Semiconductor-based Ultrawideband Micromanipulation Of Cancer Stem Cells

Dielectric characterization of brain cancer cell lines

Wesam Gamal
Bangor University

The SUMCASTEC project receives funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement N° 737164

Outline

1- Dielectric characterisation of brain cancer cells

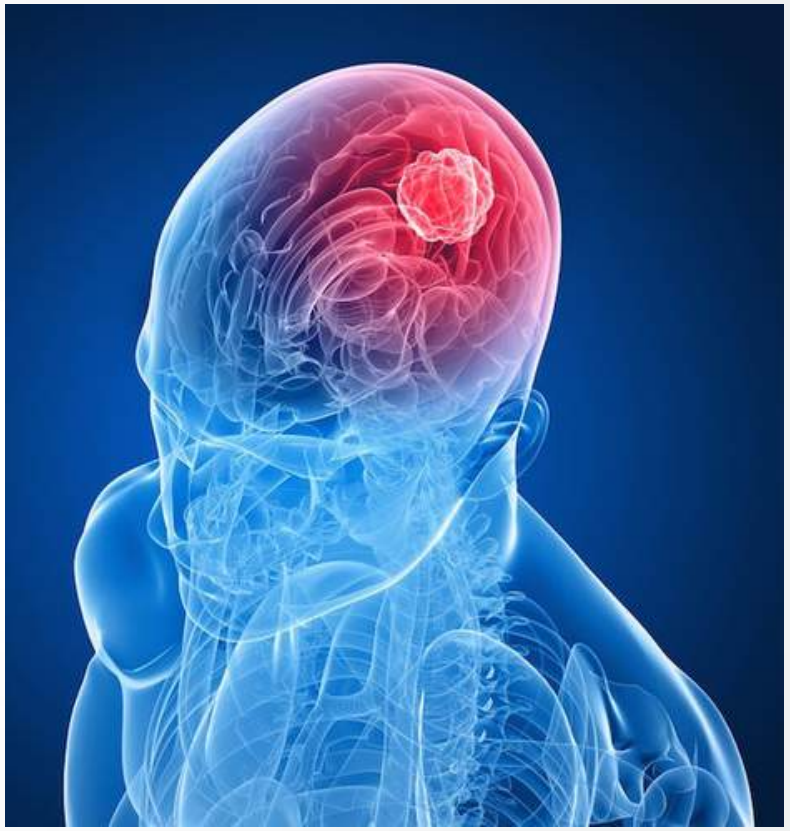
- ❑ Glioblastoma and Medulloblastoma
- ❑ Cancer stem cells (CSCs)
- ❑ A need for CSCs dielectric model
- ❑ A novel dielectric study of brain CSCs
- ❑ Work in progress

2- On-chip EF exposure

- ❑ Different methods for CSC neutralisation
- ❑ Mesoscale CPW test structures
- ❑ EF exposure and membrane permeability
- ❑ Work in progress

Glioblastoma and Medulloblastoma brain Cancers

- A leading cause of death in Europe with poor survival rate.
- High recurrence rate.
- Strong resistance to conventional cancer therapies.
- A possible role for CSCs?

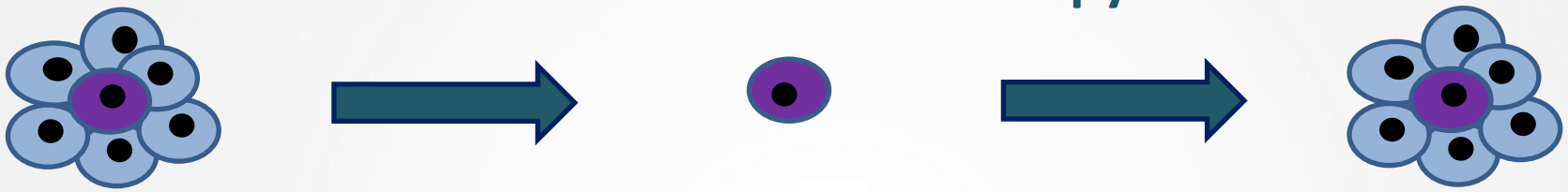


Cancer Stem Cells: A role in tumor recurrence

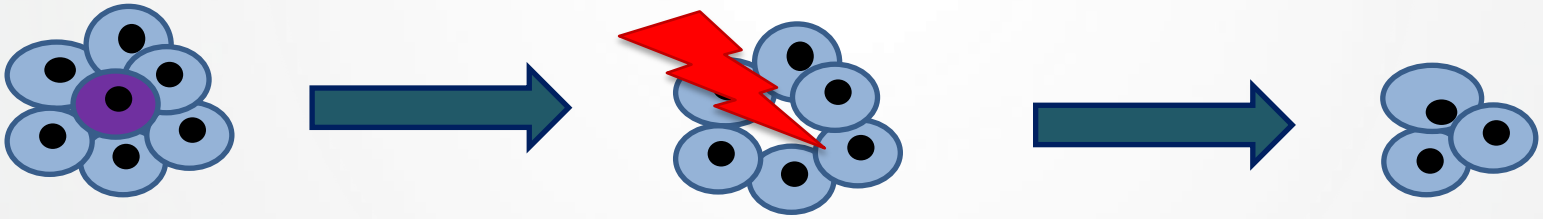
- Have been identified in various solid tumors including glioblastoma, melanoma, ovarian, gastric and lung cancers.
- Stem-like properties such as self-renewal, differentiation and their ability to migrate are believed to play a role in tumour initiation, invasion and recurrence.
- Drug resistance is behind the failure of conventional cancer therapies in many cases.

