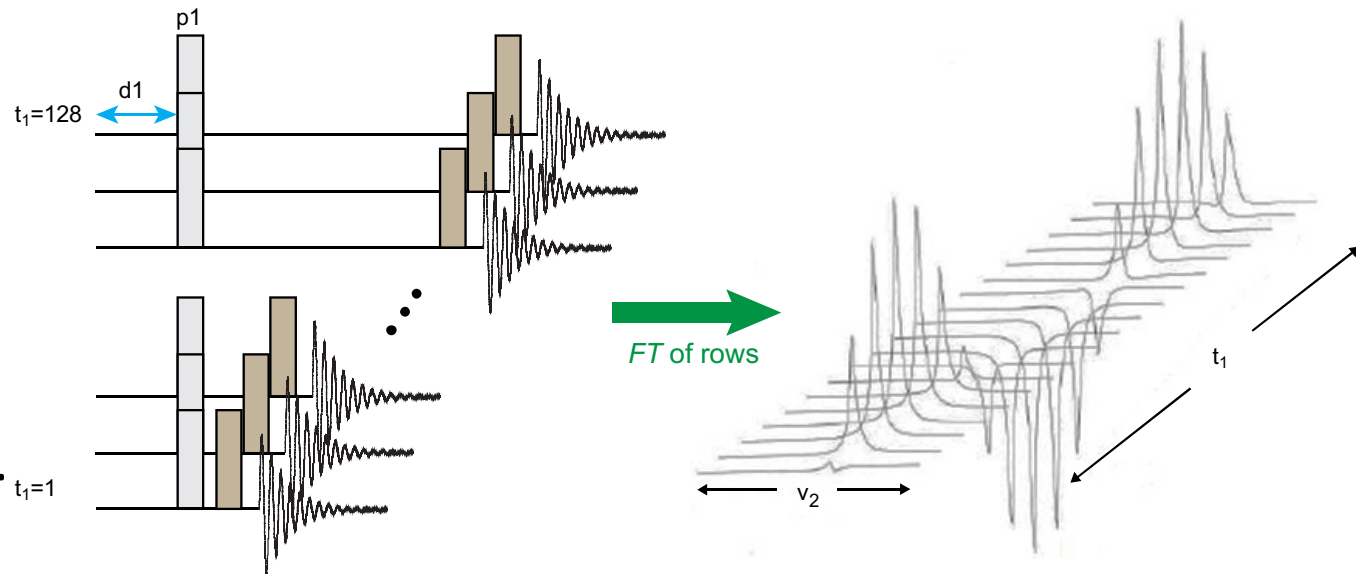


Quick Acquisition of 2D Spectra using Non-Uniform Sampling (NUS)



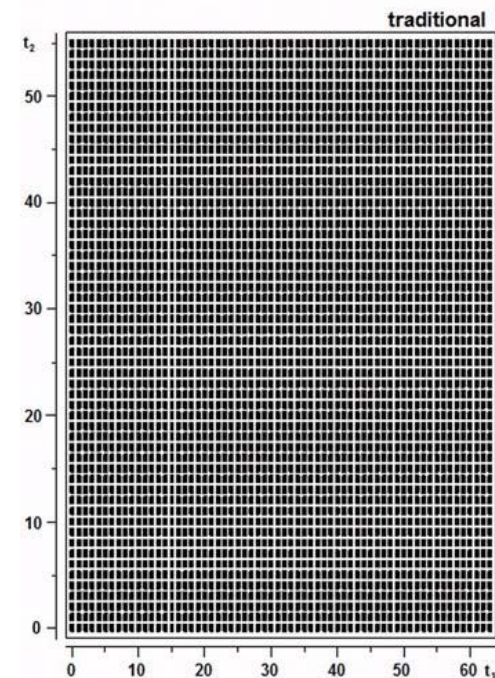
Traditional Linear Sampling

- Generally, multi-dimensional NMR data is acquired linearly in uniform time increments and is then processed using a FFT algorithm.

