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COMMUNICATIONS

1.1-GHz Continuous-Wave EPR Spectroscopy with a Frequency Modulation Method

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Continuous-wave EPR spectroscopy using a frequency modulation (FM) scheme was developed. An electronically tunable resonator and an automatic tuning control (ATC) system were used. Using the FM scheme instead of magnetic field modulation, we detected EPR absorption at the first derivative mode. We used a microwave frequency of 1.1 GHz in the present experiment. Similar signal-to-noise ratios were obtained with conventional field modulation and the FM method, and a low-quality factor EPR resonator was not necessary to suppress the significant microwave reflection from the resonator. The FM method with a tunable resonator may be an alternative solution to achieving phase-sensitive detection, when the side-effects of magnetic field modulation, such as microphonic noise and mechanical vibration, are detrimental for EPR detection.

Key Words: **continuous-wave EPR spectroscopy; frequency modulation; magnetic field modulation; tunable EPR resonator; automatic tuning control.**

Magnetic field modulation has been used to make highly sensitive measurements in continuous-wave (CW) electron paramagnetic resonance (EPR) spectroscopy (*1*). Perturbation of the DC magnetic field with an alternative magnetic field is called field modulation. After phase-sensitive detection (PSD), we can record EPR spectra with high sensitivity. PSD with magnetic field modulation is a conventional technique in CW-EPR spectroscopy (*1*). In practical CW-EPR spectroscopy and imaging, a Helmholtz coil or a saddle-type coil has been widely used for magnetic field modulation. The time-varying magnetic field generates an eddy current in components made of conductive materials, such as a resonator or shielding case. This eddy current can interact with the static magnetic field, as in an audio speaker. This side effect cannot be avoided with magnetic field modulation. In particular, high-field EPR and CW-NMR spectrometers face this problem for mechanical vibration due to $\mathbf{B} \times \mathbf{I}$, where **B** is the magnetic flux density and **I** is the current through conductive media.

There are various methods for detecting EPR signals without magnetic field modulation; pulsed EPR spectroscopy (*2–6*), multiquantum EPR spectroscopy (*7–11*), and longitudinally detected EPR spectroscopy (*12–15*), thermal modulation, and amplitude modulation of the microwave source (*1*). These are advanced methods for EPR instrumentation that require specific microwave technologies for the spectrometer setup. These methods raise technical issues in biomedical EPR applications. For example, the pulsed EPR method requires extremely high-speed switches because of the extremely short relaxation times in biological materials (*3–6*). The multiquantum EPR method requires good linearity for microwave elements and microwave generators that can emit a highly pure microwave (*7*). Longitudinally detected EPR should solve the problem of the high-level specific absorption rate (SAR) required in biological research (*13, 14*).

Another way to detect EPR signals without magnetic field modulation involves the use of frequency modulation (FM). Absorption spectra of gases with the FM method were investigated in the 1940s–1960s (*16–19*). The FM method in magnetic resonance spectroscopy was also investigated (*20–23*). In principle, magnetic field and frequency modulation may differ from each other, and Zhong and Pilbrow (*24*) have studied the theoretical investigations for this matter in detail. In fact, when small frequency modulation and magnetic field sweep are used, a difference between the EPR absorptions recorded by field and frequency modulation becomes ultimately small. Unless a tunable resonator is used in the FM scheme, a low-quality factor resonator should be used in the spectrometer setup. Otherwise, the frequency response of the resonator induces a significant reflection of the microwaves in an EPR bridge. Since EPR signal intensity is proportional to the product of the quality factor (Q) and the filling factor of the resonator, a low-Q resonator is detrimental to sensitivity. If an electronically tunable resonator is available for an EPR spectrometer, it can overcome

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the disadvantage of a low-Q resonator (*25*). An automatic tuning control (ATC) system is necessary in the FM method with a tunable resonator (*26*). Although FM detection is not a new idea, the FM method with a tunable EPR resonator and an ATC system is an alternative solution to achieving phase-sensitive detection when the side-effects of magnetic field modulation are detrimental to EPR detection.