Cancer stem cells: the need for a tailored therapy

Conventional cancer therapy



Cancer stem cell tailored therapy



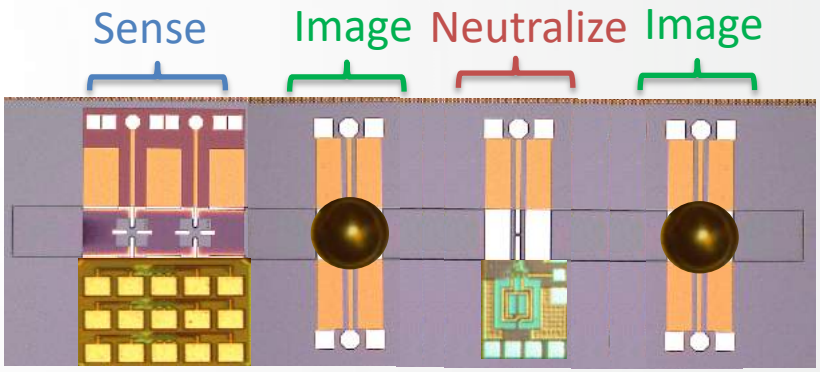
Semiconductor-based Ultrawideband Micromanipulation of CAncer STEm Cells (SUMCASTEC)

- We are developing the world's first CMOS-based micro-optofluidic lab-on-chip platform enabling

i. CSCs **isolation** via electromagnetic (EM) sensing.

ii. Nanoscale **imaging.**

iii. CSCs selective **neutralization** via EM radiations.



A need for CSCs dielectric model

- An accurate dielectric model of CSCs is an essential requirement for identifying cells physical properties and arriving at a reliable estimation of the electromagnetic (EM) field distribution within a single cell and small cell clusters.

- Studies characterizing the dielectric properties of abnormal tissues is however very limited, specially for brain cancer cells.

- A study by D. Yoo[1] on dielectric properties of cancerous tissues. Cancers were cultivated in mice applying the xenograft model of growing human cancerous tissues.

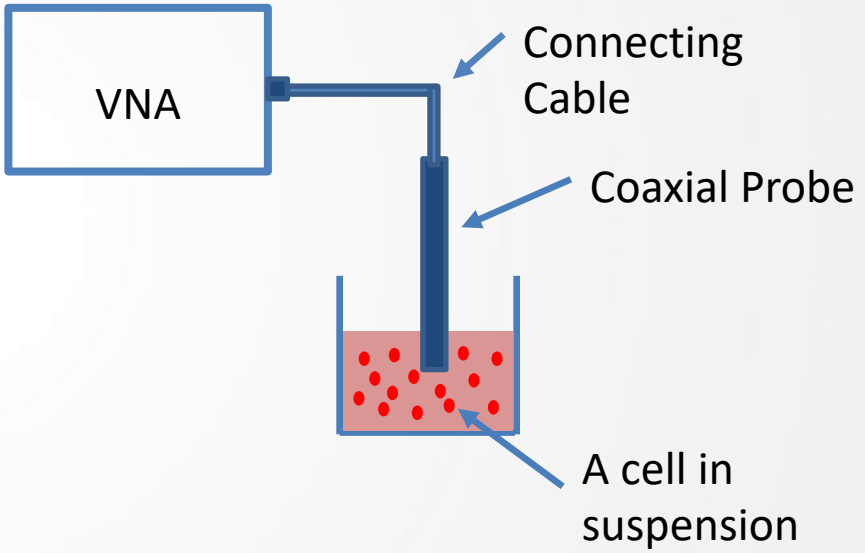
- There are no dielectric models of human brain CSCs due to the difficulty in their isolation and culture.

[1] Bioelectromagnetics25:492-497(2004)

A novel dielectric study of human brain cancer cells

- The dielectric properties of human glioblastoma (U87) and medulloblastoma (D283) cell lines with a relatively pure population of brain cancer stem cells (CSCs) were measured in the frequency range of 500 MHz to 3 GHz.

- The complex permittivity was measured using an open-ended coaxial probe (Keysight Technology 85070E dielectric probe kit) and a Keysight network analyser.



Cell electrical parameter assessment

Experimental Conditions

- Buffer (PBS+H2O+ sucrose)
- MEM
- DMEM
- D283 in MEM (5, 10, 20 mln)
- D283 in buffer (5, 10, 20 mln)
- U87 in DMEM (5, 10, 20 mln)
- U87 in buffer (5, 10, 20 mln)

Merla et al., TMTT 58:3, 2010; Denzi et al., TBME 62:6, 2015

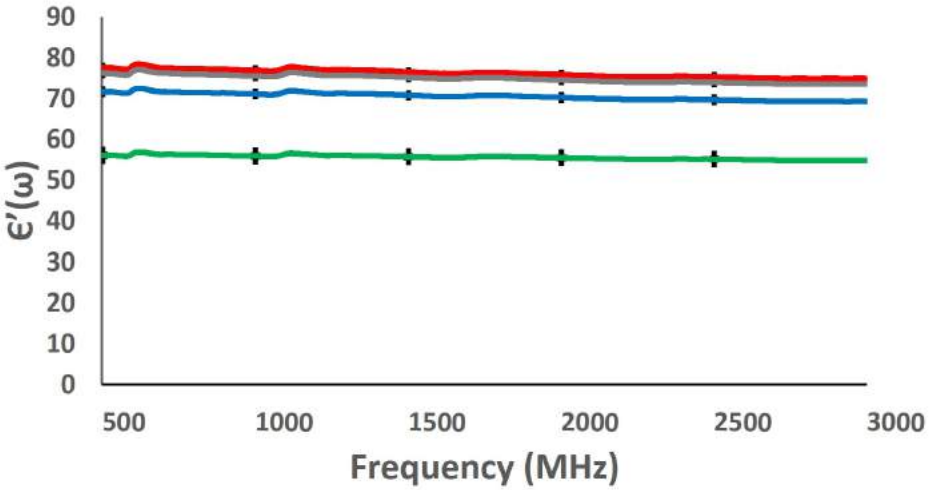
Computing average of **real and imaginary parts** (AVGs)
- 3 independent experiments
- Each experiment has 5 repetitions
- Total file averaged for each condition=15
- Comparison of AGV for the different concentrations

