Processing With Topspin

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INTRODUCTION

Topspin is a pretty vast piece of software and can do many, many things...

There's not time to teach you everything!

The picture of your spectra is not your result…

- Well, at least from a certain perspective…

- You have to process your raw result into a picture you can interpret.

- There is not necessarily a single 'correct' way to process your data… Which is one reason we don't store the processed data.
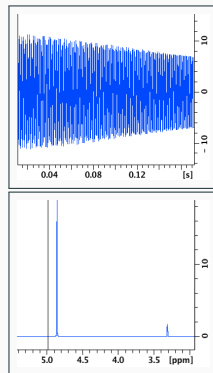
Topspins design

- Topspin is a Java application. It can be slow to start, as it is composed of many files and by default most virus scanners will scan them. I usually exclude the Topspin directory from the scan.
- You'll find there are multiple ways to do anything...
- It has a command-line interface, this can be faster than hunting through menus with the mouse.
- It can be programmed to automate tasks, via macros, or a form of the 'C' programming language.
- It has a long history, going back to SGi unix days; which gives it some quirks...
- ...but at least it has a full set of manuals in electronic format.

Here's what it looks like.
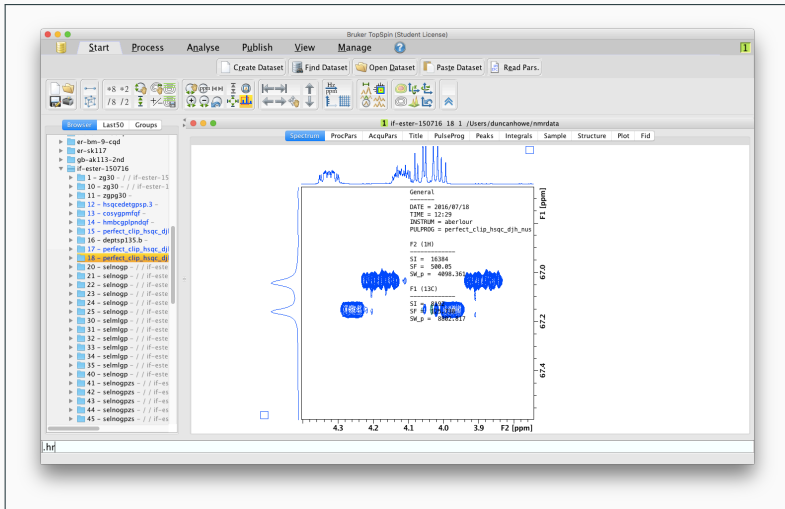
Customise Topspin.
This can be done by typing 'set' at the command line.
I do at a minimum :-

- Open last dataset used.
- Uncheck 'Automatic processing'.

This is somewhat optional; but it makes sense to have a look.
Also I add my data directory to the internal browser (via right-click).

Dealing With Data

Make a directory on your computer to copy data to.

- Topspin isn't too fussy where.

WITH IRREPLACABLE DATA, WORK WITH A COPY.

- It is possible to mess up the data, even on a processing station.

Dealing With Data

Data structure

Topspins filesystem.

Datasets consist of :-

- Numbered experiments.
  - The raw data is an fid or ser file.
- Within those, data can be processed in multiple ways.
  - Contained in the pdata directory, in numbered directories.
- To send data, you need to send the whole tree.

Its worth spending a little time looking for yourself.

Figure 1: File tree

Dealing With Data

Navigation

Opening data in Topspin.

- Drag and drop works.
- Internal browser.
- File menu.
- Command line.

From the command line.

- re *dataset expno procno*, or a subset of that.
- re 10 - will read exp. no. 10 of the dataset you are in.
- dir - will show the datasets in the same directory as the one you're looking at.
- dir *wc* - the command with 'wildcards', in this case matches all datasets with 'wc' somewhere in its filename.

1-DIMENSIONAL NMR PROCESSING

1-DIMENSIONAL NMR PROCESSING

WORKFLOW

Step-by-step.

- Manipulate the raw FID.
- Transform the data (from time to frequency based.)
- Phase correct the spectrum.
- Reference the spectrum.
- Baseline correct the spectrum.
- Peak-pick the spectrum.
- Integrate the spectrum.
- Plot the spectrum.

