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HYSCORE and DEER with an upgraded 95 GHz pulse EPR spectrometer

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ABSTRACT

The set-up of a new microwave bridge for a 95 GHz pulse EPR spectrometer is described. The virtues of the bridge are its simple and flexible design and its relatively high output power (0.7 W) that generates π pulses of 25 ns and a microwave field, B_1 = 0.71 mT. Such a high B_1 enhances considerably the sensitivity of high field double electron-electron resonance (DEER) measurements for distance determination, as we demonstrate on a nitroxide biradical with an interspin distance of 3.6 nm. Moreover, it allowed us to carry out HYSCORE (hyperfine sublevel-correlation) experiments at 95 GHz, observing nuclear modulation frequencies of ^{14}N and ^{17}O as high as 40 MHz. This opens a new window for the observation of relatively large hyperfine couplings, yet not resolved in the EPR spectrum, that are difficult to observe with HYSCORE carried out at conventional X-band frequencies. The correlations provided by the HYSCORE spectra are most important for signal assignment, and the improved resolution due to the two dimensional character of the experiment provides ^{14}N quadrupolar splittings.

1. Introduction

The scope of high field EPR is broad and, in principle, encompasses all the sub-disciplines of EPR spectroscopy, namely continuous wave (CW), time resolved and pulsed EPR, electron-nuclear-double resonance (ENDOR) and double electron-electron resonance (DEER) techniques. In the last decade numerous applications of high field CW EPR and ENDOR have been reported and the virtues of measurements at a high field have been well demonstrated and established [1–6]. Recently, it has also been demonstrated that distance measurements between paramagnetic centers by pulsed DEER techniques can also be performed at high fields [9–12]. Distance measurements have become very popular in the last few years and are carried out usually at X-band frequencies [7,8]. The high field measurements offer additional information such as the relative orientations of the g-tensors of the two coupled paramagnetic centers [10]. Among the above mentioned high resolution EPR techniques, the absence of applications of electron–spin echo modulation (ESEEM) spectroscopy is conspicuous. ESEEM spectroscopy is a well established method for measurements of small hyperfine and nuclear quadrupole interactions [13,14], and is complementary to ENDOR. The ESEEM advantages are that it does not require the application of RF (radio-frequency) pulses, and therefore it is simpler than ENDOR in terms of cavity design and hardware requirements. Furthermore, a unique and most useful property of ESEEM experiments is the possibility to generate sum combination frequencies. The frequency and the fine structure of sum combination lines are directly related to the anisotropic part of the hyperfine interaction [17] and the nuclear quadrupole interaction, respectively [15,16]. Moreover, two dimensional (2D) ESEEM experiments (such as hyperfine sublevel correlation, HYSCORE, spectroscopy) can be readily performed. These experiments often yield highly resolved spectra, disentangling overlapping signals arising from multiple nuclei and providing correlations that are essential for signal assignment. These virtues turned HYSCORE spectroscopy into a very popular technique.

In the past, due to widespread use of proton related ESEEM measurements it was often assumed that ESEEM spectroscopy is best performed at low frequencies (2–9 GHz) to decrease Zeeman frequencies of nuclei and therefore facilitate the simultaneous excitation of forbidden and allowed EPR transitions necessary for the generation of the ESEEM effect. This led to the design and construction of low frequency pulse EPR spectrometers such as S- and C-band spectrometers [18–22]. However, experience with Ku, (12–18 GHz) Ka- and Q-band (26–35 GHz) gained during the last few years showed that ESEEM spectroscopy at these frequencies can be routine and very useful [23–25], thus expanding considerably the range of multi-frequency ESEEM. We expect that ESEEM experiments carried out at even higher frequencies will make new ranges of hyperfine and nuclear quadrupole couplings accessible. This is supported by a few reports of ^{14}N ESEEM observed at W-

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band, where the hyperfine couplings were on the order of the nuclear Zeeman frequency [26–28]. In these reports, however, the frequencies observed were below 10 MHz, corresponding to the electron–spin manifold for which the hyperfine coupling and the Larmor frequency are of opposite signs. The nuclear frequencies of the second electron–spin manifold, which are higher, could not be observed due to power (B_1) limitations. Nonetheless, these reports, along with the new pulse sequences designed for the enhancement of the nuclear modulation [29–31], call for implementing ESEEM spectroscopy at W-band (95 GHz).

As mentioned above, the major obstacle for the observation of nuclear modulation at high operational frequencies is the lack of the mw (microwave) power necessary to produce mw pulses with a large enough bandwidth and amplitude that can excite simultaneously the relevant allowed ($\Delta M_S = \pm 1$, $\Delta M_I = 0$) and forbidden ($\Delta M_S = \pm 1$, $\Delta M_I \neq 0$) electron–spin transitions. At lower operational frequencies (2–34 GHz) the problem was solved by introducing high power amplifiers and constructing specially designed cavities. Unfortunately, the available power of commercial W-band spectrometers generates a B_1 amplitude of only $\sim$0.28 mT ($\omega_1 \sim 8$ MHz) [12]. This limits the number of systems amenable for ESEEM investigations. The limited B_1 also restricts W-band DEER applications. The DEER experiment, in principle, does not require very short pulses, but the pulse bandwidth does determine the upper limit of the dipolar interaction that can be accessed by the technique. Moreover, insufficient mw power requires the application of relatively long pulses ($\sim$100 ns) [12] and thereby reduces the sensitivity of the technique.