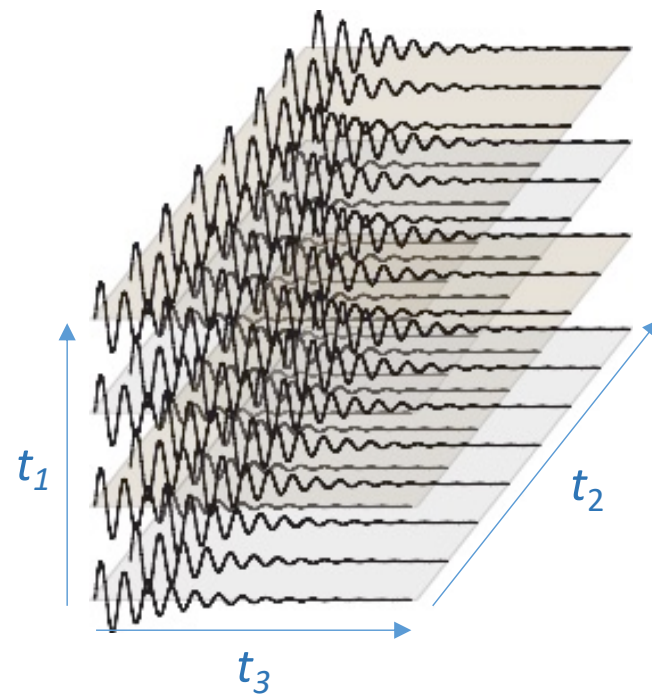
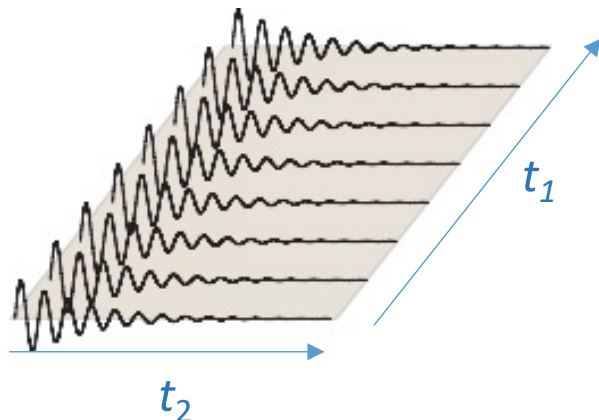
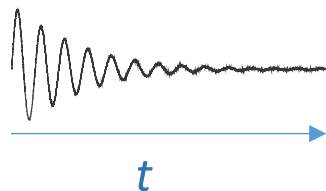


- The data points acquired in the indirect dimension(s) form a grid where the distance between the points on the grid is given by the sweep width and the number of points by the TD for each dimension respectively.

3D ($t_1 t_2$ plane)



Multi-dimensions and Experiment Time



1D: 1 FID

Expt = 1 scan x 4 sec.

2D: 256 FIDs

Expt = 1 scan x 4 sec. x 256
= 17 min.

