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Efficient Magnetic Resonance Imaging Methods for Automated Quantitation of Magnetic Resonance Parameters from Multiple Samples

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Magnetic resonance imaging methods have been developed which simultaneously measure the bulk magnetic resonance parameters of many samples contained in stacks of multi-well sample plates using either a 2.35 T, 31 cm or a 2 T, 100 cm horizontal bore magnet each with a quadrature 'birdcage' resonator. Computerized automatic edge detection software detects the position of each well within the first spin-echo image which can then determine the mean magnetic resonance parameters of the sample in each well. An example is given of the automated quantitation of T_1, T_2 and M_0 values for 2% (w/v) agar gel in four plates each with 30 wells per plate (120 samples in total). It was found that the maximum number of samples that can be analysed is limited by the homogeneity of the probe B_1 field and the B_0 field of the magnet. The applicability of this technology for future applications is discussed.

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INTRODUCTION

The purpose of this work was to develop nuclear magnetic resonance (NMR) imaging methodology which can provide convenient, automated measurement of bulk magnetic resonance (MR) parameters for a large number of samples. Existing 'bench-top' NMR devices fitted with hardware for automatic sample presentation can provide a low-cost option. However, owing to the time taken to place individual samples sequentially into a magnet, this may make the study of many hundreds of samples too time consuming. This study demonstrates a novel alternative in which a large number of samples are placed in a magnet at the same time and magnetic resonance imaging (MRI) protocols are used to derive simultaneously the bulk MR parameters of each sample.

An earlier study had demonstrated interesting variations in the spin–lattice (T_1) and spin–spin (T_2) relaxation times, the total proton content (M_0) and the magnetisation transfer (MT) characteristics of pork induced by freeze–thawing, or by brining.[1] To develop this work further, it was necessary to obtain measurements from a larger number of samples to permit a more detailed statistical study of these phenomena, in particular variations between different regions of an animal and biological variations between individual animals and, eventually, different species. This offered the daunting prospect of making accurate measurements on many hundreds of samples, with all the attendant problems of controlling the measurement conditions and analysing the data.

The problem of presenting a large number of samples for MRI at low cost, was easily solved by using plastic well counters which are commonly used in tissue culture; these can contain large numbers of specimens in a regular array. The signal-to-noise requirements for meat studies at *ca.* 2 T was satisfied by using plates with an area of 10 × 7 cm each with an array of 8 × 12 wells, each with a volume of 0.37 ml. These plates could be easily tailored to fit inside MRI probes of different dimensions; for one-dimensional measurements, a sample row could be detached using a band saw; for a set of plates, sequential, or interleaved, 2D slice imaging could be used.

The focus of this work was the development of the methodology for the fully automated analysis of multiple samples, for which purpose the well counters were filled with 2% (w/v) agar gel whose bulk MR parameters were already known, to determine the accuracy and reproducibility of this method. T_2 and M_0 measurements, which are known to be sensitive to B_0 and B_1 inhomogeneities, were made in a 31 cm bore magnet to determine the maximum number of samples for that size of magnet. Benchmark measurements of T_2 and M_0 were also made on the same phantom with a 100 cm bore 'whole body' magnet where there was no compromised B_0 and B_1 field. T_1 measurements, which are less

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sensitive to field inhomogeneites, were made on the 31 cm bore magnet to determine the effect of reducing the time domain sampling from 32 to 7 time points for the T_1 relaxation curve. The applicability of this method to study meat and other samples is also discussed.

EXPERIMENTAL

MRI hardware

All the images were acquired at room temperature (*ca.* 22 °C) using either a 2 T, 100 cm or a 2.35 T, 31 cm horizontal bore superconducting magnet connected to a Bruker BMT (Bruker Medzintechnik Biospec II) imaging console. All radiofrequency (RF) probes were based on a cylindrical eight-strut 'birdcage' design operating in quadrature mode. A 9.4 cm i.d. RF probe and 14.5 cm i.d. gradient set were used in the 31 cm bore magnet and an 18 cm i.d. RF probe and 24 cm i.d. gradient set were used inside the 100 cm bore magnet. All gradient sets and RF probes were built in-house.

Experimental protocol

Each well counter plate (Sterilin, UK) was trimmed so that it had an array of 6 × 5 wells. The wells were all filled with 2% (w/v) agar gel and magnetic susceptibility effects caused by air gaps between wells were eliminated by filling the spaces with 2% (w/v) agar gel doped with 5 mM $MnCl_2$. This doping reduced the relaxation time of the gel so that its contribution to the total MRI signal was negligible and improved the ease with which the automated software located the wells within the images.

