Published in final edited form as:

Concepts Magn Reson Part B Magn Reson Eng. 2013 August 1; 43(3): 100–109. doi:10.1002/cmr.b.21240.

Design and implementation of an FPGA-based timing pulse programmer for pulsed-electron paramagnetic resonance applications

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Abstract

The design, construction and implementation of a field-programmable gate array (FPGA) -based pulse programmer for pulsed-electron paramagnetic resonance (EPR) experiments is described. The FPGA pulse programmer offers advantages in design flexibility and cost over previous pulse programmers, that are based on commercial digital delay generators, logic pattern generators, and application-specific integrated circuit (ASIC) designs. The FPGA pulse programmer features a novel transition-based algorithm and command protocol, that is optimized for the timing structure required for most pulsed magnetic resonance experiments. The algorithm was implemented by using a Spartan-6 FPGA (Xilinx), which provides an easily accessible and cost effective solution for FPGA interfacing. An auxiliary board was designed for the FPGA-instrument interface, which buffers the FPGA outputs for increased power consumption and capacitive load requirements. Device specifications include: Nanosecond pulse formation (transition edge rise/fall times, 3 ns), low jitter (150 ps), large number of channels (16 implemented; 48 available), and long pulse duration (no limit). The hardware and software for the device were designed for facile reconfiguration to match user experimental requirements and constraints. Operation of the device is demonstrated and benchmarked by applications to 1-D electron spin echo envelope modulation (ESEEM) and 2-D hyperfine sublevel correlation (HYSCORE) experiments. The FPGA approach is transferrable to applications in nuclear magnetic resonance (NMR; magnetic resonance imaging, MRI), and to pulse perturbation and detection bandwidths in spectroscopies up through the optical range.

Keywords

FPGA; pulse programmer; pulsed EPR; EPR spectroscopy; magnetic resonance

INTRODUCTION

In pulsed-electron paramagnetic resonance (EPR) experiments, one or more short pulses of microwave (MW) electromagnetic (EM) radiation are used to manipulate electron spin coherences to generate free induction decay (FID) or electron spin echo (ESE) signals (1)

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(2). A balance among the available time resolution of the pulse-forming circuit, the MW pulse power and desired excitation bandwidth is typically met by square pulse widths of tens of nanoseconds. Continuous-wave MW radiation, of a frequency that is resonant with electron spin flip transitions in the EPR line, is created by a broad-band MW synthesizer. The most simple, 2-pulse ESE is generated by two microwave pulses, that are separated by the dephasing time, τ (2). The 2-pulse ESE is formed at a time, 2τ , after the first microwave pulse. This experiment requires a minimum of 6 logic pulses, on separate channels, that interface with spectrometer components, as follows: (a) Two pulses gate PIN diode switches that create the two MW pulses from the synthesizer output. (b) Two pulses overlap the low power MW pulses in time, to gate the MW traveling wave tube (TWT) amplifier. (c) One pulse controls a PIN diode switch that protects the receiver components from the high-power MW pulses. (d) One pulse gates the measurement device. The integrated amplitude of the ESE as a function of increments in τ , which generates the echo envelope, is modulated at the precession periods of nuclear spins, that are coupled to the unpaired electron spin. This 2-pulse electron spin echo envelope modulation (ESEEM) experiment thus requires increments in pulse timing on 5 channels. Echo modulation experiments with >2 pulses require MW phase modulation schemes to eliminate unwanted ESE signals (1, 2), and thus, additional logic pulses and output channels for gating phase modulators. The precision in timing and maximum number of logic pulses determines the overall experimental performance, and the types of experiments that can be conducted, respectively. Therefore, in addition to fast and flexible programming capabilities, the following features are essential for the timing pulse programmer in pulsed-EPR experiments: Nanosecond timing resolution, including logic transitions on the ns or shorter time scale, low jitter of a few hundred ps or less, large number of channels (at least 6), and long pulse durations extending to the ms range, that satisfy low-temperature relaxation experiments. Here, we describe a flexibly configurable, versatile and cost-effective pulse programmer, that is based on the field programmable gate array (FPGA) integrated circuit (IC) (3).

