Non-Uniform Sampling (NUS) for Everyday Use: Sharper Spectra in Less Time

Application Note

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Abstract

This application note demonstrates non-uniform sampling (NUS) acquisition and processing for routine measurement of 2D NMR spectra using tools in Agilent VnmrJ 4 software. It shows that NUS techniques can be applied easily and reliably to small molecule NMR measurements, yielding 2D spectra with comparable digital resolution to those obtained by traditional linear sampling methods, typically with a two- to four-fold reduction in experiment time. It also shows that NUS can enable higher resolution spectra to be obtained in a given measurement time compared to traditional linear sampling and Fourier Transform (FT) methods.

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Introduction

The measurement of NMR spectra commonly requires a compromise between a number of important experimental and spectral parameters, in particular sensitivity, resolution, and available measurement time. Over the years, a great deal of NMR research has been concerned with the development of techniques for circumventing NMR's inherent limitations and, in particular, reducing measurement time required for multidimensional NMR data collection. However, despite the proliferation of other methodologies for recording and processing NMR data, the Fourier Transform (FT) is still by far the most widely used technique for collecting and processing NMR data, owing to its ease-of-use and robustness. The FT needs no adjustable parameters, it is rigorously quantitative, and the results of an FT are not dependent on the details of the particular algorithm employed to perform it.

Despite their popularity, conventional FT methods are, by themselves, hampered by the relatively strict conditions that they impose on the input data. For example, the usual discrete FT demands that time-domain data points be spaced equally; missing or non-contiguous data invariably give rise to artefacts in the resulting spectrum. And critically, the line shapes produced by a discrete FT depend in part on the number of points in the measured data, such that an FT applied to a small number of points will have broad lines and large truncation artifacts, even if the input data have little or no decay and are noise-free, as shown in Figure 1.

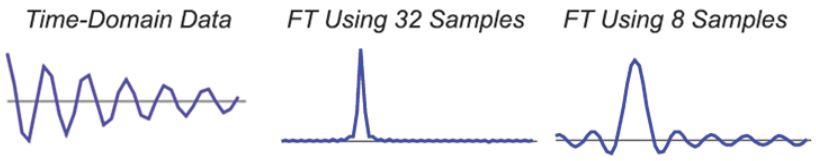

Figure 1. With conventional FT, line shapes depend on the number of time-domain data points used.

While relatively inconsequential for one-dimensional (1D) NMR experiments, the shortcomings of the Fourier Transform become more apparent for multidimensional (nD) NMR techniques. Consider the case of recording a typical two-dimensional (2D) NMR dataset: the traditional approach to data collection involves execution of the 2D pulse sequence a number of times, each time incrementing the t_1 evolution period in a linear fashion. The minimum number of t_1 increments needed to obtain a usable spectrum is usually dictated by the minimum digital resolution required in the indirect dimension (F_1). It follows that, using linear sampling, an increase in the F_1 digital resolution by a factor n necessitates an increase in the overall experiment time by the same factor. For higher dimensionality experiments, the time penalties paid for increased digital resolution can become even more severe as the total number of increments recorded is the product of the number of increments in each indirectly detected dimension. For example, to double the digital resolution in all three indirectly detected dimensions of a 4D experiment increases the experiment time eightfold!

There has recently been significant attention paid to the development and application of sparse or non-uniform sampling (NUS) techniques to NMR as a way of significantly speeding up the measurement of multidimensional datasets. With NUS, only a subset of the usual linearly sampled data is recorded, according to a user-definable sampling schedule. The resolution in the NUS dimension of the resulting spectrum is then given by the maximum t_1 acquisition time sampled. A conventional Fourier Transform of a NUS dataset does not yield a useable spectrum; special processing techniques are needed to reconstruct the spectrum from the raw data. A number of NUS data processing techniques have been introduced in recent years, including Maximum Entropy[1], MFT[2], MDD[3], CLEAN[4], and IST[5]. However, the vast majority of NUS experiments have been applied to biomolecular systems where the time savings afforded by NUS are likely to have the greatest impact. While many effective biomolecular NUS applications have been demonstrated, these have largely required specially-modified pulse sequences, special-purpose software, and complicated reconstruction schemes.

This application note demonstrates that there is much to be gained by applying NUS techniques to small molecule 2D NMR measurements, and that the acquisition and processing framework in VnmrJ 4 provides the advantages of NUS with the same ease-of-use as for a conventional measurement.

Experimental

Sample

This study used a sample containing ca. 2% 2-ethyl-1-indanone in $CDCl_3$.

