Dielectric parameters extraction and modelling of brain cancer stem-like cells

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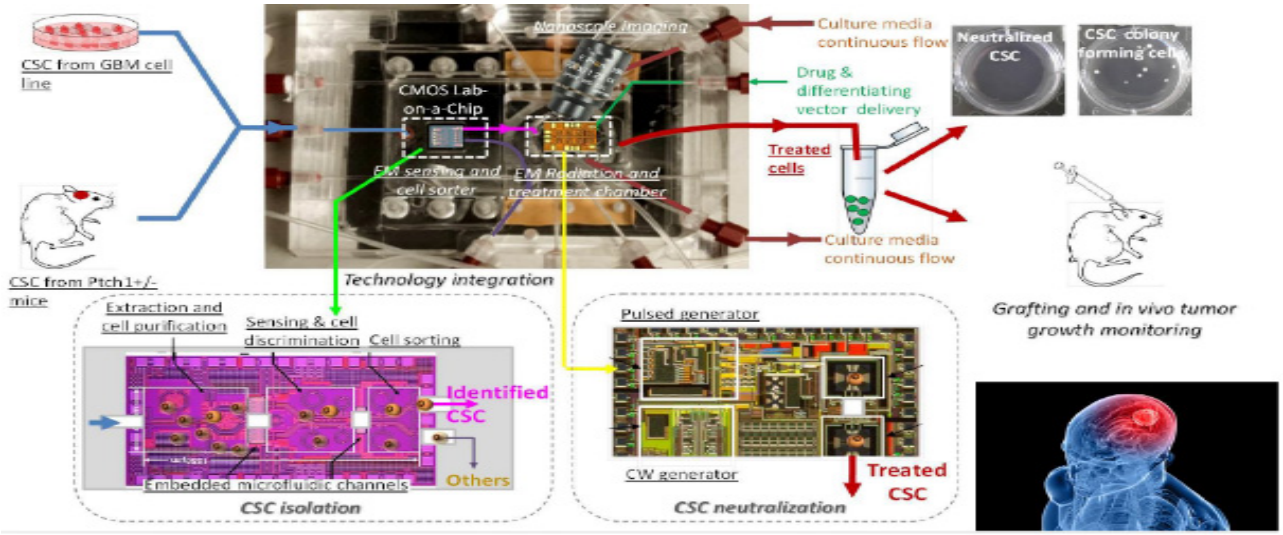
Outline

- Context and application
- Dielectric parameters extraction
  - Experimental setup and statistical approach
  - CSC content for tested cell types
  - Complex permittivity measurement results
- Compact circuit modeling approach
  - Motivation
  - Implementation approach
  - Observations
- Conclusions

Semiconductor based Micromanipulation of Cancer Stem Cells (CSCs)



EM based on-chip:

- ❑ discrimination
- ❑ neutralization
- ❑ imaging of ***CSCs***

Target: brain tumors

- *Glioblastoma Multiforme*
- *Medulloblastoma*

Sumcastec

Université de Limoges

Università degli Studi di Padova

Prifysgol Bangor University

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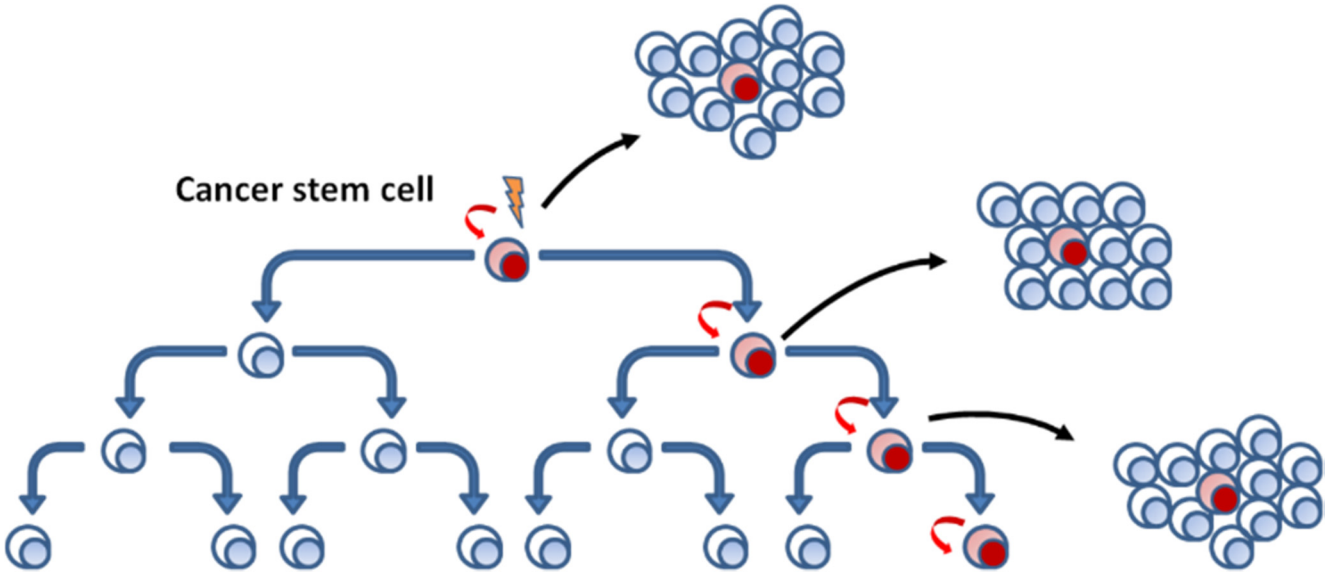
HORIZON 2020

CSCs

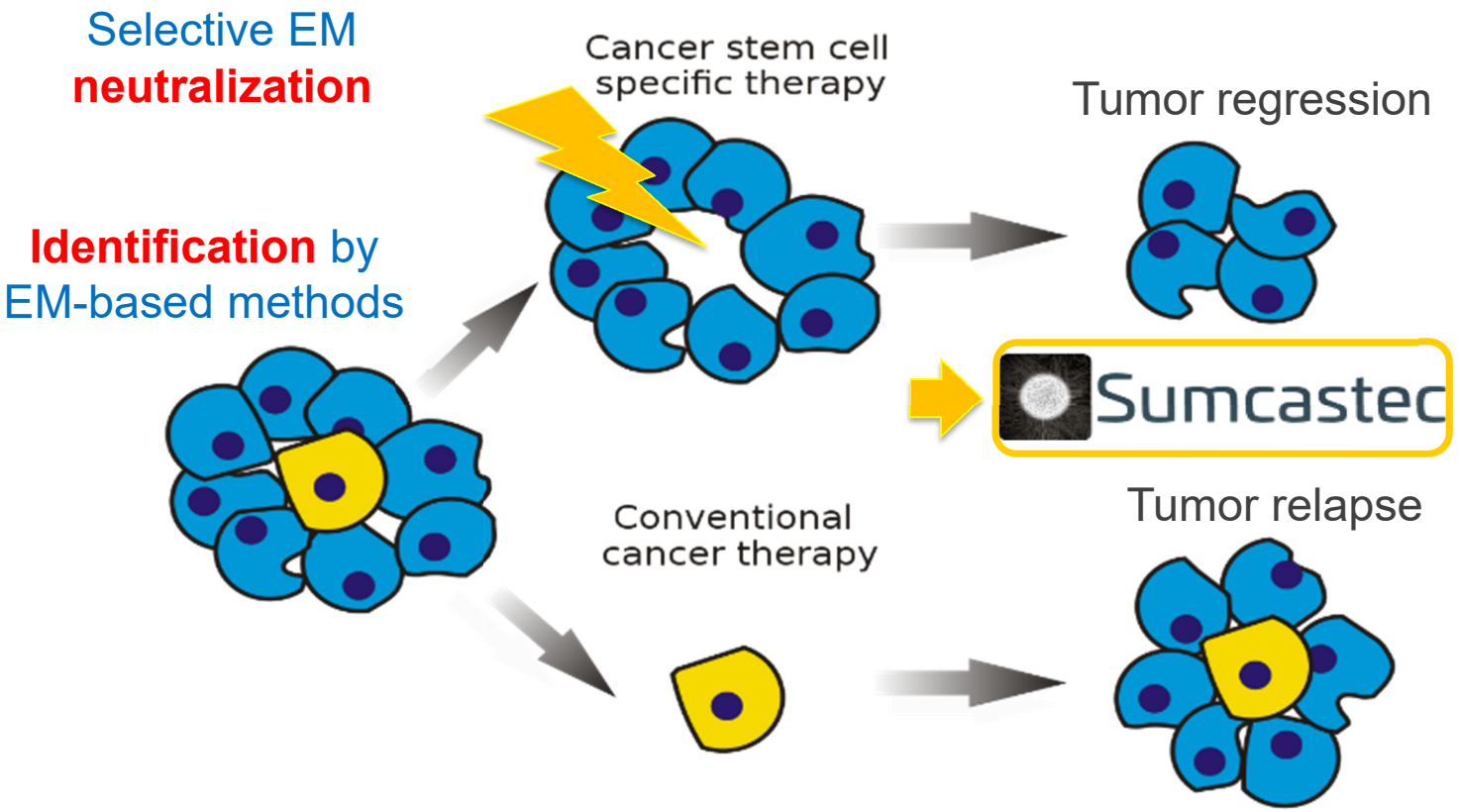
Tumorigenic cells with ability to give rise to all tumor cell type:

