Standard Operating Procedure

Task: Non-Uniform Sampling Date: 09/02/18

Background:

 Non-Uniform Sampling (NUS) allows spectra to be acquired using only a percentage of points in standard acquisition. Multi-dimensional experiments can be acquired much faster with NUS than normal acquisition methods because only a fraction of the points are collected. NUS takes advantage of mathematical fitting algorithms to fit a FID from the acquired points, and even extrapolate the FID to points beyond what was acquired to improve resolution.

Training Requirements:

- Lab safety training and any procedure-specific training for the desired sample preparation.
- NMR spectrometer training

Potential Hazards:

- Many NMR solvents are hazardous themselves and can carry dissolved materials through the gloves into the skin. Be aware of hazards associated both solvents and dissolved materials.
- Magnetic Field Warning! Older magnets have large stray fields; avoid bringing magnetic materials into the fields. Avoid strong fields if you have metal implants and a pacemaker.

Materials Needed:

- NMR tube
- Deuterated solvent
- NMR spectrometer

Procedure:

Experiment Setup

- 1. Lock, shim, and tune on your sample as normal.
- **2.** Setup a 2D NMR experiment. COSY, HSQC, HMQC, HMBC and other methods are compatible.
- **3.** Traditional planes is selected by default when a 2D experiment is setup. Select Non-Uniform Sampling from the dropdown menu.

			grais Sample Structure							
崎 🛄 S 🧧 🗄	₹ <u>1,2,</u> ▼ C 🚜	Prob	e: bbo							
Experiment		F2	F1	Frequency axis						
Width Receiver	S Experiment									
Nucleus	PULPROG	hsqcetgpsi2	E	Current pulse program						
Durations Power	AQ_mod	DQD		Acquisition mode						
Program	FnTYPE	non-uniform_sampling) –	nD acquisition mode for 3D etc.						
Probe	FnMODE		Echo-Antiecho 🔻	Acquisition mode for 2D, 3D etc.						
Lists	TD	1024	256	Size of fid						
NUS	DS	16		Number of dummy scans						
Wobble Lock	NS	2		Number of scans						
Automation	TD0	1		Loop count for 'td0'						
Miscellaneous	TDav	0		Average loop counter for nD experiments						
User Routing	🐼 Width									
3	SW [ppm]	12.9836	165.0000	Spectral width						
	SWH [Hz]	6493.506	20751.592	Spectral width						
	IN_F [µsec]		48.19	Increment for delay						
	AQ [sec]	0.0788480	0.0061682	Acquisition time						
	FIDRES [Hz]	12.682630	162.121811	Fid resolution						
	FW [Hz]	4032000.000		Filter width						

- **4.** In the menu on the left side, select NUS. This will bring you to NUS options:
 - Sampling Percentage: Determines how many points will be sampled. Usually this is a percentage from 25% to 50%.
 - Sampling Method: Proper selection of sampling methods can have tremendous impact on the acquired spectrum. Sampling methods should be sparse (<50%), weighted towards early time points, and cover the entire array of T₂ values.

NUS (Non Uniform Sampling) parameters													
	NUS Help		Show NUS help										
NusAMOUNT [%]	30		Amount of sparse sampling										
NusPOINTS	38		Number of hypercomplex points in indirect dimension										
NusJSP [Hz]		0	J-coupling										
NusT2 [sec]		1	T2 relaxation										
NusSEED	54321		Random generator seed										
NUSLIST	automatic		Name of loopcounter list for NUS (Non Uniform Sampling)										
	Calculate		Calculate point spread function										
	Show]	Display NUS point spread										
	NusAMOUNT [%] NusPOINTS NusJSP [Hz] NusT2 [sec] NusSEED	NUS Help NUS Help 30 NUSPOINTS 38 NUSJSP [Hz] NUSEED 54321 NUSLIST automatic Calculate	NUS Help NUS Help NUS Help 30 NusPOINTS 38 NusJSP [Hz] 0 NuSEED 54321 NUSLIST automatic										

 A sampling schedule is automatically generated by Topspin. The generated sampling schedule is saved as a text file, "automatic", which is then read by the experiment. Custom sampling schedules can be input by saving a text file with a custom schedule in C:\Bruker\Topspin3.5pl3\exp\stan\nmr\lists\vc. The *automatic* text in NUSLIST should be replaced with the name of the text file with the modified sampling schedule. More discussion on choosing a sampling method is provided below.

