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UHF EPR spectrometer operating at frequencies between 400 MHz and 1 GHz

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Abstract

A spectrometer was designed and constructed to facilitate measurements of T_1 , T_2 , spin echo signal-to-noise, and resonator quality factor, Q , between about 400 and 1000 MHz. Pulse patterns are generated at 250 MHz and mixed with the output from a second source to perform excitation and detection. A cross-loop resonator was constructed in which the same sample could be measured in the same resonator over the full range of frequencies. An air-core, 4-coil, water-cooled electromagnet with a large experimental volume was built.

Keywords

adjustable frequency; cross-loop resonator; frequency mixing

Introduction

Pulsed EPR imaging uses changes in electron spin relaxation times T_1 or T_2 to determine local oxygen concentrations [1, 2]. The selection of an “optimum” RF/microwave frequency for *in vivo* imaging of O_2 in tissues involves a tradeoff among electron spin relaxation times of the O_2 -sensitive probe molecule, the signal-to-noise ratio, and depth of penetration of the RF into tissues. A spectrometer was designed and constructed to facilitate measurements of T_1 , T_2 , spin echo signal-to-noise, and resonator quality factor, Q , between about 400 and 1000.

Most EPR spectrometers, whether commercial or purpose-built, have been designed to operate in a narrow band of RF/microwave frequencies, using the mixing scheme originally described by Weber et al. [3]. Many Bruker spectrometers mix from X-band to L-, S-, Q-, and W-band to take advantage of the well-developed pulse capabilities at X-band. Hyde et al. mixed from Q-band to W-band [4]. In our lab we have a 250 MHz EPR spectrometer [5]. Since we needed to explore pulsed EPR at frequencies between our currently-available 250

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MHz and 1 GHz, we built a system to use the existing pulse capabilities of the 250 MHz spectrometer [5-7] by mixing from 250 MHz to UHF frequencies between 400 and 1000 MHz.

Frequency Mixing System

The UHF spectrometer is designed to be a pulsed EPR accessory to the previously described VHF (250 MHz) spectrometer [5-7] constructed at the University of Denver. The heart of the UHF EPR spectrometer is the frequency mixing system. Output from a second frequency source is mixed with the pulse sequences of the 250 MHz system to achieve desired frequencies. Mixing with the second frequency is performed in an accessory box, whose inputs, outputs and internal construction are shown in Figures 1, 2 and 3.

Design Criteria

The design specifications for the UHF accessory were as follows:

- One relatively compact box to hold all of the RF components except the pulse power amplifier.
- It is designed to work only with a cross-loop (bi-modal) resonator in the frequency range of about 400 to 1,000 MHz (EPR field values from ca. 14 to ca. 36 mT). No circulator is provided to accommodate a UHF reflection resonator.
- No modifications to the existing VHF spectrometer are required other than a few cable connections.
- The second frequency source must not require phase lock to the VHF source. The VHF source is to be operated at a fixed 250 MHz regardless of the chosen UHF frequency.
- The accessory uses the VHF pulse forming unit for the EPR excitation pulse widths, timing, and amplitudes. All of the pulse experiments that can be performed on the VHF spectrometer will be possible at UHF using the accessory.
- The quadrature signal detection will be done in the VHF spectrometer at 250 MHz.
- The baseband video amplifier function and signal recording are done in the VHF spectrometer.
- User-friendly front panel controls.
- Simple wall-plug power supply.

Block diagram

The block diagram for the accessory is shown in Figure 4.

EPR operate mode – excitation—In the EPR operate mode, pulses at 250 MHz enter at the upper left of the block diagram. They have been formed in the VHF pulse forming unit from timing signals that originate in the Bruker console, which is a part of the DU VHF

