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Electro-permeabilization of isolated cancer stem cells with a push-pull configuration of high power MOSFETs

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Creo Medical / Bangor University

Introduction to the **Project**

Sumcastec

PRIFYSGOL BANGOR UNIVERSITY

Université de Limoges

ENEA
Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile

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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 **STE**m **C**ells or **SUMCASTEC**
- SUMCASTEC explores a new approach for real time isolation and neutralization of Cancer Stem Cells (CSCs). http://www.sumcastec.eu
- CSCs are associated with Glioblastoma Multiforme and Medulloblastoma.
- A project deliverable: to develop an off-chip pulsed Electric-Field (EF) generator for the Electroporation (EP) of CSCs.

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Project Deliverables

- To deliver a generator capable of Electroporation
- Generate pulse of amplitude in excess of 1 kV, with pulse widths of 100 ns to 300ns to a 50Ω load.
- Minimisation of overshoot and ringing (Flat-Top pulses)
- Investigate various pulse parameters of SUMCASTECs Pulse Generator (SPG) on CSCs. (parameters as: pulse duration, repetition frequency, number of pulses)
- CSCs placed into a cuvette with a 50ohm buffer solution
- Develop an Non-thermal EP approach

HORIZON 2020

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Background

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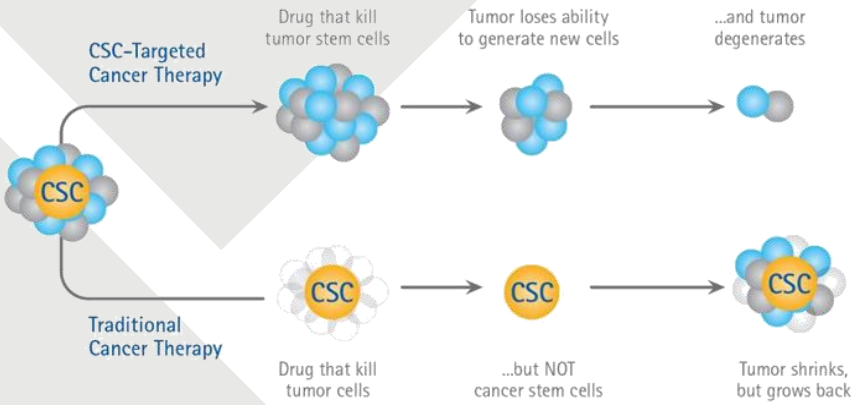
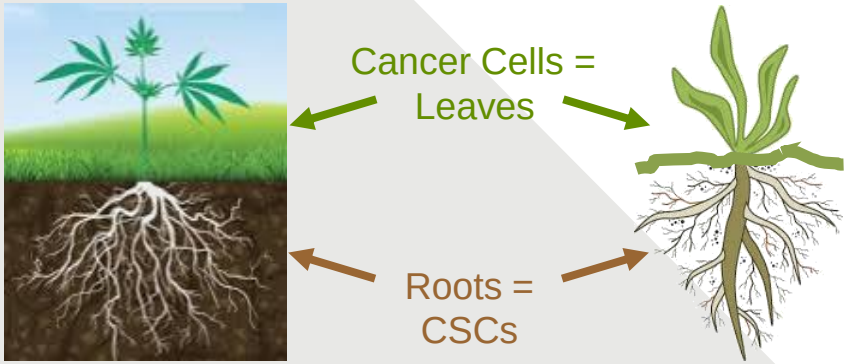
What are Cancer Stem Cells?



https://www.sigmaaldrich.com/technical-documents/articles/biofiles/the-cancer-stem-cell.html

Tumour = Weed



- Tumour growth has been linked to the presence of CSCs
- Main embodiment of a tumour with cancer cells
- Resistance to Chemotherapy (general chemotherapeutic agents) & Radiation Therapy
- Posses the ability of recovering from cytotoxic therapy and slowly repopulate the tumour, leading to relapse.

