

BIPOLAR NANOSECOND PULSE GENERATION USING TRANSMISSION LINES FOR CELL ELECTRO-MANIPULATION*

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Abstract

Design and operation of a pulse generator based on fast recovery diodes and shorted transmission lines are presented. The generator produces 3.5 ns wide, ± 350 V amplitude bipolar pulses into 50-ohm load at the maximum repetition rate of 100 kHz. Short bipolar pulses are used for the studies of biological cell response to high electric fields when the net transfer of charge is undesirable. The bipolar pulse is produced from a unipolar pulse by the parallel connection of a shorted transmission line. This transmission line delays and inverts the initial pulse, so the output is the sum of the initial and the inverted and delayed pulses. Proper terminations both at the entrance and the exit of the transmission line system are essential if one is to avoid spurious pulses. If preserving the exact shape of the pulse is not necessary, a parallel L-C circuit can replace the shorted transmission line. This L-C circuit provides near Gaussian bipolar pulse shapes.

I. INTRODUCTION

Electroperturbation of biological cells can be achieved by the influence of pulsed electric fields. The voltage induced across a cell membrane depends on the pulse length and pulse amplitude. Pulses longer than ~ 1 μ s will charge the outer cell membrane and can lead to the opening of pores, temporary or permanent, the latter usually resulting in cell death [1]. Pulses much shorter than ~ 1 μ s can affect intracellular structures without adversely affecting the outer cell membrane. An interesting effect of pulses of a few tens of ns duration and ~ 5 – 10 MV/m amplitude is triggering of apoptosis or programmed cell death [2, 3]. There is a need for shorter, higher amplitude electric pulses for cell biology research to probe and manipulate internal parts of the cell such as nuclei and mitochondria.

Observations of pulse-induced (unipolar pulses) phosphatidylserine (PS) externalization in living cell membranes led us to a model and a hypothesis which

could be tested both with shorter pulses, and with bipolar pulses.

The appearance of nanoelectropulse-translocated PS only at the anode pole of the cell is consistent with direct, electric field-driven transport of the anionic PS head group across the Born energy barrier of the lipid interior of the membrane, on the order of 100 kJ/mol for a homogeneous phospholipid bilayer. For the pulse conditions in our initial unipolar pulse experiments, the transmembrane potential calculated from the simple dielectric shell model of the cell reaches 1 V, already sufficient to provide the necessary translocation activation energy (1 eV \sim 100 kJ/mol).

One test of the field-driven translocation hypothesis calls for shorter pulses with correspondingly higher fields. If PS externalization is a consequence of the electrostatic potential developed across the cytoplasmic membrane during a 7 ns, 2.5 MV/m pulse, then a pulse which is too short to charge the membrane to the conductive breakdown potential, and which therefore produces less than the 10–20 MV/m field (0.5–1 eV) in the membrane dielectric generated in those experiments, should not cause immediate PS translocation (but may have other effects, consequences of the high field delivered to the intracellular environment). The direct field-driven translation hypothesis predicts also that a series of pulses, alternating in polarity, will translocate phospholipids at both poles of the cell.

Preliminary results obtained with the help of both a unipolar diode pulser [4], and the bipolar pulser described in this report, are consistent with the direct, electric field-driven translocation hypothesis

II. DESIGN

Existing pulse generator systems used in ultra short pulse electroperturbation research are based on spark gap switched transmission lines [5], or radiofrequency MOSFET switched capacitors [6]. The spark gap based system suffers from large size and low repetition rate, relatively short lifetime, and erratic, high jitter triggers.

* This work was primarily funded by the Compact-Pulsed Power MURI program funded by the Director of Defense Research and Engineering (DDR&E) and managed by the Air Force Office of Scientific Research (AFOSR) and was also funded by the Army Research Office (ARO).

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They also need rapid charging of the transmission line capacitance in order to overvolt the spark gap to satisfy the fast rise time requirement [5]. The MOSFET switched capacitor cannot generate faster or narrower pulses than 15 – 20 ns due to complications of the MOSFET driving circuit and inherent limitations of the MOSFET device [6]. We have designed and constructed a pulse generator conforming to the above requirements [4]. It can produce 3.5 ns wide, 600 V amplitude unipolar pulses with a maximum repetition rate of 100 kHz. The circuit is an adaptation of a design using specialized custom fabricated drift step recovery diodes [7, 8], to lower voltage, off the shelf available, standard, low-cost, fast recovery rectifiers.