spectrometer. These pulses are then mixed with a separate source frequency, designated Fluke Source #2. This is a Fluke (later Gigatronics) signal generator model 6082A. The upper or lower sideband of the mixing product is selected by the filter following the mixer, per table 1. Filter values are chosen to provide high attenuation of unwanted frequencies even if the operating frequency is attenuated by as much as 3 dB. This is needed because the separation between upper and lower sidebands is not as large as would ideally be the case. After the filter the pulse frequency is at the desired UHF frequency. Following the filter is a preamplifier that is used to bring the UHF pulse amplitude up to the level required by the external power amplifier. SW1 is a logically-controlled switch, configured as shown in the lower left of the block diagram. In the EPR operate mode SW1 is configured to connect port C to port 1. The connection configuration of each of the switches in the diagram for the operate mode and each of the tune modes is in the table at the bottom center of the block diagram. The pulses are then routed to an external power amplifier, which in this case is a Tomco model BT0300-Lambda, and an external isolator to protect the power amplifier from load mismatch damage. The amplified power pulses are then routed back into the UHF accessory unit to be routed to the excitation side of the bi-modal cross-loop resonator (CLR #1). The variable frequency resonator that we use with this UHF system is described in the resonator section.

EPR operate mode – detection—The EPR signal, either an FID or echo, is induced in CLR #2 and routed into the UHF accessory unit via port C of SW3. The SW3 configuration for EPR operate is set to connect port C to port 1. This routes the EPR signal to the input of a low-noise amplifier with 26 dB gain. The amplified signal is then routed to the RF side of a down-converting mixer. The LO side of the mixer is derived from the same “Fluke source #2” that supplied the up-conversion (VHF to UHF) for the excitation pulses described above. The IF output of the mixer is back to VHF (250 MHz). Note that it does not matter if the upper or the lower sideband was used to create the EPR operating frequency on the excitation side. The lower sideband is always used in the detection mixer, so only a fixed frequency low-pass filter at 300 MHz is needed here. Also note that while the power splitter is drawn as a balanced phase device it need not be controlled for phase. The phase between the IF (Fluke source #2) and the VHF and UHF frequencies is arbitrary so the VHF source (250 MHz) and source #2, (say 750 MHz for 500 MHz EPR), do not have to be phase locked or be derived from the same primary oscillator. The 250 MHz down-converted signal is then routed to the DU VHF spectrometer for detection to baseband followed by video amplification. After that, digitization, recording, and analysis are done in the normal Bruker Xepr hardware and software.

Resonator tune modes—Provision is made in the UHF accessory unit to tune the CLR using pulse ring-down [8]. The reflected signal from a resonator excited by a rectangular pulse of variable amplitude and frequency is used. Adjustments of each resonator of a CLR can then be made independently to tune resonator center frequency and coupling. The transmission mode response of a CLR can be observed to facilitate an isolation adjustment. In addition, the Q of each of the resonators in a CLR can be calculated from the observed ring-down time constant and the microwave frequency. The reflected responses to the tuning

pulses are observed with an oscilloscope after detection in a simple crystal detector in the accessory unit (upper right side of the block diagram)

CLR#1 tune mode: In order to observe a tune pattern for CLR#1 it is necessary to provide a rectangular pulse of microwaves to the resonator and then observe the reflection of that pulse in the time domain [8]. This path is established in the UHF accessory unit by selecting the SW1 path C-2, SW2 path C-1, SW3 path C-1 and the NO (Normally Open) path of the coaxial switch. The external power amplifier (Tomco) is not used in tune mode (except for the isolation adjustment) as the preamplifier power alone is sufficient. The SW3 path setting provides a 50 ohm termination for CLR#2 at port 2 of SW2 during CLR#1 tuning. The reflection from the CLR#1 resonator is detected by the crystal and observed on an oscilloscope. The tune pulse is formed in the VHF pulse forming unit from a control signal generated in the UHF accessory unit (see section below describing the UHF accessory logic and timing sections).

CLR#2 tune mode: The tune mode paths through the UHF accessory unit are very similar to what has been described for tuning CLR#1, above. The only difference is that the SW2 path is changed from C-1 to C-2, which causes CLR#2 to be excited by the tune pulse. Again the detection crystal and scope are used to observe the CLR#2 reflection.

“Both” tune mode: The chart at the bottom of the UHF accessory block diagram shows a “TUNE BOTH” mode. This was intended to be used with an alternating trace oscilloscope to observe the tuning patterns of both CLRs “simultaneously” on alternate traces of the oscilloscope. This is accomplished by switching the path through SW2 on alternate trials between CLR#1 and CLR#2. This feature works satisfactorily when an alternate-triggering analog oscilloscope is used. However, newer digital oscilloscopes do not employ alternate triggering mode so the “both” tune mode is no longer routinely used.