Commands to type.

Command	Description
efp	Takes you from a FID to a spectrum
apk, apks	Does automatic phase correction
abs, absn	Does automatic baseline correction
cy, mi	Sets maximum and minimum peak-picking height
ppf, pps	Peak-picking on full or visible spectrum
.hr	Shows you the whole spectrum
.ph	Enters manual phase correction
.cal	Enters spectrum referencing mode
.int	Enters manual integration

Table 1: Basic commands

1-DIMENSIONAL NMR PROCESSING

TRANSFORM THE DATA

Manipulate the FID.

The first step is to apply a weighting function (or functions) to the fid:-

- Usually (positive) exponential multiplication.
    - The 'lb' parameter.
- But can be anything, according to whether you want to enhance signal or line-width.
    - E.G. the 'gb' parameter for Gaussian.

Transform the FID.

The next step is the fourier transform; to take the data from the time to frequency domain.

Generally you type 'efp' at the command line :-

- · E – exponential multiplication.
- · F – fourier transform.
- · P – phase correction (apply dataset default.)

You should now see a picture of your result, which will probably need further manipulation...

1-DIMENSIONAL NMR PROCESSING

PHASE THE SPECTRUM

Why does the spectrum look wrong?

The result so far is very unlikely to have the correct phase; you can correct it automatically; or/and manually.

- Automatically via command line :-
    - apk
    - apk0
    - apks
    - apkm

- Manually, via the phase correction module (.ph from command line)
    - adjust ph0, (choose a large peak near one end)
    - adjust ph1
    - save and return (.sret from command line)

1-DIMENSIONAL NMR PROCESSING

REFERENCE THE SPECTRUM

Why are my peaks at the wrong ppm?
When acquired, the instrument locks onto the deuterium in the solvent; which is at a known frequency. You can reference any peak to any frequency however.

- The calibration routine can be started by typing '.cal'.
- choose a peak, click and type a value.

This gives you an 'sr' value, which can be handy to know for referencing 2d spectra.

1-dimensional NMR processing

Baseline correction

Tidy the spectrum up

Again you can automatically or manually baseline correct your spectrum. Automatically works in the majority of cases.

- Automatically via abs
    - 'abs' will also create new integral regions, or wipe existing ones...
    - 'absn' won't wipe the integrate regions present.
    - Theshold dependent on 'isen' parameter.
- Manually via the correction module (basl from the command line)
    - The aim of the game is to define your own polynomial function to subtract from your spectrum.

1-DIMENSIONAL NMR PROCESSING

PEAK PICKING

Set the parameters

Again, this can be done automatically or manually. I usually do it automatically. There are several parameters that affect the output:-

- 'cy' The height of the largest peak.
- 'mi' The minimum height of peak to be picked.
- 'maxi' The highest intensity of peaks to be picked; when I have probems, I set it to be about 10x 'cy'.
- 'pc' The amount of noise rejected; low values pick more peaks, especially on broad peaks.
- 'pscal' Set this to 'global' if you have problems.

Several commands perform the peak picking

- 'ppf' peak pick on the full spectrum
- 'pps' peak pick on the region on the screen
- 'pp' choose what type of peak peaking

Typically you change the above parameters iteratively until you get what you want.

Sometimes you won't get what you want.
For instance if there's a region you don't want to peak-pick.
'.pp' from the command line starts the manual routine.

- Pick peaks by drawing boxes around peaks or interest.
    - Dependent on the peak picking parameters; 'pc', 'maxi' etc.
- You can also manually add a peak to the list.

1-DIMENSIONAL NMR PROCESSING

INTEGRATION WORKFLOW

My workflow goes as follows :-

- .int to go into the routine…
- cut the integrals into chunks.
- delete the unwanted parts.
- add other regions.
- reference a known region.
- .sret to save and return from the routine.

2-DIMENSIONAL NMR PROCESSING

Into the *n*-th dimension...
Topspin actually supports *n*-dimensional data; but really 2 is enough to be going on with.
The workflow is pretty similar to 1-dimensional processing, but with more degrees of freedom; there can be more to fix...