Merits of the FPGA-based pulse programmer are revealed by considering the performance features of prior successful hardware approaches. In home-built pulsed-EPR spectrometer systems, three basic types of pulse programmer have been implemented, since the 1990's. The first type is based on commercial digital delay generators (4–6). Commercial digital delay generators, including the popular, 4-edge/2-pulse DG535 (Stanford Research Systems; Sunnyvale, CA), have the advantages of precise control of delay time, at picosecond resolution, and long delay durations (> 1 ms). Disadvantages include a limited number of edges (output channels) for pulse formation in each unit, and the added circuitry that is needed to combine pulse outputs of multiple, ganged units. The second type of pulse programmer is based on the logic pattern/word generator, or digital arbitrary waveform generator (AWG). Timing control on our home-built pulsed-EPR spectrometer previously utilized the 613 MHz-bandwidth HFS9003 Stimulus System (Tektronix; Beaverton, OR), with 2 expansion cards, that each incorporate 4 output channels. The outputs for each pulse cycle are stored in 64 Kbit memory per channel, and are triggered externally at rates of > 5 kHz. Drive strength and output level are specifiable for each output channel. The advantage of the logic pattern generator is extensive control over the pulse patterns. Disadvantages for pulsed-EPR experiments are as follows: (a) The output patterns of each clock cycle are pre-

calculated and stored in the finite memory. The maximum pulse pattern duration is therefore limited by the memory size. (b) Programming the pulse pattern into the memory requires substantial set-up time. For the HFS9003 system, the longest pulse pattern duration is limited to 128 μ s with a 2 ns time base, and the computer interface (General Purpose Interface Bus, GPIB) requires seconds for pulse pattern set up. A third approach to pulse programming is a customized, application-specific integrated circuit (ASIC). In the implementation of Gromov *et al.* (7), 2 ns resolution, 8 channels per chip, and a simple serial communication protocol were achieved. The purpose-designed ASIC is fast and efficient. Disadvantages of the ASIC technology include the high entry barrier for ASIC design and fabrication, and relatively inflexible architecture. Furthermore, ASIC chips require additional circuitry to function as a stand-alone instrument.

FPGA technology (3) offers a new approach to high-performance pulse programmer hardware design for pulsed-EPR spectroscopy, that is transferrable to other magnetic resonance applications. An FPGA is an IC, that is designed to be configurable “in the field.” The first commercial FPGA devices emerged in the mid-1980’s, and a wide variety of multi-channel models, with bandwidths extending into the GHz range, are now available. The re-programmability of the FPGAs provides designers with a high degree of flexibility, and reduces the time and cost associated with circuit design (3). The basic logic elements of an FPGA are Look-Up-Tables (LUT) and Flip-Flops (FF). The LUT’s can be configured to replicate the functionality of a specific logic gate [e.g., negated and (NAND), exclusive or (XOR)] or a more complex function (for example, a register or an adder). The design flexibility of an FPGA requires an increased number of transistors per logic element, and an attendant low energy efficiency. For large-scale production, an ASIC design can be considerably less expensive and more efficient than an FPGA design. However, in the laboratory, and for relatively small-scale production, the flexibility provided by an FGPA design can lead to considerable savings in time and cost.

We demonstrate a straightforward implementation of the software and support hardware of a new FPGA-based design for control of pulsed-EPR experiments. Our design realizes a significant advantage in cost over other pulse programming technologies, because we utilize an FPGA with a low-frequency internal clock (250 MHz), and apply a serialization approach to increase the timing resolution to 2 ns. The hardware system includes the FPGA (Spartan-6 LX45, Xilinx; San Jose, CA), auxiliary boards, power supply, and high-speed buffer circuitry for the FPGA device-spectrometer interface. The associated, home-built X- and Ku-band pulsed-EPR spectrometer is described. Function of the FPGA device is demonstrated for 1-D 3-pulse ESEEM and 2-D, 4-pulse hyperfine sublevel correlation (HYSCORE) spectroscopy experiments.

MATERIALS AND METHODS

FPGA logic and transition-based command protocol

A distinguishing feature of our design is a transition-based command protocol. This protocol is introduced, because, in general, timing pulses in magnetic resonance experiments occur infrequently, in comparison with the total pattern length. The protocol obviates the need for time-consuming pre-calculation and storage of output patterns for each clock cycle, as found

for the AWG. The selected FPGA was the Spartan-6 LX45 (Xilinx). Figure 1 illustrates how the transition-based command protocol is processed inside the FPGA logic, among the following 6 functional blocks: Digital clock management (DCM), Universal Asynchronous Receiver/Transmitter (UART) parser, block memory, control logic and finite state machine (FSM), command first-in/first-out (FIFO), and SelectIO output. The DCM, block memory and SelectIO are implemented by using Xilinx IP cores, for specific hardware component functions. The logic controls are synthesized and implemented from codes constructed in the Verilog hardware description language (HDL), by using the Xilinx Integrated Software Environment (ISE) software (version 14.3).