2 Acquisition finished:	C:/data/nmrsu/linesha			nplina ti	ials/1/	odata/	1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_			1	-
Spectrum ProcPa	rs AcquPars Title	🤹 nu																							X	
ю Л S 🕇 🖾	1,2, 🔻 C 🔗	C:\da	ta\nm	rsu\line	shap	e\nus\	nuss	ampl	ing tr	ials\1	\nus	list													1	
Experiment Width Receiver Nucleus Durations Power Program Probe Lists NUS Wobbie Lock Automation Miscellaneous User	VPLIST VPLIST VTLIST VTLIST NUS (Non Ur NUSAMOUNT [%] NUSPOINTS NUSISP [H2] NUST2 [sec] NUSEED NUSLIST		*			*		6 30 30		1001 3		**			* **		**				•	•	•	: 301	•	
Routing	Wobble WBSW [MHz] WBST O Lock LOCNUC SOLVENT O Automation AUNM PYIM EXP	0	5 :	.0 15	20	25	30	35	40	45	* (t1 done	60 L [poi ac	nts] tive	• tod	0	80	85	90	95	100	105	110	115	120	

- **5.** Adjust your percentage to obtain the desired acquisition time; going below 25% is not recommended for routine acquisition.
- 6. Type **expt** to check the time required. It should be significantly lower than routine acquisition.
- 7. Adjust the TD value in the F2 dimensions. TD values determine the resolution of your 2D spectrum. Larger TD values allow neighboring peaks to be resolved, but will be eventually limited by linewidth in the proton dimension. One of the most powerful ways to use NUS is to acquire far more scans or TD than time usually permits. This can generate very high quality spectra in a relatively short time frame.
- 8. Use getprosol, rga, and zg as normal. Note: In the current version of TOPSPIN at UNC (3.5pl3), NUS spectra CANNOT be processed. External software, like Mestrenova is needed to process spectra acquired with NUS. Also check out NMRbox.org* for free Mestrenova and other software able to process NUS spectra.
- 9. *Nota Bene*: Non-Uniform Sampling is ideal for samples with moderate to strong cross-peaks and similar dynamic ranges. Weak cross peaks can be lost via NUS, making traditional 2D NMR ideal when large concentration differences are present in the sample. Weighting the sampling method can alleviate this drawback.

Picking a Sampling Schedule

- **1.** Sampling schedules are important to minimize artifacts in non-uniform sampling acquisition. Three factors to consider are:
 - a. Sparsity: NUS requires sparsity to minimize artifacts. Typically, 50% of the points acquired is the recommended maximum value for acquisition. Too few points can also impact schedule. 25% is recommended for routine acquisition, though less can be used with care.
 - i. Points can be determined by taking TD/2 in the F1 dimension.
 - b. Randomness: **Sampling schedule must be random**! Non-random sampling schedules can lead to artifacts. Often, completely random schedule is not ideal. Topspin will generate a schedule that is random across input T2 values.

However, often it is best practice to favor early values where intensity is at its greatest. Thus, a weighted schedule is often preferred, an example of which is shown below.

- i. Gerhard Wagner group has an easy to used sample schedule generator: <u>http://gwagner.med.harvard.edu/intranet/hmsIST/gensched_new.html</u>
- c. Coverage: While weighting points early can increase the signal/noise ratio, the schedule should encompass the range of possible T2 values. A default T2 of 1 second works for most small molecules; however, this must be changed when T2 values become extremely long!



Processing

- 1. For this walk-through Mestrenova will be used; other software packages are available.
- 2. Mestrenova can open NUS acquired spectra as easily as normal FID's. Just drag and drop the experiment folder or open the FID through Mestrenova.
- 3. Cross peaks can now be examined and exported in the same manner of traditional 2D spectra.

Related SOPs:

• Absolute Referencing

Further information and Resources:

- Wagner's Sample Scheduler and general information about NUS
- 2012 NUS Tutorial Lecture by Jeffery C. Hoch
- <u>https://www.nmrbox.org/overview</u>