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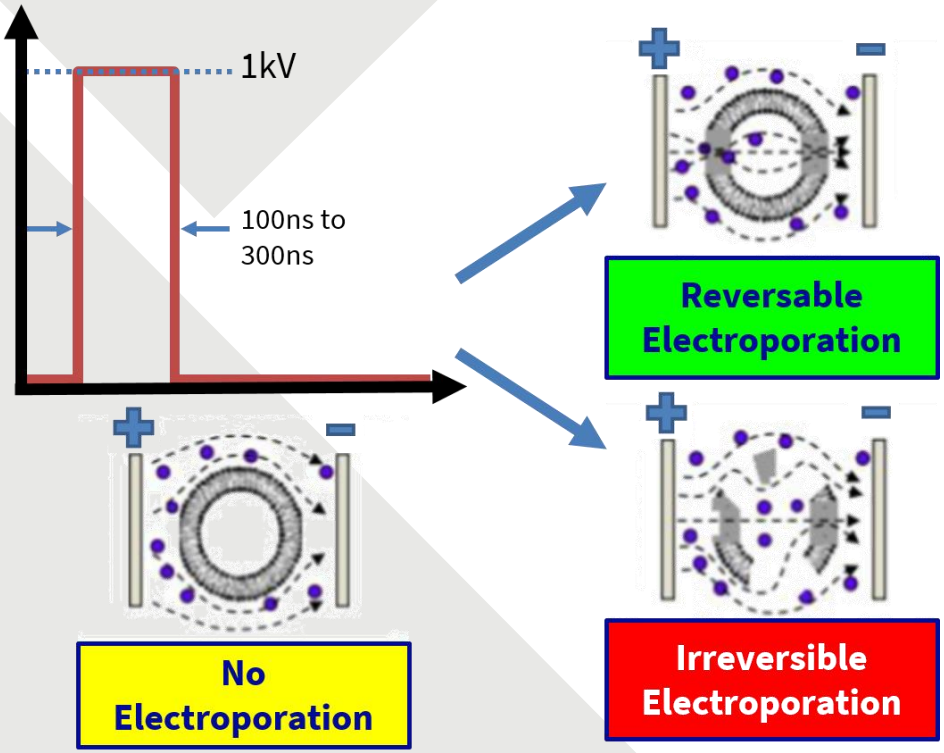
What is Electroporation?



- Alternative physical technique for non-thermal ablation
- Use precisely controlled high amplitude **pulsed electric 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

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SUMCASTEC EP Pulse Generator (SPG)

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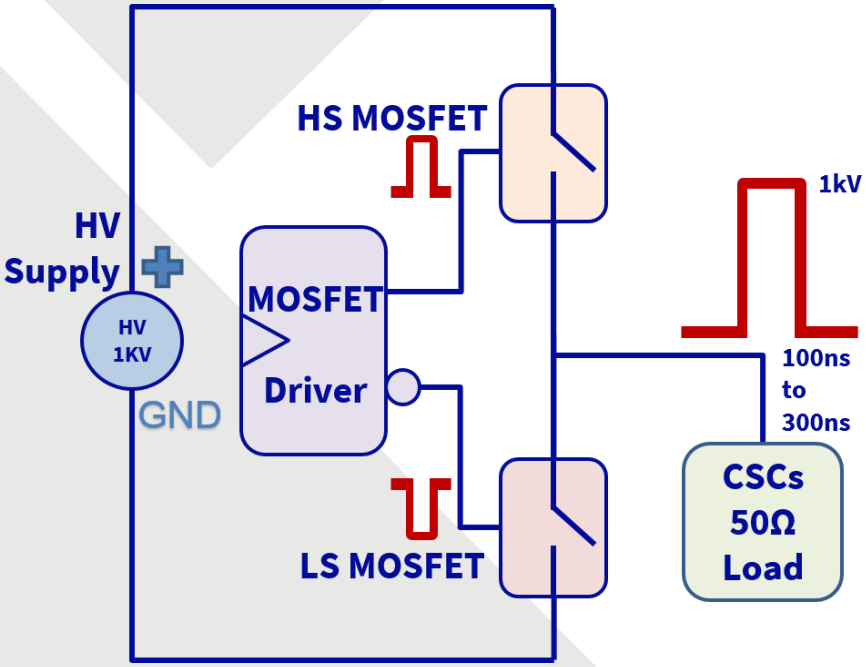
Instrumentation



