

Image-Selected *in Vivo* Spectroscopy (ISIS). A New Technique for Spatially Selective NMR Spectroscopy

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A method of spatial localization is described which is particularly suitable for the *in vivo* spectroscopic investigation of biological and medical samples. The technique overcomes most of the technical problems associated with localized NMR spectroscopy and allows the spectrum to be investigated from a cube which can be positioned by reference to an NMR image. The cube can be reduced or enlarged, and can be rapidly moved in space to investigate further volumes of interest within the sample. The first experimental results from a phantom and the human leg are presented. © 1986 Academic Press, Inc.

INTRODUCTION

During the past few years several techniques have been reported which allow the spectroscopic investigation of limited volumes of material within a sample (1-22). Topical magnetic resonance (TMR) (1, 2) was one of the first methods proposed, and while many notable results were obtained using this technique in combination with surface coils (3), its limitations are that the selected volume is poorly defined and not moveable in position. Techniques based upon imaging principles have more recently been successful in generating chemical-shift images of samples, and show the spatial distribution of the chemically shifted resonances of a particular nucleus (4-12). These methods all suffer from the disadvantage that a long experimental time is usually necessary, typically in excess of 10 min, which excludes their application in most time-course studies. Chemical-shift imaging techniques use pulsed magnetic field gradients which in the standard configuration of superconducting magnets generate sufficiently large eddy currents upon gradient removal to temporarily degrade the field homogeneity. This is one of the reasons why the implementation of high-resolution spectroscopy by most chemical-shift imaging methods is difficult. The field homogeneity is recovered after a time delay of approximately 10 ms; therefore most chemical-shift imaging experiments utilize a spin echo, and the observed spectrum has a T_2 weighting factor for each resonance. Other methods rely on rf field nonuniformity in the form of gradients (13), or localized maxima in rf field strength sometimes in combination with multipulse techniques (14-19). While these methods do not require magnetic field gradients, the volume of selected spins is usually irregular in shape, and in most cases difficult to vary in position. Selection to a cube of material has been achieved by use of the VSE method (20); however, this technique suffers from the disadvantage that high-power rf pulses are required which must produce accurate nutation angles over the whole sample. If the pulse angle is incorrectly set then the excited nuclei

outside the cube of interest contribute a far larger signal than those nuclei contained within the selected cube. Consequently this technique cannot be used with rf coils which have an inhomogeneous field distribution.

A new technique which we have called ISIS, (image selected *in vivo* spectroscopy), overcomes most of these problems. Cubic volumes can be selected by the differencing of NMR signals from eight experiments, which results in coaddition of NMR data from within the cube and cancellation of signals from all external regions. The technique does not require accurate spin nutation angles and can be implemented in combination with rf coils which show gross rf inhomogeneity, for example, surface coils. The selected volume may be easily varied in all its dimensions and may be accurately positioned and repositioned under computer control by movement of a cursor over a standard NMR image of the specimen. Selection is complete after a single sequence of eight experiments, and the NMR spectrum does not contain any T_2 distortion with only a slight dependence of signal strength upon T_1 value. The technique is particularly suitable for *in vivo* spectroscopic investigations of living samples.

THEORY

The method relies on the principle of selective inversion of the spin population prior to data accumulation. A one-dimensional version of the method has been presented elsewhere (21, 22) and, in this form, requires only two experiments. In the first experiment the total spectrum is acquired from all nuclear spins within the field of view of the rf receiver/transmitter coil following application of a single rf pulse. The second experiment commences with the inversion of the spin population in a selected slice of the specimen by application of an amplitude modulated selective pulse in combination with a magnetic field gradient. This preparation pulse is followed after a suitable time delay by a signal acquisition as performed in the first experiment. Subtraction of the two free induction decays gives only the signal contribution from the spins perturbed by the selective 180° rf pulse, while signal contribution from unperturbed spins is canceled. The dependence of selected signal strength upon nutation angle θ of the selective preparation pulse is therefore $(1 - \cos \theta)$ with the maximum signal strength being obtained for a nutation angle of 180° . Provided the selective pulse nutates the selected spins through 180° , there is twice the signal contribution from the slice as obtained in a single experiment, and therefore the full signal-to-noise ratio (S/N) is realized in the final NMR spectrum.

The method has been extended further to include three dimensions of spatial selection by incorporation of three selective rf pulses in the preparation period prior to data accumulation. Each of the three pulses is applied in conjunction with a magnetic field gradient, and the gradients are applied along the three Cartesian axes, x , y , and z , to provide three dimensions of spatial selection. Eight experiments are required to localize the spatial distribution of NMR signal to a cube of material, and the experimental sequence of selective pulses for the eight experiments is summarized in Table 1.