A. Unipolar pulse

There are two main approaches to generate bipolar pulses from unipolar ones. The straightforward method is to differentiate a unipolar square pulse with the help of a series capacitor. We have used this method successfully with the MOSFET based pulser, but the pulse width of that pulse generator is excessive for the present need. The other method, useful for any pulse shape, is the shorted transmission line method reported here.

The actual pulse is generated by a diode acting as an opening switch, interrupting the current in an inductor and commuting it into the load impedance as shown in Fig. 1.

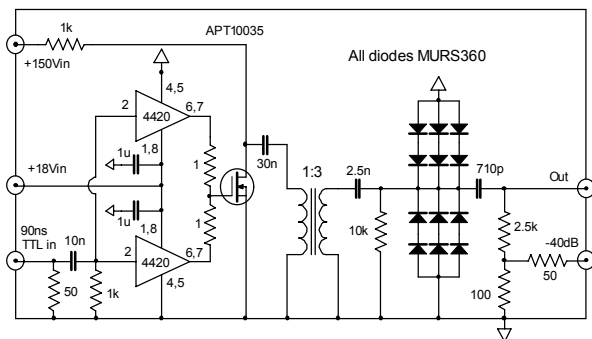


Figure 1. The “nanopulser” diode pulse generator circuit

The unipolar output pulse from this generator into a 50 Ω load is shown in Figure 2. This is the pulse we convert into a bipolar output pulse.

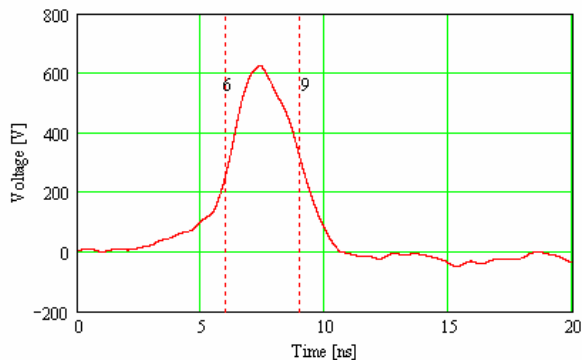


Figure 2. Unipolar generator output.

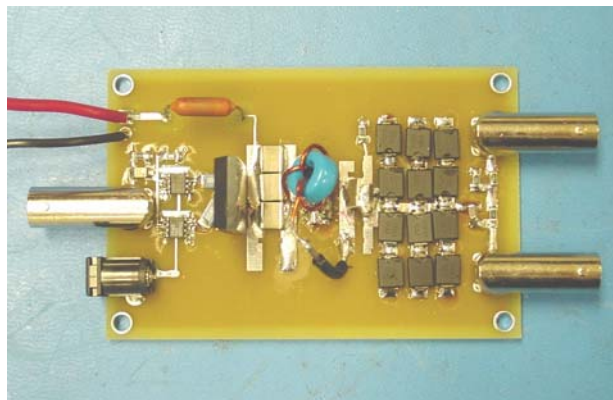


Figure 3. The unipolar diode pulser on a 3.8” x 2.5” circuit board

A built-in -26dB attenuator, calibrated by commercial attenuator sets, was used to sense the output voltage. A Tektronix P6021 current probe was used to measure the diode current. The complete diode pulser assembled on a double-sided printed circuit board is shown in Fig. 3.

B. Transmission line method

The addition of a shorted transmission line can convert any pulse generator to one with bipolar pulse output. Such a system needs careful attention to impedance matching in order to avoid spurious pulses. The proper matching requirements are easy to achieve in the diode pulse generator.

The operational principle of the bipolar pulse generation is shown in Figure 4.

