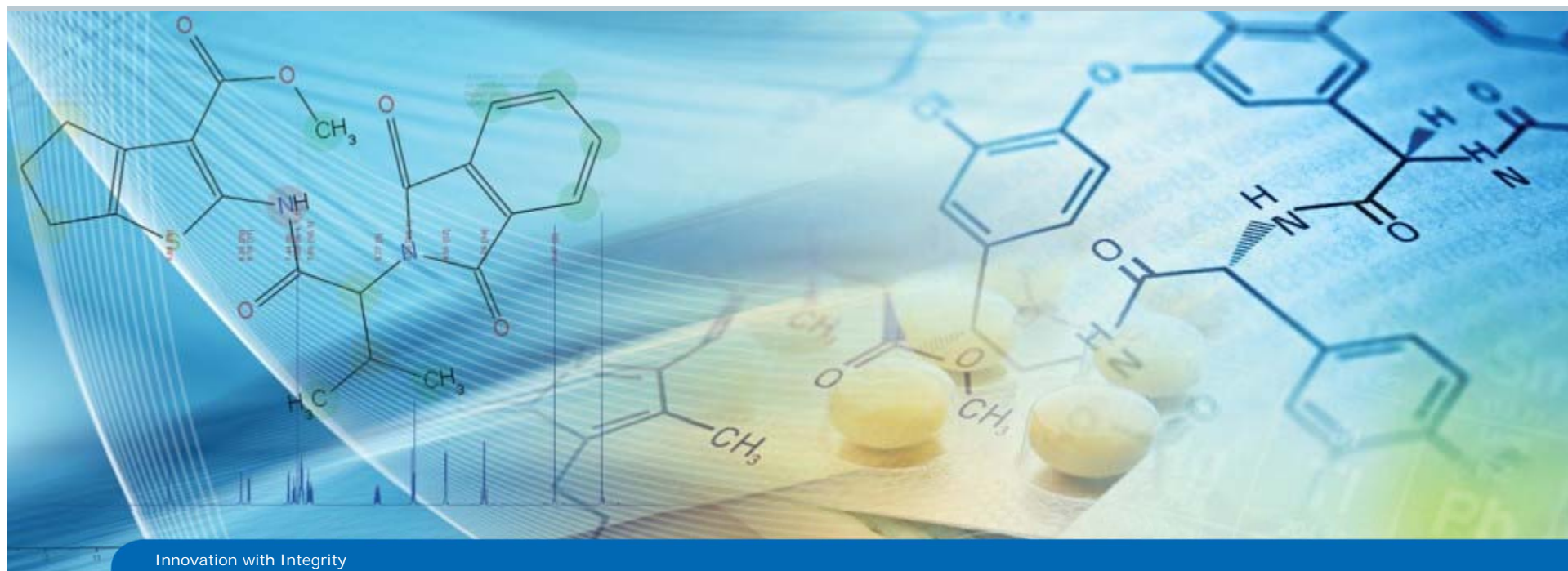


Getting the most out of your NMR



Eric Johnson
Senior Applications Scientist
Bruker Users Meeting at PANIC
La Jolla, California – March 4, 2018



Innovation with Integrity

Non-Uniform Sampling



- What is it?
- Why should I use it?
- When should I use it?
- How do I use it?



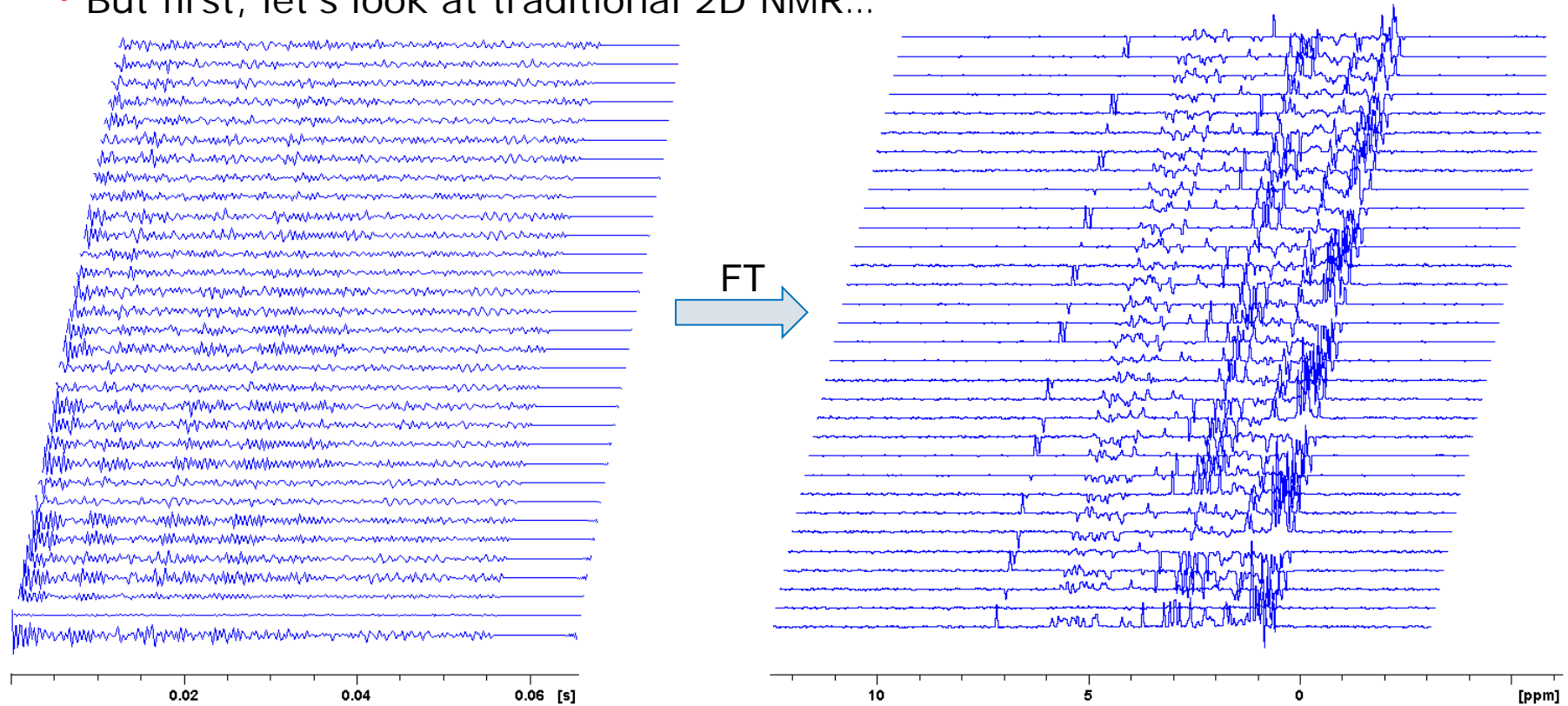


Non-Uniform Sampling

- If you're not already using it, why not?
 - Common answers:
 - I don't know what it is.
 - I don't know how/when to use it.
 - I already have 100 things on my plate and I don't have the time/resources to test/troubleshoot something new.
-

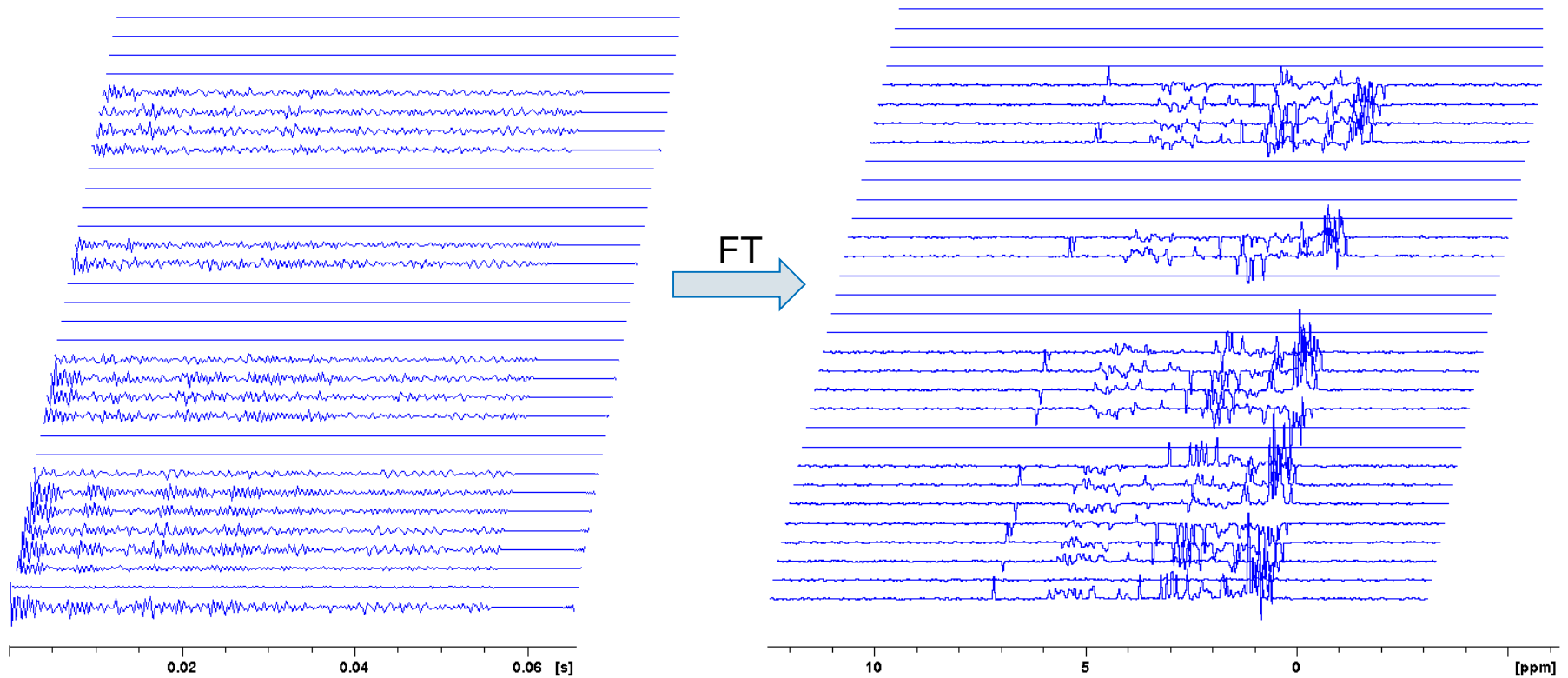
What is Non-Uniform Sampling?

- But first, let's look at traditional 2D NMR...



