CANCER RESEARCH INSTITUTE
TOMSK NRMC

IHCE SB RAS

Different sensitivity of normal and tumor cells to pulsed radiofrequency exposure

Authors:

Evtina A.A., Cancer Research Institute, TNRMC RAS;

Priputnev P.V., Institute of High Current Electronics, SB RAS

Buldakov M.A., Cancer Research Institute, TNRMC RAS

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Features of biological efficiency of radiofrequency pulses

- control of the physiological state of dividing cells, in particular, cause the death of tumor cells;
- non-thermal effect on the irradiated object, which is achieved by their short duration (from micro-to nanoseconds);
- low average absorbed energy per session;
- feed to the target without using electrodes;
- electroporation caused by high-intensity electrical impulses, which leads to an increase in the permeability of cell membranes.

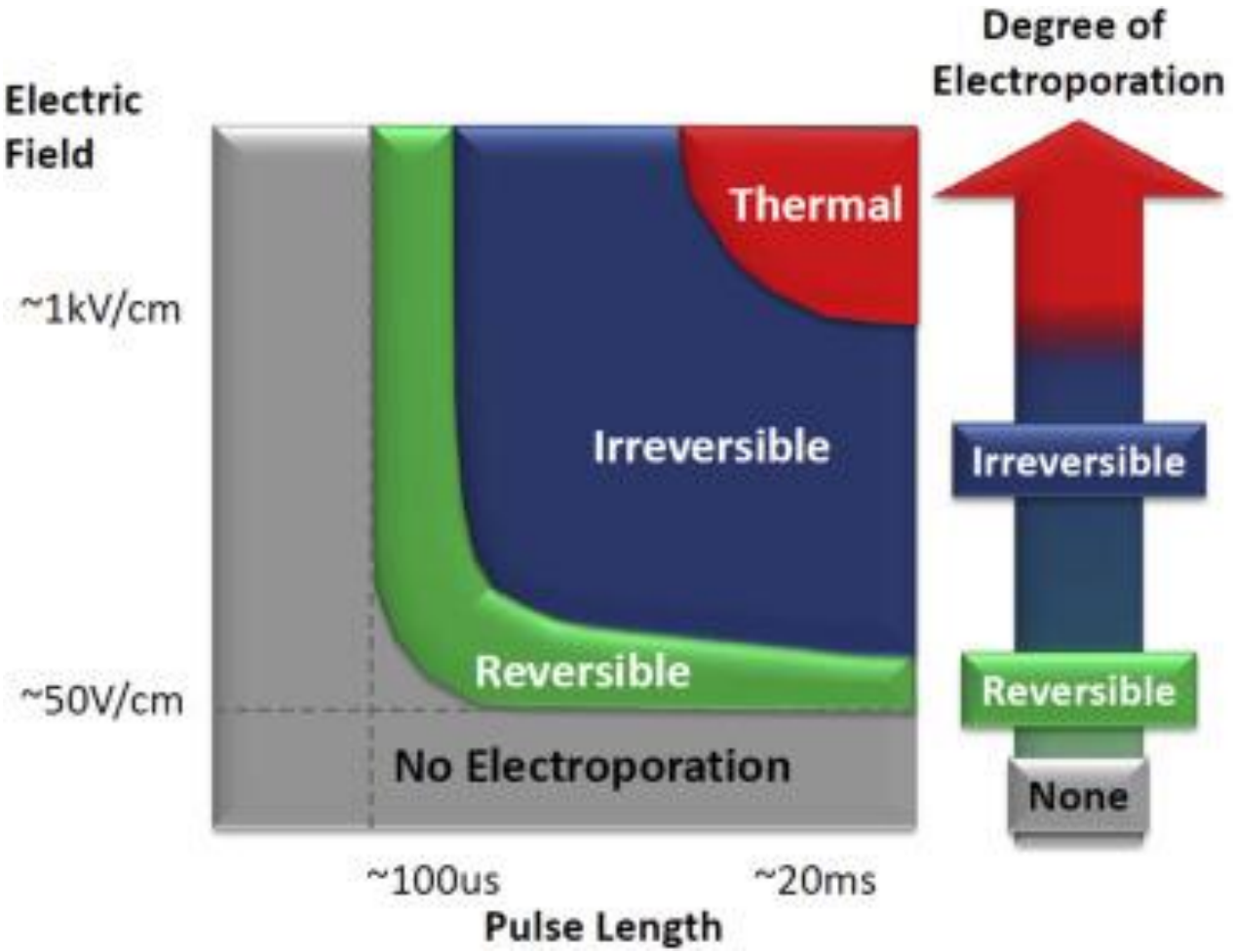


Fig. 1 – Zones of reversible, irreversible, and thermal ablation based on pulsed length and electric field; * * – Source: Hsiao C.-Y., Huang K.-W. etc. Irreversible Electroporation: A Novel Ultrasound-guided Modality for Non-thermal Tumor Ablation. Journal of Medical Ultrasound, 25 (4), 2017, pp. 195-200.

