Journal of Engineering Research and Reports

20(6): 9-16, 2021; Article no.JERR.67636
ISSN: 2582-2926

Design and Development of Mini-pulsar for Real-Time Bioelectric Experiment

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Authors' contributions

This work was carried out in collaboration among all authors. Author GK designed the study, did all the hardware work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RS and AS managed the analyses of the study. Author GK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JERR/2021/v20i617322
Editor(s):
(1) Dr. Guang Yih Sheu, Chang-Jung Christian University, Taiwan.
Reviewers:
(1) Andres Cicuttin, The Abdus Salam International Centre for Theoretical Physics, Italy.
(2) Xin Rao, Hangzhou Dianzi University, China.
Complete Peer review History: http://www.sdiarticle4.com/review-history/v67636

Short Research Article

Received 11 February 2021
Accepted 21 April 2021
Published 30 April 2021

ABSTRACT

Investigation of the effect of pulsed electric field on eukaryotic cells has been of interest to biomedical scientists and researchers since a few decades. Reversible electroporation (EP) is used for uptake of chemicals, drugs, DNA into the eukaryotic cells under low electric field (100's V/cm) of millisecond duration to few hundreds of microsecond duration. An electric field of nanosecond duration and a very high electric field (50's kV/cm) can stress to intracellular organelles of eukaryotic cells and that can trigger apoptotic pathways. In this article experimental setup has been prepared for real-time investigation of the effect of nanosecond electric field on eukaryotic cells. Pulsar producing ~1.5kV, 20ns FWHM, single shot as well as ~10Hz rep-rate with rise-time as fast as ~10ns, has been prepared using blumlein pulse forming line and avalanche transistor switches. A 300µm gap microplate chamber has been prepared to expose the electric field to eukaryotic cells.

Keywords: Electroporation; microchamber; electrical field; blumlein.

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1. INTRODUCTION

Since the 50s, investigation of the effect of the electrical field on biological cells has been carried out by many scientists. For the manipulation of biological cells, the intensity of electrical fields should be sufficiently high. Nanosecond kV/cm electric field can trigger the release of intracellular calcium, trans-locate phosphatidylserine (PS) across the cell membrane, cause chromatin rearrangement and promote programmed cell death (apoptosis) in malignant cells without permanently damaging the outer cell membrane [1-3]. These effects have, among other things, generated interest in the electro-manipulation of intracellular structures for promoting apoptotic pathways and understanding gene transfection mechanisms.

Effective manipulation of intracellular structures using electro-pulses requires pulses that are short enough to bypass the cytoplasmic membrane and deposit their energy across intracellular membrane-bound structures. Molecular dynamics simulations show electrical pulses that can create transmembrane fields of the order of 50-200kV/cm (i.e., transmembrane potential >1 V) cause nano-meter-diameter pores to form in phospholipid bilayers within 1–2 ns of application. This infers sub-nanosecond rise time 50-200 kV/cm pulses could minimize nano-poration of the cell membrane, enabling the voltage across the inner membranes to exceed that across the outer membrane and allow intracellular electro-manipulation to dominate over membrane effects [2].

The commonly used devices for in vitro biological experiments are cuvettes [4-7], needle electrodes [8], or planar electrodes [9]. A "biochip" topology of the delivery device has also been proposed which is suitable for sample exposure on the microscope stage [10].

In the work described here, the pulse generator is designed to generate an average output peak voltage of 1.5 kV. A repetition rate of ~10 pulses per second (10Hz). The system is designed to drive an effective load impedance of 100Ω. Microchamber for real-time biological investigation has been designed to get a uniform electric field ~50kV/cm in between electrodes.