- with self-renewal capabilities
- differentiation into multiple cell types (progenitors…)
- hypothesized to be the main cause of relapse and metastasis



Quiescent properties -> Resistant to conventional chemo and ionizing treatments

New therapies targeting CSCs



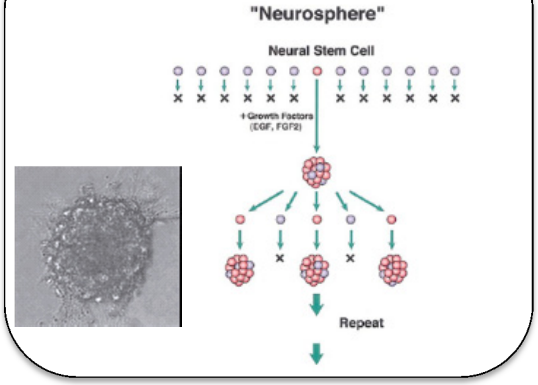
How identify CSCs?

Specific immunostaining markers are lacking:

Stemness lineament can be accessed with commonly used markers for stem cells (anti-Nanog, anti-Sox2, anti-OCT4 anti-CD133…) without 100% absolute certainty

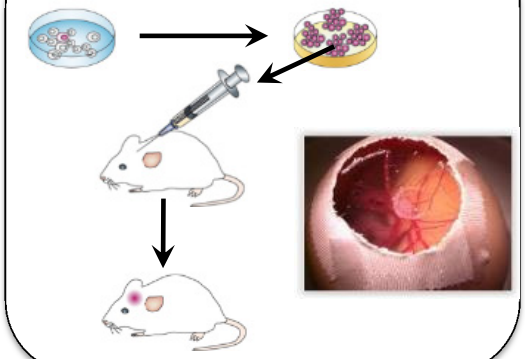
Others identification approaches available

Clonogenicity tests

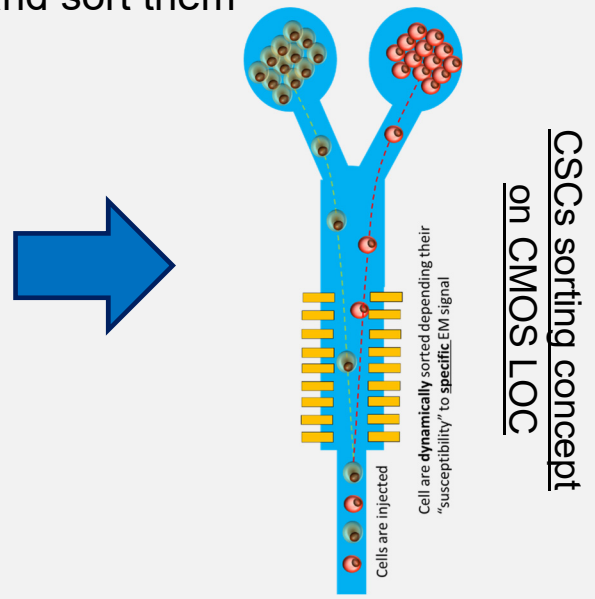


Long (~40days) and complex tests

Tumorigenicity tests



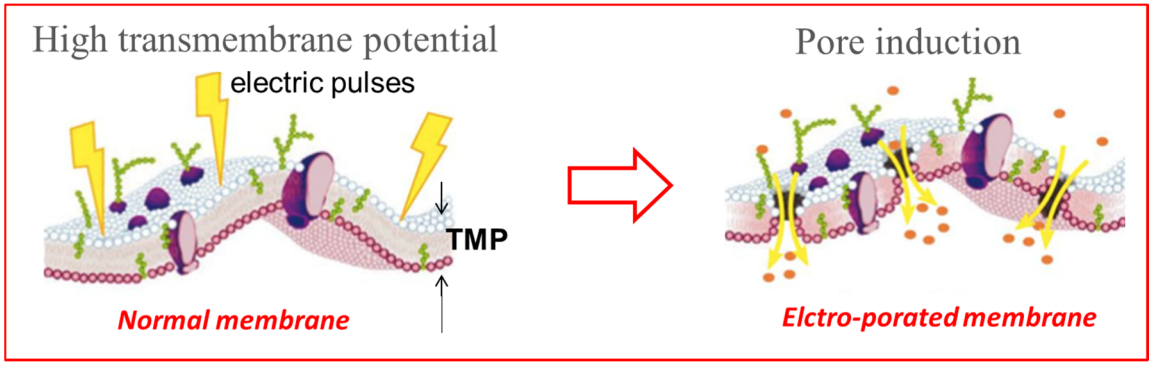
Alternative: Discriminate cells on their EM signature, recognize CSC and sort them



EM fields for cell neutralization:

µsPEF ⇒ charge displacement
⇒ main target : plasma membrane
nsPEF ⇒ charge displacement + dipole orientation
⇒ main target : organelle membranes and plasma membrane

- Non thermal effects on cells
- Observed effects are repeatable and stable among different labs
- Primary effects implies transient structural alteration of cell membranes
- Secondary effects elicited modulation of different cell functions (e.g. viability, proliferation, apoptosis, differentiation, activation of stress pathways, Ca release)



Tanori et al., Red J.
On line 2020

Why cell modelling

Experimental drive hypothesis

Experimental activity → Models to interpret and support experiments

Theoretical driven hypothesis

Models as prediction tools → for targeted experimental activity

How perform cell modelling

⇒ Dielectric properties of cells in particular CSCs assessed by:
⇒ **indirect methods**
⇒ **single cell measurements**

⇒ EM solution

Coupled to biophysical models

⇒ Electroporation
⇒ Neurodynamics
⇒ Thermal
⇒ Transport

Dielectric properties of CSCs

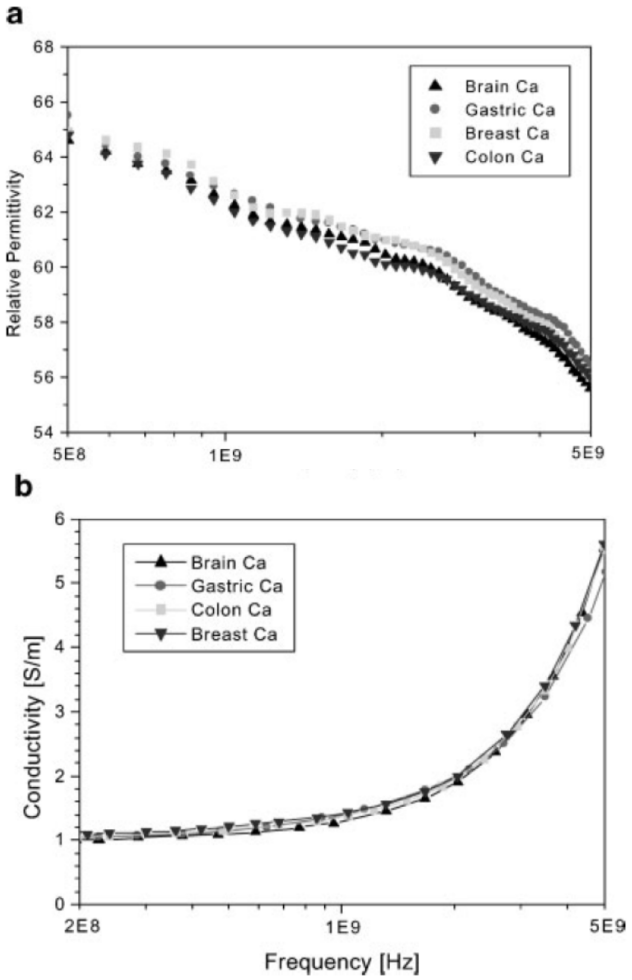
Bioelectromagnetics 25:492–497 (2004)

The Dielectric Properties of Cancerous Tissues in a Nude Mouse Xenograft Model

Done-Sik Yoo*

⇒ Lack of complex permittivity measurements on cancer tissues/cells

⇒ **Absence of complex permittivity measurements on CSCs**



Cell electrical parameter assessment

Experimental Conditions

- MEM
- DMEM
- **D283 in MEM** (5, 10, 20 mln)
- **U87 in DMEM** (5, 10, 20 mln)