This article reports the results of 1.1-GHz CW-EPR spectroscopy with the FM method instead of magnetic field modulation. In CW-EPR spectroscopy with the FM method, the magnetic field is swept to record first derivative EPR absorption, as in conventional CW-EPR spectroscopy. The resonance frequency of the resonator follows the modulated microwave frequency with the ATC system. Although the FM method can solve the problem of mechanical vibration due to $\mathbf{B} \times \mathbf{I}$, other technical issues are present, as described in the last part of this article.

A 1.1-GHz CW-EPR spectrometer with the FM method is based on a conventional CW-EPR spectrometer. Figure 1 shows a block diagram of the CW-EPR spectrometer with the FM scheme: all of the microwave elements in the EPR bridge are the same as in the conventional spectrometer. In the present study, however, the frequency of the resonator was electronically controlled by the ATC system. The uses of a varactor diode make tuning possible in the resonator circuit. While an automatic frequency control (AFC) system tunes the microwave frequency at the resonance frequency of the resonator, the ATC system tunes the resonance frequency of the resonator to the microwave frequency (*26*). In the ATC system, the microwave frequency acts as a reference for the feedback loop. The modulation signal (1.0 kHz) for EPR signal detection is added to the modulation signal (285 kHz) for the ATC system with a summing amplifier. While field modulation of 100 kHz has been used in conventional CW-EPR spectroscopy, low-frequency modulation of 1 kHz is used in the FM method. This is associated with the closed-loop function of the ATC system, as shown in Fig. 2.

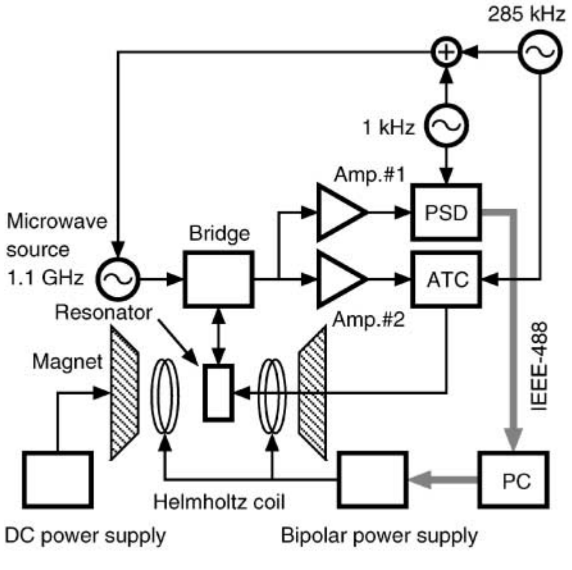

FIG. 1. Diagram of a continuous-wave EPR spectroscopy with the frequency modulation method. A free-running cavity oscillator (Magnum Microwave, FRL13-00, 1080-1220 MHz, 23 dBm) is used as a microwave source and the voltage applied to the electronic tuning port controls the carrier frequency. The automatic tuning control (ATC) system allows the tunable resonator to follow the microwave frequency modulated with a 1-kHz signal. After RF detection and amplification, the EPR signal is detected by a phase-sensitive lock-in amplifier (PSD) (NF Electronic Instruments, Japan, LI5640) associated with a reference signal from a signal synthesizer (Agilent Technologies, Palo Alto, CA, 33120A). A bipolar DC power supply (Kikusui Electronics, Japan, PBX20-5) drives the Helmholtz coil, and its maximum magnetic flux density is ±4.5 mT.

