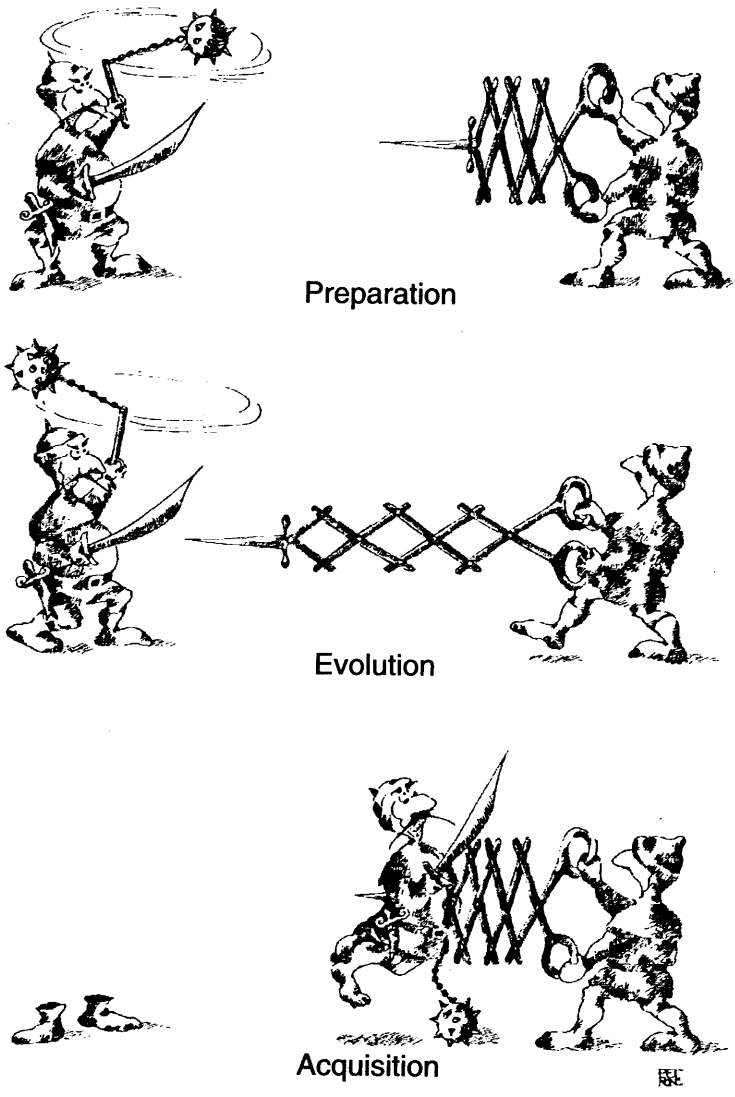
Two-dimensional Spectroscopy

The advent of Fourier transform methods in NMR (1) achieved two important advantages that were immediately exploited. The first was the dramatic improvement in sensitivity brought about by monitoring all the resonances in the spectrum simultaneously rather than one at a time – the so-called multiplex advantage. The second was the possibility of studying time-dependent phenomena such as spin–spin and spin–lattice relaxation, chemical exchange, and transient nuclear Overhauser effects. Prior to the Fourier transform revolution, the techniques available for following these transient phenomena had been very cumbersome. Imagine having to sweep through a spectrum under slow-passage conditions when the phenomenon under investigation is changing the intensities on a time scale of seconds.

A third development, of comparable importance with the other two, passed almost unnoticed at the time. At a summer school in the former Yugoslavia, Jeener (2) described a novel experiment in which a coupled spin system was excited by a sequence of two 90° pulses separated by a variable time interval t_1. Jeener realized that if t_1 were to be varied in small steps in a series of experiments, this would introduce a new time dimension, and that the signal could be Fourier transformed as a function of t_1. This variable period is now known as the *evolution time*. The other independent time dimension (t_2) is the running variable for sampling the free induction decay, but no signal acquisition takes place during the evolution time. Information about the behaviour of the nuclear spins during t_1 is only derived indirectly by detecting its influence on the set of free induction decays. This relies on the fact that the spins possess a memory of what happened to them in the past, a memory with a time constant T_2, the spin–spin relaxation time. The evolution of the spins is mapped out point by point by incrementing t_1 in suitable small steps while detecting the free induction decays.

Although Jeener obtained experimental spectra by this new two-dimensional method, the equipment available at the time was quite unstable and the spectra were consequently very noisy. He decided not to publish them for fear that they might jeopardize the acceptance of the two-dimensional concept. Much of the credit for demonstrating the generality of the idea and for extending it to many more applications is due to Ernst (3). Close parallels can be traced with the development of double-resonance methods in the previous decade; indeed most two-dimensional



experiments can be thought of as the translation of double-resonance techniques into the time domain.