Combining Re and Im parts

Evaluation of statistical significant differences of final parameters for Re and Im parts

Maximum Likelihood minimization of fitted parameters (μ and σ^2) for Im and Re parts on the basis of evaluated p

Repeat process for imaginary parts

Maximum Likelihood minimization of fitted parameters (μ and σ^2) on the basis of p
- Averaging only parameters which are statistically different

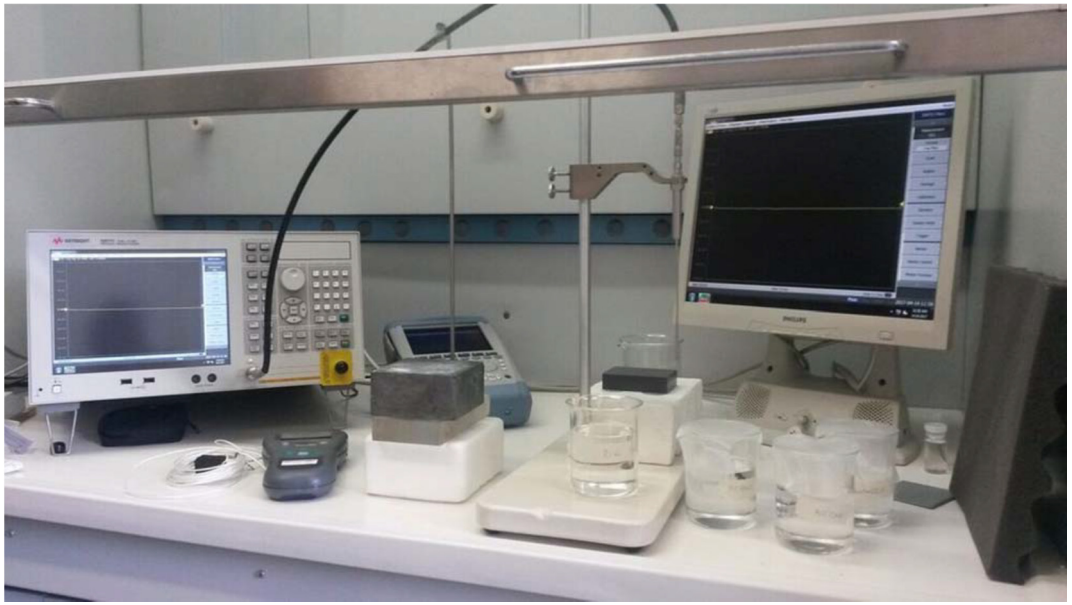
Evaluation of statistical significant differences for comparable conditions
- Fit AVG(medium), Fit AVG(20 mln), FitAVG(10mln), FitAVG(5mln) ⇒ p for each set of assessed parameters

Fitting of AVGs using inverse EMT
- 15 different fitting

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

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

Complex permittivity measurement: setup



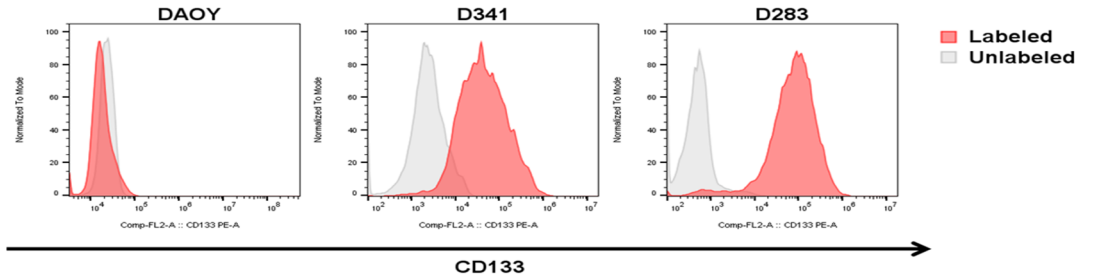
⇒ VNA, Keysight E5071C ENA + open-ended coaxial line probe Keysight 85070E slim-form 500 MHz- 3 GHz, IFBW 300 Hz. 1601 frequency points

⇒ Calibration: air, short and distilled water at 22°C. Calibration is refreshed in air

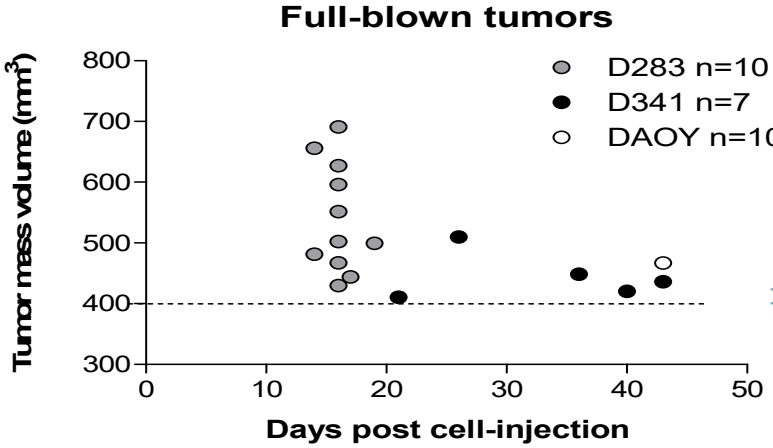
⇒ Cell viability was evaluated before and just after each measurement

D283 cells: high content of CSCs

DAOY: CD133⁺ cells *vs* blank **6%** **D341**: CD133⁺ cells *vs* blank **81%** **D283**: CD133⁺ cells *vs* blank **96%**



