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School of Chemistry

Faster NMR
Non Uniform Sampling (NUS)

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December 2017

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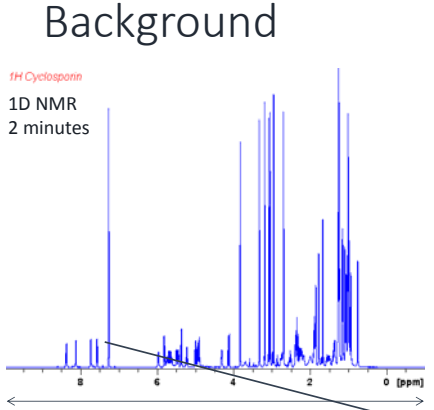
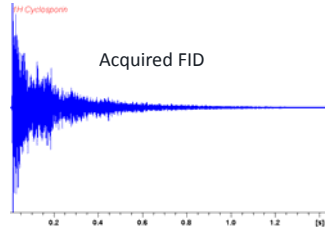
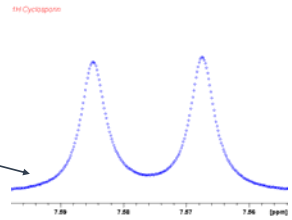
Background



1D NMR
2 minutes





Resolution depends on:
acquisition time ∝ sweep width & time domain
6 seconds – 11 ppm – 64k points

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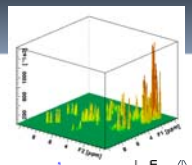
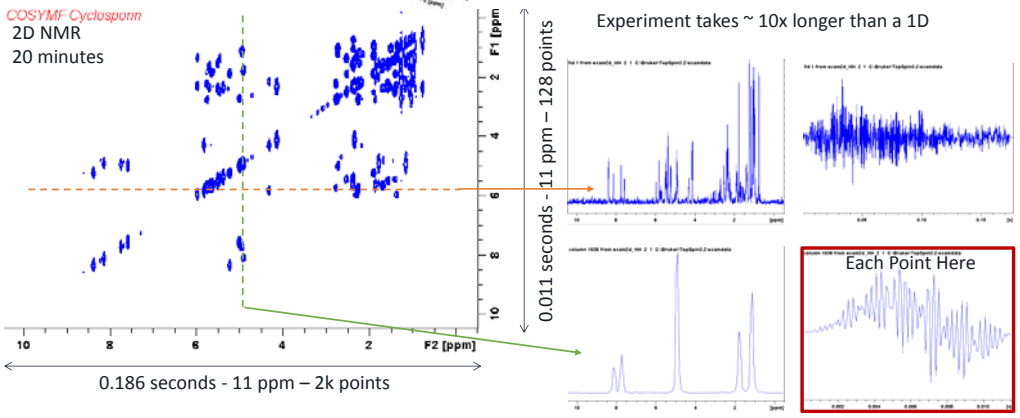


Lower resolution in each axis
F1 (y-axis, indirect dimension) only 128 points
As each point has to be measured individually!

Experiment takes ~ 10x longer than a 1D



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NMR Resolution and Sensitivity

- **Resolution** is dictated by the length of the FID
  - $res = \frac{1}{at} = \frac{2sw}{np}$
    - Digital resolution (res)
    - Acquisition time (at, *s*)
    - Sweep Width (sw, *Hz*)
    - Number of Points (np)

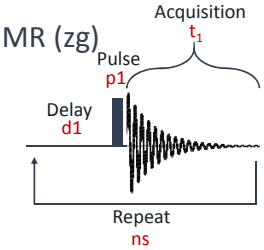
Rule of thumb:
Digital resolution should be < ½ of natural linewidth of a peak

- **Sensitivity** is improved by summing scans
  - i.e. Number of scans (ns)
    - Signal increases linearly with ns
    - Noise increases by $\sqrt{2}$
    - Double SNR takes 4x NS

