

U400 Plotting Practice on the SUNDS

This handout contains instructions on the processing and plotting of NMR data acquired from the Unity 400 spectrometer. The purpose of this exercise is to acquaint you with the new system as well as some of the most basic commands for data processing and plotting, and to prepare you for the actual training on the U400 spectrometer. You should practice as many times as is necessary until a basic understanding of the new system is acquired and an acceptable proficiency in data processing and plotting is achieved. NOTE: Even if you plan to do the majority of your data processing off-line using the NUTS program, you still need a certain proficiency using the U400 software for processing in order to check your data.

Before starting your training on the U400 spectrometer, you should be able to accomplish the following tasks without referring to the handout line-by-line:

1. changing working directory and retrieving a data file; saving and deleting a data file
2. data processing, *e.g.*, weighted Fourier transformation, phasing, referencing, *etc.*
3. integration
4. measuring signal-to-noise (S/N) and line width at half height (LW1/2)
5. displaying and plotting spectra

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Explanation of Types of Commands Found in this Handout:

1. The vnmr software and the UNIX operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (*i.e.* CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.

Example: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by a return (signified by <rtm>).

Example: **wft** <rtm>

3. Some commands are executed by clicking a mouse button with its pointer on a "button" found on the screen. The execution of these commands are indicated by a two letter designation (LC {left click}, RC {right click}, or CC {center click}) followed by a word or words in shadow or **bold** that would appear in the button.

Example: LC Main Menu

This means to click the left mouse button with its pointer on the button that says "Main Menu".

4. Some commands are executed by the mouse itself. These commands are indicated by a two letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.

Example: LC (at 6 ppm)

This means to click the left mouse button with the mouse cursor positioned at 6ppm.

5. Parameters are entered by typing the parameter name followed by an equal sign, the value, and a return.

Example: **nt=16** <rtm>

* * * * *

CHECKLIST

According to the instructions, you should have taken the BASICS test before starting on this handout. If you haven't done so, I suggest you do it now. During the course of going through this handout, several questions are asked. I do expect you to pay attention and answer them. In order to facilitate that process, I reproduce the relevant sections and their questions, below.

Part I. ^1H Sensitivity, 0.1% Ethylbenzene in CDCl_3

Compare the S/N values with $l_b=1$ and $l_b=0.3$. What is your conclusion and why?

Part II. ^1H Integration, 0.1% Ethylbenzene in CDCl_3

Why is the integral in the phenyl region ~ 7 instead of ~ 5 ?

Part III. ^1H Homodecoupling, 0.1% Ethylbenzene in CDCl_3

How can you tell if your spectrum is "well-phased"?

Part IV. ^1H Lineshape, 1.0% CHCl_3 in CDCl_3

Note the linewidth and the digital resolution. Why do they differ from each other?

Note the linewidth and the digital resolution. Did they change? Why or why not?

What conclusions do you draw with respect to zero filling and linewidth, line broadening and linewidth, and digital resolution and linewidth?

What value should you expect to obtain for the $\text{LW}^{1/2}$ of the CHCl_3 peak in this sample when you practice on the U400?

Part V. $^{13}\text{C}\{^1\text{H}\}$ Spectrum of 57% Menthol in Acetone- d_6

What do you think would happen if these spectra were re-measured using $d_1=5$?

If after 2 hours of acquisition, some of the signals in your ^{13}C spectrum are just barely visible, how long would it take to double the S/N so that these signals are more creditable?

Compare the pll printout with the dll printout. Why would you prefer one to the other? Or don't you think it matters?

To start the practice, log onto Reslog for the SUNDS and then log onto the datastation:

1. SIGN UP IN THE LOG BOOK

2. CHANGE WORKING DIRECTORY

The sample data for the plotting practice are stored in the U400 fid directory PRACTICE. After logging onto the SPARC2 via Reslog, you should change the current working directory (should be "/export/home/user1d") to the directory PRACTICE (i.e., "/export/home/data/u400/PRACTICE") as follows:

logon <rtn> or LC Log On

Select a spectrometer from the list below [1-6]:

1 <rtn> select the u400 spectrometer

Input your directory name:

PRACTICE <rtn> enter PRACTICE as the username

The screen will show the files in this directory, e.g., DECH1.fid/, INTH1.fid/, etc.