The rf pulse timing sequence and associated gradient sequence for experiment 8 is shown in Fig. 1. Each selective rf pulse is amplitude modulated with a sinc function, and negative rf powers are produced by 180° phase inversion of the applied carrier

TABLE 1
Experimental Sequence of Selective rf Pulses Applied during the Preparation Period

Experiment number	x-selective pulse	y-selective pulse	z-selective pulse	Contribution to total spectrum
1	OFF	OFF	OFF	+1
2	ON	OFF	OFF	-1
3	OFF	ON	OFF	-1
4	ON	ON	OFF	+1
5	OFF	OFF	ON	-1
6	ON	OFF	ON	+1
7	OFF	ON	ON	+1
8	ON	ON	ON	-1

Notes. OFF refers to the absence of a selective pulse, and ON refers to the application of a selective pulse of sufficient power to cause 180° spin nutation.

frequency. This gives an excitation spectrum with an approximately rectangular distribution of frequency components and a spectral width related to the duration of the pulse. The width of the cube along any axis may therefore be varied either by adjustment of the selective rf pulse length, or by adjustment of the size of the respective field gradient.

Let us consider the effect on the spatial distribution of z magnetization following each of the experiments in Table 1. We can readily see the effect of the pulse train by considering three planes of spins, corresponding to a plane above the desired cube, a plane below the cube, and the central plane containing the cube. Figure 2 shows the three planes diagrammatically, and each plane has been further divided into three rows and three columns, corresponding to rows either side and including the cube along the y axis, and columns either side and including the cube along the x axis. The cube to be localized is therefore represented by the center square of the center plane, and each square we can assume has an equal net spin magnetization of unity.

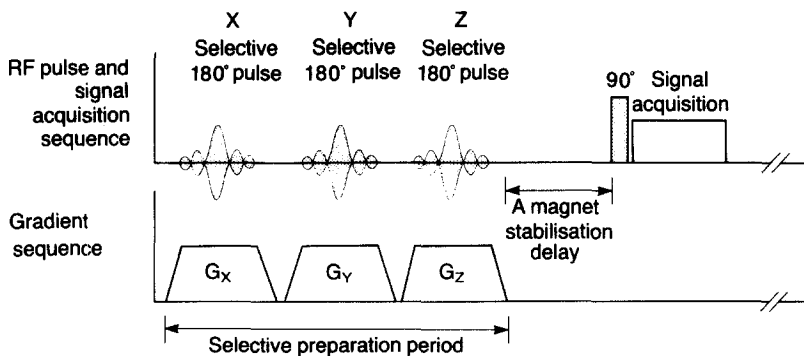
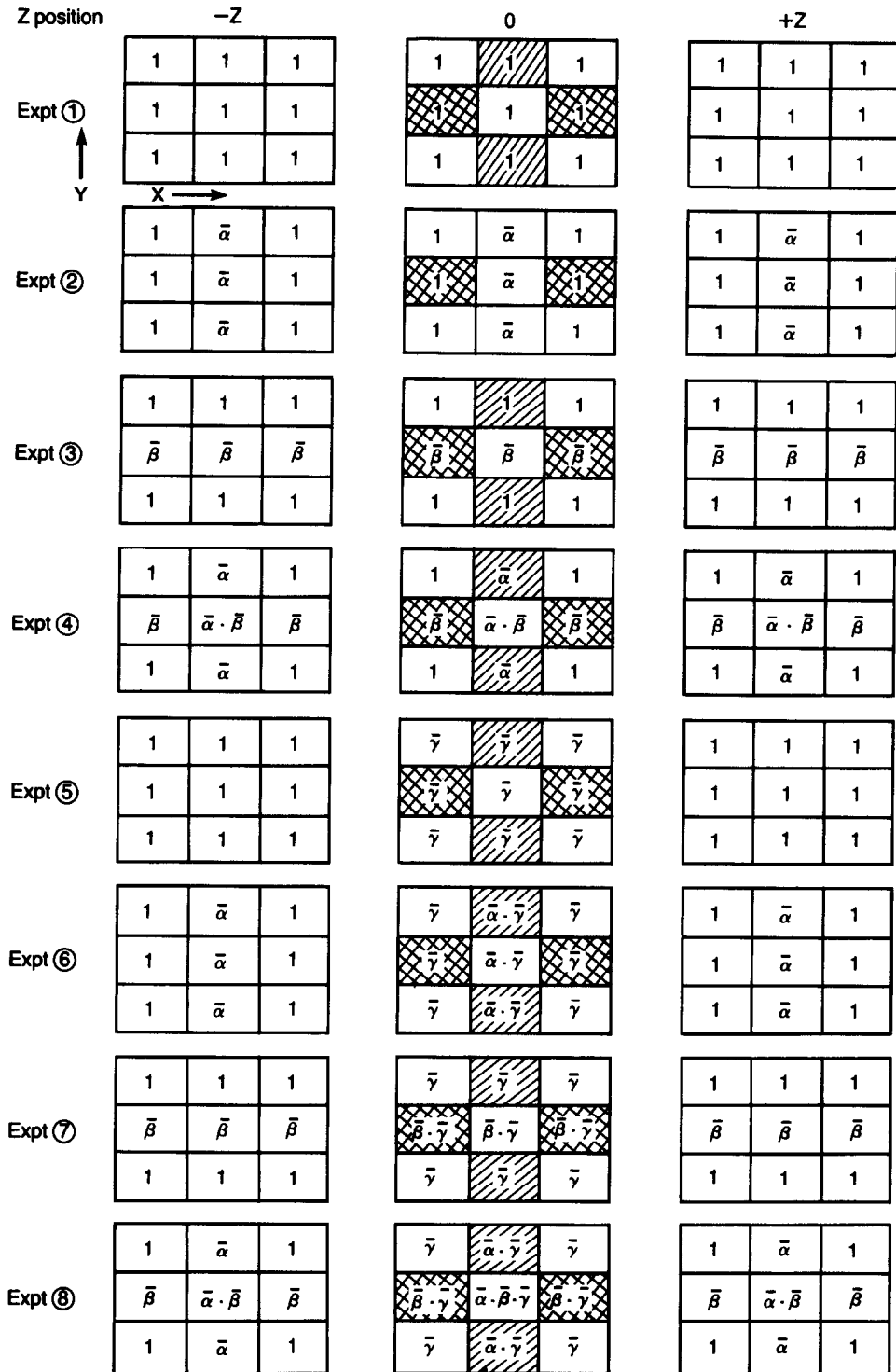