It is convenient to think of the experimental data (3–6) as a two-dimensional array $S(t_1, t_2)$. In the simplest case where there is only one frequency present, this array might look like Fig. 1. The signal follows a decaying cosine wave in both time dimensions. We could imagine converting this array directly into a two-dimensional spectrum $S(F_1, F_2)$ through two-dimensional Fourier transformation, but in practice this operation is carried out in two stages. First of all, each free

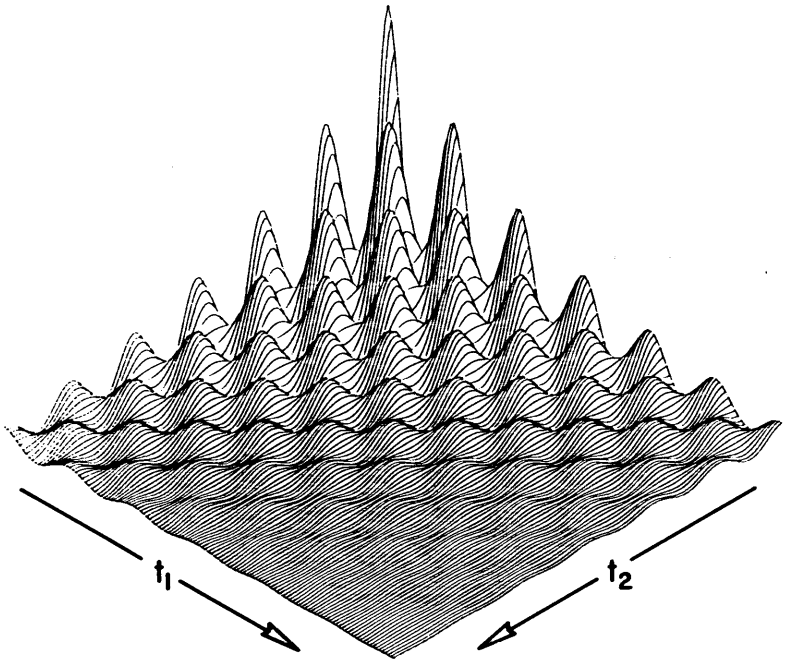


Fig. 1. Schematic diagram of the data array $S(t_1, t_2)$ when there is a single decaying cosine wave in both time dimensions.

induction decay $S(t_2)$ is Fourier transformed, one for every increment t_1, creating a new array $S(t_1, F_2)$ which has spectra in one dimension and an oscillatory signal, sometimes called an *interferogram*, in the other (Fig. 2). It is now necessary to read this array in the other sense, that is to say, to follow the modulation in the t_1 dimension for each and every point in the spectrum $S(F_2)$. Because the array $S(t_1, F_2)$ is usually quite large, it is often stored in a linear fashion on a disc, so it may be necessary to rearrange the data set $[S(t_1, F_2) \rightarrow S(F_2, t_1)]$ so that it can be read in the form of interferograms (Fig. 3). Then the second Fourier transformation can be carried out as a function of t_1 to give the two-dimensional spectrum $S(F_1, F_2)$. For this very simple example, the spectrum is only a single peak (Fig. 4) which happens to have the characteristic shape of a two-dimensional Lorentzian because we assumed that the signal decayed exponentially in both time dimensions.

An important factor that has contributed to the general acceptance of two-dimensional spectroscopy has been its inherently high sensitivity – comparable with that of a one-dimensional experiment accumulated for the same total time. As far as the signal-to-noise ratio is concerned, there is little to choose between time averaging an amplitude-modulated time-domain signal, and time averaging an equal set of identical signals. Looked at from a different viewpoint, this multiplex advantage comes about because two-dimensional spectroscopy spreads the noise