2. SYSTEM DESIGN

2.1 Theory of Operation

Coaxial cable can be used as an energy-storing element. Blumlein Pulse forming line [11] consists of a coaxial cable divided into two parts such that the only sheath is disconnected and in between sheath a resistance having a value of $2Z_0$ is inserted between sheath as in Fig.1, Z_0 is characteristics impedance of the cable. One end of the cable is connected to the supply through charging resistance R_S and another end remains open. R_S is to limit the charging current as well as current through the switch when the switch is ON. Output is obtained across $2Z_{0.}$ Cable introduces a delay time in signal propagation which depends upon the length of line and permittivity and permeability of insulation used in it. Delay time is given by

$$\tau = \frac{l}{v} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (1)$$

Where; l is the length of cable and v is the velocity of the wave in transmission line Also; velocity of the wave in the cable.

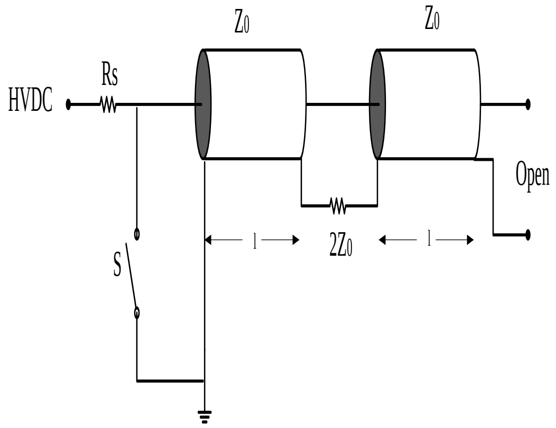

Fig.1. Circuit schematic diagram

$$v = \frac{c}{\sqrt{(\mu_r \varepsilon_r)}} \quad (2)$$

Where; c is the velocity of light and ε_r is the relative permittivity and μ_r the relative permeability of transmission line insulation.

When supply is turned ON both part of cable gets charged up to supply voltage V and the initial voltage across 2Z0 is zero. As soon as switch S is turned ON at the source end, the cable gets short-circuited at the source end and started to discharge. A –V voltage wave starts travelling towards the open end. The source end part of the cable is started to discharge from +V to 0Vsince in between cable parts (after a delay time) there is impedance change in the way of voltage wave, which causes reflection and transmission of the wave as per the equation below:

$$\rho = \frac{Z_l - Z_0}{Z_l + Z_0} \quad (3)$$

$$t = 1 + \rho \quad (4)$$

Where; Z_l is impedance at the break, Z_0 is characteristics impedance of line in eq. (3). In eq (4) t and ρ are transmission and reflection coefficients respectively.

And at that point reflection coefficientρ will be 0.5 (at this point $Z_l = 3Z_0$) therefore wave $-\frac{V}{2}$ will reflect (cable now get charged to $-\frac{V}{2}$) and $-\frac{V}{2}$wave transmits to the second part of the cable (Now the cable at the source end is charged by $-\frac{V}{2}$another end by $+\frac{V}{2}$). At the end of the second part of the cable, the reflection coefficient will be 1 (at this point $Z_l = \propto$) as it is open and wave,$-\frac{V}{2}$ is coming at this point will reflectto the source end that makes the load end part of the cable discharged. At the same instant reflected wave in part 1 of the cable will reach the source end and since the switch is still on (short circuit), the reflection coefficient at this end is −1(at this point $Z_l = 0$), $+\frac{V}{2}$ will start travelling towards load end that makes source end cable discharged too thus part 1 and part 2 of cable get discharged completely in two delay times. Therefore, pulse obtained across $2Z_0$ will be of the duration 2τ and process repeats if HVDC supply is turned ON and switch S is triggered repeatedly, it should be kept in mind that switch should beOFFfor the time required to charge the cable. The length of the cable theoretically decides the pulse duration at the output pulse.

2.2 Design of Pulse Generator

Our circuit consists of 15kV (DC) 2.5kV (RF) coaxial cable (RG58), having the length of each part is 100cm and $2Z_0$=100Ω, the permittivity of cable (here it is equal to 2.1), permeability is 1 (nonmagnetic) and R_S=250kΩ to limit the charging current and also to limit the current through the switch. All above parameters gave theoretically calculated pulse width ~9.66ns. the switch should be fast enough for the desired pulse generation. We have selected avalanche transistors (FMMT417) for this operation as they are fast enough for the operation in nanosecond. Each transistor is rated for 320V therefore a string of seven avalanche transistors is used in this circuit. the voltage applied to the string should be less than 2.2kV. A suitable dc power supply is required to supply the circuit.

