Non Uniform Sampling in Routine 2D Correlation Experiments

INTRODUCTION

Data obtained from two-dimensional NMR experiments is incredibly useful for structure elucidation of complex molecules, especially when their one-dimensional spectra feature overlapping peaks. However, some experiments require significant amounts of time in order to yield data with adequate resolution or signal to noise for unambiguous interpretation. Any means of reducing the total acquisition time is useful. In this Note, we'll explore a technique known as Non Uniform Sampling (NUS), demonstrate how it can be used to speed up data collection, and highlight how it can be employed in Delta™ on JEOL Spectrometers.

SAMPLING OF INDIRECT DIMENSIONS

In typical 2D correlation experiments the indirect dimension is sampled as a series of 1D measurements where a delay (sometimes called the evolution time) is incremented so that the effects of a desired interaction, such as J-coupling between protons in a COSY or the proton and carbon chemical shifts in an HSQC, can be observed. The example below (Figure 1) is from an HSQC data set sampled with 128 points. The size of the steps is the inverse of the spectral width. In this case, a time step of 0.05 ms corresponds to a sweep width of 20,000 Hz, or approximately 200 ppm at 100 MHz (the frequency of ^{13}C on a 400 MHz system). Similarly, the resolution is determined by the total time elapsed. In this particular case, 6.4 ms of total acquisition time (i.e., the longest time interval) corresponds to a resolution of 157 Hz in the indirect dimension.

As an alternative, we can collect a smaller sample of the 1D spectra in the indirect dimension in order to speed up the experiment. This technique is known as Non Uniform Sampling (NUS). In this scheme, we collect only a portion of the points (in Figure 2 below, 25%) and fill in the missing 1D increments with zeroes.

Total Y acquisition time: 6.4 milliseconds
Time between data points: 0.05 ms
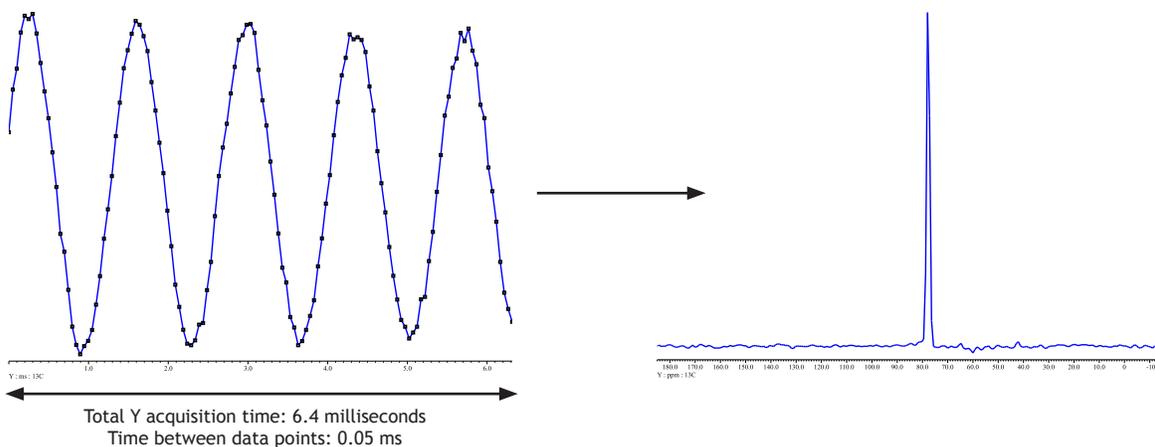
Figure 1. (Left) A time-domain Y slice of an HSQC. (Right) Result of the Fourier Transform. Black squares correspond to the actual data points.
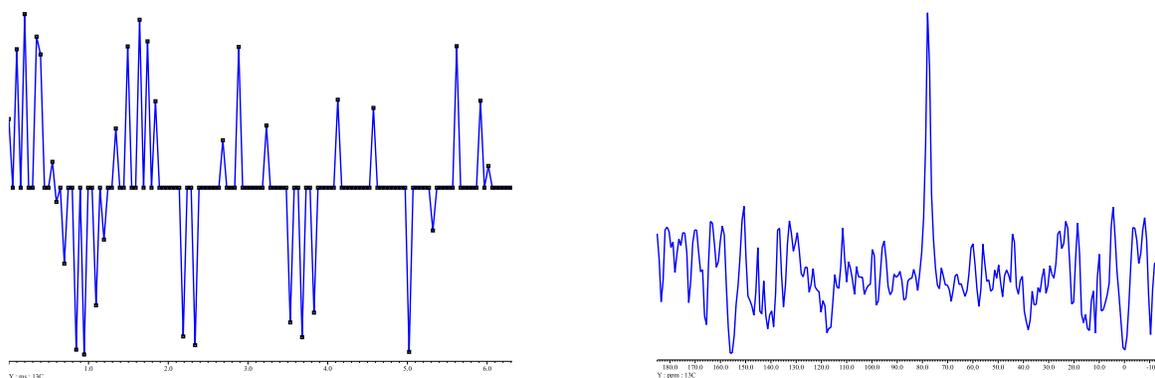
Figure 2. (Left) A time-domain Y slice of an HSQC collected with 25% sampling. Missing increments are replaced with zeroes. (Right) Result of a Fourier Transform of the data.