To maintain a synchronized logic among the block memory, control logic and the command FIFO, the main operating frequency is kept low, at 250 MHz. A serialization approach is utilized with the SelectIO output block to achieve the nanosecond timing resolution required for pulsed-EPR experiments. The SelectIO block functions as a serializer, with a wider input width at a lower clock speed and a narrower output width at a higher clock speed. The integral ratio of the input width to the output width, which equals the ratio of the higher clock speed to the lower clock speed, is denoted as the serialization factor. In our design, a serialization factor of 2 is used to establish a 2 ns timing resolution. In order to realize 1 ns resolution on 16 channels with 250 MHz input clock, a serialization factor of 4 with an output serialization clock of 1 GHz, and an input de-serialized data width of 64 bits would be required.

The DCM block generates the clock signals. An on-board, external 100 MHz oscillator signal is buffered and fed into an on-device phase locked loop (PLL) unit to generate low-jitter (<150 ps) 500 MHz, 250 MHz, and 100 MHz clock signals. These clock signals are distributed through global clock lines to allow low-skew and low-delay propagation, in the ISE implementation. The UART parser block, which operates at 100 MHz, receives incoming transmission of user instructions and parses them into operations that write specific bytes into selected memory addresses. A dual port block memory with an 8-bit write port and a 64-bit read port is used to store the transition commands. The 64-bit transition commands, required for the 2 ns, 16 channel configuration, is organized as follows: The first 32 bits (T_T) are the transition time in the 4 ns (250 MHz) time base, the next 16 bits (D_0) are the output of 16 channels prior to transition, and the last 16 bits (D_1) are the output at the transition time. With a serialization factor of 2 for the SelectIO, the 32-bit SelectIO input is D_0D_0 before the transition, and D_0D_1 at the transition time. The block memory read port width can be configured up to 256 bits, and more than one block memory unit can be configured on one FPGA. This allows a wide range of configurations of transition commands to be used for different timing and channel number requirements. For example, if 1 ns timing resolution and 16 channels are desired, a 128 bit read width can be configured for the block memory. The first 64 bits (T_T) represent the transition time, and next four 16 bits represent the data (D_0 , D_1 , D_2 , and D_3), thus accommodating a serialization factor of 4. In this case, the 64-bit SelectIO input is $D_0D_0D_0D_0$ before the transition, and $D_0D_1D_2D_3$ at the transition.

The read address input of the block memory is registered in our design, resulting in the read data lagging two clock cycles behind a change on the read address input. In hardware tests,

the actual read data output from the block memory was not available exactly after two clock cycles, and behavior simulation by using Xilinx ISIN software indicates a 100 ps delay. For this reason, a command FIFO, with four 64-bit registers, which buffers the block memory readout, is implemented in the design.

Figure 2 illustrates how the transition-based command protocol and the control algorithm work. This example is based on a serialized 500 MHz time base, with 2 ns time resolution and 2 output channels. The function of this example pulse program is to create a pulse on channel 1 from 20 ns to 62 ns, and another pulse on channel 2 from 42 ns to 64 ns. This arrangement could correspond to the gating of the TWT amplifier (channel 1), which has a relatively long rise-time, and the formation of the low power pulse by a PIN diode switch (channel 2). The pulse pattern translates into 4 memory instructions as listed in Figure 2A. The main algorithm resides in the control logic and FSM block. When a falling edge on the trigger is detected, the internal state proceeds from state 0 to state 6, and during those states, memory instructions are buffered into the command FIFO sequentially, as shown in Figure 2B. After command FIFO is prepared, a timing counter starts, and is compared with the T_T field of the current command in the FIFO. The current command in the FIFO is indexed with the “FIFO Top” register. When a transition is reached (at 10 ns, 20 ns, 22 ns, and 32 ns in Figure 2B), this index is incremented by one, and the FIFO consumption flag (“FIFO Full_n”) is set, resulting in the next memory instruction being pushed into the command FIFO. The algorithm continues until a total number of transitions (stored in M0) is reached.

