Development of a novel pulsed electric field generator and a handheld syringe and needle holder with embedded electrodes for electroporation and electroextraction of biological molecules from the tissue

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Current solid tissue sampling methods for diagnostics mostly use tissue biopsy, which could lead to localized tissue injury, bleeding, and scarring. Tissue biopsy is followed by pathological examination of the sampled tissue and a decision based on that examination. Recently, we demonstrated novel technology for harvesting biomolecules *in vivo* based on extraction using vacuum assisted electroporation – termed e-biopsy. During the period of this research we accomplished the following specific aims. (1) Develop a novel pulsed electric field generator for tissue electroporation and electroextraction. (2) Develop a novel handheld applicator for skin molecular biopsy with electroporation (3) Validate e-biopsy devices in human non-melanoma skin cancer. In this study, we used 100 excised human skin samples with known pathology and initially characterized by biomarkers for protein biomarker extraction with the novel equipment. Our approach is based on the temporary tissue permeabilization using non-thermal pulsed electric fields. The permeabilized tissue releases proteins which are harvested by the same e-biopsy handheld device, pumped out and analyzed for tissue profiling. Profiling consist of shotgun proteomics. Bioinformatics is applied to the resulting data to assess and optimize technology performance and to detect differentially expressed proteins and pathways. In addition, a classifier is under development that will provide a basis for the potential future use of the proposed technology in diagnostics.

