

Transforming NMR spectroscopy: adding new dimensions

Richard Ernst, this year's winner of the Nobel prize for chemistry, is responsible for several revolutions in NMR spectroscopy.

The phenomenon of nuclear magnetic resonance (NMR) in bulk matter was discovered by Felix Bloch and Edward Purcell in 1946, an event quickly recognized by the award of the Nobel prize for Physics in 1952. In the four decades since, NMR has become indispensable in the practice of chemistry, grown into the most powerful technique for determining three-dimensional structures of biological macromolecules in solution, and developed into an invaluable, non-invasive diagnostic tool in medicine. The evolution of NMR from an esoteric phenomenon in physics to a central technique in chemistry, biology

and medicine has been shaped by three major revolutions. The standard-bearer of these revolutions, Richard R. Ernst, of the Eidgenössische Technische Hochschule (ETH), Zürich, has now been recognized by the award of this year's Nobel prize for chemistry for his contributions to developments in the techniques of high-resolution NMR. Ernst has earned the nickname 'father of FT NMR'; he indeed transformed NMR into one of the most powerful spectroscopic techniques. NMR as it was practised in the pre-Ernst era up to the mid-sixties had already been recognized as an important analytical tool

with many applications in chemistry. High-homogeneity magnets had been developed but NMR being a low-frequency method continued to be plagued by low sensitivity, an inevitable consequence of the use of the continuous-wave (CW) method of its inventors of the late forties.

During the sixties Ernst transformed NMR from a frequency-domain method to a time-domain method, changing completely the practice of NMR, dramatically increasing its use, opening up applicability to biomolecules and to low-sensitivity nuclei, and to the design of many new experiments. During the

seventies he literally added a second dimension to NMR, making it one of the most versatile physical techniques. His multitudinous pioneering contributions to the development of two-dimensional (2D) NMR—over 150 papers on 2DNMR and its applications with every major technique originating from his laboratory—are responsible for bringing the field to a state of full maturity. It has now become possible for several groups using the 2D algorithm to obtain three-dimensional structures of biomolecules in solution by use of two-dimensional NMR. Ernst's Fourier method of NMR imaging, which itself has created an excitement in medical diagnostics, is the preferred basic algorithm for medical magnetic resonance imaging.

Ernst, having brought about most significant changes in the technology and methodology of NMR, is an undoubted leader in the field and is still continuing at a feverish pace, and most likely will be responsible for future breakthroughs. The following is an account of his most significant contributions, an incomplete and subjective selection influenced by personal involvement.

Fourier transform (FT) NMR in one dimension

Often the interplay of interdisciplinary forces has a profound impact on the practice and applicability of science. Ernst, a chemist, picked up enough fundamentals of electrical engineering, which he applied to the physics of magnetic resonance. His brilliant demonstration that the excitation of all resonances of a spectrum by an impulse and analysis of the response using a Fourier transform is a much more efficient way of doing spectroscopy first revolutionized the practice of NMR and later other fields. The impact of this finding was felt rather slowly—because it required a dedicated minicomputer with every NMR machine—but eventually it proved to be the much-needed breakthrough in the field. It changed the practice of NMR from the frequency to the time domain, leading to the design of many new experiments, opening the field to low sensitivity nuclei such as naturally occurring carbon-13 and application of NMR to biomolecules, areas that were earlier in a nascent state

owing to severe sensitivity limitations. Ernst not only demonstrated the superior sensitivity of the FT technique in a most emphatic paper in *Review of Scientific Instruments*¹, (now a citation classic), but also completed the full story by documenting the optimum way of doing these experiments; calculating from signal-to-noise ratio considerations the optimum flip angle of the impulse—now famous as the Ernst angle—; developing the algorithm for automatic phase correction and the use of matched filters for enhancement of resolution and sensitivity; documenting by detailed analysis advantages of signal averaging; demonstrating decoupling by noise modulation of radio frequency; etc.—all the nuts and bolts required for the full



Major advances in theory and practice of NMR

fledged practice of the field. NMR, which had revolutionized the practice of chemistry, was itself revolutionized. Soon the multimillion-dollar industry turning out high-resolution NMR spectrometers largely gave up making conventional spectrometers and switched to FT NMR spectrometers with dedicated minicomputers as integral components. The first of the revolutions charted by Ernst was thus complete.