2-DIMENSIONAL NMR PROCESSING

TRANSFORMATION

2d Transformation.

n-d data is actually comprised of successive 1d experiments; from which the additional dimensions are derived.

- With 2d data 'xf2' will transform all these 1d experiments and 'xf1' will derive the second dimension.
- The command 'xfb' will do it all at once.

Processing parameters are perhaps more important than 1d data. Your data acquired from our default experiments should give valid results though.

2-dimensional NMR processing

Some guidelines for processing parameters

Define the resolution.
You will need to examine what the experimental conditions
were used to give some boundaries on what is possible.
It is usual to interpolate the data up to a higher resolution
than was acquired.

- 2048 x 2048 resolution is usually sufficient.
- Forwards prediction in *F1* is usually used :-
    - Set 'Memod' to 'LPfc'
    - Set 'NCOEF' to 32 (Larger values don't really seem to help
      much; but you can try it)

Window functions.

The 'window functions' (WDW in the Procpars tab) used for the transform, (or weighting function) are often sine or qsine.

- The 'ssb' parameter affects how the sine function operates.
- SSB is often 2 or more.

Ultimate resolution.

Sometimes I do change the F2 function to be 'em'; if I want to extract some high quality 1d data from the 2d dataset.
You'll need to type 'xfb' after each adjustment to see the result.

2-DIMENSIONAL NMR PROCESSING

NON-UNIFORM SAMPLING

You don't get something for nothing...
If you think about a 2d spectrum, its mostly empty space, and it takes a lot of acquiring to see that. You might think there is a better faster way. There is, with some proviso's...

How it works.

- One way. You acquire a sub-set of the full sampling space.
- You then do a lot of signal processing to reconstruct the spectrum...
- ...this takes a lot of computation.

Processing an NUS spectrum

The latest version of Topspin will do this, earlier versions need a special license.

- Set the 'mdd_mod' flag to be 'cs' ('Compressed-sensing')
  - Unless its a 3d dataset or doesn't work or gives a poor result…Then set it to 'mdd' ('Multi-dimensional deconvolution')
- Type 'xfb'
- Wait…

There are processing software options...
Other software may have algorithms that are faster; this is a very active area of scientific development.

- MNova.
- NMRPipe–With qMDD and MDDNMR 'plugins'

These either cost money, or are difficult to use/export data from.

2-dimensional NMR processing

2d Phase correction

Now the tricky bit...

A bit more complicated than 1d, but not every 2d needs to be phased; examine the pulseprogram.

- Choose a diagonal peak from one end; maybe one from the middle and then one from the other end of the spectrum.
- Choose the rows to phase first.
    - Choose the upper-most 1d spectrum (Somewhere towards the end of the spectrum)
    - Adjust 'ph0' so that the spectrum is phased under the red line.
    - Adjust 'ph1' so that the spectrum furthest in ppm from the one you just selected comes into phase.
- Now do the same for the columns.

2-DIMENSIONAL NMR PROCESSING

2D BASELINE CORRECTION

There's not much to say, I've only ever used two commands...

- 'abs1'
- 'abs2'

2-dimensional NMR processing

Integration and peak-picking

I find its somewhat unusual to do this, and its best done manually.

- Automated peak-picking can produce thousands of peaks.

    - The trick is to set the lowest level to that of the contours.

- It doesn't always make sense to integrate 2d data anyway.

2-dimensional NMR processing

Finishing touches

Now make it look pretty!

There's a couple more things to set up…

- Define 1d projections by right-clicking.
- Define the number of contours used.
    - Type 'nlev 32' (32 contour levels are useful; more than 32 aren't…)
    - Type 'clev' to recalculate the levels.

The data should now be ready to plot!

Taking spectra out of Topspin

Taking spectra out of Topspin

Plotting and printing

Plotting and printing

Clicking the 'Plot' tab or typing 'plot' takes a layout defined in the dataset (see the processing tab, automation section) and starts the routine. This is really quite an in depth topic; but a few key points.

- The editor behaves a little like Powerpoint; in that the document is composed of objects; that can be manipulated.
- These layouts can be saved and loaded from the editor.
- The plot you've made can be exported to a variety of formats.

You can get a WYSIWYG print of the main work area by typing 'prnt'.