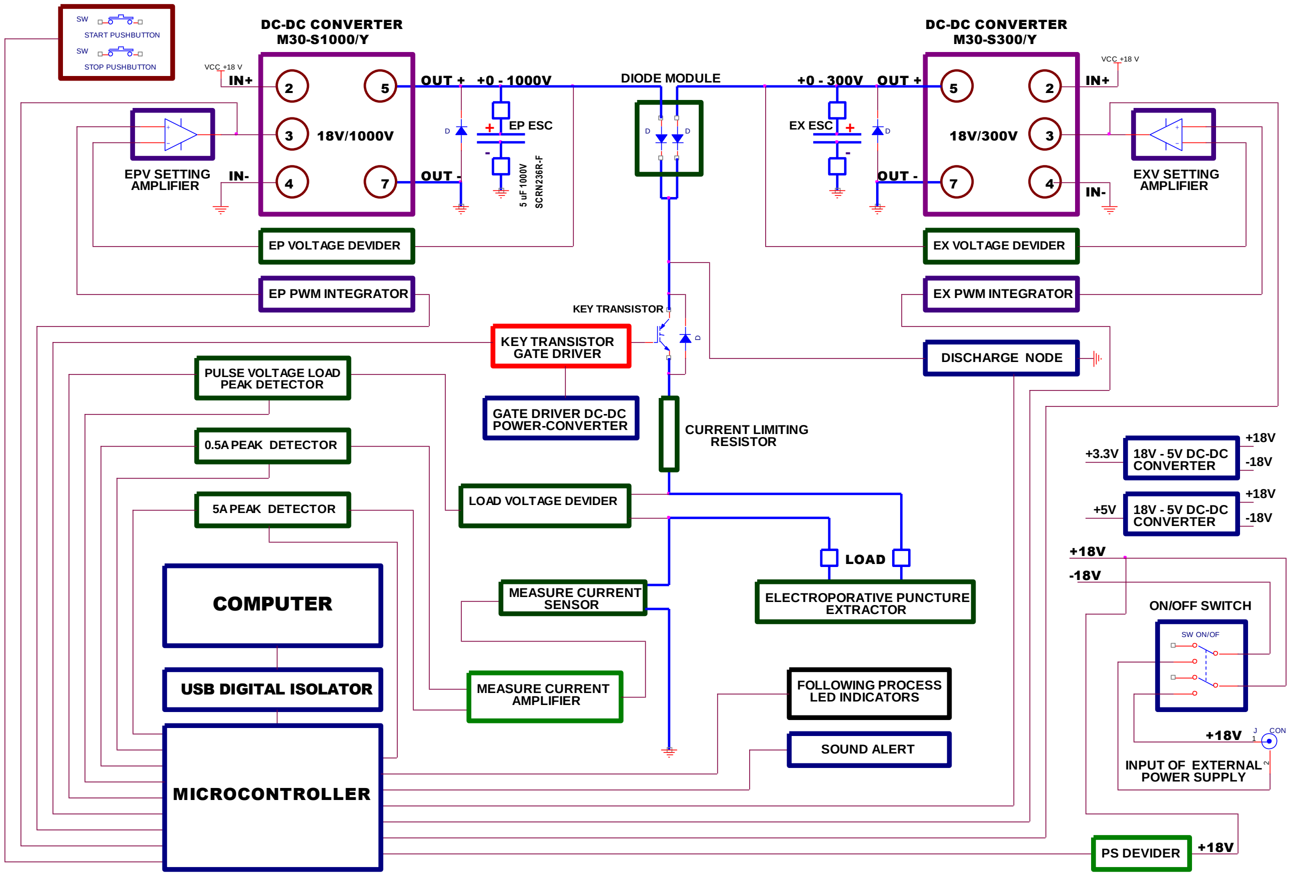
Publications

Genish I, Gabay B, Ruban A, Goldshmit Y, Singth A, Wise J, Levkov K, **Shalom A**, Vitkin E, **Yakhini Z**, **Golberg A.** Electroporation-based proteome sampling ex vivo enables the detection of brain melanoma protein signatures in a location proximate to visible tumor margins. **PLoS ONE.** 2022. 17(5):e0265866.

Two additional manuscripts are submitted and two are in preparation.

Results

Description of the functional diagram of the device of the electroporative punctional biopsy.



FUNCTIONAL DIAGRAM OF THE DEVICE OF THE ELECTROPORATIVE PUNCTIONAL BIOPSY.

Fig. 1.

A functional diagram of the hardware of an electroporation puncture biopsy device with an electroporation puncture extractor (EPE) connected to its output is shown in **Fig. 1.**

Hardware part is a variant of the high voltage pulse generator (HVPG) with mains power supply, which is controlled by an external computer via a built-in microcontroller.

The load of hardware part is the electroporative puncture extractor (**Fig. 2**), which is connected to the corresponding output of the device.

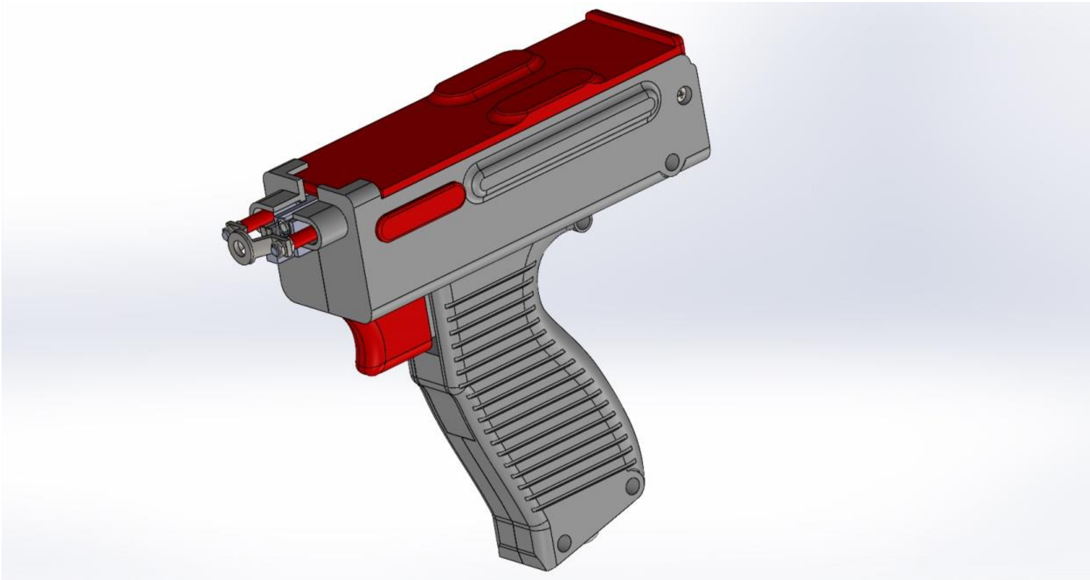


Fig. 2

The main functional purpose of the hardware part of the device is the formation on the load of high-voltage current pulses of a quasi-rectangular shape with an exponential decay for carrying out the processes of electroporation and extraction.