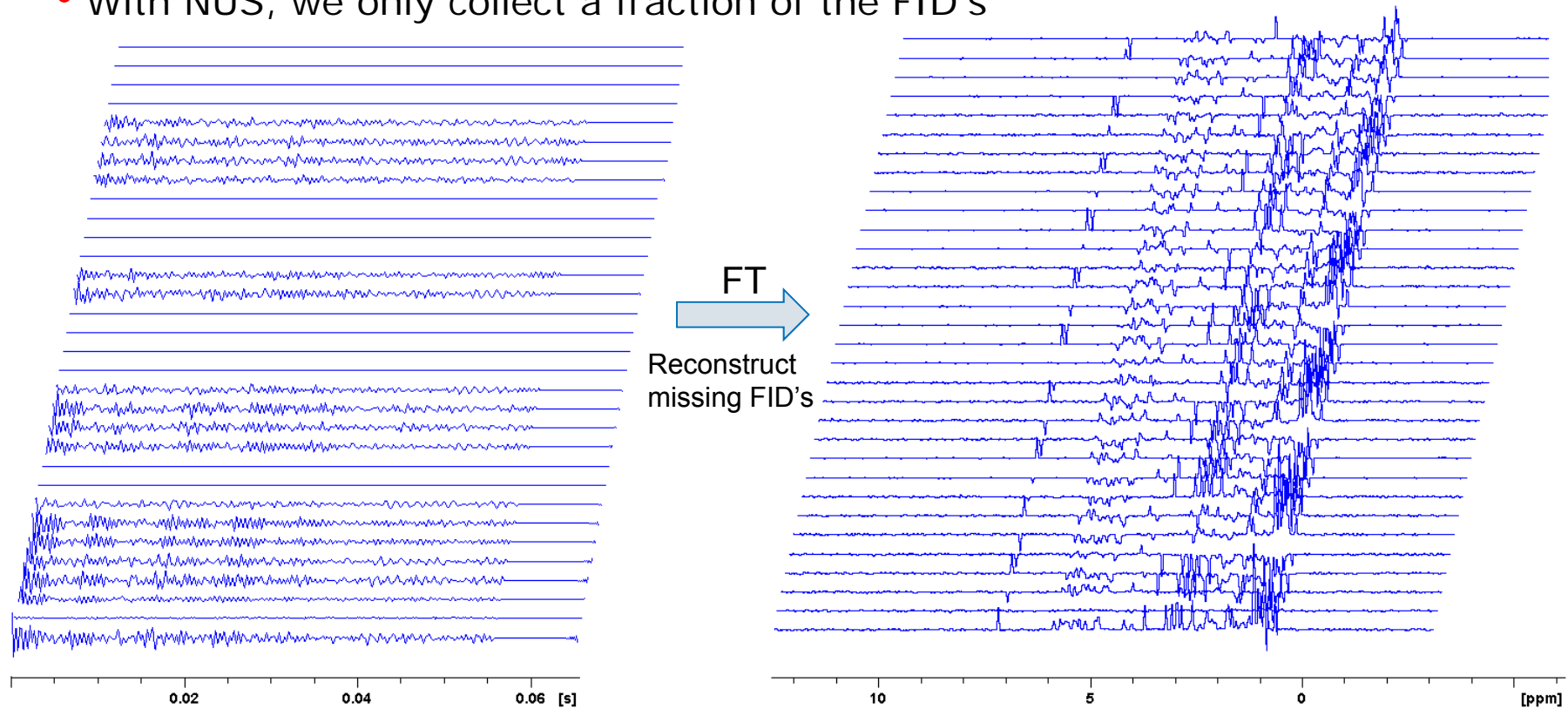
What is Non-Uniform Sampling?

- With NUS, we only collect a fraction of the FID's



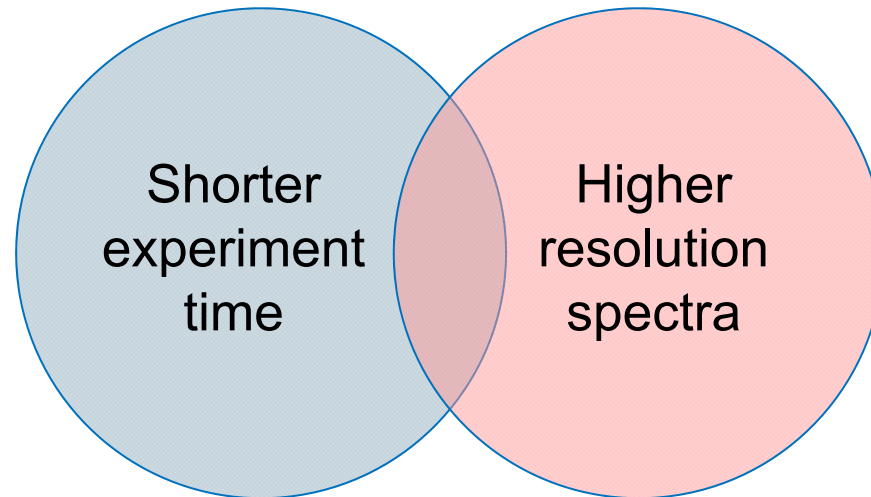
What is Non-Uniform Sampling?

- With NUS, we only collect a fraction of the FID's





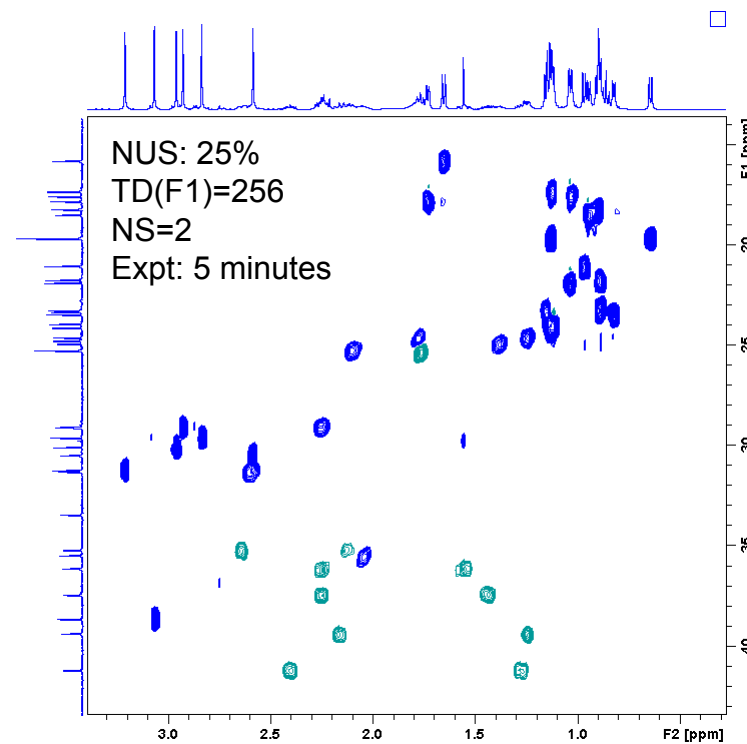
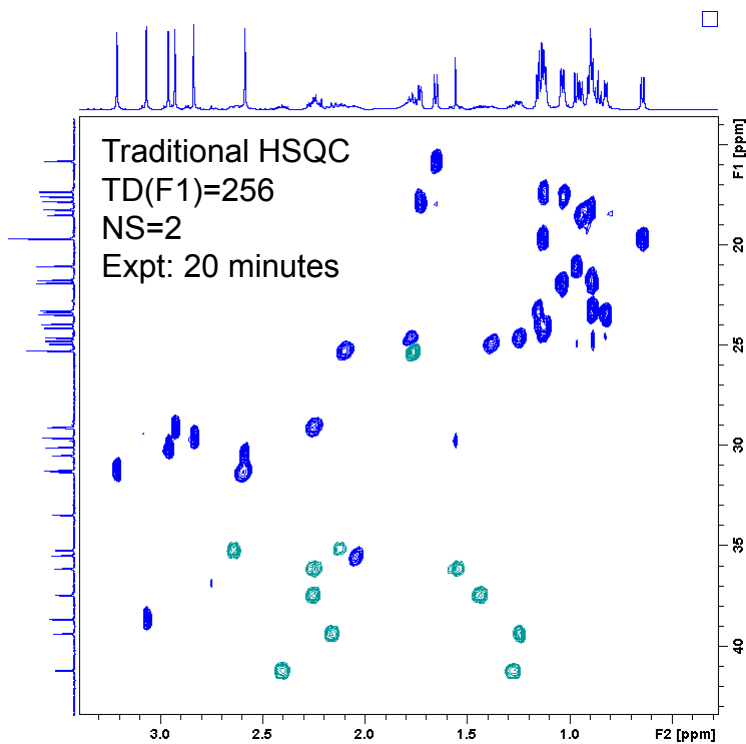
How can I benefit from using NUS?



- Acquire an nD spectrum in less time
or
- Acquire a spectrum with much higher resolution in the indirect dimension(s)
or
- Some combination of the above

... and more!

NUS for faster acquisition

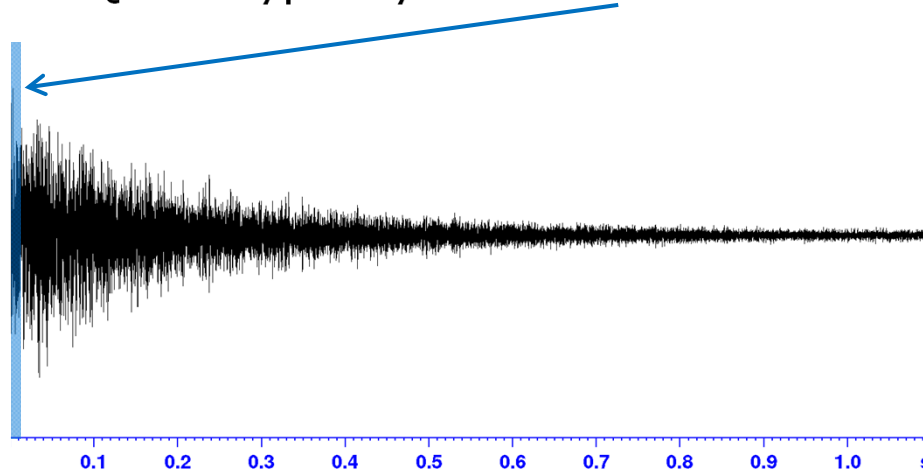


50mM cyclosporine in benzene-d6

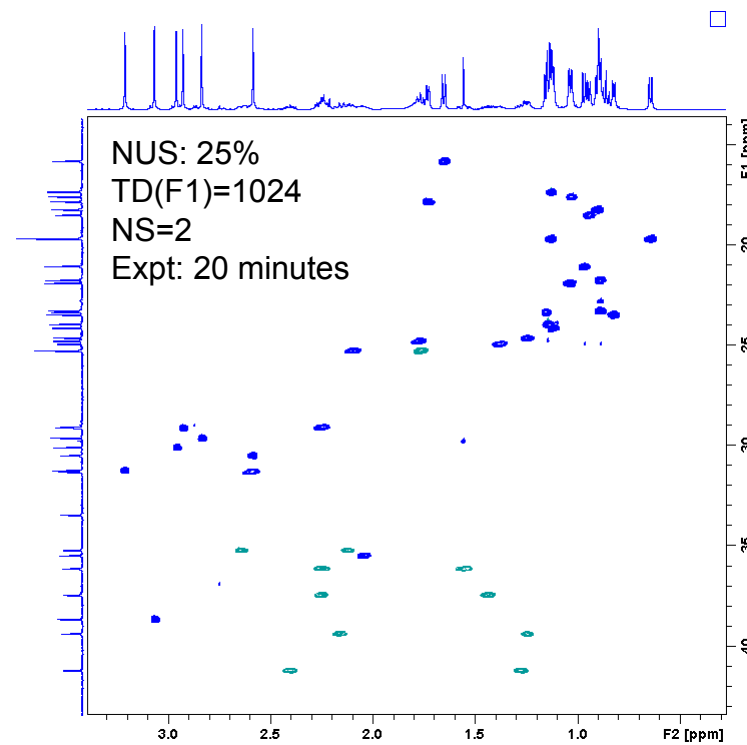
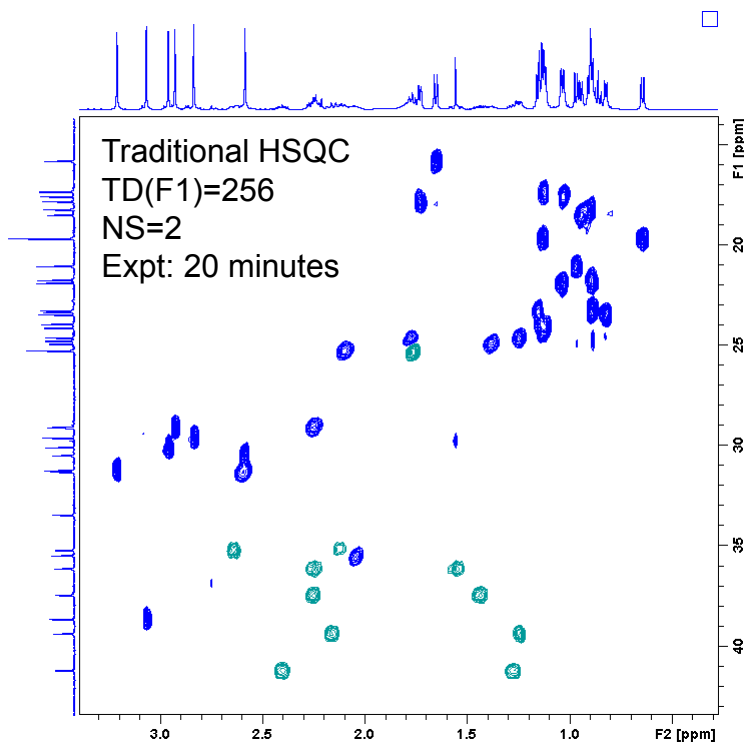