NS	Signal	Noise	SNR
1	1	1	1
4	4	2	2
16	16	4	4
64	64	8	8
256	256	16	16

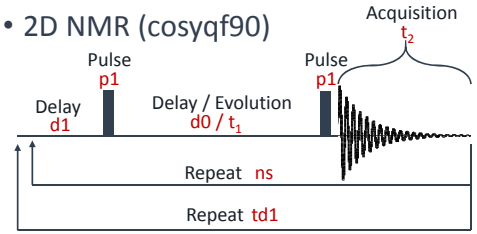
Background

- 1D NMR (zg)



  - d1, t1 – seconds
  - p1 – microseconds
  - ns – 1+
  - Time taken for experiment
    - (d1+t1)*ns
  - **1D as 1 time dimension (t_1)**

- 2D NMR (cosyqf90)



  - d1, t_1, t_2 – seconds/miliseconds
  - ns – 1+
  - **td1** – 64 – 2048 – *number of points in F1 (y-axis).*
    - *Directly related to resolution in y-axis!*
  - Time taken for experiment
    - ((d1+t1+t2)*ns)*td1
    - Dictated by resolution in 2 dimensions!
  - **2D as 2 time dimensions (t_1,t_2)**

Interferogram

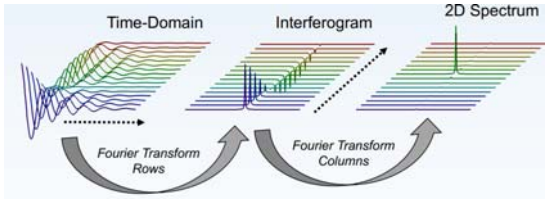
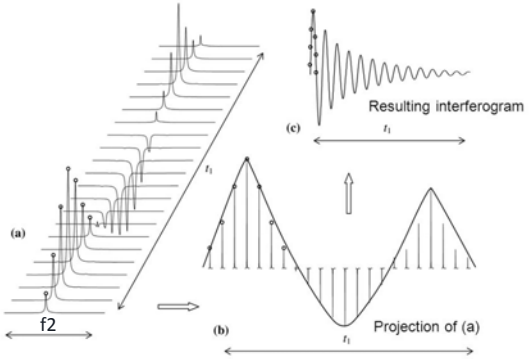


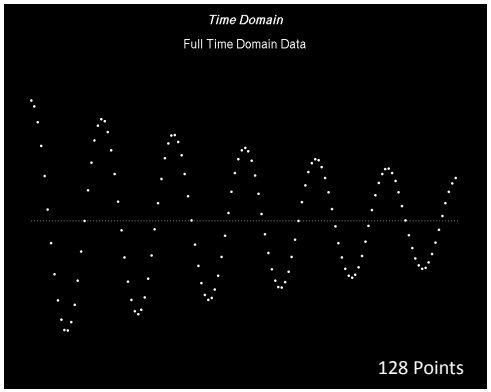
Figure from Frank Delalgio

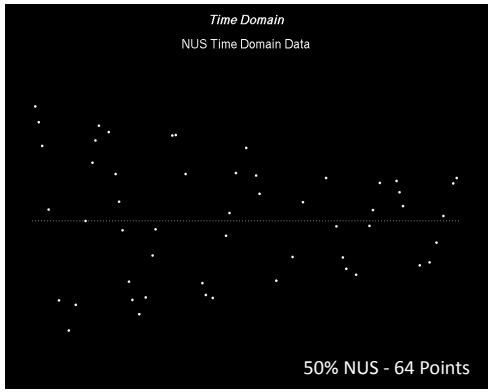


The major time cost in *n*D NMR experiments is sampling the indirect dimension(s) – e.g. t_1

NMR signals are simply superpositions of sine waves – predictable?
Do we need to measure every point?