- Push-pull switching of High Voltage, fast switching MOSFETs
- Driven by opto-isolators with comparable switching times
- High enough current to charge up gate-source and gate drain capacitances

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

- High-side (HS) MOSFET determine pulse width. Low-side (LS) MOSFET complimentary of High-side MOSFET

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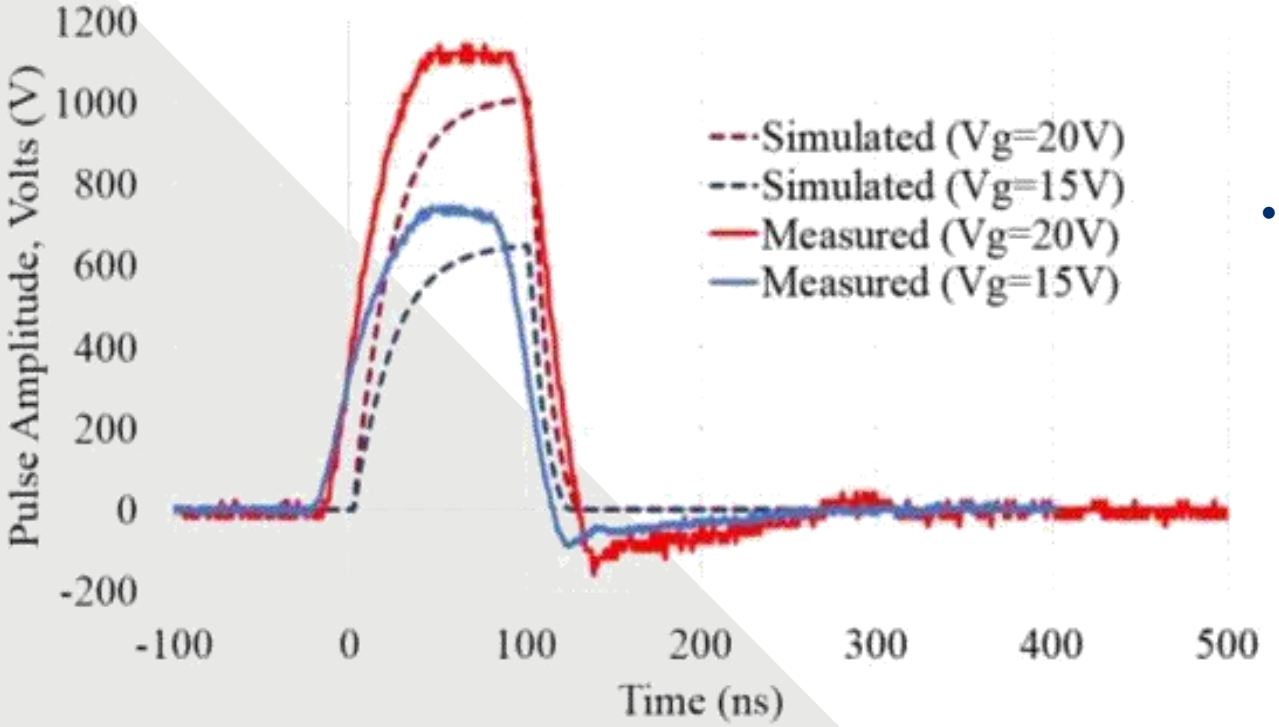
Characterization

- Flat pulses free from ringing and overshoot
- Developed EP generator performance exceeds the LTSpice simulation



- Increase of gate voltage from 15 V to 20 V results in increased pulse amplitude.
- Pulse amplitude is unaffected through-out its operating repetition frequencies (1-50 Hz)
- Optimized for pulses of 100 ns to 300 ns in width, and amplitudes in excess of 1kV

