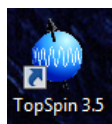


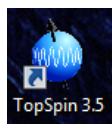
# 600 MHz IconNMR Instructions

NEVER turn off the PC or the NMR console!

If there is a problem, stop and restart Topspin or IconNMR!

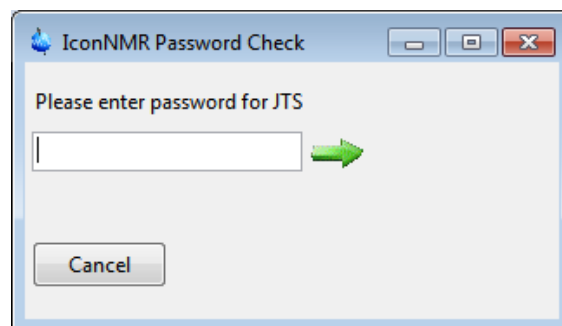
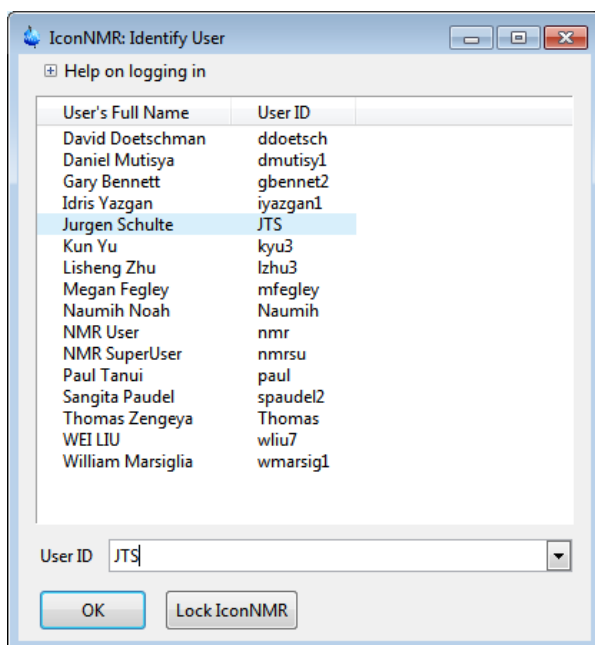
1. Check the task bar at the bottom of the screen whether TOPSPIN  is already running.



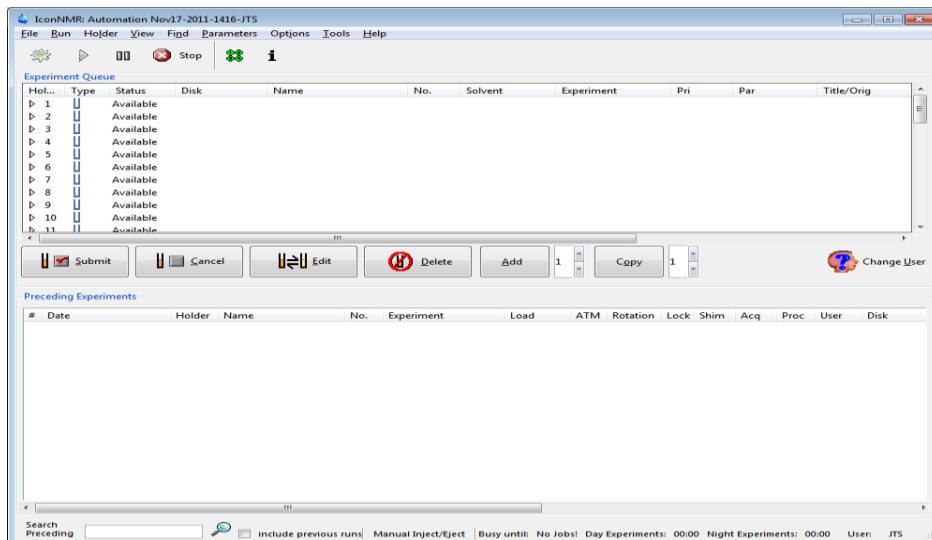
If it is not running, you can start it with the  button on the desktop.

2. Type **"icona"** in the command line to start the IconNMR Automation:

3. Select **your** username, click OK and enter your password in the Password Check window.



4. The Automation window will appear momentarily:



- Select any available holder number(s) in the automation window.  
Click the "Add" button to plan the experiments for your first sample.

Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Pri	Par	Title/Orig
1		1 Available								
		Available	C:\ICON_User		1					

- Enter the following information:

Name: sample name (the folder where all spectra for one sample will be stored)  
 No.: experiment number, must be unique for each experiment. **DO NOT CHANGE!**  
 Solvent: select the correct solvent (mixture) from the list  
 Experiment: select the experiment(s) you want to run from the list

→ I have added two options for a **COMPLETE NMR characterization** at the top of the list, consisting of  $^1\text{H}$ , COSY, HSQC, (and  $^{13}\text{C}$  if desired). ←

You can choose to run individual experiments. Here is a small selection of the most common ones:

PROTON for a proton spectrum (5 minutes)  
 C13CPD for a carbon spectrum (1 hour)  
 C13DEPT135 for a DEPT spectrum (20 minutes)  
 COSYGPSW for a COSY spectrum (10 minutes, includes  $^1\text{H}$ )  
 HSQCEDETGP for a HSQC spectrum (25 minutes, includes  $^1\text{H}$ )  
 NOESYPHSW for a NOESY spectrum (1 hour, includes  $^1\text{H}$ )  
 P31CPD for a  $^{31}\text{P}$  spectrum (10 minutes)

Title: Enter the title for the printout, i.e. name of the compound.