Because the total acquisition time in the 2nd dimension as well as the time steps between the samples are kept the same, the spectral width and resolution are identical to the uniformly sampled data.

Next, we can use a reconstruction technique known as iterative soft thresholding to reconstruct the "missing" data points and yield a conventional FID (Figure 3). See the additional reading for more information on the details of reconstruction.
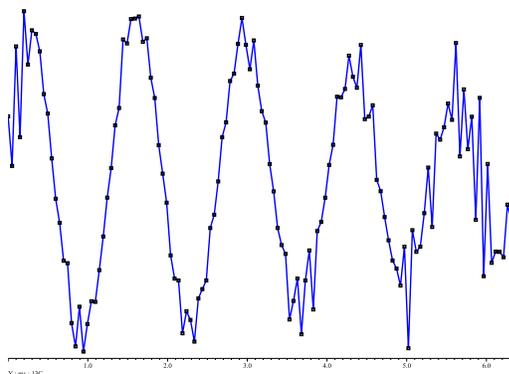
Figure 3. A reconstructed time-domain Y slice of an HSQC collected with 25% sampling.
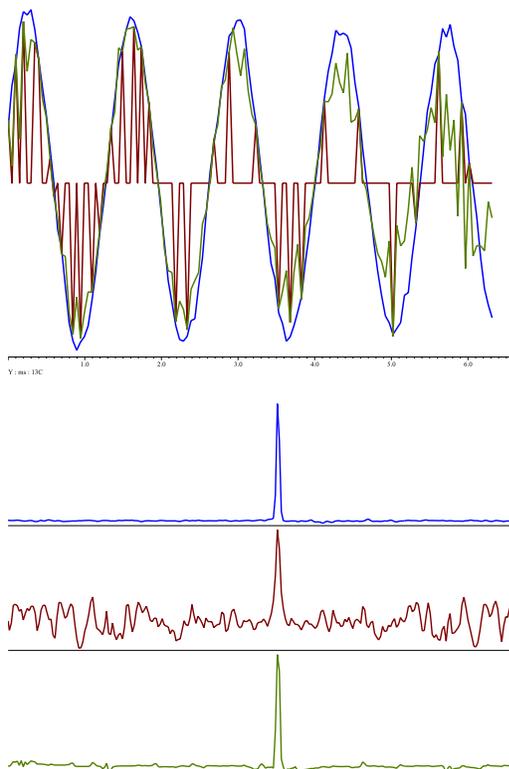
Figure 4. (Top) Overlay of the time-domain Y slice of conventionally sampled data (blue), 25% sampled data with zerofilling of missing points (red) and the reconstruction of the 25% sampled data (green). (Bottom) Result of the Fourier Transform of the data.

