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A Submicrosecond Resonator and Receiver System for Pulsed Magnetic Resonance with Large Samples

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We describe a submicrosecond resonator and receiver system for use in pulsed magnetic resonance at 220 MHz. This new resonator and receiver system design enables a reduction of the dead time, and in principle its complete elimination. We show experimentally that the resonator and receiver system permits the detection of free induction decay signals 300 ns from the end of the transmitting pulse, even with large (55-ml) saline samples. This apparatus was specifically developed for *in vivo* Fourier transform electron paramagnetic resonance detection of free radicals; however, it can also be used in pulsed nuclear magnetic resonance imaging of solids with applications in materials research and the polymer industry. © 1998 Academic Press

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The ability of electron paramagnetic resonance (EPR) to detect and image free radicals *in vivo* has important implications for the study of many physiological and pathological conditions (*1*). Free radicals are involved in a number of conditions (*2*) such as myocardial ischemia, inflammation, ageing and cancer. Conventional continuous wave (CW) X-band (9 GHz) EPR has been limited to model systems, such as aqueous solutions and biological samples of less than 200 μl volume, because of the high dielectric losses in the samples (*1*). However, the development of *in vivo* low-frequency (100-MHz to 1-GHz) CW EPR has permitted the study of larger samples (*1*) such as plants, tissue specimens, perfused organs, and whole rats. This has recently allowed the metabolism of exogenous stable nitroxide free radicals to be monitored in whole rats (*3–4*) and the detection of nitric oxide in mice using spin trapping techniques (*5–6*).

One of the limitations of *in vivo* low-frequency CW EPR is the slow instrumental acquisition rate. Typically, EPR spectra are acquired by irradiating the sample continuously with a radio frequency (RF) field and sweeping the main magnetic field over a suitable range in about 30 s. For imaging purposes, a stationary magnetic field gradient (typically 20 mT/m) is applied and a projection along the gradient is acquired. A three-dimensional reconstruction requires at least 64 projections at different spatial orientations. The total acquisition time (more than 30 min) is not compatible with the biological half-lives of the paramagnetic probes of interest.

Recently RF pulsed (time domain) EPR has been proposed as a means to overcome this problem (*7*). However, with pulsed magnetic resonance there are many technical issues to be addressed: magnetic field homogeneity and stability, pulse power level, pulse width, amplitude of the irradiating magnetic field for a given power level, receiver dead time, time resolution, and digitization rate. In previous work, these aspects have been optimized for the particular experimental applications and the operating frequency (*8*). The receiver dead time determines the fundamental limit for the detection of fast-decaying signals and many instrumental developments have been proposed to reduce it (*9–13*). To illustrate the concept of dead time, Fig. 1 shows the basic sequence of a pulsed magnetic resonance experiment. To observe fast free induction decay (FID) signals and also to optimize the signal-to-noise ratio (SNR), the receiver dead time following the application of the transmitter pulse has to be very short compared to the longitudinal and transverse relaxation times of the sample (T_1, T_2). For *in vivo* EPR studies exogenous nitroxide free radicals are often used with typically submicrosecond (100 ns to 1 μs) relaxation times (*14*). NMR (and NMR imaging) of solids is increasingly being used in materials research. T_2 values as short as 3 μs are observed in materials such as glassy polymers, which are therefore difficult to study with existing apparatus (*15*).

From the previous examples, it can be seen that a resonator and receiver system for pulsed magnetic resonance (EPR/NMR) with submicrosecond dead time capable of accommodating large samples is a major challenge. Here we propose a resonator and receiver system that operates at 220 MHz and overcomes many of the current limitations. The apparatus is based on a new and efficient design which reduces the receiver dead time to about 300 ns, and in principle could eliminate it completely. Moreover, it permits a considerable increase in the size of the observable samples and can be used with high quality factor transmitter coils.

