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Triangular Sampling of Multidimensional NMR Data Sets

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The experimental acquisition of large multidimensional (three- and four-dimensional) data sets is very time-consuming and it is not uncommon that an experiment requires several days of acquisition time. Most of this time is required for good resolution on all spectroscopic axes, rather than for signal-to-noise reasons. Some schemes have been proposed to reduce this acquisition time while retaining high resolution, but all these schemes require a sophisticated deconvolution processing to restore to a normal data set. We present here a simple acquisition protocol which consists in acquiring those points in the (t_1, t_2) plane which lie in the lower triangle. We show here that such a scheme reduces only very slightly the overall resolution, while permitting the reduction of the duration of a three-dimensional experiment by a factor of two. The same principle can be used to reduce the acquisition times by a factor of four for four-dimensional experiments and eight for five dimensions while retaining the same quality of resolution. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Acquisition of a multi-dimensional experiment is always time-consuming. This is mostly due to two principal independent factors. First is the need to acquire a certain number of points along each spectroscopic axis, to obtain a minimum spectral resolution permitting the analysis of the spectrum. Second, there is the need to acquire a certain number of scans to insure a minimum phase cycling and a sufficient signal-to-noise ratio (S/N). However, in many cases, sensitivity is not a limiting factor, and the number of scans can be reduced to a minimum by the use, for instance, of pulsed field gradients.[1,2]

The set-up of an *n*-dimensional experiment is therefore usually the result of a compromise between the resolution along each spectroscopic axis, the desired S/N ratio and the overall acquisition time. Quite often the optimum resolution cannot be achieved because of the prohibitive acquisition time that would follow.

There is no simple way of reducing the number of points along each spectroscopic axis. The two incremental delays of a three-dimensional sequence (e.g. t_1 and t_2), have to be regularly varied from 0 to the maximal chosen evolution time which determines the maximum resolution that can be obtained. The value of t_1 and t_2 can be described to lie on a plane, and a sampling of this (t_1, t_2) plane is to be done. This sampling has to be regular and complete to permit the use of fast Fourier transform algorithms. The spectral resolution along each spectral axis is directly related to the inverse of the maximum delay used for this sampling. Doubling the resolution along one spectral axis thus doubles the total acquisition time, while doubling the overall three-dimensional resolution quadruples the total acquisition time.

In the case of two-dimensional experiments, several solutions have already been proposed to optimize the acquisition. All approaches are based on the fact that the S/N is not constant in the (t_1, t_2) plane.[3] In most experiments (with the notable exception of COSY-type experiments), the signal decays with a T_2 law from a maximum located at (t_1, t_2) = (0, 0). Since the acquisition noise is constant, the S/N is decaying in the same manner.

This decay has been exploited for instance by the triangular zero-setting technique proposed by Blümler and Blümich,[4] to optimize the S/N of the experiment. In this technique, the upper half of the (t_1, t_2) plane of the two-dimensional experiment is set to zero, thus rejecting the most noisy part of the detected signal. This procedure can be seen as a triangular apodization of the two-dimensional experiment.

Another possibility is to partially sample the evolution of the signal, acquiring more points along the t_1-axis when the S/N is higher, and less points when the S/N is lower. Typically an exponential law is chosen for the sampling,[5] but other sampling laws have been explored.[6]

In three-dimensional spectroscopy, because of the presence of the two independent sampled delays, the same partial sampling technique has been extended to the complete three-dimensional (t_1, t_2) plane,[7] thus permitting gains in total acquisition time as much as 20 h *vs.* nearly 4 days. However, the experimental set-up and