Evaluation of stemness markers

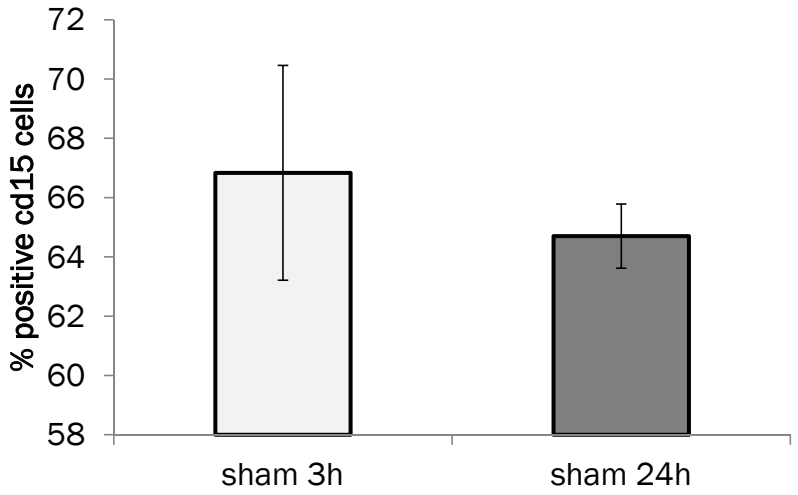


⇒ 16 days post injection

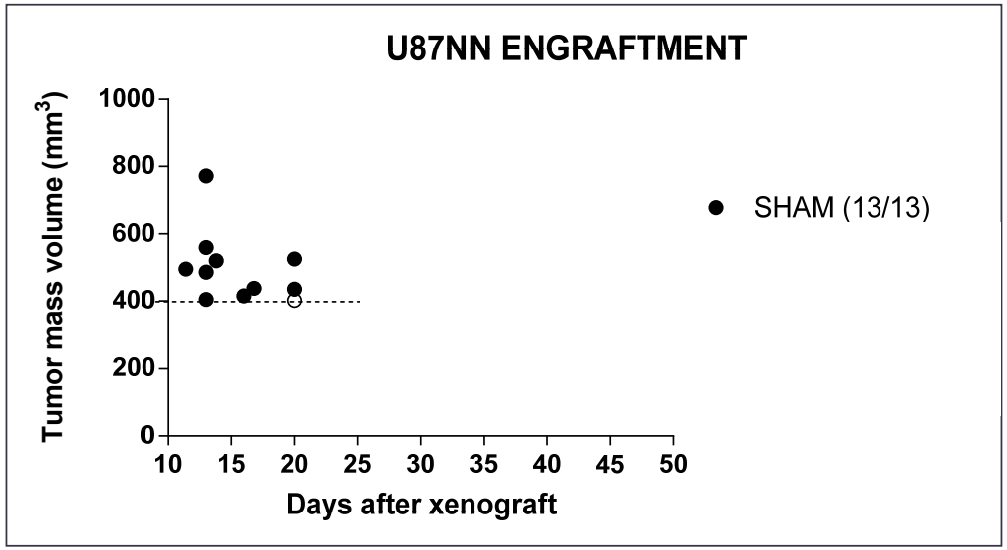
Tumor engrafment in vivo

Casciati et al., Cancers, 2019

U87 cells: medium content of CSCs



Evaluation of stemness markers



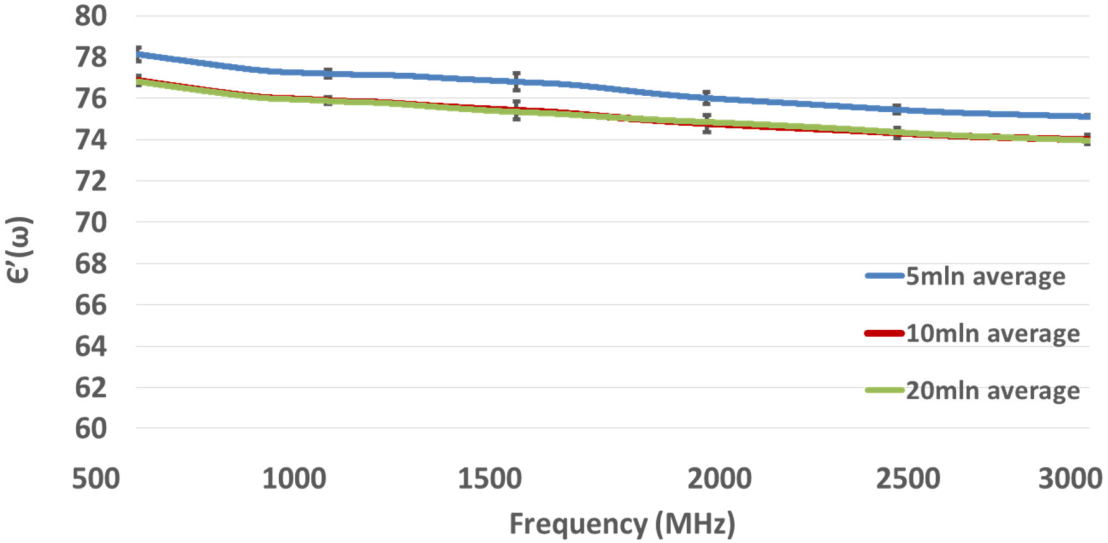
⇒ 20 days post injection

Time of tumor engrafment in vivo

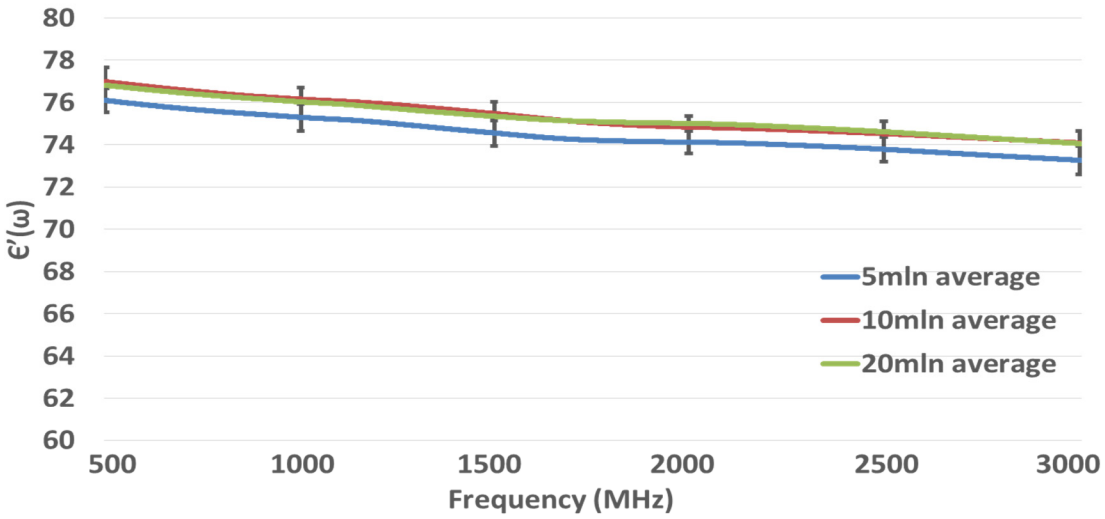
Casciati et al., under submission

Complex permittivity measurement: results

⇒ D283 cells in MEM

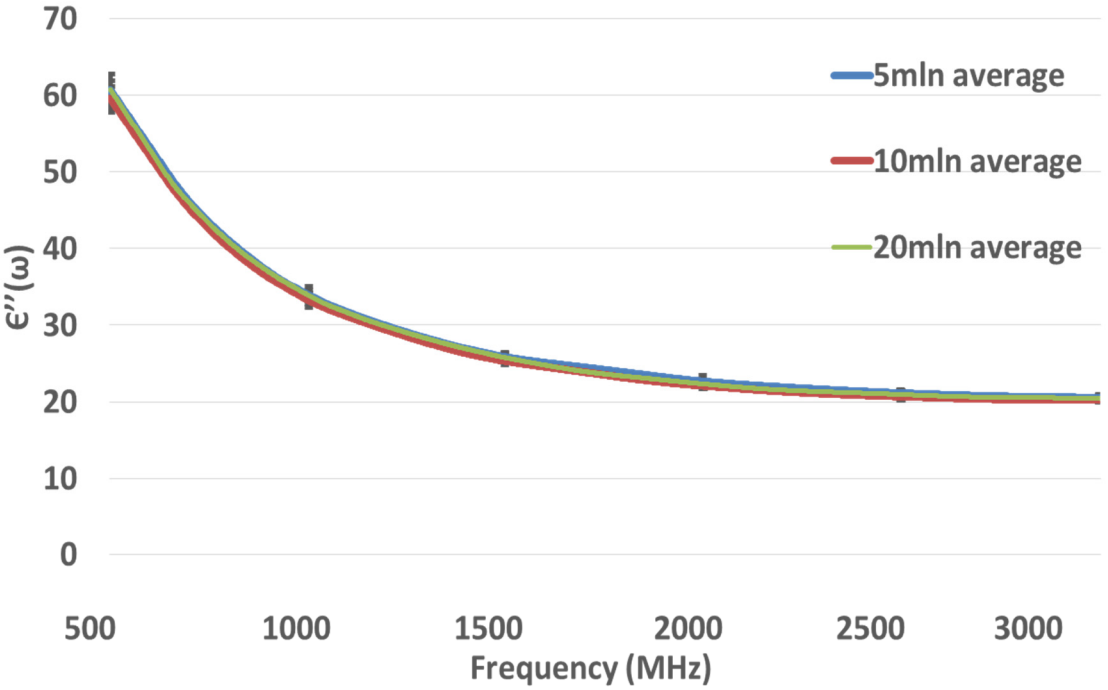


⇒ U87 cells in DMEM

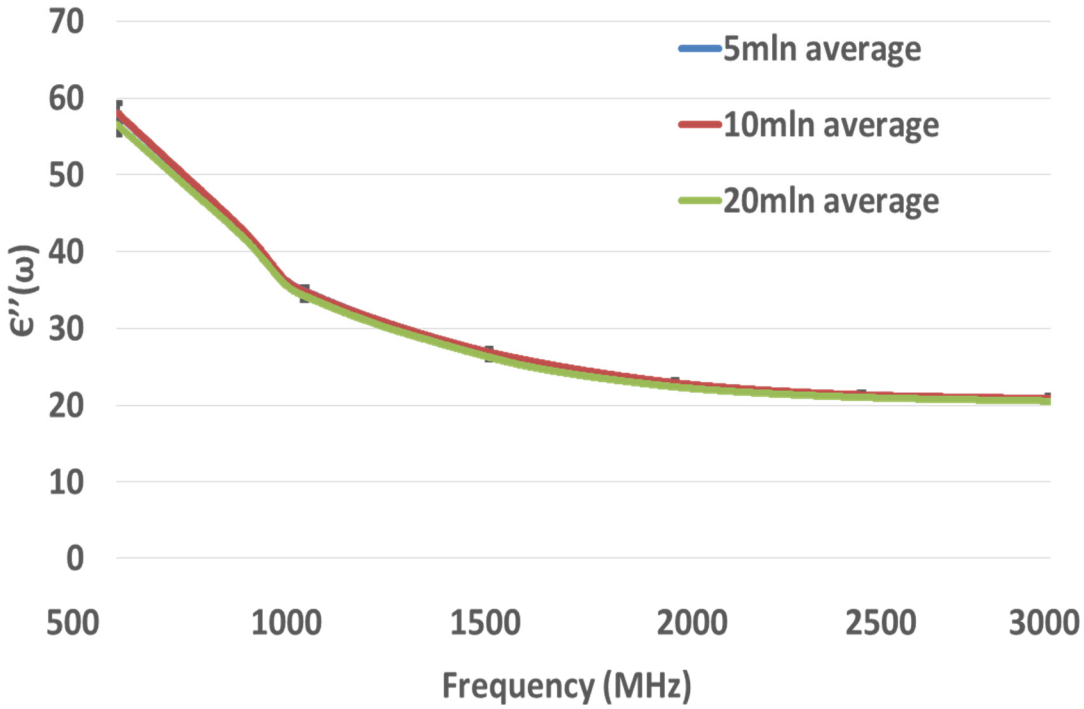


Complex permittivity measurement: results

⇒ D283 cells in MEM



⇒ U87 cells in DMEM



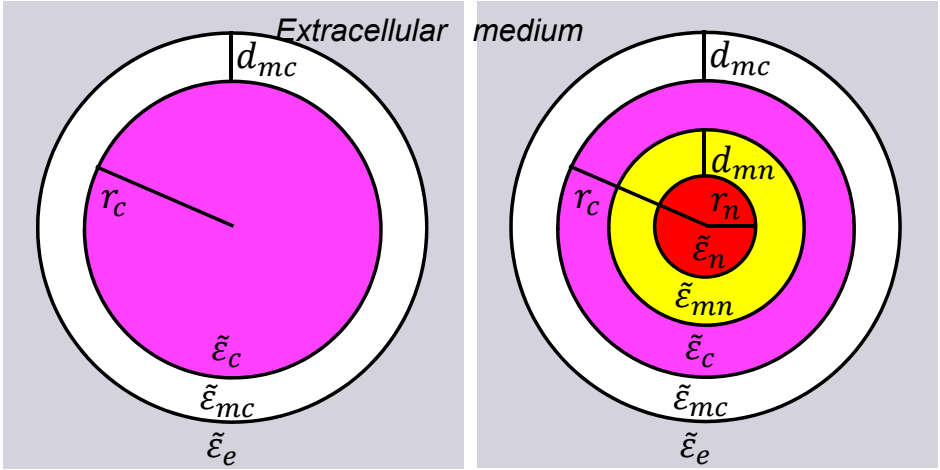
Circuit Model Implementation & Results

- Motivation
- Implementation approach
- Observations

Motivation

- Support cell characterization, design of experiment and end-point interpretation
- Allows compact, iterative representation for multilayer structure for which analytical expression are cumbersome
- Can take advantage of circuit simulators (e.g. SPICE, ADS) optimizers and time/frequency domain analysis tools

Implementation approach



2 layers, 1 membrane
Single Shelll

4 layers, 2 membranes
Double Shelll

Complex permittivity in AC domain:

$$\tilde{\varepsilon}_x = \varepsilon'_x - j\varepsilon''_x = \varepsilon_{x\infty} + \frac{\varepsilon_{x\infty} - \varepsilon_{xs}}{1 + j\omega\tau} - j\frac{\sigma_{xs}}{\omega\varepsilon_0}$$