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Project Setup

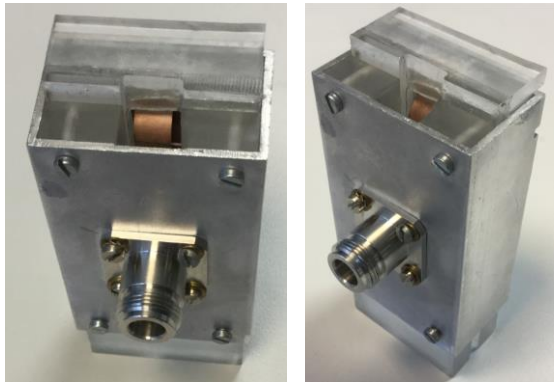
Instruments

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*

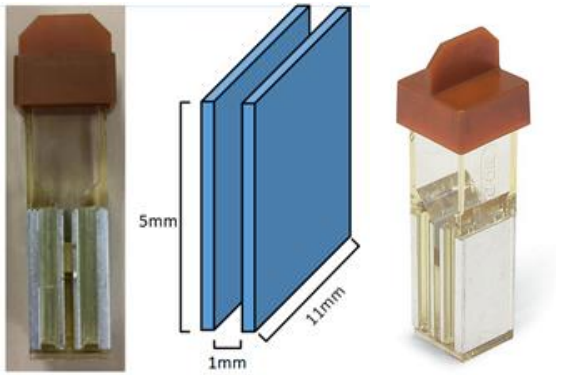


Exposes CSCs in cuvette to EP
Compatible with CSCs and standard N-connectors

Allow real time monitoring of EP HV pulse

*Designed and constructed by A. Zambotti and ENEA

ENEA 50Ω artificial buffer solution



Butten (4)PBS + H2O + Sucrose [1]
Proportions for 100 mL of 0.3 S/m (50Ω) buffer solution:
- 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

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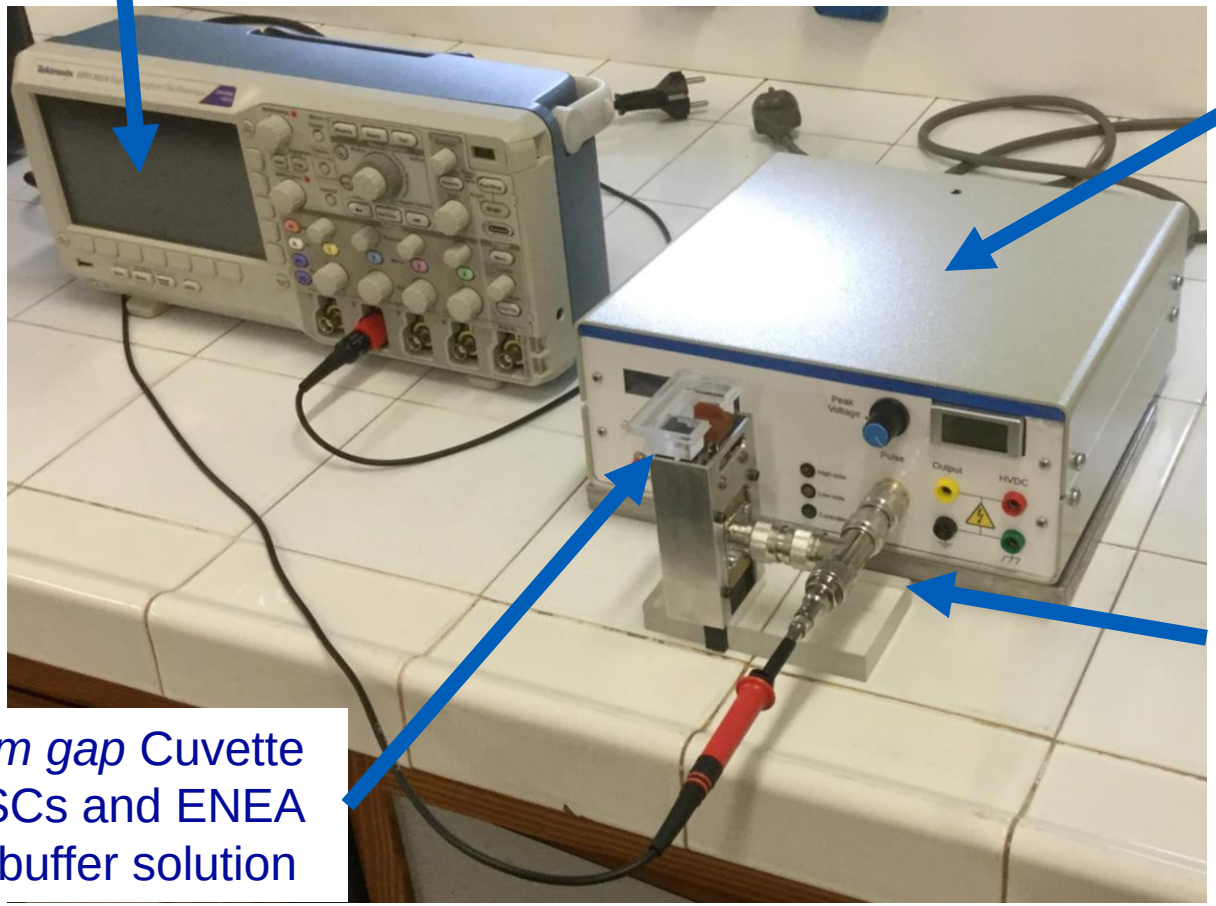
Bench Study Set-up