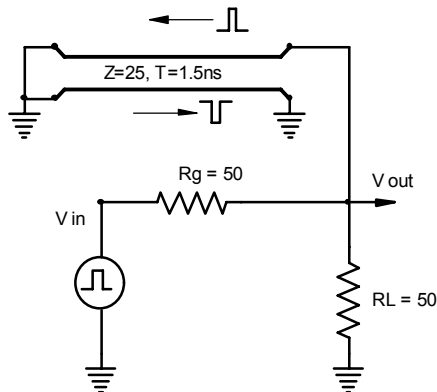


Figure 4. Principle of bipolar pulse generation by a shorted transmission line

The pulse initiated at the generator output is reduced in amplitude by the voltage divider consisting of the generator internal impedance, R_g , and the parallel combination of the load impedance, R_L , and the transmission line characteristic impedance, Z . The reduced pulse enters the transmission line and reflects from the shorted end with inverted shape. After the inverted pulse returns to the load, delayed by twice the

electrical length of the line, it is appended to the end of the original pulse.

The returning inverted pulse is properly terminated by the parallel combination of the generator source and the load impedances. Hence, if the source and load are both 50Ω , the characteristic impedance of the line must be 25Ω . In possession of all impedances we can calculate the voltage division ratio: V_{out} is a quarter of V_{in} , or half the amplitude of the unipolar output pulse.

The electrical length of the transmission line is equal to half the unipolar pulse width to ensure a proper bipolar pulse shape. In our case, the 25Ω transmission line consists of two RG174 type, 50Ω coaxial cable segments in parallel. The physical length of the segments is 36 cm, adjusted to give a round trip delay of approximately 4 ns. The transmission line is connected parallel to the load, at the end of a 2 m long RG58 cable between the Unipolar “nanopulser” and the instrumented slide.

SPICE simulation of the transmission line system is shown in Figure 4. The input is a trapezoidal pulse with 1 ns rise and fall times. In this ideal case perfect matching of the forward and reflected waveform are possible and the result is a single bipolar period. In reality, transmission line losses and skin effect induced dispersion broadens the inverted pulse and reduces its amplitude.

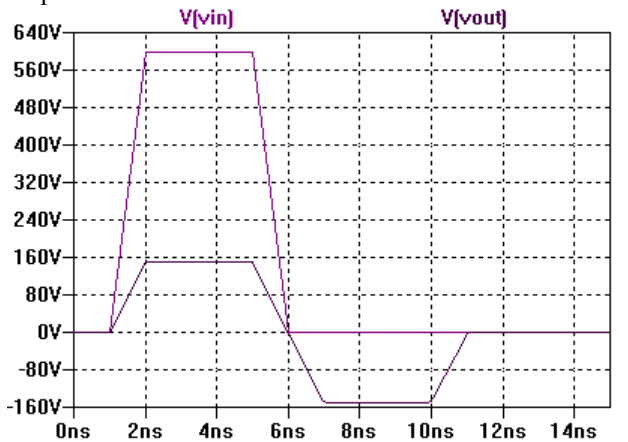


Figure 4. SPICE result of transmission-line waveforms

C. L-C pulse forming circuit

It is possible to replace the shorted transmission line by a lumped element equivalent circuit. The simplest such circuit is the low-pass Π -network. When shorted on one end it reduces to a parallel L-C resonant circuit as shown in Figure 5.

The circuit parameters are calculated from the equivalent transmission line parameters. To first order, we approximate the total inductance $L = T Z$, or $L = 62.5$ nH. Likewise, the total capacitance is $2C = T / Z$, or $C = 50$ pF.

The SPICE result of this L-C circuit is shown in Figure 6.

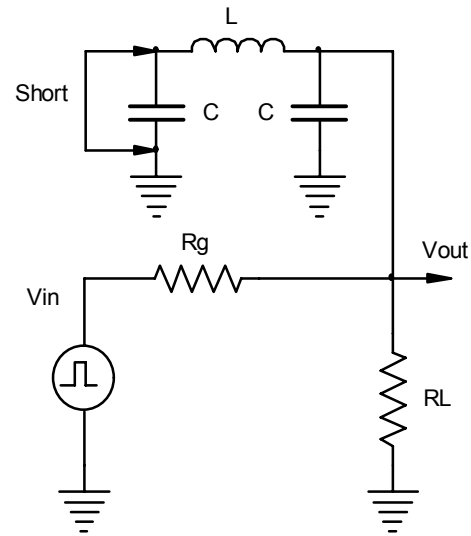


Figure 5. Bipolar pulse generation by a synthetic shorted transmission line represented by a parallel resonant LC circuit