Isolation adjustment mode: Resonators of the CLR type require a mechanical adjustment to minimize the transmission of microwave power from the excitation resonator to the detection resonator. This is done by adjusting the orthogonality of the resonators. The UHF accessory unit provides a tune path to facilitate this adjustment. The isolation adjustment is basically to minimize the transmission mode path from CLR#1 to CLR#2. For this mode the external power amplifier is brought into the power path since the object is to measure and minimize an intrinsically very small signal. The path settings for this mode are: SW1 is set to path C-1, SW2 is set to path C-2, SW3 is set to path C-2 and the coaxial switch is set to the NC (Normally Closed) path. As with the other tune modes the result is observed on the oscilloscope after detection by the crystal. The best isolation is obtained when this transmission mode signal is minimized or eliminated.

Circuit Card Schematic Diagram

Figures 5 and 6 are schematic diagrams for the DU-designed circuit card that is in the UHF accessory unit. This card provides the selection logic for various functions, an oscillator for the tune display, and current drive amplifiers and switches for the coaxial relay and microwave amplifiers. A brief description of these circuits follows. Referring to Figure 5,

U1A and U1B form a dual one-shot oscillator in which a repeating pulse is produced at an adjustable repetition rate. The timeout of U1A determines the repetition interval, which can be adjusted by trimmer pot R5. The repetition interval can be adjusted over a range of approximately 300 μ s to 1 ms. A setting of approximately 600 μ s is used. The second one-shot, U1B controls the tuning pulse width. Trimmer pot R2 adjusts the pulse width. A setting of approximately 1 μ s is commonly used. This pulse width becomes the on-time of the microwave pulse applied to the resonator for tuning purposes. The pulse is also output to the oscilloscope to trigger the tune mode display (U3D and J5).

The pulse gate formed in the Bruker PatternJet module that normally goes to the VHF pulse forming unit is re-routed through the UHF accessory so that a selection can be made between the Bruker pulse gate and the UHF tune mode pulse. This selection is made by gates U3A, U3B, U3C and U4A. The Bruker pulse gate is selected for operate mode and the UHF tune pulse is selected for all tuning modes including isolation. After this selection, the pulse gate or UHF tune pulse is returned to the VHF pulse forming unit via connector J2 to gate the microwave pulses. Each path through the electronic microwave switches SW1, SW2 and SW3 requires a separate control signal. The logic matrix is shown in the table of switch paths in Figure 4. Gates U4A, U4B, U5B and U5C implement this switching matrix. Gate U5A decodes the control signal for the coaxial relay switch that is logically identical to the SW1 C-2 path control signal.

4-coil air-core magnet

At RF/microwave frequencies below about 1 GHz, and especially for imaging, it is convenient to use an open, air-core magnet instead of an electromagnet of the type that is familiar in EPR, where an iron core and yoke enhance and constrain the magnetic field to achieve the large uniform fields used for X-band and Q-band EPR. A large air-core magnet for 250 MHz EPR imaging was described by Rinard et al. [6]. The same design principles can be used for magnets with many different tradeoffs of cross-section, spacing, relative number of turns, etc. For the magnet described here, the experimental volume relative to outside dimensions was enhanced by the use of four square coils. The two inner coils have fewer turns than the two outer coils. This magnet was designed to achieve wide magnetic field scans for L-band (ca. 1.07 GHz) EPR spectroscopy [9], and is in the same lab as the 250 MHz spectrometer, so this magnet was used for the UHF EPR spectroscopy. The parameters are given in the following list. The magnet is constructed of AWG-10 square wire: $R = 2.8$ ohms, $L = 154$ mH. The magnet, including the frame that positions the water-cooled coils, is 52×52×43 cm. For L-band we normally operate at 37.2 mT (1.07 GHz). The coil constant is 1.67 mT/A so we require 22.3 A. The power required is thus approximately 1400 watts. The uniformity is 40 ppm over about 3 cm. Cooling water flows through a plastic jacket around each of the coils (Figure 7). A drawing of the four coils is shown in Figure 8 and a photograph of the assembled magnet is shown in Figure 9. Magnetic field homogeneity was adjusted by small changes in the spacing of the four coils, by using the brass threaded rod and nuts visible in the corners of the magnet. A small resonator was carefully positioned in three dimensions with an array of scales as shown in Figure 10.