Dynamic monitoring of pulse during cell EP or IRE



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

ENEA Cuvette Housing Unit

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

Results and **Analysis**

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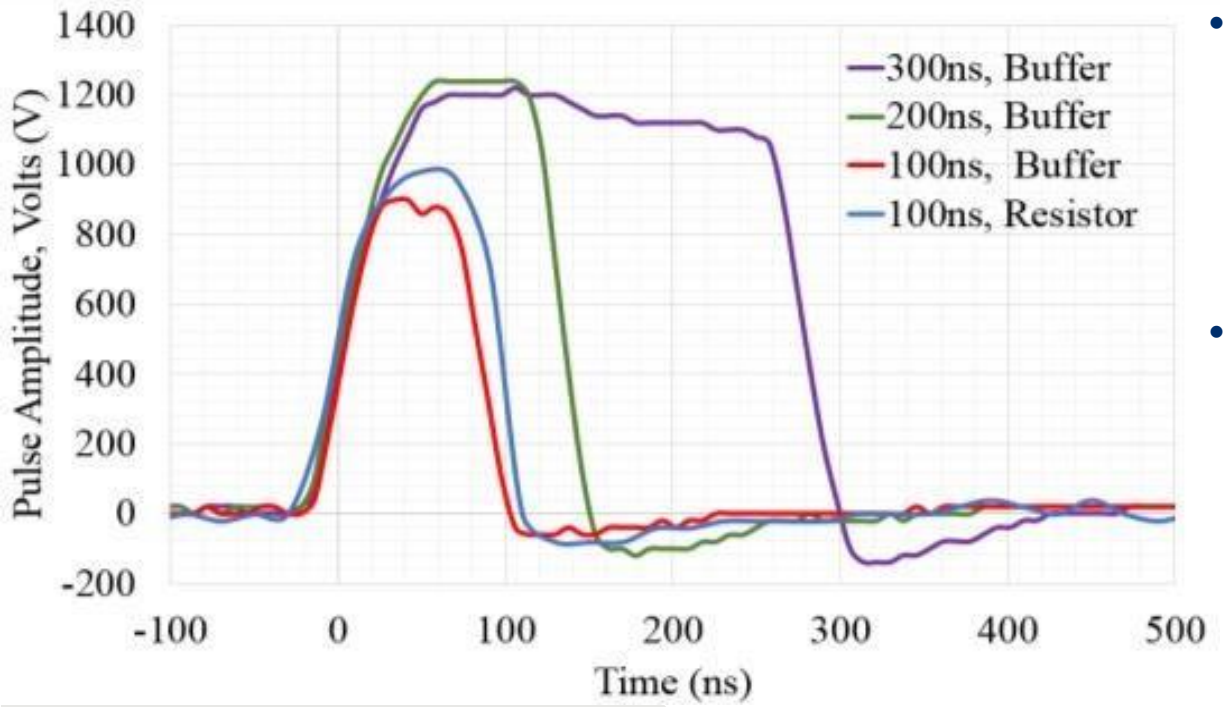
Pulsed Electric-Field on CSC