Taking spectra out of Topspin

Data backup

Processed data can be arbitrarily large

You can fill up your hard-disk quite rapidly processing *n*D data. This is another reason we don't save it.

- You may need to delete old processed data
- Or archive your work

Open Data

The 'Open Data' initiative requires that the data be available. Although probably not strictly necessary, you may wish to :-

- Provide the processed data.
- Convert the data to the 'open-source' JCamp format.

Taking spectra out of Topspin

JCamp Data

The problem of propietry data.

Brukers data is in a binary (non-ascii) format, that may change with Topspin version; so there's no guarantee that you'll be able to:-

- Import it to other reprocessing software.
- Import it to older versions of Topspin. (Particularly for acquisition.)

JCamp-DX

- The JCamp-DX format has been defined and designed by IUPAC committee to be an electronic data standard.
- There are definitions for NMR, IR, MS, IMS data; along with chemical structure.
- Bruker can export both your raw and processed data. Your mileage on importing it to other software may vary…

The commands.
To export to Jcamp, at the command line type :-

```
tojdx
```

For help type:-

```
tojdx?
```

You should regard this in the same way as you would any other backup. As with all backups, you should ensure its doing what you think its doing and that you can actually use the backup as you intended.

Optimising your work flow

Optimising your work flow

Parameter sets

Dictate your own conditions.

You can save a default experiment type to a parameter set, then load them again for any experiment of that type.

- Type 'wpar *par-name*' at the command line.
- Choose a combination of 'proc', 'outd', 'title' and 'clevels'. WARNING! WRITING 'ACQU' PARAMETERS IS PROBABLY NOT A GOOD IDEA.

Writing 'aqcu' parameters.

These are frequency dependent. So say you write parameters from a 500 MHz dataset; then load them into a 400 data set... You've just broken your 400 data!

To read a parameter set.

- rpar *par-name*
- Or rpar *par-name par-part*
    - e.g. rpar proton.std proc — Will read in the processing parameters of the parameter set.

OPTIMISING YOUR WORK FLOW

AUTOMATION

...and now some clever bits...

- Macros
    - A list of commands. Type 'edmac'
- 'AU' programs
    - 'C' code. Type 'edau'
    - There are service programs on the server.

Using some of the service programs.

Command	Description
service-def-proc	Does default processing on the experiment no. you are in.
service-master-proc	As above, but goes through *all* the experiments.
service_nohump *n*	Chops the baseline into *n* chunks and baselines corrects. If it looks wrong, do 'efp' and use different *n*.

Table 2: Service AU programs

Other useful features

- Working with multiple windows.
- Multiple display.
    - Works within a 2d dataset also.
    - Load to portfolio in plot editor.
- Structure Editor.
- Export peaks with multiplet information in journal format.
    - 'managuide' and 'mana' (Multiplet Analysis)
    - ...see online help!
- Dynamics package.

Other useful features

Multiplet analysis

Draw or load a .mol file.

The multiplet analysis tool can annotate a stucture; Topspin has a built in structure editor acessable from the 'Structure' tab. This seems fairly standard to me, just takes a bit of playing with to get to work. Some points though...

- Topspin has a default directory for structures; find it via the 'set' menu.
- The plot layout saves the location of the structure file.
  - The service plots have this set to '../structure.mol'
  - So to get these layouts to work do 'save as...Choose current EXPNO and the filename ../structure.mol'

Multiplet Analysis

This tool aims to find multiplets; link them together, annotate a structure and then output a journal-ready report.. Started by typing 'mana' or 'managuide' from the command line. The 'managuide' tries to walk you through the process. But the basic process is as follows…

- Make sure all the peaks in the multiplets you want to analyse are picked.
- Define the multiplets.
- Link the multiplets.
- Annotate the spectrum.
- Generate the report.

Other useful features

The analysis of things that change...

The 'Dynamics Centre' package.
This is a separate program and reads in processed data from Topspin. It can do a variety of analyses; for instance relaxation, kinetics and diffusion.

- The first time you run it, set the default directory in the preferences.
- Process all your data correctly in Topspin first.
- Click through the work-flow in the software.

(THE END)

(The end)

Send an email to djh35@cam.ac.uk with any questions.