Two-dimensional NMR and NMR imaging

On his return from the US in the late sixties Ernst set up his laboratories at ETH, Zürich, to continue his work on

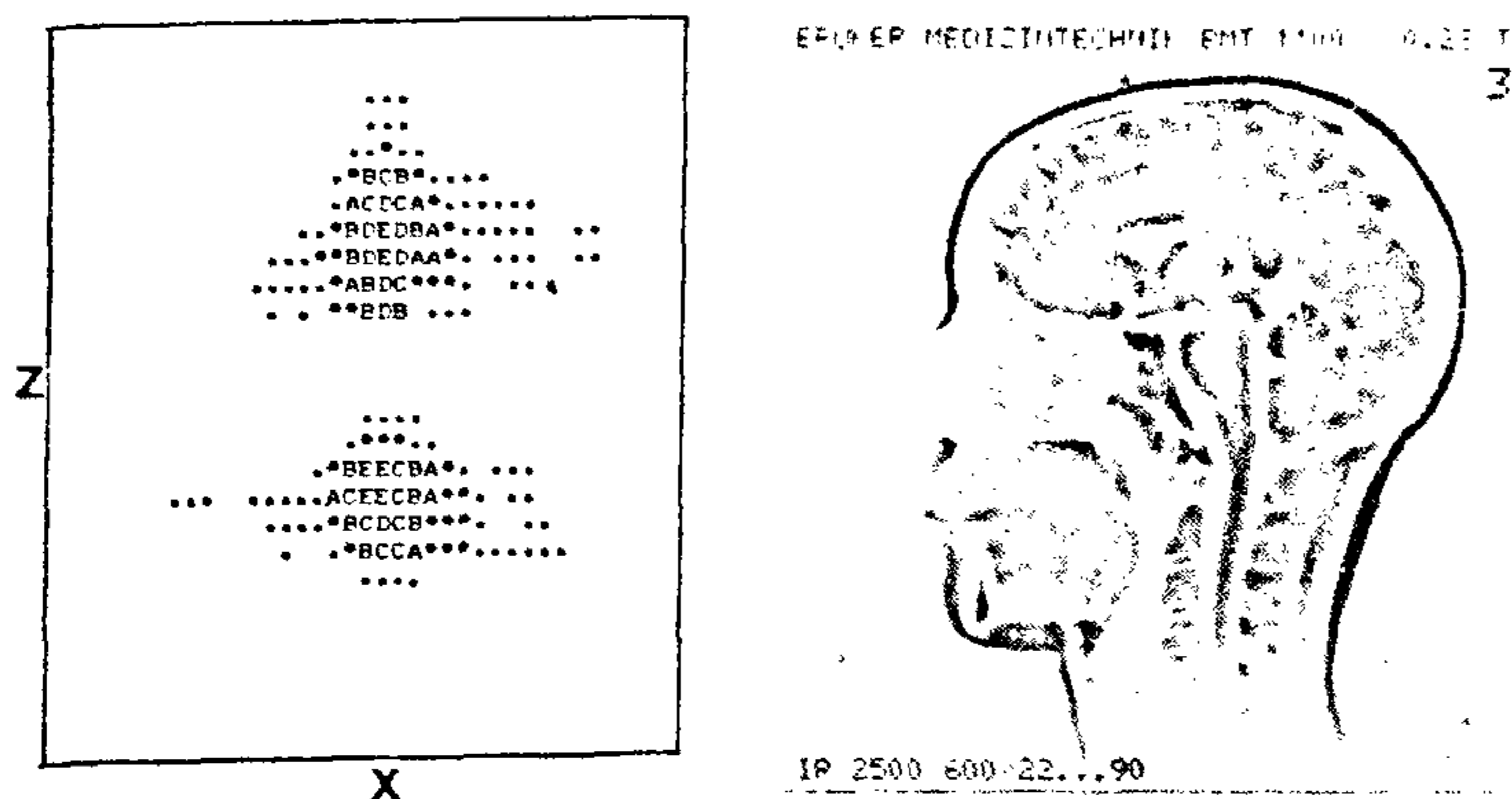
various techniques and methodology of NMR. During the seventies two major developments took place in the field of NMR, both benefitting tremendously from the work of Ernst.