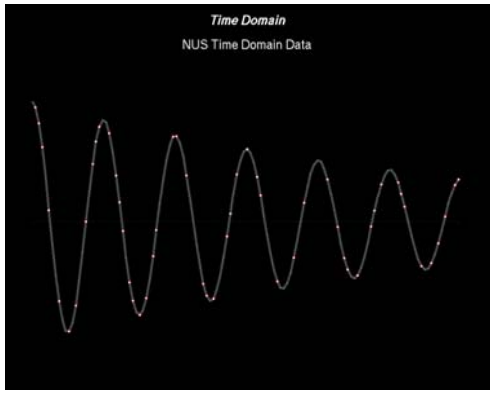
Non Uniform Sampling



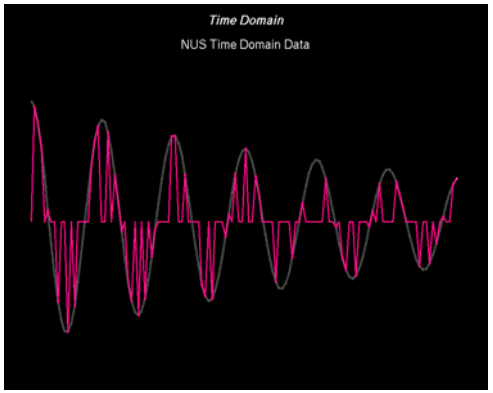


Figures from Frank Delalgio

Non Uniform Sampling



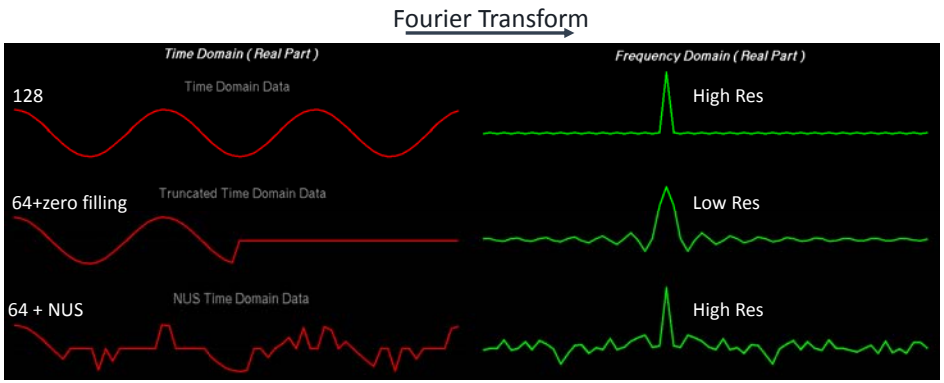
To "reconstruct" this signal



Set missing points to zero

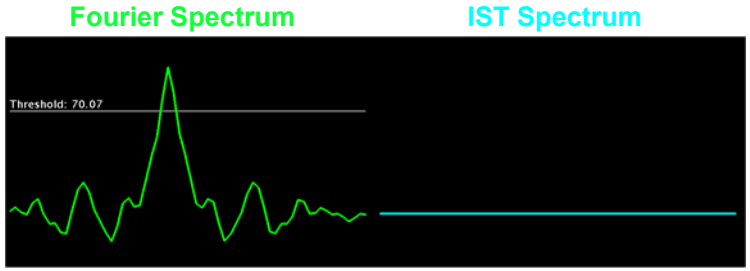
Figures from Frank Delalgio

Processing NUS Data



Figures from Frank Delalgio

Iterative Soft Thresholding

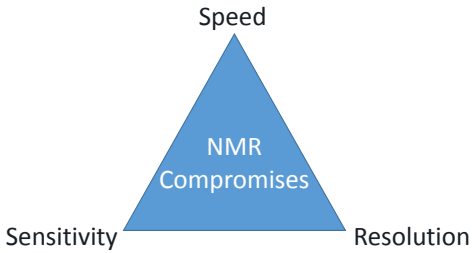


Noise in the FT NUS data is proportional to the signal
Iteratively processing the data allows for a high quality reconstruction
IST is just one of many NUS algorithms

Figures from Frank Delalgio

Why do NUS?

