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Randomized Acquisition for the Suppression of Systematic F_1 Artifacts in Two-Dimensional NMR Spectroscopy[1]

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2D spectra, particularly for homonuclear correlation, can show a variety of artifactual signals in the F_1 domain. Common sources include carry-over of signal modulation from one transient to the next ("rapid pulsing artifacts") and systematic variations in room temperature ("parallel diagonals"). In both cases there is one very simple expedient which can greatly reduce the impact of these sources of error. Multidimensional data sets are almost invariably recorded by simply incrementing or decrementing evolution periods, largely for reasons of convenience and historical precedent. If instead the sampling of the evolution periods is carried out in random order, the perturbations responsible for the sharp F_1 signals in the conventional experiment manifest themselves as t_1 noise. Since the randomized acquisition redistributes coherent artifactual signals randomly in F_1, the maximum artifactual signal is substantially reduced in the randomized experiment and no longer appears in the form of misleading distinct peaks. © 1999 Academic Press

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Almost all two-dimensional (2D) spectra exhibit imperfections in the F_1 domain. Perhaps the most familiar is t_1 noise—bands of apparently random fluctuations that run parallel to the F_1 axis at the F_2 frequencies of strong signals (*1, 2*). t_1 noise arises where irreproducibilities in the experiment, which may be random or systematic in character, cause apparently random modulation of signals as a function of t_1. Arguably more damaging, however, are coherent artifacts which appear as well-defined peaks, since these can more easily be misinterpreted as real signals. The two commonest examples of coherent artifacts are parallel diagonal signals, arising from t_1 modulations caused by room temperature oscillations, and rapid pulsing artifacts, which arise from incomplete relaxation between measurements. This Communication concerns a simple method for the conversion of coherent artifacts into incoherent t_1 noise, with the aim of reducing the danger of spectral misinterpretation.

The term t_1 noise is often used loosely to cover almost any additions to or distortions of the expected signals in the F_1 domain of a 2D NMR spectrum. Here the term will be used in its more restrictive, and original, sense of a weak, apparently random modulation of F_1 signals. This includes both the effects of truly random perturbations, for example noise in the transmitter channel leading to phase and amplitude irreproducibility of pulses, and coherent perturbations which can give rise to apparently random results, such as mains (line) 50 (60) Hz modulation. It is helpful to view coherent F_1 artifacts as a separate problem; coherent modulations as a function of t_1 lead to distinct signals in F_1, which can easily be misinterpreted as cross-peaks. Of the two common sources of such artifactual signals, parallel diagonals arise because air conditioning systems generally cause room temperature to oscillate over a range of 1–2°C. This temperature variation in turn causes the phase and gain of the spectrometer system (and, if probe temperature regulation is inadequate, relative chemical shifts) to oscillate during the course of a 2D experiment (*3, 4*). If the evolution time is incremented linearly during a 2D experiment of duration D, room temperature oscillation with a period P will generate F_1 sidebands which are displaced from the true signals by multiples of a fraction P/D of the F_1 spectral width (*4, 5*). This leads to the characteristic appearance of "parallel diagonals" straddling the main diagonal.

Rapid pulsing artifacts arise because of incomplete relaxation between transients in multidimensional experiments, where the term "transient" is used loosely to denote the application of a pulse sequence followed by the measurement of a free induction decay. Transverse relaxation, main field inhomogeneity, and diffusion together generally lead to relatively complete loss of transverse magnetization between the end of one transient and the beginning of the next, but incomplete spin–lattice relaxation means that the longitudinal magnetization M_z at the start of a given transient depends on the sign and amplitude of the residual M_z at the end of the preceding one. Since the sign and amplitude of M_z here depend on the value of t_1 for the preceding transient, in a conventional 2D experiment this will produce a coherent amplitude modulation of M_z. It is this amplitude modulation of M_z, together with any carryover of transverse magnetization where very short recovery delays are used, which is responsible for spurious signals at zero frequency and at multiples of F_1 (*6–11*). A number of techniques have been proposed for the suppression of rapid

