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.

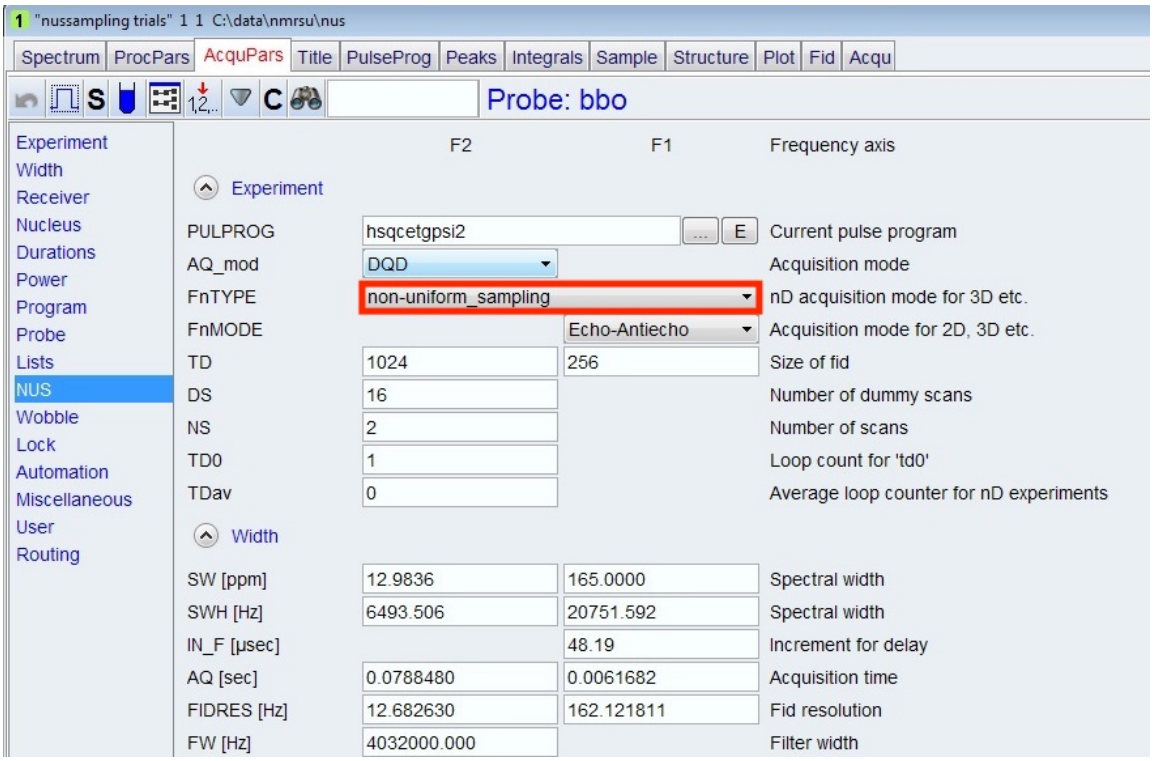
Materials Needed:

- NMR tube
- Deuterated solvent
- NMR spectrometer

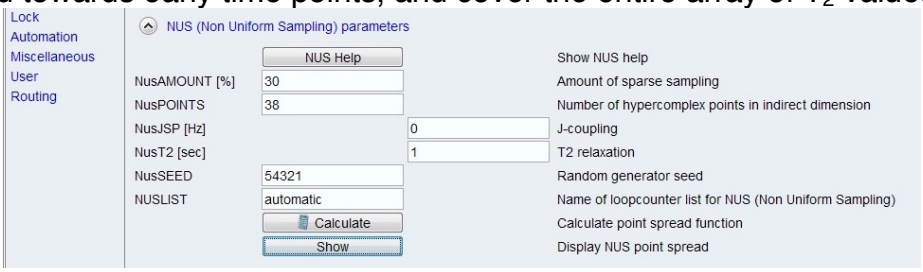
Procedure:

Experiment Setup

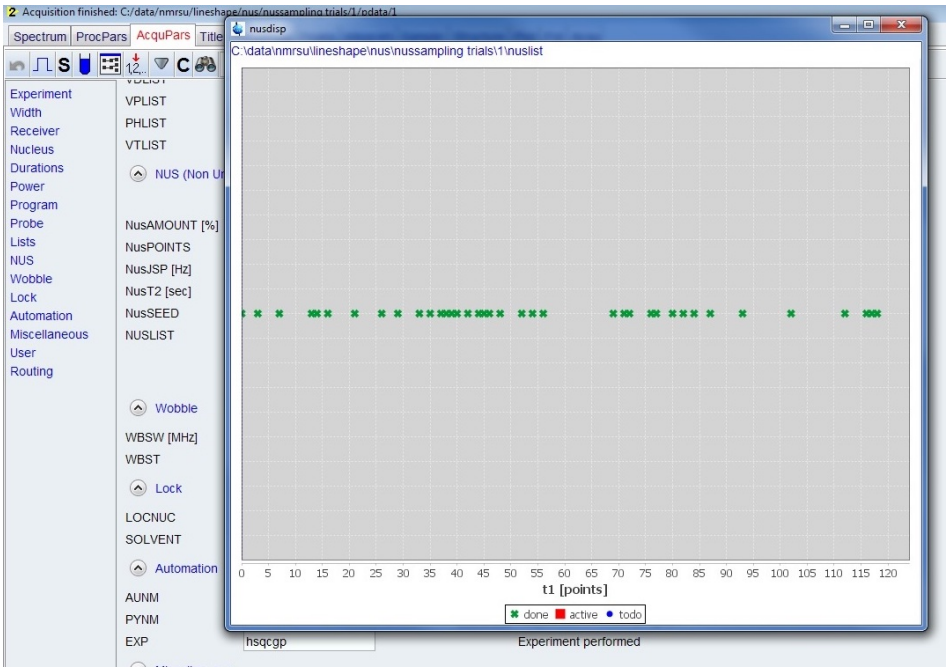
1. Lock, shim, and tune on your sample as normal.
2. Setup a 2D NMR experiment. COSY, HSQC, HMQC, HMBC and other methods are compatible.
3. Traditional planes is selected by default when a 2D experiment is setup. Select Non-Uniform Sampling from the dropdown menu.



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 T_2 values.



   - 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.



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

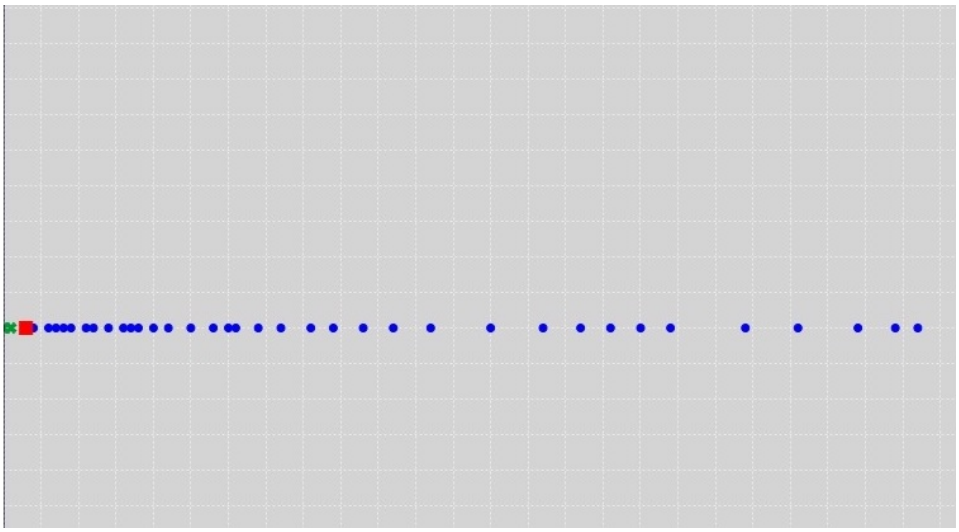
1. Sampling schedules are important to minimize artifacts in non-uniform sampling acquisition. Three factors to consider are:
    a. Sparsity: NUS requires sparsity to minimize artifacts. Typically, 50% of the points acquired is the recommended maximum value for acquisition. Too few points can also impact 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.

*NMRbox.org is an NIH virtual machine web platform with the aim to "*simplify and integrate dissemination, maintenance, support, and application of NMR data processing and analysis software packages".* It contains a free suite of NMR processing and analysis packages, including Mestrenova and other NUS reconstruction programs that the user may find useful.

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: http://gwagner.med.harvard.edu/intranet/hmsIST/gensched_new.html

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**
- **https://www.nmrbox.org/overview**