Electropermeabilization of Isolated Cancer Stem Cells with a Novel and Versatile Nanosecond Pulse Generator

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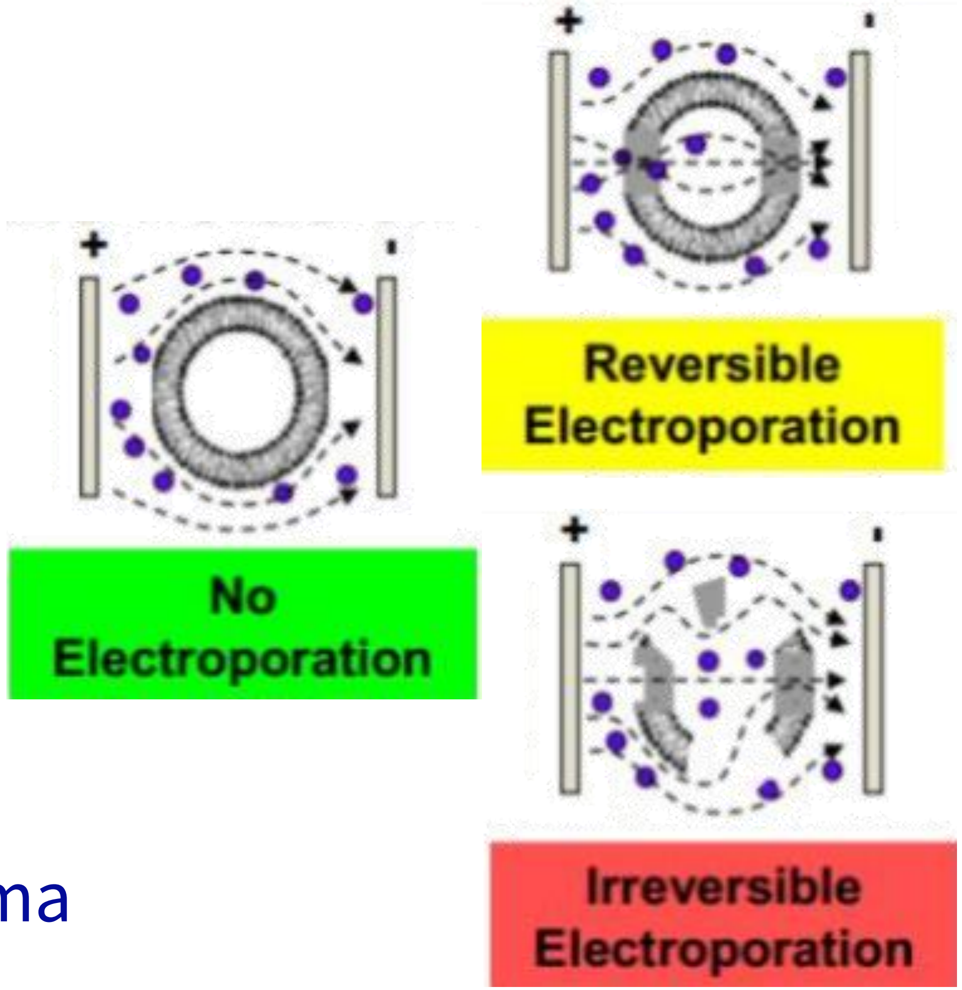
Introduction: To the **Project**

- Part of European Union's Horizon 2020 research and innovation program: **S**emiconductor based **U**ltrawideband **M**icromanipulation of **CA**ncer **ST**em **C**ells or **SUMCASTEC**
- SUMCASTEC explores a new approach for real time isolation and neutralization of Cancer Stem Cells (CSCs). http://www.sumcastec.eu
- CSC are associated with Glioblastoma Multiforme (GBM) and Medulloblastoma (MB).
- A project deliverable: to develop an off-chip pulsed Electric-Field (EF) or Electroporation (EP) or Irreversible Electroporation (IEP) generator.

Introduction: What is Electroporation (EP)?

- Alternative physical technique for non-thermal ablation
- Use precisely controlled **high amplitude pulsed electric pulsed fields** of **short duration** to alter a cell’s transmembrane potential.
- Results in permeabilizing the cell’s plasma membrane and disturbing intercellular homeostasis.
- The resultant permeabilization of cell plasma membrane can be reversible or irreversible.