3D: 256 x 256 FIDs

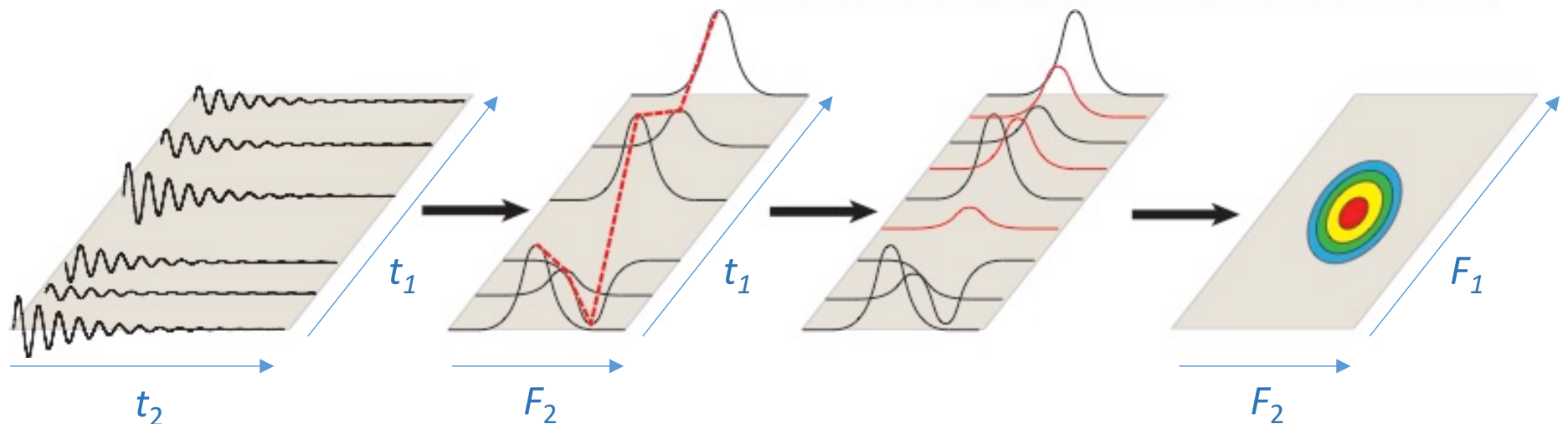
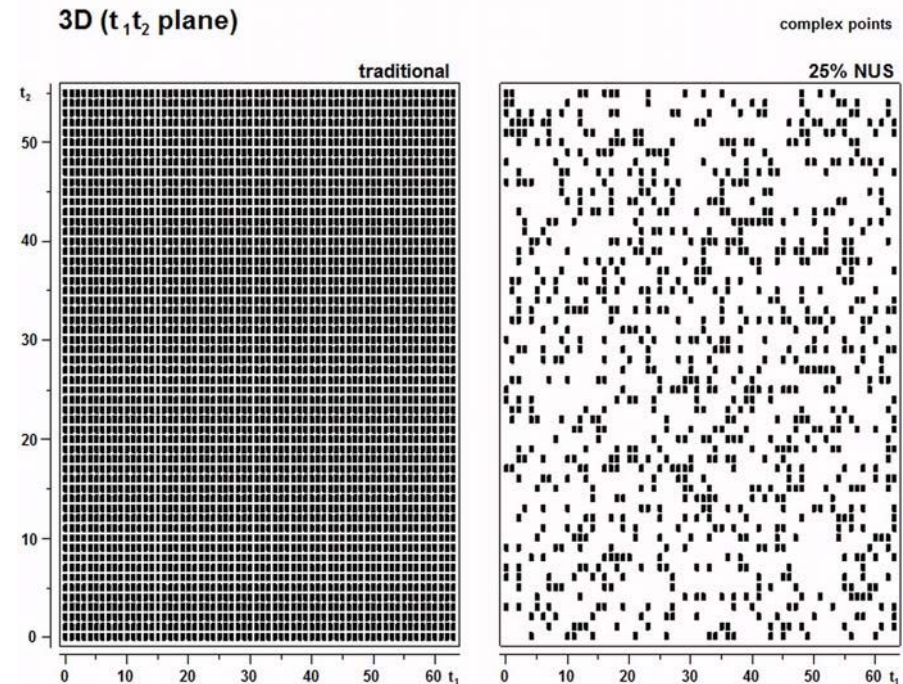
Expt = 1 scan x 4 sec. x 256 x 256
= 73 hours

- Experiment time depends on the number of samples in the indirect dimension and the number of scans per FID.
 - Increasing the number of experiments will improve resolution