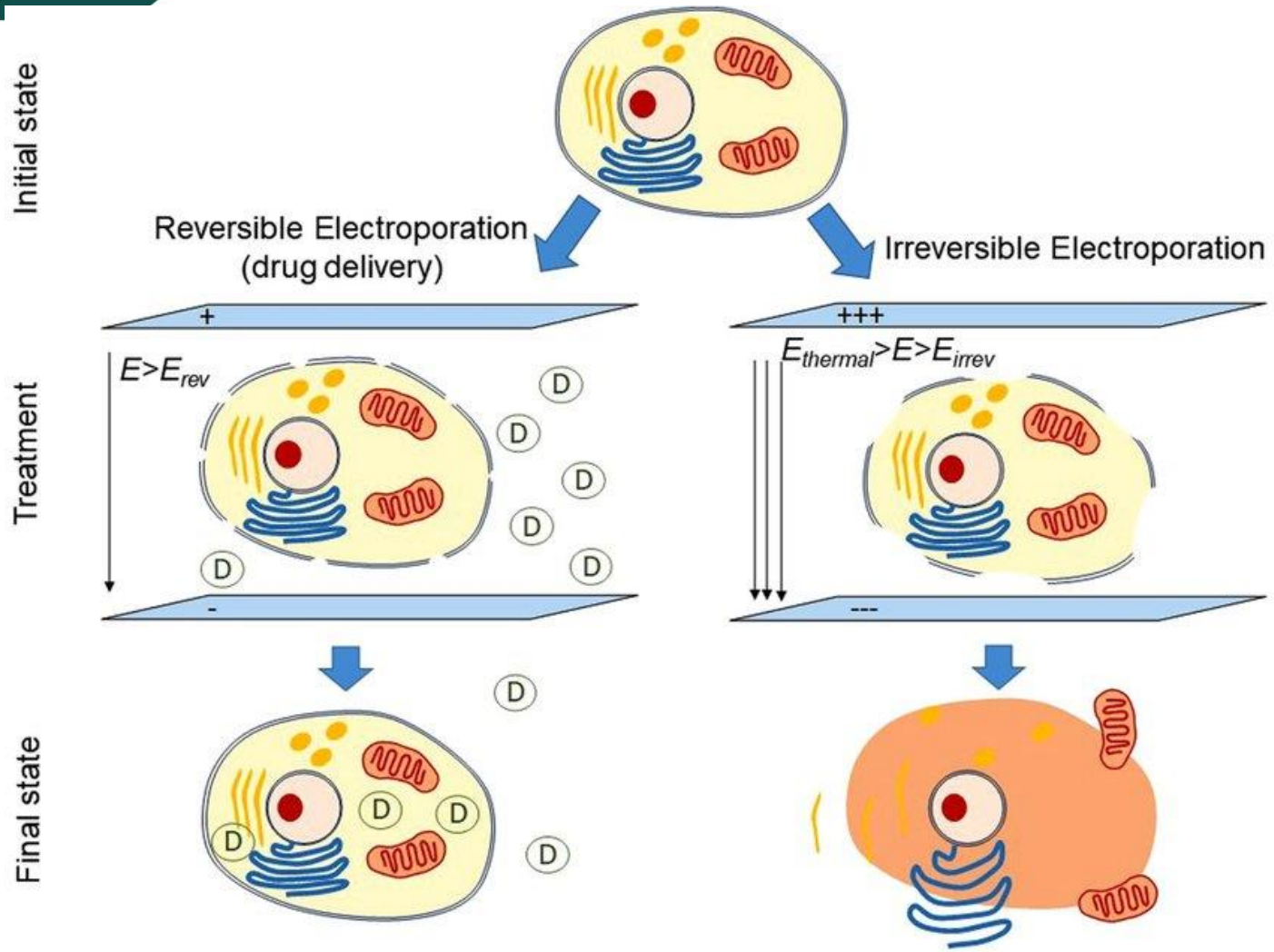


Fig. 2 – Electroporation. Reversible (RE, left) and irreversible (IRE, right) processes; *

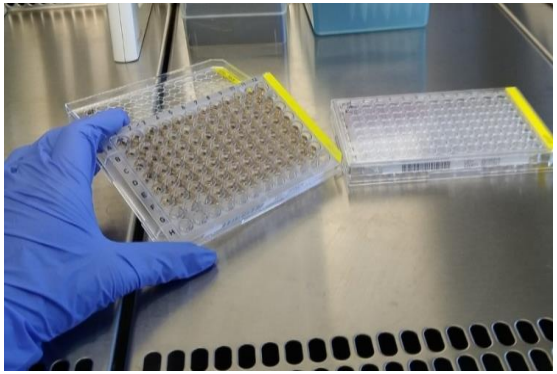
* – Source: Lopez-Alonso B., Hernaez A., Sarnago H. etc. Histopathological and Ultrastructural Changes after Electroporation in Pig Liver Using Parallel-Plate Electrodes and High-Performance Generator. Scientific Reports, 9, 2019, P. 2647.