NUS for higher resolution

- Typical benefit of NUS for small molecules:
 - Increasing the resolution of indirect dimensions
 - Example: $^1\text{H}/^{13}\text{C}$ = HSQC on a typical small molecule
 - **Carbon magnetization is present for over 1 second**
 - But in an HSQC we typically measure 5 - 10 msec of it

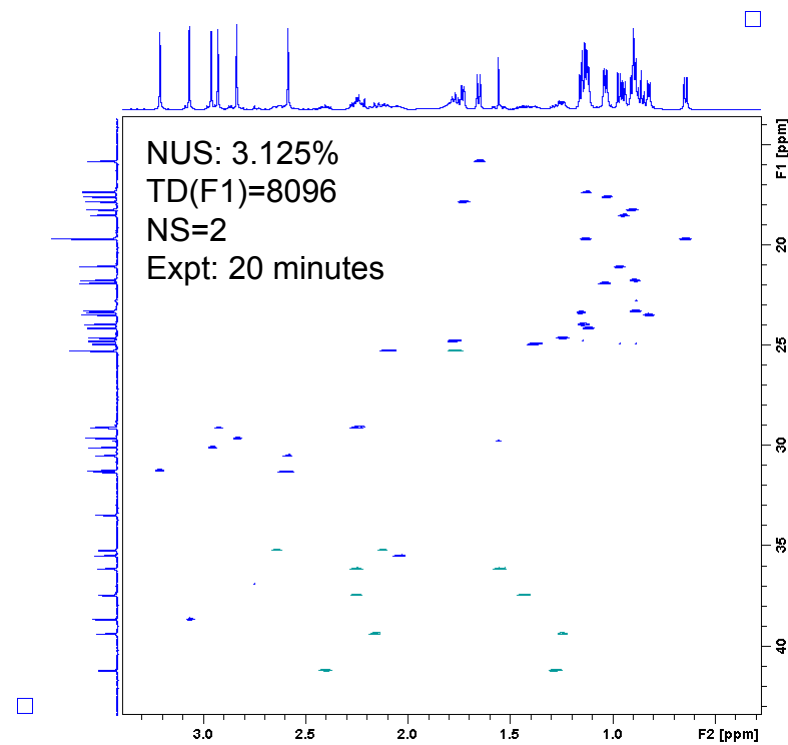
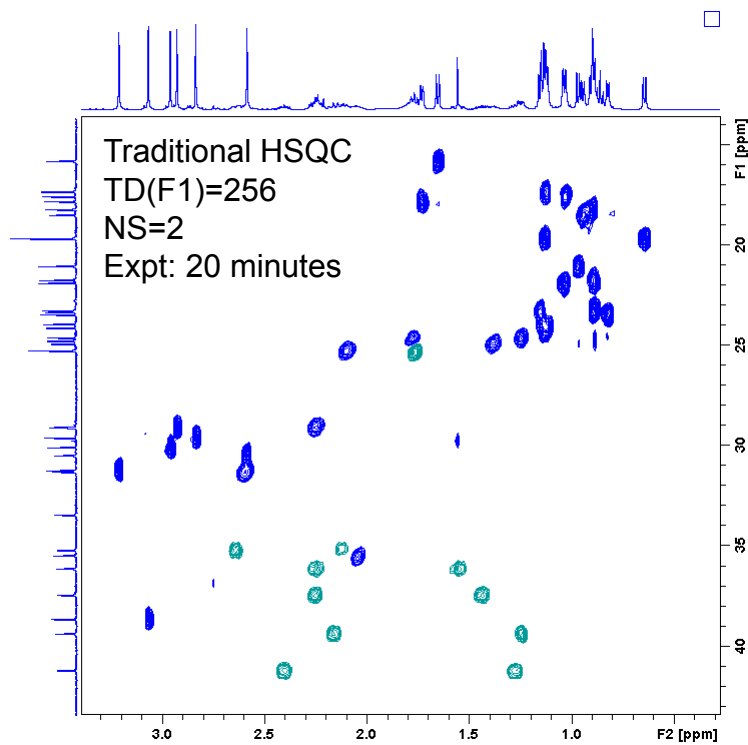


NUS for higher resolution



50mM cyclosporine in benzene-d6

NUS for higher resolution

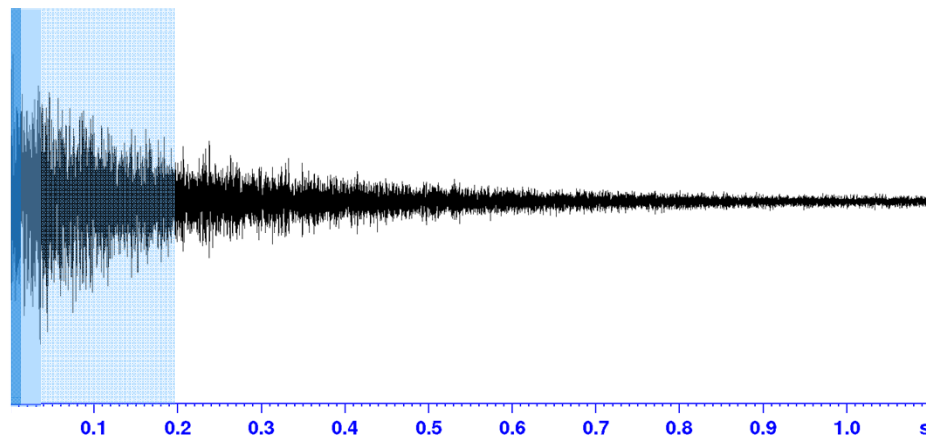


50mM cyclosporine in benzene-d₆



NUS for higher resolution

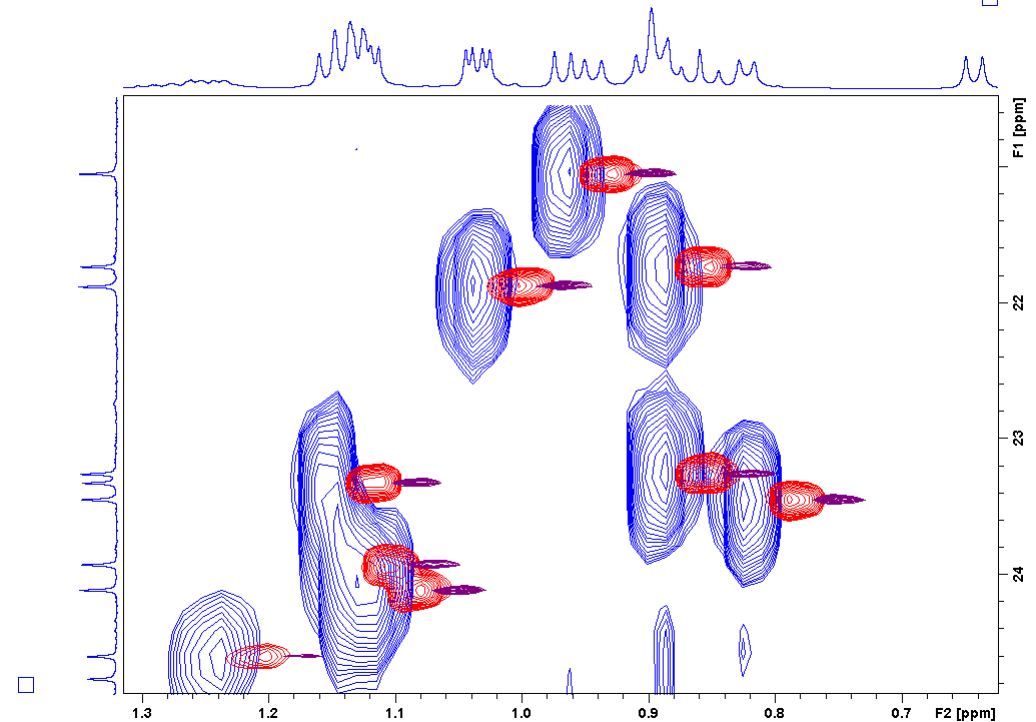
- Typical benefit of NUS for small molecules:
 - Increasing the resolution of indirect dimensions
 - Example: $^1\text{H}/^{13}\text{C}$ = HSQC on a typical small molecule
 - 1k/25% and 8k/3.125% NUS sampling



NUS for higher resolution

- Same experiment time for all 3 spectra to the right
~ 20 minutes

- Traditional 2D: TD=256
- NUS: 25% of TD=1k
- NUS: 3.125% of TD=8k

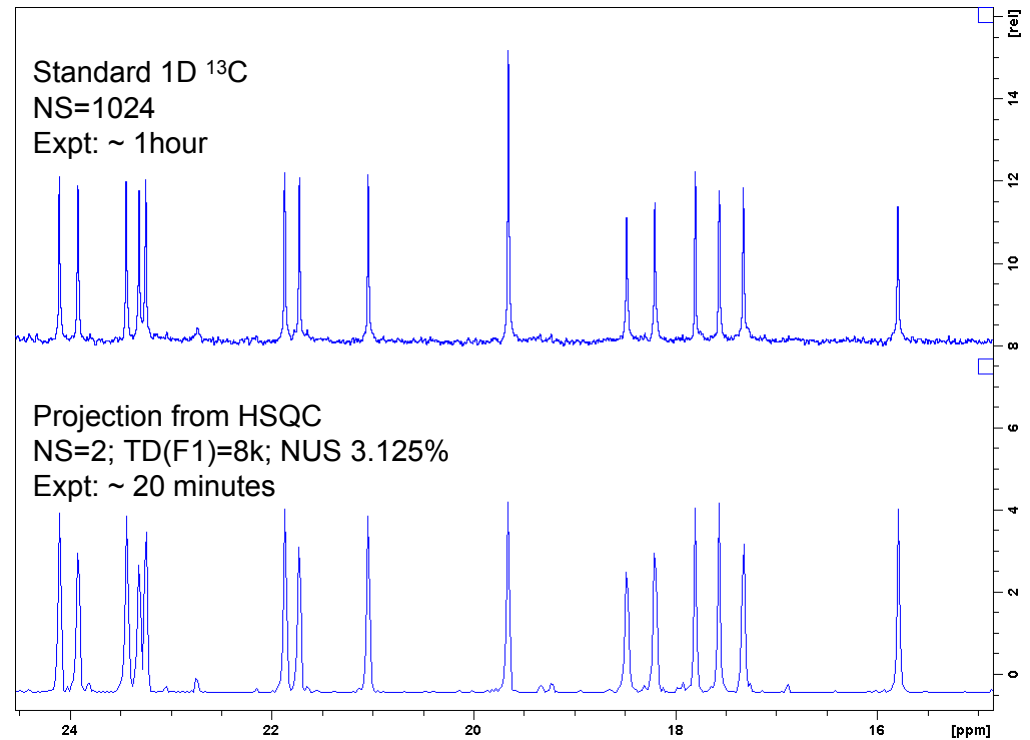


50mM cyclosporine in benzene-d6



NUS for higher resolution

- Resolution in indirect dimension approaching that of a standard 1D ^{13}C spectrum