Electroporation pulses are higher voltage and are generated in the range
250 - 950V.

The extraction pulses are relatively low voltage and are generated in the range of 50 - 250V.

The energy source that provides pulses of electroporation current to the load is
non-polar pulse the energy storage capacitor EP ESC.

The EP ESC charge is made during a pause before feeding the next pulse.

The EP ESC charging process is carried out by a DC-DC converter that converts the 19V input voltage from an external DC source into an adjustable EP ESC charging output voltage in the range of 0 - 950V.

The circuit for regulating the output voltage of the charging DC-DC converter for EP ESC includes the corresponding PWM output of the microcontroller, PWM integrator EP PWM INTEGRATOR, amplifier for voltage regulation EP AMPLIFIER and a divider EP DEVIDER of charge voltage EP ESC.

When programmatically setting predermined electroporation voltage, by mean a microcontroller formed PWM signal, which is supplied to one of its corresponding outputs. The integrator EP PWM INTEGRATOR converts the PWM signal of setting the electroporation voltage to a predetermined analog voltage level, which is fed to the direct input of the voltage regulating amplifier EP AMPLIFIER.

The inverse input of this amplifier receives feedback voltage from the output of the divider EP DEVIDER of charge voltage EP ESC.

The EP AMPLIFIER is a comparison element that compares the voltage set by the microcontroller with the voltage at the EP ESC and, if it is lower than required, it continues to provide a high voltage control signal for charging the EP ESC.

When the voltage on the EP ESC reaches the set voltage, the output of the EP AMPLIFIER is set to a low signal level, which stops the charging process of the EP ESC.

The output of the EP AMPLIFIER is connected to one of the digital inputs of the microcontroller to block the innings impulses until the EP ESC charging process is complete.

During the working cycle, the control circuit maintains the constancy of the set voltage on the EP ESC.

The energy source that provides pulses of electroporation current to the load is the energy storage capacitor EX ESC.

As an energy source EX ESC can be used an electrolytic capacitor with an operating voltage of at least 350V.

To increase the steepness of the leading edge of the pulses and mechanical durability, the EX ESC energy storage capacitor can be shunted by a low-inductance non-polar capacitor.

The EX ESC charging process is carried out by a DC-DC converter that converts the 19V input voltage from an external DC source into an adjustable EP ESC charging output voltage in the range of 0 - 250V.

The circuit design and charging process of the extraction energy storage capacitor EX ESC are similar to the electrical circuit and charging process of the EP ESC energy storage capacitor.

In addition to the DC-DC converter, the electric circuit for charging the EX ESC energy storage capacitor contains an EX PWM INTEGRATOR integrator, an EX DEVIDER voltage divider and an EX AMPLIFIER comparison amplifier.

The decoupling of two energy storage devices and their charge sources with different voltages is carried out using two power pulse diodes, which are combined into a single "DIODE MODULE" module.

Pulse discharges of the energy storage capacitors EP ESC and EX ESC to the connected load are carried out using a corresponding switch transistor «KEY TRANSISTOR», which is connected to the discharge circuits of these capacitors.

The process of generating control pulses by this transistor with a given duration and repetition rate is performed by the microcontroller program.

From the corresponding digital output of the microcontroller, control pulses are fed to the input of the driver of the key transistor "KEY TRANSISTOR GATE DRIVER".

The output of this driver is connected to the control electrode of the key transistor - its gate.

The first purpose of the "KEY TRANSISTOR GATE DRIVER" driver is to convert the TTL level of the digital output of the microcontroller into the level of the control signal of the key transistor.