The receiver dead time (T_D) can be expressed as (8)

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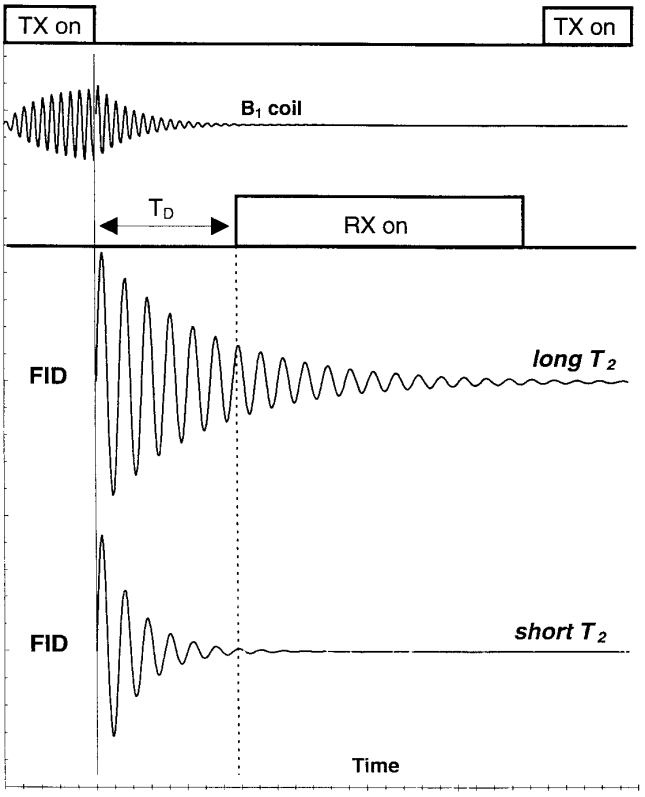

FIG. 1. Basic sequence of a pulsed magnetic resonance experiment. A radio frequency pulse is applied to the transmitter (TX) coil producing the ringing down of the magnetic field B_1. To observe fast free induction decay (FID) signals and to optimize the signal-to-noise ratio, the receiver (RX) dead time (T_D) has to be very short compared to the relaxation time (T_2) of the sample.

$$T_D = \tau \cdot \ln\left(\frac{P^*_{TX}}{P_n}\right), \qquad [1]$$

where P^*_{TX} is the fraction of transmitter (TX) power that leaks to the receiver (RX), P_n is the intrinsic detector noise, and τ is the coil ringing time. The power ratio in Eq. [1] is a measure of the isolation between the transmitter and the receiver. As a rule of thumb, in pulsed magnetic resonance apparatus the desirable isolation is assumed to be 130 dB and from Eq. [1] we obtain $T_D \cong 30 \cdot \tau$. The ringing time τ is a consequence of the applied TX pulse (Fig. 1) to the coil (an inductive/capacitive circuit with high quality factor) and it is given by (9)

$$\tau = \frac{Q}{2\pi \cdot f_0}, \qquad [2]$$

where Q is the loaded quality factor and f_0 is the resonant frequency. The dependence of τ on the inverse of the frequency penalizes low-frequency applications. For example, we may compare T_D at high frequency (9 GHz) and low frequency (220 MHz) assuming a quality factor of 1000. In the first case we have $T_D \cong 500$ ns, while at low frequency $T_D \cong 20$ μs.

At high frequency a simple and widely adopted technique to decrease T_D is to deliberately reduce the quality factor (or alternatively to overcouple the coil) (9). The trade-off for the reduction in τ is a decrease of the SNR. However, the reduction of Q is not particularly suitable at low frequency because the level of the applied transmitter power can became prohibitive. The efficiency factor Λ of the coil is given by (8)

$$\Lambda = \frac{B_1}{\sqrt{P}} \propto \sqrt{\frac{Q}{V_c \cdot f_0}}, \qquad [3]$$

where B_1 is the amplitude of the rotating component of the RF magnetic field, P is the power incident on the TX coil, and V_c is the effective volume of the coil. From this equation we observe that, to compensate for the reduced Q, we should decrease the coil volume. Unfortunately, this is not compatible with low-frequency applications where large samples of about 20 to 200 ml are studied, requiring a higher TX power level to produce a 90° flip angle and this again increases the dead time. For these reasons, to date the majority of pulsed magnetic resonance apparatus at high and low frequencies adopted a classical duplex (single TX/RX coil) or crossed (separate TX and RX coils) configuration with very small coil volumes (less than 10 ml), low quality factor (less than 200), and suitable electronic circuits for the quenching of the TX coil (*7–12, 16–18*).

The resonator and receiver system proposed here, shown in Fig. 2A, is based on the two following main features:

(a) Use of separate TX and RX coils (crossed configuration) to produce high isolation (25 to 40 dB) and a consequent reduction of T_D. The TX coil (Fig. 2B) is a loop–gap resonator (*19*) (LGR, diameter 59 mm, length 20 mm) tuned to 220 MHz.