FPGA auxiliary circuit

The FPGA and associated components of the pulse programmer, and the layout, are shown in Figure 3. In the timing pulse programmer, an Atlys development board (Digilent; Pullman, WA) was employed with the Xilinx Spartan-6 LX45 FPGA. The Atlys platform provides the serial flash memory device to program and configure the FPGA. Configuration files are loaded into the FPGA through an on-board USB transceiver. An auxiliary board is connected to the Atlys board via a Very-High-Density Cable Interconnect (VHDCI) connector. In the current implementation, the outputs of timing pulses are buffered with an IC (OPA693; Texas Instruments; Dallas, TX), which is an 800 MHz high-speed op-amp that provides a slew-rate of 2,500 V/ μ s and can supply or sink currents as high as 200 mA to drive high capacitive loads (>100 pF). If higher slew-rate or output current are desired, other op-amp buffers, such as the OPA2670 (Texas Instruments), with a slew-rate of 5,000 V/ μ s and max output current of 700 mA, can be implemented. The timing pulses are routed through U.FL connectors and WRL-09145 cable (Sparkfun Electronics; Boulder, CO), into SubMiniature version A (SMA) connectors on the front panel, which provide the FPGA device-spectrometer interface. The FPGA board and the auxiliary board are powered by a dual-voltage external power supply (TML 40205C, Traco; Zurich, Switzerland), and mounted in a 19” aluminum rack-mount case. The total cost of components for the FPGA-based pulse programmer was less than 500 USD. The cost is 10- to 30-fold less, relative to the costs of current commercially available logic pattern generators [for example: PG3A, PG3L (Tektronix); PXIe-7961R, 654 $\times$, 655 $\times$ (National Instruments; Austin, TX)], and the FPGA-based pulse programmer exhibits superior overall performance for the pulsed-EPR application.

Pulsed-EPR Spectrometer

The home-built pulsed-EPR spectrometer incorporates broadband components that allow operation over the full X-band (8.2–12.4 GHz) and Ku-band (12.4–18.0 GHz) MW frequency ranges. The design is novel, but it is based on prior home-built pulsed-EPR spectrometers (4, 5, 8, 9). This pulsed-EPR spectrometer (with the HFS9003 Stimulus System as pulse programmer) has been applied in biophysical studies (10–16). A schematic diagram of the pulsed-EPR spectrometer, and associated instrumentation, is shown in Supporting Information, Figure S1. Model numbers and sources of the spectrometer components are provided in Supporting Information. Computer control of the spectrometer utilizes software, that was developed by using the MATLAB (MathWorks; Natick, MA) Instrument Control Toolbox.

The MW circuit in the spectrometer includes capabilities for performing both ESE and Fourier transform (FT) -EPR experiments. The continuous-wave output of a broadband MW synthesizer (HP83752A, 0.01–20 GHz; Hewlett-Packard; Palo Alto, CA) is divided into reference and signal channels, for a homodyne detection scheme. The signal channel enters the pulse-forming network, which is divided into two arms, as follows: (1) The “A” arm includes a PIN diode switch and biphasic modulator, for performance of standard 2- and 3-pulse ESE experiments. A continuously variable phase shifter and a variable attenuator are present in the ESE arm for manual adjustment of the MW phase and power. This accommodates phase adjustments between the arms, for experiments that require sophisticated phase cycling schemes, or pulses with different MW power. (2) The “B” arm includes a PIN diode switch, for MW pulse formation, followed by the combination of a 90° hybrid coupler, parallel biphasic modulators, and combiner. This block allows microwave phase alternations of 0, 90, 180 and 270°, for phase-cycling sequences that correct for imbalances of the phases and amplitudes in the two quadrature channels (17). The low power MW pulses are pre-amplified by a GaAs FET amplifier (40 dB), ALM/180–5040, CTT; Sunnyvale, CA), and then elevated to a power of 1 kW by a pulsed-traveling wave tube (TWT) amplifier (117X/Ku, 54303–1, Applied Systems Engineering; Fort Worth, TX). The high power MW pulses are attenuated, and interact with the sample in a reflection MW resonator arrangement. The high power pulses, and the delayed EPR signal, then enter the receiver.

The receiver components are protected from the high power pulses by a PIN diode switch and a limiter. A broadband band-pass filter attenuates switch transients. The receiver incorporates a quadrature mixer and a double-balanced mixer, which are used for different experiments. The intermediate frequency (IF) signal from mixing of the MW signal [pulse channel radio frequency (RF) port] and reference [local oscillator (LO) port] arms is amplified in two stages, attenuated, and then enters the digital sampling oscilloscope (DSO; TDS 620B, Tektronix). Signals are averaged, stored temporarily, and combined in the DSO, and the data is then transferred to the operating computer.