The development of 2DNMR can rightly be called the second revolution in NMR. At the end of the sixties and in the early seventies it looked as if the development in NMR technology had been completed, with double-resonance techniques reaching a state of full maturity and FTNMR experiments becoming routine. Applications in biophysics were picking up rapidly, even though the algorithm tended to be tedious. 2DNMR infused fresh blood into application of NMR to biological systems.

The beginning of 2DNMR was rather slow. The idea, an esoteric one at first sight, was put up by Jeener in a summer school in 1971, and nothing happened for a couple of years. I remember Ernst telling me in late 1973 that he had been thinking about the idea but did not want to step on Jeener's toes if he was continuing on that theme—a case of high, and these days rare, scientific ethics. I was of the opinion that a two-year period was long enough. Even then Ernst waited for some more time; then, in 1974, he first chose an experiment in which there was a very different kind of application of the 2D Fourier-transform algorithm from the one envisaged by Jeener. He developed a powerful technique for NMR imaging².

NMR imaging had been just discovered by Paul Lauterbur, who introduced a projection-reconstruction algorithm. Ernst immediately showed that it is much better to do imaging by using Fourier spectroscopy in two as well as three dimensions², and introduced an algorithm which is now used in all modern human and other magnetic resonance imaging (MRI) instruments.

Ernst then focused his attention on spectroscopy, and in 1975 he embarked on the elaborate development of 2D NMR spectroscopy. The very first experiment, published in late 1975, which resolved the multiplets of heteronuclear coupled spin systems into two dimensions, demonstrated the power of the technique³. This was followed by a comprehensive paper on 2DNMR spectroscopy (another citation classic), which still is the last word on the subject⁴. It is