(b) The RX coil is composed of two identical pairs of saddle-shaped coils (Fig. 2B) tuned to 220 MHz, perpendicular to each other and perpendicular to the LGR. In principle, the two receiving coils (RX_S and RX_R) have identical ringing times. The RX_S coil is perpendicular to the main magnetic field and is used for FID detection. The RX_R coil is parallel to the main field and is used to reduce the receiver dead time. Because of the finite isolation between the TX and the RX coils (25 to 40 dB) the same ringing voltages (amplitude and phase) are induced in RX_S and RX_R. By using a dual channel resonator and receiver system, when the signals in the two arms are exactly out-of-phase a marked increase of the isolation is produced.

As shown in Fig. 2A, the output of the RX_S coil is preamplified (Mini Circuit, MAN-1-HLN) and connected to a power combiner (Mini Circuit, ZFSCJ-2-1), while the RX_R

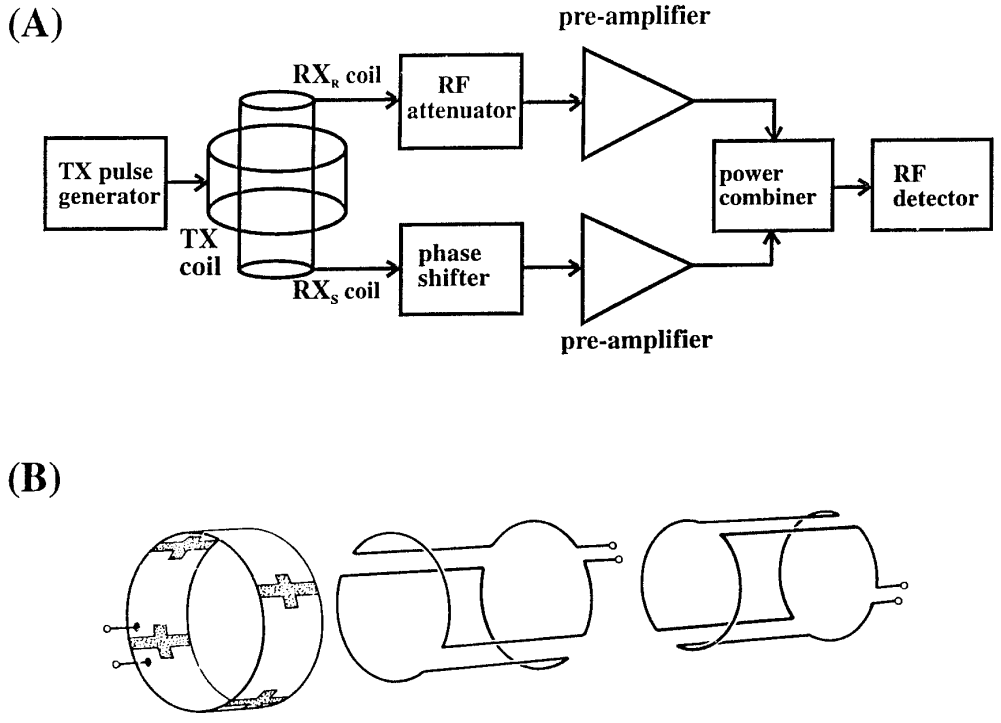

FIG. 2. (A) Overall schematic diagram of the submicrosecond resonator and receiver system operating at 220 MHz. The RF detector is composed of an RF switch (Mini-Circuits, ZYSWA-2-50DA) connected to cascaded amplifiers (Research Communication Ltd 9004 and Mini-Circuit Man-1HLN) for a total gain of 50 dB. A balanced mixer (Mini-Circuits, ZAD-1) is used for the detection of the FID. The reference signal (+10 dBm) for the local oscillator input of the mixer was obtained from the main transmitting RF source (HP8640B) via a power splitter (Mini-Circuit, ZFSC-2-2). The mixer output, after 20 dB amplification with a home-built video amplifier, is connected to a digital storage oscilloscope (Tektronix, TDS 540) for signal capture and display. (B) The loop–gap resonator used as transmitter coil and the two pairs of perpendicular saddle-shaped used as receiver coils.

output is connected to a variable phase shifter (Microlab-FRX Model ST-05), then amplified by a low-noise preamplifier (Mini Circuit, MAN-1-HLN), and finally connected to the other input of the power combiner. For FID detection the power combiner output is connected, via a standard $\lambda/4$ line with crossed diodes (*20*) and an RF switch (Mini-Circuit, ZYSWA-2-50DA) to the RF detector. A digital storage oscilloscope (Tektronix, TDS 540) was used for signal capture and display. A full description of the pulsed EPR spectrometer will be presented elsewhere.