It was found that conventional multi-slice methods were not suitable for acquiring the data for quantitation of the MR parameters from each well plate. Although that would have minimized the time for data acquisition, the production of unwanted echoes at the slice edges and RF imperfections[2] which partially saturated the MR signal from the slice of interest led to reduced image contrast and errors in the estimation of the relaxation times. In addition, although a multi-slice protocol could be used for the acquisition of T_1 data, the shortest repetition time for four slices was 270 ms, which was too long for the relatively short repetition required to define the early part of the relaxation curve.[3] In addition, interference was caused by the off-resonance effects from pulses intended for other slices which attenuated the signal through the equivalent of magnetization transfer. It has been shown that these sources of interference are more pronounced with magnetization transfer imaging of multiple slices and cannot be rectified by modification of the pulse sequence.[4]

Based on the above considerations, it was decided to acquire MR parameters sequentially from each slice; accordingly, a slice selection lobe was introduced in the pulse sequence to reduce unwanted echoes produced by imperfections in the sequence. The stack of well plates was placed in the probe so that five wells were along the bore of the magnet (*z*-axis), six wells were oriented horizontally (*x*-axis) and four plates were stacked vertically (*y*-axis). Figure 1 shows the sagittal-*z* image of the stack of multi-well plates which was used to determine the position of each slice for image acquisition in the coronal-*z* plane. All images had a field of view of 7 cm, 4 mm slice thickness, 128 pixels in the read direction (*z*-axis) with a field of view extension factor of four and a phase reduction of two; this gave images with 512 × 64 pixels, which were later processed to give 128 × 128 pixels with a resolution of 547 μm per pixel. This protocol was chosen to give the optimum pixel resolution and a signal-to-noise ratio (SNR) of *ca.* 150 necessary for the automated image analysis, with the minimum time for the full acquisition of the MR parameters.

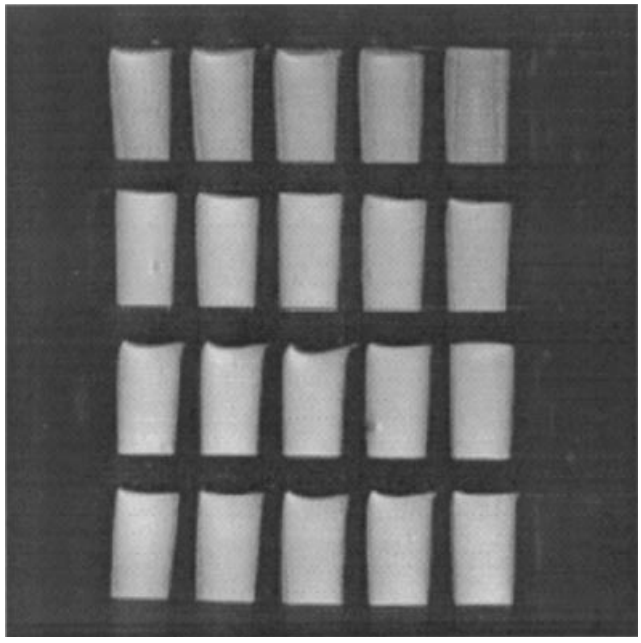

Figure 1. Sagittal-*z* image of stack of four well plates used to determine position of each slice for quantitation.

Data acquisition

T_1 and T_2 images were acquired from the plates by varying the repetition (TR) and echo time (TE) in the multi-echo Carr–Purcell–Meiboom–Gill MRI sequence.[5] T_1 values were calculated from images with either 7 or 32 different TR values (between 0.12 and 10 s based on a logarithmic scale). T_2 was quantified by varying TE (16 images, inter-echo time of TE 12 ms, TR 10 s with the 31 cm bore magnet; eight images, inter-echo time of TE 15 ms, TR 10 s with the 100 cm bore magnet).

The raw data were transferred from the Bruker Aspect 3000 computer via a fast data transfer board (designed by A. A. Wilkinson and Dr N. Dillon) to a network of Unix workstations. The images were zero-padded in the phase direction, baseline corrected and a Gaussian filter applied before Fourier transformation. The fitting of the data was performed using a curve fitting program written by Dr J. J. Attard and visualized using image display software written by Dr N. J. Herrod in the Herchel Smith Laboratory.