[=] Par: Change parameters if desired, i.e. scan number (NS) or plot limits (F1P and F2P).

If you want to run additional experiments with the **same sample**, click "Add" and repeat step 6.  
 If you have **another sample**, select the next available sample holder, click "Add" and repeat step 6.  
 You can schedule to run your experiments at a later time or let them start immediately.

- Select **all** your experiments with the mouse. Make sure you don't just select one experiment.  
Most two-dimensional experiments (i.e. COSY, NOESY, HSQC) **REQUIRE** running a proton spectrum first.

- Remove the cap from the magnet before you click the **Submit** button.

Click OK to eject the standard sample, replace it with your own sample, and click OK again to insert it.

- To finish your scheduling session, please click the "Change User" button and do not log on again.

No further interaction is required. Probe tuning and sample shimming will be done automatically.  
 The experiment(s) will start, and the data will be processed and printed automatically, if selected.  
 You can follow the progress of the experiments in the bottom half of the automation window.

Preceding Experiments																
#	Date	Holder	Name	No.	Experiment	Load	ATM	Rotation	Lock	Shim	Acq	Proc	User	Disk	Title/Orig	Remarks
2	2011-11-17 14:48:57	1	CBOA-HSQC	2	HSQCGP		✓	✓					JTS	C:\ICON_Users\data\JTS\nmr	Cellulose Octaacetate in CDCl3 - HSQC	
1	2011-11-17 14:34:58	1	CBOA-HSQC	1	PROTON	✓	✓	✓	✓	✓	✓	✓	JTS	C:\ICON_Users\data\JTS\nmr	Cellulose octaacetate in CDCl3 - $^1\text{H}$	

After the experiments have finished you will be prompted again to eject your sample.

**Please remove your samples as soon as possible and replace them with the standard sample.**

**Minimum sample concentrations for the AV 400  
using default settings:**

**$^1\text{H}$ ,  $^{19}\text{F}$ ,  $^{31}\text{P}$ : 1 mM**

**COSY, NOESY, TOCSY: 1 mM**

**HSQC, HMBC: 20 mM**

**$^{13}\text{C}$ : 200 mM**

**$^{15}\text{N}$ : 20,000 mM**

**$^{15}\text{N}$ -HSQC: 200 mM**

**Processing and printing are not permitted at the spectrometer workstation.**

Please use one of the processing workstations in SN-0200 or CE-B028 to work up your data.

All new data will be available online within minutes of completion of the experiments.

<\\NMR400Proc1\400mhz> in the Center of Excellence Building  
[\\B10035411\\_4\nmr\lcon400](\\B10035411_4\nmr\lcon400) in the Smart Energy Building

Ask the NMR specialist for the user-id and password.

Alternatively, you can bring a flash drive and retrieve your data manually from any processing PC.

The 400 MHz data is stored in several layers of folders:

```

\\ NMR400Proc1\400mhz\data\username\nmr\samplename\experiment_number\pdata\1\
                        ↑           ↑    ↑           ↑
Backup your data from this folder or from this folder.    ↑           ↑
                                                ↑           ↑
To find the correct spectrum, you should use consistent numbering in step 6.    ↑           ↑
                                                                    ↑
                                                                    ↑
                                                                    The processed data will be in this folder.
                                                                    If you use ACD-NMR, open the spectrum file:
                                                                    1r is a one-dimensional spectrum.
                                                                    2rr is a two-dimensional spectrum.
```

**Free NMR processing programs are available to work up the data on your own PCs:**

Bruker's **Topspin** software is now free for academic users:

<https://www.bruker.com/freetopspin>

MESTREC (simple program, good for most basic 1D processing, reads various formats):

<http://chemiris.chem.binghamton.edu/staff/schulte/MestreC.exe>

(Program with instructions will be installed in: C:\\Work\Mestrec\)

ACD-NMR (reads many data formats, can produce detailed NMR reports):

Software: <http://chemiris.chem.binghamton.edu/staff/schulte/ACDNMR.exe>

Instructions: <http://chemiris.chem.binghamton.edu/staff/schulte/ACDNMR-Manual.doc>

Programs for Mac OS (I have never tried them):

matNMR: <http://matnmr.sourceforge.net>

iNMR: <http://inmr.net>

**NMR Operation Manuals:**

Bruker Avance 600: <http://chemiris.chem.binghamton.edu/staff/schulte/AV600-Manual.pdf>

Bruker Avance 400: <http://chemiris.chem.binghamton.edu/staff/schulte/AV400-Manual.pdf>

Bruker Tecmag 300: <http://chemiris.chem.binghamton.edu/staff/schulte/AM-Tecmag-Manual.doc>

Bruker AM 360: <http://chemiris.chem.binghamton.edu/staff/schulte/AM-AC-Manual.doc>

## NMR Sample Preparation:

### 1. Find a clean, dry NMR tube.

- After washing NMR tubes with acetone, lay the tubes flat on a layer of paper towels in the oven for at least **two hours at max. 50 °C** before using them again. A vacuum oven is preferred.
- Standing the tubes upright in a flask or beaker in an oven is not recommended, as the tubes might warp or paramagnetic rust particles might fall into the tubes.
- Compressed air contains mineral oils and should **never** be used to dry NMR tubes.
- Store clean NMR tubes uncapped, preferably laying down.
- If your proton spectra consistently show a peak near 2.05 ppm, the tube wasn't dry enough. (Traces of acetone may be present, even if the tube appears to be dry!)