Materials and methods

Cell lines: cervical cancer tumor cells (HeLa) and normal rat fibroblasts (3T3). The cells were incubated in Petri dishes in a humid environment containing 5% of CO_2 at a temperature of 37 °C. Irradiated cells located in test tubes with a nutrient medium in a concentration of 1 million/ml.

Experimental setup: the cells were irradiated using a pulsed high-power microwave generator based on gyromagnetic nonlinear transmission line (NLTL)

- electric field strength 25-30 kV/cm;
- central frequency of the microwave pulses 0.6 GHz-1 GHz;
- pulse duration 5-20 ns;
- pulse repetition rate 13 Hz.



Assessment of viability of cells:

1. MTT-test.
2. Non-invasive cell culture analysis system iCELLigence (ACEA Biosciences Inc., USA);

Materials and methods

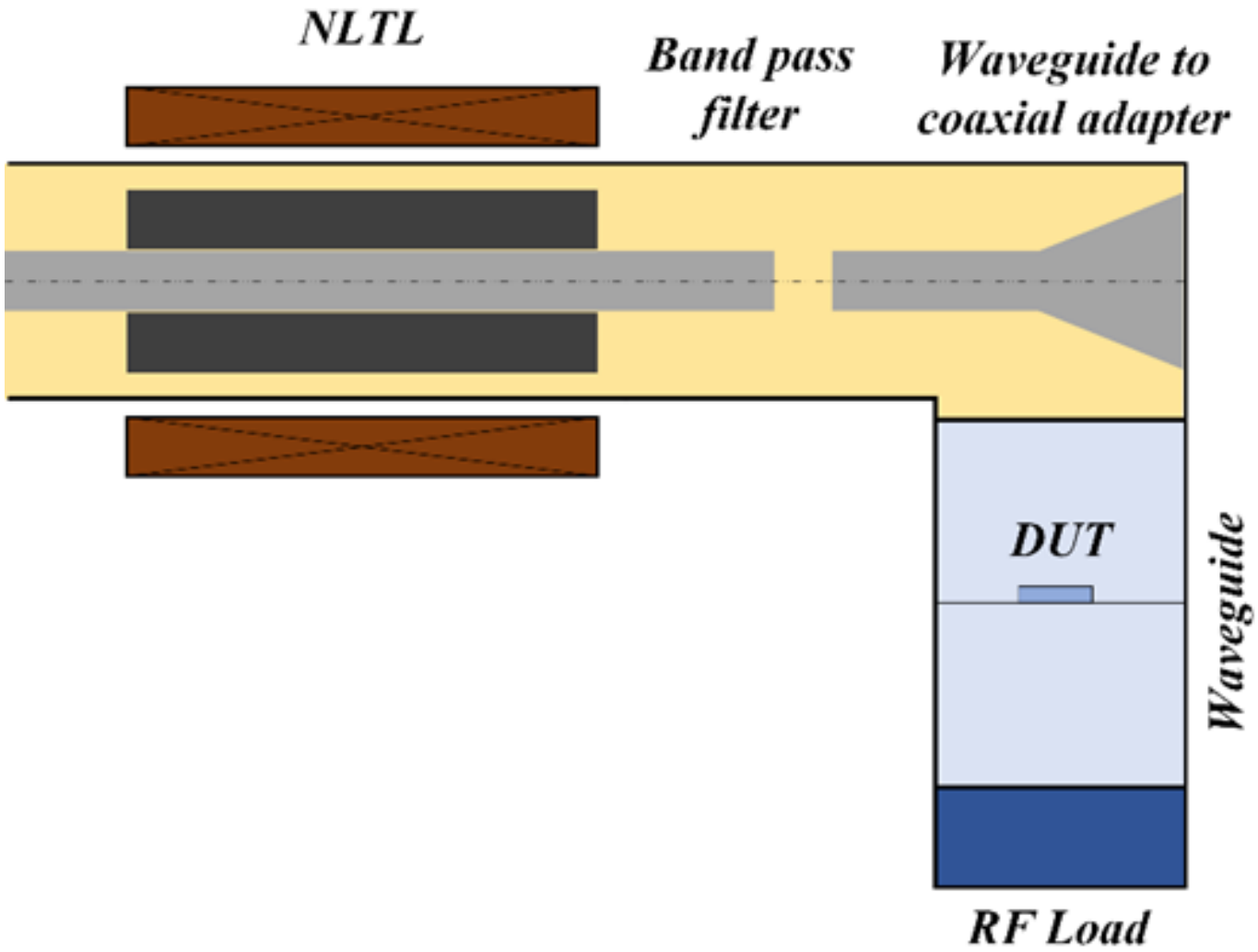


Fig. 3 – Scheme of the experimental setup.