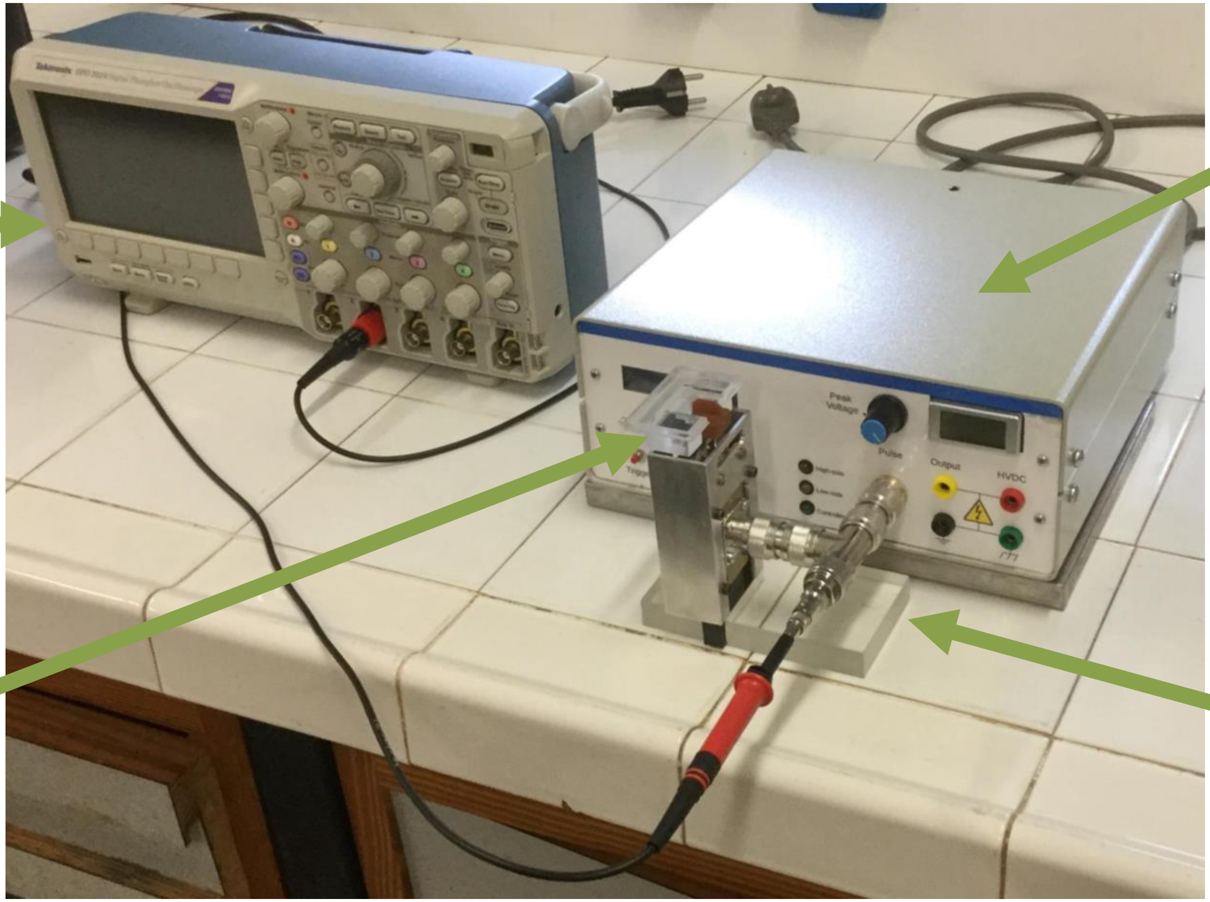


Introduction: Project Goal

- To deliver a generator capable of pulse amplitude in excess of 1 kV, with pulse widths in the 100 ns regime for electroporation.
- Minimisation of overshoot and ringing (Flat-Top pulses)
- Investigate various pulse parameters associated with the SUMCASTEC Pulse Generator (SPG) on CSC. (pulse duration, repetition frequency, number of pulses)
- To developed an Non-thermal EP approach
- Investigate SPG effects on CSC suspended in a 50 Ω, 0.3 S/m buffer and other conductive solutions.

Instrumentation: Project Set-up

Dynamic monitoring of pulse during cell EP or IRE



SUMCASTEC **P**ulse **G**enerator (SPG)

Bio-rads 0.1cm gap Cuvette containing CSC and ENEA 50Ω artificial buffer

ENEA Cuvette Housing Unit

Instrumentation: Project Set-up

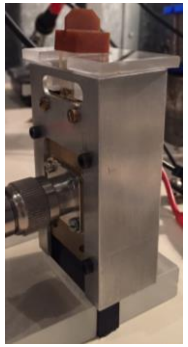
SUMCASTEC Pulse Generator (SPG)

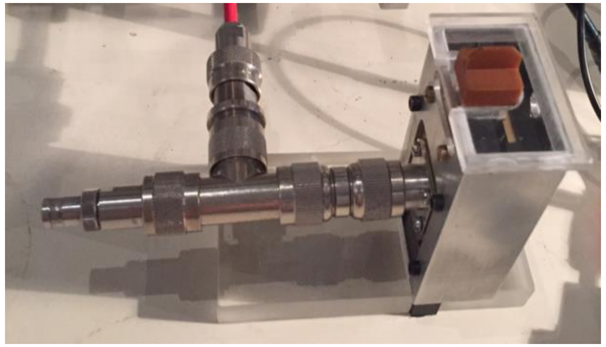
Pulse repetition Frequency: 1 - 50 Hz
Pulse Width Generated: 80 ns - 1 µs*
Number of pulses generated: 1 - ∞ infinite (continuous wave)
Pulse amplitudes: 280V - 1100V**
N-connector and banana sockets output.