IMPORTANT: You should stay in this directory for the duration of the practice. Do not delete or save over the data in this directory!

Part I. ^1H Sensitivity, 0.1% Ethylbenzene in CDCl_3

Load the SNH1.fid Directory

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

LC SNH1.fid

select the SNH1.fid directory

LC 3:Load

load the selected data to current experiment

Enter Text, Transform, Display, and Phase the Spectrum

text('month/day/year, your last name, advisor's initials, SNH1') <rtn>

wft <rtn>

weighted Fourier transformation

f <rtn>

display full spectrum

full <rtn>

display spectrum to a full screen

aph <rtn>

autophase

dscale <rtn> or LC Dscale

display scale (it's in ppm because $axis='p'$)

Resize Spectral Display Window

LC Resize Window

resizes the spectral display window to a larger

ds <rtn>

display

display spectrum

Reference the Spectrum (setting the CHCl_3 to 7.26 ppm)

NOTE: You are referencing this spectrum using the residual CHCl_3 peak. You should always expand around the peak to be referenced and make sure that the cursor is on the proper peak before typing the reference command, rl(##p).

LC (at the left side of the phenyl region, ~7.5 ppm)

RC (at the right side of the phenyl region, ~7 ppm)

LC 3:Expand

vsadj <rtn> (or CC on top of peak at the desired height)

LC (at the center of the highest peak)

nl <rtn>

rl(7.26p) <rtn>

expand region inside cursors

adjust vertical scale

place cursor on the CHCl₃ signal

select nearest line

reference the selected line to 7.26 ppm

Measure S/N and Store Value in Register

LC 3:Full

LC (at 6 ppm, or type **cr=6p** <rtn>)

RC (at 2.5 ppm, or RC to bring up a second cursor and type **delta=3.5p** <rtn>)

LC 3:Expand

vsadj <rtn> (or CC on top of peak at the desired height)

LC (at 5 ppm, or type **cr=5.0p** <rtn>)

RC (at 3.5 ppm, or RC to bring up a second cursor and type **delta=1.5p** <rtn>)

dsn <rtn> (The value should be ~125.)

dsn:r1 <rtn>

display full spectrum (same as typing **f** <rtn>)

expand region inside cursors

adjust vertical scale

measure signal-to-noise, with the quartet as the signal and 5.0 - 3.5 ppm as the noise region

NOTE: This number should be ~ 120

store signal-to-noise value in register 1 in the dgs parameter group

Set Plotter and Printer Output

plotter? <rtn>

printer? <rtn>

plotter='PS_AR'

printer='LaserJet_600'

Plot Spectrum with Scale and Parameters

pl <rtn>

pscale <rtn>

pap <rtn>

page <rtn>

or LC 8:Plot, LC 1:plot, LC 8:Return

plot spectrum

plot scale

plot parameters

submit above plotter commands to the plotter and change paper after the plot has been completed

Reprocess the data with a different line broadening value

lb? <rtn>

lb=0.3 <rtn>

wft <rtn>

dscale

LC (at 5 ppm, or type **cr=5.0p** <rtn>)

RC (at 3.5 ppm, or RC to bring up a second cursor and type **delta=1.5p** <rtn>)

dsn <rtn>

dsn:r2 <rtn>

shows current value of the line broadening

NOTE THE VALUE _____

change value of line broadening

measure signal-to-noise, with the quartet as the signal and 5.0 - 3.5 ppm as the noise region

store signal-to-noise value in register 2 in the dgs parameter group

Resize Spectral Display Window

LC Resize Window

resizes the spectral display window to the smaller display
display spectrum**ds** <rtn>

NOTE: Always resize the spectral display window to the smaller display before logging off.

dg <rtn>

display acquisition and processing parameters

Compare the S/N values with lb=1 and lb=0.3. What is your conclusion and why?