The second purpose of the KEY TRANSISTOR GATE DRIVER of the key transistor is to provide galvanic isolation between the high-voltage circuits of the discharge circuit and the low-voltage control circuits.

Power supply of the key transistor driver is carried out from a separate DC-DC converter "GATE DRIVER DC-DC POWER-CONVERTER" with high-voltage galvanic isolation.

The common element for both discharge circuits is the load connected to output of hardware part.

The main requirements for the load parameters are its minimum possible reactivity and resistance value, which should not be lower than 200 Ohms.

In case of an unacceptable decrease in the load resistance in the process of impulse action on the object, a proportional decrease in the voltage of the acting energy storage capacitor occurs to a level that determines the specified level of current limitation.

In the event of a sudden short circuit, a passive current limiting process is carried out using a current limiting resistor
"CURRENT LIMITING RESISTOR", which is connected in series to the circuit of both discharge circuits.

In the course of the impulse action, the amplitude values of the voltage and current of each of the impulses are measured.

Measurement of the amplitude value of the voltage of the pulses at the load is carried out using the voltage divider "LOAD VOLTAGE DEVIDER", from the output of which the signal voltage is fed to the peak detector "PULSE VOLTAGE LOAD PEAK DETECTOR".

A peak detector is needed to temporarily store an analog signal with a voltage value that is proportional to the amplitude value of the voltage or current of each of the pulses.

Using the ADC of the microcontroller, to one of the inputs of which the peak detector "PULSE VOLTAGE LOAD PEAK DETECTOR" is connected, the voltage at the output of the peak detector is measured by converting an analog value into its digital meaning.

The measurement of the amplitude value of the current is carried out using the current sensor "MEASURE CURRENT SENSOR", to the output of which the signal amplifier of the sensor "MEASURE CURRENT AMPLIFIER" is connected with two gain levels for measuring current in the ranges of 0.5A and 5A.

Signals from each output of the "MEASURE CURRENT AMPLIFIER" are fed to the inputs of the corresponding peak detectors “0.5A PEAK DETECTOR” and “5A PEAK DETECTOR”.

The values of the maximum voltages of signals that relevant to the values of the measured currents are stored by the peak detectors until they are converted using the ADC of the microcontroller, to the inputs of which the outputs of the peak detectors are connected.

Further, the program determines and fixes the measured current value that is measured with the highest resolution and is in the middle of the measurement range.

After completing the procedure for measuring the amplitudes of the received signals, the microcontroller from one of its digital outputs sends a reset signal to the corresponding inputs of the peak detectors to reset their analog memories.

The Teensy 3.2 board module with the MK20DX256 microcontroller is used as an operational control device directly mounted in the electrical scheme (**Fig. 3**).