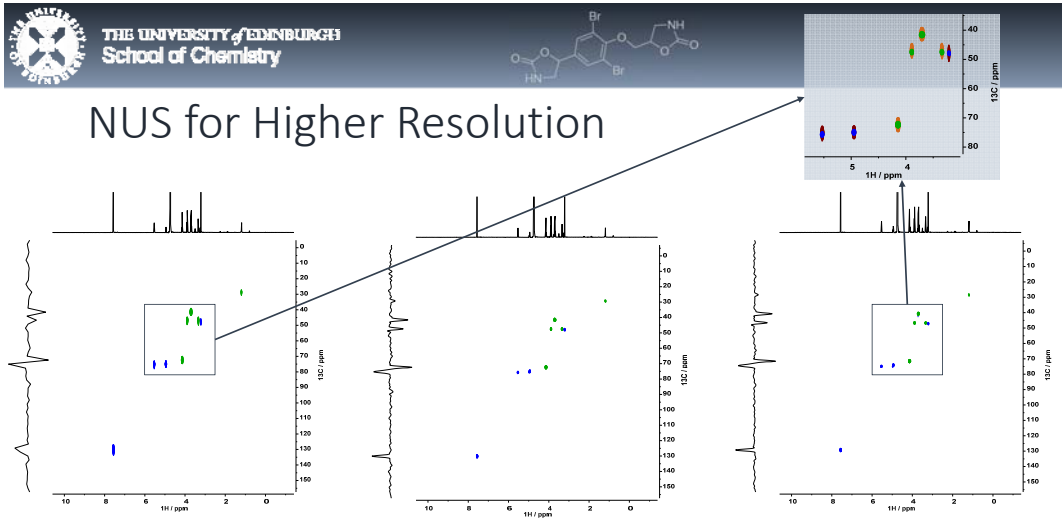
- Increase **resolution** with no time cost (Time Domain)
  or
- Increase **sensitivity** with no time cost (Number of Scans)
  or
- Acquire spectra **faster** with no resolution or sensitivity penalty



For example, if you have 60 minutes of instrument time:

- TD1 = 256, NS = 4, Uniformly Sampled – Low Resolution, Normal Sensitivity — Res. = 10 Hz, S/N = 1
- TD1 = 256 (1024), NS = 4, NUS (25%) – High Resolution, Normal Sensitivity — Res. = 2.5 Hz, S/N = 1
- TD1 = 64(256), NS = 16, NUS (25%) – Low Resolution, High Sensitivity — Res. = 10 Hz, S/N = 2