FIG. 1. Radiofrequency pulse and magnetic field gradient sequences for the ISIS experiment. Selective inversion of three orthogonal slices is shown during the preparation period (i.e., Table 1; experiment 8). All other experiments in the ISIS sequence may be constructed by omission of one or more of the selective inversion pulses.



The spin nutation angles for the x , y , and z selective pulses are defined as α , β , and γ , and the measured signal during the subsequent acquisition period has the magnitude and sign of the residual z magnetization following the selective preparation pulse train. We can also assume that any xy components excited by application of the selective pulses dephase either between pulses or before signal acquisition. The residual z magnetization is simply the product of the cosines of the individual nutation angles experienced during the preparation period by the spins in the various regions of the sample. Figure 2 therefore shows the three-dimensional signal distribution in each of the eight experiments, that will appear upon investigation by a subsequent nonselective 90° pulse.

To achieve the desired localization, a linear combination of the eight sets of data must be sought in which all signals cancel except in the selected cube. For the areas in the sample indicated in Fig. 2 by shaded squares the total signal is

$$(C_1 + C_3) + (C_2 + C_4)\cos \alpha + (C_5 + C_7)\cos \gamma + (C_6 + C_8)\cos \alpha \cdot \cos \gamma \quad [1]$$

where C_i denotes the coefficient of the i th experiment in the linear combination of the data from all eight experiments. Expression [1] is zero for all values of α and γ only when

$$(C_1 + C_3) = (C_2 + C_4) = (C_5 + C_7) = (C_6 + C_8) = 0. \quad [2]$$

Analogously for the cross-hatched squares in Fig. 2 we find the total signal is

$$(C_1 + C_2) + (C_3 + C_4)\cos \beta + (C_5 + C_6)\cos \gamma + (C_7 + C_8)\cos \beta \cdot \cos \gamma \quad [3]$$

which vanishes for all values of β and γ if

$$(C_1 + C_2) = (C_3 + C_4) = (C_5 + C_6) = (C_7 + C_8) = 0. \quad [4]$$

Arbitrarily choosing $C_1 = +1$ we find from Eqs. [2] and [4]

$$C_1 = C_4 = C_6 = C_7 = +1 \quad [5]$$

and

$$C_2 = C_3 = C_5 = C_8 = -1 \quad [6]$$

as indicated in the last column of Table 1.

Inspection shows that with this linear combination of the eight experiments of Fig. 2, the signals cancel in all squares except in the one corresponding to the selected cube. This cancellation occurs independent of the values of α , β , or γ and therefore the residual signal must always come from the central cube. A further consequence of this cancellation property is that the technique may be used in combination with rf coils which have a highly nonuniform rf distribution and intensity, e.g., surface coils. The overall signal intensity I , from the linear combination of all eight experiments is given by

FIG. 2. Matrices representing three-dimensional space and showing planes of material below, including, and above the selected cube. Each plane is further divided into rows and columns on either side and including the volume of interest. The selected cube is therefore the central region of the center plane, and the diagram shows the z magnetization in the different regions following the preparation periods of the eight experiments of Table 1, $\bar{\alpha}$, $\bar{\beta}$, and $\bar{\gamma}$ represent $\cos \alpha$, $\cos \beta$, and $\cos \gamma$, respectively, and therefore $\bar{\alpha} \cdot \bar{\beta} \cdot \bar{\gamma}$ represents the product of these three cosines.