Instrument

NMR spectra were recorded on an Agilent 400-MR DD2 spectrometer equipped with a 5 mm OneNMR Probe, ProTune, and a 7510-AS sample changer. All data were collected with active sample temperature regulation at 25 °C.

Experiments and parameters

In order to provide a comparison between results obtainable using linear and non-uniform sampling methods, two pairs of 2D experiments were performed using two different pulse sequences, adiabatic HSQC (HSQCAD) and zTOCSY.

HSQCAD

Linearly-sampled experiment

- **Acquisition:** 128 t_1 increments were recorded. Each increment comprised two transients of 524 complex data points. The spectral widths in F_1 and F_2 were 20.1 and 3.5 kHz, respectively, and a relaxation delay of 1 second was used, giving a total experiment time of 11 minutes.

- **Processing:** The t_1 data were extended to 256 complex points using linear prediction. The data were multiplied by matched Gaussian functions and zero-filled to 1k complex points in both t_1 and t_2 dimensions.

NUS experiment

- **Acquisition:** All parameters were the same as for the linearly-sampled experiment, except that randomized, sequential NUS was used. A maximum t_1 acquisition time was chosen to match that of the linearly-sampled experiment, with a sampling density of 25%, giving an overall experiment time of 3.5 minutes.

- **Processing:** The data were then inflated to give 128 t_1 increments and processed using the CLEAN algorithm. The resulting reconstructed time-domain dataset was then processed per the conventional experiment.

zTOCSY

Linearly-sampled experiment

- **Acquisition:** 128 t_1 increments were recorded. Each increment comprised two transients of 524 complex data points. The F_1 and F_2 spectral widths were both 3.5 kHz and a relaxation delay of 1 second was used, giving a total experiment time of 12 minutes.

- **Processing:** The t_1 data were extended to 256 complex points using linear prediction. The data were multiplied by matched Gaussian functions and zero-filled to 1k complex points in both t_1 and t_2 dimensions.

NUS experiment

- **Acquisition:** All parameters were the same as for the linearly-sampled experiment, except that randomized, sequential non-uniform sampling was used. A maximum t_1 acquisition time double that of the linearly-sampled experiment and a sampling density of 50% were used, giving an overall experiment time of 12 minutes.

- **Processing:** The data were inflated to give 256 t_1 increments and processed using the IST algorithm.

Results and Discussion

Setup of NUS Experiments in VnmrJ 4

Non-uniform sampling is available for almost all 2D experiments in VnmrJ 4 and is enabled by checking the **Enable non-uniform sampling** option on the **NUS** panel (Figure 2). Once enabled, it is just a matter of setting the desired number of t_1 increments in the reconstructed dataset and the sampling density to be used. The number of t_1 increments actually recorded is the product of the sampling density and the final number of increments.

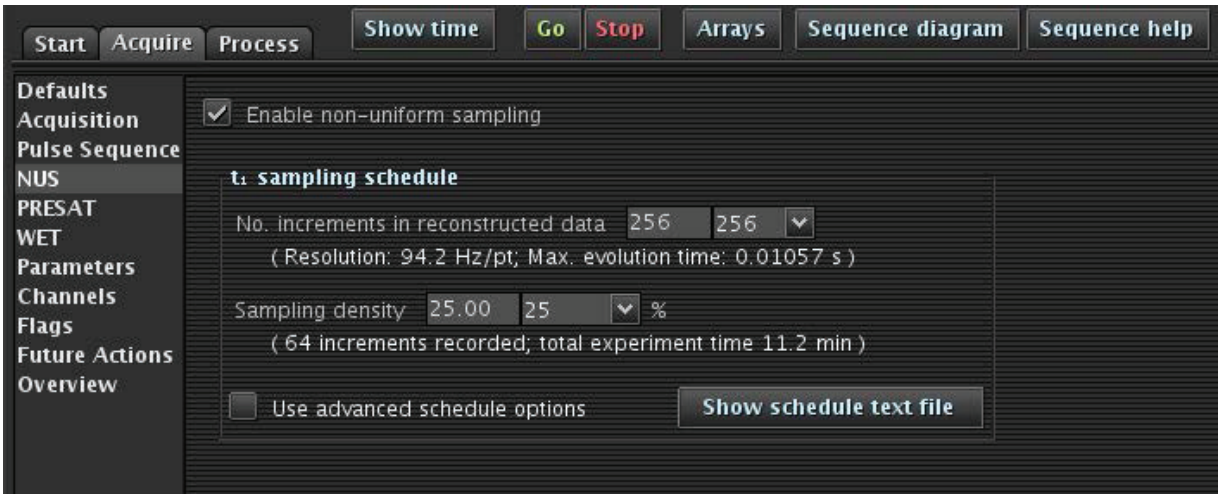

Figure 2. The NUS panel used for setting up non-uniform sampling.