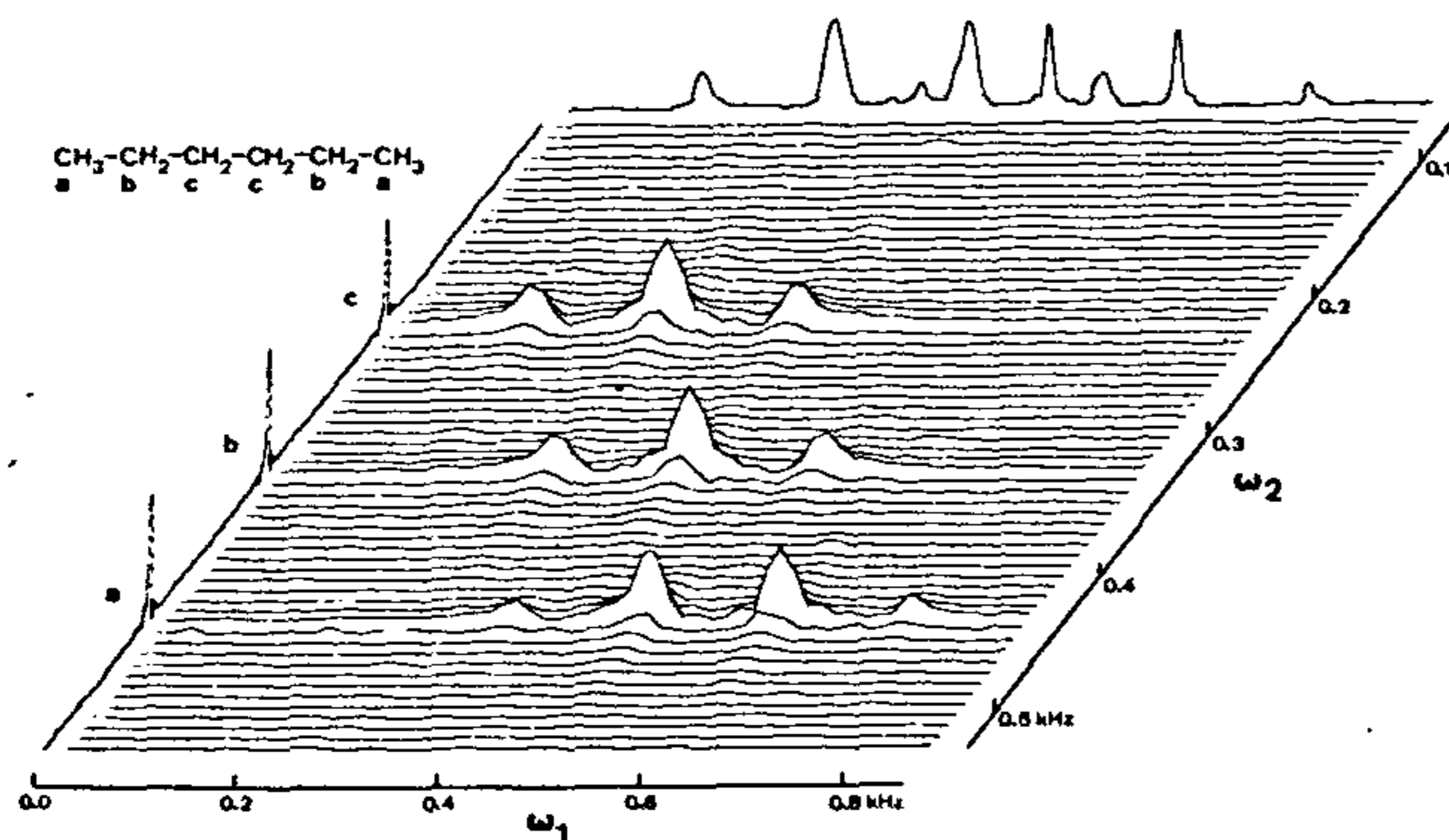


Magnetic resonance imaging. *Left:* The first 2D Fourier NMR image, obtained in 1974, of two 1-mm-diameter glass capillaries filled with water and surrounded with D_2O , and placed so that their axes were parallel to the y -axis and the line joining their centres parallel to the z -axis. The figure, called a 2D Fourier zeugmatogram, plots signal intensity as a function of x and z , increasing in the order $\bullet, *$, A, B, C, D, E. The figure shows only the central portion of the complete zeugmatogram, clearly 'imaging' the samples in cross-section. (From ref. 2)

Right: A modern magnetic-resonance image of a human head. (Courtesy, Bruker Instruments)

amazing how much insight he reached in that paper: it not only contains the basic theoretical framework of 2D spectroscopy, with full analysis of intensities, phases and connectivity of transitions, but also contains details on line shape, flip angle, strong coupling, relaxation and field-inhomogeneity effects. The

paper also contains the most original ideas on multiple-quantum transitions, their excitations in pulsed spectroscopy by coherence transfer from single-quantum transitions and indirect detection using the 2D algorithm—all supplemented with detailed experimental spectra. The foundation of 2D NMR