50mM cyclosporine in benzene-d6



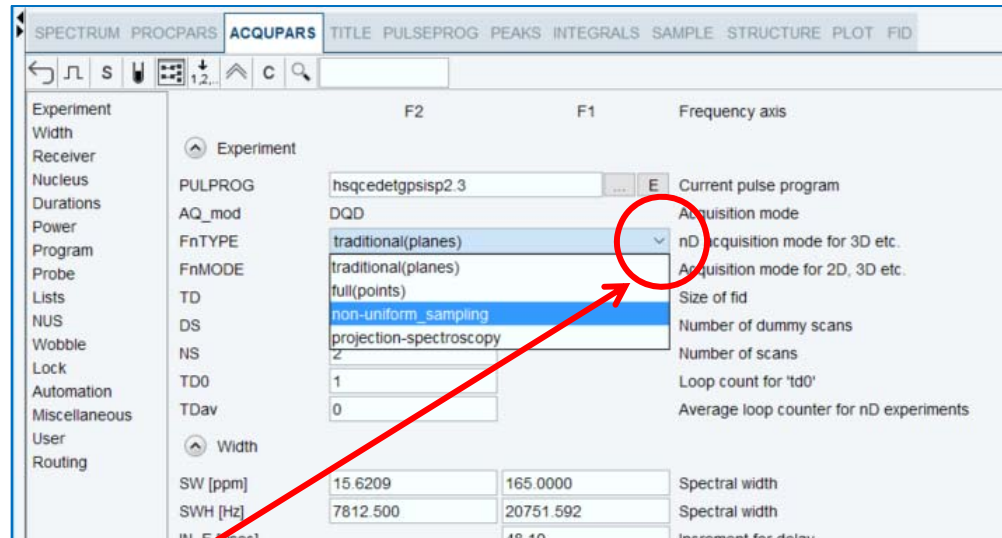
How sparsely can I sample?

- Not really the right question...
- A better question is: How many FID's do I need to acquire?
 - It largely depends on complexity of sample/spectrum:
 - How many expected frequencies (peaks)
 - What kind of dynamic range of expected peaks
- When should I use NUS?
 - HSQC of pure compound: definitely!
 - NOESY of mixture: more challenging



NUS acquisition in TopSpin

- How do I do it?
- Acquisition and processing built into Topspin3.0 and newer



- change FnTYPE from “traditional(planes)” to “non-uniform_sampling”



NUS acquisition in TopSpin

- Acquisition parameters

How sparsely do you want to sample?

- Effective TD = 256 (128 complex points)
- You can set either NusAMOUNT[%] or NusPOINTS

Parameter	F2	F1	Frequency axis
Experiment	Experiment		
PULPROG	hsqcetdgpsisp2.3		
AQ_mod	DQD		
FnTYPE	non-uniform_sampling		
FnMODE	Echo Antiecho		
TD	2048	256	
DS	32		
NS	2		
TD0	1		
TDav	0		
SW [ppm]	15.6209	165.0000	Spectral width
SWH [Hz]	7812.500	20751.592	Spectral width
IN_F [µsec]		18.10	Increment for
AQ [sec]	0.1310720	0.0061682	Acquisition time

Parameter	Value
NusAMOUNT [%]	25
NusPOINTS	32
NusJSP [Hz]	0
NusT2 [sec]	1
NusSEED	54321
NUSLIST	automatic

- Indirect acquisition time



NUS acquisition in TopSpin

- Acquisition parameters

How sparsely do you want to sample?

- Effective TD = 8k (4096 complex points)
- You can set either NusAMOUNT[%] or NusPOINTS

Parameter	F2	F1	Frequency axis
PULPROG	hsqcetgpcisp2.3		
AQ_mod	DQD		
FnTYPE	non-uniform_sampling		
FnMODE	Echo Antiecho		
TD	2048	8192	
DS	32		
NS	2		
TD0	1		
TDav	0		
SW [ppm]	15.6209	165.0000	
SWH [Hz]	7812.500	20751.592	
IN_F [µsec]		18.19	
AQ [sec]	0.1310720	0.1973825	

Parameter	Value
NusAMOUNT [%]	3.125
NusPOINTS	128
NusJSP [Hz]	0
NusT2 [sec]	1
Lists	54321
NUS	automatic
NUSLIST	

- Indirect acquisition time



NUS acquisition in TopSpin

- Looking at the NUS schedule

The screenshot shows the TopSpin ACQUPARS window with the following parameters:

Parameter	Value	Description
NusAMOUNT [%]	3.125	Amount of sparse sampling
NusPOINTS	128	Number of hypercomplex points in indirect dimension
NusJSP [Hz]	0	J-coupling
NusT2 [sec]	1	T2 relaxation
NusSEED	54321	Random generator seed
NUSLIST	automatic	

The 'Show' button under the NUSLIST parameter is highlighted with a red box and a red arrow.

Point	Value
1	0
2	2087
3	751
4	3312
5	3841
6	121
7	3215
8	671
9	1777
10	2352
11	888
12	3402
13	1226

The 'nuslist' window title is 'nuslist' and the menu is 'File Edit Search'.

The 'nusdisp' window shows a plot of the NUS schedule over time. The x-axis is 't1 [points]' ranging from 0 to 4,200. The plot shows a series of points in different colors: green (done), red (active), and blue (todo). The legend at the bottom indicates: * done, ■ active, • todo.

NUS schedule is in a text readable file "nuslist" in the EXPNO directory

- After we start an acquisition, we can see the NUS schedule of FID's



NUS acquisition in TopSpin

- Controlling the NUS schedule

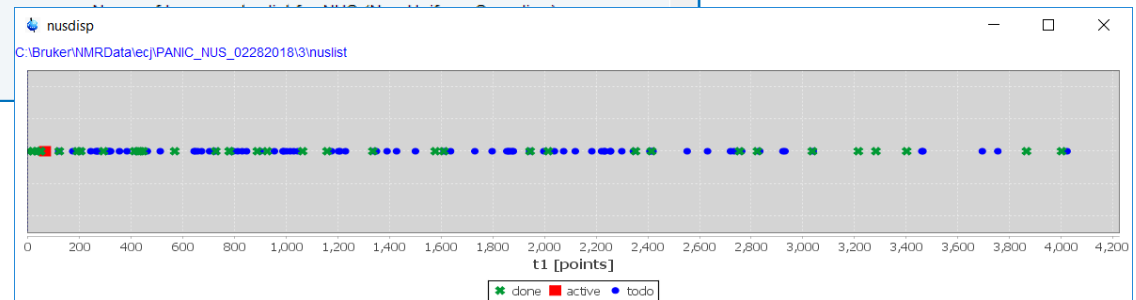
ACQUPARS

NUS (Non Uniform Sampling) parameters

NusAMOUNT [%]	3.125	Show NUS help	Show NUS help
NusPOINTS	128		Amount of sparse sampling
NusJSP [Hz]	0		Number of hypercomplex points in indirect dimension
NusT2 [sec]	0.1		J-coupling
NUSSEED	54321		T2 relaxation
NUSLIST	automatic		Random generator seed

Buttons: Calculate, Show

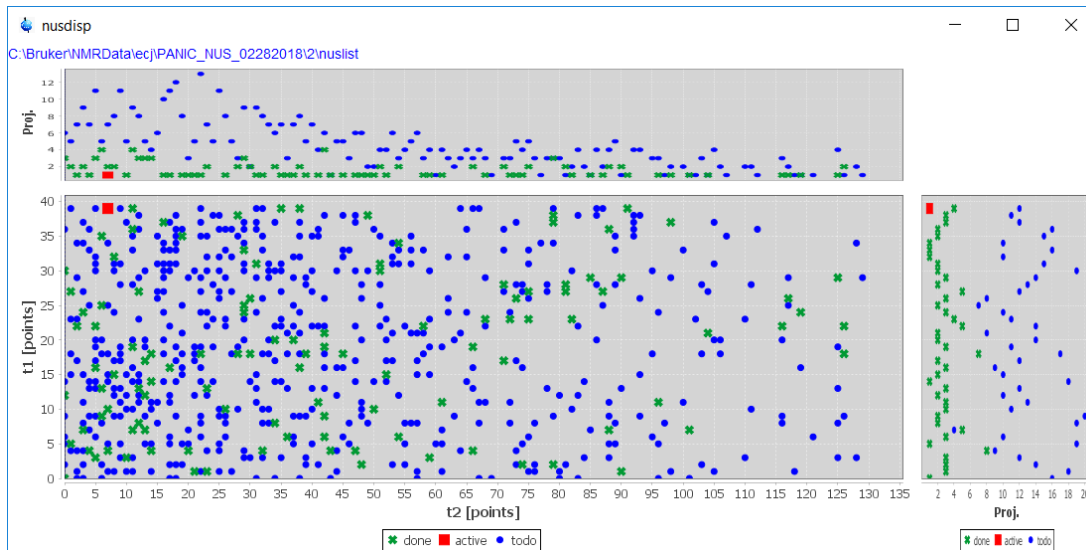
- We can bias the sampling towards shorter indirect evolution times
- Default value of NusT2 = 1 second
→ effectively no weighting





NUS acquisition in TopSpin

- Example NUS schedule for a 3D dataset



The figure shows a software window titled 'nuslist' with a menu bar 'File Edit Search'. It contains a list of acquisition points with columns for point number, t1, and t2. A status bar at the bottom shows '625 : 1'.

1	0	0
2	1	23
3	31	51
4	22	126
5	33	29
6	27	1
7	39	11
8	37	98
9	13	6
10	38	79
11	30	51
12	11	41
13	14	14
14	15	52
15	4	47
16	16	38
17	4	20
18	26	117
19	32	8
20	29	85



NUS acquisition in TopSpin

- We can use NUS schedules generated outside of TopSpin

The screenshot shows the TopSpin ACQUPARS interface. The left pane shows the 'NUS (Non Uniform Sampling) parameters' section with the following values:

NusAMOUNT [%]	3.125
NusPOINTS	128
NusJSP [Hz]	0
NusT2 [sec]	0.1
NusSEED	54321
NUSLIST	my_nus_list

The right pane shows the 'Experiment' section with the following values:

PULPROG	hsqcetdgpisp2.3
AQ_mod	DQD
FnTYPE	non-uniform_sampling
FnMODE	Echo-Antiecho
TD	2048
DS	32
NS	2

The 'NUSLIST' field is highlighted with a red box, and the 'Calculate' button is crossed out with a red 'X'. The 'FnMODE' dropdown menu is also highlighted with a red box, showing 'Echo-Antiecho' selected.

1. Put the NUS schedule in the vc list directory:
 - <Topspin>/exp/stan/nmr/lists/vc/
2. Enter the name of the file in NUSLIST
3. Enter the effective TD
4. Start your acquisition

- DO NOT click Calculate!

NUS processing in TopSpin



- But first a couple words on Licenses...
- No special NUS licenses are needed for data acquisition
- Prior to TopSpin3.5pl3, a special NUS license was required for processing in Topspin
- In TS3.5pl3 and newer, basic 2D processing is free
... but please make sure you have at least TS3.5pl6!



NUS processing methods

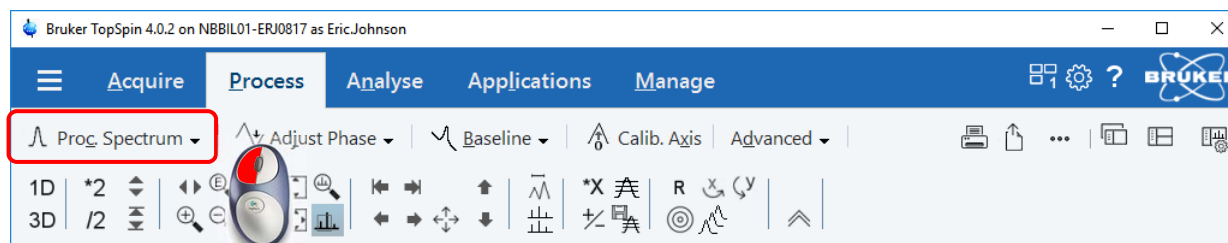


- Inside Topspin:
 - MDD – Multi Dimensional Deconvolution
 - CS – Compressed Sensing
 - IST – Iterative Soft Thresholding
 - IRLS - Iterative Reweighted Least Squares
- Outside of Topspin:
 - hmsIST: <http://gwagner.med.harvard.edu/intranet/hmsIST/>
 - NESTA: <http://nestanmr.com/>
 - Rowland Toolkit: <http://rnmrtk.uchc.edu/rnmrtk/RNMRTK.html>
 - NMR-Pipe: <http://spin.niddk.nih.gov/bax/software/NMRPipe/info.html>
 - And more...