COMPARISONS

The following spectra of Lasalocid (acetone-d6) were all obtained on an ECZ400S equipped with a ROYAL probe.
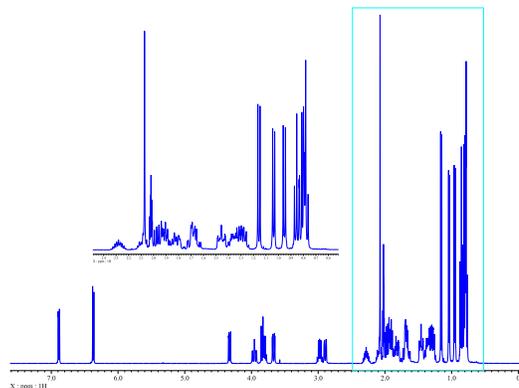
Figure 5. ¹H spectrum of lasalocid at 400 MHz.

Spectrum A in Figure 6 is a traditional, uniformly sampled C2HSQC with 128 Y points, a 200 ppm sweep width, 8 scans, and multiplicity editing. The total runtime of the experiment that produced the spectrum was approximately one hour. Note the congestion of the aliphatic region even in the 2D spectra (Figure 6). Spectrum B was acquired with a 25% sampling rate and all other parameters kept constant. Thus, the total acquisition time was cut down to 15 minutes. Spectra C and D maintained the 25% sampling, but increased the scans (C, to 32) or the Y points (512), in order to keep the total experiment time at one hour like spectrum A. Expansions of the aliphatic regions for these four spectra can be found in Figure 6. Note the increase in Y resolution (128 to 512 Y points) that is necessary to distinguish the overlapped peaks at 0.8 ppm as well as 1.3 ppm.
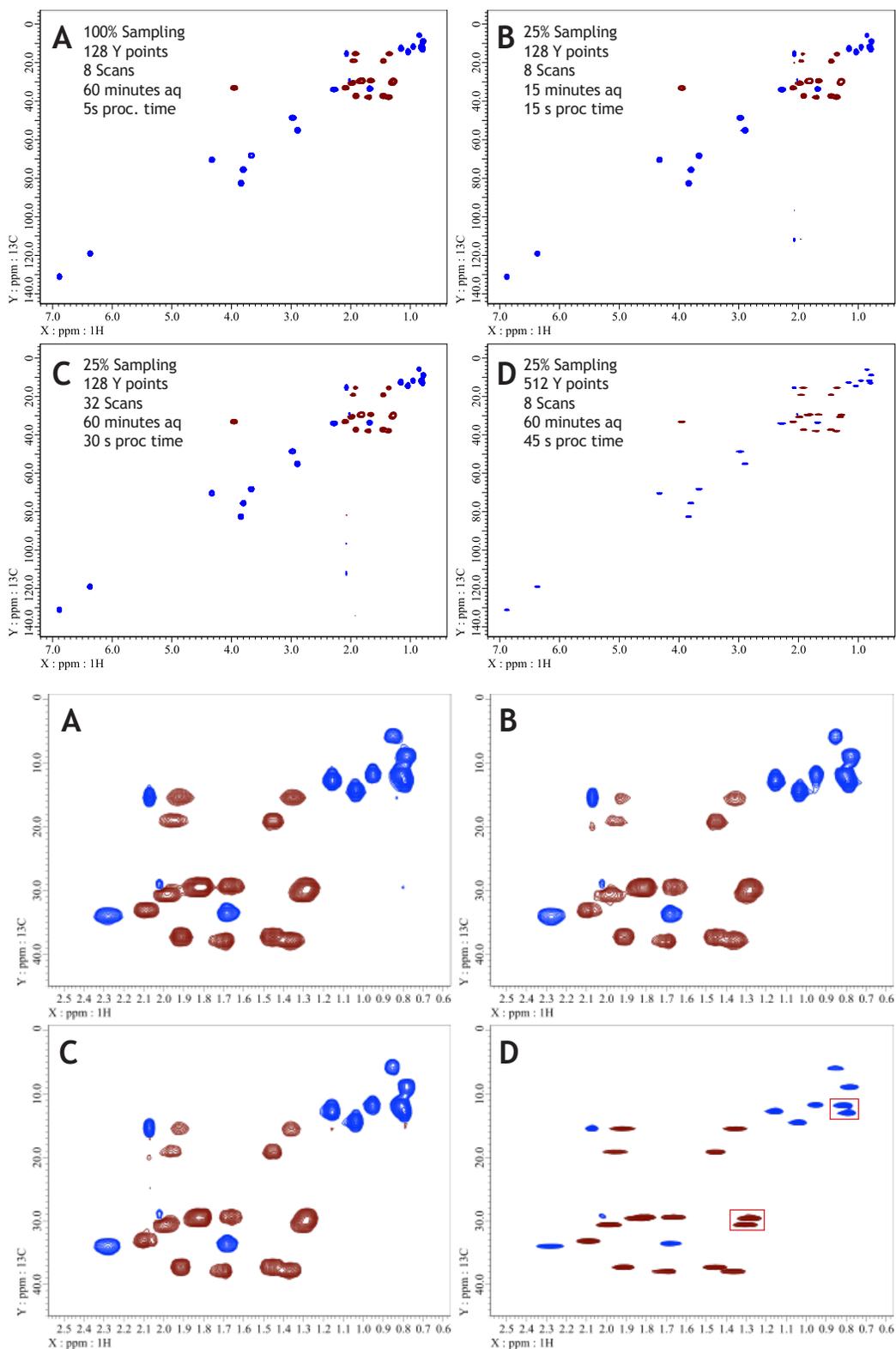
Figure 6. *(Top) C2HSQC spectra of lasalocid at 400 MHz (Bottom) Expansion of the aliphatic region. Note the peaks that are resolved with 512 Y points (Spectrum D)*