### 2. Choose an NMR solvent appropriate to your compound.

- Only use deuterated solvents (i.e. CDCl<sub>3</sub>, D<sub>2</sub>O, C<sub>6</sub>D<sub>6</sub>).
- Chloroform is the standard solvent to try first. Be aware that it can be acidic.
- If your compound is not soluble in chloroform, try benzene (nonpolar or average polarity compounds), acetone (dissolves almost anything), DMSO or methanol (polar compounds).

### 3. Dissolve your sample.

- Make sure your sample is free of solvent; if your compound is not volatile, placing the flask on a high vacuum line for 5-30 minutes is a good idea.
- Measure the correct amount of sample into a vial.
- For a solid, use 10-20 mg for a routine <sup>1</sup>H and 50-100 mg for a routine <sup>13</sup>C NMR. You can use less, but your experiments will take longer.
- For an oil or liquid, dip a glass pipette into the liquid (one drop is enough for <sup>1</sup>H NMR).
- Dissolve the sample in 0.6 ml of the NMR solvent.

### 4. Put the solution into the NMR tube.

- If any solid remains, filter the sample into the tube through a pipette with a cotton plug.
- The filling height should be 4-5 cm. Less volume will make shimming more difficult.

### 5. De-gas your samples (for NOE or NOESY experiments).

- Use several "Freeze – Pump – Thaw" cycles to remove dissolved oxygen.
- Easy alternative: bubble some N<sub>2</sub> or Ar gas through your solution.

### 6. Acquire your spectrum.

**Minimum sample concentrations for the AV 600  
using default settings:**

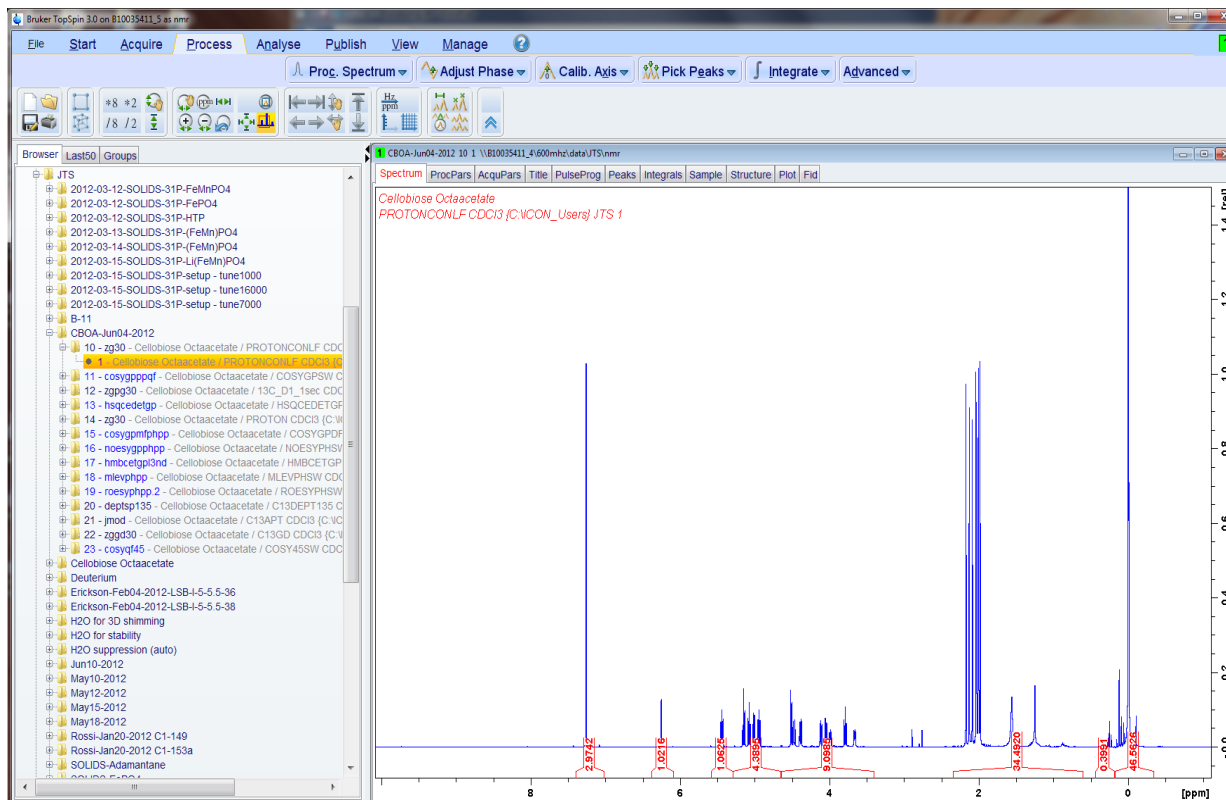
<b>1H, 19F, 31P:</b>	<b>0.1 mM</b>
<b>COSY, NOESY, TOCSY:</b>	<b>1 mM</b>
<b>HSQC, HMBC:</b>	<b>10 mM</b>
<b>13C:</b>	<b>100 mM</b>
<b>15N:</b>	<b>10,000 mM</b>
<b>15N-HSQC:</b>	<b>100 mM</b>