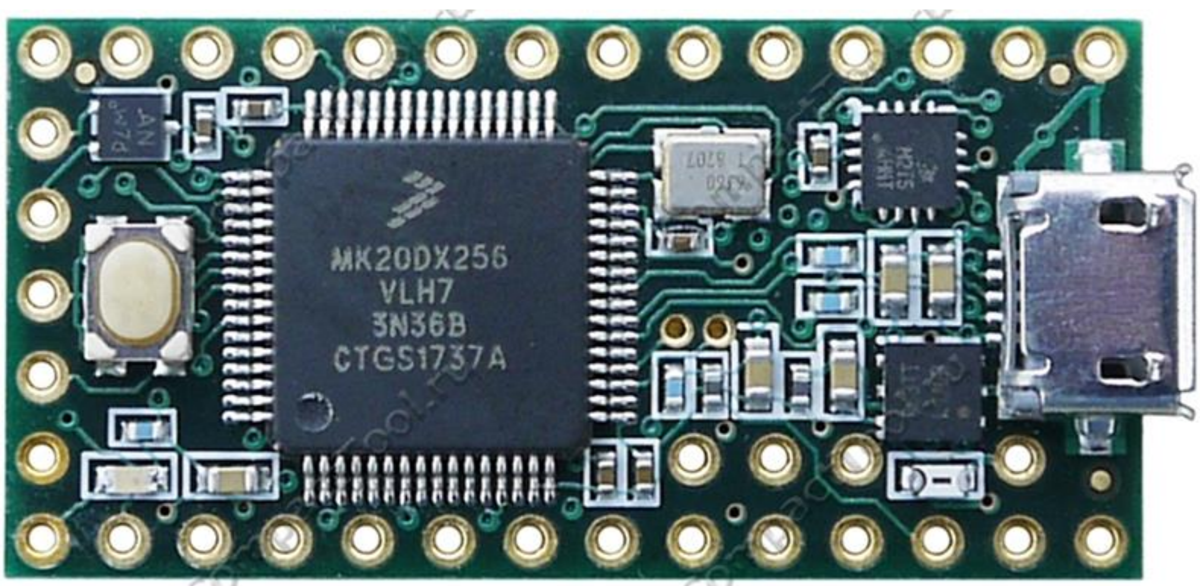


Fig. 3

Input of the initial parameters of high-voltage impulse action, as well as visualization and registration of the process is carried out using a computer and his program.

The graphical user interface (GUI) of this program is shown on **Fig. 4.**

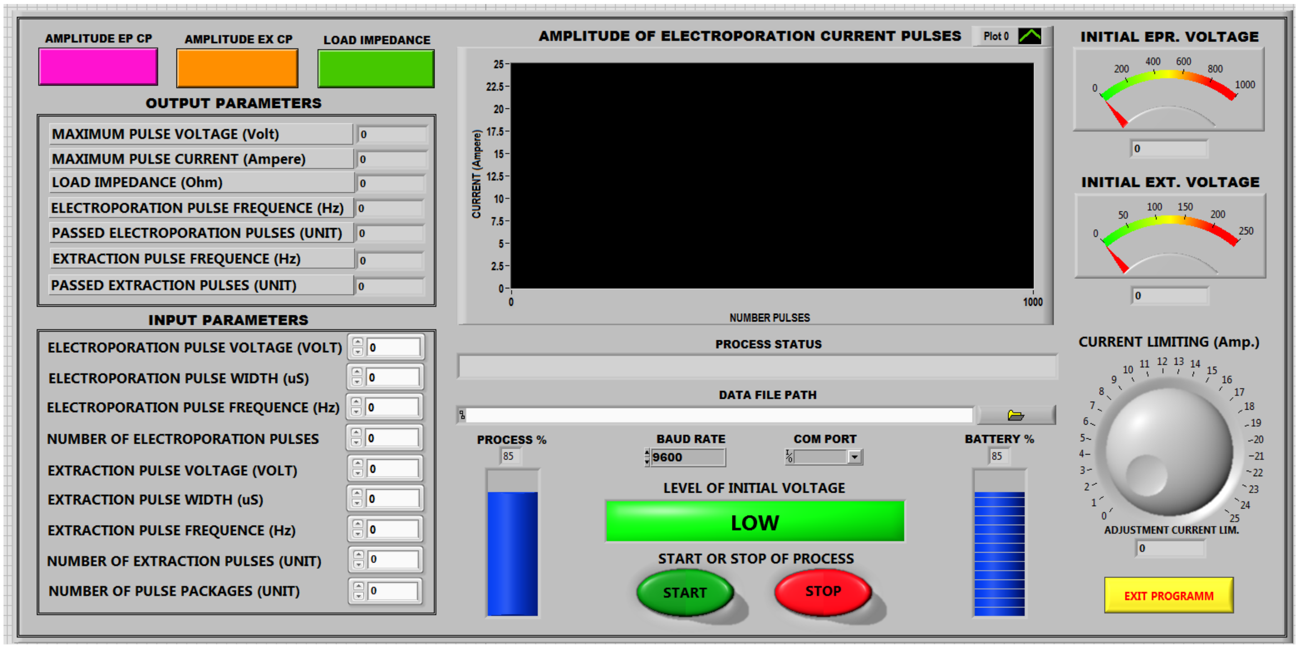


Fig. 4

The computer communicates with the hardware microcontroller via a USB interface.
To ensure high-voltage galvanic isolation of the hardware part of the device and the computer, a USB opto-isolator "USB DIGITAL ISOLATOR" is used.