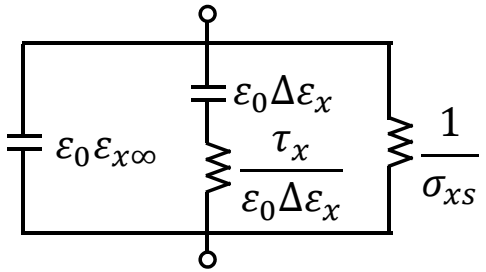
- Debye relaxation model with finite static conductivity
- Spherical single shell model assumed to start. Double shell through iteration, later
- Indexes: x= *i* (cytoplasm), *mem* (membrane), *m* (medium)

Circuit implementation

$$j\omega\tilde{\varepsilon}_x = j\omega\varepsilon_{x\infty} + j\omega\frac{\Delta\varepsilon_x}{1+j\omega\tau} + \frac{\sigma_{xs}}{\varepsilon_0}$$

This is the admittance of a frequency variable, lossy capacitor with unit area and spacing

$$Y_{C_x} = j\omega\varepsilon_0\tilde{\varepsilon}_x\frac{A}{d}$$



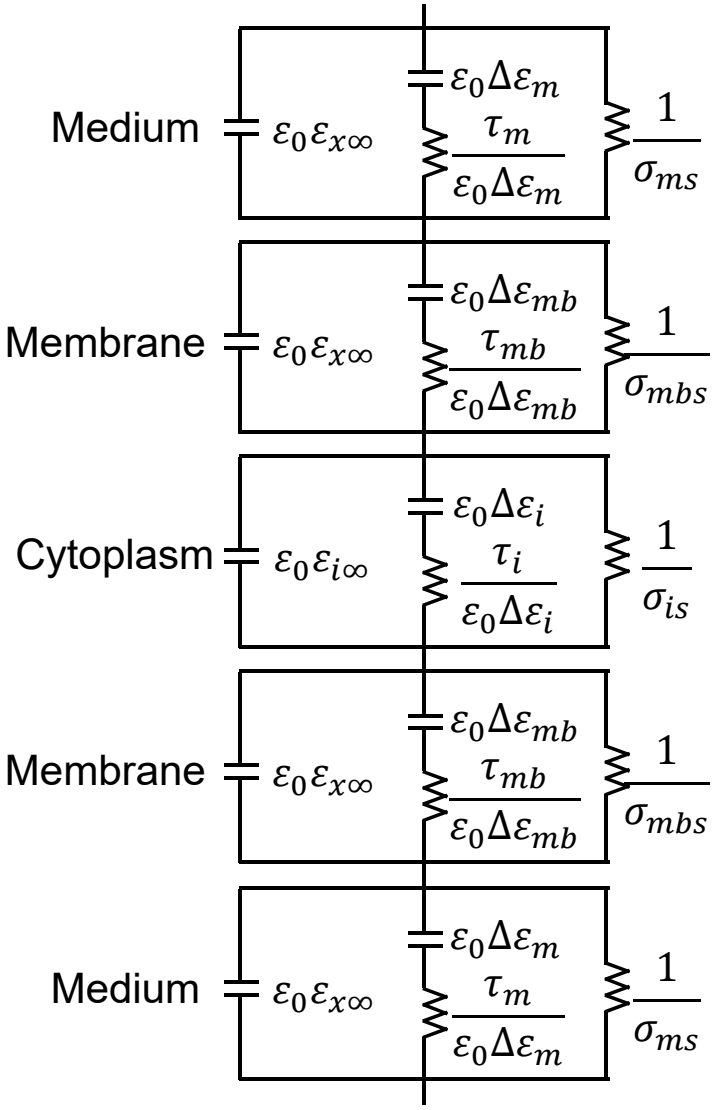
$$\Delta\varepsilon_x = \varepsilon_{x\infty} - \varepsilon_{xs}$$

$$A/d = 1$$

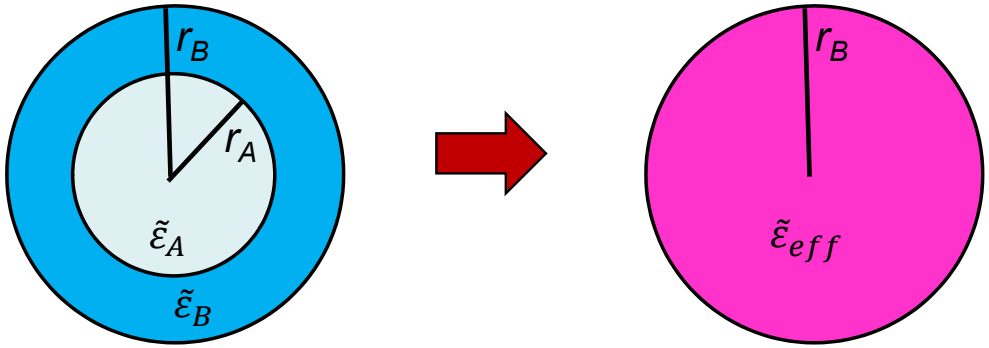
- 3 constituents of Debye relaxation model seen as admittances
- Extended to all compartments

Single cell circuit

- Mapping is straightforward and exploits symmetry
- 12 parameter model for entire cell
- Further circuit implementation required for:
  1) effective cell parameters and
  2) cell suspension (mixture)



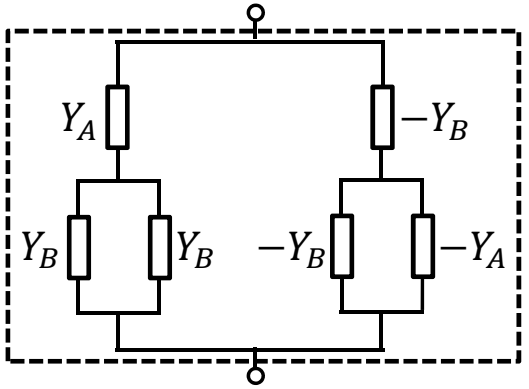
Effective Cell (1/2)



$$\tilde{\varepsilon}_{eff} = \tilde{\varepsilon}_B \left[\frac{\left(\frac{r_B}{r_A}\right)^3 + 2\left(\frac{\tilde{\varepsilon}_A - \tilde{\varepsilon}_B}{\tilde{\varepsilon}_A + 2\tilde{\varepsilon}_B}\right)}{\left(\frac{r_B}{r_A}\right)^3 - \left(\frac{\tilde{\varepsilon}_A - \tilde{\varepsilon}_B}{\tilde{\varepsilon}_A + 2\tilde{\varepsilon}_B}\right)} \right]$$