The magnetic field of an air-core magnet is defined by the current, without the hysteresis and non-linearity problems of an iron-core magnet. Magnetic field gradients for imaging applications make it difficult to locate a Hall feedback device for magnetic field control. To circumvent these problems, yet remain compatible with the widely-used Bruker spectrometers, a current-sensing system was developed that looks like a Hall probe to the Bruker field controller. A block diagram is shown in Figure 11.

Resonator

The variable-frequency resonator is a key component of the UHF spectrometer. Most EPR resonators are designed to operate over a relatively narrow range of frequencies. The CLR for the multi-frequency measurements (Figure 12) was designed such that the frequency could be adjusted from about 400 to 1000 MHz with the same 16 mm od sample tube and the same filling factor, thus maintaining everything constant except RF frequency and resonator Q throughout the series of relaxation and S/N measurements. The variable-frequency resonator was designed to implement the constant size case described in [10] (case 1 in table 1). The frequency adjustment mechanism is shown in Figure 12B. The inductance of the circular loop is estimated to be about 6.8 nH and the gap capacitance is estimated to be about 1.8 pF, which would give a maximum resonant frequency of about 1.4 GHz. Movement of the disk capacitor changes the net capacitance of the gap, which adjusts the frequency of the resonator. The maximum capacitance of the circular disk capacitor is about 29 pF. This capacitance, combined with the gap capacitance, is about 31 pF which would give a minimum frequency of 350 MHz. The actual tuning range depends on the lossiness of the sample solution. Isolation is changed by mechanical adjustment of the cross-loop resonator to make the respective fields orthogonal. The filling factor with samples in 16 mm o.d., 0.9 mm wall, is about 30-35%.

The resonator Q was measured using a network analyzer (model HP 8719D) which has an operating range of 50 MHz to 13.5 GHz. To minimize dead time in pulsed operation, the resonator Q was switched as described in figure 5 of [7]. The Q values of both CLR#1 and CLR#2, empty and without Q-spoiling diodes were about 500 at 442 MHz and 700 at 984 MHz, which is very close to the predicted dependence on square root of frequency [11]. The frequency dependence of Q for the two resonators, with the Q-spoiling diodes installed, is shown in Fig. 13. The Q-spoiling diodes, when reverse biased (the high Q condition), have a loss that increases with frequency, which decreases resonator Q relative to that for the same resonator without the diodes installed (Fig. 13) to a greater extent at high frequency than at low frequency. When an aqueous saline sample (NaCl, 0.9% wt/vol) was inserted into the resonator, with these Q-spoiling diodes installed, the Q was decreased to a much larger extent. Thus for the aqueous saline solutions the Q of the resonator was primarily determined by the conductivity of the sample itself. The EPR signal, at a given frequency, is proportional to the square root of Q. Calculations indicate that the effect of the diodes reduced the amplitude of the EPR signal in an aqueous saline solution less than 1% at all frequencies studied, relative to that which would be observed if the diodes were not in the resonator.

Pulsed EPR operation

Pulsed EPR was performed with a Tomco BT0300-Lambda pulsed power amplifier. The operating specifications included an output power of 300 W over the frequency range 400 to 1000 MHz, with up to 10% duty cycle. The peak power output at 0 dB is 480 W at 600 MHz, and 435 W at 700 MHz. An example of the pulse shape, including rise and fall times, is in Figure 14. Figure 15 shows the free induction decay (FID) obtained with the UHF spectrometer for a sample of the symmetric deuterated trityl variously called Finland-d or trityl-CD₃. A two-pulse spin echo for the same sample is in Figure 16. These results are in excellent agreement with those expected based on prior work at 250 MHz.

Inversion recovery curves for the trityl radical OX063 in aqueous saline solution at frequencies between 400 and 980 MHz are shown in Fig. 17. The values of T_1 calculated by fitting an exponential to the recovery curves increase from 7.4 μ s at 500 MHz to 10 μ s at 980 MHz. The increase in T_1 with increasing frequency is consistent with published results at 250 MHz and 1 GHz [12]. The dependence of T_1 and T_2 for OX63 on frequency, radical concentration, and salt concentration are described in detail in ref. [13].