Materials and methods

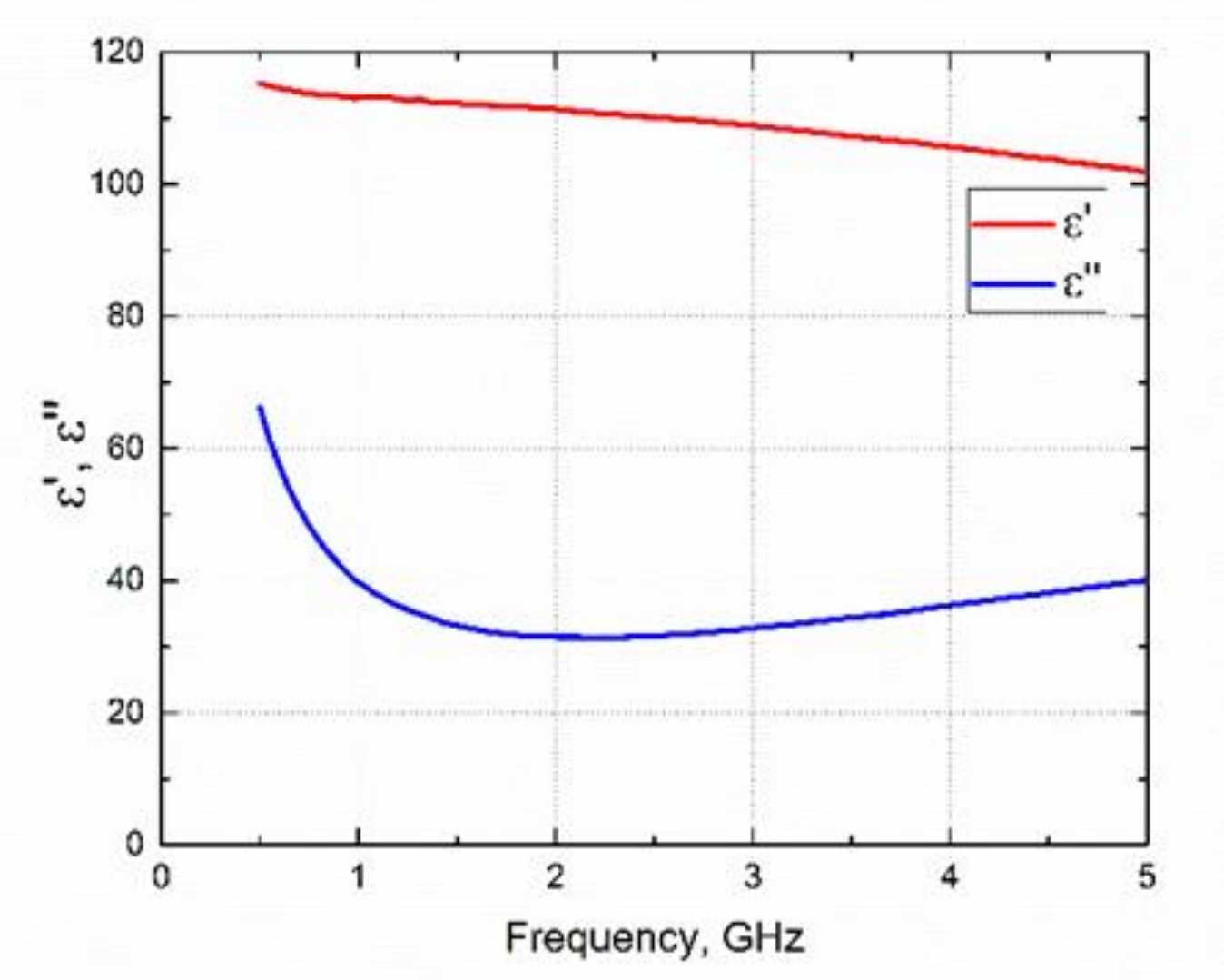


Fig. 4 – Frequency dependence of the components of the dielectric constant of the irradiated medium.