NUS processing in TopSpin



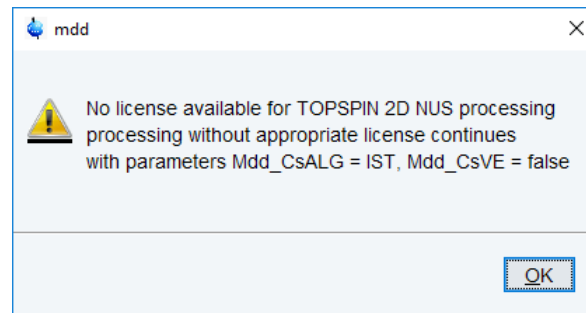
- That's nice, but what do I really need to know?
- Usually no need to change the NUS processing parameters.
- Just process the way you would any other dataset
 - ***xfb*** or ***xf2*** for 2D's
 - ***ftnd*** for nD experiments





NUS processing in TopSpin

- Just process the way you would any other dataset – *xfb*
- I did that, but I keep getting this error message!



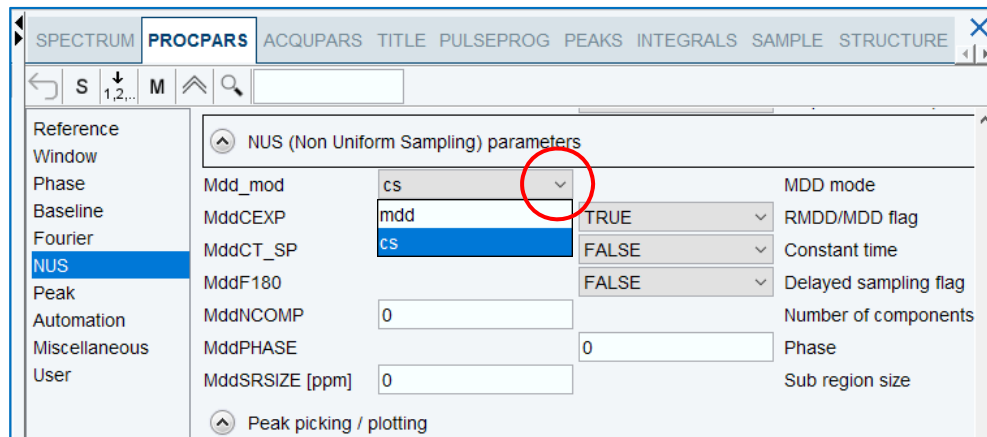
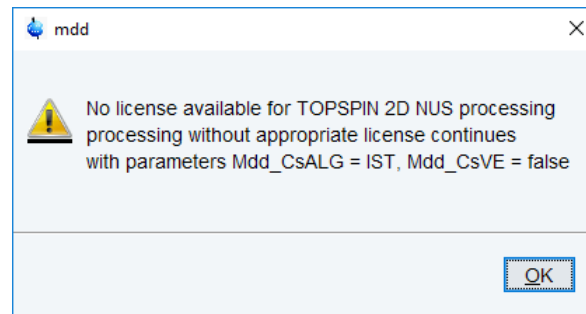
- "In TS3.5pl3 and newer, basic 2D processing is free."



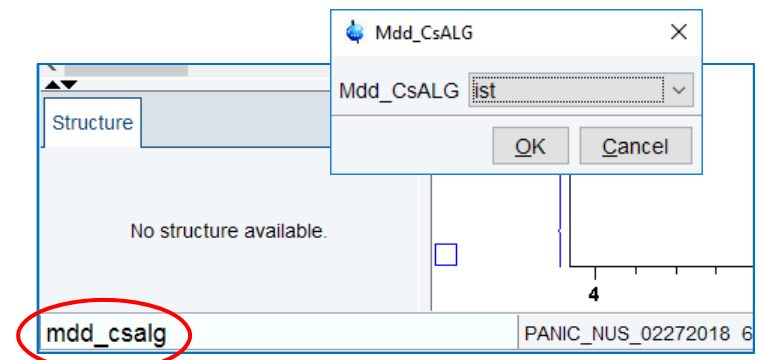


NUS processing in TopSpin

- Getting rid of the NUS license message...

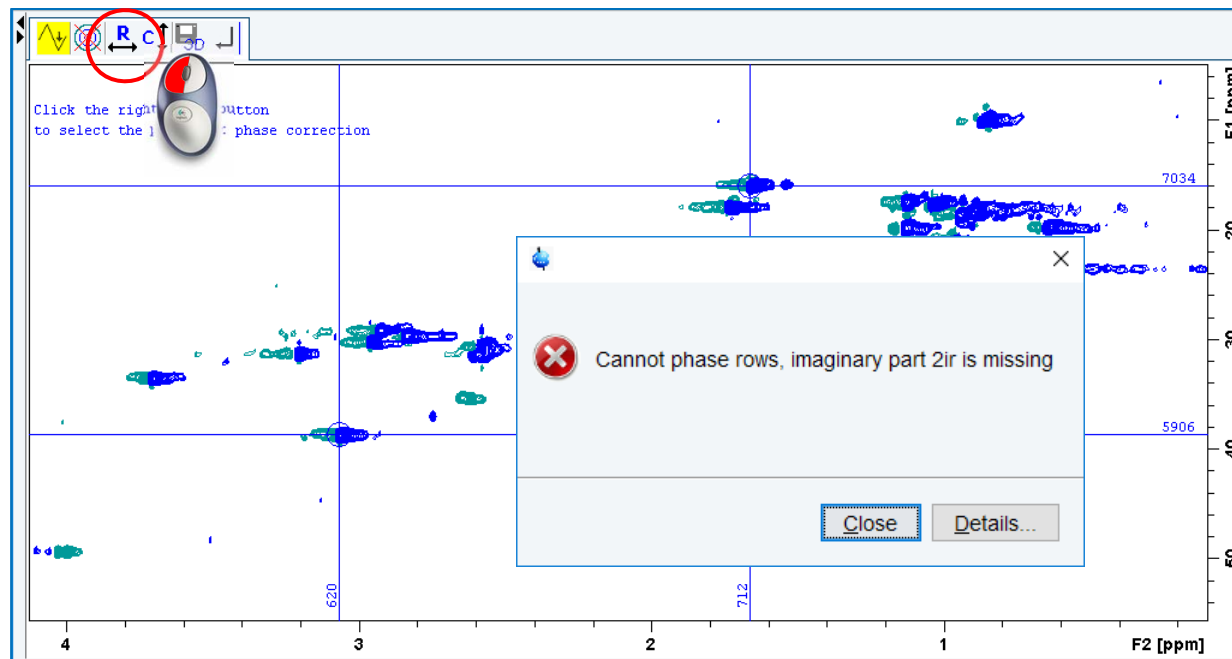


- Make sure **Mdd_mod** = cs
- Set the “hidden paramters” from Topspin command line:
 - **Mdd_CsALG** = ist
 - **Mdd_CsVE** = false



NUS processing in TopSpin

- OK, now I can process my spectrum, but I can't phase!





NUS processing in TopSpin

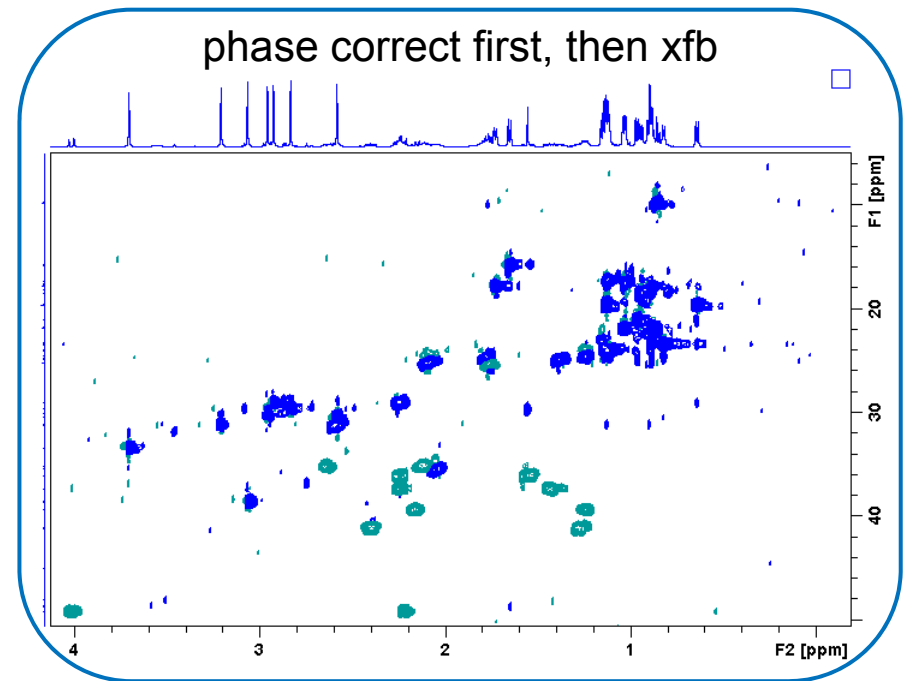
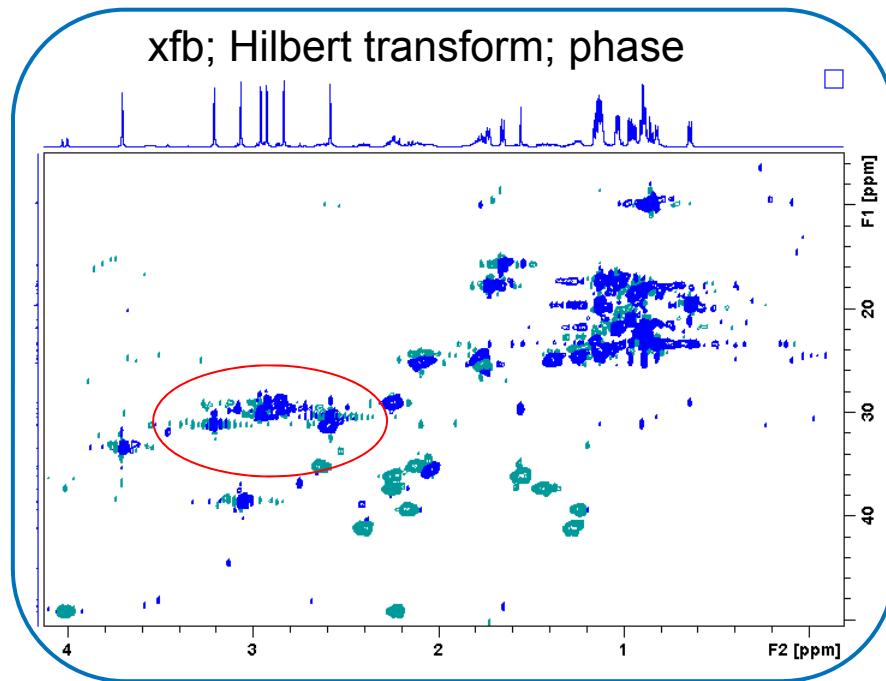
- OK, now I can process my spectrum, but I can't phase!
- Imaginary data isn't kept after the NUS reconstruction.
 - But we can re-create it with a Hilbert transform

The screenshot shows the Bruker TopSpin 4.0.2 software interface. The 'Advanced' menu is open, and the 'Special Transforms' option is highlighted. Within this menu, 'Hilbert in F2 (xht2)' is also highlighted. The interface includes a menu bar with 'Acquire', 'Process', 'Analyse', 'Applications', and 'Manage'. Below the menu bar is a toolbar with various icons for spectrum processing. The title bar indicates the user is Eric Johnson.