$$I = 1 - \cos \alpha - \cos \beta + \cos \alpha \cdot \cos \beta - \cos \gamma + \cos \alpha \cdot \cos \gamma + \cos \beta \cdot \cos \gamma - \cos \alpha \cdot \cos \beta \cdot \cos \gamma. \quad [7]$$

If $\alpha = \beta = \gamma = 180^\circ$ then $I = 8$, and the overall signal is eight times the signal contribution from the central cube in a single data acquisition, which is equivalent to a signal average of eight performed on the central cube in isolation. The variation of signal from the cube with spin nutation angle when $\alpha = \beta = \gamma$ is shown in Fig. 3.

The selected volume may be easily moved in three-dimensional space along any direction by either adjustment of the spatial position of the gradient zero-crossing point, or more conveniently, by adjustment of the rf frequency carrier during the respective selective rf pulse. In this manner, the desired location for the selected cube may be encoded as three frequency offsets corresponding to the three spatial axes. A standard NMR image provides spatial information encoded along two orthogonal frequency axes; therefore the position of the cube can be easily selected by reference to the NMR image.

The technique is insensitive to the T_2 values of the resonances under investigation, but does show a slight T_1 weighting in the spectrum, caused by the delay between selective preparation and signal acquisition. This delay is to allow for the decay of magnet eddy currents following gradient switching, and can be as short as 10 ms. Since most biological T_1 values are of the order of several hundreds of milliseconds, this should not present any problem for the investigation *in vivo* of spectra from living samples. Furthermore, T_1 relaxation will not affect the cancellation of signal upon differencing of data, provided a sufficiently long inter-experiment delay time is used to allow for complete relaxation between experiments. If it is necessary to perform signal averaging with a short delay time, this can be performed efficiently by signal averaging of each of the eight experiments separately, with a sufficient relaxation delay time between sequences to allow for complete cancellation of saturation effects.

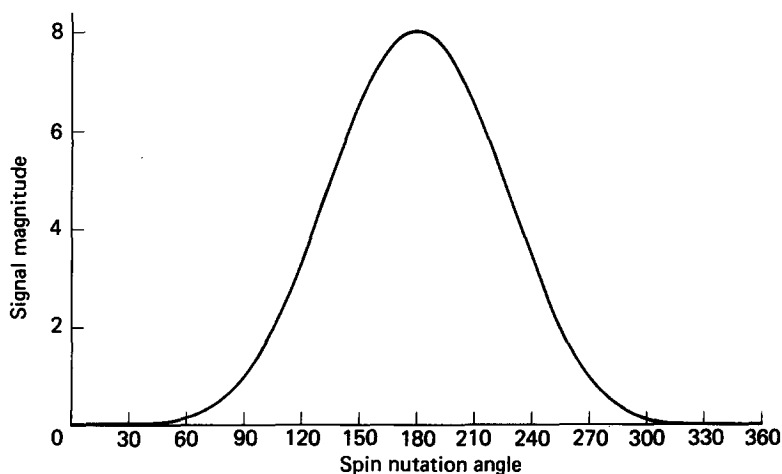


FIG. 3. Dependence of the signal intensity from the selected cube on the spin nutation angle for the full three-dimensional ISIS experiment. The spin nutation angles for the inversion pulses in all three directions are assumed to be identical. The maximum signal occurs when the spin nutation angles are 180° .

EXPERIMENTAL RESULTS

All experiments were performed on a standard Biospec 1.89 tesla imaging/spectroscopy system, with a clear bore of 26 cm. The images were obtained as 128×128 matrices, using a spin-echo 2D FT imaging technique, with a spectral width of 16 kHz and an imaging time of 3 min. All selective pulses were of 6 ms duration producing a spectral width of 1600 Hz. They were applied in a field gradient of 0.25 G/cm giving a 1.6 cm slice thickness along all three directions. The spectroscopy experiments were performed by using the standard imaging coil, (a 12 cm diameter slotted tube resonator), as a transmitter/receiver coil. The Biospec can be programmed to modify the transmitter frequency within a period of 400 μ s, which allows a separate frequency offset for each of the three selective rf pulses. In this manner, the selected cube can be moved in three dimensions to investigate a volume of material outlined on the NMR image. It is essential that following the frequency adjustments during selective preparation, the rf synthesizer does not introduce any phase errors upon returning to the frequency offset for on-resonance signal acquisition. A cursor is used to center the cube on the desired position, and a box is displayed for any given setting of the rf pulse length to show the extent of the volume to be selected. The positional information is translated into frequency offsets which are then automatically stored in a frequency list to be used by the ISIS localization sequence.