*(increment of 10ns between 80 - 400 ns and 20ns increment 400ns - 1µs)
** With 50Ω Load

Designed and Build by Creo Medical

ENEA Cuvette Housing Unit

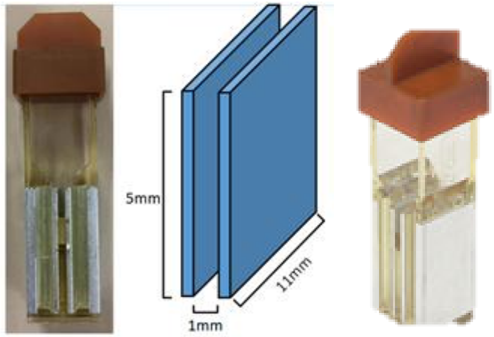




Exposes CSC in cuvette to EP
Compatible with CSC and standard N-connectors
Allow real time monitoring of EP HV pulse

Designed and constructed by A. Zambott and ENEA

ENEA 50Ω artificial buffer

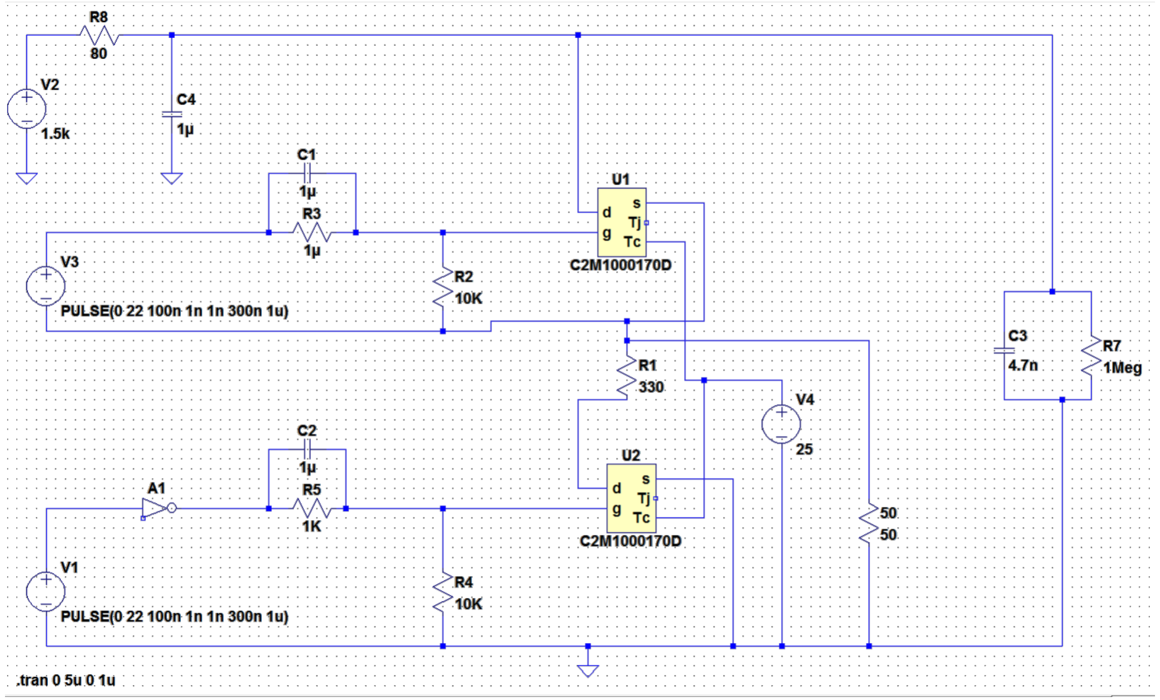


Butten (4)PBS + H2O + Sucrose [1]
Proportions for 100 mL of 0.3 S/m (50Ω) buffer:
- 20 mL of PBS (phosphate saline buffer)
- 80 mL of H2O (distilled water)
- 8.2 g of sucrose (to balance out Osmatic Pressure with the cells)