T. B. Jones, *Electromechanics of Particles.* Cambridge University Press, 1995

$$"Y_{AB}" = Y_B \frac{Y_A - Y_B}{Y_A + 2Y_B} = Y_B \frac{j\omega\tilde{\varepsilon}_A - j\omega\tilde{\varepsilon}_B}{j\omega\tilde{\varepsilon}_A + j\omega 2\tilde{\varepsilon}_B} = Y_B k \quad \rightarrow \quad k_i = \frac{Y_{EQ}}{Y_B}$$



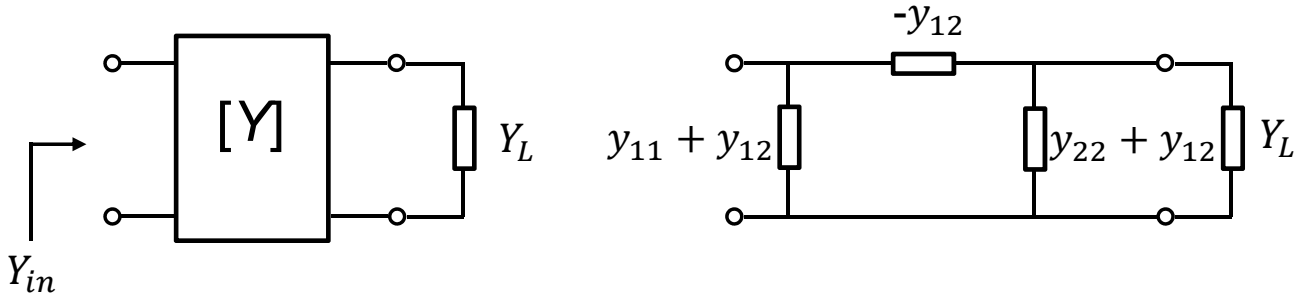
k_i (similar to but not= Clausius-Mossotti Factor) can be expressed as a normalized admittance!

Effective Cell (2/2)

Both Y_{AB} and Y_{eff} are of the form :

$$Y_{in} = y_{11} - \frac{y_{12}y_{21}}{y_{22} + Y_L}$$

which is the input admittance of a loaded a 2-Port pi network :



Same form but different coefficients for loaded π-network-1 (Y_{AB}) and -2 (Y_{eff}):

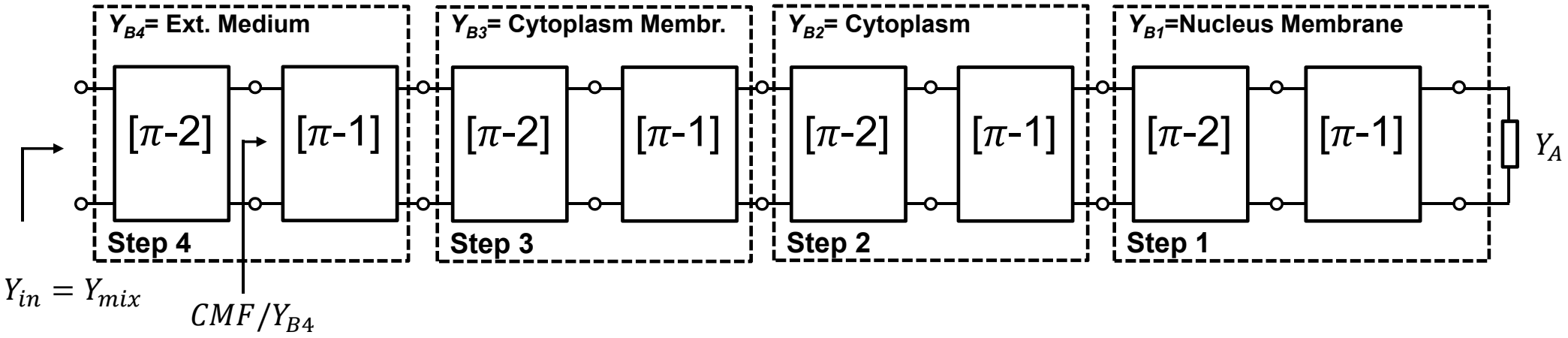
Coefficient depend on elementary Y_B and $(r_A/r_B)^3 = r$ but not k_i . This allows cascaded approach!

Mixture and Iterative Model

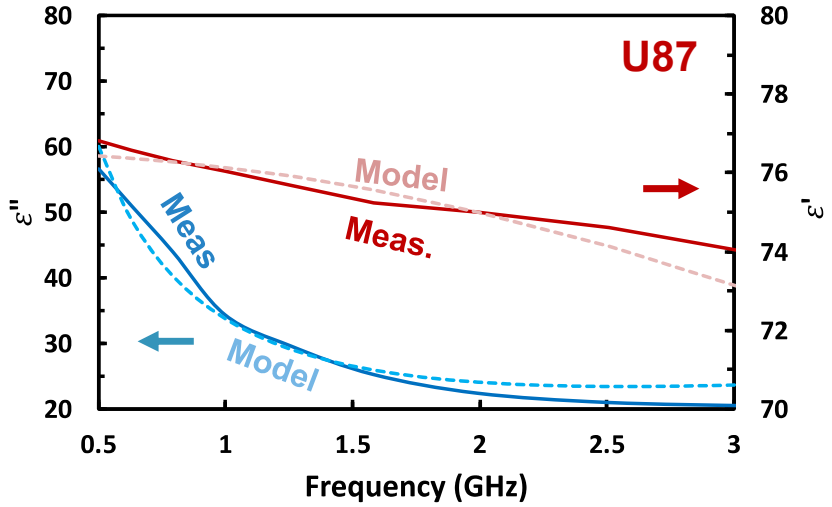
$$K_o = \frac{\tilde{\varepsilon}_{eff} - \tilde{\varepsilon}_m}{\tilde{\varepsilon}_{eff} + 2\tilde{\varepsilon}_m} \quad CMF = Re\{K_o\} \qquad \tilde{\varepsilon}_{mix} = \tilde{\varepsilon}_m \left[\frac{1 + 2\varphi K_O}{1 - \varphi K_O}\right]$$

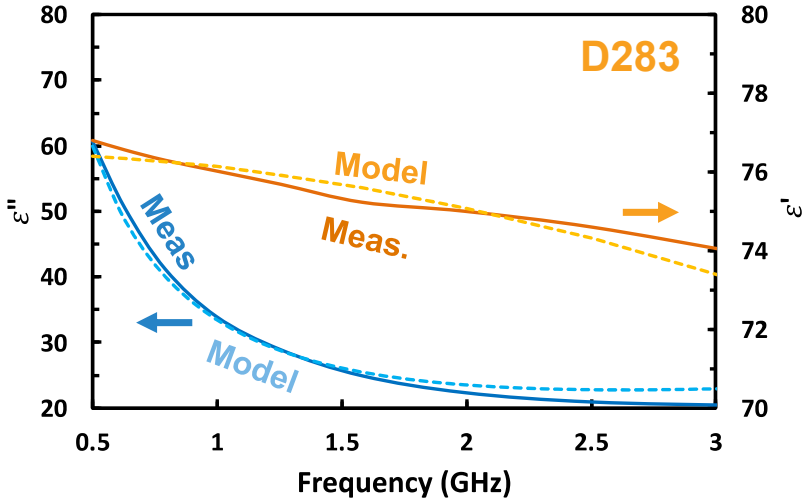
where $\boldsymbol{\varphi}$ is the fractional volume

- The mixture parameters is just another π-network-2 (Y_{AB}) with r=1/$\boldsymbol{\varphi}$
- Then a double shell model can be iteratively implemented as:



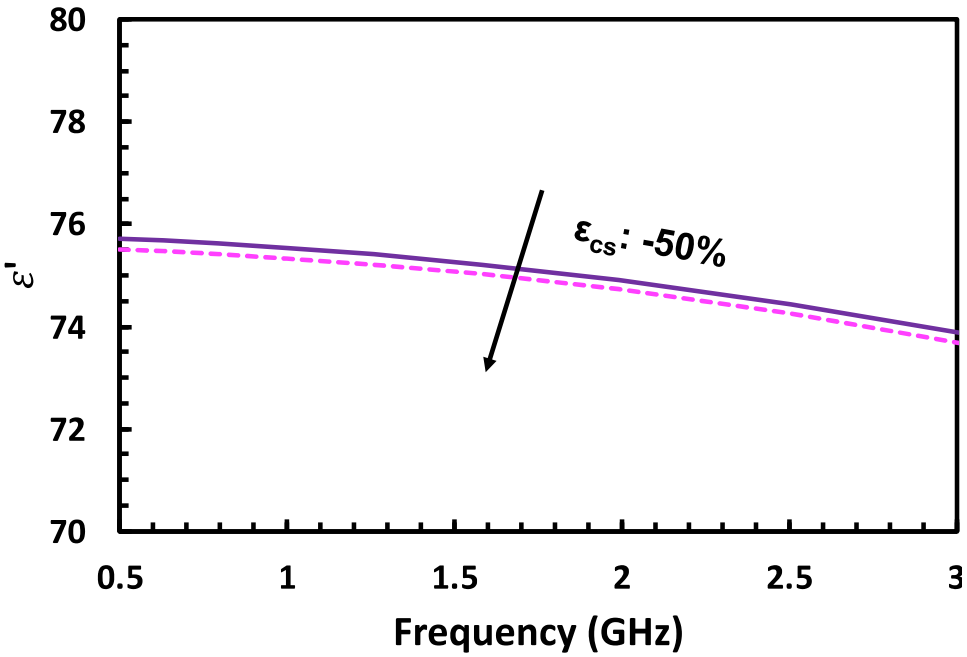
Extracted parameters: U87/D283 Cells (single shell)

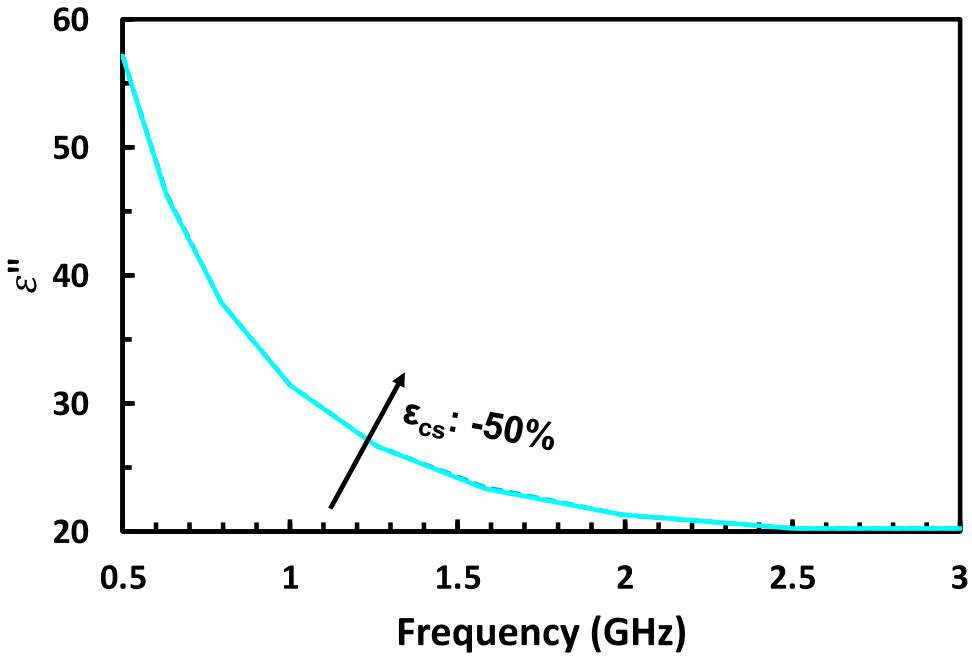




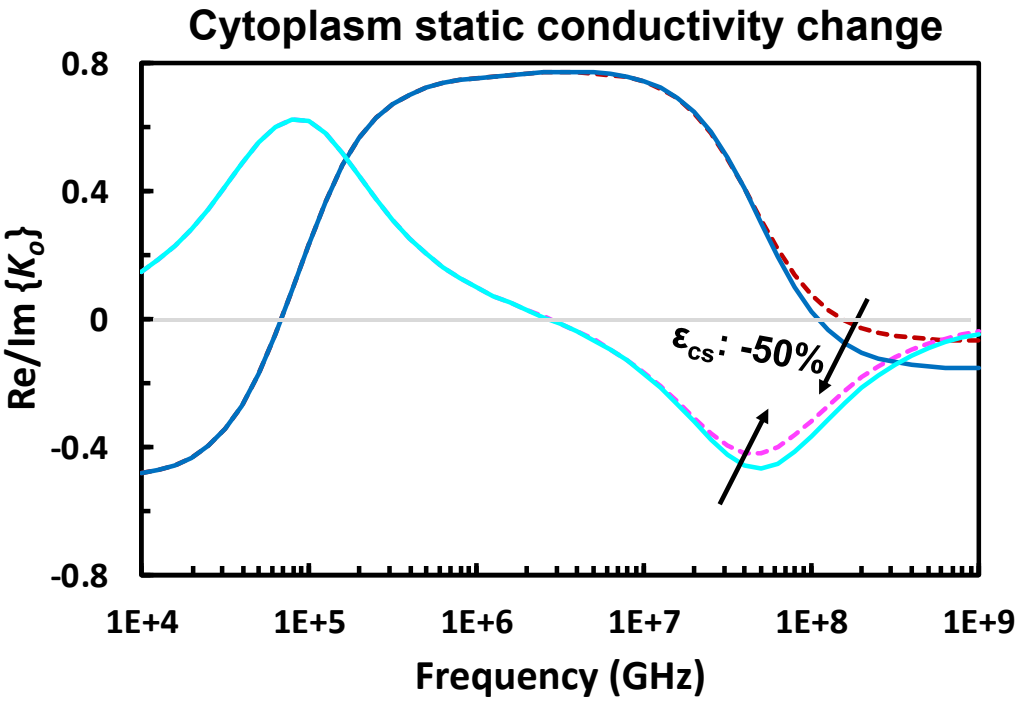
	ε_{xs}	$\varepsilon_{x\infty}$	τ_x [s]	$f_x = 1/2\pi\tau_x$ [Hz]	σ_{xs} [S/m]
R [µm]			7.5		
d [nm]			5		
ϕ			$1 \cdot 10^{-4}$		
x=c	65.9	7.5	55.4/56.2 $\cdot 10^{-12}$	2.87/2.83 $\cdot 10^{9}$	0.68/0.66
x=mc	15	1.7	35.9/42.3 $\cdot 10^{-9}$	443/378 $\cdot 10^{6}$	71.5/75.5 $\cdot 10^{-9}$
x=e	76.52/76.49	15	12.7/14.4 $\cdot 10^{-12}$	12.5/13.1 $\cdot 10^{9}$	1.55

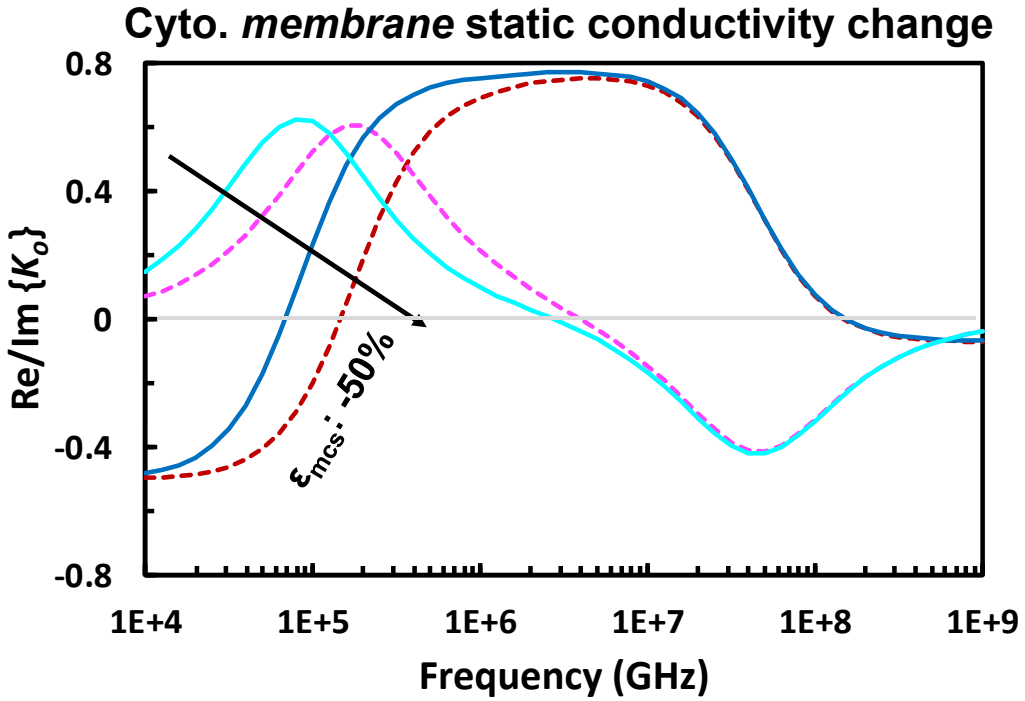
Weak Dependence on Cell Parameters for Low Fractional Volume (φ=10^{-4})