NUS for Higher Resolution

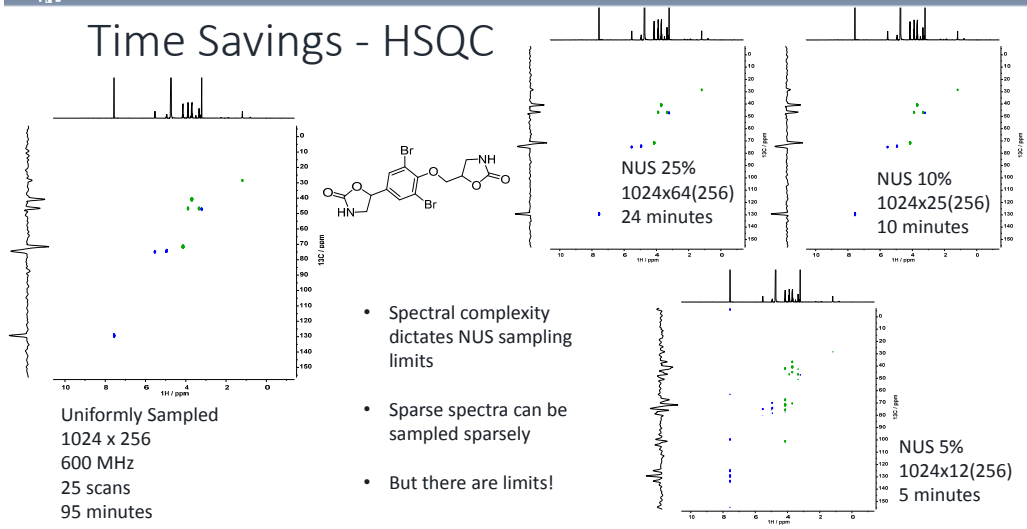


Uniformly Sampled
TD1 = 64, NS = 25
Time = **24 minutes**

Uniformly Sampled
TD1 = 256, NS = 25
Time = **95 minutes**

NUS 25%
TD1 = 64 (256), NS = 25
Time = **24 minutes**

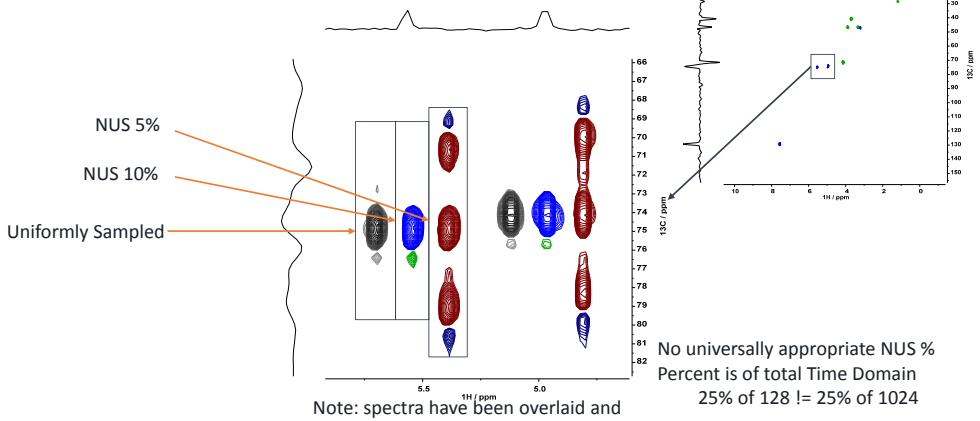
Time Savings - HSQC



Uniformly Sampled
1024 x 256
600 MHz
25 scans
95 minutes

- Spectral complexity dictates NUS sampling limits
- Sparse spectra can be sampled sparsely
- But there are limits!

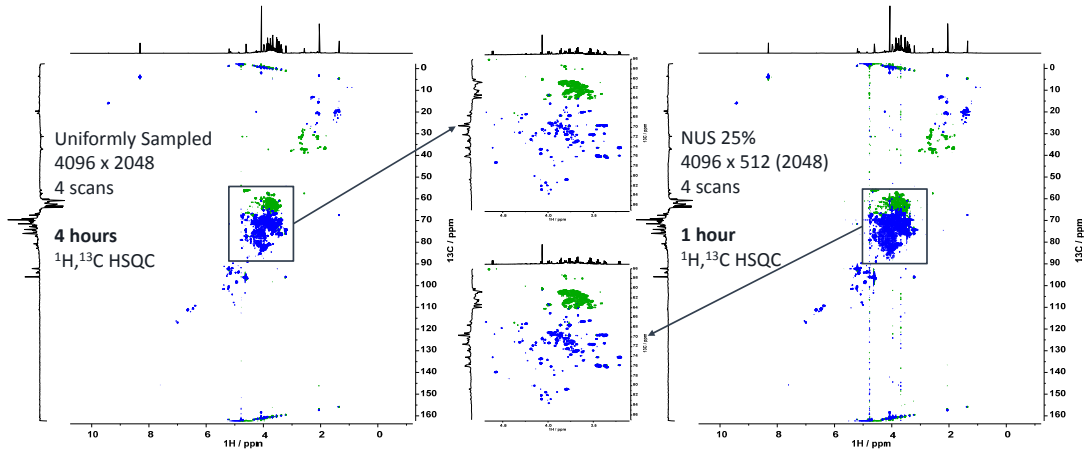
NUS Sampling Limits



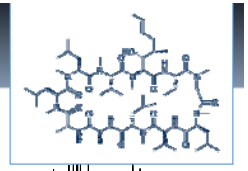
Note: spectra have been overlaid and shifted on x-axis for visualisation