Made by ENEA

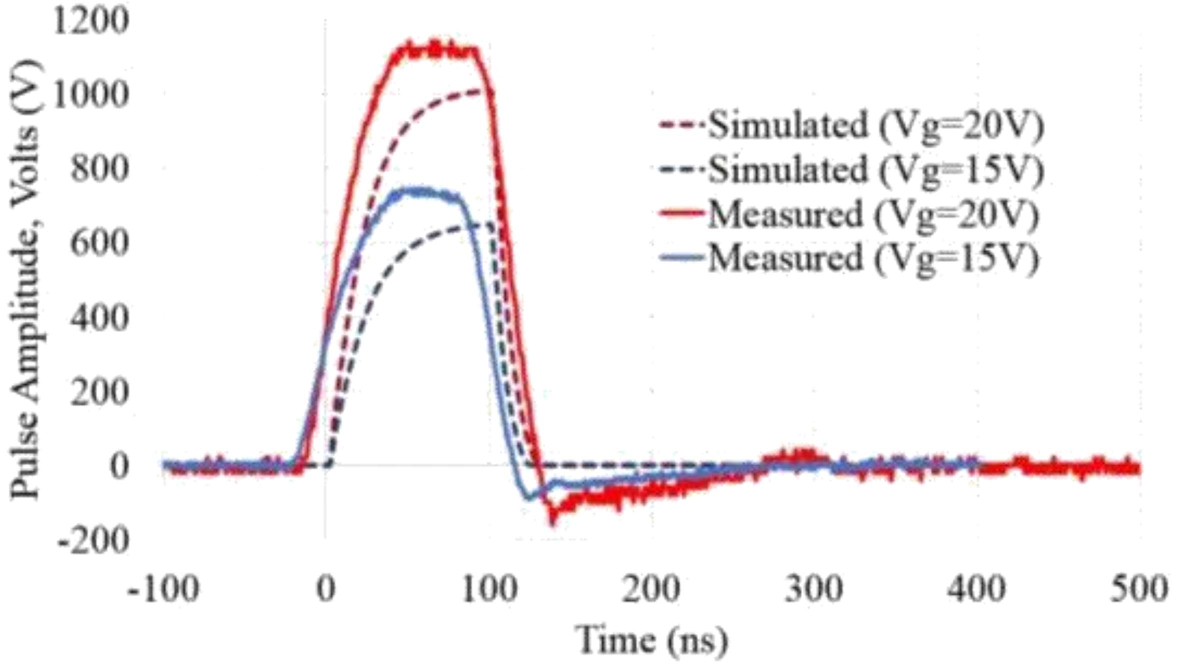
Instrumentation: SPG Hardware

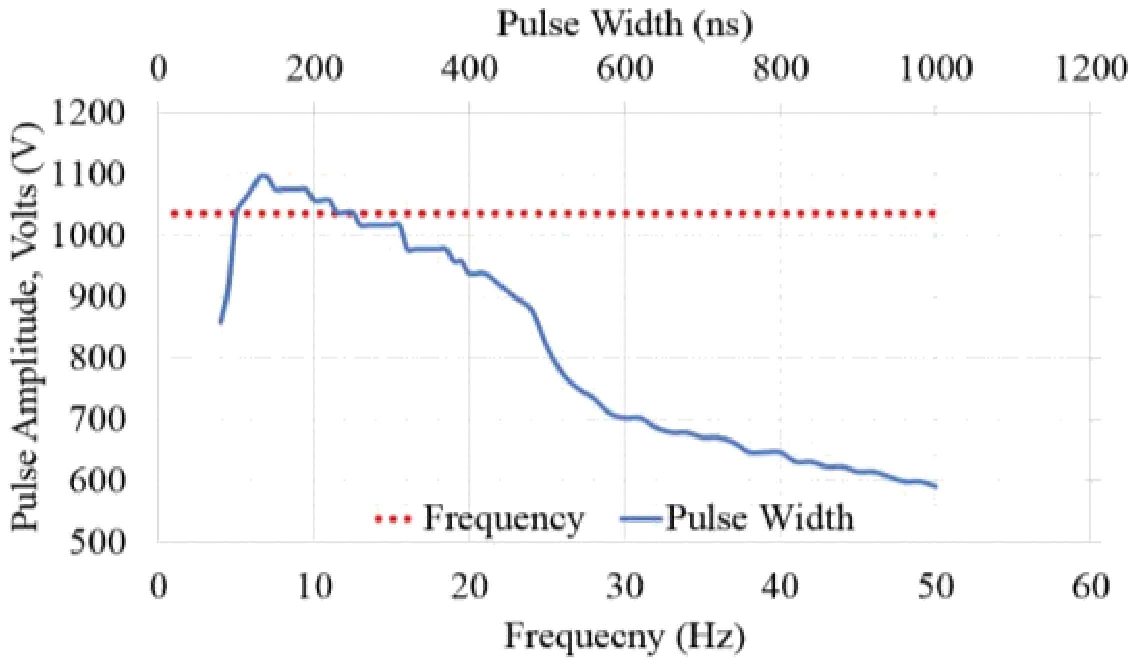
- Push-pull switching of High Voltage (1700v), fast switching (70.5ns) MOSFETs
- Driven by opto-isolators with comparable switching times – high enough current to charge up gate-source and gate drain capacitances
- High Side MOSFET determine pulse width. Low-side complimentary of High-side MOSFET : to ensure symmetrical fall time.