**Minimum sample concentrations for the AV 400  
using default settings:**

<b>1H, 19F, 31P:</b>	<b>1 mM</b>
<b>COSY, NOESY, TOCSY:</b>	<b>1 mM</b>
<b>HSQC, HMBC:</b>	<b>20 mM</b>
<b>13C:</b>	<b>200 mM</b>
<b>15N:</b>	<b>20,000 mM</b>
<b>15N-HSQC:</b>	<b>200 mM</b>

# Processing 1D Spectra with Topspin

Start Topspin and open your file from your folder by double-clicking it in the Topspin browser:



You can use any combination of icons in the toolbar to zoom, shrink, magnify, move, etc. the spectrum:



The icons with a hand require you to hold down the left mouse button while moving the mouse.

Start processing by selecting the **Process** tab from the toolbar:



This will open a new toolbar with processing functions:




If you don't like the result of the automation's default processing, you can start processing from scratch: Typing "EF" on the command line will require you to do everything manually.

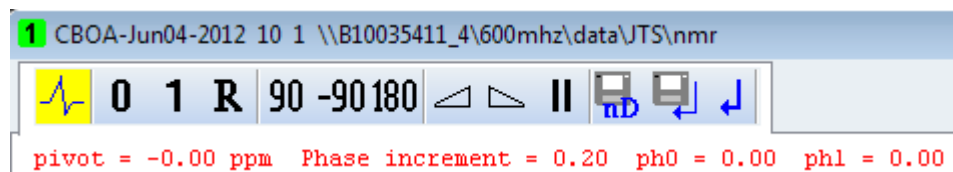
You can always revert to the automatic processing by selecting the **Proc. Spectrum** tab.

This will undo all processing steps you did manually, i.e. phasing, calibration, peak picking, integration. (good for fixing manual processing mistakes).

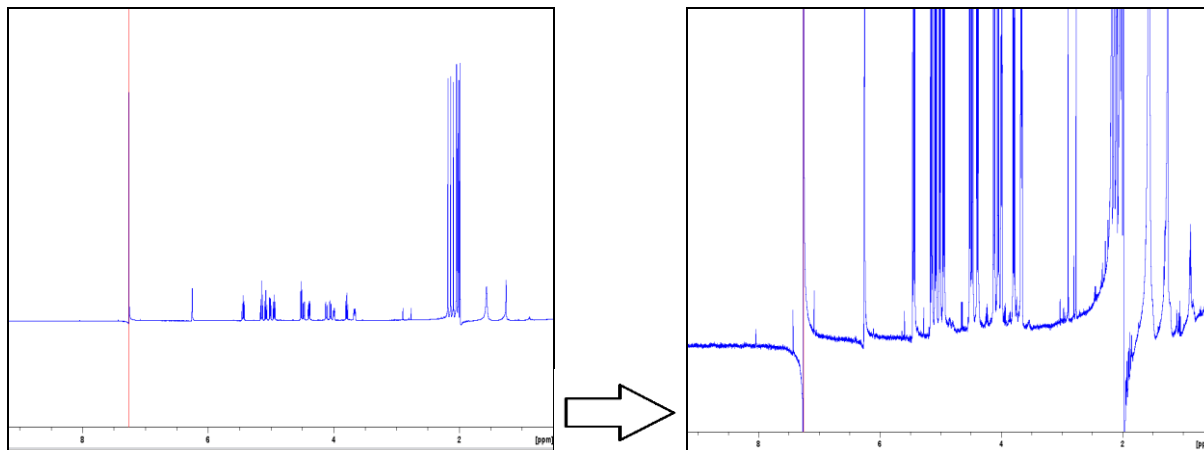
If the spectrum needs further improvement, begin by adjusting the phase:

## PHASE ADJUSTMENT:

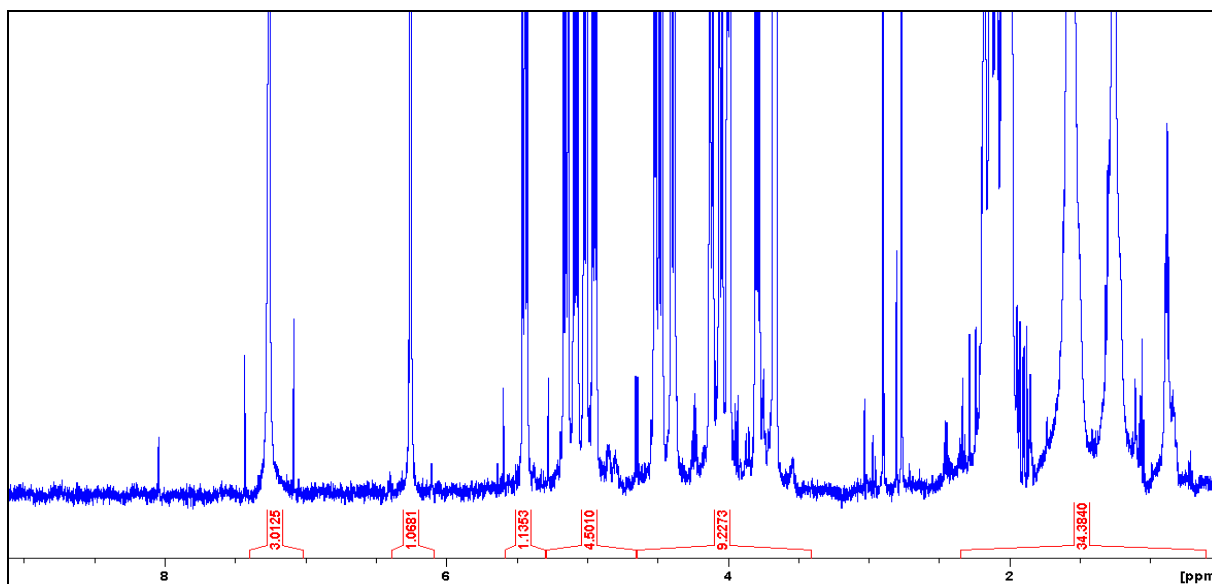
Click  and a new row of buttons will appear on top of the spectrum:



Make the peaks taller to better see their phase distortions:



The icons 0 and 1 in the toolbar can be used to do the 0<sup>th</sup> and 1<sup>st</sup> order phase corrections: Hold down the 0 icon and move the mouse to make the peak with the red line symmetrical. Hold down the 1 icon to make the other peaks symmetrical and the baseline flat. 180 will flip the spectrum upside down, < and > will increase and decrease the mouse sensitivity.




When finished save the phased spectrum by clicking .

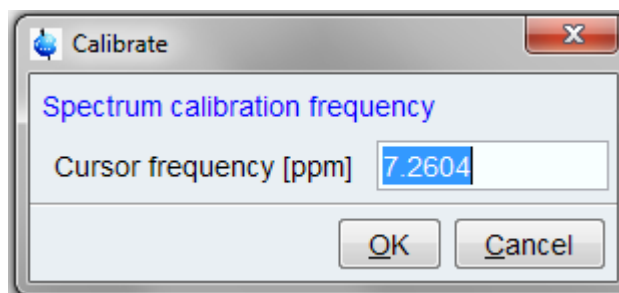
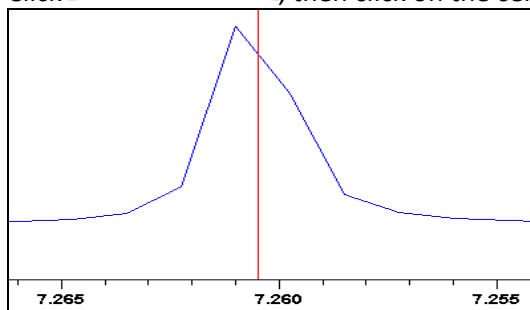
When the phasing is perfect, type “ABS” on the command line to further flatten the baseline.



### **CALIBRATION:**

Zoom into the solvent signal (or the TMS signal, if your sample contains TMS).

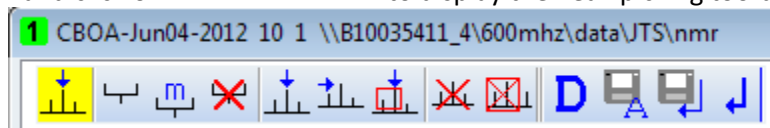
Click , then click on the center of the signal:



Enter the correct chemical shift for the signal.

### **PEAK PICKING:**

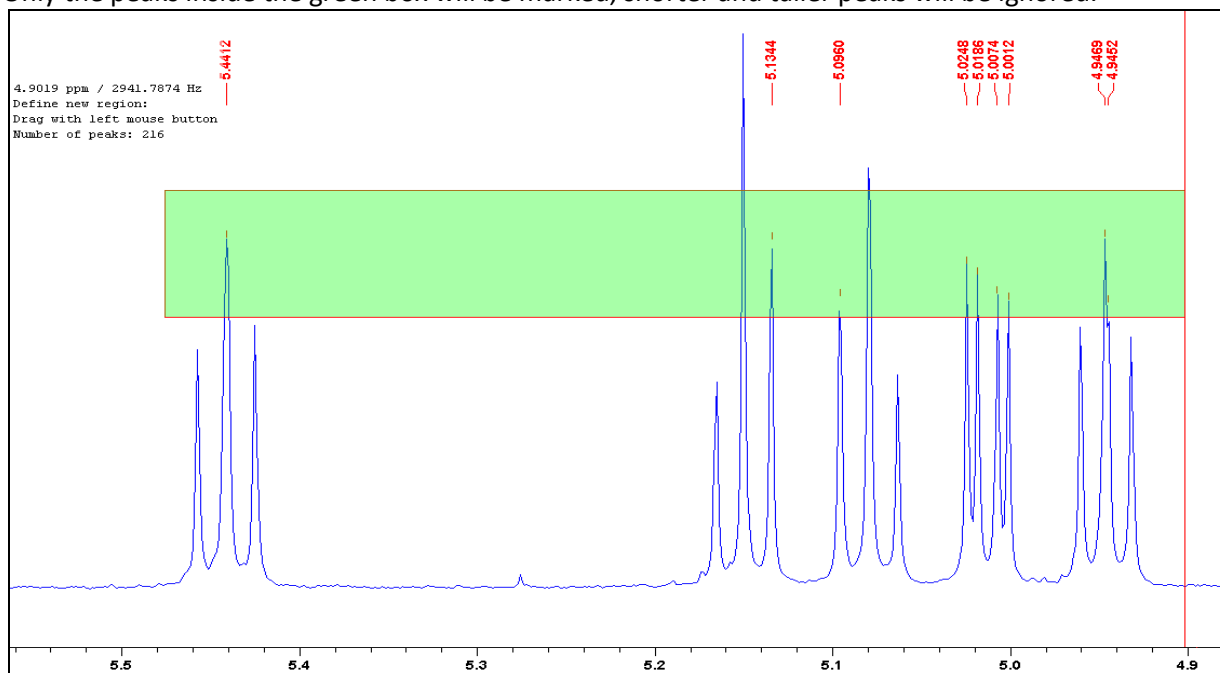
Display the full spectrum and click on  to display the Peak picking toolbar:



Define peaks with this button. ↑

↑ This button erases all peak information.

Only the peaks inside the green box will be marked, shorter and taller peaks will be ignored.



When finished save the picked peaks by clicking .

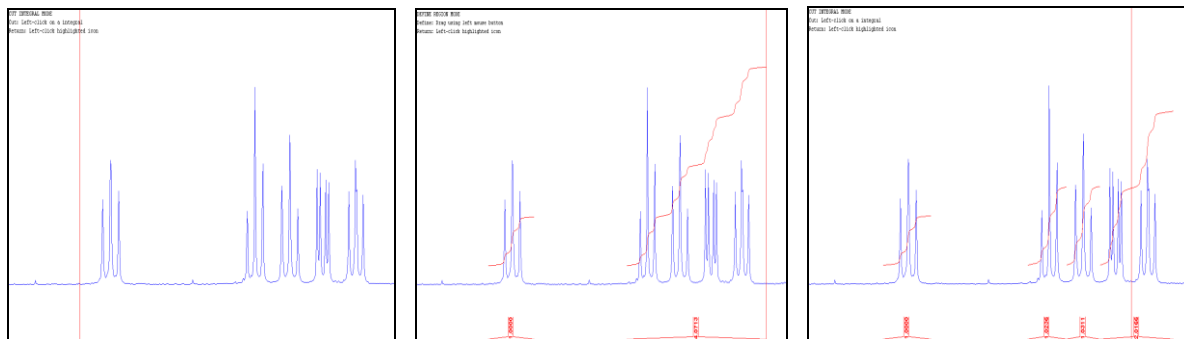
## **INTEGRATION:**

Click  to show the integration toolbar:



Zoom into a group of peaks you want to integrate.


It is usually best to first delete all integrals from the automatic integration and to define your own.



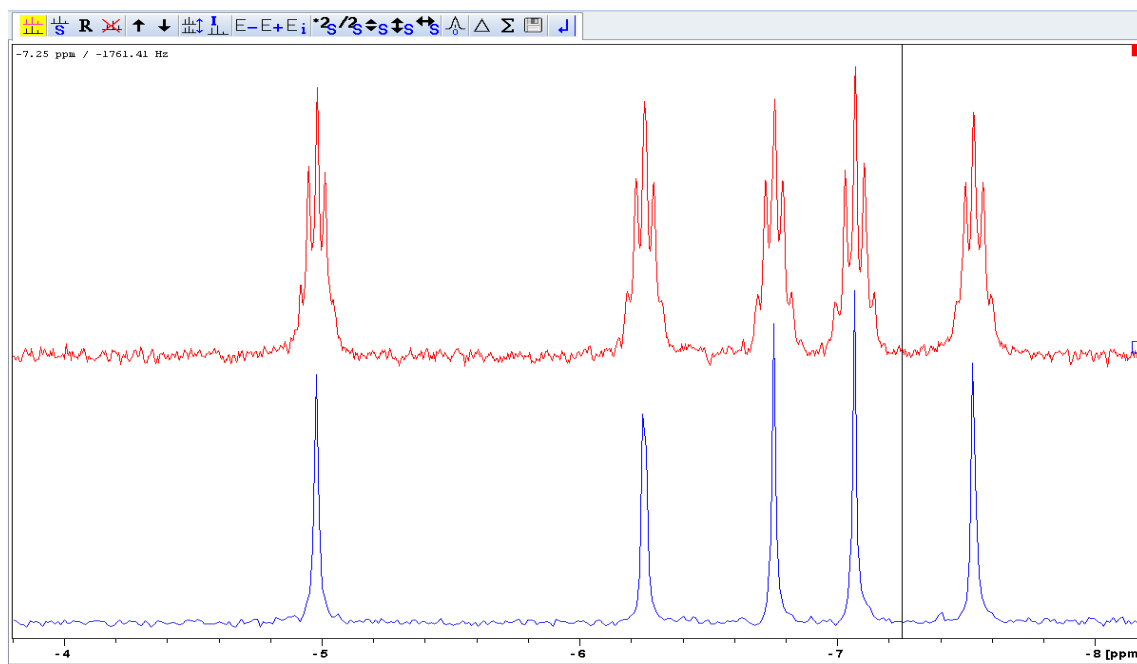
Define integrals of individual signals or groups of signals ↑ then cut integrals into pieces. ↑

When finished save the selected integrals by clicking .

## **MULTIPLE DISPLAY:**

After fully processing a series of spectra, open one of them and click the  icon.