Non Uniform Sampling can be employed in other types of 2D experiments as well, like ^{1}H-^{13}C and ^{1}H-^{15}N HMBCs. The ^{1}H-^{15}N HMBCs (or HSQCs) in particular are good candidates for NUS due to their relatively simple 2D spectra in small molecules and low signal to noise, allowing for quick "scout" type experiments to find ^{15}N resonances when only their approximate chemical shifts are known (Figure 7). This is particularly useful when the sample concentration is low or the sample is not ^{15}N enriched, making direct detection of ^{15}N impossible.
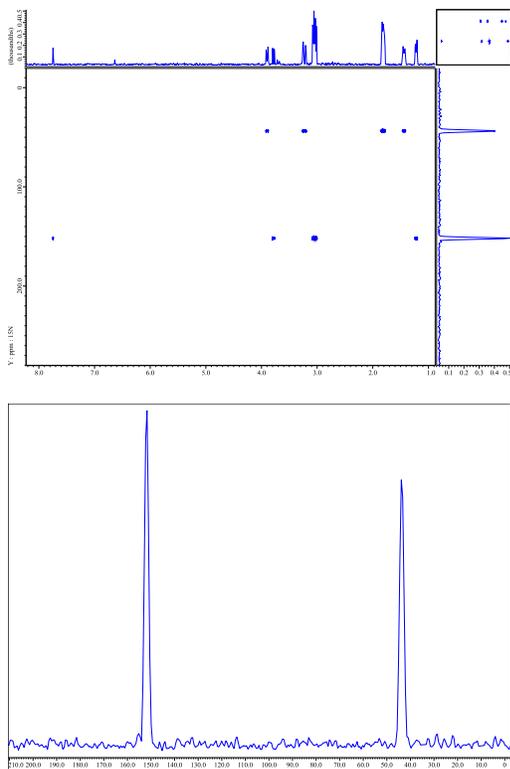
Figure 7. (Top) H-N gHMBCAD of brucine (50 mg, chloroform-d). Sampling: 50%. Total acquisition time approximately 20 minutes. (Bottom) Projection of the Y-dimension as a 1D 15N spectrum.