Additional options for creating nonsequential and weighted sampling schedules are also available using the **Use advanced schedule options** check box (Figure 3).

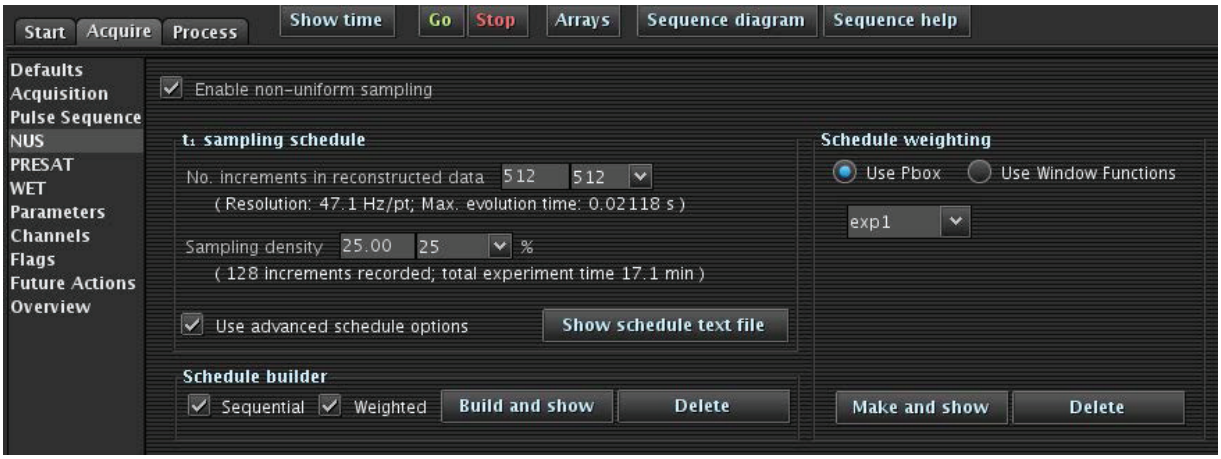

Figure 3. Advanced NUS schedule options.

NUS data processing tools are available on the **Default** panel from the **Process** tab (Figure 4, panel A).

VnmrJ 4 currently supports both CLEAN and IST NUS algorithms. Default NUS processing can be applied in just a couple of button clicks. More advanced options can be accessed, if required, by the more experienced user. CLEAN processing of a typical 2D dataset (Figure 4, panel B) is quick to complete (typically less than 5 seconds), while IST (Figure 4, panel C) usually takes 1–3 minutes to complete.