- ... or type ***xht2*** at the TopSpin command line
- Phasing now works normally!

NUS processing in TopSpin

- Recommendation: re-process spectrum after phasing
 - NUS reconstruction works better when 1D spectra are properly phased

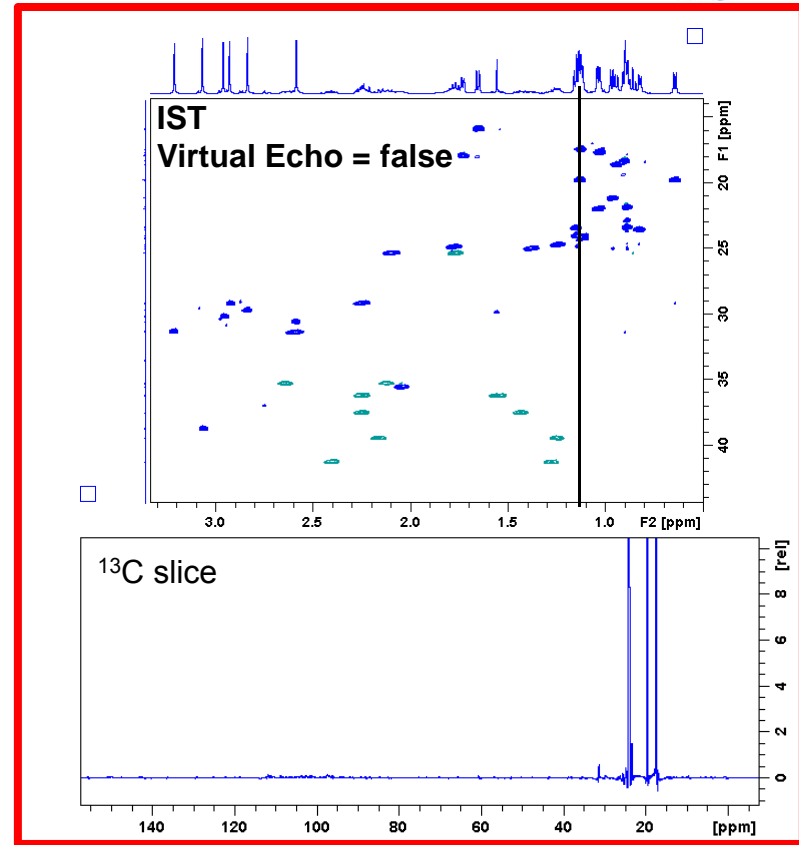
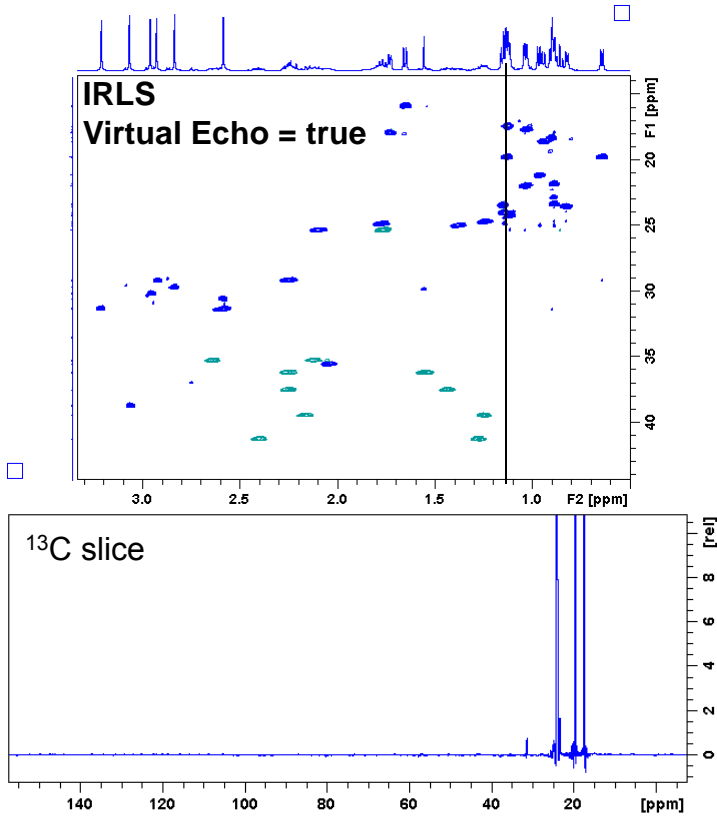




Comparing processing algorithms

25% of TD=1k

Free method

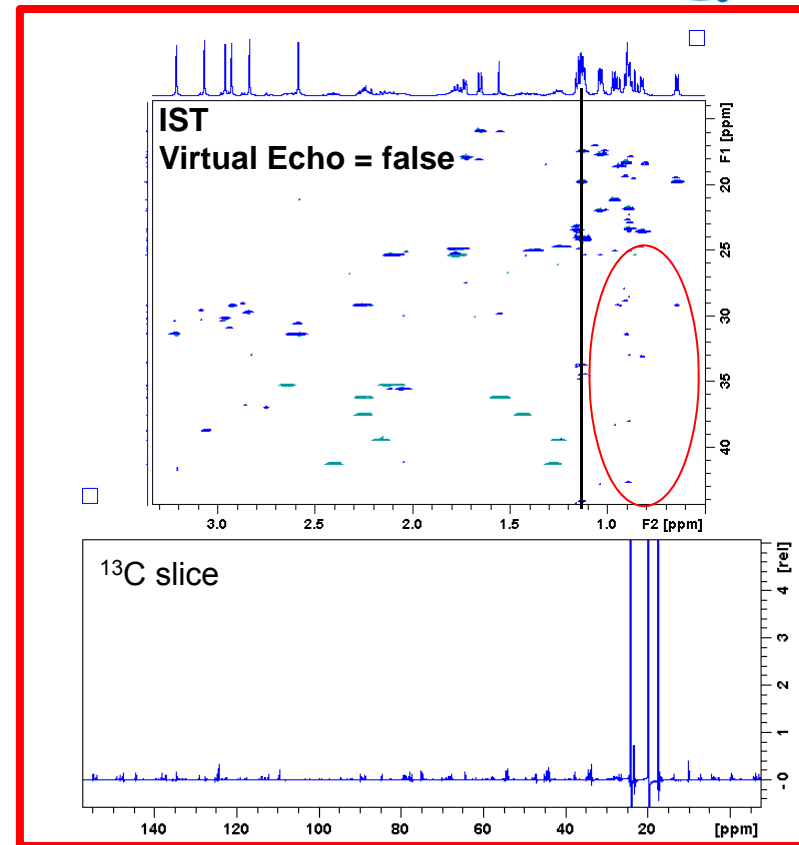
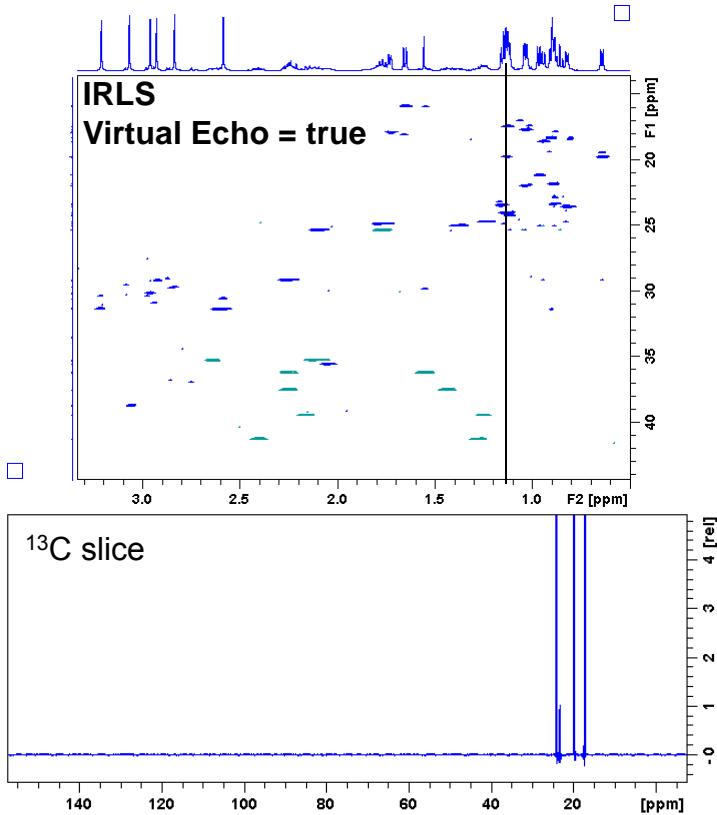