The ability to acquire multiplicity edited HSQCs (like the CRISIS-HSQCAD) with significantly reduced experiment time can be very valuable. For example, the spectrum of 50 mg of brucine (Figure 8) was acquired with 25% sampling, leading to a total experiment time of just under 5 minutes. In addition to giving ^{1}H-^{13}C connectivities, it also yields carbon multiplicity (methyls and methines phased opposite of methylenes, represented by blue and red peaks, respectively), rendering the acquisition of a slower, less sensitive ^{13}C-detected DEPT unnecessary.
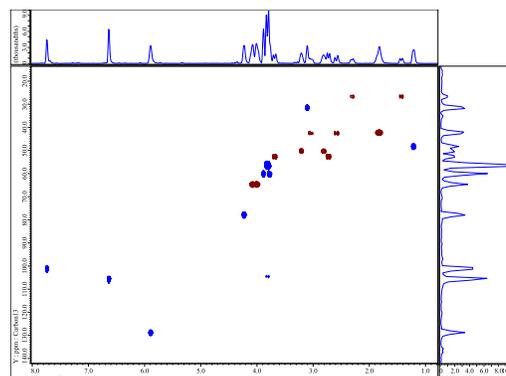
Figure 8. CRISIS-HSQCAD of brucine (50 mg, chloroform-d). Sampling: 25%. Total acquisition time approximately 5 minutes.

However, one should use caution regarding the sampling percentage chosen. For example, a system with multiple frequencies per Y slice (like an HMBC, or very congested HSQCs) can produce spectral artifacts during the reconstruction process when sampling rates are too low. In practice, this means ~25% for HSQCs and ~50% for HMBCs. While also possible, NUS is not currently recommended for homonuclear 2Ds like COSYs where there may be large number of correlations per Y slice, and NOESYs where the diagonal may be significantly more intense than the correlation peaks. As NUS is a field of active research, the reader is encouraged to check the literature for new strategies in experimental design as well as data processing.

NUS IN DELTA

Note: NUS is currently available in Delta v5.1 and onwards. Please contact JEOL Applications for specifics.

Experiment setup:

Option 1: Automation with generic schedules
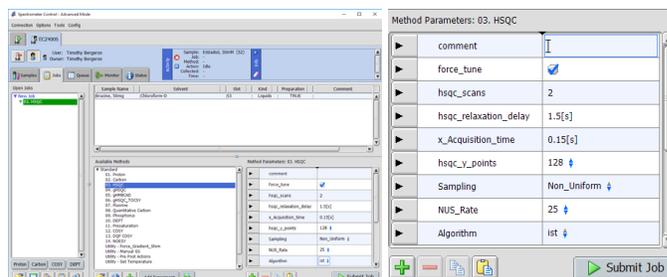
Figure 9. A sample automation method. Note the simple toggles for sampling type, number of Y points, and sampling rate. These can be customized as the user desires.

Schedules can be built ahead of time and used as an include file in an experiment. This allows for quick choices through automation mode, such as the number of Y points, sampling percentage, and even a preferred reconstruction method.

Option 2: Generating an NUS list with sampling tool in experiment mode

Figure 10. The acquisition tab of an HSQC experiment.

Click Add Parameters
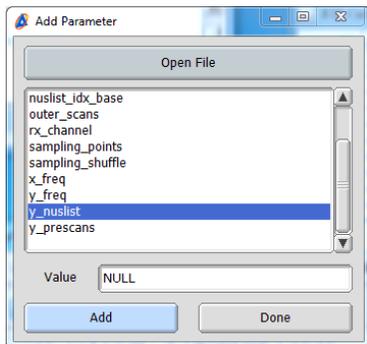
Figure 11. Additional parameter choices.

Choose y_nuslist, click Add

Figure 12. y_nuslist added to the experiment parameter list.