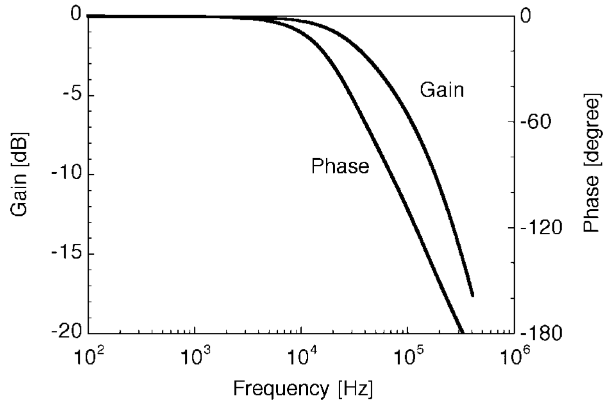

FIG. 2. Estimated closed-loop function of the automatic tuning control (ATC) system. The system bandwidth of the ATC system is 28 kHz in the calculation. A phase delay of 0.4° and an error of 1.2% are expected at a modulation of 1 kHz, so that the system can tune the resonance frequency of the resonator to the microwave frequency without significant error or delay. The numerical simulation was performed based on linear control theory (*27, 28*). In this calculation, we assumed that the microwave power applied to the resonator is 13 mW, the quality factor of the resonator is 260, and the deviation of the frequency modulation is 12.4 kHz (zero-to-peak).

The ATC system is a kind of negative feedback control, and its stability depends on the open-loop function of the system. There are some analogies between AFC/ATC and phase-locked loop (PLL) techniques (*27*), and the latter have been well established with the assistance of control theory. While PLL locks the phase of the output signal to the reference phase, ATC locks the resonance frequency of the resonator to the frequency of the microwave source. The closed-loop and open-loop functions can be calculated based on linear control theory (*28*). This allows us to predict the behavior of the feedback control system. Figure 2 shows the closed-loop function for the ATC system for given parameters for the microwave and electronic circuits in the experimental setup. In a numerical simulation, we assumed that the microwave power applied to the resonator is 13 mW, the quality factor of the resonator is 260, and the deviation of the frequency modulation is 12.4 kHz (zero-to-peak).

Since the ATC system in the spectrometer needs to provide a minimum frequency error, the system bandwidth was controlled at 28 kHz. Since the modulation frequency (1 kHz) is only 3.6% of the system bandwidth, the phase delay of the resonance frequency is 0.4° at a modulation of 1 kHz, as shown in Fig. 2. To maintain a broad bandwidth in the ATC system, we used higher frequency modulation (285 kHz). In practice, the electronic circuits of the ATC system are the same as in a conventional AFC system. After RF detection and amplification, an EPR signal is detected by a digital lock-in amplifier (NF Electronic Instruments, Japan, LI5640) associated with a reference signal from a signal synthesizer (Agilent Technologies, Palo Alto, CA, 33120A).

An electronically tunable surface-coil-type resonator was used in the present experiments (*29, 30*). The resonator has functions for tuning and impedance-matching by applying reverse-bias potentials to the control ports (a schematic diagram of the resonator has been shown in Fig. 1 of Ref. 29). The resonant system of the tunable resonator consists of a single-turn loop and a parallel transmission line. The mean diameter of the single-turn loop, i.e., a surface coil, was 10 mm, and this was made of 1.0 mm-thick copper wire. The resonance frequency of the resonator can be adjusted in the range of ±6 MHz from the center frequency of 1125 MHz in the absence of a sample. The resonance frequency is a function of the biased voltage with a coefficient of 1.26 MHz/V in the tunable resonator used in the present experiments. In the resonator, varactor diodes (Toshiba, Japan, 1SV186) have been used in tuning and matching circuits to make automatic control possible. The varactors do not significantly affect the quality factor of the resonator, because their loss is essentially small in the energy dissipation of the resonator. To avoid interference between magnetic field modulation and the biased voltages applied to the varactors, the tuning and matching circuits of the resonator are in a copper shielding case (5 mm thick) which may isolate magnetic field modulation. The generation efficiency of RF magnetic flux density is 77 μT/W$^{1/2}$, and the quality factor of the resonator is 260 in the absence of the sample. The surface coil has 16% of the energy stored in the resonator (*30*). The filling factor of the resonator becomes less than 0.01, when a small sample is placed at the center of the surface coil.