50mM cyclosporine in benzene-d6

Comparing processing algorithms

3.125% of TD=8k

Free method

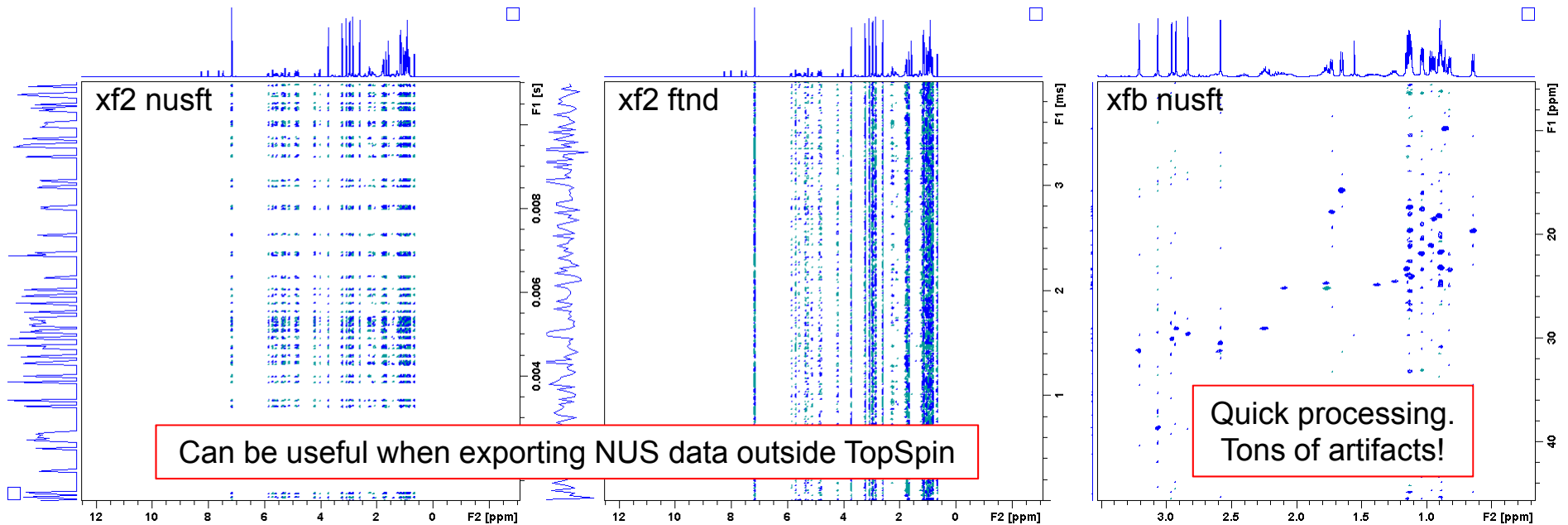


50mM cyclosporine in benzene-d6



A few other NUS procession options

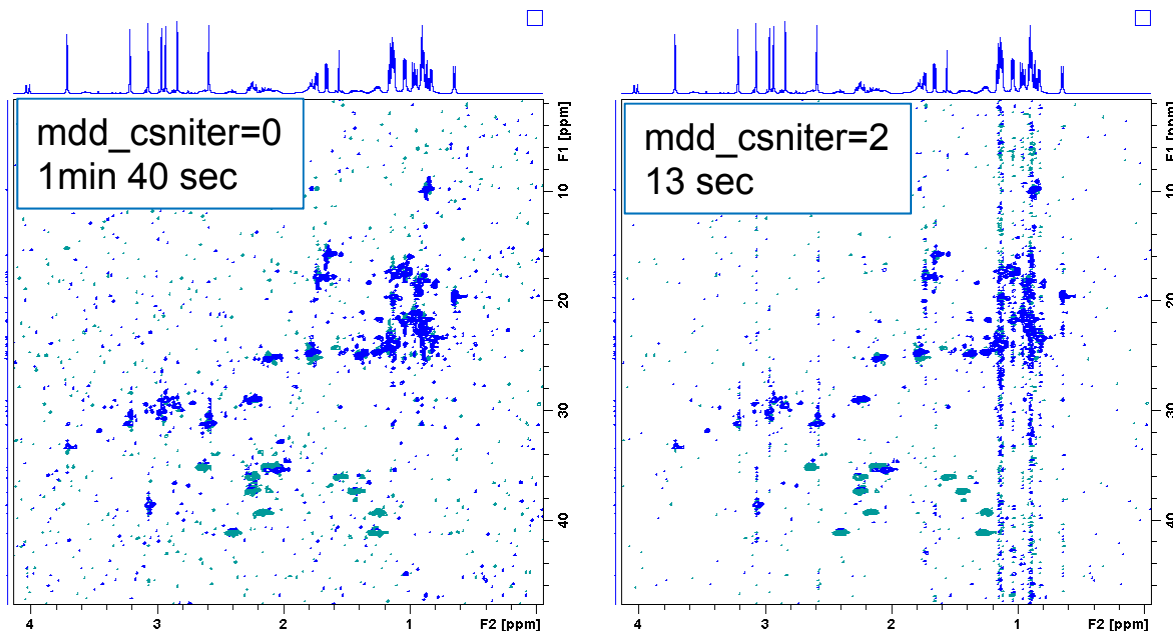
- Special options:
 - nusft – sort FID's, leaving blanks where no data was collected (xf2, xfb, ftnd)
 - nd2d – leave FID's in the acquired order (xf2 only)



A few other NUS procession options

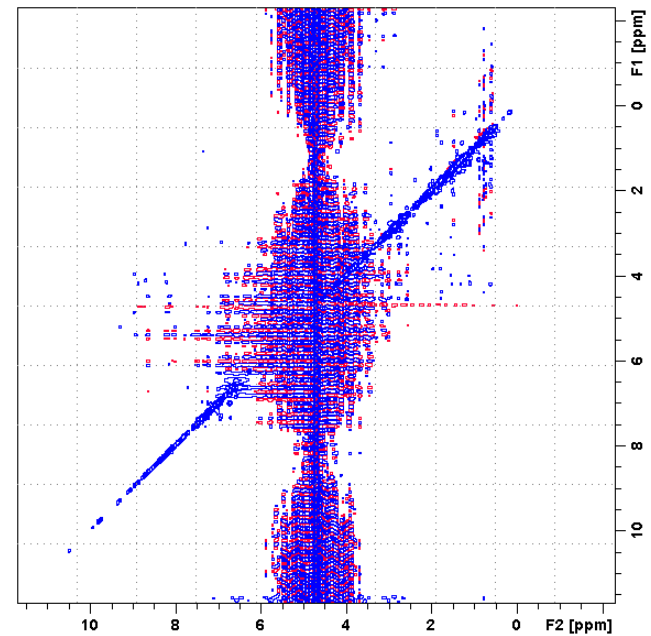
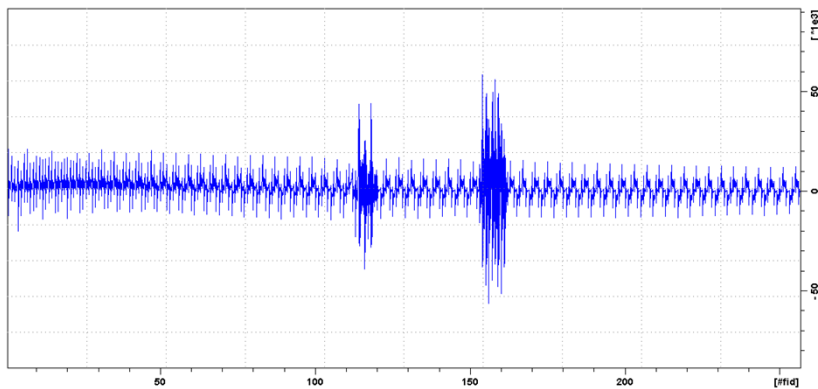
- `mdd_csniter` - number of iterations performed in reconstruction.
 - Smaller value \rightarrow faster processing, but more artifacts
 - Default value 0: process until convergence

- NOT in ProcPars GUI
 \rightarrow set via command line



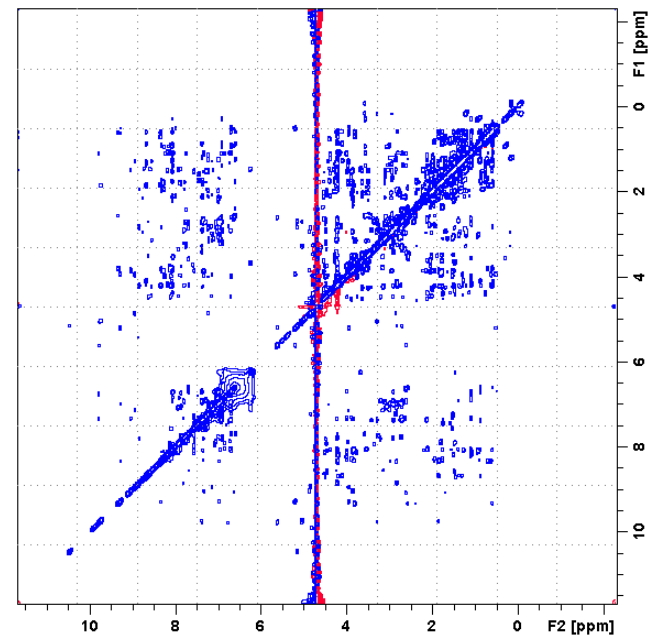
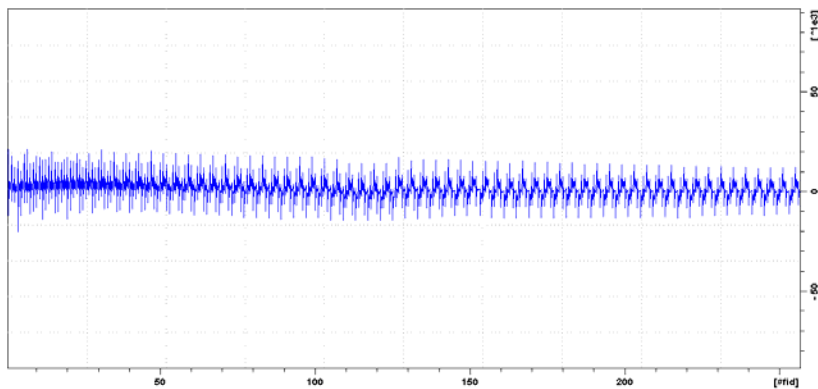
What else can I do with NUS?

- Fix bad data points!
 - Many external factors can disturb an acquisition.
 - This can lead to individual FID's in a 2D that are corrupted.



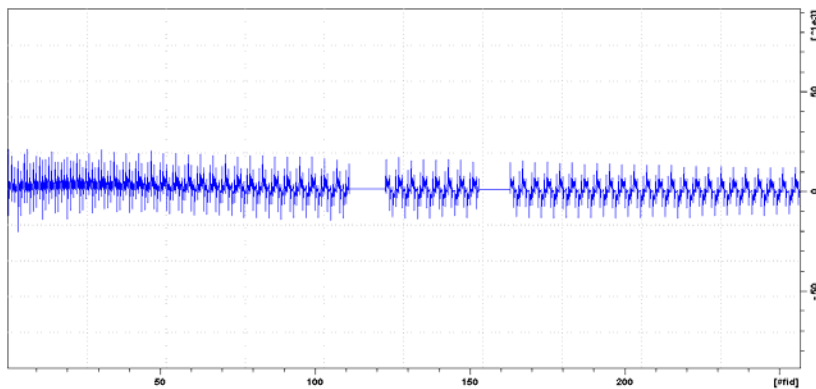
Repairing data with NUS

- 1) Determine the index of corrupted FID's
- 2) Create NUSLIST of only corrupted FID's
- 3) Run experiment
- 4) Replace damaged FID's with good ones
- 5) Normal processing

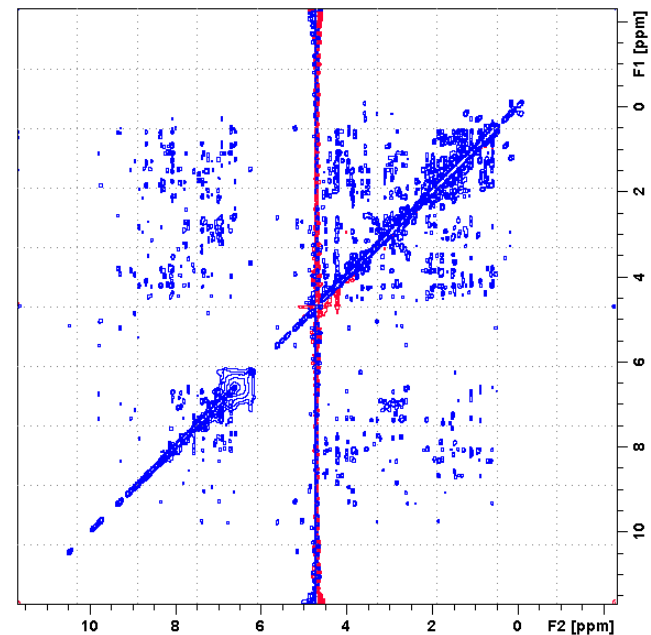


Repairing data with NUS

- 1) Determine the index of corrupted FID's
- 2) Create NUSLIST of all FID's except those corrupted
- 3) ~~Run experiment~~
- 4) Remove damaged FID's
- 5) Process with NUS



xfb
NUS





What else can I do with NUS?