Fitting of AVGs using inverse EMT
- 15 different fitting for real and imaginary parts
- Standard deviation of fitted parameters

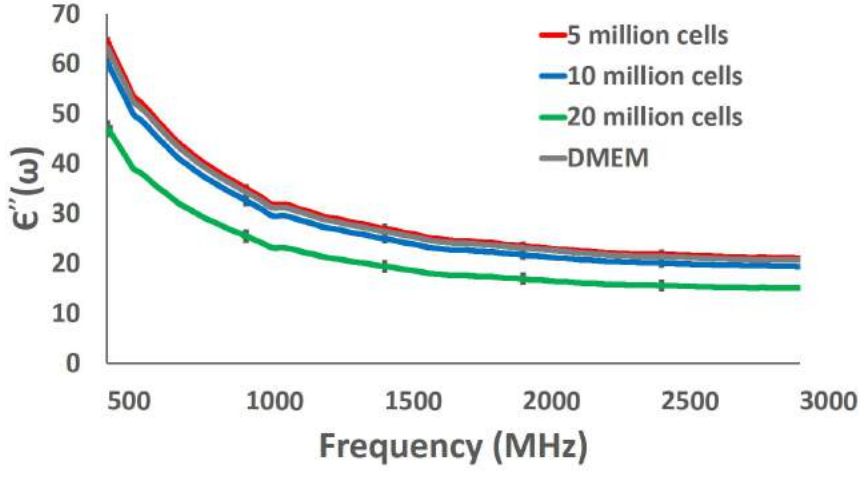
Evaluation of statistical significant differences for comparable conditions
- Fit AVG(medium), Fit AVG(20 mln), FitAVG(10mln), FitAVG(5mln) $\Rightarrow$ p for each set of assessed parameters

Maximum Likelihood minimization of all fitted parameters (μ and σ^2)
- Weighted mean value and standard deviation of weighted mean

Permittivity measurements of Glioblastoma cell line (U87)

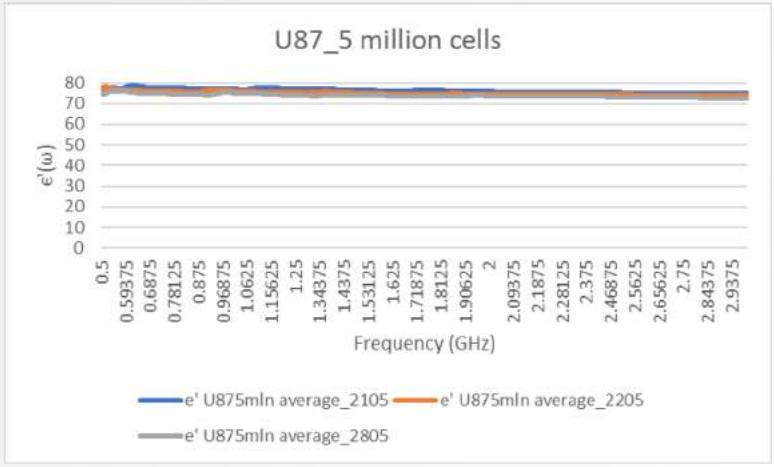


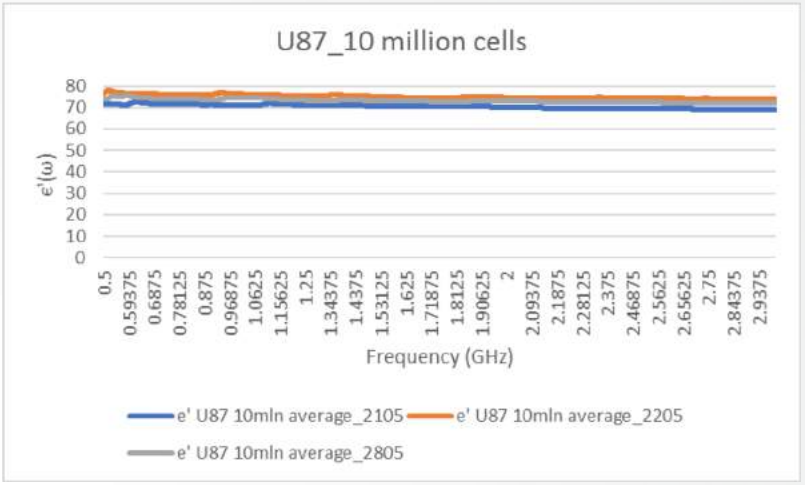
(a)

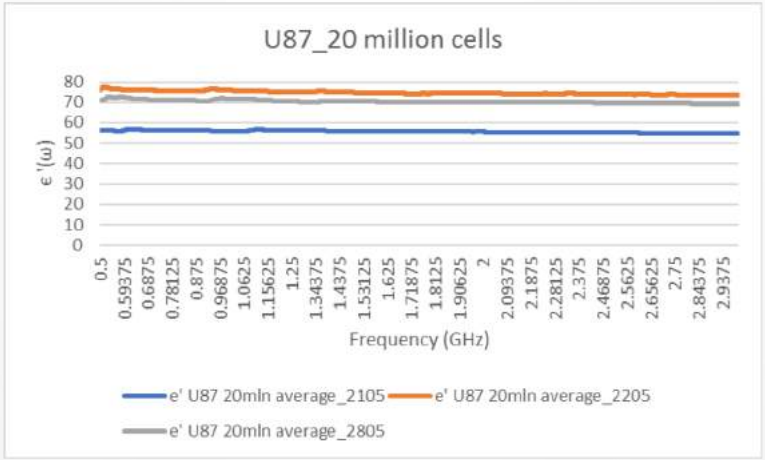


(b)