The magnet system consisted of a DC resistive magnet and a Helmholtz coil (radius of 50 mm). The Helmholtz coil was located at the center of the main magnet and was used for magnetic field scanning. The main magnet supplied the center field of EPR absorption. A bipolar DC power supply (Kikusui Electronics, Japan, PBX20-5) drives the Helmholtz coil, and its maximum magnetic flux density is ±4.5 mT. Although the field modulation for EPR signal detection is replaced by frequency modulation, magnetic field scanning is still used in the present experiments. Another Helmholtz coil was used for magnetic field modulation, and was wound on the bobbin of the field-scanning Helmholtz coil.

The EPR sample was 20 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) powder in a disposable polypropylene test tube (1.5 ml). The signal-to-noise ratio (SNR) in the FM method was compared with that in CW-EPR spectroscopy with magnetic field modulation. All of the microwave elements in the spectrometers were identical, except for the bandwidths of the operational amplifiers after RF detection (see Fig. 1). The bandwidth of amplifier #1 in the spectrometer with magnetic field modulation was from 2.4 kHz to 354 kHz, and that in the spectrometer with the FM method was from 180 Hz to 4 kHz. In the magnetic field modulation method, we used a modulation frequency of 90 kHz for EPR detection. The ATC system tuned the resonance frequency in both the magnetic field and frequency modulation experiments. However, a signal of 1 kHz (see Fig. 1) was not applied to the microwave source, when magnetic field modulation was used to detect the EPR absorption. For 4-hydroxy-2,2,6,6-tetramethyl-piperidinooxy (4-hydroxy-TEMPO) solution in a test tube, the minimum number of detectable spins was found to be 1.5×10^{16} with magnetic field modulation when the scan time is 30 s and the time constant of the lock-in amplifier is 30 ms. The dominant noise source in the experimental setup is a low-noise amplifier (Mini-Circuits, NY, ZEL-0812LN, noise figure 1.5 dB).

We recorded EPR spectra of the sample using the 1.1-GHz CW-EPR spectrometers that used either the FM or the magnetic field modulation techniques. The sample tube was placed at the center of the surface coil. The upper trace in Fig. 3 was recorded using the FM method when the frequency deviation and the microwave frequency were 28 kHz (peak-to-peak) and 1125 MHz, respectively. The modulation range is extremely small, and the modulation index is equivalent to 1 μT (peak-to-peak) at 40.2 mT in the magnetic field modulation method. The lower trace in Fig. 3 was recorded using magnetic field modulation, and the amplitude of the field modulation was 1 μT.

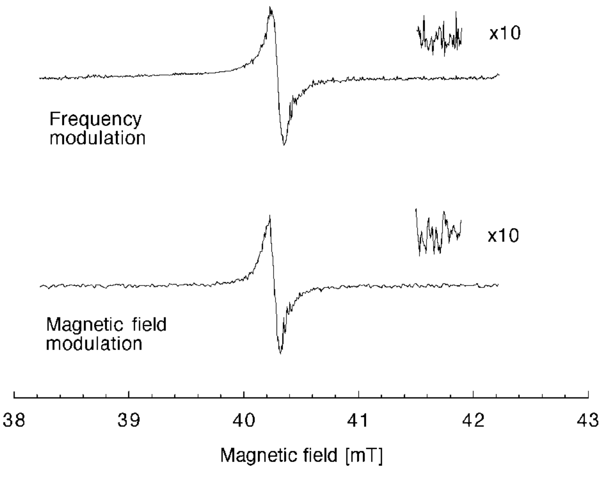

FIG. 3. EPR spectra recorded using frequency modulation (FM) (upper trace) and field modulation (lower trace). The sample was 20 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) powder. In the FM method, the frequency deviation was 28 kHz (peak-to-peak), and the modulation frequency was 1 kHz. In the magnetic field modulation method, the field modulation was 1 μT (peak-to-peak) and the modulation frequency was 90 kHz. In both methods, the scan width was 4.0 mT, the scan time was 30 s, the time constant of the lock-in amplifier was 30 ms, and the microwave power applied to the resonator was 13 mW. The signal-to-noise ratios in both traces are about 60.

Both traces have an SNR of about 60; the SNR for the FM method is almost the same as that with magnetic field modulation.