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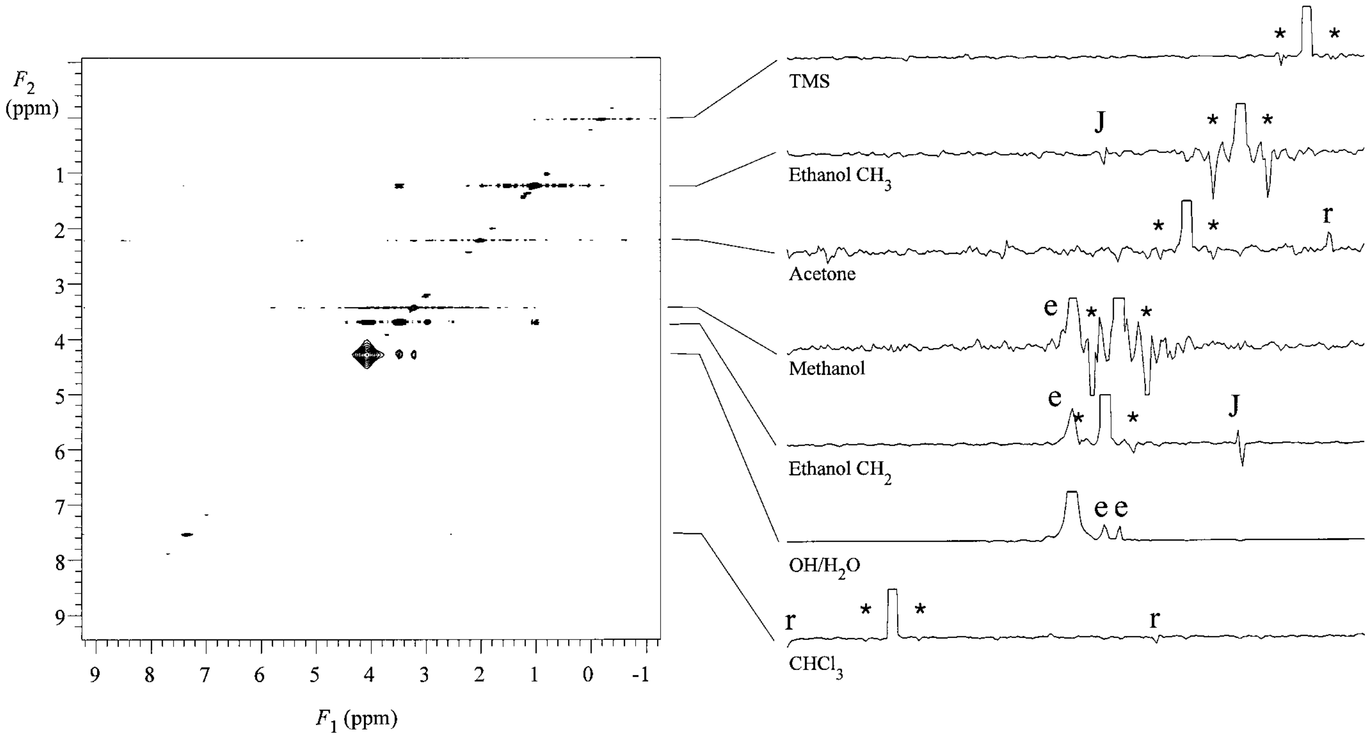

FIG. 1. Phase-sensitive NOESY spectrum of a mixture of ethanol, methanol, acetone, chloroform, and TMS, acquired on a Varian Unity INOVA 300 spectrometer. 256 increments of four transients of 4096 complex data points were acquired using a mixing time of 500 ms over a period of 4 h. The data were processed using Gaussian weighting and a single zero-filling in both dimensions. A relaxation delay of 4 s was used between the end of data acquisition of one transient and the first pulse of the next. Rapid pulsing artifacts are marked **r**, exchange cross peaks **e**, and zero quantum (J) cross peaks **J**, while sidebands due to air-conditioning temperature oscillations are marked with asterisks.

pulsing artifacts, mostly involving modifications to phase cycling (*6–10*). Thus Derome and Williamson (*8*) described two phase cycles (an eight-step cycle and a 16-step cycle) for rapid pulsing artifact suppression in the double-quantum filtered (DQF) COSY experiment. A different approach was described by Marion *et al.* (*11*), who used a "saturation" pulse at the end of each t_2 acquisition period to ensure the same initial state for each transient.