The primary microwave resonator design used is the folded stripline, half-wave resonator (18). These resonators are held in a teflon block, which is inserted into a section of WR-90 (X-band) or WR-62 (Ku-band) brass waveguide. An adjustable short is used to optimize resonator coupling to the waveguide mode. Microwave power is admitted to the cavity by

using a Gordon coupling arrangement (19), essentially as described by (20). Other types of loop-gap (21) and bridged loop gap (22) resonators can also be incorporated. The set of folded stripline resonators, with selected resonant frequencies in the X- and Ku-band ranges, have operating, coupler-adjustable loaded Q values of ~ 100 . The resonator assembly affords dead times of ~ 100 ns, and sensitivity of $\sim 10^{14}$ spins/Gauss for $\text{Cu(II)(H}_2\text{O)}_6$ at X-band.

Variable temperature control is accomplished by using a SuperVaritemp liquid helium-flow cryostat (Janis Research Co., Wilmington, MA), that was custom-designed to accommodate the X- and Ku-band waveguide header-resonator assemblies. The cryostat allows stable temperature control over the range from 6 K to room temperature, by using different cryogens. External magnetic fields at the sample of up to 1.2 T are created by an electromagnet (HF12H, Walker Scientific, Worcester, MA), with Hall-effect magnetic field teslameter/controller (BH15, Bruker, Billerica, MA).

Sample Preparation

The ring-2,3,5,6- $^2\text{H}_4$ -L-tyrosine was obtained from Cambridge Isotope Laboratories (Cambridge, MA) and used without further purification. A 20 mM solution of ring-2,3,5,6- $^2\text{H}_4$ -L-tyrosine in 40% w/v NaOH was placed in a 4 mm outer diameter quartz EPR tube (Wilmaad-LabGlass; Buena, NJ). The sample was then degassed (3 freeze-pump-thaw cycles, with argon gas back-fill), and frozen in liquid nitrogen. The sample was irradiated with a 650 W mercury arc lamp UV for 90 s, to induce tyrosine radical formation (23).

Pulsed-EPR Spectroscopy

All pulsed-EPR experiments were performed at 170 K, at a MW frequency of 8.3335 GHz, a magnetic field of 297.6 mT, microwave pulse power of 90 W, τ value of 256 ns, and initial T value (T_0) of -106 ns. The 3-pulse ESEEM sequence, $P_{90}-\tau-P_{90}-T-P_{90}-\tau$ —ESE, with MW pulse-swapping (24) was used, where P_θ is the MW pulse and θ is rotation angle, in degrees. The value of $P_{90}=20$ ns, and a 4-phase cycle sequence was used (24, 25). Dead-time reconstruction and Fourier transformation of the 3-pulse ESEEM waveform were performed by using the OPTESIM ESEEM simulation and analysis software suite (26). The HYSCORE pulse sequence, $P_{90}-\tau-P_{90}-t_1-P_{180}-t_2-P_{90}-\tau$ —ESE, was used, with $P_{90}=20$ ns, $P_{180}=40$ ns, and 4-phase cycling (27). Acquisition parameters were as specified above, with initial values for t_1 and t_2 of 80 ns. HYSCORE waveforms were baseline corrected in both dimensions.

RESULTS and DISCUSSION

The FPGA pulse programmer was used to conduct two standard pulsed-EPR experiments (2), 3-pulse ESEEM and 4-pulse HYSCORE (28), by using the home-built pulsed-EPR spectrometer. In the simple 3-pulse ESEEM experiment, the value of τ is fixed, the “waiting time” interval between the second and third pulses, T , is varied, and a 2-phase cycle sequence can be applied (29). A variation of the experiment, which is used here, employs values of $T < 0$ to reduce the effective dead time, and requires a 4-phase cycle sequence (24). Ten logic pulses on 7 channels were required for the MW switches, biphasic modulators,

TWT amplifier, receiver protection switch, and DSO detection gating. The experiment proceeded by collecting integrated 3-pulse ESE amplitude at each pulse-time point, sequentially. The delayed time base of the DSO is incremented to maintain a constant position of the 3-pulse ESE, for screen presentation and integration measurement.

Figure 4 shows the 3-pulse ESEEM waveform and corresponding cosine Fourier transform for the ring-2,3,5,6-²H-(L)-tyrosine radical in 40% w/v aqueous NaOH glass at a temperature of 177 K. Figure 4A shows a single collected waveform of 400 points. The data acquisition parameters included 32 pulse sequence repetitions per phase per time point (128, total), at a repetition rate of 100 Hz (the maximum possible repetition rate is 14 kHz, which corresponds to a 36 ns trigger delay and the pulse sequence duration), which leads to a dwell time at each data point of 1.28 s. The FPGA programming time is 50 ms. Communications between the console computer and the DSO over the GPIB interface require 0.9 s. The total time per data point is therefore 2.3 s, which represents a 3-fold decrease in waveform acquisition time for the FPGA-based system, relative to the previous Tektronix HFS9003, digital AWG-based system. The rate-limiting steps in data acquisition are currently associated with communications between the console computer and DSO. The throughput can be improved by using a DSO with a high speed communication port.