Trigger pulse applied to the base of 7th transistor as all other has base-emitter short-circuited (required for the operation in avalanche mode). To trigger the transistor, ~9V square pulse of nanosecond duration pulse is suitable to turn on the transistor. DC supply source, High Voltage Unit (HV4800E, ECIL, India) is suitable. The complete setup is as shown in Fig. 3.

2.3 Design of Electrodes

We have designed the microchamber required for the bioelectric experiment Fig. 4. The gap in between the two-copper plate is kept at 300µm. Its impedance should be ~100Ω under load as it had been observed and found that resistivity of mammalian cells in culture medium is about 100Ω-cm [2], for this microchamber;

Separation and height of electrodes = 300µm
Width of electrodes = 1cm
Volume occupied by electrode= 1cm×300µm×300µm=1×0.03×0.03 cm^3 = 9×10^{-4} cm^3
Also 1 cm^3 = 1ml
Thus, the volume occupied by electrodes = 0.9µl ~ 1µl
The resistance offered by electrode when filled with cells in culture

$$R = \rho\frac{l}{A} = \rho\frac{l^2}{Al} = \rho\frac{0.03\times0.03}{1\times0.03\times0.03} = \rho = 100\Omega$$

That exactly matches with pulsar circuit impedance.

Complete arrangement schematic of the pulsar and microchamber is shown in Fig. 5. The microchamber gap is filled by the biological cells in suspension (Media e.g., DMEM) of volume 1µl and can be investigated under a laser microscope for the effect of electrical pulses in the presence of a fluorescent agent.

3. RESULTS AND DISCUSSION

OrCad Simulation Fig 6, shows the circuit and output voltage waveform in the ideal case, with no parasitic elements the output voltage waveform, will be square pulse however, in practical circuit voltage waveform measured using high voltage probe (North Star, PVM-5) and displayed on a digital oscilloscope (Infinii Vision DSO7034A, Agilent Technologies). A probe is connected across the load. Fig. 7 shows the output of a single shot by a single trigger pulse applying at the base of the lowest transistor, and in Fig 8, the rep-rate pulse output at 10Hz. Having a single shot of pulse complete width of the pulse is about ~15ns and the Full Width at Half Maxima (FWHM) is 20ns. We obtained negative pulse of amplitude $\sim -1.5kV$ and minute reflections are can be observed due to a slight impedance mismatch. The rise time of the pulse is ~10ns (in the negative direction).