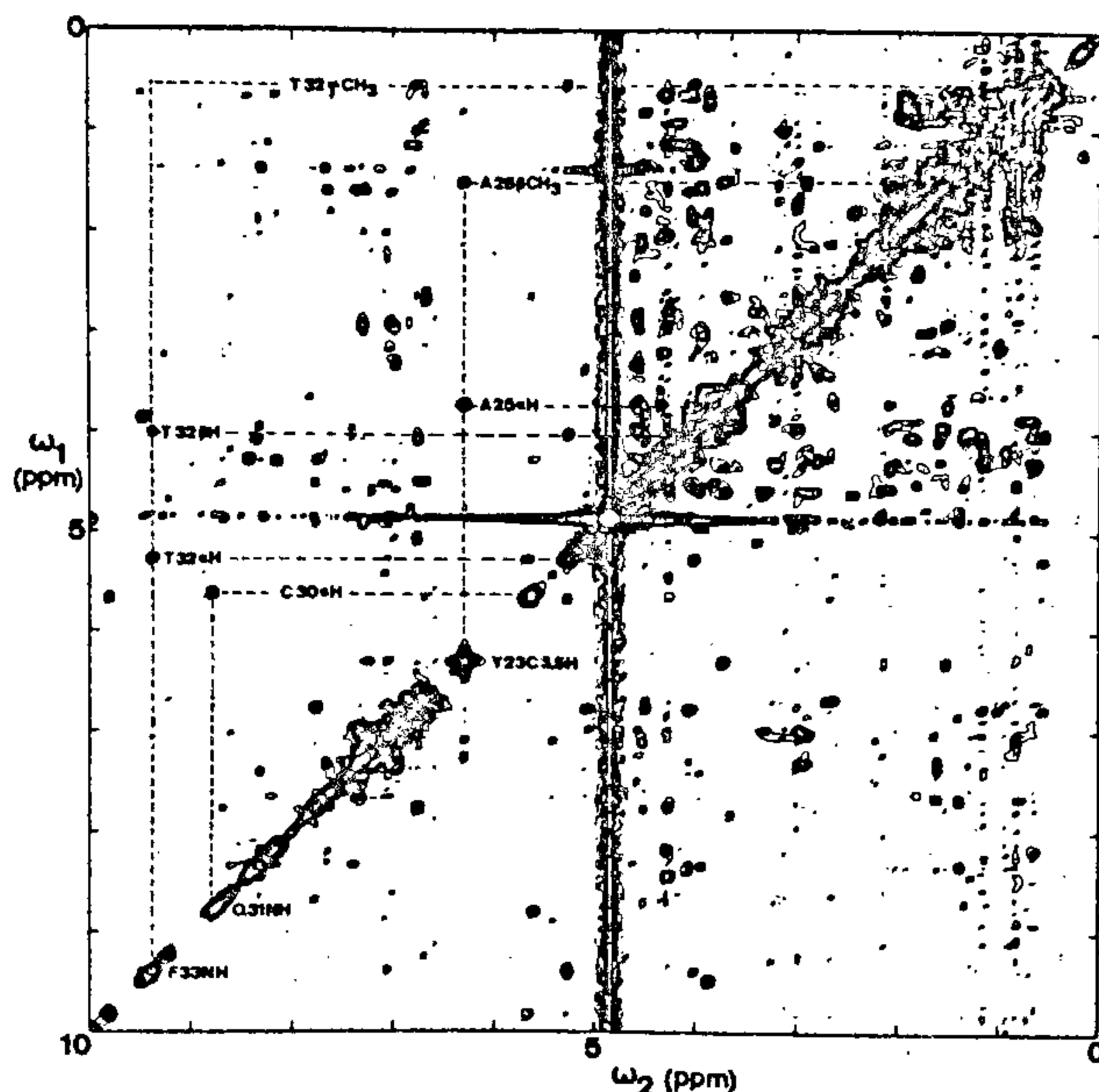


Double resonance. The first 2D NMR spectrum, recorded in 1975: 2D-resolved ^{13}C spectrum of n -hexane. In such an experiment, called proton noise decoupling, the range of proton frequencies is irradiated with a strong field while simultaneously irradiating the ^{13}C carbon nuclei with the 'observe' frequency. This 'decouples' the carbon nuclei from the protons (hence heteronuclear decoupling), and results in ^{13}C signals that are effectively an average of the different signals due to coupling with different spin states of nearby protons. Proton decoupling in ^{13}C NMR results in simplification of the spectra: doublet and multiplet peaks are reduced to single, sharp peaks. The figure shows a resolution in two dimensions of a 2D ^{13}C spectrum. The undecoupled 1D spectrum is shown along the ω_1 axis and the proton-decoupled spectrum along the ω_2 axis. Each peak in the resolved 2D plot corresponds to one multiplet line of the undecoupled spectrum (ω_1 axis) displaced in the ω_2 direction according to the corresponding carbon. There are three pairs of equivalent carbons a, b, c in the molecule. (From ref. 3).

spectroscopy was thus firmly laid.

Development of 2D NMR spectroscopy rapidly took off, in Ernst's laboratory and in many others the world over. In Ernst's laboratory, in particular, the heteronuclear J -resolved spectroscopy was modified to remove redundancies; the 2D resolved spectroscopy was extended to homonuclear coupled spin systems and for the first time self-decoupled proton spectra were obtained. Correlated 2D spectroscopy was extended to heteronuclear coupled spins and the basic algorithm for shift-correlation spectroscopy established. Coherence-transfer processes were studied in detail and the foundation for sensitivity enhancement by coherence transfer established. Great strides were made in the study and use of multiple-quantum 2D spectroscopy. The usefulness of multiple-quantum transitions was highlighted with respect to their relaxation properties and their phase dependences exploited for selective detection of various multiple-equation orders. At this point a crucial collaboration began between Ernst and his biophysicist colleague Kurt Wüthrich, also at ETH Zürich. It was quickly recognized that the powerful 2D techniques can be of great value in simplifying the complex spectra of biomolecules and in obtaining additional information from them. This collaboration provided some of the most significant applications of 2D spectroscopy and in turn fuelled its phenomenal growth. 