Since all the positional information is encoded in the selective pulse frequency offsets, it is essential that readout gradients in the NMR imaging experiment are identical to the plane selection gradients in the ISIS technique. Also the frequency and duration of the imaging plane selection pulse and the size of its associated gradient, must match the corresponding parameters in the subsequent ISIS experiment. This assumes, of course, that combined proton spectroscopy and proton NMR imaging are to be performed.

Figure 4 illustrates the volume-selection procedure and shows localized spectra from a phantom consisting of three spheres of 4 cm diameter. Sphere 1 contains water, sphere 2 methanol, and sphere 3 toluene. In Fig. 4A a standard image of the phantom is shown together with its total proton spectrum. In the spectrum, the respective peaks are from the protons in the aromatic ring of toluene (I), water (II), the methanol methyl group (III), and the toluene methyl group (IV). The resonance peak of the methanol OH group is obscured by the strong water signal.

In Figs. 4B and C localization is illustrated in the methanol and toluene spheres, respectively. To the left are images of the phantom after preparation pulses corresponding to experiment 4 in Table 1. The effect of this preparation is inversion of the z magnetization in two slices parallel to the xz and yz planes. This results in the appearance of black lines in the image, corresponding to zero crossings of the z magnetization in space, which clearly mark the position of the cube. By choosing the appropriate frequency offsets for the preparation pulses, the cube may be positioned at will. Selection along the z axis is performed by the standard selective excitation technique. The experimental time for these "crossed" images is the same as for a standard image, i.e., approximately 3 min.

The result of differencing eight images using the full ISIS experimental sequence is shown to the right of the crossed images. These images demonstrate the excellent

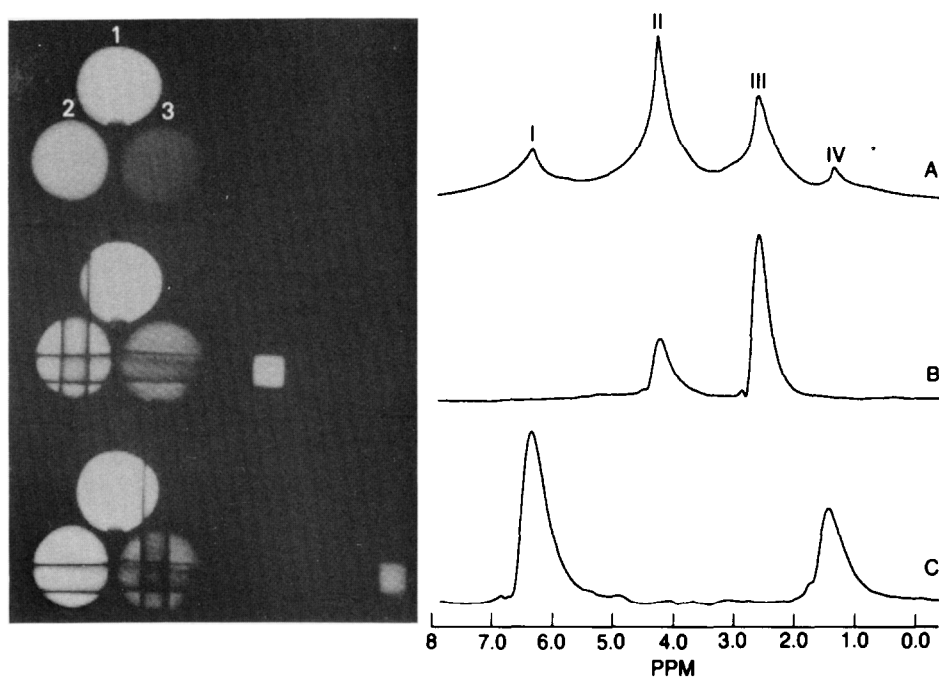


FIG. 4. (A) An 80 MHz proton image of a phantom of three 4 cm diameter spheres containing H_2O (1), CH_3OH (2), and $\text{C}_6\text{H}_5\text{CH}_3$ (3), and the corresponding total spectrum. Separate resonances can be observed from the protons in the aromatic ring of toluene (I), water and methanol OH (II), methanol CH_3 (III), and toluene CH_3 (IV). (B) On the left, an image of the phantom where "crossed" lines mark planes of zero z magnetization created during the preparation period using the pulse sequence of experiment 4. The lines show the xy boundaries of the selected cube in the methanol sphere. In the middle, an image of the selected cube, and on the right, the corresponding ISIS spectrum. (C) As above but with the cube positioned in the toluene sphere of the phantom.

cancellation of signals from spins outside the selected volume, which constitute 95% or more of the total signal acquired during each of the eight experiments in the ISIS sequence. On the far right of Figs. 4B and C, are shown the ISIS spectra of methanol and toluene from the corresponding cubes. Each spectrum is the result of one cycle of the ISIS sequence, corresponding to eight scans of the selected cube, and the total experimental time was 40 s. Each cube is 1.6 cm along all three sides, enclosing a total volume of 4.1 cm^3 . The spectra are reproduced with different scaling factors to aid comparison.