Materials and methods

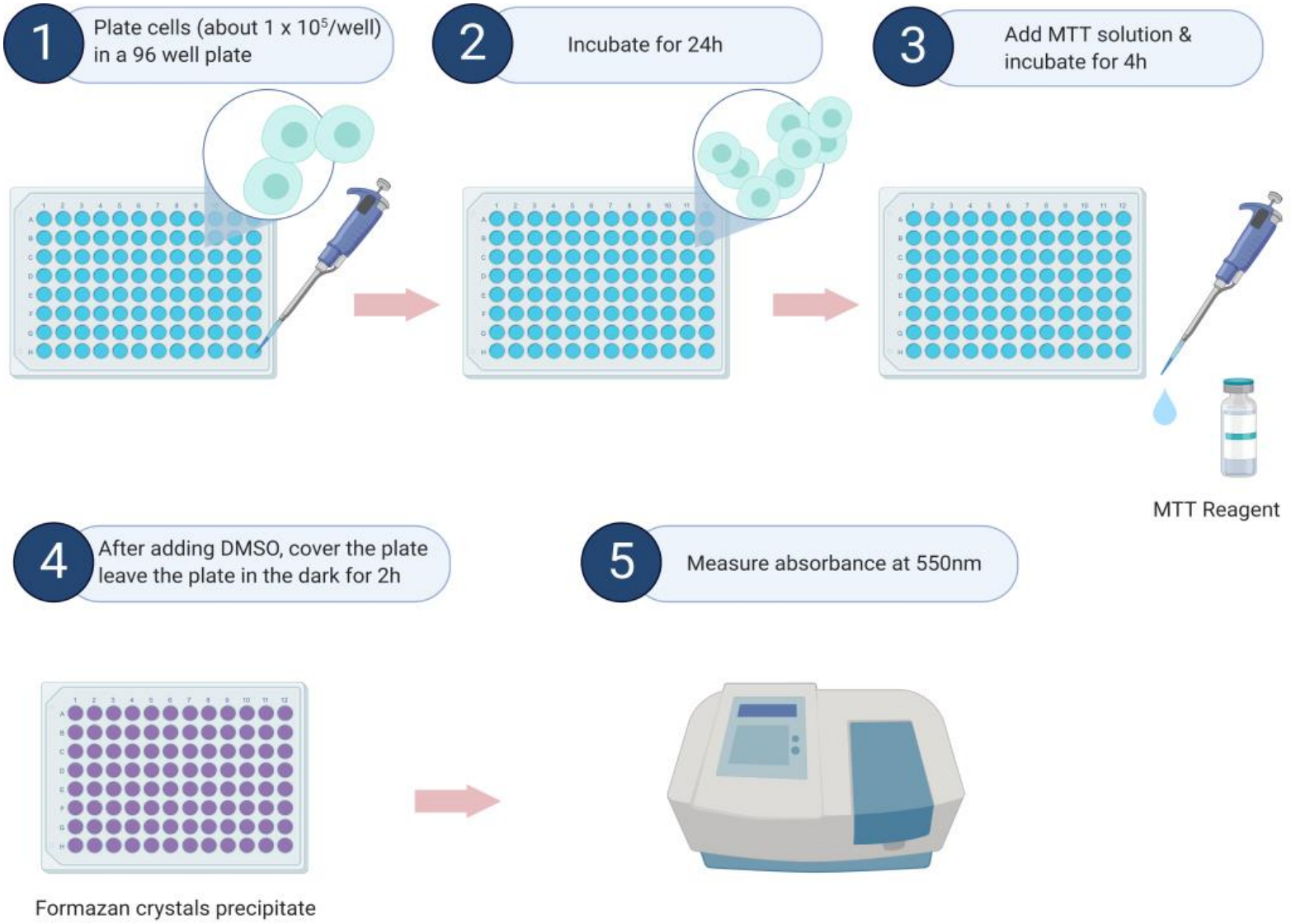


Fig. 5 – Diagram of the MTT assay; *

* – Source: https://theferalexplorer.wordpress.com/2019/07/05/understanding-mtt-assay/