Common to the generation of both air conditioning sidebands and rapid pulsing artifacts is the carryover of undesirable modulation from one transient to the next. The overwhelming majority of 2D NMR experiments are carried out by steadily incrementing the evolution period over the course of the experiment, with the remainder instead decrementing t_1. The alternative approach suggested here is to acquire the data for the different values of t_1 in random order; any time averaging is carried out in interleaved mode, by acquiring a single transient for each t_1 value, then repeating and co-adding results until the desired number of transients has been averaged. The randomization ensures that any carryover of modulation from one value of t_1 to the next highest t_1 has random phase, irrespective of whether the modulation derives from incomplete T_1 relaxation between transients or from coherent modulation of instrumental conditions in real time. The coherent artifact signals are effectively randomized, and appear simply as a slight increase in t_1 noise. At first sight, the suppression of one type of artifact at the expense of another may seem a rather contentious way of tackling this problem. However, as already mentioned, coherent artifacts are potentially more damaging than t_1 noise, since they are open to misinterpretation. Also, for constant artifact signal power the randomized experiment will reduce the peak artifact signal seen, in proportion to the square root of the number of t_1 increments.

The advantages of randomized data accumulation are illustrated here for the basic phase-sensitive NOESY experiment. Figure 1 shows a NOESY spectrum of a mixture of ethanol, methanol, acetone, chloroform, and TMS in deuteriochloroform, measured on a standard 300 MHz INOVA instrument with a 5 mm dual probe. The T_1 values for the protons in this sample were mostly in the region of 3.5–4 s, with the exception of chloroform (6.3 s) and HDO/OH (1.3 s). The data were acquired in the conventional manner, using the hypercomplex method for quadrature detection in F_1 (*12*). The relaxation delay of 4 s was sufficiently short to generate rapid pulsing artifacts at the F_2 frequencies of most resonances. As can be seen from the cross sections displayed, there are clear F_1 sidebands, which can be attributed to the room temperature cycling (1–2°C peak-to-peak) caused by air conditioning, on