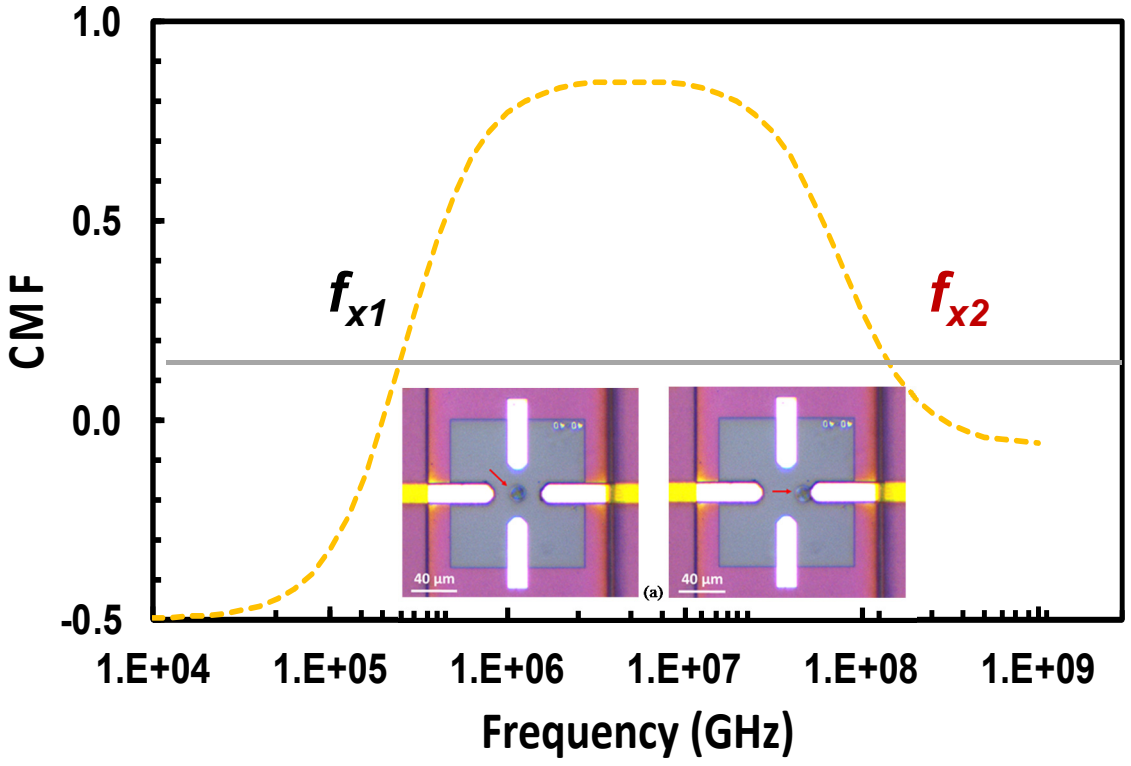


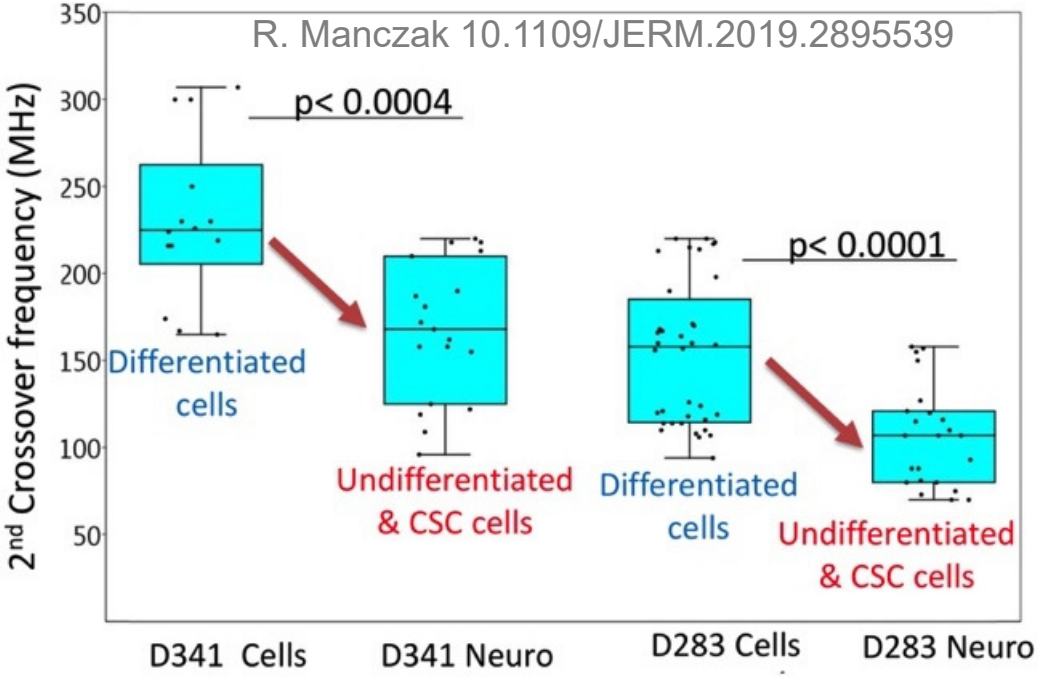
But CMF Sensitive to Internal Parameters! (even for $\varphi=10^{-4}$)





Parameters Extracted at 0.5-3 GHz Match Crossover Frequencies in Lower Range!

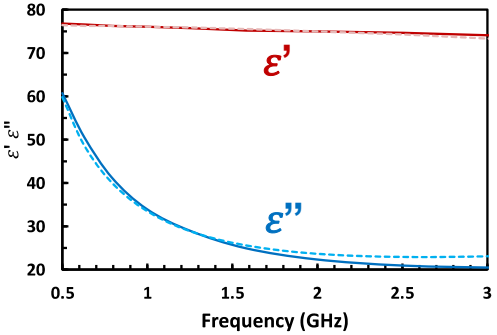




Non-dispersive Compartments Reduce Model Cost

	ε_{xs}	$\varepsilon_{x\infty}$	τ_x [s]	$f_x=1/2\pi\tau_x$ [Hz]	σ_{xs} [S/m]
R [µm]	7.5				
d [nm]	5				
ϕ	$1\cdot10^{-4}$				
x=c	65.9	7.5	55.4/56.2 $\cdot10^{-12}$	2.87/2.83 $\cdot10^{9}$	0.68/0.66
x=mc	15	1.7	35.9/42.3$\cdot10^{-9}$	443/378$\cdot10^{6}$	71.5/75.5$\cdot10^{-9}$
x=e	76.52/76.49	15	12.7/14.4$\cdot10^{-12}$	12.5/13.1$\cdot10^{9}$	1.55

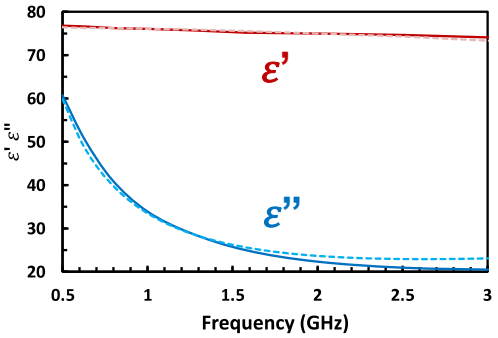
→ ***15 Parameters (S-shell)***
→ ***23 Parameters (D-shell)***



No difference!!

	ε_{xs}	$\varepsilon_{x\infty}$	τ_x [s]	$f_x=1/2\pi\tau_x$ [Hz]	σ_{xs} [S/m]
R [µm]	7.5				
d [nm]	5				
ϕ	$1\cdot10^{-4}$				
x=c	65.9	7.5	55.4/56.2 $\cdot10^{-12}$	2.87/2.83 $\cdot10^{9}$	0.68/0.66
x=mc	15		NA		
x=e	76.7		NA		1.55

→ ***11 Parameters (S-shell)***
→ ***15 Parameters (D-shell)***



Circuit Model: Lessons Learned

- Computationally efficient but limited to simple spherical shapes for now
- 1-2GHz frequency dependence captured but can be improved
- Low fractional volumes challenging for modeling
- (High fractional volumes challenging for logistics…)
- The approach could be equally applied to single cell settings
- Predicted to be useful for time domain analysis, too

Conclusions

- Effort to fill lack of dielectric parameters for brain cancer stem-like cells articulated in two approaches
- Statistical modeling across different fractional volumes
- Construction of a model for agile f- and t-domain simulation
- Model and extracted parameters match relevant experimental observations