2D J -resolved spectroscopy was first applied to proteins and then spin-echo correlated 2D spectroscopy was developed for assignment of resonance to specific atoms in the case of biomolecules.

A key breakthrough took place in the application of 2D techniques to biomolecules at this juncture. A very significant development was published from Ernst's laboratory in 1979, namely a 2D experiment in which the transfer of magnetization takes place via incoherent processes such as chemical exchange or nuclear Overhauser effects (NOE)⁵. NOE provide key structural information to biophysicists, who had been doing literally hundreds of such experiments using one-dimensional FT NMR. The application of 2D techniques to the study of biomolecular NOE proved to be a turning point in application of 2D NMR to biology⁶. The conventional 2D correlated experi-



Structure determination. The first 2D NOE spectrum of a macromolecule recorded in 1980: the proton 2D NOE spectrum of the protein bovine pancreatic trypsin inhibitor at 360 MHz. The informative features are the off-diagonal 'cross-peaks' seen throughout. These represent coupling of magnetization due to NOE between nearby protons. A few such connectivities are indicated in the figure by broken lines and the cross-peaks are identified by the one-letter code for the amino acids whose protons are responsible for the signal, their position in the amino-acid sequence of the protein, and the type of protons generating the signal. A complete map of NOEs is thus useful in determination of three-dimensional structure.

ment, by now named COSY, was also rejuvenated, and an algorithm for using 2D NOE and COSY in an interactive manner was developed for resonance assignments and for obtaining structural information on biomolecules. The initial build-up rate of the 2D NOE signal provided distance information and was used for obtaining structural information. Detailed analysis of 2D NOE and exchange experiments in systems with spin-spin coupling were carried out. A great revolution thus started is flourishing even today and is providing three-dimensional structures of proteins and oligonucleotides in solution⁷.