Experimental results

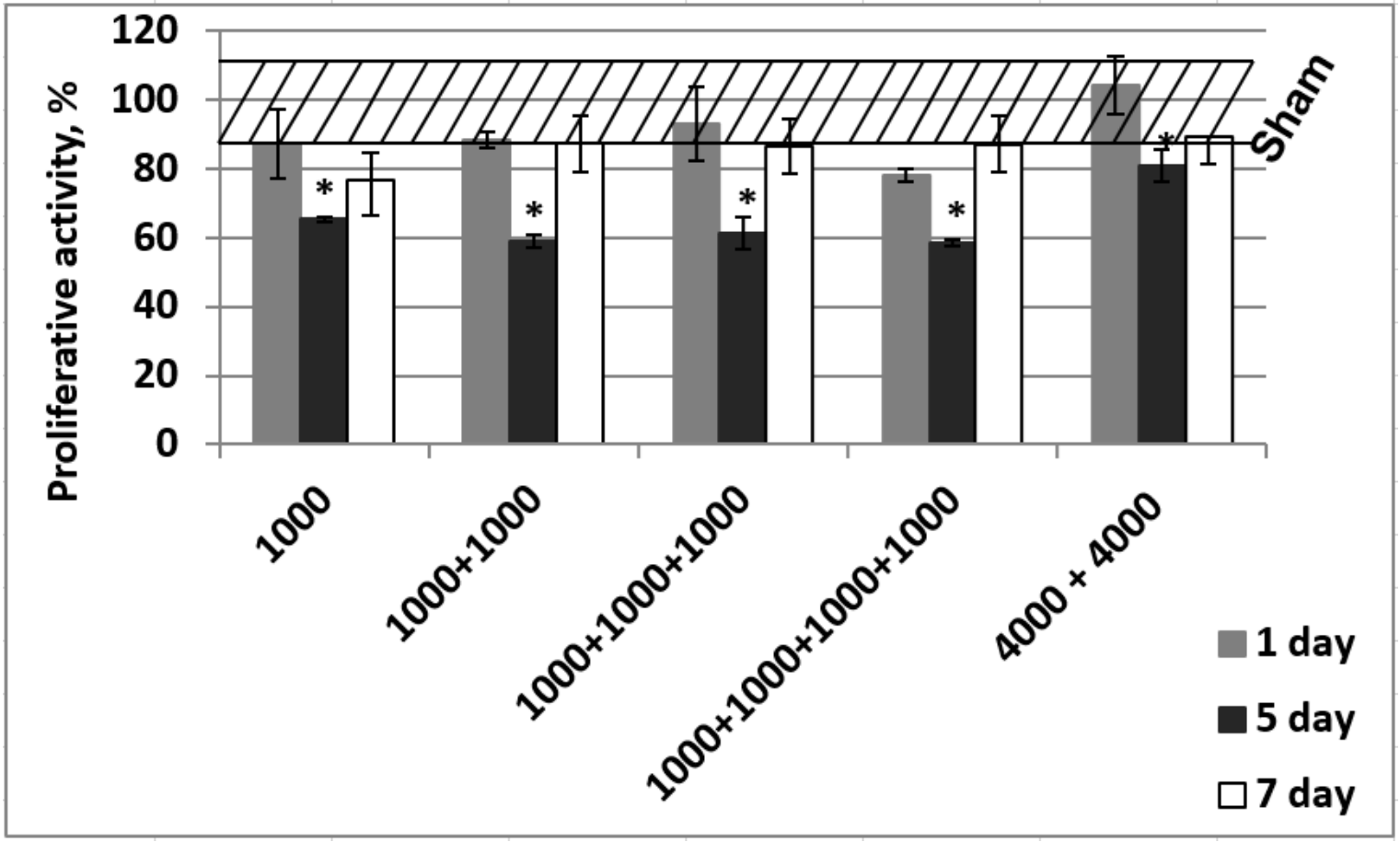


Fig. 6 – The decrease in the proliferative activity of Hela cell after exposure to nanosecond radio frequency pulses. The shaded region refers to the 95% confidence interval of the average proliferative activity of sham cells taken as 100%, * means that the difference from the sham is significant ($p \leq 0.05$)