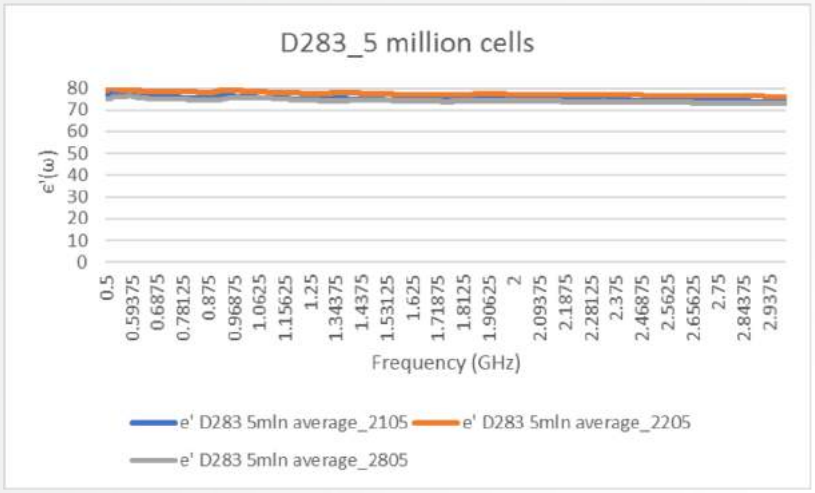
Measurement Repeatability for Glioblastoma cell line (U87)

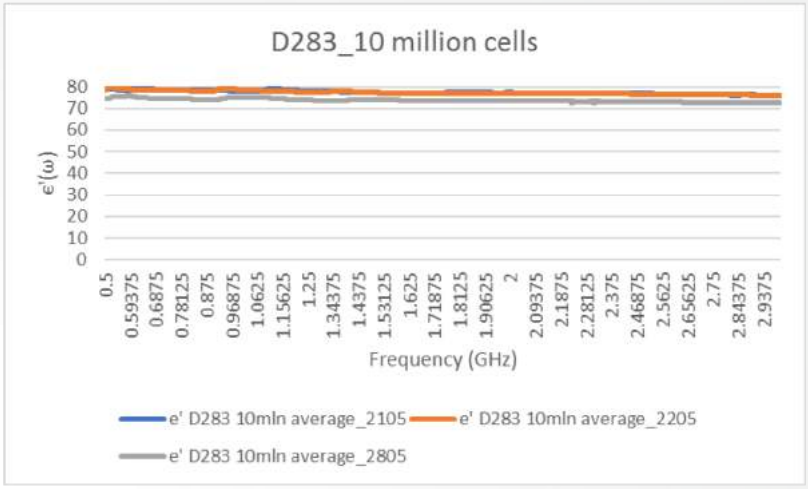


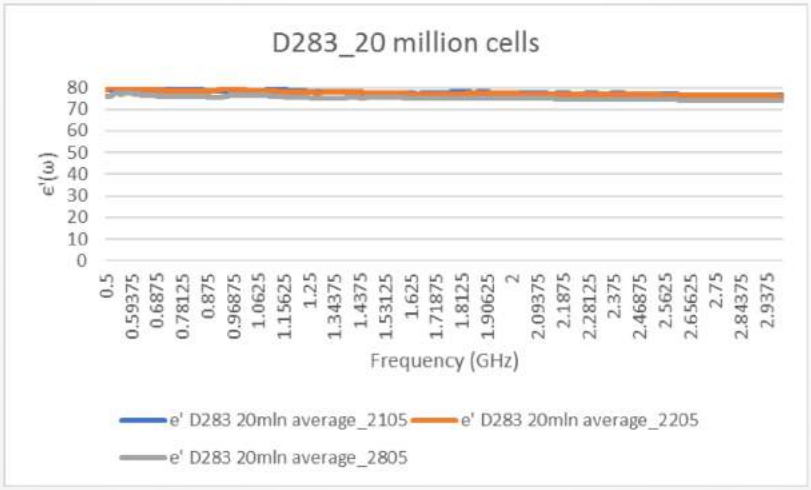




Measurement Repeatability for Medulloblastoma cell line (D283)

