follow the instructions in section F below.



**E. gDQCOSY processing (hypercomplex, phase sensitive data: works also with DQCOSY, NOESY, ROESY, TOCSY, etc.) from Varian's VNMR**

- i. Open the file named *fid* inside the folder of data that is the gDQCOSY you want to process.
- ii. Start the macro by typing **RU** .
- iii. Choose the macro **varian\_dqcosy.mac** .  
 This macro will process both dimensions, and leaves NUTS in the two dimensional display mode with the direct axis on the x-axis (horizontal) and the indirect axis on the y-axis (vertical).
- iv. You typically should not have to phase dqcosy data; typically the diagonal is phased positive with the crosspeaks being antiphase (both positive and negative, half one way, half the other). For NOESY, ROESY, etc., see “Phasing 2D data” in section F below.
- v. To save the data, follow the instructions in section F below.

**F. Useful commands for 2D data processing in NUTS:**

- CP** (contour plot) displays the peaks as contours  
**IP** (intensity plot) displays the peaks as intensities

**Phasing 2D data:**

- a) *left-hold* to get red crosshairs thru the data, then while keeping the *left-hold* do a *right-drag* (left and right mouse buttons concurrently held down) to see the trace of the data where the mouse is located. The vertical scale of the trace can be increased / decreased by hitting the PAGE UP / PAGE DOWN keys (while still doing the *left- and right-hold*).
- b) Stop on a trace that has a peak or peaks in the upper right of the data set.
- c) Type **1D** (the same trace should now be displayed in 1D mode).
- d) *left-click-and-hold*, then press **P** to set toggle point while still holding the mouse button down. Choose a pivot point on a peak on the right side of the spectrum.
- e) Use **PH** to enter phase mode, and correct the phase of the pivot peak with a *left-drag* (0-order phase). Hit enter when you're satisfied with the phase adjustment (this applies the new phase to the entire 2D matrix)
- f) Type **2D** to enter the 2D display mode, and now choose a trace with a peak or peaks in the lower left of the data set and type **1D** .
- g) Enter phase mode using **PH** and with *right-drag* adjust the phases of the peaks on the left side (1<sup>st</sup> order phases); hit **enter** to save and apply the phases to the 2D matrix
- h) Type **2D** , and you now should be phased (this phases the dimension that is on the x-axis (horizontal)). If the other dimension needs phasing, transpose the matrix with **TD** and repeat the phasing procedure above.

**Referencing the spectrum:**

- a) *left-click* to get the red crosshairs, and bring them over a peak that you want the spectrum referenced to (make sure the crosshairs are exactly on the diagonal for COSY or any 1H,1H data).
- b) Type **O** (or **R**) and the referencing dialog box appears.
- c) Enter the appropriate chemical shift values in both the horizontal and vertical dimensions.
- d) Click OK in dialog box and correct referencing should appear in the spectrum
- e) **TD** will reverse the axes (switch x and y); this is needed for phasing the different dimensions of phase sensitive data.

**Saving 2D data sets:**

- a) Exit 2D mode by typing **1D**.
- b) Click on the save icon in the toolbar or go to **File → Save As**.
- c) When the dialog box appears, choose the appropriate directory to save the data in, e.g., **P:\temp**.
- d) At the bottom of the dialog box choose “2D file” in the *Save as Type* box.
- e) Enter an appropriate file name in the *File Name* box.
- f) When reopening the data it will first appear in the 1D mode, then type **2D** or **IP** to get the 2D display.

**Zooming in on regions:**

- a) **ZO** will enter zoom mode, as will a *double-left-click*.
- b) Highlight the desired zoomed region with a *left-drag*.
- c) Put the cursor over the region and *right-click* to expand.
- d) To get the full display, exit **ZO** (with **enter**) and *double-right-click*.

**Zooming in on regions:**

- a) **ZO** will enter zoom mode, as will a *double-left-click*.
- b) Highlight the desired zoomed region with a *left-drag*.

**Drawing lines on the 2D spectrum (LL mode)**

In making assignments using 2D spectra, being able to draw lines on your spectrum can be very valuable. The new LL command provides this capability.

- a) *left-click* to get crosshairs to display, place them at an appropriate position then type
  - **V** to add a vertical line or

- **H** to add a horizontal line
- *left-click* and **L** deletes the last entered line
- *left-click* and **C** clears all the entered lines

b) **LL** enters the 'Edit Line List' routine

- Move the cursor over a line to select it (you should see the line blink) then type **E** and a dialog box appears, allowing you to edit features of that line.
- **D** will delete the currently selected line
- **C** will delete all the lines

Note that in LL mode, you can add vertical (V) and horizontal (H) lines without bringing up the crosshairs, just place the cursor in the desired position and type V or H.

c) As with any subroutine, use ↵ to exit LL mode.

- Unfortunately the lines can not be saved for your next NUTS session.