Print Parameter Groups**printon** <rtn>

send output from the following commands to printer

dg <rtn>

print acquisition and processing parameter groups

dg1 <rtn>

print displaying parameter groups

dgs <rtn>

print "special" parameter groups including the shims

printoff <rtn>

stop output to printer and start printing

Part II. ¹H Integration, 0.1% Ethylbenzene in CDCl₃**Load the INTH1.fid Directory**

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

LC INTH1.fid

select the INTH1.fid directory

LC 3:Load

load the selected data to current experiment

Enter Text, Transform, Display, and Phase Spectrum**text('month/day/year, your last name, advisor's initials, INTEGRATION')** <rtn>**wft** <rtn>

weighted Fourier transformation

Switch Plotter to 11 x 17 in paper**ps_br** <rtn>

macro for plotter='PS_BR'; set plotter output to 11 x 17 inch page

Display, and Phase Spectrum**f full** <rtn>

display full spectrum to a full screen

aph <rtn>

autophase

| |
|---|
| Reference the Spectrum (setting the solvent peak to 7.26 ppm) |
|---|

NOTE: This is exactly the same type of procedure used in Part I.

| | |
|--|--|
| LC (at the left side of the phenyl region) | |
| RC (at the right side of the phenyl region) | |
| LC 3:Expand | expand region inside cursors |
| LC (at the center of the CHCl ₃ solvent peak) | |
| nl <rtn> | select nearest line |
| rl(7.26p) <rtn> | reference the solvent peak to 7.26 ppm |

| |
|-------------|
| Integration |
|-------------|

| | |
|-----------------------------|--|
| LC 2:Integration | enter integration submenu |
| LC 4:Part Int | enter integration routine with integral blanking |
| f <rtn> or LC 2:Full | display full spectrum |
| cz <rtn> | clear previous integral reset points, if any |
| cdc <rtn> | cancel previous drift correction, if any |
| dc <rtn> | apply drift correction |

NOTE: When setting integrals, try to include only the peaks of interest and to be consistent in the amount of baseline cut on either side of peaks of interest.

Expand around the region of interest (place the two cursors near each region to be integrated, then LC 2:Expand); I recommend that you start with the phenyl region.

Select the areas to be cut by using

LC 6:Resets

LC on the **left and right** sides of the signal(s) to set integral zero points

Repeat this process for the rest of the signals you want to integrate in this area; then

| | |
|-----------------------------|-----------------------|
| f <rtn> or LC 2:full | display full spectrum |
|-----------------------------|-----------------------|

Continue expanding and making your integral cuts in this way until the cuts for all areas of interest (the phenyl region at ~7 ppm, the quartet at ~3 ppm and the triplet at ~1 ppm) are set. NOTE: Every time you switch to another expansion, you will need to LC 4:Resets

| | |
|-----------------------|-----------------------|
| wp=10.5p <rtn> | set width of plot |
| sp=-0.5p <rtn> | set start of plot |
| vsadj <rtn> | adjust vertical scale |

| | |
|--|--------------------------------|
| isadj <rtn> (or CC on top of an integral at desired height) | adjust integral vertical scale |
| ds <rtn> | display spectrum |

Place the cursor on the QUARTET.

LC 2:Integration

LC 7:Normalize

Current integral is 100.00. New value?

| | |
|----------------|---|
| 2 <rtn> | set integral normalization scale to 2 protons for the |
|----------------|---|

quartet and displays *normalized* integral values
(should give values ~ 7, 2, and 3)

Why is the integral in the phenyl region ~7 instead of ~5?