$$i = C\frac{dV}{dt}$$

Instrumentation: SPG Characteristic

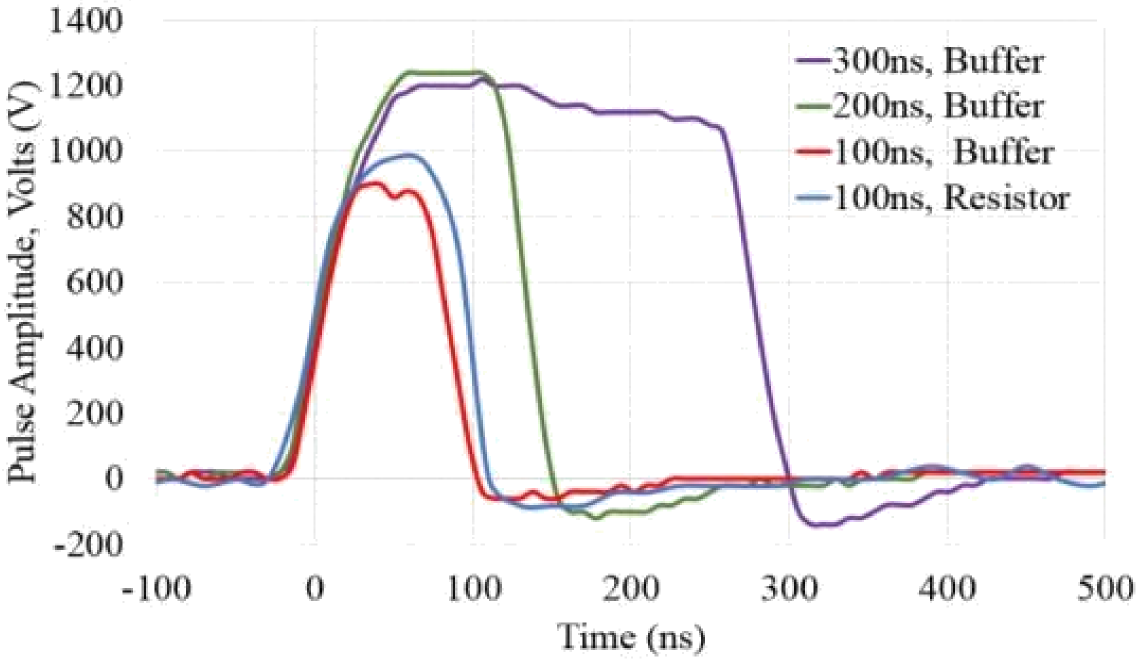


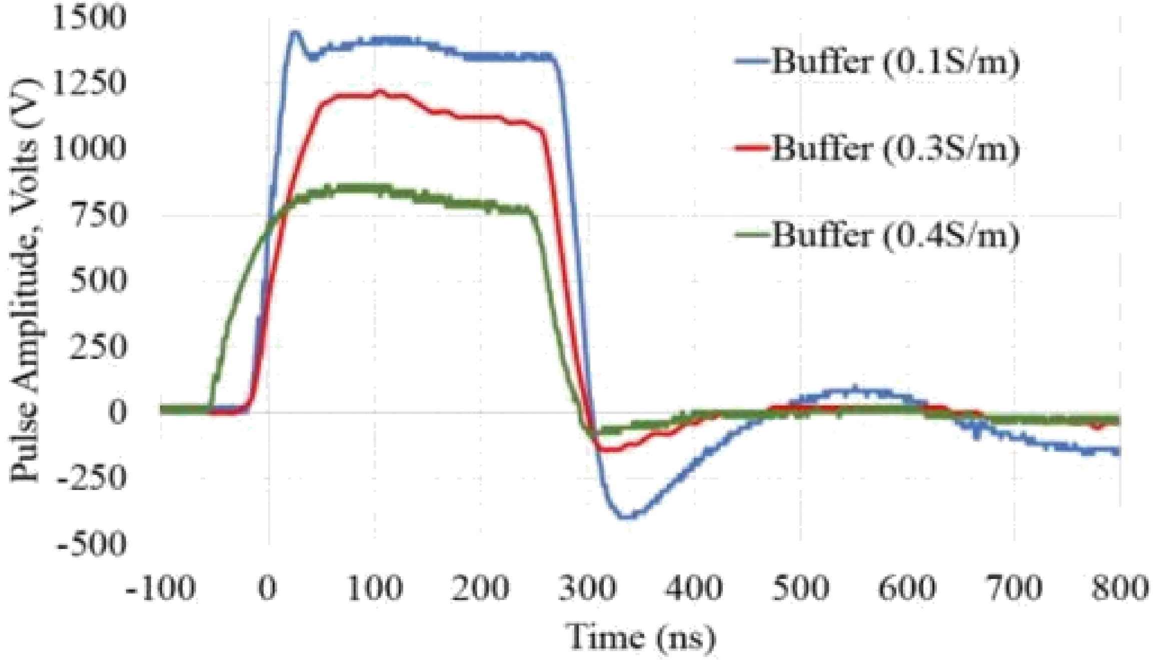


- Flat pulses free from ringing and overshoot
- Increase of gate voltage from 15 V to 20 V results in increased pulse amplitude.
- Developed EP generator performance exceeds the LTSpice simulation.