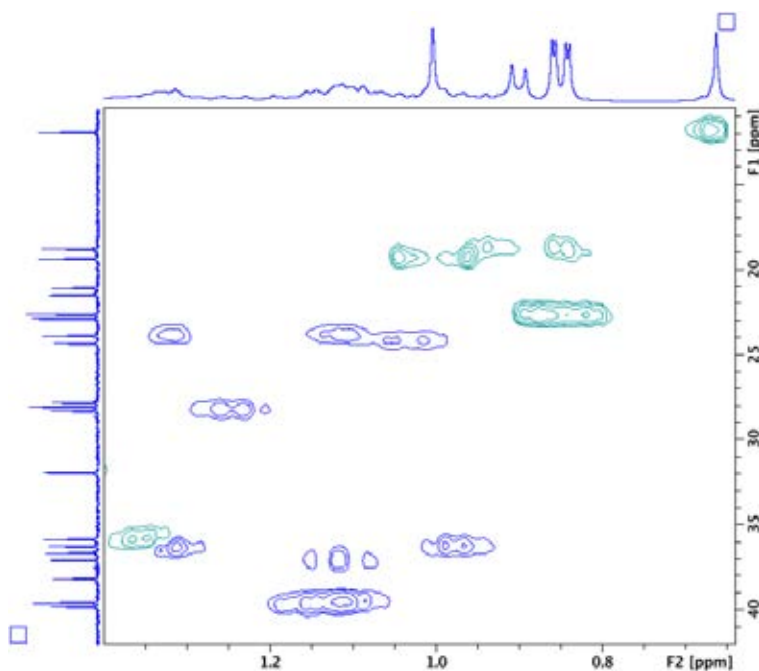


Basic Concept of Non-Uniform Sampling

- The key principle of NUS is to acquire only a subset of data points in a random manner while still using the same grid.
- As seen in the figure to the right, only 25% of the data points are collected.
- With the reduced acquisition time in the direct dimension, the overall experimental time will be much lower.



Benefits of NUS



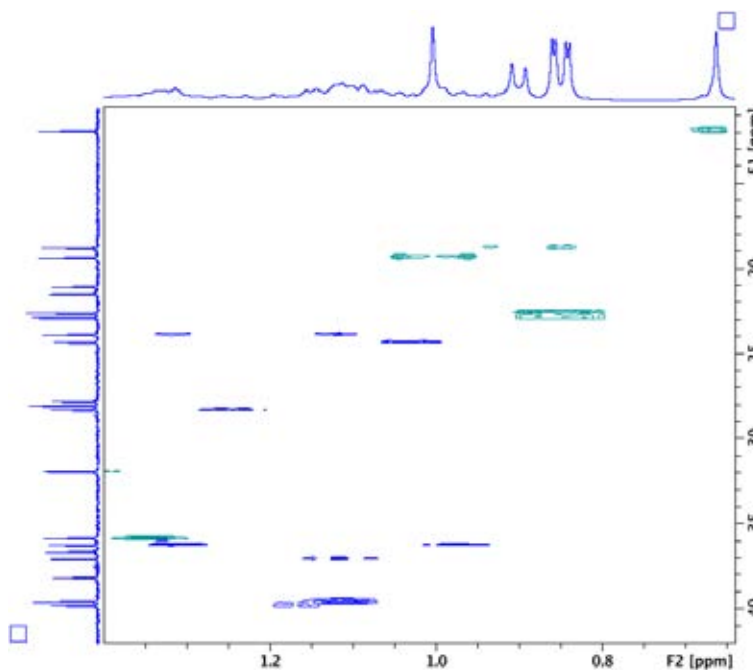
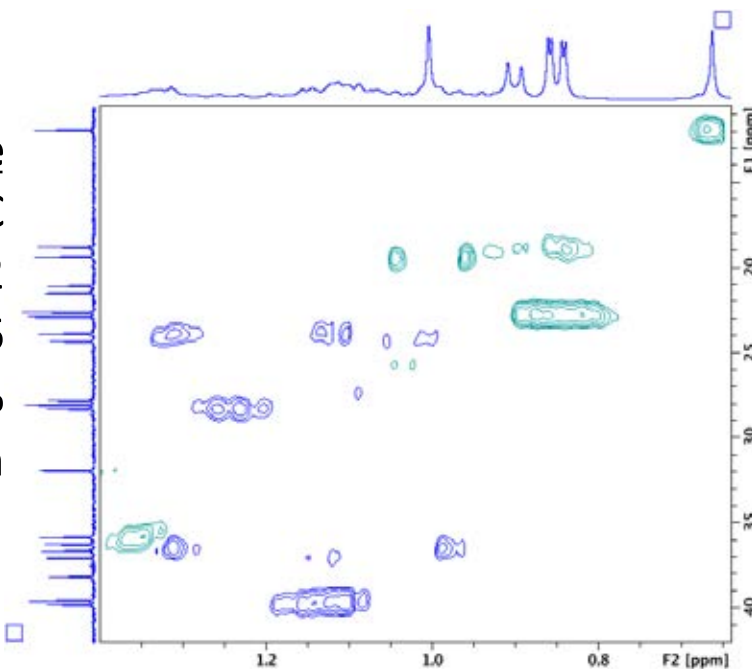
Standard HSQC