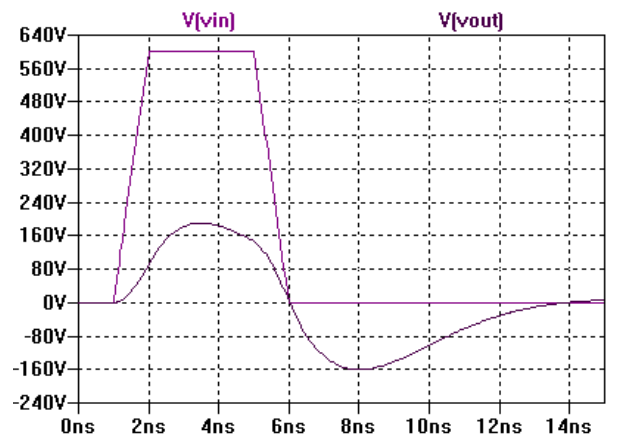


Figure 6. SPICE waveforms of the L-C pulse forming circuit

The output approximates a differentiated Gaussian monopulse. As can be seen, there is an asymmetry between the positive and negative side of the waveform, the negative side is broadened and is reduced in amplitude. However, in situations where this distortion is acceptable significant reduction in size are possible using surface mount inductor and capacitor components.

D. Electrical Load

The load is an instrumented microscope slide. Several gold deposited electrode lines separated by $100\mu\text{m}$ and covered by a second glass slide hold the cell solution [6]. The electrical load impedance is about 200Ω . However, the pulse generator must be able to work into a load of 50Ω , as the generator is connected to the slide by a coaxial cable of 50Ω characteristic impedance. The cable is terminated at the slide by a 50Ω surface mount resistor

connected parallel with the electrode structure. We have opted for this brute force solution to establish a $\sim 50 \Omega$ electrical load impedance instead of the more efficient, but troublesome transmission line transformer approach, as efficiency is less important than good match over a range of variable cell impedances.

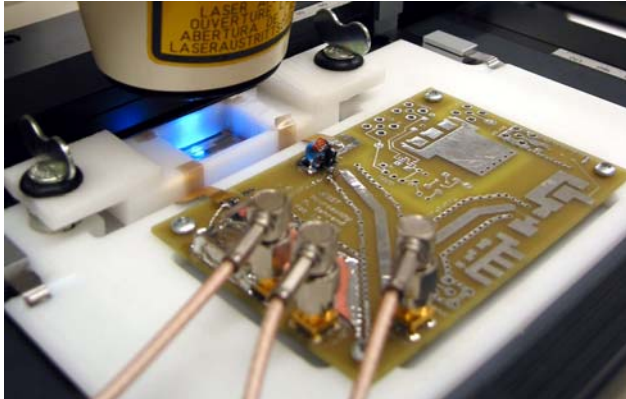


Figure 7. The instrumented microscope slide and cable connections

III. OPERATION

The pulser has been tested at repetition rates up to 100 kHz with resistive charging, higher repetition rates can be achieved with resonant charging methods at the cost of increased complexity, especially in the area of optimizing core reset. Ultimately the repetition rate is limited to 5 MHz by the duration of the charge transfer sequence.

Typical output into 50Ω using the parallel, shorted RG174 cables is shown in Fig. 8. The pulse amplitude is ± 350 V, and the FWHM is 3.5 ns.

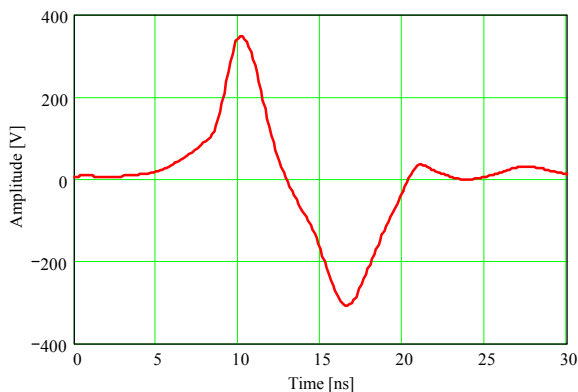


Figure 8. Output of the bipolar diode pulser into a 50 Ohm load

The pulse generator is presently being used for real-time microscopy of cell electroperturbation [9]. It has been in operation for over two months with no problems with reliability, drift, or change in output characteristics.

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