The following equations were used to quantify T_1 and T_2:

$$M_{xy} = M_0 \rho(1 - e^{-(T_R/T_1)})$$

and

$$M_{xy} = M_0 e^{-(T_E/T_2)} + C$$

respectively, where M_{xy} is the magnetization at a particular pixel, ρ and C are the average noise in the images and M_0, T_1 and T_2 are initial estimates for starting points in the curve-fitting process based on the Levenberg–Marquardt method.[6] M_0 was quantified by back-projection of the T_2 decay curve to where it intersected the y-axis at time zero.

Automatic edge detection

The estimation of the mean MR parameters for each well was performed by fully automated computerized analysis. The location of the circular boundaries of the wells was determined by using an edge-detection program based on a recursive filtering algorithm originally developed for automated measurements of the thickness of finger joint cartilage.[7] The first echo image [Fig. 2(a)] was used to produce a map of the circular boundaries of the wells [Fig. 2(b)]. The map of data delineating the edges was then used to locate the centre of each well using a modified Hough transform algorithm for circles,[8] which gave a Hough space map of the original image [Fig. 2(c)]. The local maxima of the Hough space indicated the centres of the wells [Fig. 2(d)]. Since the radius of each well is constant and is known beforehand, the problem of locating circles in Hough space is reduced to two dimensions, namely the x- and y-coordinates of the centres of the circles. This method gave a very fast search in Hough space to locate the wells (*ca.* 33 s per slice as evaluated by the