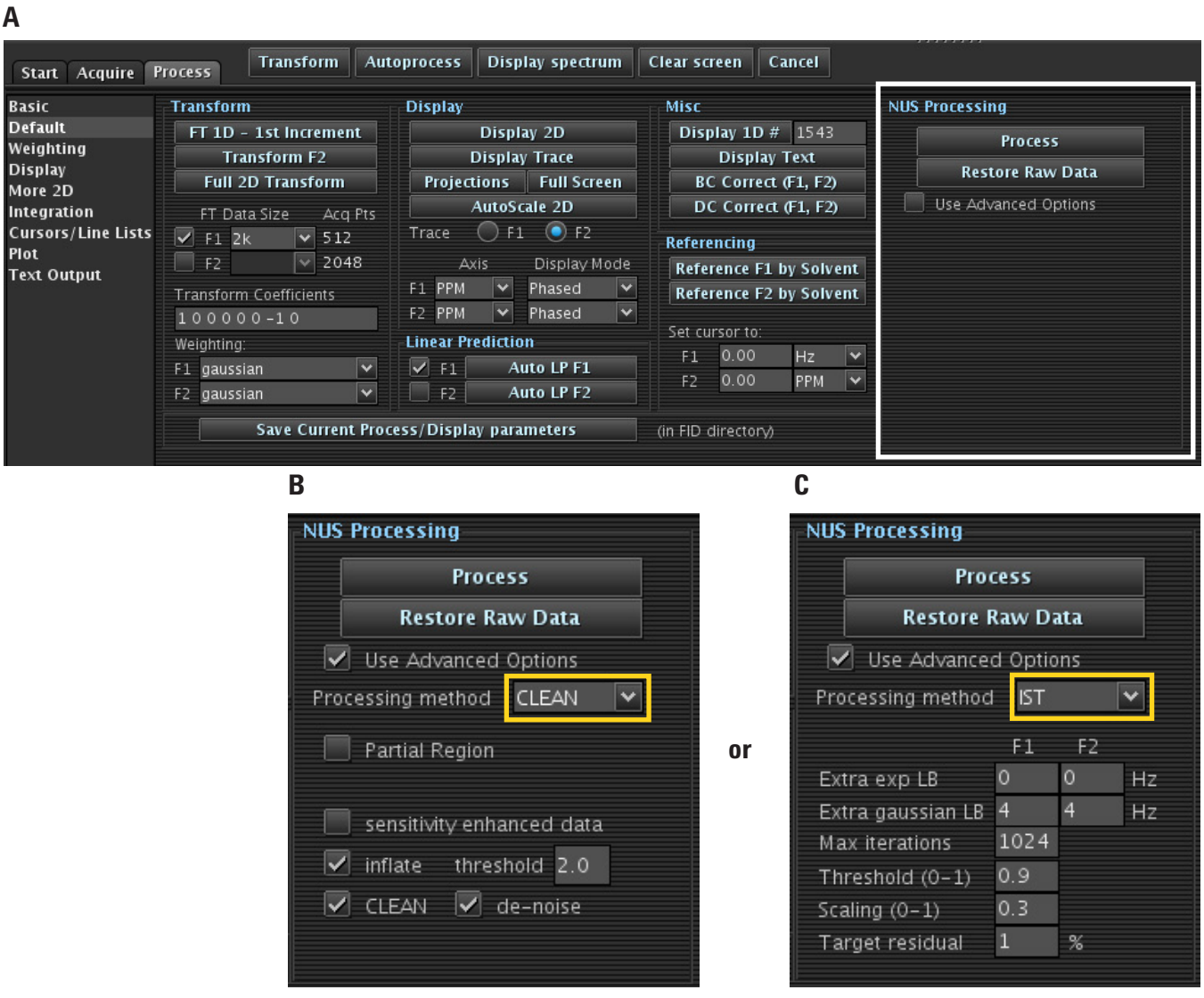

Figure 4. Basic and advanced NUS processing tools on the Default panel.

Figure 5 shows a comparison of a region of the HSQCAD spectrum measured using traditional uniform sampling with the same region of the spectrum measured using NUS as described above.

As Figure 5 shows, the spectrum measured using NUS with a maximum t_1 acquisition time equivalent to the linearly-sampled data shows comparable resolution to that of the conventional spectrum, yet it was recorded in a quarter of the time.

Figure 6 shows a comparison of a region of the zTOCSY spectrum measured using linear sampling with the same region of the spectrum measured using NUS.

The results clearly show the NUS spectrum has higher F_1 resolution than the linearly-sampled one, with additional fine structure apparent in the cross-peaks. Note that both datasets took the same amount of time to acquire.

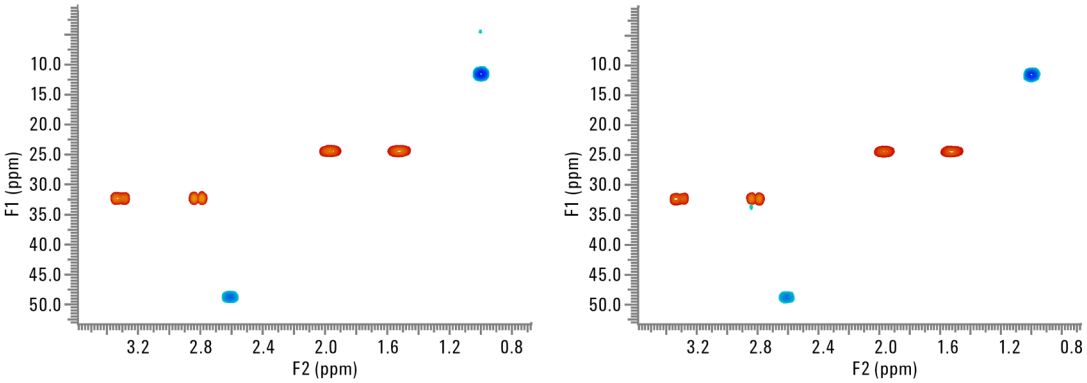

Figure 5. Comparison of (left) conventionally acquired HSQCAD spectrum with (right) spectrum measured using NUS and processed using CLEAN with the same maximum t_1 acquisition time but with a sampling density of 25%. The NUS dataset was recorded in a quarter of the time of the conventionally acquired dataset.