ns = 2

TD = 256

Expt = 20 min

Less Time
NUS HSQC
ns = 2
TD = 256
NUS @ 10%
Expt = 2 min



Higher Resolution

NUS HSQC

ns = 2

TD = 2048

NUS @ 10%

Expt = 16 min



NUS Setup

- Bruker has made NUS acquisition standard in TOPSPIN 3.0. **This is only available on the UTL400 spectrometer.**
- **Steps:**
 1. Read in the desired 2D sequence that you would like to acquire.
 2. Under the *AcquPars* tab in the *Experiment* section, set **FnTYPE** to “non-uniform_sampling”

The screenshot shows the Bruker TOPSPIN 3.0 software interface, specifically the **AcquPars** tab. The window title is "1 test 1 1 C:\Bruker\TopSpin3.0\examdata". The **Probe** is set to "5 mm QNP 1H/15N/13C/31P". The **PULPROG** is "b_hncogp3d". The **AQ_mod** is "DQD". The **FnMODE** is "Echo Anticcho" and the **States TPPI** is "States TPPI". The **FnTYPE** is set to "non-uniform_sampling", which is circled in red. The **TD** is "2048", **NS** is "8", **DS** is "32", and **TD0** is "1". The **Width** section shows a table of parameters: SW [ppm], SWH [Hz], IN_F [μsec], AQ [sec], FIDRES [Hz], and FW [Hz]. The **Receiver** section is also visible.

Parameter	Value
PULPROG	b_hncogp3d
AQ_mod	DQD
FnMODE	Echo Anticcho
States TPPI	States TPPI
FnTYPE	non-uniform_sampling
TD	2048
NS	8
DS	32
TD0	1
SW [ppm]	14.0019
SWH [Hz]	7002.801
IN_F [μsec]	0.0731850
AQ [sec]	0.0163808
FIDRES [Hz]	13.664002
FW [Hz]	125000.000



NUS Setup

3. On the left list, click on **NUS** to get to the NUS parameter section

1 test 1 1 C:\Bruker\TopSpin3.0\examdata

Spectrum ProcPars **AcquPars** Title PulseProg Peaks Integrals Sample Structure Plot Fid

Probe: 5 mm QNP 1H/15N/13C/31P

Experiment
Width
Receiver
Nucleus
Durations
Power
Program
Probe
Lists
NUS
Wobble
Lock
Automation
Miscellaneous
User
Routing

VTLIST E Variable temperature list
NUSLIST automatic Name of loopcounter list for NUS (Non Uniform Sampling)

^ NUS (Non Uniform Sampling) parameters

NusAMOUNT [%]	25			Amount of sparse sampling
NusPOINTS	512			Number of hypercomplex points in indirect dimension
NusJSP [Hz]	0	0	0	J-coupling
NusT2 [sec]	1	1	1	T2 relaxation
NusSEED	54321			Random generator seed
<input type="button" value="Calculate"/>				Calculate point spread function
^ Wobble				
WBSW [MHz]	10.0000000			Wobble sweep width
WBST	1024			Number of wobble steps
^ Lock				
LOCNUC	2H			Lock nucleus
SOLVENT				Sample solvent