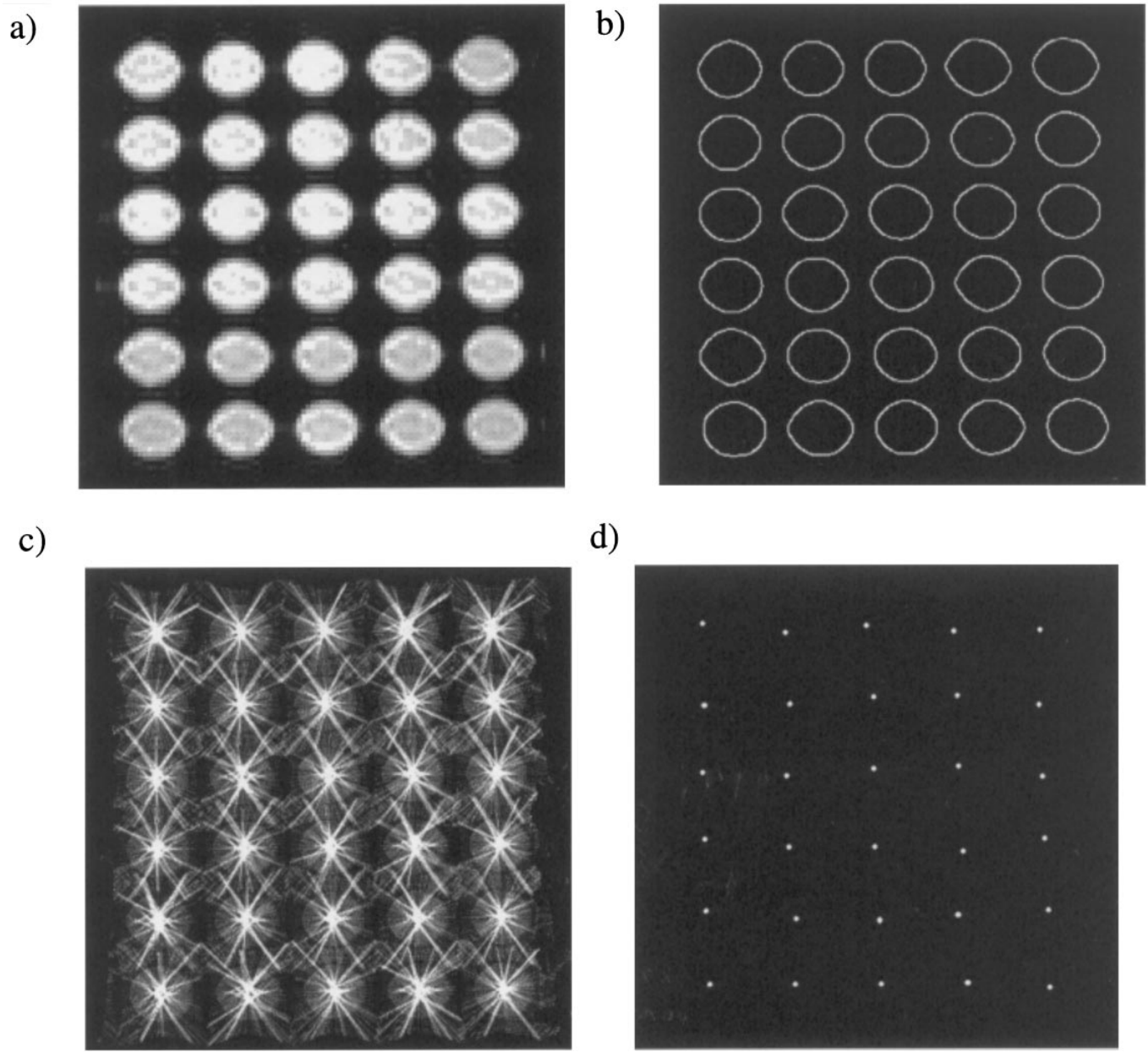

Figure 2. (a) First echo image, (b) edge-detected image, (c) transform of edge data into Hough space and (d) local maxima in Hough space indicate centres of wells

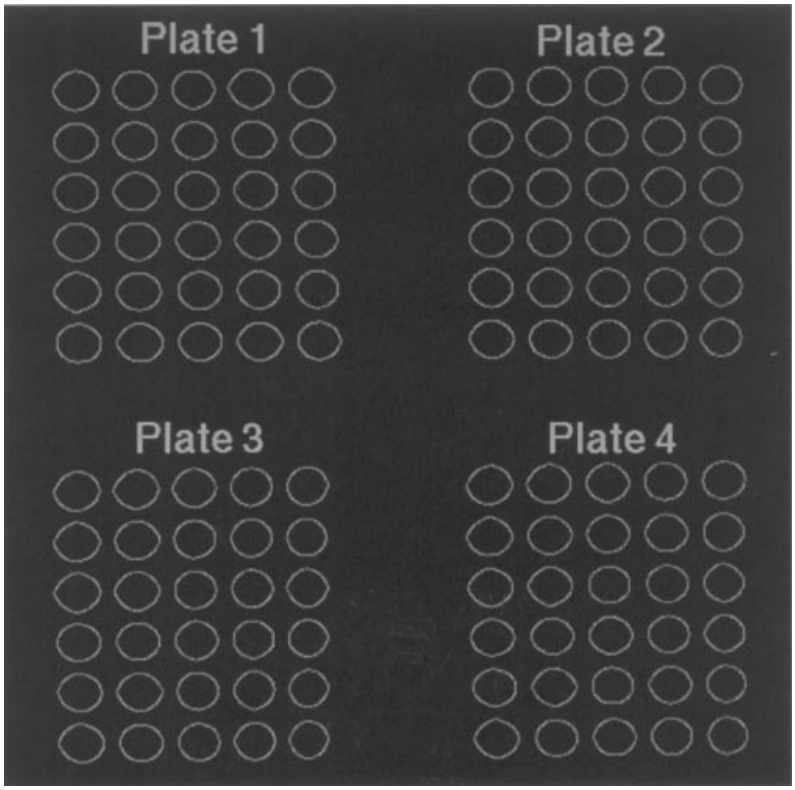

Figure 3. Edge-detected images from all four plates.

UNIX 'time' command on a 133 MHz Intel Pentium running i486-pc-solaris2). A binary mask was then generated to remove unwanted signals surrounding the wells and also to determine the mean and standard deviation of all the pixels enclosed within the boundary of each well in the fitted images. The accuracy of the mean MR value per well determined by this method was validated using image viewing software (Cmrview) written by Dr N. J. Herrod.

RESULTS AND DISCUSSION

The edge detection software was capable of detecting the position of all the wells from each plate (Fig. 3). It was also found that the automatically quantified MR parameters were identical with those calculated for regions determined by hand using the image visualization software Cmrview. The T_2 and M_0 results from the 31 cm bore magnet were more variable than those measured with the 100 cm bore magnet, particularly for plates in positions 1 and 4 (Table 1). This is due to the larger volume of B_0 and B_1 field homogeneity in the larger bore magnet. It was found that by eliminating data for wells on the top and bottom plates (to a 4×5 well matrix) the mean T_2 values were closer to the mean values of the middle two plates. This demonstrates that the maximum number of samples for this combination of gradient set and probe in a 31 cm bore magnet is 100. If T_2 measurements need to be made with greater accuracy, then the samples would have to be confined to the middle two plates, in which case 60 samples could be accommodated. This still represents a significant time saving over conventional bulk sampling or MRI methods.