Since magnetic field modulation was not used in the present experiments with the FM method, there is essentially no microphonic noise. However, the SNR of the resultant spectrum depends on the degree of impedance matching of the resonator and the frequency characteristics of the microwave bridge. While the ATC system automatically tunes the resonance frequency of the resonator to the instantaneous microwave frequency, the phase delay of the ATC system is still present. The ATC system has a phase delay of 0.4° at a modulation of 1 kHz according to our calculation (see Fig. 2). This phase delay and the frequency characteristics of the microwave bridge affect the output signal at the modulation frequency (1 kHz) at RF detection. Even if there is no EPR sample, there is still output at the modulation frequency. This effect is the same as microphonic noise, in which the output signal has the same frequency as the modulation for PSD. This background signal is associated with the frequency characteristics of the oscillator, the low-noise amplifier, and reflections of the transmission lines in the spectrometer. Sealy *et al.* have pointed out that modulation should be transferred to the microwave carrier only at the resonance condition (*31*). The background signal resulted in by the violation of the "transfer of modulation" scheme. If frequency modulation is used, the error and delay of the ATC system cause baseline instability. While the sensitivity with the FM method was comparable to that in EPR spectroscopy with magnetic field modulation, the sensitivity with the FM method should decrease more due to the lack of an open-loop gain for the ATC system in the present experimental setup. To achieve more stable and sensitive spectroscopy with the FM scheme, we have to optimize the baseline stability.

The FM scheme can be applicable to both high-field EPR and CW-NMR spectroscopy. If the modulation coil is located in a higher magnetic field, the driving force due to interaction between the magnetic field and the conducting current becomes significant, and consequently the SNR is reduced. The FM method may be an answer to the problem of mechanical vibration in high-field EPR and CW-NMR spectroscopy.

With our experimental setup, the FM scheme would have limited application, since the microwave oscillator's frequency control is within ±1.5 MHz. When a microwave oscillator is tuned in a broad frequency range, one can apply the maximum modulation for the sample to obtain the maximum signal intensity. To decrease the spectral frequency by 1 kHz in the absence of the EPR sample, we need to use microwave components with frequency characteristics as flat as possible. In the present experiments, $1/f$ noise did not seem to be significant, and thus the SNR of the FM method was comparable to that with magnetic field modulation. While we demonstrated the FM method with the tunable resonator and the ATC system, the noise source for limiting the sensitivity was a low-noise amplifier in the experimental setup. This noise source is a key issue to make the comparison in depth of frequency and field modulation.

The FM method has several advantages: (i) freedom from microphonic noise and mechanical vibration due to the absence of magnetic field modulation, (ii) avoidance of modulation coils and an amplifier to drive the coils, and (iii) homogeneous modulation throughout the sample volume. For magnetic field modulation, homogeneity of the time-varying magnetic field in a volume of interest is an issue in recent spectroscopy and imaging techniques. This is because the modulation amplitude directly affects the intensity of EPR signals. Nevertheless, the FM method can essentially yield homogeneous modulation in a sample, since the deviation of the frequency modulation over the volume-of-interest is the same as that of the RF magnetic field. In contrast, the FM method also has some disadvantages: (i) baseline instability when the open-loop gain of the ATC system cannot minimize microwave reflection, and (ii) the strong influence of impedance matching between the resonator and the transmission line on the noise level of the baseline. Since all microwave elements have unique frequency characteristics, these may affect the amplitude of the incident microwaves at the RF detector. This makes the microwave bridge a frequency discriminator. To address this problem, an automatic matching control system may help to improve the baseline stability.

We have described a new CW-EPR spectroscopy with an FM scheme. The sensitivity of FM-EPR detection was comparable to that of EPR with conventional magnetic field modulation in our experiments. CW-EPR spectroscopy with the FM method is free from microphonic noise, and avoids the use of a modulation coil and an amplifier to drive the coil. However, the FM method also has undesired side-effects. The open-loop gain of the ATC system is a key factor for improving the baseline stability, and the limiting noise source has to be investigated to interpret the comparison of frequency and field modulation.

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