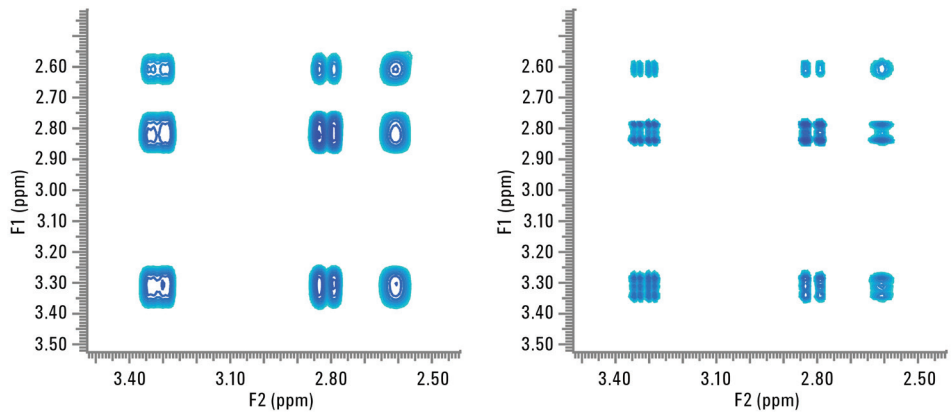

Figure 6. Comparison of (left) conventionally acquired zTOCSY spectrum with (right) spectrum measured using NUS and processed using IST with double the maximum t_1 acquisition time but with a sampling density of 50%. Note that both datasets were recorded in the same time.

Simulating NUS

In addition to tools for acquiring and processing NUS data, VnmrJ 4 also features facilities for simulating NUS on a linearly-sampled dataset (Figure 7). This is a powerful feature that allows the user to tailor the NUS schedule to suit the type of data and application without the need for potentially wasteful trial and error experiments.

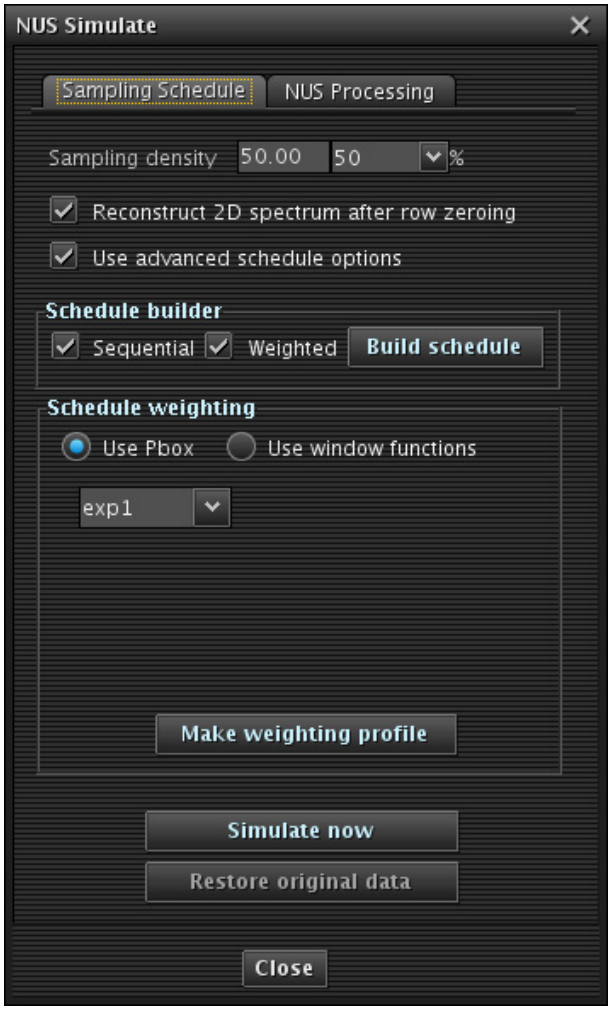

Figure 7. The NUS Simulate panel.

Conclusions

This application note demonstrates that the non-uniform sampling (NUS) tools in VnmrJ 4 can facilitate significant reductions in the time needed to record high resolution 2D NMR spectra of small molecules in solution, with two- to four-fold reductions in experiment time being typical. In addition, it shows that comparable gains in spectral resolution gain can also be obtained without paying the usual penalty of increased measurement time. The NUS tools are robust, and can be applied to most 2D experiments. Furthermore, the simplicity of NUS measurement and reconstruction setup in VnmrJ 4 means that NUS 2D experiments can be as routine as their linearly-sampled counterparts, so that NUS is no longer a specialized and esoteric approach to the collection of NMR data.

References

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