A comparison between T_1 measurements made with 7 and 32 TRs (Table 2) confirmed that more sampling points on the T_1 relaxation curve reduced the standard deviation of measurements, albeit at a greater cost of time (*ca.* 2 h per slice for 32 TRs, with *ca.* 20 min per slice for 7 TRs). In fact, 32 TRs gave a standard deviation of 0.025 for all samples, which was approximately half of that measured with 7 TRs (SD = 0.047). This demonstrated that compromising the number of TRs to minimize the image acquisition time reduced the accuracy of the T_1 measurements (Fig. 4). The acceptability of this error will depend on several factors: the nature

Table 1. T_2 and M_0 measurements made with 31 and 100 cm bore magnets (mean ± SD)

Plate position in stack	31 cm bore magnet		100 cm bore magnet	
	T_2 (s)	M_0	T_2 (s)	M_0
1	0.057 ± 0.002	212800 ± 11500	0.057 ± 0.001	200100 ± 9000
2	0.058 ± 0.003	209000 ± 10000	0.056 ± 0.001	200100 ± 7000
3	0.062 ± 0.002	205400 ± 8000	0.058 ± 0.001	200400 ± 8000
4	0.053 ± 0.002	227800 ± 11500	0.057 ± 0.001	200200 ± 7000

Table 2. Mean T_1 (± SD) relaxation measurements made with either 7 or 32 repetition times (TR) in the decay curve

Plate position stack	T_1 (s) 7 TRs	32 TRs
1	2.17 ± 0.03	2.16 ± 0.02
2	2.17 ± 0.04	2.16 ± 0.03
3	2.13 ± 0.05	2.14 ± 0.03
4	2.14 ± 0.06	2.15 ± 0.02

of the material being studied, the number of samples and the magnitude of difference between sample means.

The acquisition time required for quantification of the MR parameters could be reduced to *ca.* 1 min if one-dimensional profiles were taken. For example, five samples could be quantified at a time inside the 31 cm bore magnet. An increase in the bore size of the magnet and hence the homogeneity of the B_1 field would enable more samples to be accommodated; indeed, in the 100 cm bore magnet, several hundred samples could have been quantified automatically with the 2D protocol described here.

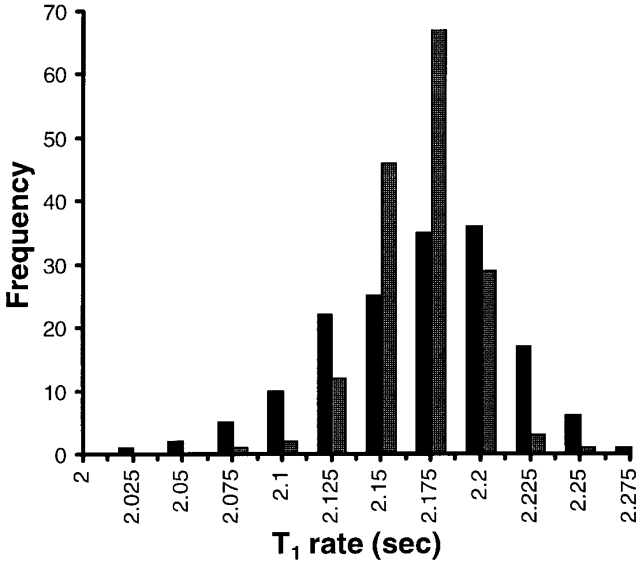

Figure 4. Frequency distribution of T_1 rates calculated using 7 (dark bars) or 32 (light bars) repetition times from all sample plates.

CONCLUSIONS

The development of MRI methods for measuring the bulk MR parameters of many samples has great potential, yet until now MRI has often been prohibitively time consuming and hence expensive to justify studies in non-medical areas. The combination of inexpensive multi-well sample plates, MRI and edge detection software used here has been demonstrated to provide robust and reproducible quantification of up to 100 separate samples in a 31 cm bore magnet. With this technology, it is now possible to acquire data from a large number of samples within a relatively short period of time and without any operator intervention. Indeed, we envisage that samples could be presented on a conveyor belt to the magnet, so that an even larger throughput could be achieved under automated control. For those on-line processing situations for which the length of time required for two-dimensional imaging would be prohibitive, one-dimensional profiles would have to be acquired instead; this would reduce the time for data acquisition from several hours to several minutes.

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