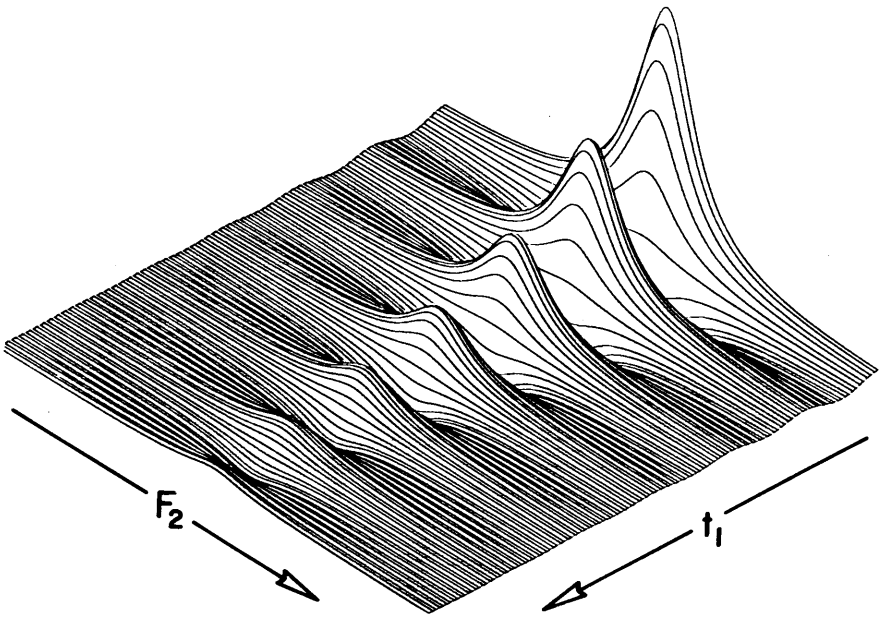


Fig. 2. The intermediate data array $S(t_1, F_2)$ obtained from Fig. 1 by Fourier transformation with respect to t_2.

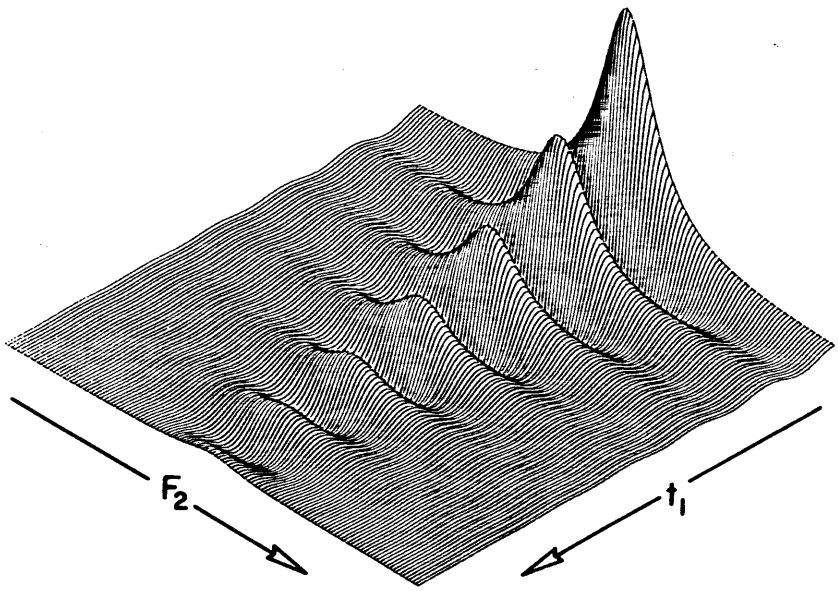


Fig. 3. Transposition of the data array shown in Fig. 2 so as to provide rapid access to t_1 traces in preparation for the second stage of Fourier transformation.