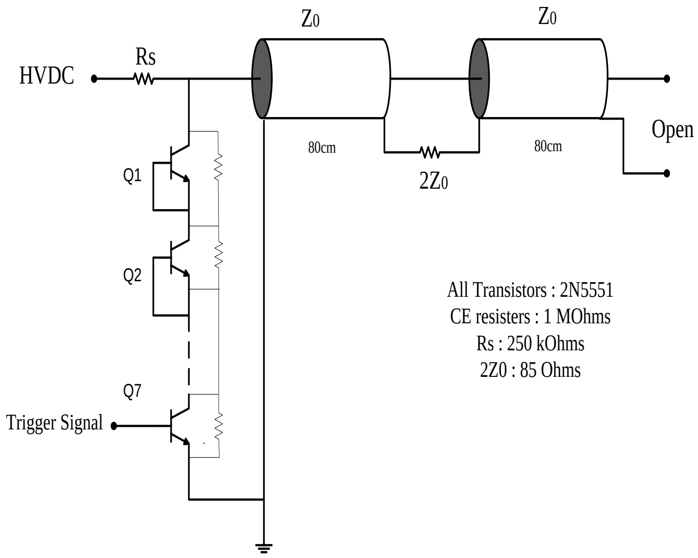

Fig. 2. Practical circuit schematic

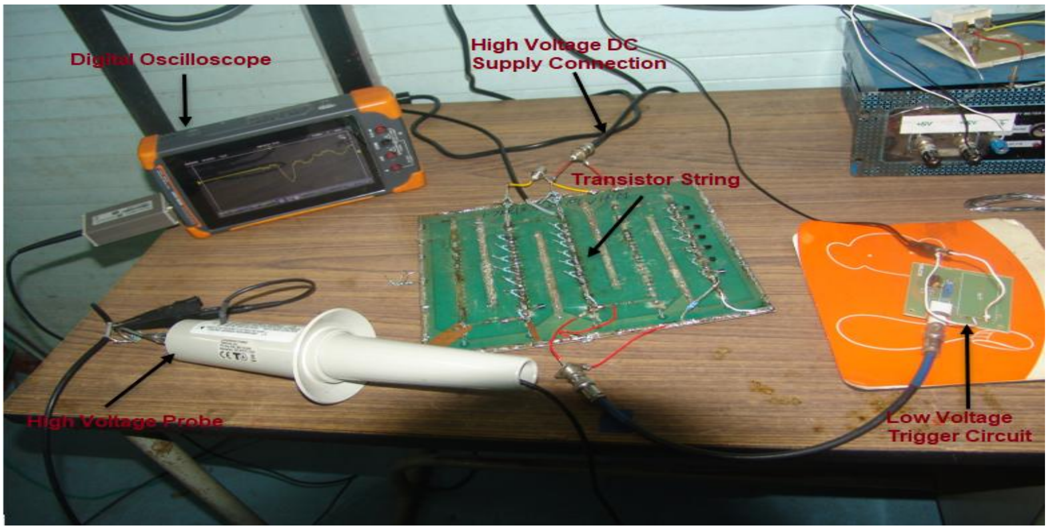

Fig. 3. Practical Circuit of the pulse generator (DC supply source is not shown in the figure

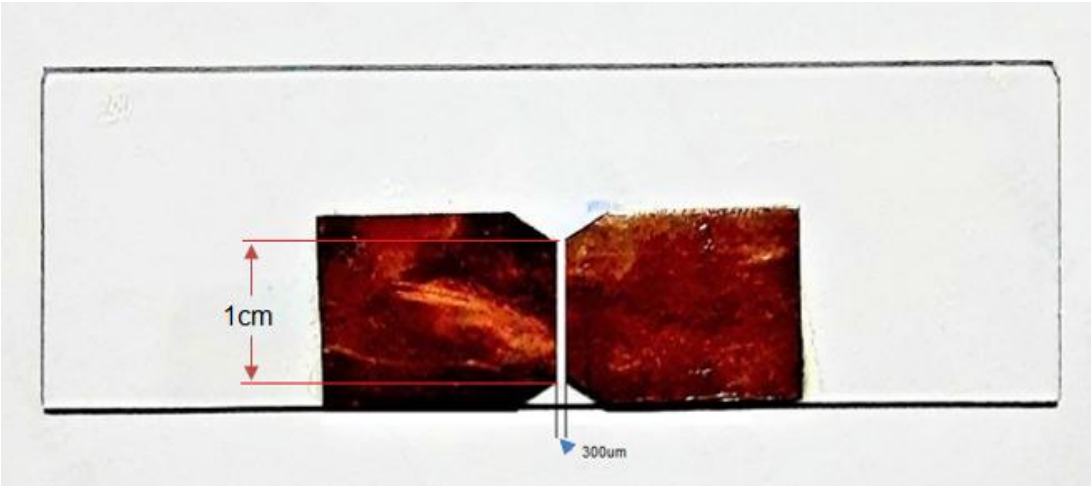

Fig. 4. Microchamber (microplates are made of a copper plate with having a thickness of 300µm)