- Pulse amplitude is unaffected throughout its operating repetition frequencies (1-50 Hz)
- Optimized in the pulse width range of 100 ns to 300 ns, for pulse amplitudes in excess of 1kV

Result and Analysis: Electric pulse across cuvette electrodes





- ENEA artificial 50Ω is comparable to waveform measured with a 50 Ω resistor.
- 100 ns, 200ns and 300 ns pulse waveforms measured across the EP cuvette containing CSCs suspended in 50Ω, 0.3 S/m buffer solution

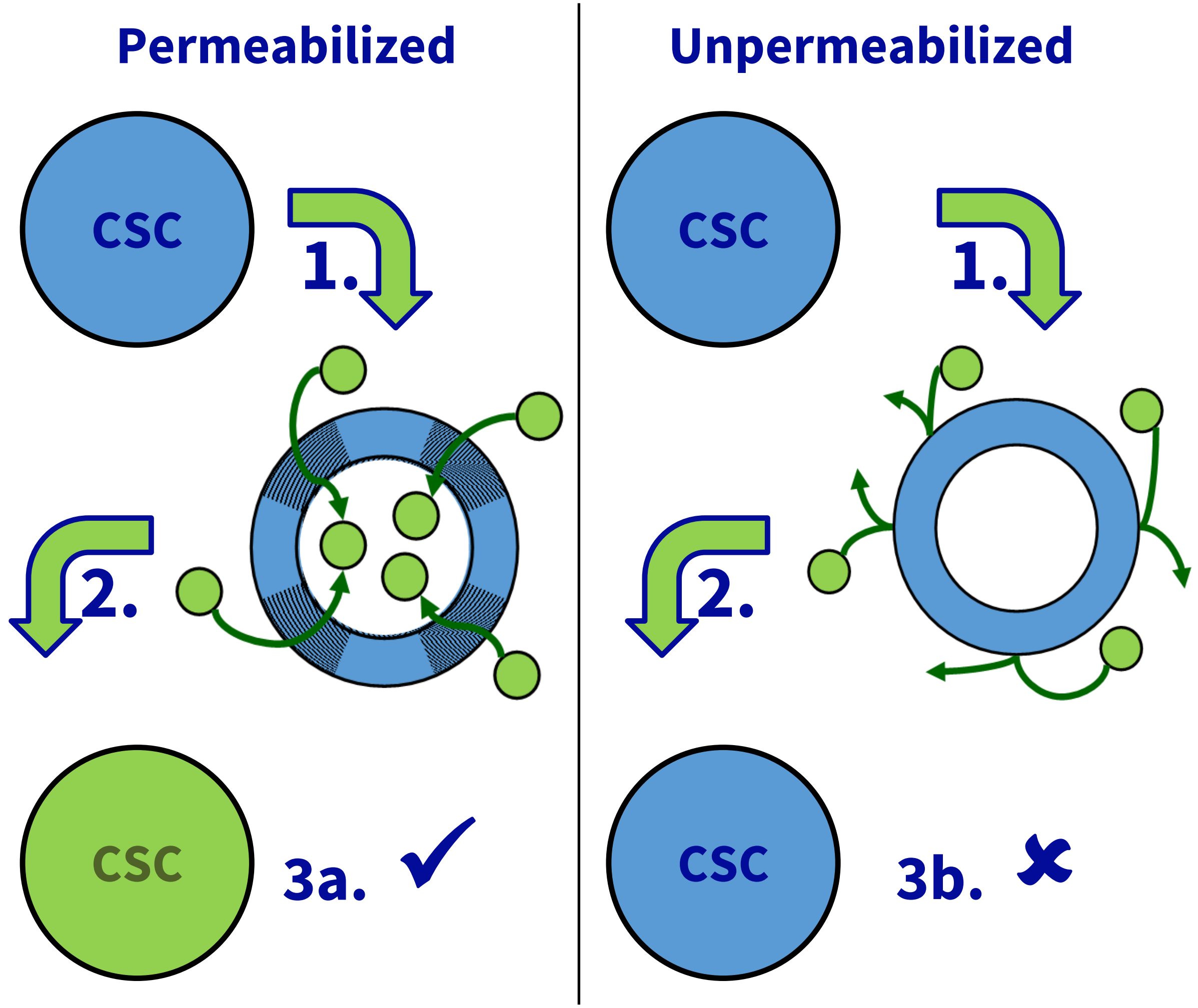
- 300ns pulses with various buffer solution at load
- Shape of the pulse is non-affected
- Demonstrating broadband matching performance