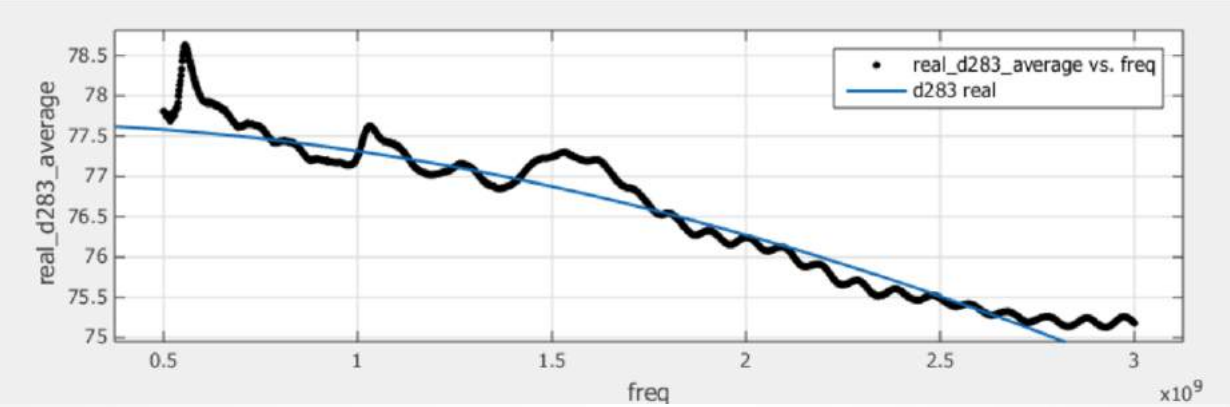


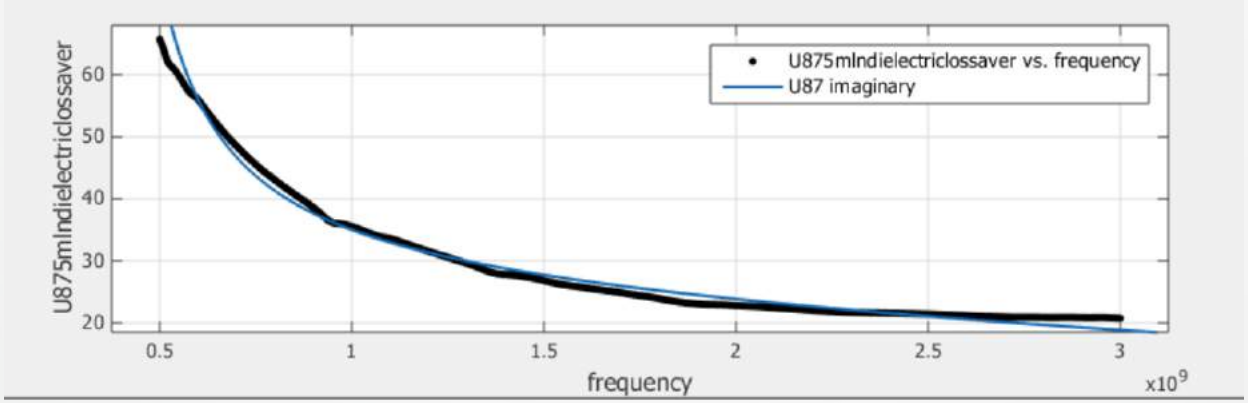




Work In Progress

- Experiment repeats.
- Curve fitting and extraction of cell parameters (Effective Medium Theory).
- Statistical analysis.

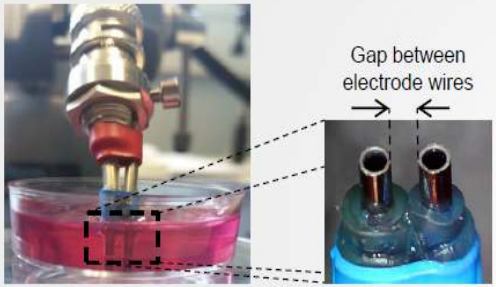




On-chip EF Exposure

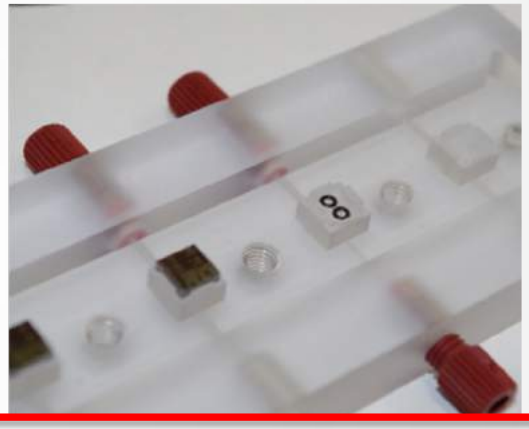
On-chip neutralization of brain cancer stem cells