NUS Setup

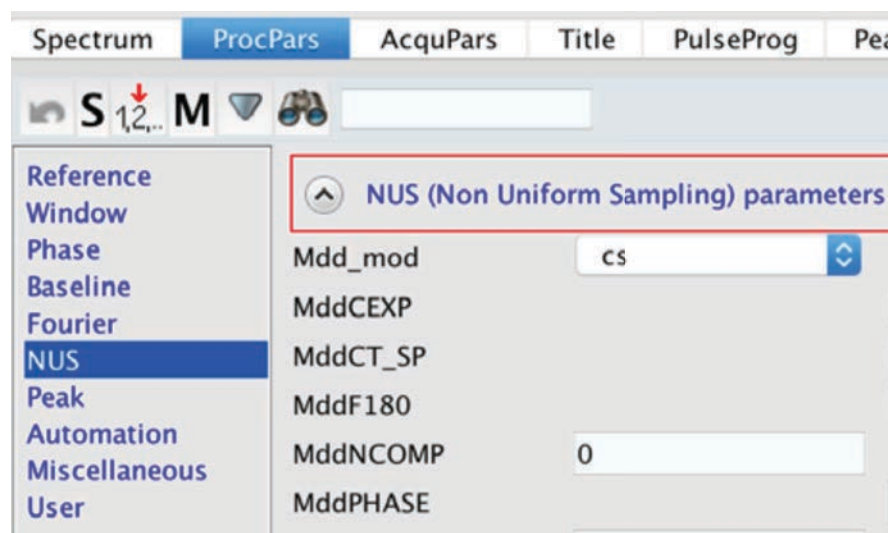
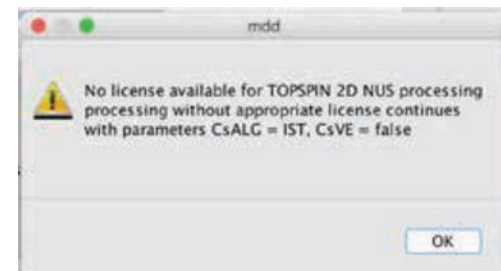
- Acquisition parameters:
 - **NusAMOUNT[%]** - percentage amount of sparse sampling, default is 25
 - **NusPOINTS** - number of complex data points to be recorded, for nD experiment it is $[(td1*td2 \dots *tdn) *amount/100] / 2(n-1)$
 - Note: As a rule of thumb the number of hypercomplex points should be at least the same as the number of frequencies (signals in the spectrum).
 - **Jsp [Hz]** - J coupling, default is 0. In the case of J evolution in an indirect dimension the points acquired can be matched to the maxima of such a FID by setting this coupling constant.
 - **T2 [s]** - T2 relaxation time, default is 1. For indirect dimensions with so called real time evolution the FID in the indirect dimension will decay according to the T2 relaxation time of the spins evolving in this dimension. By setting the T2 parameter according to the relaxation time, parts of the FID with more intensity will be strengthened (exponential weighting of sampling scheme)
 - Note: If an evolution period is implemented as constant time in the pulse program, exponential weighting must not be used!
 - **seed** - random number generator seed, responsible for the different distribution of data points, default is 54321
 - **Calculate** - allows to calculate and then view the distribution of points without starting the experiment.



NUS Processing

- 2D NUS processing is available on Topspin 3.5pl6 or higher
- Usually no need to change the NUS processing parameters
- Use the typical command “**xfb**” to process the 2D data
 - If you get an mdd error stating no license is available, some parameters will need to be adjusted:

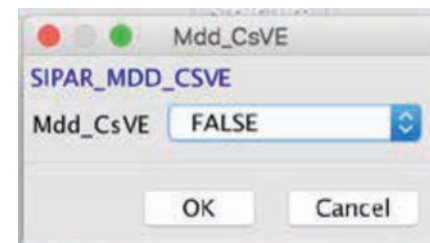
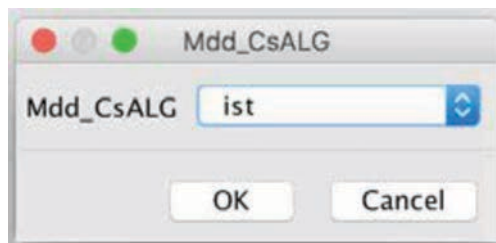
1. Make sure **mdd_mod = cs** in the procpars list



2. Set the “hidden parameters” from Topspin command line:

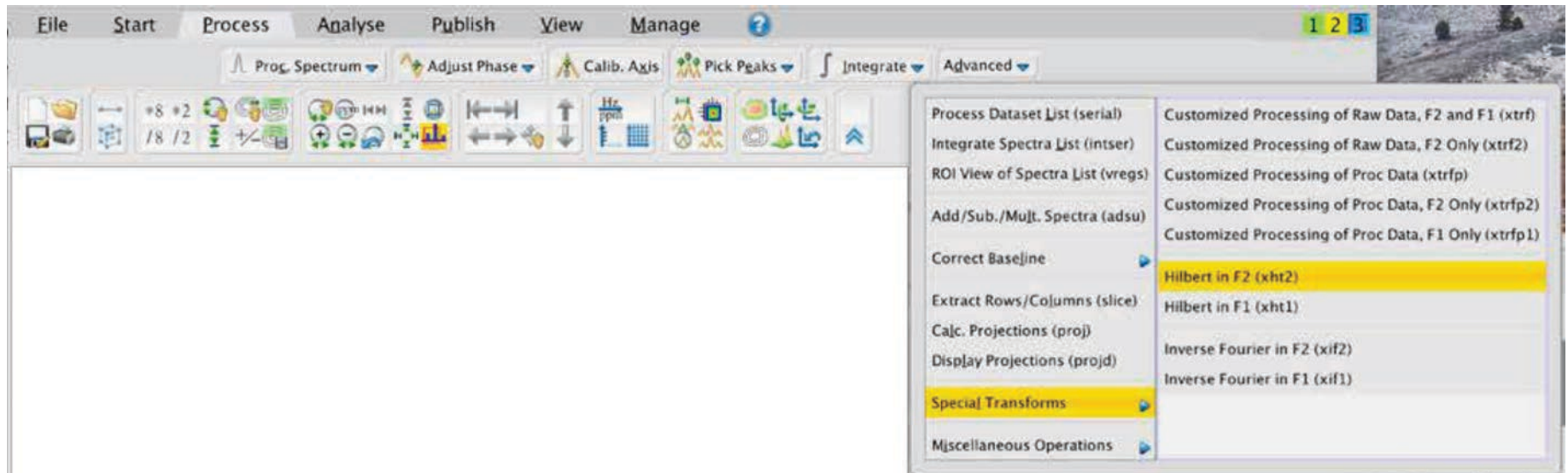
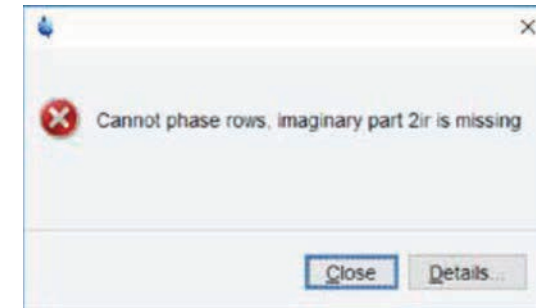
> **mdd_csalg = ist**

> **mdd_csve = false**



NUS Processing

- Now that it's Fourier transformed the data can be processed.
- An error might arise when trying to phase the spectrum
- Imaginary data isn't kept after NUS reconstruction. The imaginary component could be re-created with a Hilbert transform using the menu options...



- ... or type **xht2** in the command line.
- Phasing should now work properly.
- Recommendation: re-process spectrum (**xfb**) again after phasing