Result and Analysis: CSC Permeabilization Test

1. CSC placed in buffer with YOPRO-1
- YOPRO-1 a fluorescent dye (green in colour)

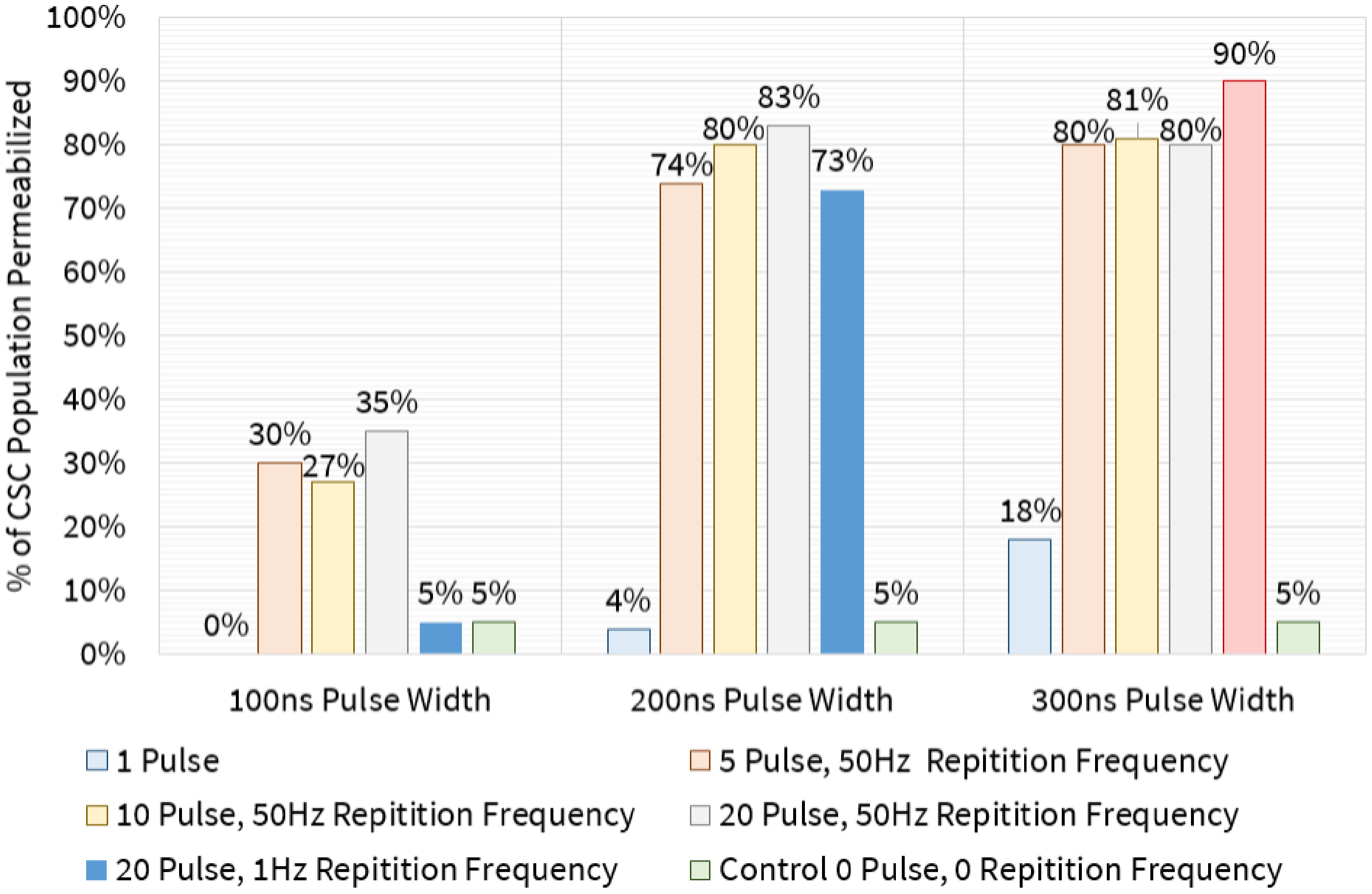
2. CSC exposed to EP
3. Exam CSC under fluorescent light:
   - 3a. Permeabilized: CSC green
   - 3b. Unpermeabilized: CSC transparent