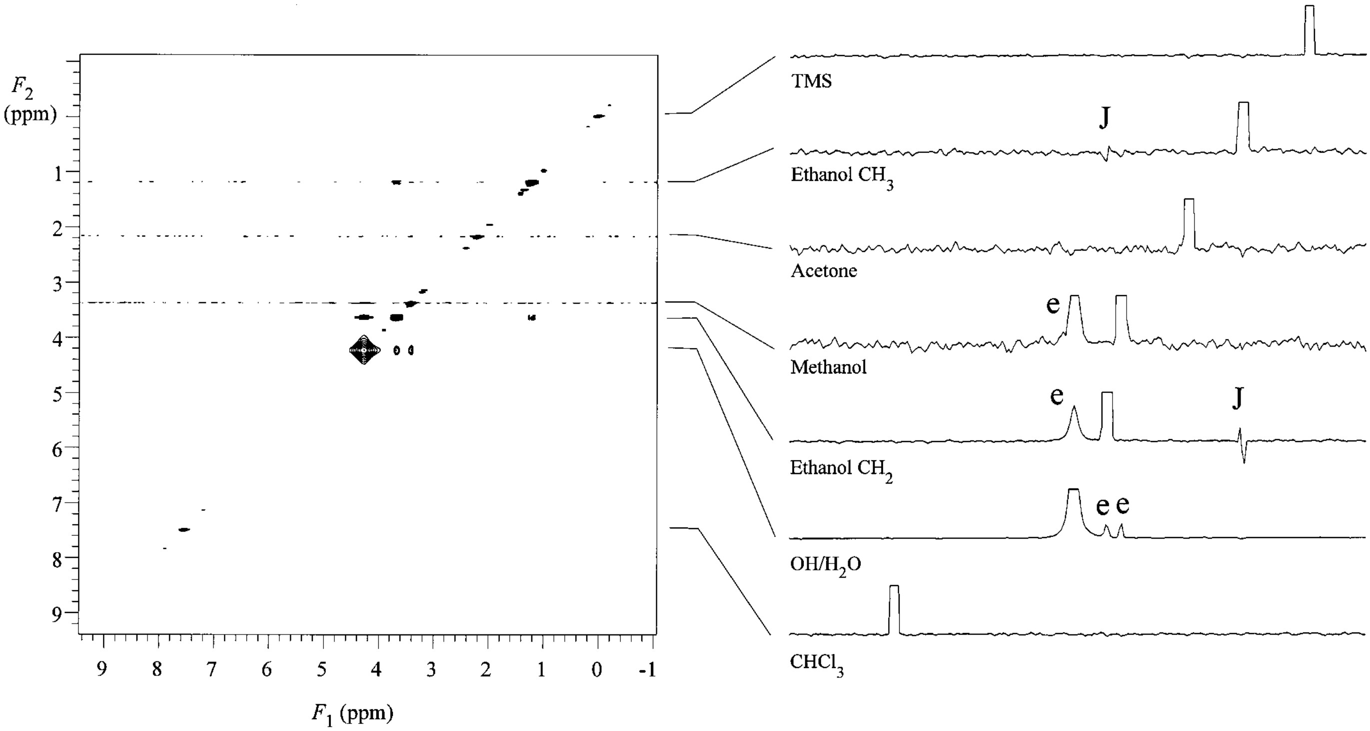

FIG. 2. Phase-sensitive NOESY spectrum of the same sample as Fig. 1, recorded and processed with identical parameters except that the t_1 increments were recorded in random order. Only genuine peaks (diagonal peaks, exchange cross peaks, and *J* cross peaks) are visible.

all of the diagonal peaks. In addition, rapid pulsing artifacts are clearly visible at the F_2 frequencies of the chloroform, acetone, and methanol methyl signals.

Figure 2 shows a spectrum of the same sample as used for Fig. 1, acquired and processed identically, except that the t_1 increments were recorded in random order in interleaved mode. The only changes required to the standard instrument software were a modified NOESY pulse sequence and a short macro to reorder the t_1 increments stored; copies of both are available from the authors on request. In contrast with the spectrum in Fig. 1, air conditioning sidebands and rapid-pulsing artifacts are completely absent from the spectrum in Fig. 2. The penalty paid for this is, as expected, very slightly increased t_1 noise on all resonances. However, the increase in t_1 noise is more than compensated for by the elimination of the potentially misleading peaks seen in the conventionally acquired spectrum.

This example shows that randomized acquisition can greatly reduce the impact of two sources of coherent artifacts in 2D spectra. Perhaps the most appealing feature of this technique is its sheer simplicity: it can, in principle, be applied successfully to almost any multidimensional experiment. t_1 noise is usually regarded as an unmitigated evil; as the results presented here demonstrate, in some circumstances it may at least be the lesser of two evils.

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REFERENCES

1. A. F. Mehlkopf, D. Korbee, T. A. Tiggelman, and R. Freeman, *J. Magn. Reson.* **58,** 315–323 (1984).
2. R. Freeman, “Spin Choreography,” Spektrum Academic Publishers, Oxford (1996).
3. G. A. Morris, *J. Magn. Reson.* **100,** 316–328 (1992).
4. Varian Associates, “Magnetic Moments,” Vol. 4, No. 4, p. 6, Palo Alto, California (1990).
5. D. Braun, G. Wider, and K. Wüthrich, *J. Magn. Reson. B.* **110,** 313–315 (1996).
6. C. J. Turner and S. L. Patt, *J. Magn. Reson.* **85,** 492–505 (1989).
7. C. J. Turner, *J. Magn. Reson.* **86,** 420–425 (1990).
8. A. E. Derome and M. P. Williamson, *J. Magn. Reson.* **88,** 177–185 (1990).
9. M. Bourdonneau and B. Ancian, *J. Magn. Reson.* **132,** 316–327 (1998).
10. C. J. Turner and W. C. Hutton, *J. Magn. Reson.* **100,** 469–483 (1992).
11. D. Marion, M. Ikura, R. Tschudin, and A. Bax, *J. Magn. Reson.* **85,** 393–399 (1989).
12. D. J. States, R. A. Haberkorn, and D. J. Ruben, *J. Magn. Reson.* **48,** 286 (1982).