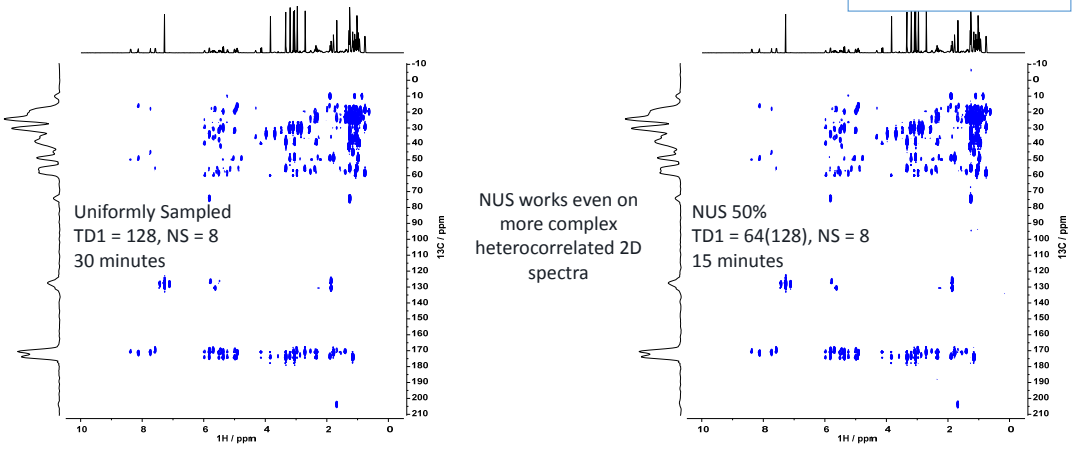
No universally appropriate NUS %
Percent is of total Time Domain
25% of 128 != 25% of 1024

Real World – Very Complex Mixture HSQC

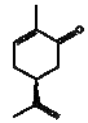


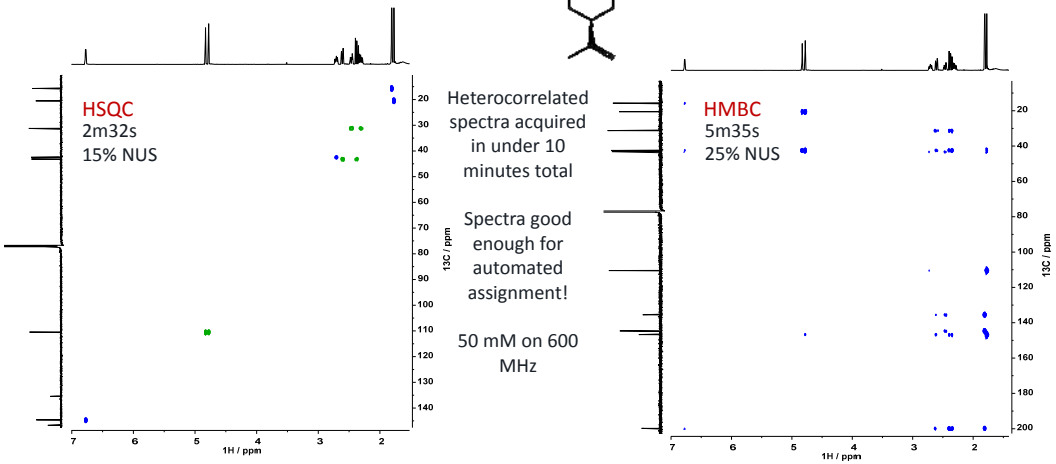
NUS for HMBC - Cyclosporine





Another Fast Example





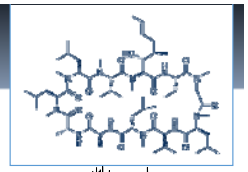
Heterocorrelated spectra acquired in under 10 minutes total