Method 1: Wire Electrodes

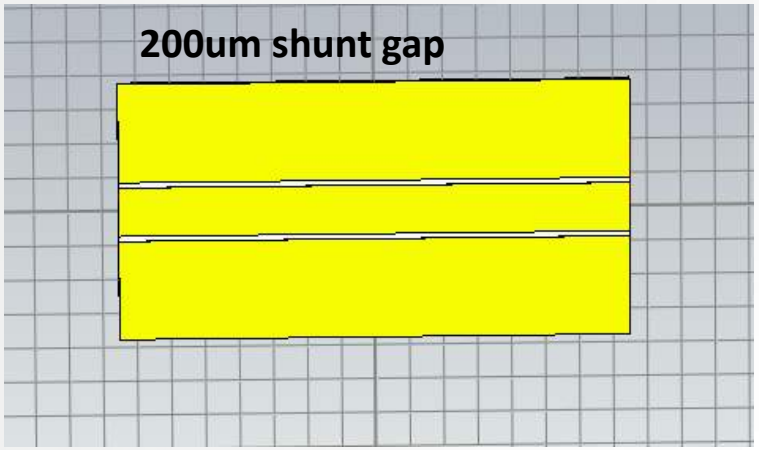


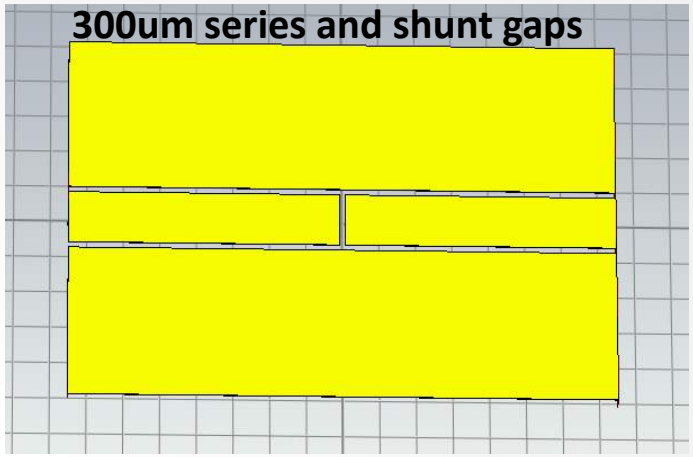
Method 2: Electroporation Cuvette

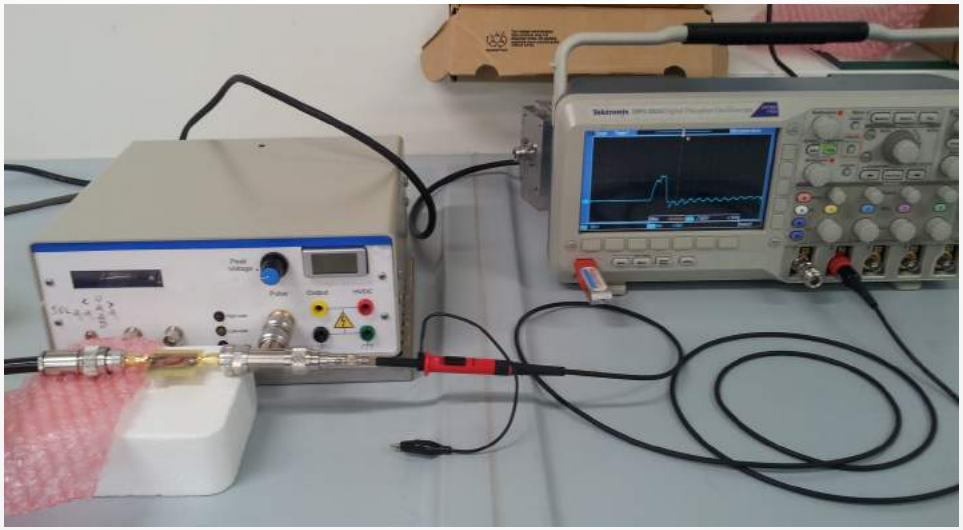
Method 3: On-chip EF exposure



Mesoscale CPW test structures







EF Exposure and membrane permeability

- 35 um copper Shunt and series CPW structures were fabricated in Bangor on FR-4 and connected to the Creo generator to expose cells to 8 pulses of 200ns, 1Kv, 1Hz.

- Cells were cultured on cover slips which were put on top of the CPW structure for EF exposure.

- Cell lines exposed were:

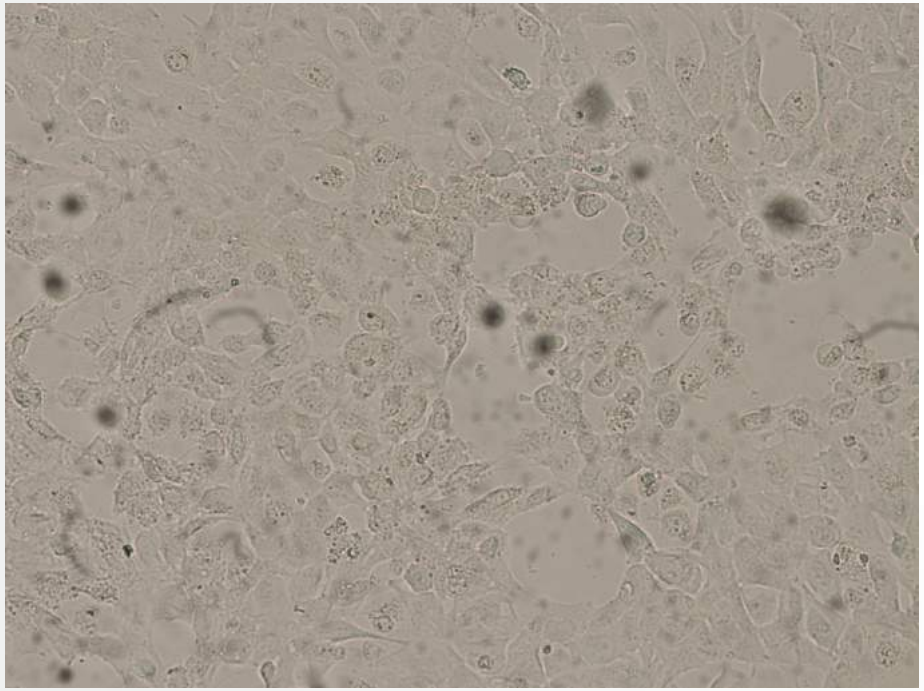
1- Daoy (medulloblastoma cell line).

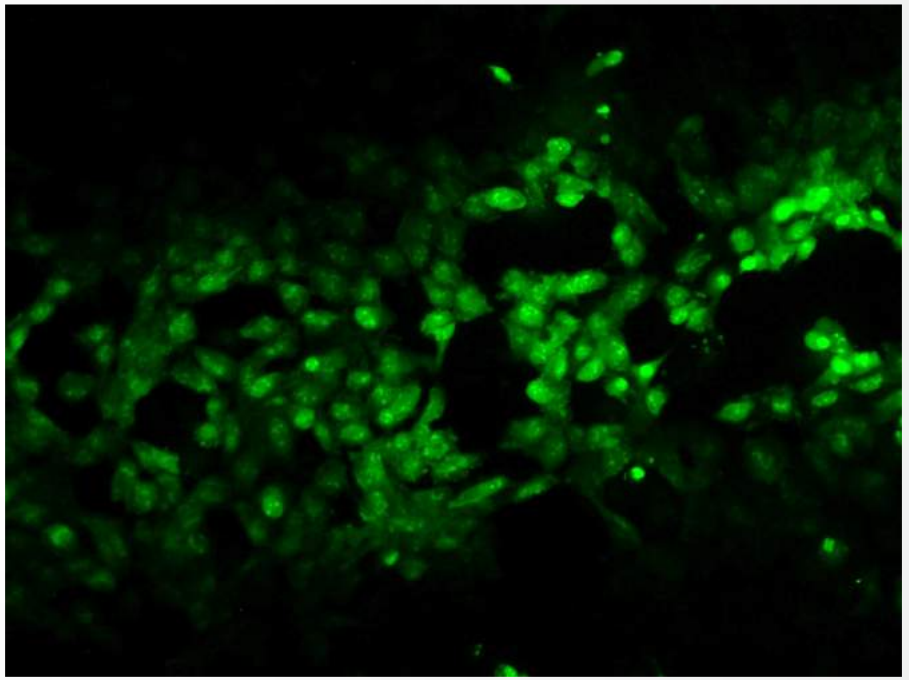
2- D283 (Medulloblastoma cells, 95% stem cells).