The eighties saw a continued upsurge in the research emanating from Ernst's laboratory, perhaps partly contributed by the coming together under his umbrella of several outstanding scientists, three of whom he fondly referred to as his 'pulse engineers'. Not only was the 2D activity continued at a feverish pace, but work on solid-state NMR and electron spin resonance also picked up significantly and many important contributions were made to the practice

of magnetic resonance. Notable in the work of this period are: 2D 'accordion' spectroscopy; uniform excitation of multiple-quantum coherences and their use in resonance assignments; development of multiple-quantum filters, leading to the famous double-quantum-filtered COSY experiment; and development of versatile digital RF phase shifters. Ideas such as coherence level diagram were extremely helpful in elucidating the coherence-transfer phenomenon, in design of phase shifts, and in obtaining pure-phase 2D spectra. The coherence-transfer process was extended to remote spins, first by a 'relay' later by 'isotropic mixing'. A very elegant 'product-operator' formalism⁸, which makes the gymnastics of the spin under chemical shift, spin coupling and RF pulses easy to calculate, fuelled rapid development of many new 2D experiments, and is widely used in research laboratories and classrooms.

The hallmark of Ernst has been to complete the revolution begun. He provides every nut and bolt, pointing out every pitfall and remedy. To 2D

spectroscopy also he provided the most solid foundation. A natural extension to three-dimensional NMR had to await its necessity and an intervention by the master⁹.

A very fertile, though somewhat difficult, field is NMR of solids. Ernst was the first to observe, in 1974, transient oscillations in the cross-polarization process arising from a dominant dipolar coupling¹⁰. He investigated shift tensors, resolving them in two dimensions, and studied modulation of solid echoes. More recently he has reexamined spin dynamics in solid-state cross-polarization experiments and has given a better interpretation of the dynamics. Several papers dealing with multiple-pulse line narrowing sequences and composite pulses have thrown additional light on these topics. A consequent extension to rotating-frame coherence transfer and NOE transfer has been studied in detail in his laboratory. Work on time-domain electron spin resonance (ESR) spectroscopy has been initiated, first by building the fast electronics needed for these studies and then by monitoring the electron spin-echo amplitudes. Many 2D experiments first discovered in NMR have now been extended to the much more difficult field of ESR.

Ernst has written a monumental monograph on principles of one- and two-dimensional NMR¹¹. It took him nearly a decade to complete the book, primarily owing to his critical nature and his desire to bring out an everlasting document in a subject that was undergoing a metamorphosis during that period. The book contains detailed chapters on one- and two-dimensional NMR—the first such comprehensive analysis of these spectroscopies, particularly of 2D NMR. Undoubtedly this book is going to be the last word on the subject, and in my opinion will remain so as long as NMR spectroscopy is practised in the present form.

Born in 1933 in Winterthur, Switzerland, Ernst obtained a PhD in physical chemistry at ETH Zürich in 1962. After five years in the USA, Ernst joined the faculty at ETH, and became professor in 1976. Ernst's preeminence as the foremost worker in the field of magnetic resonance has been well recognized and during recent years he has been a popular invited speaker at all major conferences in the field. In

1983 Ernst was awarded a gold medal of the Society of Magnetic Resonance in Medicine. In 1985 he was honoured with an honorary doctorate by ETH Lausanne, and was awarded the Marcel-Benoist Prize in 1986. During 1988 he was R. B. Woodward visiting professor at Harvard University, USA. In 1991 he was awarded the prestigious Wolf Prize. Ernst has visited India twice in recent years, to lecture at the Tata Institute of Fundamental Research, Bombay, in 1984 and the Indian Institute of Science, Bangalore, in 1986.

Ernst is one of those fine people who combine humility with knowledge. He is an extremely polite person and a thorough gentleman. This is exemplified by his characteristic style of putting the names of all his associates prior to his own name in all his publications even though his own personal contribution may be the largest. He also has the rare quality of combining perfection with

polite persuasion. Like all Swiss, he loves mountains and is an expert guide. He has deep interest in Western classical music and is a lover of fine arts. He has a large collection of Tibetan *tankas* (religious scroll paintings), and his wife once remarked (when they did not own a car in the early seventies) that whenever they had enough money (to buy a car) he went and bought a painting. I wonder what he will do with the Nobel money!