Double-clicking on additional spectra in the browser window will add them above the first spectrum:



Each spectrum can be individually selected, moved, shrunk, magnified, etc.

Individual spectra can be added or subtracted and the sum/difference can be saved as a new data set.

**PRINTING:**

Printing is **not allowed** at the spectrometer console.

You will have to generate a PDF file and import it into your reports or print it in your own lab.

Please always use the processing PCs in SN-0200 or CE-B028 or your own PC.

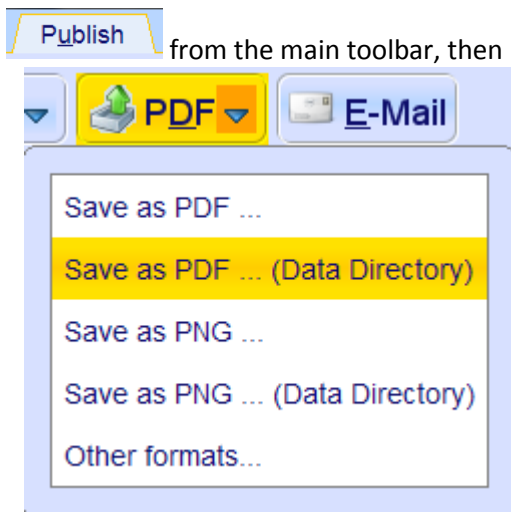
Display the spectrum on the screen exactly as you would like it to appear on paper.

*The quick and easy way to print:*

---

To create an exact image of the spectrum as it appears on screen:

Select **Publish** from the main toolbar, then pull down the PDF menu:



Select: **Save as PDF ... (Data Directory)**

Move up three folder levels (to the sample's main folder) and save the pdf file here.

*The advanced, interactive way to print:*

---

Select the "plot" tab to open the plot layout.

Click on the spectrum or any other item you want to resize, modify, or delete.

**Axis, Grid, Curve...** allows you to resize the width, height, and position of the spectrum.

**Peaks, Integrals...** allows you to modify peaks and integrals.

The ^ icon allows you to insert new elements, i.e. expansions, drawings, labels, even other spectra ...

When finished, select: **Save as PDF ... (Data Directory)**

Move up three folder levels (to the sample folder) and save the pdf file here.

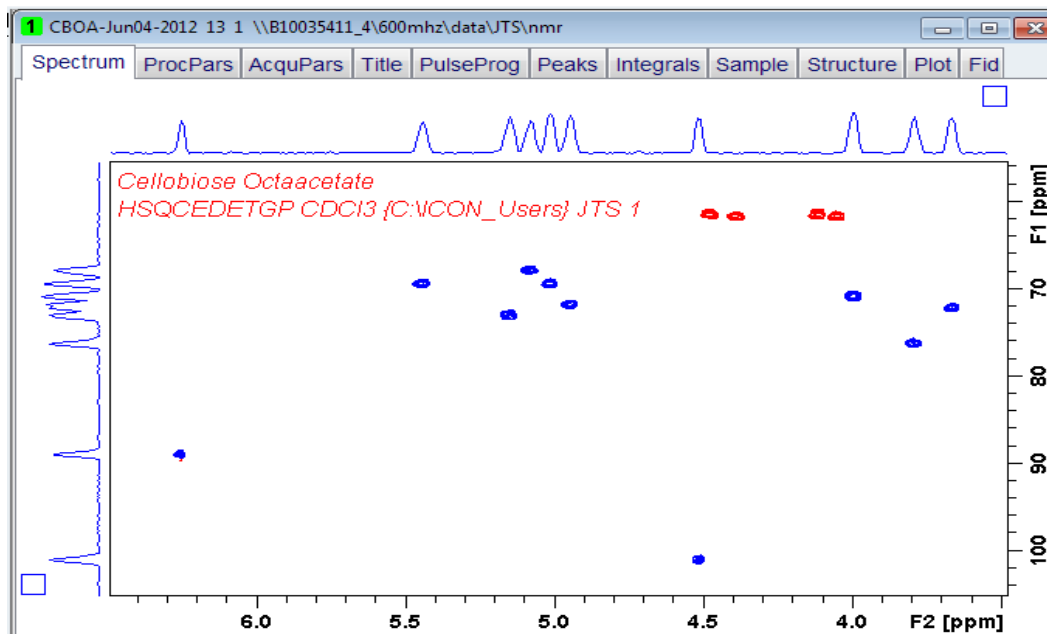
## Processing 2D Spectra with Topspin

(i.e. COSY, NOESY, TOCSY, HSQC, HMBC, ...)

Open the 2D spectrum. If no spectrum appears, go to the command line and type: "xfb".

HMBC spectra need one more step: type on the command line: "xf2m".