3- U87 (glioblastoma cells).

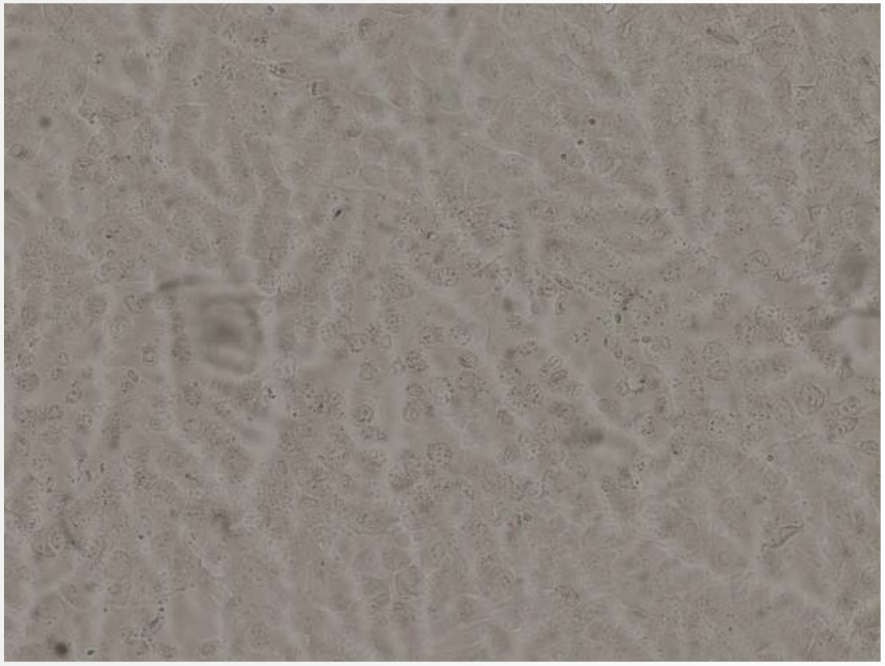
- Yo-pro, a green dye, was used to check the permeability of the membrane after pulse application.
- Cells were fixed and mounted on a glass slide using a mounting gel with DAPI (to stain cell nuclei blue).