1. Ernst, R. R. and Anderson, W. A., *Rev. Sci. Instrum.*, 1966, 37, 93.
2. Anil Kumar, Welti, D. and Ernst, R. R., *J. Magn. Reson.*, 1975, 18, 69.
3. Muller, L., Anil Kumar and Ernst, R. R., *J. Chem. Phys.*, 1975, 63, 5490.
4. Aue, W. P., Bartholdi, E. and Ernst, R. R., *J. Chem. Phys.*, 1976, 64, 2229.
5. Jeener, J., Meier, B. M., Bachmann, P. and Ernst, R. R., *J. Chem. Phys.*, 1979, 71, 4546.

6. Anil Kumar, Ernst, R. R. and Wuthrich, K., *Biochem. Biophys. Res. Commun.*, 1980, 95, 1.
7. Wuthrich, K., *NMR of Proteins and Nucleic Acids*, Wiley, New York, 1986.
8. Sorensen, O. W., Eich, G. W., Levitt, M. H., Bodenhausen, G. and Ernst, R. R., *Prog. NMR Spectrosc.*, 1983, 16, 163.
9. Griesinger, C., Sorensen, O. W. and Ernst, R. R., *J. Magn. Reson.*, 1987, 109, 7227.
10. Muller, L., Anil Kumar, Baumann, T. and Ernst, R. R., *Phys. Rev. Lett.*, 1974, 32, 1402.
11. Ernst, R. R., Bodenhausen, G. and Wokaun, A., *Principles of Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford, 1987.

Anil Kumar, Department of Physics and Sophisticated Instruments Facility, Indian Institute of Science, Bangalore 560 012

Membrane ion channels singled out

This year's Nobel prize for physiology or medicine celebrates a technique that allows detailed study of membrane function.

The study of ion transport across biological membranes is a paradoxically old field: Nasse described the exchange of anions across red blood cell membranes a decade before Arrhenius proposed his ionic theory. Nasse actually talked of the acids rather than anions *per se*. The area has remained quirky ever since, with the study of ion channels proceeding full steam ahead at a time when the existence of the biological membrane itself was under debate. It has had its share of luminaries, such as Luigi Galvani who demonstrated that electrical stimulation could mimic nervous excitation for muscle contraction, Hodgkin and Huxley who dissected the ionic components of the action potentials of nerve, and, more recently, Neher and Sakmann who devised techniques to study the movement of ions through individual channels in biological membranes.

The biological membrane is oil-like (lipophilic) in composition and presents

a formidable barrier to passage of charged species like sodium or calcium ions. Most membranes have structures that form molecular conduits through the membrane, filled with water, which permit easy passage of ions. The channels responsible for the action potential in nerve are capable of passing many millions of ions per second while retaining the ability to distinguish between species as similar as sodium and potassium ions. The traditional method of studying these molecules was to insert electrodes into cells and monitor the passage of ions through the entire ensemble of channels in the membrane as an electrical signal that could be detected with appropriate electronics (currents of the order of nanoamperes to microamperes were seen).

A very few channels could be purified to homogeneity and incorporated into artificial bilayer lipid membranes (BLMs). Electrodes could then be placed in the aqueous compartments on either

side of the BLM and currents measured. This limited study to a few well-characterized membrane channel proteins and was carried out in an artificial setting. However, by playing around with the concentration of the channels in the BLM, it was sometimes possible to observe transport through a single open channel at a time (single-channel currents). This provided a wealth of information that forms the basis of our current thinking about the molecules that make up such channels. Neher and Sakmann pioneered a method (the patch clamp) for study, at a similar resolution, of channels in their native setting.

The patch-clamp technique involves pressing a pipette against a cell membrane and generating a seal that is tight enough to prevent the passage of ions between the pipette and the membrane. If an electrode is introduced into the pipette, the only current that can be detected will be that passing through