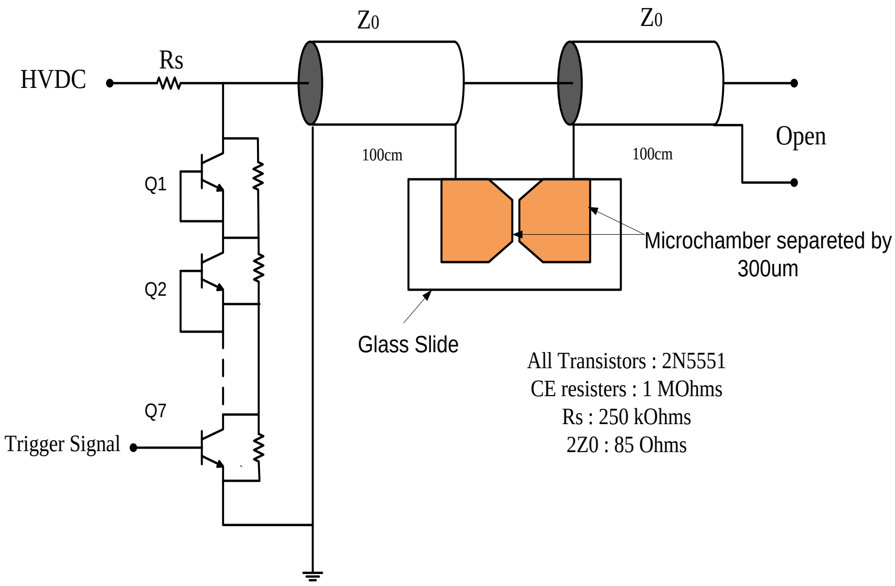

Fig. 5. Complete arrangement schematic

The difference in theoretical value (Also, Simulated) and actual value of pulse duration is because of parasitic electrical parameters. Unwanted parasitic element i.e., cable inductance, switch connection inductances, switch capacitances, line inductance, metallic plate inductance etc. increases the overall system inductance. This deteriorates the shape of the output pulse and increases the duration compared to theoretically calculated. Also, this causes impedance mismatching which further introduces unwanted reflections in the system which reflect in the output pulse shape.

To investigate the biological cell, it requires to put 1µl in the microchamber having the width of 300µm, electrical field intensity ($E = V/d$) in between electrodes will be50kV/cm. The design of pulsar is simple and can operate frequencies up to 1kHz safely [12].

Fig 9 shows the electric field distribution simulated in infolytica elecnet software, in between the electrodes electric field is uniform except at the sharp edges. At sharp edges electric field is not uniform because of fringing, making edges smoother can minimize this to some extent.