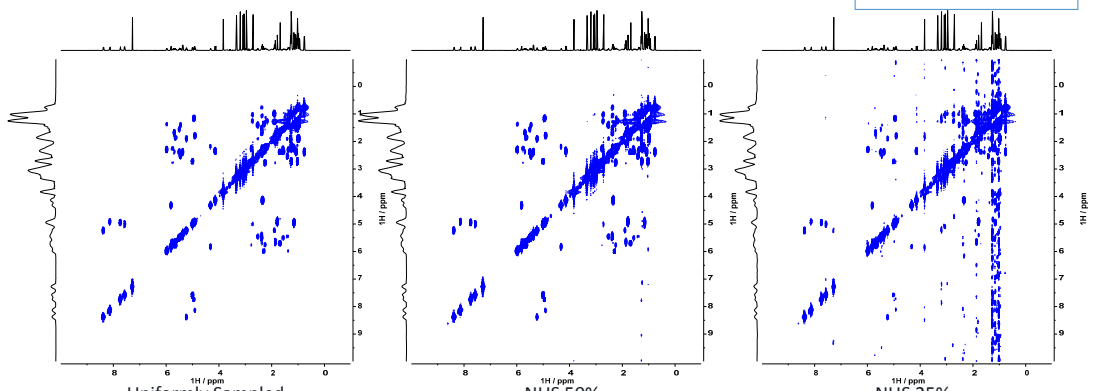
Spectra good enough for automated assignment!

50 mM on 600 MHz

Less common to perform NUS on homocorrelated spectra (COSY, NOESY, TOCSY)

Real World – COSY of Cyclosporine





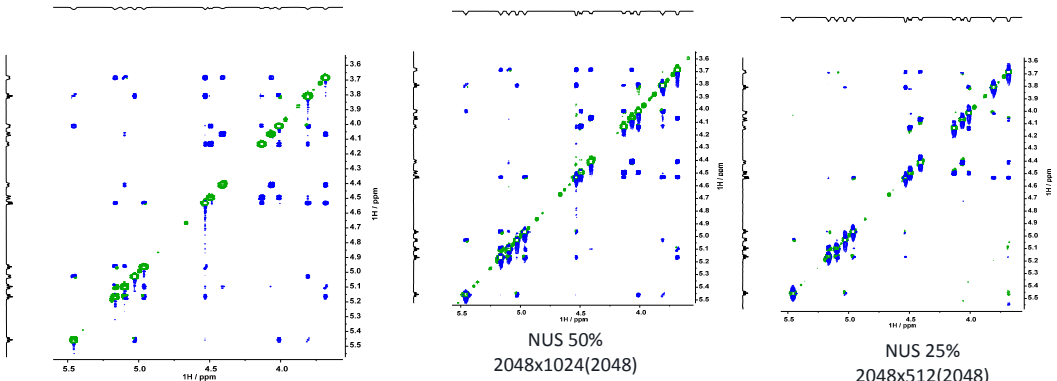
Uniformly Sampled
TD1 = 128
20 minutes

NUS 50%
TD1 = 64(128)
10 minutes

NUS 25%
TD1 = 32(128)
5 minutes

Less common to perform NUS on homocorrelated spectra (COSY, NOESY, TOCSY)

NUS – NOESY of a disaccharide

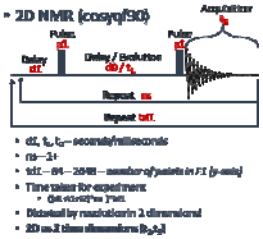


Uniformly Sampled
2048x2048
6.5 hours

NUS 50%
2048x1024(2048)
3.25 hours

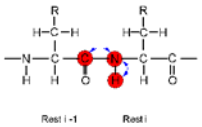
NUS 25%
2048x512(2048)
1.75 hours

High Dimensionality?

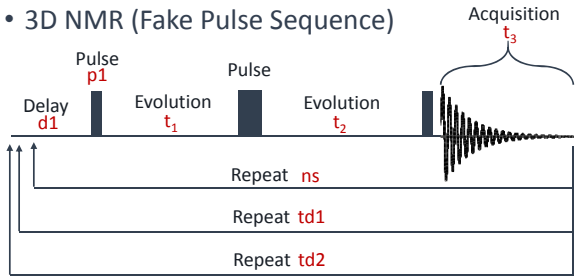


Example Triple Resonance 3D NMR Experiment

HNCO



- 3D NMR (Fake Pulse Sequence)