The modulation in the waveform in Figure 4A is dominated by contributions from the 3,5-²H hyperfine coupling with the unpaired electron spin, which is delocalized over the phenol side chain of tyrosine. The Fourier transform in Figure 4B shows the two broad hyperfine features from the 3,5-²H coupling, that are centered around the free ²H Larmor frequency of 2.1 MHz. The electron spin density at the ring-carbon 2- or 6-positions is equivalent ($\rho_{\pi} = -0.06$) (30), and is less than the equivalent electron spin density at the 3- and 5-positions ($\rho_{\pi} = -0.25$) (23). This leads to the narrow lines and reduced splitting about the ²H Larmor frequency of the 2,6-²H features, as shown in Figure 4B. The ESEEM and Fourier transform in Figure 4 is comparable to the 3-pulse ESEEM results reported earlier for the 3,5-²H labeled tyrosine radical (23), and to results obtained on the pulsed-EPR spectrometer, but by using the Tektronix HFS9003 pulse programming. This verifies the performance of the FPGA-based pulse programmer.

HYSCORE is a 2-D experiment, and therefore involves a significant increase in acquisition time, relative to 1-D experiments (the number of points increases from n for 1-D to n^2 for 2-D) (2). Two time intervals, t_1 and t_2 , are varied, and either 4- or 8-phase cycle sequences are required to remove unwanted coherences. Twelve logic pulses on 7 channels were required for the MW pulse formation, the 4-phase cycling sequence, and gating of the TWT amplification, receiver protection, and detection events. The experiment proceeded by collecting the 4-pulse ESE amplitude at each pulse-time point, sequentially. The 2D waveform was constructed by fixing a t_2 value, and varying t_1 , and then incrementing to the next t_2 value, and again varying t_1 . The increment was 80 ns, and 128 iterations were performed for each dimension (16,384 iterations, total).

Figure 5 shows the HYSCORE spectrum for the 2,3,5,6-²H-(L)-tyrosine radical in 40% w/v aqueous NaOH glass at 6 K. The time-domain data acquisition parameters included 32 pulse sequence repetitions per phase per time point (128, total), at a repetition rate of 100 Hz. The

dwel time at each point is 1.28 s, which, including the 0.9 s per point for console computer-DSO communications, leads to a total acquisition time of 10 h. This represents a 3-fold decrease in 2D waveform acquisition time, relative to the previous Tektronix HFS9003-based system, for which the run-time and associated liquid helium cost were prohibitive. The acquisition time would be reduced to 6 h, without the DSO communication bottleneck. The diagonal of the 2-D Fourier transform in Figure 5 shows the two broad hyperfine features from the 3,5-²H coupling, and the narrower features from the 2,6-²H coupling, around the free ²H Larmor frequency of 2.1 MHz.

CONCLUSIONS

A pulse programmer, that is based on FPGA technology, is demonstrated to provide a low-cost, high-performance solution to the synchronization and control of logic pulses required for pulsed-EPR experiments. The performance of the FPGA pulse programmer is benchmarked for 3-pulse ESEEM, by comparison with data obtained by using a different pulsed-EPR spectrometer (23), and by comparison with data obtained by using a wide bandwidth, digital AWG-based pulse programmer, operating on the same home-built spectrometer. The FPGA system accelerates data acquisition by >3-fold for 1-D and 2-D measurements. The throughput is limited by other spectrometer components. The FPGA-based design obviates the complexity and cost of pulse programmer configurations that are based on parallel commercial digital delay generators, and the programming and transition times are comparable with those of ASIC devices, at significantly reduced cost and greater flexibility. The specifications of the FPGA pulse programmer, as described, can be enhanced. For example, by utilizing step recovery diodes, the output pulse transition time can be reduced to the order of 50 ps. The FPGA IC also has a large number of I/O pins (232, for the Xilinx Spartan-6 LX45, CSG324 package), and thus, the number of output channels can be correspondingly large. Outputs could be routed to additional digital delay chips on individual channels, to achieve picosecond timing resolution. Therefore, the versatility of the FPGA, and the novel transition-based command protocol, can be used to satisfy a wide range of timing requirements, from MHz to THz frequencies, for pulsed experiments in spectroscopy, relaxation and imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Bud Puckett and Horace Dale, of the Department of Physics Machine Shop at Emory University, for their contributions to the construction of the pulsed-EPR spectrometer. Research reported in this publication was supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number 5R01 DK054514.