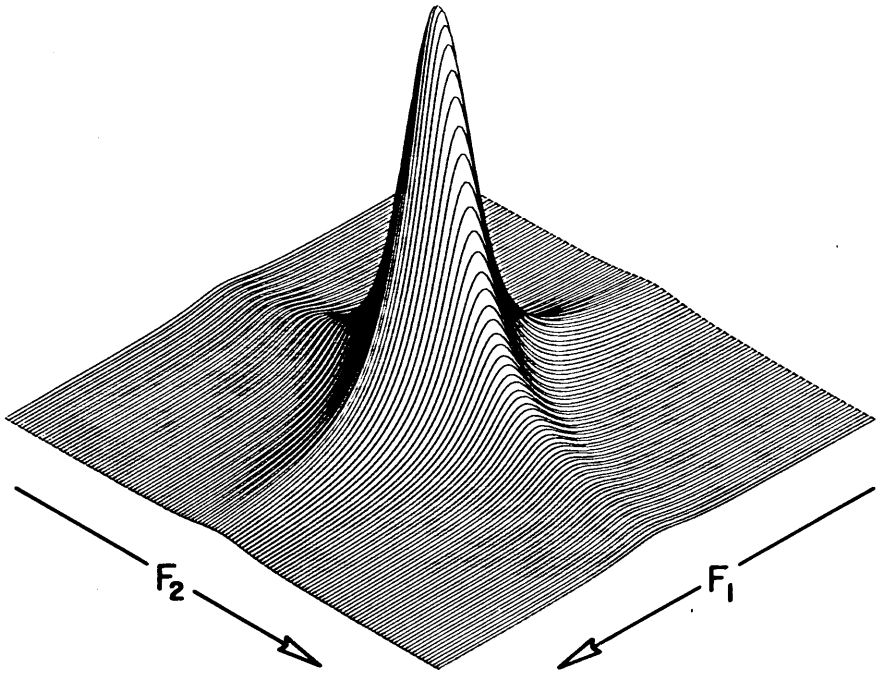


Fig. 4. A two-dimensional Lorentzian line obtained by Fourier transformation of the data array in Fig. 3 with respect to t_1. The Lorentzian shape originates in the assumption that the signal in Fig. 1 decayed exponentially in both time dimensions.

out over a plane, without reducing the signal amplitudes. Jeener in fact anticipated this key feature in the abstract of his summer school presentation (2).

So far this is not too exciting. The importance of Jeener's idea stems from the fact that the conditions governing the motion of the spins during the evolution period may be different from those prevailing during the detection period; indeed we must arrange for this to be so. Furthermore, the precession occurring during t_1 may represent a formally forbidden transition, since we only detect it indirectly. Consequently there are very many possible two-dimensional experiments, limited mainly by the spectroscopist's ingenuity. They may be divided into three broad categories, although it can be dangerously unproductive to insist on hard-and-fast rules about two-dimensional spectroscopy. It makes surprising leaps into quite new fields, for example Fourier zeugmatography (7), one of the most powerful methods for magnetic resonance imaging.

SEPARATION OF PARAMETERS

Chemical shifts can be separated from spin–spin coupling effects by performing a spin-echo experiment during the evolution time t_1:

$$90° - \tfrac{1}{2}t_1 - 180° - \tfrac{1}{2}t_1 - \text{acquisition } (t_2). \qquad [1]$$

The chemical shifts are refocused by the 180° pulse but the divergence due to spin coupling persists, because the term $J_{IS}I_ZS_Z$ remains unaffected by the 180° pulse. The resulting 'J-spectrum' has spin multiplets displayed in the F_1 dimension and the conventional (coupled) spectrum in the F_2 dimension. This technique is treated in detail under J-spectroscopy*. It can be used to obtain high-resolution proton spectra without spin–spin splittings (8).

CORRELATION EXPERIMENTS

If the polarization or coherence that exists at a given chemical site during the evolution period is transferred to another site before detection, then this is said to be a *correlation* experiment, because it identifies the two sites as having a spin–spin interaction. This is the ubiquitous COSY experiment (3), treated in detail under Correlation spectroscopy*. It provides, in a single spectrum, all the information that would be obtained from a large number of selective decoupling experiments, and presents it in the convenient form of a contour diagram (9) in which pairs of cross-peaks indicate spin–spin interactions.

We might be tempted to generalize correlation experiments to include two-dimensional nuclear Overhauser spectroscopy (NOESY) (10) and chemical exchange spectroscopy (EXSY) (11) where, although the interactions are different, the basic principle is the same. These two-dimensional experiments include a 'mixing period' t_m between evolution and detection to allow time for cross-relaxation or chemical exchange. The nuclear Overhauser effect* has proved to be of enormous importance for the structure determination of biological macromolecules in solution, where information about internuclear distances is invaluable.

FORBIDDEN TRANSITIONS

Multiple-quantum coherence* is said to be 'invisible' in the sense that it does not induce any voltage in the receiver coil. We might think of it as two diametrically opposed vectors, rotating in the XY plane at the same rate, and always having a zero resultant. Multiple-quantum coherence must be detected indirectly, by allowing it to precess during the evolution period of a two-dimensional experiment. Just before the detection stage, a radiofrequency pulse converts it into observable transverse magnetization. Multiple-quantum spectra can offer some important

simplifications compared with conventional high-resolution spectra of complex spin systems. There are fewer transitions, and the spin multiplet structure is simpler because the active spin–spin splittings do not appear in these spectra (12). Furthermore, multiple-quantum spectra can be separated according to the order of coherence, either through the excitation scheme or by the use of the appropriate multiple-quantum filters (13).