Plot Spectrum with *Integrals* and *Integral values*

vp=12 <rtn>

set vertical position of spectrum to 12 mm so that integral values can be plotted under the spectrum plot with *integrals* on the spectrum and integral values under the spectrum

pl pir pscale pap page <rtn>

(or **ds** <rtn>, LC 8:Plot, LC 3:PlotI, LC 9:Return)

print *integral table* and parameter groups

rinton dli dg dg1 dgs printoff <rtn>

ps_ar <rtn>

macro for plotter='PS_AR'; set plotter output to 8.5 x 11 inch page

Turn off Integral Display

ds <rtn> (if 2:Full Integral is not visible on the menu)

LC 2:Integration

LC 3:No Int

vp=0 <rtn>

display spectrum

turn off partial integral display

turn off integral display

set vertical position of spectrum back to zero

Part III. ¹H Homodecoupling, 0.1% Ethylbenzene in CDCl₃

Load DECH1.fid Directory

LC Main Menu

LC File Menu

LC **DECH1.fid**

LC 3:Load

select main menu

select file storage/retrieval menu

select the DECH1.fid directory

load the selected data to current experiment

Enter Text, Transform, Display, and Phase the Spectra

text('month/day/year, your last name, advisor's initials, DECOUPLING') <rtn>

wft <rtn>

weighted Fourier transformation

ds(1) <rtn>

display spectrum 1 (the control spectrum)

f full aph <rtn>

display full spectrum to full screen and autophase

NOTE: Autophasing may not work properly due to the glitch at 5 ppm – this is not a "real" peak. **THIS PEAK WILL NOT PHASE.** If the other peaks in the spectrum are properly phased, then go on to plotting. Manually phase the spectrum if it is required – see instructions below.

How can you tell if your spectrum is "well-phased"?

LC 6:Phase enter the interactive phasing mode
 LC (click on a signal toward the left side of the spectrum about halfway vertically up the screen and adjust the phase by moving the mouse vertically while holding down the left button for coarse adjustment, or the right button for fine adjustment, of the zero-order or frequency-independent phase parameter *rp*)
 LC (click on a signal toward the right side of the spectrum and adjust the phase as above to change the first-order or frequency dependent phase parameter *lp*)
 LC 1:Box exit the interactive phasing mode

NOTE: if you can't seem to phase the spectrum manually, reset both zero order and first order phases to zero by typing "**lp=0** <rtm>" and "**rp=0** <rtm>", and try again.

Set Plot Limits

wp=2p <rtm> set width of plot
sp=1p <rtm> set start of plot

Display Stacked Spectra Vertically

vsadj <rtm> adjust vertical scale
vs=vs/4 <rtm> set vertical scale to 1/4 of current **vs**
dssa <rtm> display stacked spectra vertically

Stacked Plot of Spectra as Displayed

pl(1,3) pscale page <rtm> stacked plot of spectrum 1 to 3 with scale

Print Parameter groups

printon dg dg1 dgs printoff <rtm> print parameter groups

Part IV. ¹H Lineshape, 1.0% CHCl₃ in CDCl₃

Load LSH1.fid Directory

LC Main Menu select main menu
 LC File Menu select file storage/retrieval menu
 LC **LSH1.fid** select the LSH1.fid directory
 LC 3:Load load the selected data to current experiment

Enter Text, Transform, and Phase the Spectra

text('month/day/month, your name, advisor's initials, LINESHAPE') <rtm>
wft <rtm> weighted Fourier transformation
f full <rtm> display full spectrum to a full screen
aph <rtm> autophase (manually phase it if necessary)
dscale <rtm> display scale in Hz (because *axis = 'h'*)

| |
|--|
| Reference the CHCl_3 signal to 0 Hz |
|--|

LC (at the left side of the peak)
 RC (at the right side of the peak)
 LC 3:Expand
 LC (at the center of the peak)
nl <rtn>
rl(0) <rtn>

expand region inside cursors

select nearest line

reference the selected line to 0 Hz

| |
|---|
| Determine $\text{LW}_{1/2}$ and Store Value in Register |
|---|

dres <rtn>
dres:r1 <rtn>

measure linewidth at half-height for selected signal
 store linewidth value in register 1

Note the linewidth and the digital resolution. Why do they differ from each other?