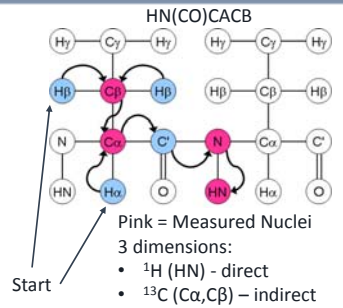
For each loop, increment one of td1 or td2

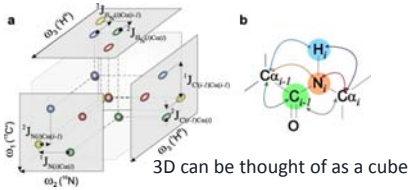
3D experiments have another indirect dimension which must be sampled
Experiments take much longer!
4D… up to 8D NMR reported!

NUS for *n*D experiments

- 3D HN(CO)CACB
  - 2048 x 40 x 128
    - 5120 indirect dimension points
    - 16 scans per increment
  - 1 day
- 4D HNCOCA
  - 2048 x 32 x 32 x 64
    - 65536 indirect dimension points
    - 16 scans per increment
  - 16 days

Uniformly Sampled these experiments are extremely long
Do they need to be?
How quickly can we do them?

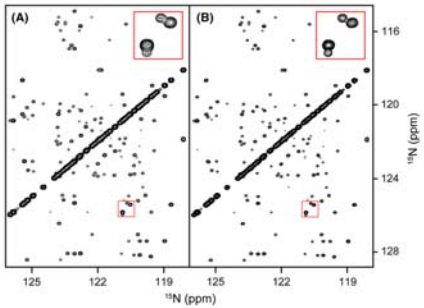




NUS on 3D NMR of α-synuclein

2D Plane from the 3D Cube

Uniformly Sampled



NUS 2.6%
(Effectively 1.15% with zero filling)

38x faster to acquire

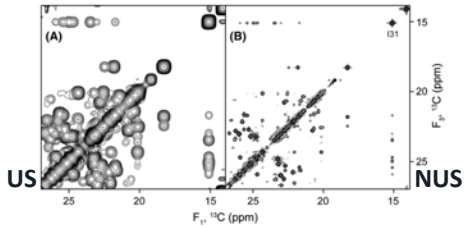
Uniformly Sampled = 5 days
NUS = 3 hours

600 MHz
3D (H)N(COCO)NH
928 x 928 x 464
2D ^{15}N,^{15}N plane

J Biomol NMR (2017) 68:101–118
DOI 10.1007/s10858-016-0072-7

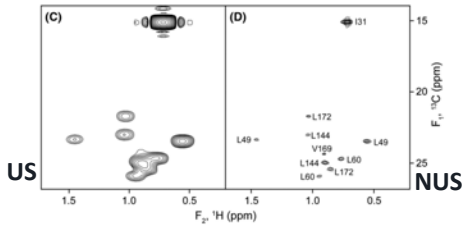
NUS on 4D NMR - Protein

HMQC-NOESY-HMQC
600MHz



NUS 1.56 %
(Effectively 0.46%)

Uniformly Sampled
(Caveat not same TD as NUS data)



50% zero filling (each indirect D) to:
646 x 512 x 512 x 512
134,217,728 indirect dimension points

If fully sampled (no zero filling or NUS), 1 second per FID, would take > 4 years!

Practical Aspects of NUS

- Try to avoid thinking in % sampling only
  - 25% of 128 is very different to 25% of 2048
  - Likewise 2.6% of 107,648 points is still a lot of points!
- Complex data requires more sampled points
  - Still can use NUS to boost sensitivity or resolution, if not cut time directly
- > 2D NMR data tends to be sparse
  - NUS comes into its own for 3D,4D+ experiments

Practical Aspects of NUS

- NUS fully supported on Bruker instruments
  - Easy to change parameter to NUS, even in automation
- NUS processing available in:
  - TopSpin (even free TopSpin)
  - MestreNova
  - NMRPipe
  - + more
- In Edinburgh:
  - "Speedy" and "NUS" in the experiment name
  - "Highres NUS" gives increased resolution relative to non-NUS in same time
- In general, all 2D experiments of small, single, 'pure' molecules will benefit from NUS with no significant downside

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