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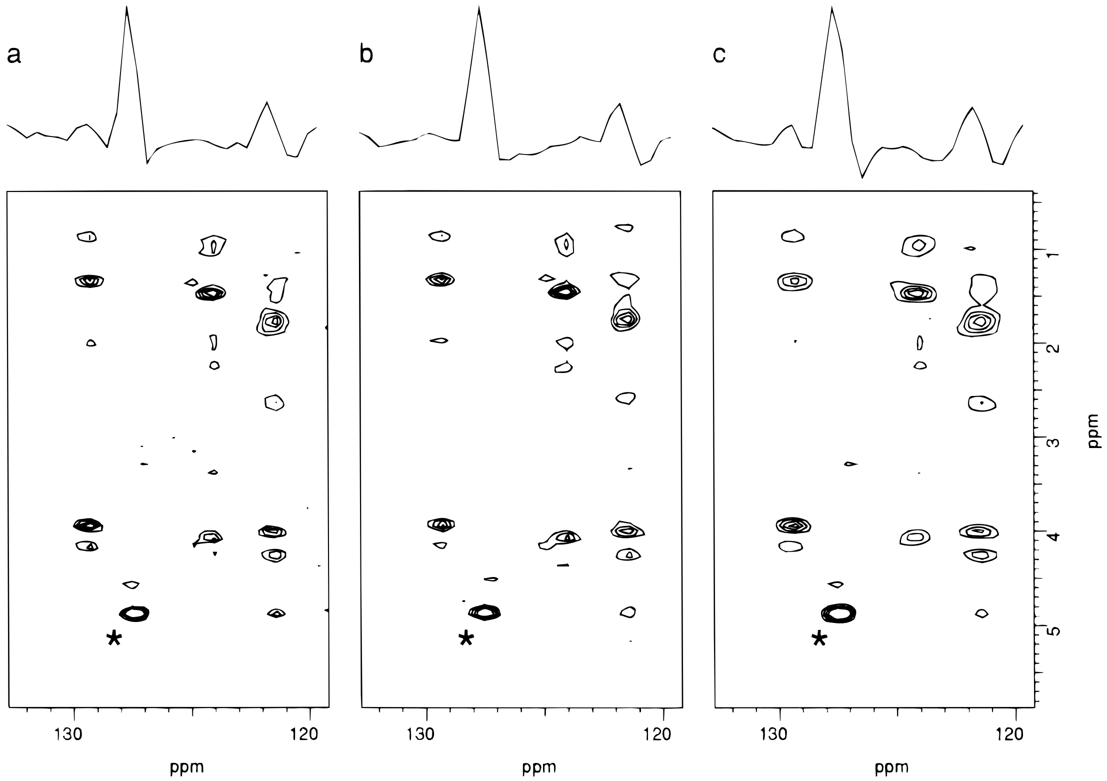

Figure 1. The F_1–F_2 plane extracted from the three-dimensional data sets at $\delta^1H = 8.3$ ppm. (a) The regular 20 h experiment, the line noted with a star, assigned to HN-Hα His 60, was fitted to a two-dimensional gaussian lineshape, and was found to have a width of 31.7 ± 2.5 Hz (F_1) and 24.5 ± 2.5 Hz (F_2). (b) The triangular sampled 10 h experiment, the fitted line was found to have a width of 33.2 ± 4.6 Hz (F_1) and 29.1 ± 2.3 Hz (F_2). (c) The faked regular 10 h experiment, the fitted line was found to have a width of 43.7 ± 4.8 Hz (F_1) by 33.2 ± 3.3 Hz (F_2). In all three cases, the contours are chosen to start at three times the noise level (3σ) and are geometrically spaced by powers of two. The one-dimensional spectra displayed at the top of the picture is a ^{15}N row extracted at the location of the HN-Hα His 60.

the subsequent processing is a bit complicated in this case.

These latter approaches, having abandoned the regular sampling scheme, require a special processing, typically done by a maximum entropy processing. This additional burden has probably considerably restricted the use of such techniques.

We are presenting here a simple extension of the partial sampling technique, which consists in measuring only the lower triangle of the (t_1, t_2) plane of a three-dimensional experiment. This is very similar to the triangular apodization, as was proposed for two-dimensional processing, but since it is done here directly at the acquisition stage, a gain on the total acquisition time is permitted.

This triangular sampling permits to measure only half of the points that would be needed for a complete acquisition, thus a reduction of the acquisition time by a factor of two for a three-dimensional experiment can be obtained.

EXPERIMENTAL

For this study we chose to work with a ^{15}N labelled sample of the N-terminal DNA binding domain of FruR.[8,9] The studied protein has 65 residues including 57 primary residues and eight extension residues with six histidines on the C-terminal. FruR is an *E. coli* transcriptional regulator that belongs to the LacI DNA-binding protein family.

On this sample, we realized two ^{15}N-^1H HSQC-NOESY[1] on our Bruker AMX 400 spectrometer at 300K. The first experiment was acquired with a regular acquisition protocol, with 64 points along the ^{15}N axis and 256 points in the ^1H direction. A total of 512 points were acquired on the classical ^1H dimension and four scans were accumulated. The spectral windows were 1121 Hz for the ^{15}N axis, and 4807.7 Hz for the ^1H spectral axes. The NOESY mixing time was 200 ms. The ^{15}N dimension was scanned first in the three-dimensional protocol. A States-TPPI[10] acquisition mode was used on both non-classical spectral axes. The total acquisition time was of 20 h.