| |
|--------------------------------------|
| Reprocess the Data with Zero-filling |
|--------------------------------------|

fn=2*np <rtn>
wft <rtn>
 LC (at the center of the peak)
nl dres <rtn>
dres:r2 <rtn>

Note the linewidth and the digital resolution. Did they change? Why or why not?

| |
|--|
| Plot Spectrum and Print Parameter groups |
|--|

wp=50 <rtn>
sp=-25 <rtn>
vsadj <rtn>
pl pscale pap page <rtn>
 (or **ds** <rtn>, LC 8:Plot, LC 1:plot, LC 9:Return)

set width of plot to 50 Hz

set start of plot to -25 Hz

adjust vertical scale

plot spectrum with scale and parameters

| |
|---------------------------------------|
| Reprocess the Data with $\text{lb}=1$ |
|---------------------------------------|

lb=1 <rtn>
wft <rtn>
 LC (at the center of the peak)
nl dres <rtn>
dres:r3 <rtn>
printon dg dg1 dgs printoff <rtn>

print parameter groups with r1, r2, and r3 values

What conclusions do you draw with respect to zero filling and linewidth, line broadening and linewidth, and digital resolution and linewidth?

Reprocess the Data from SNH1.fid

At this point, you should know how to load the SNH1.fid file. Do so, process with wft, check the LW½ of the CHCl₃ peak with dres, then store this value in register 1 (dres:r1). Note the value of the line broadening (lb? or dg).

Change the line broadening to 0.3 Hz (lb=0.3 <rtn>). Reprocess the spectrum with wft. Check the LW½ of the CHCl₃ peak with dres, then store this value in register 2 (dres:r2). Reprocess the spectrum with ft (this processes the fid with no line broadening). Check the LW½ of the CHCl₃ peak with dres, then store this value in register 3 (dres:r3). Zerofill the spectrum by setting fn = 2*np, then process with ft. Check the LW½ of the CHCl₃ peak with dres, then store this value in register 4 (dres:r4).

What value should you expect to obtain for the LW½ of the CHCl₃ peak in this sample when you practice on the U400?

printon dg printoff <rtn>

print parameter group with register values

Part V. ¹³C{¹H} Spectrum of 57% Menthol in Acetone-d₆

Load MEN.fid Directory

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

LC **MEN.fid**

select the MENd11.fid directory (with d1=1 sec)

LC 3:Load

load the selected data to current experiment

Enter Text, Transform, Phase, and Reference the Spectra

text('month/day/year, your name, advisor's initials, \\MENTHOL S/N COMPARISON: lb=0.5') <rtn>

wft <rtn>

weighted Fourier transformation

ds(1) <rtn>

display spectrum 1 (nt=1)

f full aph <rtn>

display full spectrum to a full screen and autophase

vsadj <rtn>

adjust vertical scale

dscale <rtn>

display scale

nl rl(29.8p) <rtn>

reference the methyl septet of the solvent to 29.8ppm

Remember what I said about referencing!!!

Select Noise Region and Measure S/N for Spectrum 1

f <rtn>

display full spectrum

LC (at 120 ppm, or type **cr=120p** <rtn>)

RC (at 80 ppm, or RC to bring up a second cursor and type **delta=40p** <rtn>)

dsn <rtn> (should be ~100)

measure S/N ratio using the largest peak in display

dsn:r1 <rtn>

as the signal and 120 - 80 ppm as the noise region
store S/N value in register 1 in dgs parameter group

Measure S/N for Spectrum 2

ds(2) <rtn>

LC 1:Box

dsn <rtn>

dsn:r2 <rtn>

display spectrum 2 (nt=4)
brings up last cursor positions
measure S/N (120 - 80 ppm)
store S/N value in register 2

Measure S/N for Spectrum 3

ds(3) <rtn>

LC 1:Box

dsn <rtn>

dsn:r3 <rtn>

display spectrum 3 (nt=16)
brings up last cursor positions
measure S/N (120 - 80 ppm)
store S/N value in register 3

Measure S/N for Spectrum 4

ds(4) <rtn>

LC 1:Box

dsn <rtn>

dsn:r4 <rtn>

display spectrum 4 (nt=64)
brings up last cursor positions
measure S/N (120 - 80 ppm)
store S/N value in register 4

Notice that with $d1=0$, quadrupling the number of transients does not quite double the signal-to-noise ratio of the spectrum, due to incomplete relaxation of the nucleus between pulses.