The first homebuilt pulsed W-band EPR spectrometers were based on Ukrainian technology, using IMPATT amplifiers and the maximum output power of the W-band microwave bridge was about 200–250 mW, producing π pulses with a minimum length of 80 ns [32–34,28]. This is higher or comparable to the power available in most commercial W-band spectrometers [12]. A pulse EPR spectrometer, operating at 140 GHz, utilizing a 300 W extended interaction klystron reported a $\pi/2$ pulse duration of $\sim$14 ns with full power that was estimated as 30 W incident on a Fabry-Perot cavity [35]. Recently, a unique high power W-band spectrometer has been reported by Freed and coworkers [36]. It's high mw power (1 kW) originate again from a specialized mm-wave amplifier tube, an extended interaction klystron. This spectrometer features pulses of 5 ns with a spectral width of 200 MHz and a deadtime of about 50 ns. This particular spectrometer was specifically designed for Fourier transform (FT) EPR measurements at ambient temperatures. It is primarily used for the investigation of the dynamics of complex systems of biological relevance in aqueous solutions, where the paramagnetic entity is usually a nitroxide spin probe. This spectrometer employs quasioptical techniques in the high-power stages to reduce power losses and a compact Fabry-Pérot resonator. In principle, such a spectrometer could be duplicated and accommodated for low temperature ESEEM/DEER measurements, but due to the uniqueness of the mw source this idea is impractical. A more practical approach is to upgrade the design of the old W-band bridge, taking advantage of the progress made in the last decade in the development of commercially available mw components.

Here we present a new W-band bridge with a relatively high power, constructed from available commercial components, based on waveguide technology. It is designed primarily for low temperature electron spin echo spectroscopy that require the application of short powerful mw pulses, such as ESEEM and DEER. The virtues of this bridge are its simple design and flexibility, which makes any modification straightforward. It has an output power of 0.7 W with long term power and phase stability and it features a π pulse of 25 ns. This new W-band bridge replaced our old microwave bridge, which had a maximum power of 250 mW, obtained using IMPATT amplifier technology [24].

In the following we describe the new bridge and some other minor changes made to our homebuilt spectrometer. The spectrometer performance is demonstrated on DEER measurements of a rigid nitroxide biradical and on HYSCORE measurements of two systems in frozen solutions. The first is the NO complex of ferrous myoglobin, revealing ^{14}N frequencies and resolving quadrupolar splittings, and the second is $V^{17}O(H_2{}^{17}O)_5^{2+}$ showing ^{17}O frequencies. The range of nuclear frequencies observed is 8–40 MHz, demonstrating the potential of such measurements for deciphering transition metal coordination spheres in a variety of systems.

2. The MW bridge

The design of the mw bridge is simple and similar to standard pulse X-band spectrometers. Except for the source, all operations, such as pulse formation and phase modulation are carried out at 95 GHz. The bridge utilizes a homodyne detection scheme, without the involvement of an intermediate frequency. The scheme of the bridge is given in Fig. 1. It consists of two identical channels, each of which can generate pulses with controlled independent frequency, phase and amplitude. Channel I uses a computer controlled frequency synthesizer (7.3 ± 0.1 GHz, with a frequency step of 1 kHz, Herley, CTI XS-7311). The source output is amplified to produce 20 mW power that is fed into a x13 multiplier followed by a bandpass filter, producing a 94.9 ± 1.3 GHz continuous wave signal. Part of this signal is directed, via a 3 dB directional coupler, to the receiver arm and the other part passes through a narrow band isolator that suppresses possible leakage of a reflected amplified signal to the reference arm. The signal is then fed into a digital phase modulator (0/180°) and to a fast PIN switch to form pulses. The output pulses are then preamplified and fed into a voltage controlled variable PIN attenuator for power adjustment.

Channel II is similar to channel I and has a manual phase shifter for adjusting the relative phase of the two channels. At the moment it lacks a digital phase modulator that will be installed in the near future. The current source of the second channel is based on a ADF4113 chip (BA Microwave, 0.4–8 GHz, resolution of 20 kHz). This will be replaced shortly with a source similar to channel I. The sources of the two channels are not commonly locked, but with a fast switch (Eyal microwaves, 35 ns switching speed) and a splitter it is possible to connect channel II to the source of channel I. The pulses formed in the two different channels are combined using a 3 dB directional coupler and then amplified by a gated 23 dB solid state amplifier with a saturated power output (P_s) of 1 W. The amplified pulses pass through a mechanical attenuator for further adjustment of the output power. The pulses are finally fed into the cavity via a circulator. The maximum power available at the output of the circulator is 700 mW. The probehead houses a cylindrical TE_{011} cavity and has been described previously [34]. Due to losses in the waveguide leading to the cavity, the power at the entrance to the cavity is 300–350 mW.

The transient signals reflected from the cavity are fed into the receiver, which consists of a 20 dB directional coupler connected to a diode detector for viewing the reflected pulses from the cavity for diagnostic and tuning purposes. The main power then goes through a protect PIN switch, similar to those used in channel I and II for pulse forming, a variable mechanical attenuation, a low noise amplifier (LNA) and a mixer. The LO (local oscillator) input of the mixer comes from channel I. To adjust the phase of the receiver and to optimize the LO power level needed for the best mixer response, the input power passes through a mechanical variable attenuator, a 20 dB power amplifier, a 6 dB fixed attenuator and a mechanical phase shifter. The video signal (IF output of the mixer) is preamplified by a broadband video amplifier (0–500 MHz) and is directed to the acquisition system of the spectrometer via