The TX LGR is contained in a brass shield (internal diameter 75 mm, length 90 mm). A balanced capacitive network (*20*) was adopted to optimize the LGR tuning/matching. The LGR has an unloaded Q of 172 when empty and 90 when loaded with 55 ml of physiological saline solution. The efficiency factor (Λ) on the central axis of the empty LGR was measured by the perturbing sphere method (*21*). A maximum value of about 19 $\mu T/\sqrt{W}$ was found at the center of the LGR and it decreased to about 16 $\mu T/\sqrt{W}$ at the edges of the resonator. Because of this small variation of the efficiency factor we have assumed, as a first approximation, the effective volume of the TX resonator to be equal to its geometrical volume. A 90° flip angle then requires an RF pulse of 300 ns in width and 2.4 W peak power. With the physiological solution we measured $\Lambda = 14\ \mu T/\sqrt{W}$, giving a required peak power of 4.7 W. Each saddle-shaped coil (*20*) was made of adhesive copper strip (RS components, UK) 4 mm wide. The angular aperture of the saddle is 82° and the length is 50 mm. Each saddle-shaped resonator was laid on a Teflon cylinder of external diameter of 36 mm. The Λ at the center of the empty RX_S resonator (or RX_R resonator) was found to be about 16 $\mu T/\sqrt{W}$ and it decreased to about 9 $\mu T/\sqrt{W}$ at the ends. The unloaded Q of each RX coil was 105 when empty and 35 with 55 ml of physiological solution in place.

A network analyzer (HP8753A) was used to measure the isolation between the TX LGR and the RX coils in the presence of 55 ml of physiological saline solution. As reported in Fig. 3, using the single-channel receiver RX_S only (or RX_R only) the measured isolation was about 22 dB, but with the double-channel out-of-phase combination of RX_S and RX_R the isolation increased considerably to about 50 dB, within a bandwidth of a few MHz centered at the resonant frequency of 220 MHz. The resonator and receiver system was tested with high-power pulses at 220 MHz. A standard quenching technique (*11*) employing a composite TX pulse was used to reduce the ringing of the TX coil. The high-power TX pulse (typical 4 W rms) was composed of the

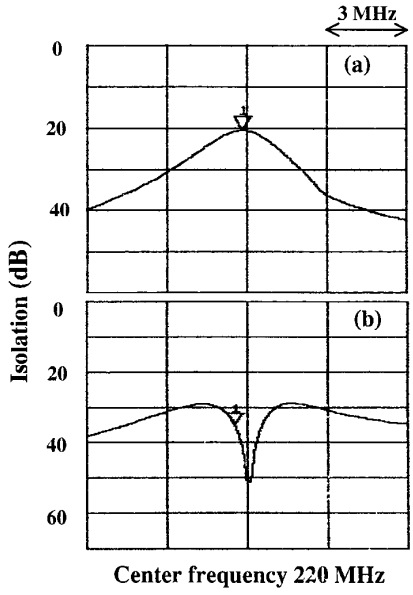

FIG. 3. Measured isolation between the TX and the RX coils in the presence of 55 ml of physiological saline solution: (a) with single-channel RX_S coil only (or RX_R only); (b) with double-channel out-of-phase combination of RX_S and RX_R.

main RF pulse (typical width 300 ns) and by an auxiliary pulse of opposite phase (typical width 60 ns). The start of the auxiliary pulse could be shifted with respect to the main pulse, to optimize the reduction of the TX resonator ringing. Figure 4b shows the decay of the ringing voltage obtained with single-channel detection using RX_S only (or RX_R only). Figure 4c shows the result obtained by subtracting the ringing voltages produced by RX_S and RX_R using a power combiner and a 180° phase shifter. The results of Fig. 4 show that the double-channel resonator system produces a dead time T_D of about 300 ns, giving rise to a reduction of about 10 with respect to the single channel detection mode. It is worth noting that the apparent faster decay time of Fig. 4c is due to the preamplifier overloading at the start of the ringing. To the best of our knowledge, this is the shortest dead time reported for these operating frequencies with large volume samples.