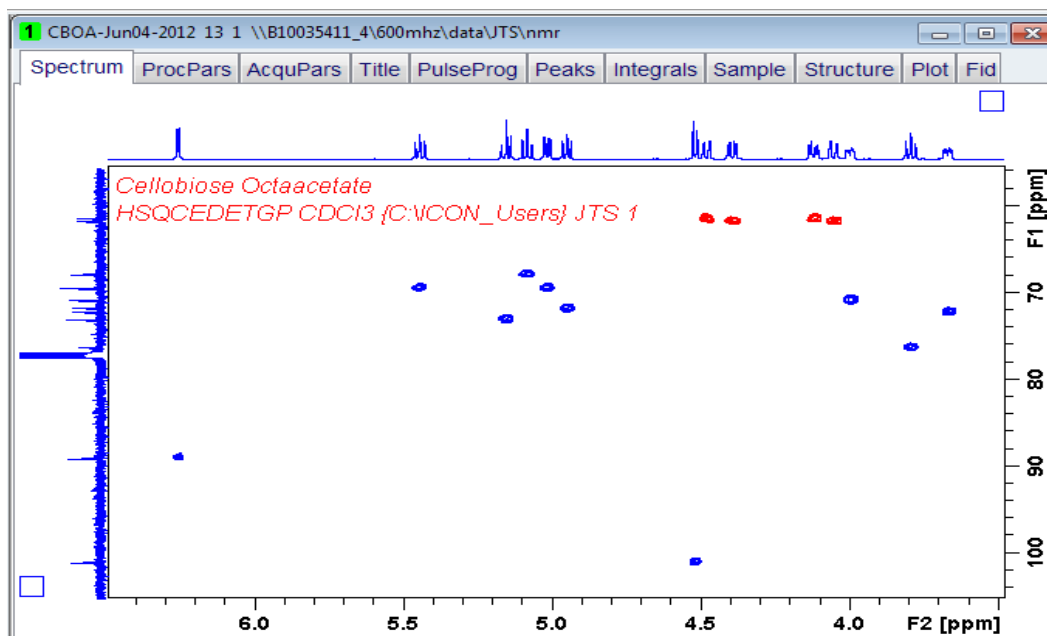
Zoom into the region you are interested in:



If the spectrum is displayed with internal projections (broad peaks on top and/or on the left side), right-click on the corresponding  $^1\text{H}$  and/or  $^{13}\text{C}$  spectrum in the browser and select "Display as 2D Projection".

In COSY, NOESY, & TOCSY: F1 + F2 are  $^1\text{H}$  ----- HSQC and HMBC spectra have  $^1\text{H}$  in F2 and  $^{13}\text{C}$  in F1.

The resulting projections should have sharper peaks:

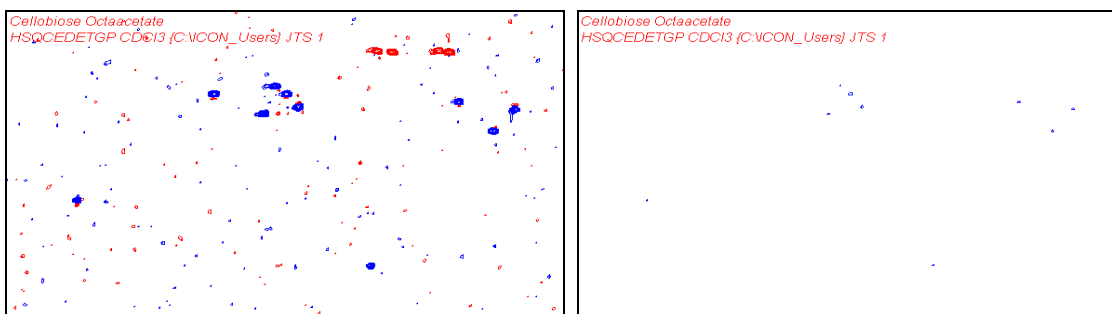


You can adjust the height of the projections by clicking them and moving the mouse wheel.

The default number of displayed levels (6 positive + 6 negative levels, if present) is far too coarse for a detailed analysis of most 2D-spectra. Most computers are powerful enough to handle many more levels (up to 64 are available) and will allow you to adjust the levels with much more detail:

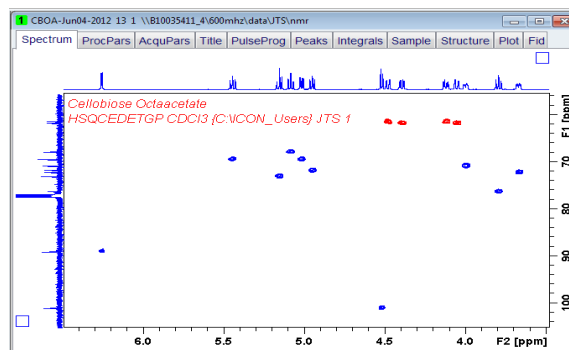
Right-click and select "Edit Contour Levels" or type ".lv" to bring up the level window.  
 Set the level increment to "1.2" for positive and negative levels. (Don't use negative numbers!)  
 Set the number of levels to 64. (A combination of 1.4 and 20 also works fine on older PCs.)  
 Click "Fill", then "Apply", and finally "OK".

Adjust the height of the cross peak levels with the mouse wheel.  
 Find a suitable level that shows all relevant cross peaks with minimal noise.



Too much noise - level too low

missing signals - level too high



correct level

Right-click and toggle between square and rectangular display.

The 2D spectrum may require re-calibration in both dimensions. As there may not be a solvent signal in the 2D spectrum, use one of the signals from your compound to calibrate both dimensions or:

For the most accurate way to calibrate a 2-dimensional spectrum use the Spectrum Reference (SR):  
 Open the properly calibrated one-dimensional carbon and/or proton spectra, type "SR" and write down the numbers. Then return to the two-dimensional spectrum and enter the correct SR values.  
 (f2 is the horizontal dimension, f1 is vertical)

Zoom into and out of the spectrum to expand or shrink the region(s) you are interested in.