Experimental results

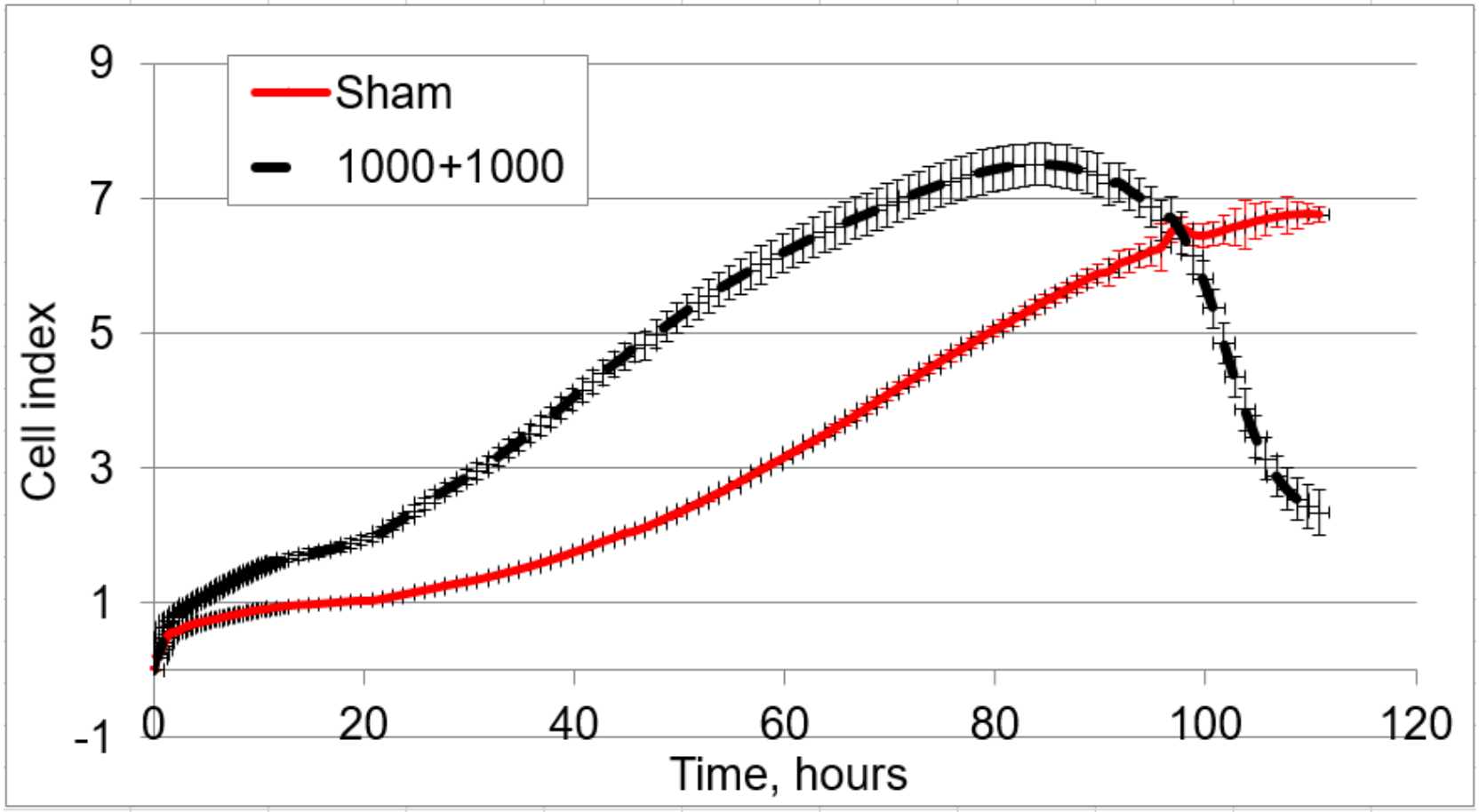


Fig. 7 – The Cell Index kinetics of HeLa cells were monitored for 120 hours after nanosecond radio frequency pulses exposure (1000+1000 pulses sessions with a 80 second interval between sessions, with a repetition rate of 13 Hz) with iCELLigence system.

Experimental results

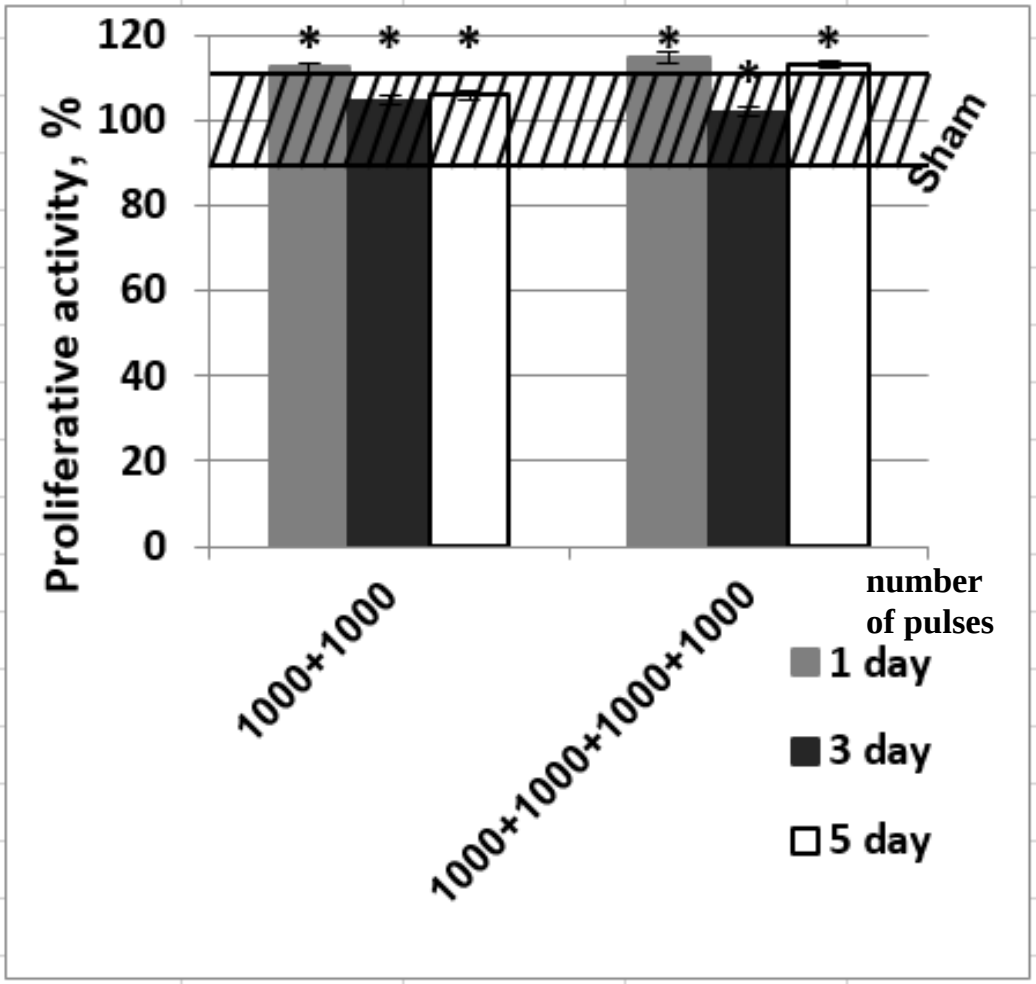


Fig. 8 – The decrease in the proliferative activity of 3T3 cell after exposure to nanosecond radio frequency pulses. The shaded region refers to the 95% confidence interval of the average proliferative activity of sham cells taken as 100%, * means that the difference from the sham is significant ($p \leq 0.05$)