REFERENCES

1. Schweiger A. Pulsed Electron-Spin-Resonance Spectroscopy - Basic Principles, Techniques, and Examples of Applications. *Angew. Chem. Intl. Ed. Eng.* 1991; 30:265–292.

2. Schweiger, A.; Jeschke, G. Principles of pulse electron paramagnetic resonance. Oxford, UK: Oxford University Press; 2001.
3. Sadrozinski, HF-W.; Wu, J. Applications of Field-Programmable Gate Arrays in Scientific Research. Taylor and Francis Boca Raton; 2010.
4. McCracken J, Dye JL, Shin D-H. Pulsed-EPR studies of electriles and sodides. *Appl. Magn. Reson.* 1992; 3:305–316.
5. Sturgeon BE, Britt RD. Sensitive pulsed-EPR spectrometer with an 8–18 GHz frequency range. *Rev. Sci. Instrum.* 1992; 63:2187–2192.
6. Shane JJ, Gromov I, Vega S, Goldfarb D. A versatile pulsed X-band ENDOR spectrometer. *Rev. Sci. Instrum.* 1998; 69:3357–3364.
7. Gromov I, Glass B, Keller J, Shane J, Forrer J, Tschaggelar R, Schweiger A. Pulse programmer for electron paramagnetic resonance spectroscopy. *Concept Magnetic Res. B Magn. Reson. Engineering.* 2004; 21B:1–10.
8. Gorcester J, Freed JH. Two-dimensional Fourier transform ESR correlation spectroscopy. *J. Chem. Phys.* 1988; 88:4678–4693.
9. Gorcester, J.; Millhauser, GL.; Freed, JH. Two dimensional electron spin resonance. In: Kevan, L.; Bowman, MK., editors. *Modern Pulsed and Continuous-wave Electron Spin Resonance*. New York: Wiley; 1990. p. 119-193.
10. Ke S-C, Torrent M, Museav DG, Morokuma K, Warncke K. Identification of Dimethylbenzimidazole Axial Coordination and Characterization of ¹⁴N Superhyperfine and Nuclear Quadrupole Coupling in Cob(II)alamin Bound to Ethanolamine Deaminase in a Catalytically-Engaged Substrate Radical-Cobalt(II) Biradical State. *Biochemistry.* 1999; 38:12681–12689. [PubMed: 10504238]
11. Ke S-C, Warncke K. Interactions of substrate and product radicals with CoII in cobalamin and with the active site in ethanolamine deaminase, characterized by ESE-EPR and ¹⁴N ESEEM spectroscopies. *J. Am. Chem. Soc.* 1999; 121:9922–9927.
12. Warncke K, Utada AS. Interaction of the Substrate Radical and the 5'-Deoxyadenosine-5'-Methyl Group in Vitamin B12 Coenzyme-Dependent Ethanolamine Deaminase. *J. Am. Chem. Soc.* 2001; 123:8564–8572. [PubMed: 11525664]
13. Canfield JM, Warncke K. Geometry of Reactant Centers in the CoII-Substrate Radical Pair State of Coenzyme B12-Dependent Ethanolamine Deaminase Determined by using Orientation-Selection-ESEEM Spectroscopy. *J. Phys. Chem. B.* 2002; 106:8831–8841.
14. Sun L, Groover OA, Canfield JM, Warncke K. Critical role of arginine 160 of the EutB protein subunit for active site structure and radical catalysis in coenzyme B12-dependent ethanolamine ammonia-lyase. *Biochemistry.* 2008; 47:5523–5535. [PubMed: 18444665]
15. Gunderson WA, Hernandez-Guzman J, Karr JW, Sun L, Szalai VA, Warncke K. Local structure and global patterning of Cu²⁺ binding in fibrillar amyloid- β protein. *J. Am. Chem. Soc.* 2012; 134:18330–18337. [PubMed: 23043377]
16. Dong J, Canfield JM, Mehta AK, Shokes JE, Tian B, Childers WS, Simmons JA, Mao Z, Scott RA, Warncke K, Lynn DG. Engineering 20 metal ion coordination to regulate amyloid fibril assembly and toxicity. *Proc. Natl. Acad. Sci.* 2007; 104:13313–13381. [PubMed: 17686982]
17. Hoult DI, Richards RE. Critical factors in the design of sensitive high resolution nuclear magnetic resonance spectrometers. *P. Roy. Soc. Lond. A.* 1975; 344:311–340.
18. Lin CP, Bowman MK, Norris JR. A folded half-wave resonator for ESR spectroscopy. *J. Magn. Reson.* 1985; 65:369–374.
19. Gordon JP. Variable coupling reflection cavity for microwave spectroscopy. *Rev. Sci. Instrum.* 1960; 32:658–661.
20. Britt RD, Klein MP. A versatile loop-gap resonator probe for low temperature electron spin-echo studies. *J. Magn. Reson.* 1987; 74:535–540.
21. Froncisz W, Hyde JS. The loop-gap resonator: A new microwave lumped circuit ESR sample structure. *J. Magn. Reson.* 1982; 47:515–521.
22. Pfenniger S, Forrer J, Schweiger A, Wieland T. Bridged-loop gap resonator for pulsed-EPR experiments. *Rev. Sci. Instrum.* 1988; 59:752–760.