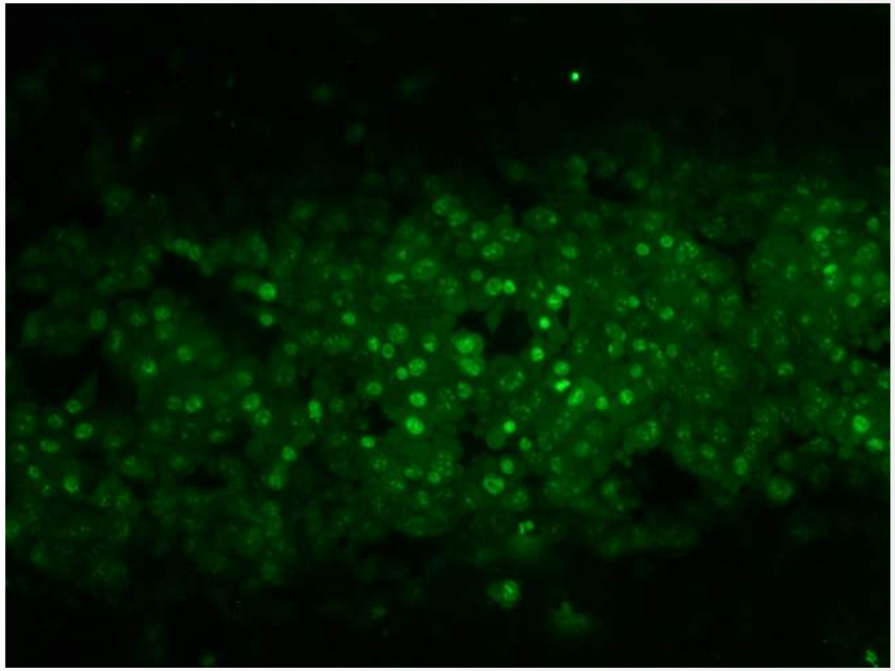
Daoy, 200ns, 8 pulses, 1kV



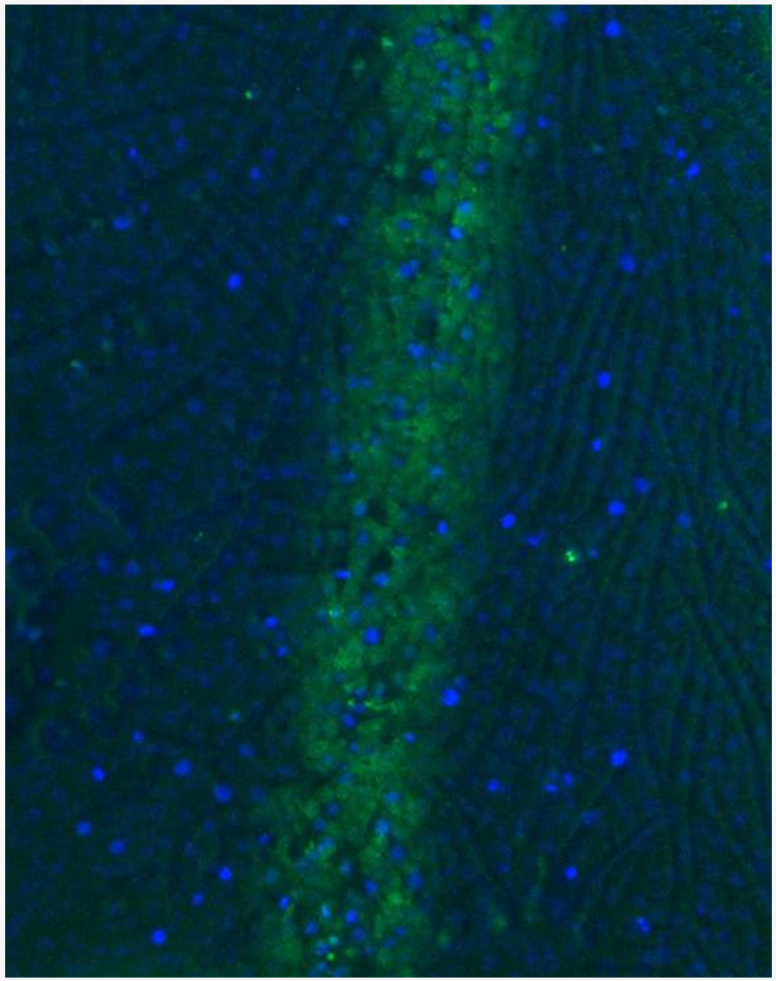


Daoy, 200ns, 8 pulses, 1 kV

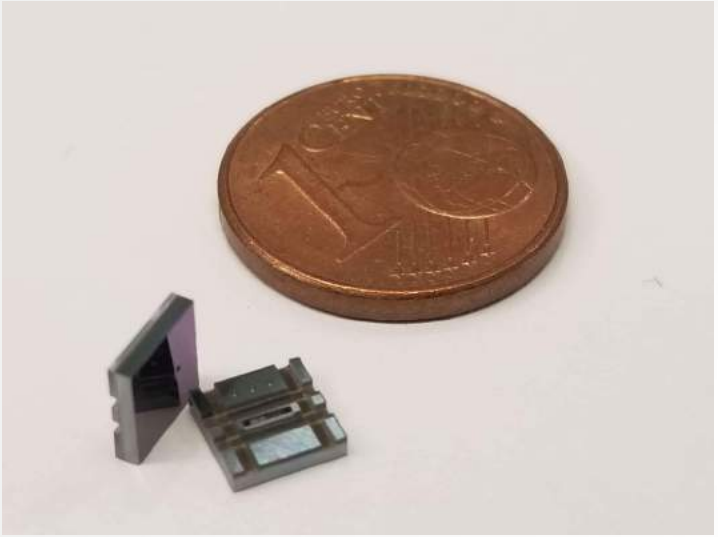


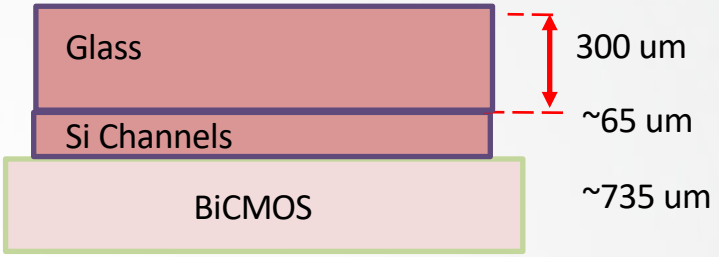


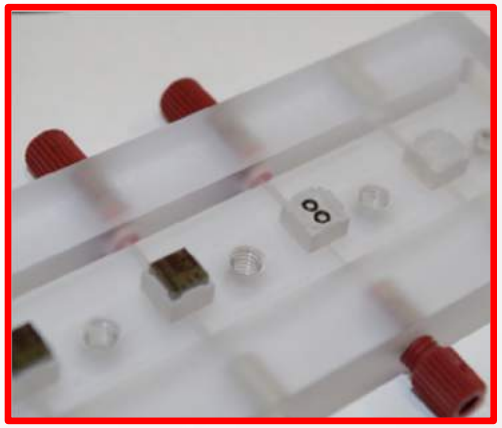
Daoy, 200ns, 8 pulses, 1kV, fixed in 4% PFA



Work in Progress
Moving from meso- to micro- scale







Acknowledgment

ENEA, Italy
- Caterina Merla
- Rosanna Pinto
- Mirella Tanori
- Arianna Casciati
- Mariateresa Mancuso

Bangor University, UK
- Cristiano Palego
- Jake shearwood

Creo Medical, UK
- Ilan Davies
- Cris Hancock
- George Hodgkins

Padova University, Italy
- Luca Persano
- Giampietro Viola

University of Limoges, France
- Sofiane Saada
- Barbara Bessette
- Fabrice Lalloue
- Arnaud Pothier

IHP, Germany
- Mesut Inac
- Cannan Baristiran-Kaynak
- Mehmet Kaynak

Funding
- European Union's Horizon 2020 research and innovation programme
- Sêr Cymru National Research Network in Advanced Engineering and Materials

Thank you


Our project SUMCASTEC
was made possible
thanks to #H2020 funding
€30 billion is still
available in the 2018-20
Work Programme!
#InvestEUresearch
European
Commission
www.sumcastec.eu