- Al 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

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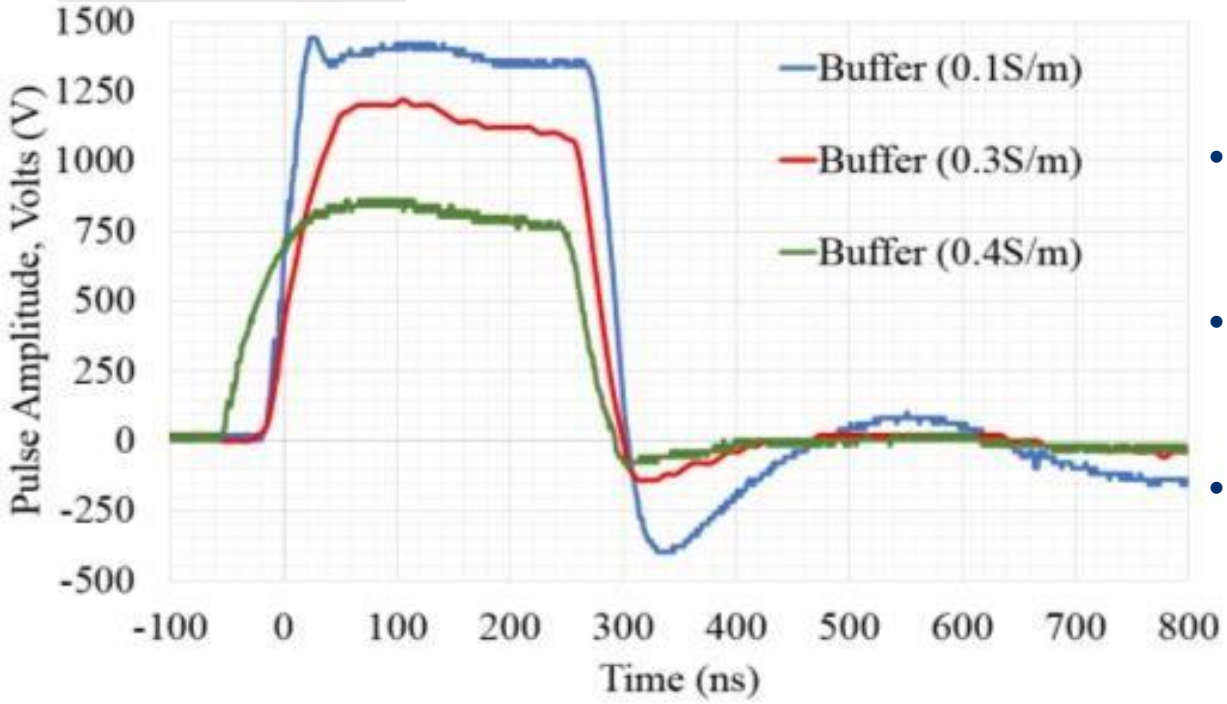
Pulsed Electric-Field on CSC