Click on y_nuslist to bring open the Sampling Scheduler. Enter the desired Y Points and Sampling Rate to calculate the number of NUS points, as well as the Sampling Method, then click Schedule to generate a list.
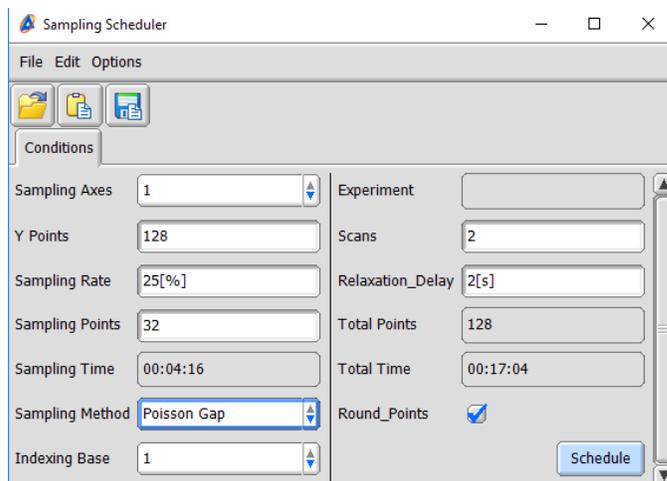
Figure 13. Sampling Scheduler.

After the list is generated, click Apply to update the values for y_nuslist in the experiment.
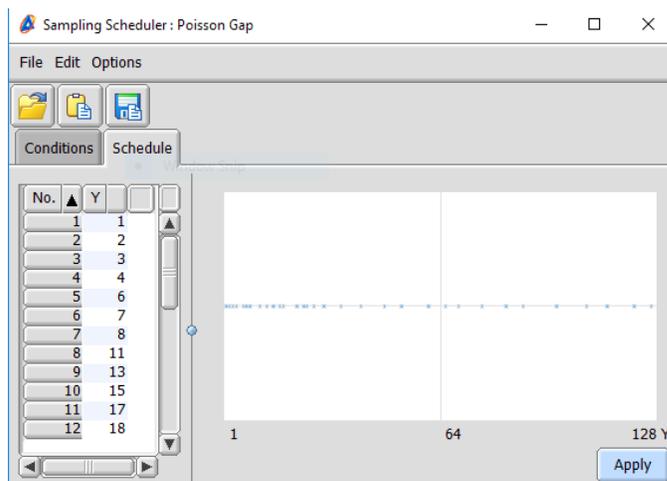
Figure 14. Sampling Scheduler results.

Option 3: Loading schedules from other sources
One can also load a defined list through the Sampling Scheduler:
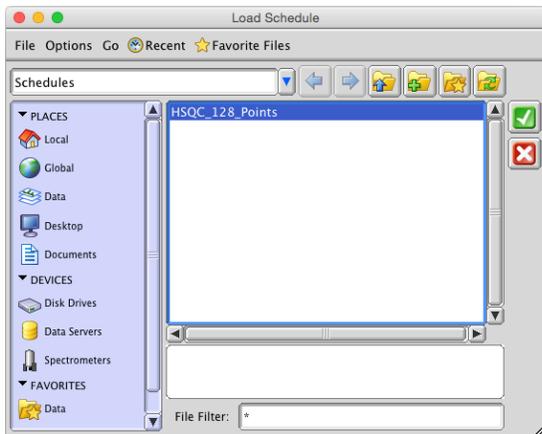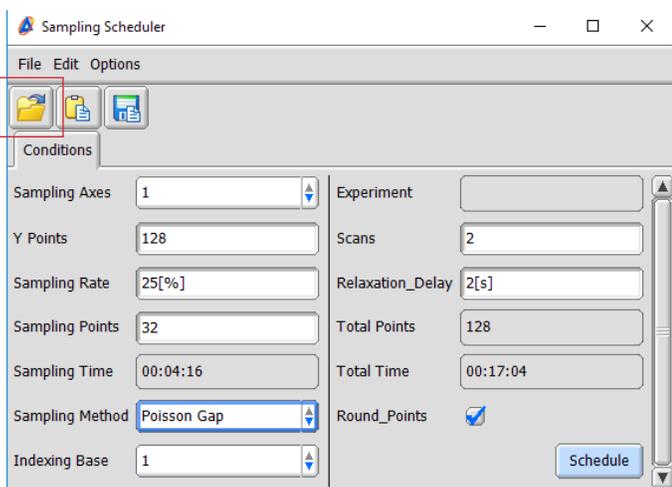
Figure 15. (Top) Open a file browser to load schedules. (Bottom) A previously constructed schedule.