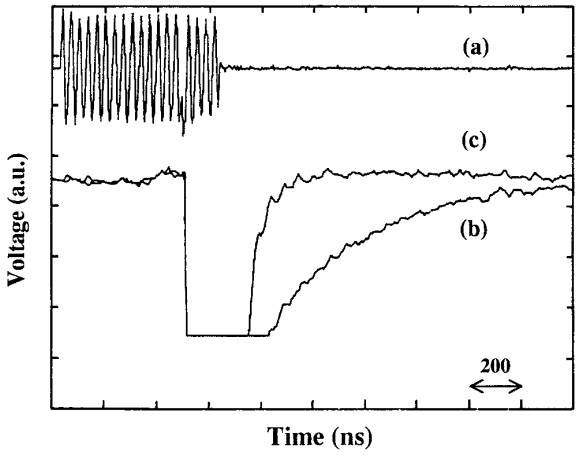

FIG. 4. (a) The composite high-power pulse at 220 MHz applied to the TX resonator. (b) The voltage decay measured with single-channel RX_S detection only (or equivalently RX_S only). (c) The result of the subtraction of the ringing voltages with double-channel out-of-phase combination using RX_S and RX_R.

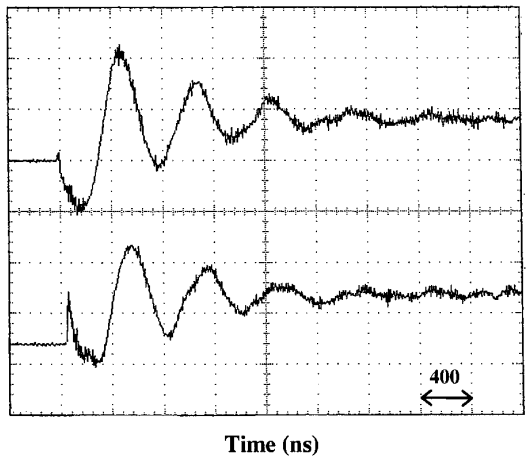

FIG. 5. Pulsed (time domain) EPR signal detection at 220 MHz using a small sample (1.5 g) of deoxygenated lithium phthalocyanine powder. Each spectrum (average of 10,000 FIDs) was acquired in 50 s with the main field of 8.18 mT and the TX power of 4 W (top) and 2 W (bottom).

Finally, we show experimental data obtained with the pulsed (time domain) EPR instrument using a small sample of deoxygenated lithium phthalocyanine (LiPtc) powder (*22*). The LiPtc powder was sealed in a small cylinder and inserted in a tube containing 55 ml of physiological saline solution. The 90° RF pulses (length 300 ns) were applied at a repetition rate of about 200 Hz (the long time between pulses was necessitated by the slow refresh rate of the oscilloscope). Figure 5 shows the FID signals, which were acquired at two different power levels. An SNR of about 20 was measured even with a low quality factor ($Q = 35$) receiver resonator. From these spectra the measured spin–spin relaxation time for the LiPtc was about 800 ns. Previous *in vivo* low-frequency CW EPR studies (*3–6*) have been performed with nitroxide free radicals at concentrations of about 4 mmol/kg in rats of 50 g, corresponding to an absolute number of spins in the rat of about 10^{20}. It has also been shown that, after administration, most of the nitroxides accumulate in the abdomen of rats (*3–6*). The LiPtc powder (1.5 g), sealed in a small cylinder and inserted into a cylindrical tube containing about 55 ml of physiological saline solution, used in the present work contains approximately 10^{21} spins. We believe that this is a reasonable model to simulate typical *in vivo* conditions. The actual speed limit of the spectrometer is determined by the low repetition rate

(200 Hz) of the digital oscilloscope. However, fast digitizer/averager systems have been reported (*23*, *24*) and we are currently developing a fast acquisition system with a repetition rate of about 200 kHz. This will allow 10,000 FIDs to be acquired in 50 ms, giving rise to a decrease in the acquisition time by a factor of 1,000, with respect to the CW EPR detection.

In conclusion, the resonator and receiver system presented here is suitable for pulsed magnetic resonance detection of very fast-decaying signals (dead time of about 300 ns) produced by large samples. We have shown that free radicals with very short relaxation times can be detected with a good SNR. The resonator and receiver system was especially designed for *in vivo* pulsed EPR spectroscopy and imaging. In addition to the biological applications, however, it can be used for pulsed nuclear magnetic resonance (NMR) imaging of solids (*25*). The current pulsed NMR detection techniques are restricted to solids with spin–spin relaxation times longer than approximately 3 μs. The use of a submicrosecond resonator and receiver system would considerably expand the applications of NMR imaging in materials research and the polymer industry.

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