Experimental results

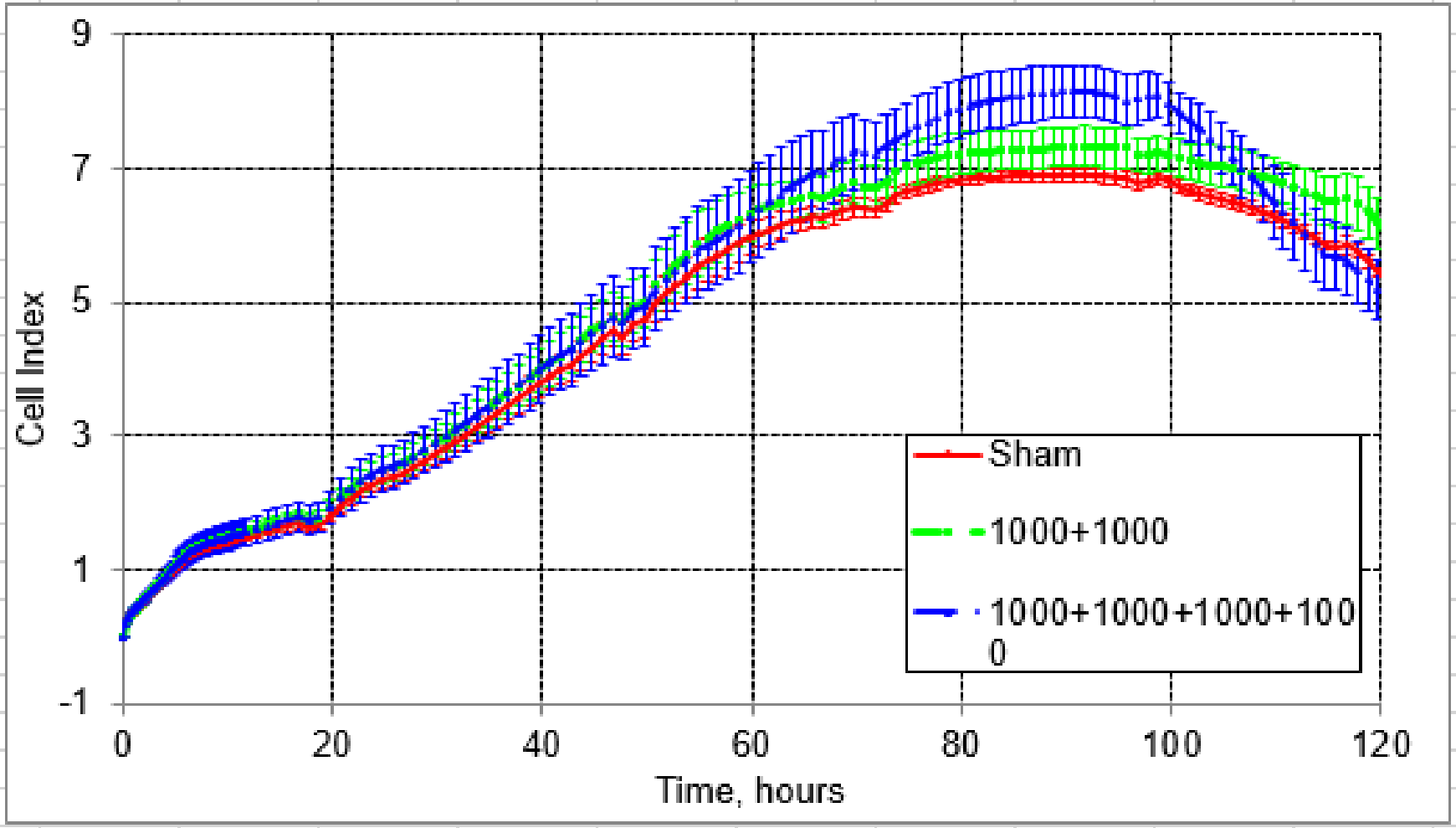


Fig. 9 – The Cell Index kinetics of 3T3 cells were monitored for 120 hours after nanosecond radio frequency pulses exposure with a repetition rate of 13 Hz with iCELLigence system.

Conclusion

- Results of calculation of the heating of the irradiated medium and electric field strength inside it allow us to establish that the effect on cell cultures is non-thermal;
- Exposure to nanosecond radiofrequency pulses under certain operating conditions affects the proliferation of both tumor and normal cells;
- Inhibition of the proliferative activity of cells can be caused by reversible electroporation, which leads to the death of cells, especially tumor cells;
- Inhibition of the proliferative activity of HeLa cells was maximal after double exposure to 1000 ns RF pulses with a pulse repetition rate of 13 Hz and an electric field strength of up to 30 kV/cm. It was most pronounced on the 5th day after exposure and reached 40% ;
- After exposure with the same parameters the maximum value of inhibition of proliferative activity of normal cells was observed on day 3rd, where it reached 10%.

Thank you for your attention!

anastasiya10152@gmail.com

CANCER RESEARCH INSTITUTE
TOMSK NRMC

5 Kooperativny Street, Tomsk, 634009, Russia
tel.: +7 (3822) 51-33-06
e-mail: onco@tnimc.ru