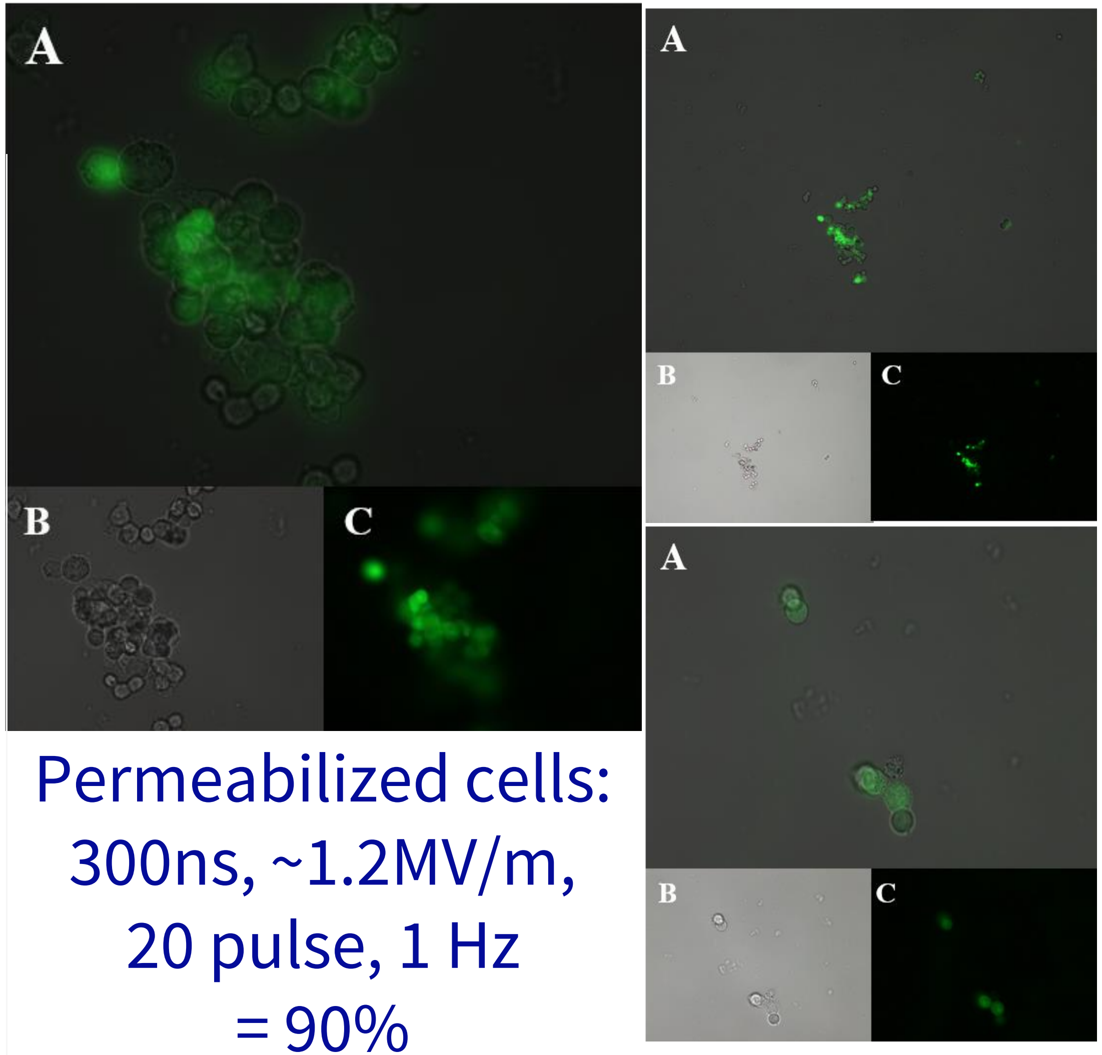
- CSC can be sensitized by the E pulses even if they are not permeabilized



Result and Analysis: CSC Permeabilization Test

- Electroporation Occurred





Permeabilized cells: 300ns, ~1.2MV/m, 20 pulse, 1 Hz = 90%

Result and Analysis: Non-thermal Effects

Pulse Width, P_w (ns)	Amplitude V (kV)	Load, Z (Ω)	Power (kW)	Repetition Frequency, f (Hz)	Energy, E (mJ)	Temperature Change, $\Delta T\ (\mu^0 C)$
100	1.0	50	20.0	1	2.00	4.8
100	1.0	50	20.0	50	100.00	239.2
200	1.2	50	28.8	1	5.76	13.8
200	1.2	50	28.8	50	288.00	689.0
300	1.2	50	28.8	1	8.64	20.7
300	1.2	50	28.8	50	432.00	1033.0

Non-thermal effect. of $1.0.\mathrm{x}10^{-3}\ {}^0C\ (100\mu^0 C)$

- D is duty cycle (*ratio*)
- E is energy (*J*)
- C is heat coefficient, 4.18 J/g/^0C, as buffer mainly consists of water
- L (*indicating volume*) is millilitres (the cuvette can hold 0.1 *mL* of solution)

$$\Delta T = \frac{\left(\frac{V^2}{Z}\right).P_w.D}{C.L}$$

Discussion and Conclusion

- ✓Successful permeabilization of the CSCs
- ✓Real-time pulse visualization
- ✓Positive results obtained in matching strategy
- ✓Non-thermal permeabilization of CSC

- ? Results do not reflect whether the CSCs are dead or alive
- ? Unknown if EP was reversible or irreversible
- ? CSCs exposed to 100 ns pulses could have been permeabilized
- ? Viability studies are required to complement these preliminary experiments in the future

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References:

[1] C. Merla et al. “SUMCASTEC_180123_NA_protocolWP3_protocol _.pdf_Rome_C.M.Merla_Partners and public_NA”, Zenodo, 2018.

Thank you for Listening

Any Questions