PROCESSING:

To process NUS data, there are two processing parameters that need to be added: NUS Zerofill, and NUS Reconstruction. These can be found in the "PreTransform" dropdown menu in the nD processor.
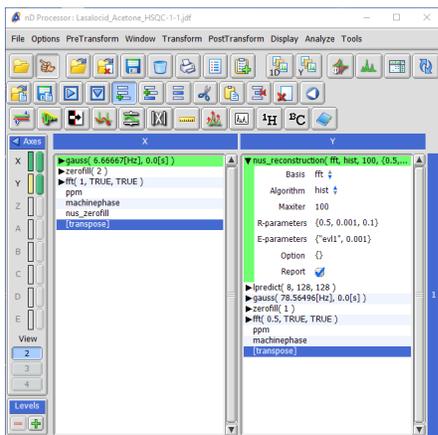
Figure 16. An nD processor with NUS processing parameters.

The NUS Reconstruction parameter gives options, such as the maximum number of iterations and reconstruction algorithm to use, as well as the generation of a report that contains information like total processing time that will be displayed in the main Delta window.
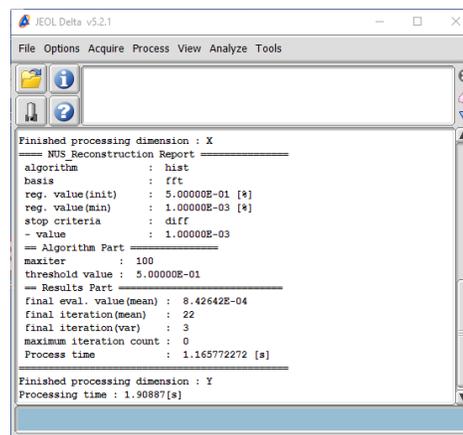
Figure 17. NUS processing results.

Currently available reconstruction methods include:

Irls: K. Kazimierczuk, V. Y. Orekhov, Angew. Chem.-Int. Edit. 2011, 50, 5556- 5559.

Ist: Hyberts, S.G., Milbradt, A.G., Wagner, A.B. et al. J Biomol NMR (2012) 52: 315

Hist: http://gwagner.med.harvard.edu/intranet/hmsIST/

Once the data has been processed, it can be exported to other tools (Data Slate, Viewer, etc) and manipulated like a traditional data set.

CONCLUSION

Employing Non Uniform Sampling allows for additional flexibility in total experiment time. One can increase scans to build up signal to noise or collect higher resolution data without sacrificing the other. As an alternative, one can obtain quicker "scout" 2Ds trading the potential for artifacts for quicker results. This can be useful to obtain a rough spectrum to see if running a much longer experiment is justified. NUS is included in Delta. To download a free copy, please visit nmrsupport.jeol.com.

ADDITIONAL READING

Exponential sampling: An alternative method for sampling in two-dimension NMR experiments, Barna, J.C., Laue, E. D., Mayger, M. R., Skilling, J. Worrall, S. J. P. (1987) J Magn Reson 73 69-77

Nonuniform sampling: bandwidth and aliasing, Bretthorst, G. L. (2008) Concepts Magn Reson 32A 417-435

Sensitivity enhancement for maximally resolved two-dimension NMR by nonuniform sampling, Rovnyak D, Sarcone M, Jiang Z. Magn Reson Chem 49 483-491 (2011)

Novel Sampling Approaches in Higher Dimensional NMR, Billeter, M. & Orekhov, V. eds., Topics in Current Chemistry, Springer, Berlin (2012)

Sparse Sampling Methods in Multidimensional NMR, Mobli, M., Maciejewski, M. W., Schuyler, A. S., Stern, A. D., & Hoch, J. C. Physical Chemistry Chemical Physics (2012)

Perspectives in magnetic resonance: NMR in the post-FFT era. SG Hyberts, H Arthanari, SA Robson, and G Wagner: J Magn Reson 241 60-73 (2014)

Sensitivity of Nonuniform Sampling NMR, MR Palmer, CL Suiter, GE Henry, J Rovnyak, JC Hoch, T Polenova, and D Rovnyak J Phys Chem B 119 6502-6515 (2015)