In Fig. 5 an image is reproduced of a cross-sectional slice through the lower leg midway between knee and ankle. Figure 5A shows the total spectrum of the volume of leg within the field of view of the rf coil. The spectrum from a cube of material positioned within the soleus muscle is shown in Fig. 5B, and the spectrum in Fig. 5C was obtained from a cube positioned within the bone marrow of the tibia. The positions of the cubes are indicated by boxes superimposed on the standard image. As in the previous experiment, the volume of each cube is 4.1 cm^3 . However, in view of the much larger dimensions of a human shin, only approximately 1% of the total signal is derived from material within each of the selected cubes. The overall spectrum A

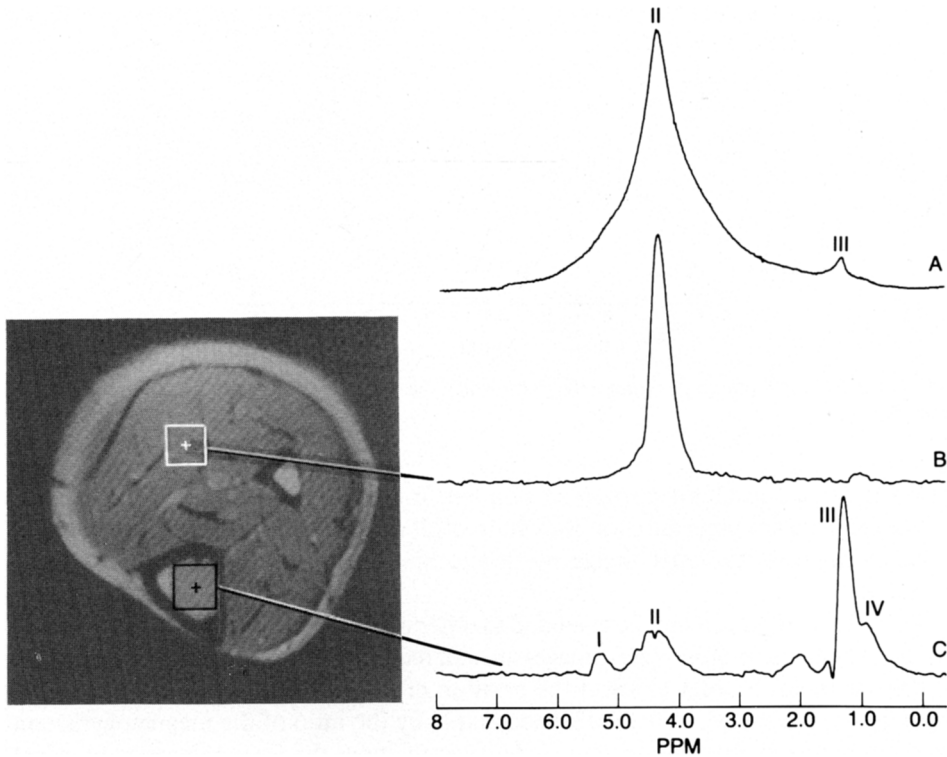


FIG. 5. An 80 MHz proton image of a cross-sectional slice through the lower leg midway between knee and ankle, with the overall spectrum shown in (A). The ISIS proton spectrum of soleus muscle is shown in (B), and the ISIS proton spectrum of bone marrow in the tibia is shown in (C). Proton resonances arise from fatty acid ($-\text{HC}=\text{CH}-$) (I), water (II), fatty acid ($-\text{CH}_2-$)_n (III), and fatty acid ($-\text{CH}_3$) (IV). The ISIS spectra are plotted at a scale which is two orders of magnitude larger than that of the total spectrum.

shows only two signals, which originate from water (II) and the protons in fatty acid chains (III). In the spectrum of the muscle in Fig. 5B the latter peak has virtually disappeared. In the bone marrow spectrum of Fig. 5C the water signal (II) is greatly reduced and several signals are visible from fatty acid chains. These are the protons in unsaturated bonds at 5.4 ppm (I), the protons in saturated bonds between 2.3 and 1.2 ppm (III), and the protons in terminal methyl groups at 0.8 ppm (IV). For comparison, reproduced in Fig. 6 is the *in vitro* spectrum of fat tissue from Ref. (23).