The second experiment was acquired in exactly the same experimental conditions except that a triangular sampling was used. Because of the States-TPPI protocol on both axes, the triangular sampling was done on group of four (t_1, t_2) points, to insure a correct complex sampling. So, for every eight increments in t_1, the loop counter for the t_2 increment was decremented by two. The total acquisition time of the triangular experiment was 10 h.

The first experiment was processed in a regular way using sine-bell apodization in t_1 and t_2 and zero padding of the data in both axes.

To process the triangular data set, an empty three-dimensional data set was first created, and the acquired FIDs was copied to the empty data in due place, and the missing data points were consequently replaced by zeros. The resulting complete three-dimensional data set was processed with the same protocol as described above.

A second processing of the regular data set was performed, by truncating each of the t_1- and t_2-axes at 44 and 180 points, respectively, followed by equivalent sine-bell apodization and zero-padding prior to Fourier transform. The spectrum obtained from this latter processing corresponds to an experiment that would have been regularly acquired in 10 h only.

Figure 1 shows the same F_1–F_2 plane extracted from the three data sets, showing the F_1–F_2 line shape in the three cases. The line noted with a star in the figure has been fitted to a two-dimensional gaussian shape, linewidths obtained in the fit are given in the figure caption. Confidence intervals are given for 90% confidence from a χ^2-analysis performed independently on each parameters. Gaussian line-shape was chosen because it is the analytical form that most closely matches the compact line-shapes obtained either by sine-bell apodization or by triangular sampling. The residuals after fit did not show any important shape miss-match (data not shown). All data processing was done using the Gifa software.[11,12]

RESULTS AND DISCUSSION

As expected naturally, out of the three data sets, the regular data set acquired in 20 h shows the highest resolution and signal-to-noise ratio. Of the two data sets acquired in half the acquisition time, the regular data set displays the worse signal. On the other hand, the triangularly sampled experiment shows a quality which is nearly comparable to the first experiment, even though it was acquired in a delay equivalent to the latter. The gaussian line-fitting on the three spectra (see figure caption) shows that for the same acquisition time, a better resolution is obtained with the triangular sampling *vs.* the regular one. The resolution thus achieved is nearly as good as the one observed for the experiment acquired in twice the time. Of course, no difference among the three experiments can be observed along the acquisition axis F_3.

The triangular sampling, concentrating more on that part of the data set which possesses the higher S/N, gives rise to a global sensitivity optimization on the experiment. Additionally, this process does not modify significantly the actual peak width because the maximum sampled t_1- and t_2-values remain equivalent to those obtained with a complete sampling. It should also be noted that the points in the (t_1, t_2) plane which are ignored by this sampling protocol are the points which are mostly decreased by a regular sine-bell apodization, as used here, thus having a small impact on the final spectrum.

In their original paper, Blümler and Blümich proposed the triangular apodization as a mean to optimize the S/N of homonuclear two-dimensional experiments. The purpose here is different, since the main benefit of the triangular sampling is to optimize the spectral resolution for a given acquisition time. In our case, the gain in S/N comes as an additional benefit. In this respect, the procedure can be easily applied unmodified on a heteronuclear experiment, in contrast with the original proposition.

Compared with other sampling protocols, one of the main advantages of this procedure is that no special processing is required for the spectral analysis of the data set. Rather, simple data management is enough to unshuffle the acquired raw data in order to fit them to a regular data set, filling the missing parts with zeros.

The triangular sampling experiment shows a small distortion of the line shape of the peaks which is a consequence of the convolution of the experiment by the triangular apodization used. This distortion appears as a faint star oriented at 45° of the main spectral axes and does not appear to be a major problem in the case studied here, for it was nearly of the same intensity as the experimental noise.

CONCLUSION

The simple technique which is presented here permits, in the general case, to reduce the total acquisition time of a three-dimensional experiment by a factor of two, with a marginal loss of sensitivity and resolution. The same technique, when applied to four- or five-dimensional experiments, should permit gains of four and eight, respectively. It does not require any sophisticated processing technique, but is implemented in a straightforward manner by a small modification of the acquisition sequence, and a simple unshuffling of the data followed by a regular processing.

This technique is very general, and can be used in almost all three-dimensional experiments, homonuclear as well as heteronuclear. For a given total acquisition time, it is possible to obtain a neat gain in both signal-to-noise ratio, and in resolution. Of course, the results will be better if the three-dimensional signals decay away from the point ($t_1 = 0$, $t_2 = 0$), but other experiments, such as constant-time experiments, should be possible too.

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