What do you think would happen if these spectra were re-measured using $d1=5$? Why?

If after 2 hours of acquisition, some of the signals in your ^{13}C spectrum are just barely visible, how long would it take to double the S/N so that these signals are more creditable?

Set Plot Limits

wp=100p <rtn>

sp=0p <rtn>

set width of plot
set start of plot

Set Threshold for Peak Picking and Display Frequencies on Screen

LC 6: Set Th

LC (drag the horizontal cursor up or down to set the threshold level, excluding the solvent signals)

dpf <rtn>

select threshold menu
display peak frequencies above the spectrum

Plot Spectrum with Parameters and Peak Frequencies

pl ppf pscale pap page <rtn>

printon dll dg dg1 dgs printoff <rtn>

plot spectrum *with peak picking* in ppm
print parameter lists *with peak frequencies*
in ppm only

Plot Peak Listing in Hz and ppm

pll page <rtn>

plot peak frequencies *in both Hz and ppm*

Compare the pll printout with the dll printout. Why would you prefer one to the other? Or don't you think it matters?

Stacked Plot the Spectra Horizontally

dssh <rtm>

display stacked spectra horizontally

pl('all') pap page <rtm>

stacked plot all spectra with parameters

Reprocess the Data with lb=1

lb=1 <rtm>

set line broadening weighting function

wft <rtm>

weighted Fourier transformation

ds(1) <rtm>

display spectrum 1

f full aph <rtm>

display full spectrum to a full screen and autophase

dscale <rtm>

display scale

Measure the S/N for these four spectra as above. Then

printon dg printoff <rtm>

print parameter groups (the dsn values are contained in the dg parameter group)

Notice that the S/N ratios for these spectra are significantly lower than those processed with $lb=0.5$. *In general, the best S/N ratio for a spectrum is obtained when $lb = LW_{1/2}$, measured on the relevant line after an ft, i. e., the natural linewidth.* The 13C linewidths for this sample range from 0.3 to 0.7 Hz.

Part VI. Saving and Deleting a File

Change to Another Directory

logon <rtm>

change to another directory

Select a spectrometer from the list below [1-6]:

1 <rtm>

select the u400 spectrometer

Input your directory name:

myname <rtm>

enter your directory name, e.g., doej

The screen will show the files in this directory. At this point, you should have no files in this directory.

NOTE: If you get an error message or can not otherwise change to your user directory, check with Tracie or Vera.

ds <rtm>

display spectrum that was last processed in exp1

Saving a File

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

LC 4:Save

save the selected data to current directory

File name (enter name and <return>)?

filename <rtn>

You should think about strategies for naming files, such as III283H, where III is the notebook number, 283 is the page number, and H indicates a proton spectrum.

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

Is the file in your directory? If not, try again!

mysize (or LC 3:Dir Size)

macro to tell you the size of your data files

Input your directory name:

myname <rtn>

enter your directory name, e.g., doej

At the bottom of the screen appears:

The size of your directory in KBytes:

1058 /export/home/data/u400

0 /export/home/data/u500

0 /export/home/data/ui500nb

Deleting a File

LC Main Menu

select main menu

LC File Menu

select file storage/retrieval menu

LC filename

select a file

LC 5:Delete

save the selected data to current directory

Are you sure you want to delete this file?

LC Confirm

Part VII.

Before logging off, make sure that the spectral display window has been resized to the "small" display.

logoff <rtn> or LC Log Off

This macro changes the working directory to "home/user1d" and displays a message on the graphics screen

Sign off in the log book and log off on the Reslog