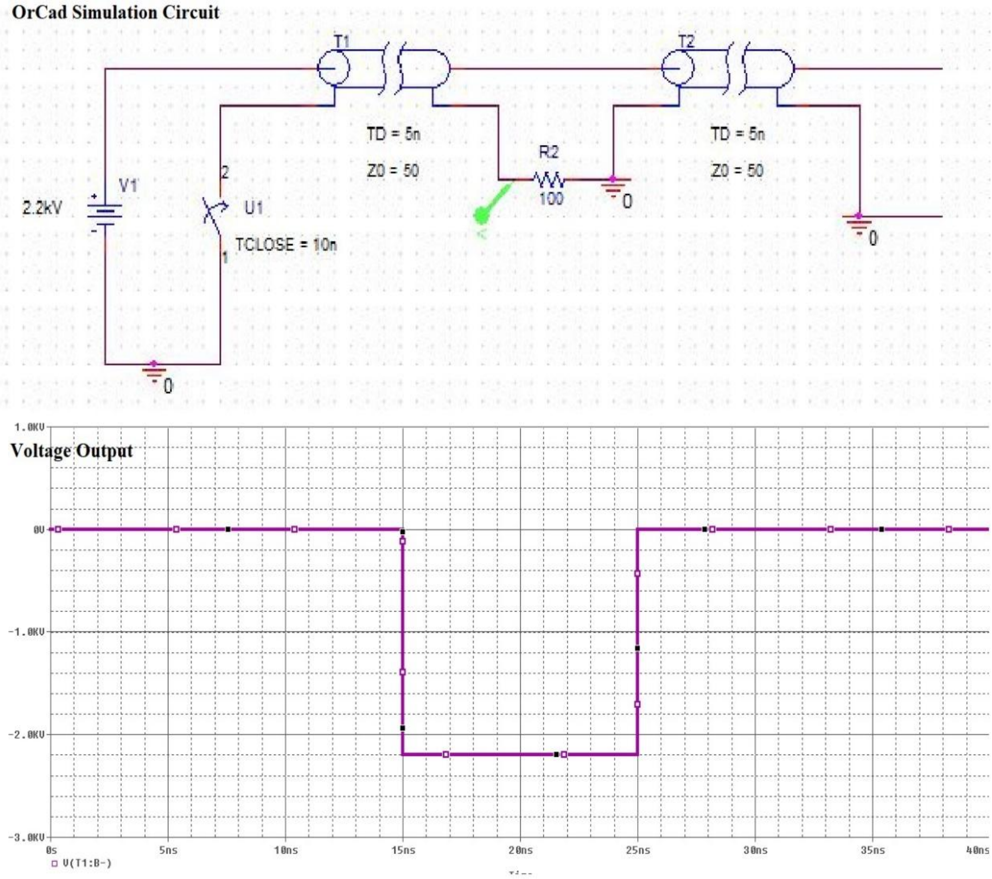

Fig. 6. OrCad Simulation and simulation result

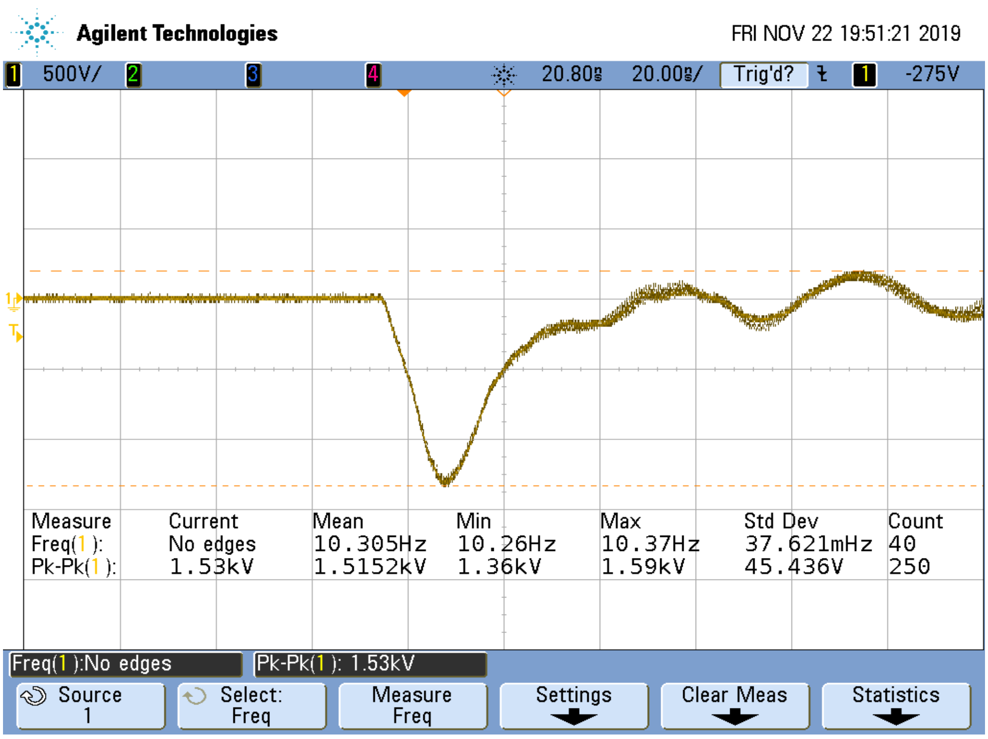

Fig. 7. Single shot output (DSO output waveform voltage vs time)