23. Warncke K, McCracken J. 2H electron spin echo envelope modulation spectroscopy of strong, a-hydrogen hyperfine coupling in randomly oriented paramagnetic systems. *J. Chem. Phys.* 1994; 101:1832–1841.
24. Fauth J-M, Schweiger A, Braunschweiler L, Forrer J, Ernst RR. Elimination of unwanted echoes and reduction of dead time in three-pulse electron spin-echo spectroscopy. *J. Magn. Reson.* 1986; 66:74–85.
25. Gemperle C, Aebli G, Schweiger A, Ernst RR. Phase Cycling in Pulse EPR. *J. Magn. Reson.* 1990; 88:241–256.
26. Sun L, Hernandez-Guzman J, Warncke K. OPTESIM, a versatile toolbox for numerical simulation of electron spin echo envelope modulation (ESEEM) that features hybrid optimization and statistical assessment of parameters. *J Magn Reson.* 2009; 200:21–28. [PubMed: 19553148]
27. Stoll S, Kasumaj B. Phase Cycling in Electron Spin Echo Envelope Modulation. *Appl. Magn. Reson.* 2008; 35:15–32.
28. Höfer P, Grupp A, Nebenführ G, Mehring M. Hyperfine sublevel correlation (HYSCORE) spectroscopy: A 2D ESR investigation of the squaric acid radical. *Chem. Phys. Lett.* 1986; 132:279–283.
29. Angerhofer A, Massoth RJ, Bowman MK. Fourier-transform epr measurement of homogeneous electron transfer rates. *Isreal J. Chem.* 1988; 28:227–238.
30. Warncke K, Babcock GT, McCracken J. Structure of the YD tyrosine radical in Photosystem II as revealed by 2H electron spin echo envelope modulation (ESEEM) spectroscopic analysis of hydrogen hyperfine couplings. *J. Am. Chem. Soc.* 1994; 116:7332–7340.

Figure 1. Schematic diagram of FPGA logic blocks. The processing of the transition-based command protocol is illustrated.

(A)

Channel	On Time (2 ns base)	Off Time (2 ns base)
1	10	31
2	21	32

→

Instructions	T_i, D_0, D_1
M1	10, 00, 00
M2	20, 01, 01
M3	22, 01, 11
M4	32, 11, 10

(B)

Figure 2.

Transition-based command protocol and control algorithm. The function of the example program is to create overlapping pulses on two channels. (A) Pulse program and instructions. (B) Timing diagram of control logic and FSM block.

(A)

(B)

Figure 3.
FPGA pulse programmer and associated components, in rack-mount case configuration. (A) Front panel perspective view. (B) Case interior view.

Figure 4.

Three-pulse ESEEM of the ring-2,3,5,6- $^2\text{H}_4$ -tyrosine neutral radical in a basic aqueous glass at 177 K. (A) Three-pulse ESEEM waveform from single collection. The integrated ESE amplitude is normalized to the constant amplitude of the ESE envelope. Vertical bar length represents 10% of the constant amplitude of the ESE envelope. (B) ESEEM spectrum. The molecular structure of the radical is shown in the inset.

Figure 5.

Hyscore spectrum of the ring-2,3,5,6- $^2\text{H}_4$ -tyrosine neutral radical in a basic aqueous glass at 177 K. The first quadrant of the complex Fourier transform of the 2-D waveform is illustrated. Scale bar portrays relative amplitude.