DISCUSSION

The ISIS technique can be further improved to extend its usefulness and application to biological and medical samples. The previous experimental results have shown that the selected cube can be moved along the *x* and *y* axes. Using multislicing, it is a simple extension of the method to provide a third axis of translation. Multislicing produces a series of cross-sectional slices of the sample by using the technique of rapidly updating the transmitter frequency for the slice-selective rf pulses. Each slice of the image therefore has a well-defined frequency offset along the *z* axis compared

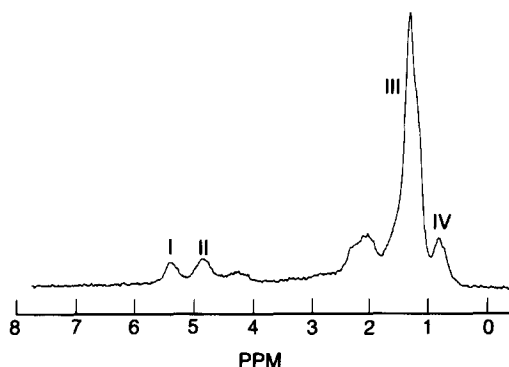


FIG. 6. A 100 MHz proton spectrum of fat tissue which was obtained *in vitro*. Reproduced by permission from Ref. (23).

with the center slice, and movement of a cursor over the three-dimensional data set is a straightforward procedure for evaluation of all three frequency offsets. Furthermore, a three-dimensional NMR image by this technique can be obtained within a few minutes.

The ISIS method can also be extended to other nuclei, e.g., to ^{31}P NMR spectroscopy on living systems. Proton NMR images are still required to provide spatial information of the specimen in order to select the position of the cube. However, by multiplying the gradient magnitudes for the ISIS technique by the ratio of the magnetogyric constants of hydrogen and the nucleus under investigation, the frequency encoding evaluated by spatial positioning in the proton image is still valid for localization of the cube for spectroscopic examination of any desired nucleus. Furthermore, the cube is the same size at both resonant frequencies. The only assumptions are that the water signal is on resonance during the imaging experiment, and for spectroscopy the carrier frequency is centered in the spectrum of the resonant nucleus.

The most common receiver coil for the detection of *in vivo* ^{31}P NMR spectra is a surface coil (3, 14) which may be easily fixed within the slotted tube resonator used for NMR imaging. The position of the surface coil may be established on the NMR image by attaching a proton marker to the coil, and it is then a simple procedure to move the ISIS cube into the field of view of the surface coil.

One problem with investigations of nuclei other than protons is that the larger ranges of chemical shifts which can occur may degrade the spatial selectivity of the ISIS technique. There is a slight spatial displacement of the cube for each of the chemically shifted resonances, which can be evaluated by the following equation:

$$\Delta F = \frac{\gamma}{2\pi} \cdot \Delta\chi \cdot G_x \quad [8]$$

where ΔF is the chemical shift offset with respect to the carrier frequency, γ is the magnetogyric ratio, G_x is the magnetic field gradient strength, and $\Delta\chi$ is the spatial displacement error. Clearly, this error can be minimized by using large field gradients. The maximum displacement error for the ATP resonances in a phosphorus spectrum using, for example, a field gradient of 1.5 G/cm is less than 2 mm.

For carbon spectroscopy the larger chemical shifts involved mean that the straightforward ISIS technique cannot be applied without the use of very large field gradients, unless a significant positional error can be tolerated. A simple solution to this problem is the combination of a polarization transfer experiment with ISIS, similar to the approach adopted by Aue *et al.* (24). This allows spatial selection to be achieved using proton spectroscopy, since the technique limits signal observation to only those carbon nuclei coupled with the selected protons.

A simple experimental check can be performed to ensure that the inaccuracy of spatial selection caused by the aforementioned displacement error, is small enough for the investigation of a spectrum from a volume of particular size. The full ISIS experiment is repeated a second time with negative field gradients and negative frequency offsets, and the resulting spectrum subtracted from the original data with positive gradients and offsets. All spatial displacement errors are reversed in the second sequence which means that the two spectra are derived from slightly different cubes. If the spectra differ substantially, this clearly means that the specimen does not show sufficient spatial homogeneity in spectral components to make reasonably accurate deductions from the data. If any spectral component shows a subtraction error greater than a preset limit, when compared to the original experiment, one may therefore decide to repeat the investigation with a smaller selected volume.

As previously mentioned, spatial selection is effectively independent of rf nutation angle; however, S/N ratio obviously deteriorates as the rf pulses vary from either their 180 or 90° conditions. The selective 180° pulses can be generated using an amplitude and phase waveform proposed by Silver *et al.* (25). This has the tremendous advantage that selective inversion of the spin population occurs over an extremely wide range of rf pulse powers although the pulse length required is quite long. If the nonselective 90° read pulse is also replaced by an adiabatic half-passage pulse (26), the ISIS method can be implemented to obtain ideal S/N ratio with virtually no dependence upon rf pulse power, making the technique very easy to apply.