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

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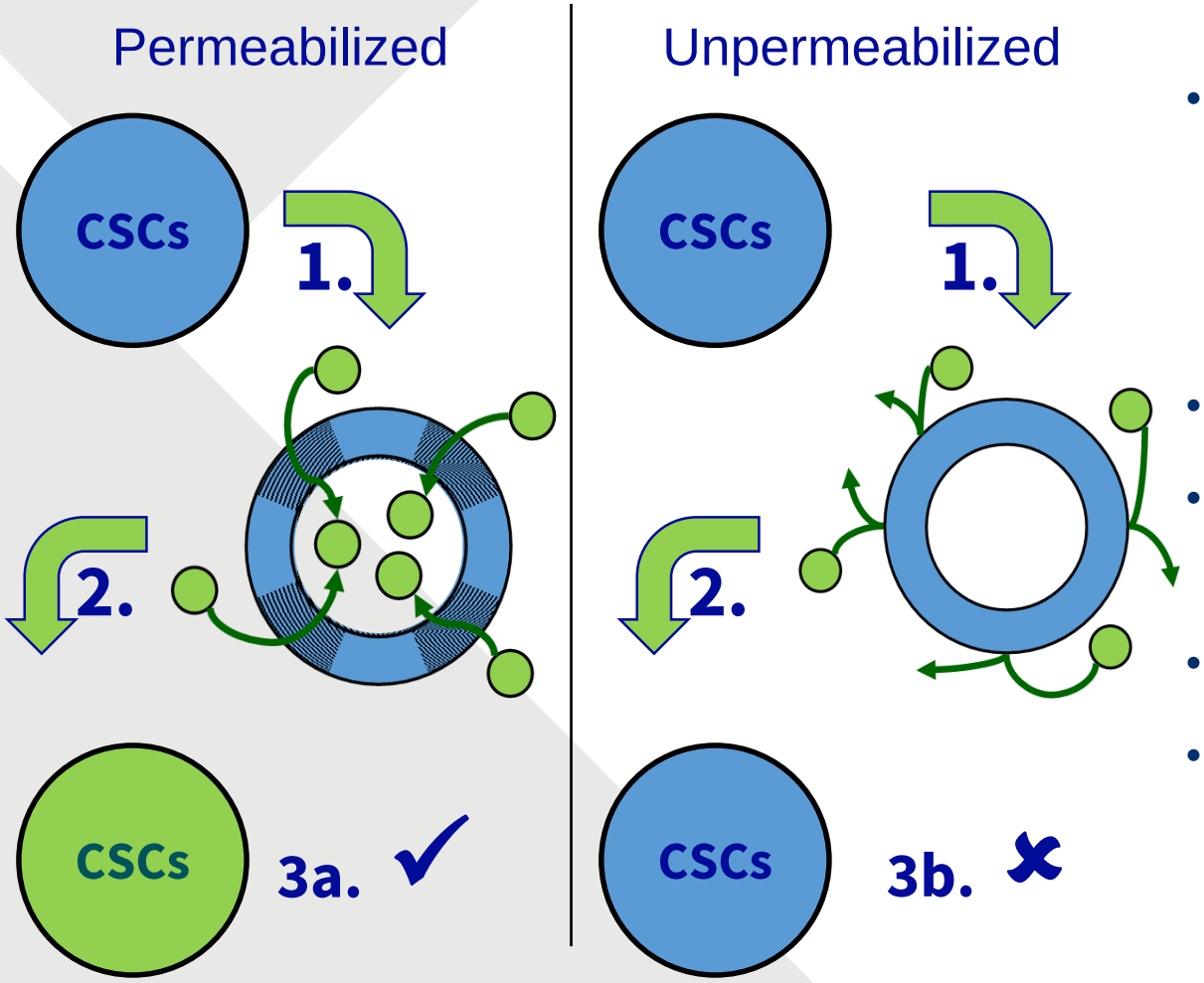
Permeabilization Test on CSCs



- 1. CSCs placed in buffer solution with YOPRO-1, a fluorescent dye (green in colour)
- 2. CSCs exposed to EP
- 3. Exam CSCs under fluorescent light:
- a. Permeabilized CSCs green
- b. Unpermeabilized: CSCs transparent