- Monitor reactions or exchange with Continuous NUS

Two-Dimensional NMR Spectroscopy with Temperature-Sweep,
Wolfgang Bermel, Rupashree Dass, Klaus-Peter Neidig and Kazimierczuk, Krzysztof
ChemPhysChem, 15,11, 2217–2220, 2014

Time-resolved multidimensional NMR with non-uniform sampling,
Maxim Mayzel, Joakim Rosenlo, Linne Isaksson, Vladislav Y. Orekhov
J Biomol NMR (2014) 58:129–139

Analysis of Complex Reacting Mixtures by Time-Resolved 2D NMR,
Rupashree Dass, Wiktor Koźmiński and Krzysztof Kazimierczuk
Anal. Chem. 2015, 87, 1337–1343

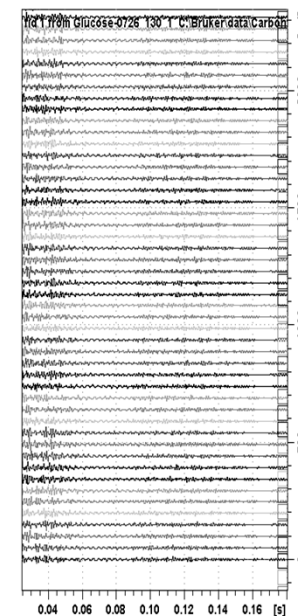


Continuous NUS



- because each subset of FID's of a NUS experiments is a complete experiment
- Start by creating a very long list for NUS sampling:

```
0 45 117 112 39 77 80 16 46 54 26 87 71 29 37 14 44 69 38  
13 82 116 48 72 35 56 118 42 93 76 55 4 32 124 14 67 49  
120 24 84 27 7 116 107 43 127 39 76 51 25 47 33 94 59 80  
82 3 64 44 103 41 26 5 91 0 6 57 52 25 34 50 70 28 37 22  
56 3 59 63 109 80 5 27 65 20 69 21 9 7 112 100 86 29 125  
53 114 74 47 17 2 124 12 66 78 40 ....
```
- Acquire data as long as your reaction lasts

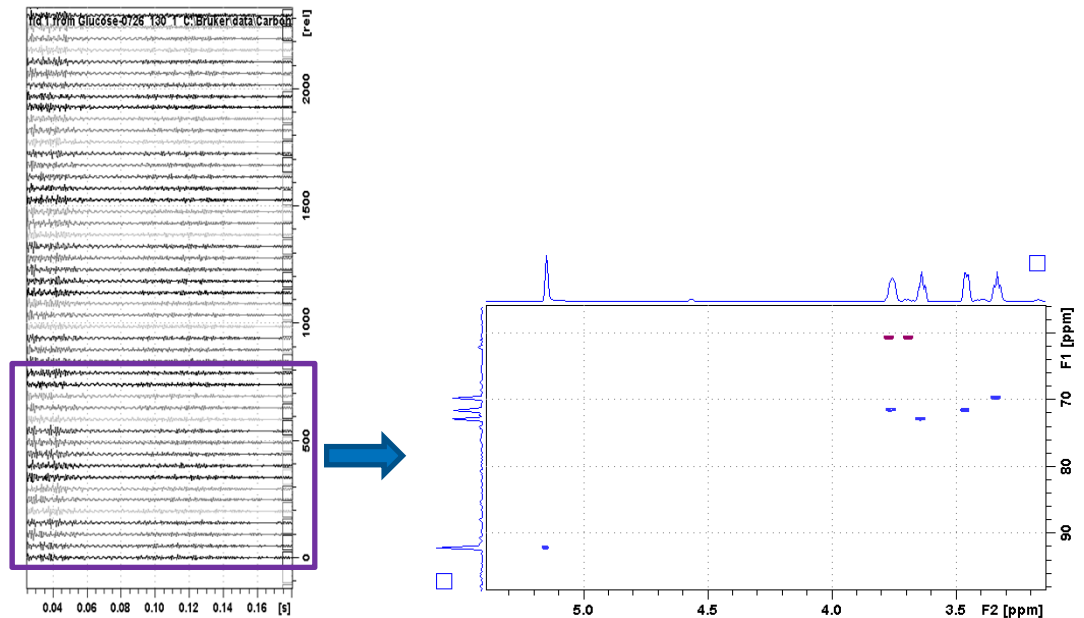




Continuous NUS

- Extract a subset and process

0 45 117 112 39 77 80 16 46 54 26 87 71
29 37 14 44 69 38 13 82 116 48 72 35 56
118 42 93 76 55 4 32 124 14 67 49 120
24 84 27 7 116 107 43 127 39 76 51 25
47 33 94 59 80 82 3 64 44 103 41 26 5
91 0 6 57 52 25 34 50 70 28 37 22 56 3
59 63 109 80 5 27 65 20 69 21 9 7 112
100 86 29 125 53 114 74 47 17 2 124 12
66 78 40

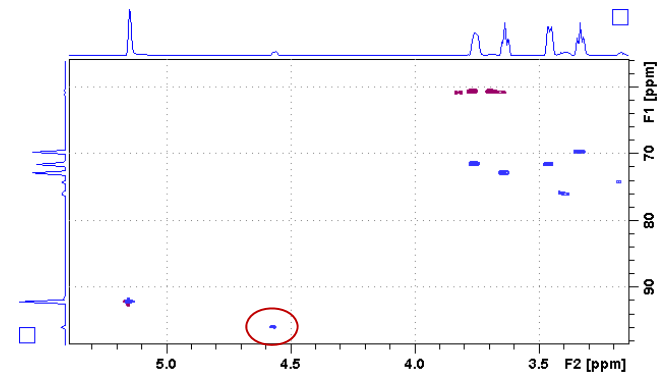
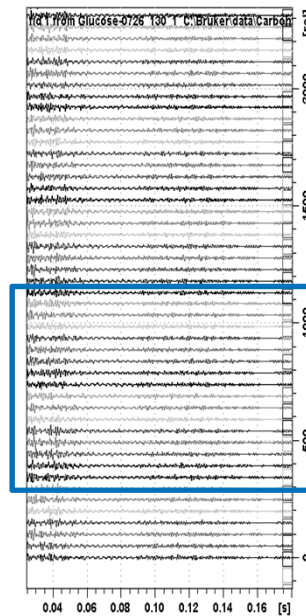


Continuous NUS



- Extract the next subset and process

0 45 117 112 39 **77 80 16 46 54 26 87 71**
29 37 14 44 69 38 13 82 116 48 72 35 56
118 42 93 76 55 4 32 124 14 67 49 120
24 84 27 7 116 107 43 127 39 76 51 25
47 33 94 59 80 82 3 64 44 103 41 26 5
91 0 6 57 52 25 34 50 70 28 37 22 56 3
59 63 109 80 5 27 65 20 69 21 9 7 112
100 86 29 125 53 114 74 47 17 2 124 12
66 78 40

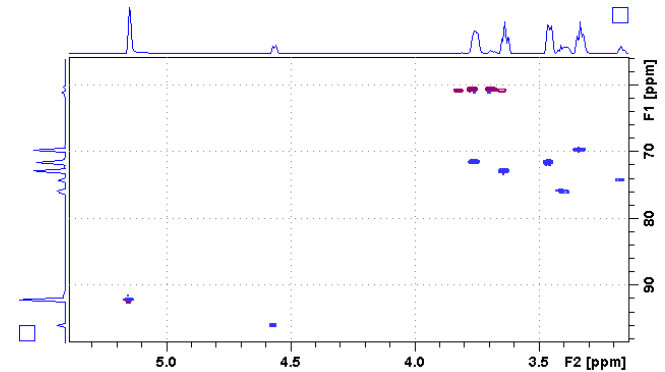
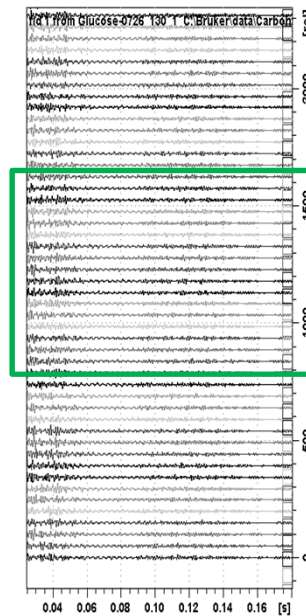




Continuous NUS

- Continue extracting and processing subsets

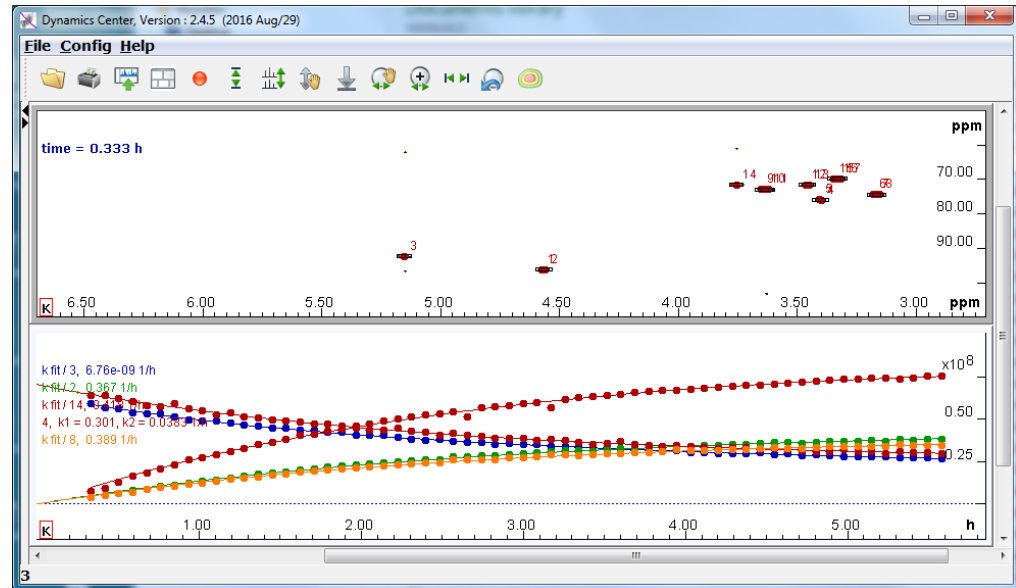
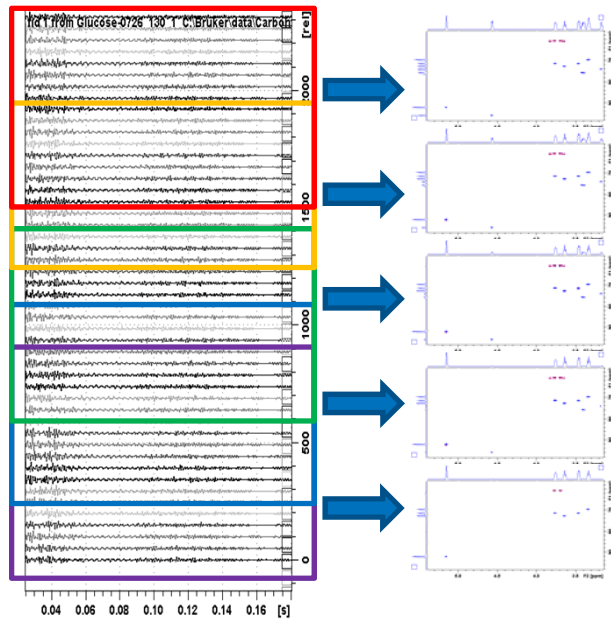
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29 37 14 44 69 38 13 82 116 48 72 35 56
118 42 93 76 55 4 32 124 14 67 49 120
24 84 27 7 116 107 43 127 39 76 51 25
47 33 94 59 80 82 3 64 44 103 41 26 5
91 0 6 57 52 25 34 50 70 28 37 22 56 3
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66 78 40



Continuous NUS



- Analyze and extract reaction rates.

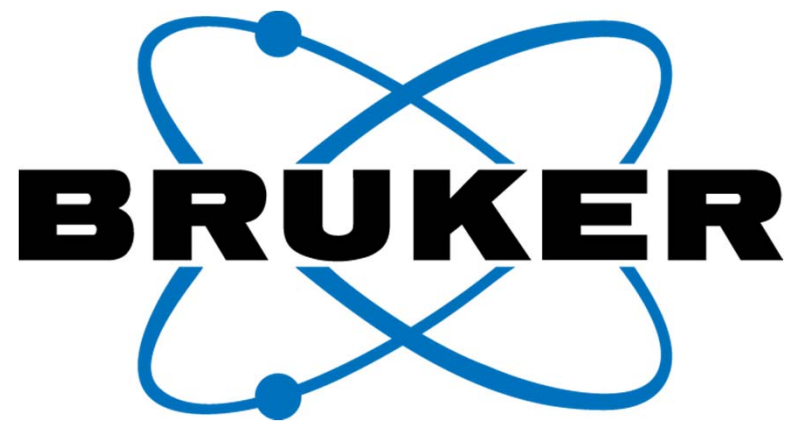


Additional application of NUS



- Repairing bad FID's
- Continuous NUS
 - AU programs available by request.





Innovation with Integrity