The ISIS method can also be extended to the simultaneous selection and investigation of multiple cubes, with the minimum number of required experiments doubling for each extra cube. The cubes may be made more rectangular to form columns, and subsequently be coadded to produce selected volumes of irregular shape. The procedure may also be terminated after the first four experiments, to achieve selection to a column. This can be subsequently investigated by a chemical-shift imaging method to give a 2D matrix showing the distribution of chemical-shift information along the column, with a considerable saving in time over 3D and 4D methods. Alternatively the first two experiments may be used as a slice-selection technique for 3D chemical-shift imaging, or spectroscopy.

CONCLUSION

The ISIS technique is a method of spatial selection which can be followed by most standard NMR experiments. It is capable of producing high-resolution NMR spectra of limited volumes within the sample with minimum distortions from the switching of magnetic field gradients. It can be applied in combination with coils showing gross rf field inhomogeneity, and provides optimum S/N performance from spins within

the selected volume. The cube can be moved in three-dimensional space, and its position can be selected by reference to a standard NMR image. The method is particularly easy to use, and should find major application in the *in vivo* investigation of living systems by NMR spectroscopy.

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REFERENCES

1. R. E. GORDON, P. E. HANLEY, D. SHAW, D. G. GADIAN, G. K. RADDI, P. STYLES, P. J. BORE, AND L. CHAN, *Nature (London)* **287**, 367 (1980).
2. R. E. GORDON, P. E. HANLEY, AND D. SHAW, *Progr. NMR Spectrosc.* **15**, 1 (1982).
3. J. J. H. ACKERMAN, T. H. GROVE, G. G. WONG, D. G. GADIAN, AND G. K. RADDI, *Nature (London)* **283**, 167 (1980).
4. A. A. MAUDSLEY, S. K. HILAL, W. H. PERMAN, AND H. E. SIMON, *J. Magn. Reson.* **51**, 147 (1983).
5. L. D. HALL AND S. SUKUMAR, *J. Magn. Reson.* **50**, 161 (1982).
6. J. C. HASELGROVE, V. H. SUBRAMANIAN, J. S. LEIGH, JR., L. GYULAI, AND B. CHANCE, *Science* **220**, 1170 (1983).
7. L. D. HALL, V. RAJANAYAGAM, AND S. SUKUMAR, *J. Magn. Reson.* **61**, 188 (1985).
8. M. L. BERNARDO, JR., P. C. LAUTERBUR, AND L. K. HEDGES, *J. Magn. Reson.* **61**, 168 (1985).
9. J. F. MARTIN AND C. G. WADE, *J. Magn. Reson.* **61**, 153 (1985).
10. P. A. BOTTOMLEY, *J. Magn. Reson.* **50**, 335 (1982).
11. K. N. SCOTT, H. R. BROOKER, J. R. FITZSIMMONS, H. F. BENNETT, AND R. C. MICK, *J. Magn. Reson.* **50**, 339 (1982).
12. T. H. MARECI AND H. R. BROOKER, *J. Magn. Reson.* **57**, 157 (1984).
13. S. J. COX AND P. STYLES, *J. Magn. Reson.* **40**, 209 (1980).
14. A. HAASE, W. HAENICKE, AND J. FRAHM, *J. Magn. Reson.* **56**, 401 (1984).
15. P. A. BOTTOMLEY, T. B. FOSTER, AND R. D. DARROW, *J. Magn. Reson.* **59**, 338 (1984).
16. M. R. BENDALL AND R. E. GORDON, *J. Magn. Reson.* **53**, 365 (1983).
17. M. R. BENDALL, *J. Magn. Reson.* **59**, 406 (1984).
18. M. R. BENDALL, J. M. MCKENDRY, I. D. CRESSHULL, AND R. J. ORDIDGE, *J. Magn. Reson.* **60**, 473 (1984).
19. A. J. SHAKA, J. KEELER, M. B. SMITH, AND R. FREEMAN, *J. Magn. Reson.* **61**, 175 (1985).
20. W. P. AUE, S. MUELLER, T. A. CROSS, AND J. SEELIG, *J. Magn. Reson.* **56**, 350 (1984).
21. R. J. ORDIDGE, M. R. BENDALL, R. E. GORDON, AND A. CONNELLY, "Magnetic Resonance in Biology and Medicine," (Govil, Khetrpal, and Saran, Eds.), Tata McGraw-Hill, New Delhi, 1985.
22. R. TYCKO AND A. PINES, *J. Magn. Reson.* **60**, 156 (1984).
23. R. E. GORDON, Ph.D. Thesis, Aberdeen University, 1975.
24. W. P. AUE, S. MUELLER, AND J. SEELIG, *J. Magn. Reson.* **61**, 392 (1985).
25. M. S. SILVER, R. I. JOSEPH, AND D. I. HOULT, *J. Magn. Reson.* **59**, 349 (1984).
26. T. FARRAR AND E. BECKER, "Pulse and Fourier Transform NMR," Academic Press, London, 1971.