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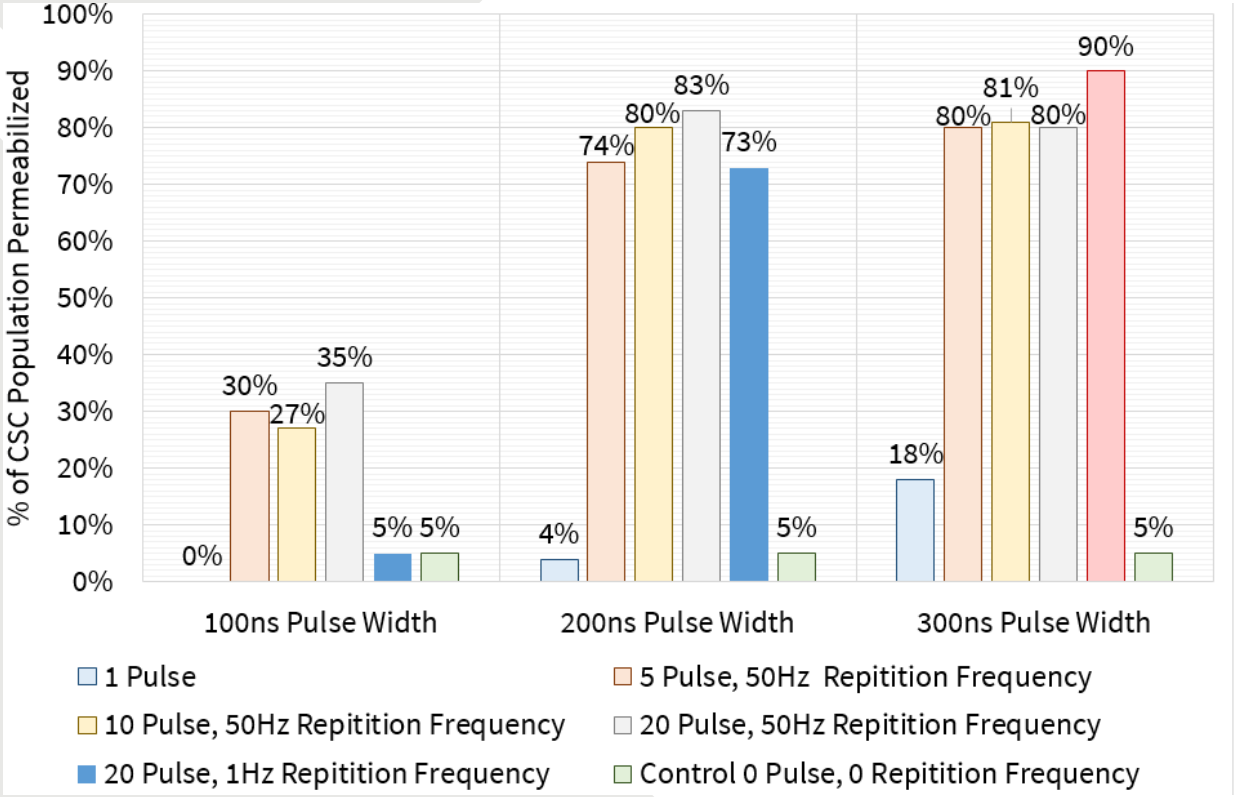
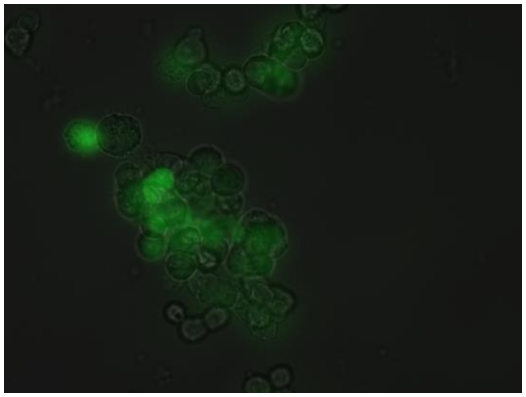
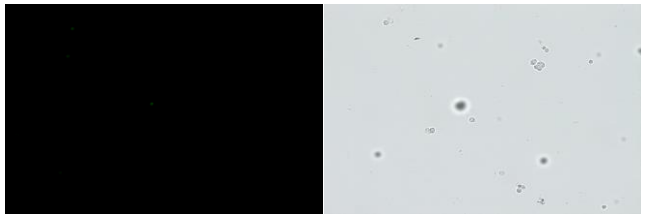
Permeabilization Test Result





300 ns, 1.2 MV/m, 20 Pulses
1 Hz = 90 % permeabilized



100 ns, 1.0 MV/m, 20 Pulses
0% permeabilized

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Non-thermal Effect

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

Non-thermal effect. of 1.0x10^{-3} $^{\circ}C$ (100 $\mu^{o}C$)

- D is duty cycle (ratio)
- E is energy (J)
- C is heat coefficient, 4.18 J/g/^{o}C, same as water
- L (indicating volume) is millilitres (the cuvette can hold 0.1 mL of solution)

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

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Discussion and **Conclusion**

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Project Deliverables

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

- 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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Referneces:

[1] C. Merla et al. "SUMCASTEC_180123_NA_protocolWP3_protocol_.pdf_Rome_C.M.Merla_Partners and public_NA", Zenodo, 2018.

For more information visit the SUMCASTEC website:

www.sumcastec.eu

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Our project SUMCASTEC
was made possible
thanks to #H2020 funding
€30 billion is still
available in the 2018-20
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