One useful application is to detect pairs of directly bound carbon-13 spins in molecules of samples with the natural isotopic abundance, in spite of the fact that molecules with isolated carbon-13 nuclei outnumber them by two orders of magnitude. The experiment relies on the fact that only pairs of coupled spins can support double-quantum coherence, so a suitable phase cycle, or pulsed field gradient sequence, can be employed to filter out the desired response, even though it is very weak. This is the two-dimensional 'INADEQUATE' experiment (14) which has proved to be a powerful tool for determining the structure of the carbon 'skeleton' of an organic molecule by building up a picture of the carbon–carbon connectivity one bond at a time. Its main drawback is the inadequate sensitivity.

MULTIDIMENSIONAL SPECTROSCOPY

It was some time before anyone ventured to tack two two-dimensional pulse sequences together to implement a three-dimensional experiment (15,16). But, as larger and more complex molecules come under investigation, the ability to spread the information into yet another frequency dimension seems more and more attractive. We might write a three-dimensional experiment schematically as

$$\text{preparation} - \text{evolution } (t_1) - \text{mixing} - \text{evolution } (t_2) - \text{mixing} - \text{detection } (t_3). \quad [2]$$

The basic building blocks are often the popular two-dimensional techniques such as COSY, NOESY or TOCSY (17). The duration of the experiment is primarily determined by the product of the number of increments in t_1 and t_2, and by any phase cycling that is used.

It is not really feasible to make useful measurements from cross-peaks distributed in three frequency dimensions, so plane sections are normally displayed. Consider the case of a three-dimensional correlation experiment performed on a coupled three-spin (ISR) system. We might investigate transfers of the type $I \rightarrow S \rightarrow S$, which give rise to peaks on the first mixing plane (Fig. 5(a)), or transfers of the type $S \rightarrow S \rightarrow R$, which give rise to peaks in the second mixing plane (Fig. 5(b)), or back-transfers of the type $I \rightarrow S \rightarrow I$, giving peaks in the back-transfer plane (Fig. 5(c)). The true three-dimensional cross-peaks, generated by two consecutive transfers of the type $I \rightarrow S \rightarrow R$, are not located on any of these planes.

Three-dimensional spectroscopy really comes into its own when biosynthetic isotopic enrichment is used to simplify two-dimensional spectra, for example the

NOESY spectra of protons in macromolecules such as proteins. These experiments rely on a 'round-trip' coherence transfer (18) of the kind

protons → carbon-13 → protons

or

protons → nitrogen-15 → protons.

(See Polarization transfer*.) The third dimension serves to separate a set of proton NOESY subspectra, one for each chemical shift of the heteronuclear species.

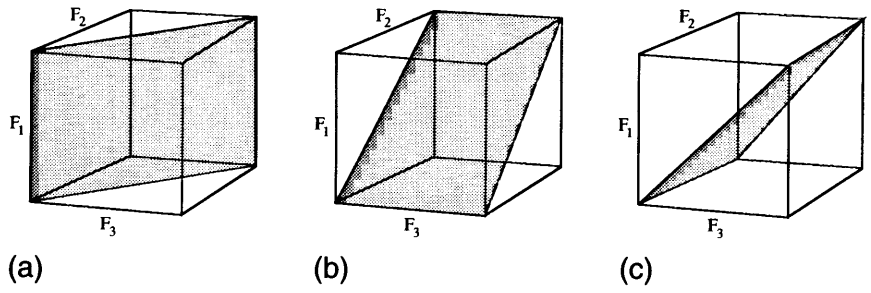


Fig. 5. Selection of particular sections through a three-dimensional spectrum representing two stages of coherence transfer. (a) The first mixing plane, showing transfers of the type I → S → S. (b) The second mixing plane, showing transfers of the type S → S → R. (c) The back-transfer plane showing transfers of the type I → S → I.

IMPACT ON CONVENTIONAL NMR

The ramifications of Jeener's original idea are not limited to multidimensional spectroscopy. It stimulated the new science of 'spin choreography' in which spin systems were manipulated in the most ingenious ways in order to extract useful information. The polarization transfer experiment INEPT (19) is a direct descendant of heteronuclear correlation schemes. Multiplicity determinations in carbon-13 spectroscopy employing spin-echo modulation can be thought of as a projection of the corresponding two-dimensional J-spectrum onto the F_2 axis (20). Many two-dimensional experiments have been converted into one-dimensional counterparts by employing selective radiofrequency pulses (21,22). Even the concept of broadband decoupling* with 180° pulses can be traced back to ideas first advanced in the context of two-dimensional correlation spectroscopy (23). It seems that Jeener's idea, rather tentatively proposed in 1971, has proved one of the most important stimuli for innovation in high-resolution NMR spectroscopy.

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