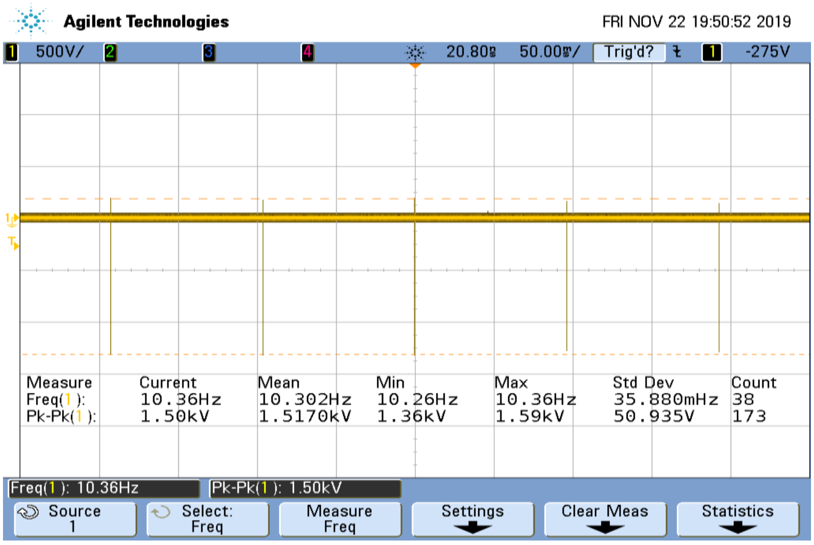

Fig. 8. Rep. rate output waveform (DSO output waveform voltage vs time)

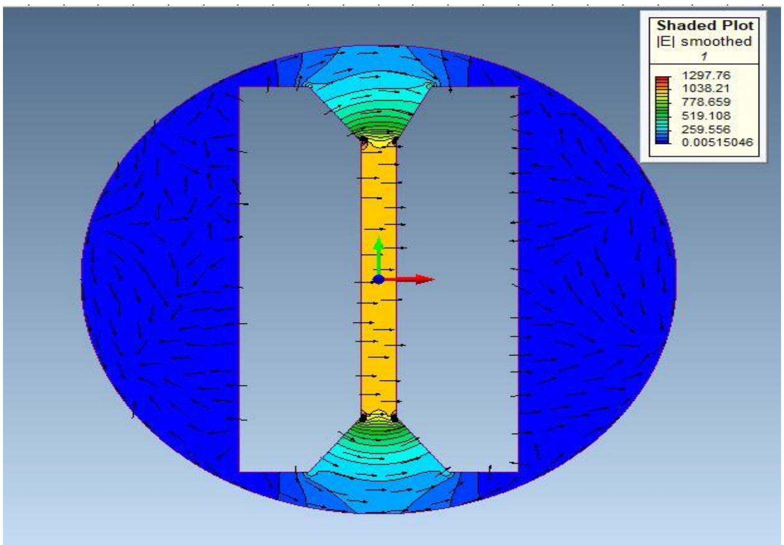

Fig. 9. Electric field pattern

Based on the Blumlein line principle but using water as dielectric, a compact pulse generator was presented for use under a microscope [13]. The length of the Blumlein line reduced significantly as the dielectric constant of water is quite high. But this requires a pulsed charging system because water is not a very good insulator, therefore the system may be charged with a dc power supply.

4. CONCLUSION

We have designed an avalanche transistor switch base pulse generator to produce 1.5 kV pulses with a rise time of 10nsand pulse width of ~20ns. The generator is operated at ~10Hz. Microchamber has been also designed such that there should be impedance matching under load (here mammalian cells in culture), also microchamber has electrode separation of 300µm when connected to pulse generator produces a uniform electric field of 50kV/cm.

ACKNOWLEDGEMENT

The author would like to thank Acelerator And Pulse Power Division BARC India for providing required material and instrument. We are also thankful to Dr. Amitava Roy and Mr. Ankur Patel for their guidance and co-operation and help throughout the work and for showing interest in through discussion in between the experimental work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/v67636