The start and stop of the process is carried out using the "START" and "STOP" buttons.

After the end of the working cycle, the energy storage devices are discharged using the corresponding "DISCHARGE NODE" discharge electric circuit.

The power supply to the hardware is carried out using an external power supply, which is connected to the device through a special connector.

MEAN WELL GSM90 90W High-Performance Medical Adapters is used as an external power source for the device (**Fig. 5**)

Power supply of 3.3V and 5V electric circuits of the device is provided by two DC-DC converters with galvanic isolation.

Fig. 5

The design of the electrical part of the device is implemented in the form of two printed circuit boards.

Fig. 6

The printed circuit board of the high voltage power unit is shown in **Fig. 6,** and the control node board is shown **in Fig. 7.**

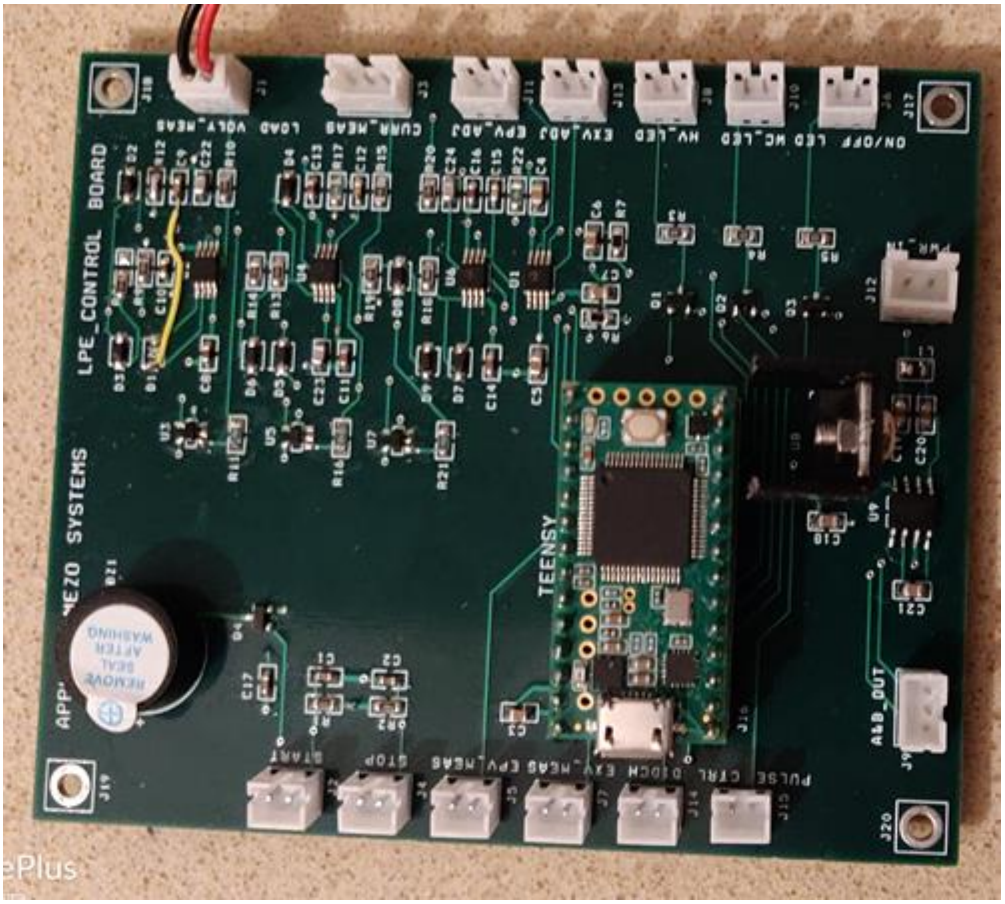


Fig. 7