Summary

The frequency mixing system, air-core magnet, Hall probe simulator, and variable frequency cross-loop resonator create a spectrometer system in which measurements of free radicals can be made over a factor of almost 2.5 in frequency while the sample size and resonator size (and hence, filling factor) are kept constant. Extensive applications to trityl radical relaxation will be published separately.

Although our focus in this paper is on pulsed EPR, the same style UHF mixing system could be used for CW EPR and for rapid scan EPR [14, 15].

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Figure 1. Front panel controls for the UHF accessory box with (A) the high-pass or (B) low-pass filter mounted.

Figure 2.
Rear panel connectors for the UHF accessory.

Figure 3.
Internal construction of the UHF accessory.

Figure 4.
Block diagram for the UHF accessory.

Figure 5.
Schematic for tuning modes in the UHF accessory, part 1.

Figure 6.
Schematic for tuning modes in the UHF accessory, part 2.

Figure 7.
Expanded view of the plastic water jacket around one of the magnet coils.

Figure 8.
Drawing of magnet.

Figure 9.
Photograph of magnet.

Figure 10.
Apparatus for positioning small resonator inside the magnet to measure field uniformity.

Figure 11.

Block diagram for current-sensing system that was developed to look like a Hall probe to the Bruker field controller.

Figure 12. Variable frequency cross-loop resonator at UHF. (A) Front and back perspective drawings of resonator with sample tube. (B) Isometric view showing the capacitor disks used to vary the resonant frequency.

Figure 13.

Frequency dependence of resonator Q for (A) CLR #1 and (B) CLR #2 with the Q-spoiling diodes installed: (▲) empty resonator, (◆) with 16 mm ID tube containing deionized water, (●) with 16 mm ID tube containing 0.2 or 0.4 mM OX063 and 154 mM NaCl in 10 mM aqueous HEPES buffer (pH = 7.4).

Figure 14.

A 52 ns pulse at 586 MHz recorded with 1 dB attenuation. Rise time is ca. 5-10 ns.

Figure 15.

FID for trityl-CD₃ obtained from the UHF spectrometer. Microwave frequency = 702 MHz, 80ns $\pi/2$ pulse, center field = 24.54 mT.

Figure 16.

Two-pulse spin echo of trityl-CD₃ obtained with the UHF spectrometer. Microwave frequency = 702 MHz, 80ns $\pi/2$ pulse, center field = 24.51 mT.

Figure 17.

Inversion recovery curves obtained at 500 to 800 MHz for 0.4 mM OX063 in 10 mM aqueous HEPES (pH 7.4) containing 154 mM NaCl. Data were acquired with a shot repetition time of 4 ms, 16 averages/point, 5 scans, 1500 points per scan, and 10-point smoothing.

Table 1

Mixing VHF to variable frequencies 400 to 1100 MHz

Mixing Up										
Operating Frequency Range (MHz)			Inputs			Unwanted Frequencies		Filter Frequency	Minicircuits P/N ^a	
	To Resonator		Source 1		Source 2					
	Lower	Upper	f1	f2 lower	f2 upper		Lower	Upper		
Lower SB	400	500	250	650	750		900	1000	400-LP	VLFX-400
	500	600	250	750	850		1000	1100	470-LP	VLFX-470
	600	700	250	850	950		1100	1200	500-LP	VLFX-500
	700	800	250	950	1050		1200	1300	540-LP	VLFX-540
Upper SB	800	900	250	550	650		300	400	640-HP	SHP-700+
	900	1000	250	650	750		400	500	820-HP	SHP-900+
	1000	1100	250	750	850		500	600	900-HP	SHP-1000+
Mixing Down										
	From Resonator		Source 2		To detector					
	f2 lower	f2 upper	f1 lower	f1 upper	Mixer Output					
Lower SB	400	500	650	750	250	1050	1250	300-LP	VLFX-300	
	500	600	750	850	250	1250	1450	300-LP	VLFX-300	
	600	700	850	950	250	1450	1650	300-LP	VLFX-300	
	700	800	950	1050	250	1650	1850	300-LP	VLFX-300	
Upper SB	800	900	550	650	250	1350	1550	300-LP	VLFX-300	
	900	1000	650	750	250	1550	1750	300-LP	VLFX-300	
	1000	1100	750	850	250	1750	1950	300-LP	VLFX-300	

^aLow-pass filters are type VLFX and the high-pass filters are type SHP; both from Mini-Circuits.