FRAC-3

Discrimination of Glioblastoma Cancer Stem Cells by Measuring Their UHF-Dielectrophoresis Crossover Frequency

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Outline

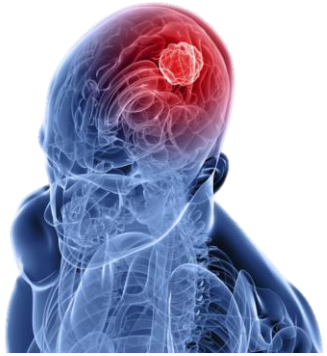
- Motivation and pursued approach
- How characterizing biological cells with high frequency DEP
- Strategy for characterizing CSC's cells
- Example of characterization of GBM cell lines
- Conclusion and futures developments

Motivation

- Need for new therapeutic strategies dedicated to poor outcome diseases

Ex: Glioblastoma:

- *Tumor with high recurrence*
- *Strong resistance to existing treatments*
- *Highly heterogeneous brain tumors*



Resulting efficiency from standard therapies is very low

- *Poor patient survival rate*
- *Frequent relapse*

Role of some hidden tumor-initiating cells ?

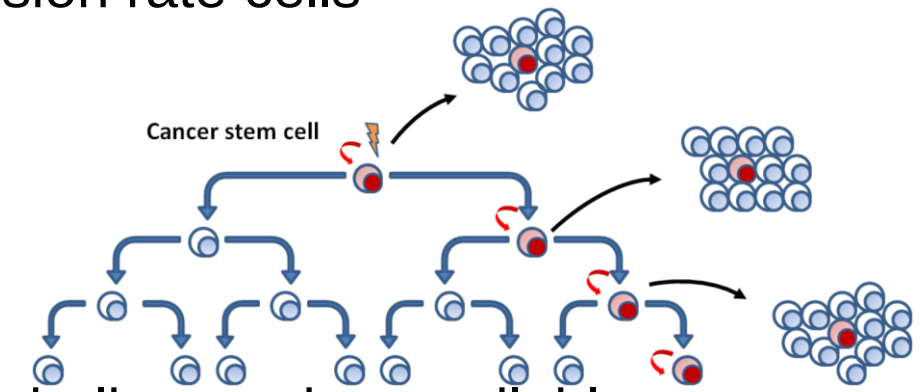
How fight them more efficiently?
What they look like?
How many are they?
Where are they?

Motivation

- Need for alternative tools able to track such specific and rare cells

Cancerous Stem Cells: *Tumorigenic cells with ability to give rise to all tumor cell type*

- Quiescent cells: escape from therapies targeting high division rate cells
- Differentiation into multiple cell types (progenitors…)
- Self-renewal capabilities
- Low number, Hidden in the tumor
- Undifferentiated cells: No specificity: lacking for specific labeling marker available

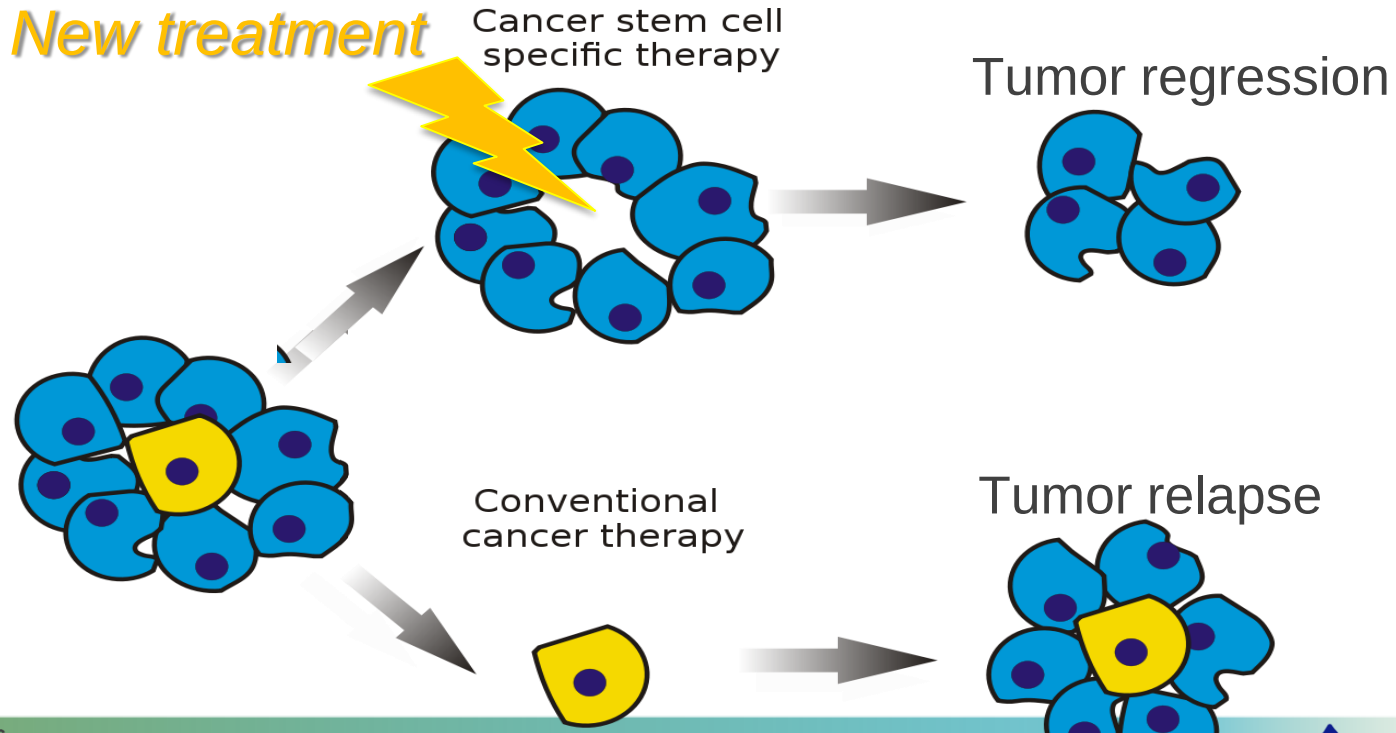


➡ Currently hypothesized to be the main cause of relapse and metastasis

Motivation

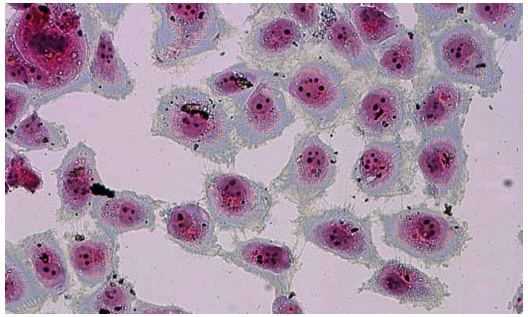
Tools able to identify CSC's in/outside the tumor might contribute to:

- help diagnosis and favor more appropriated treatment
- promote to the development of more efficient therapies

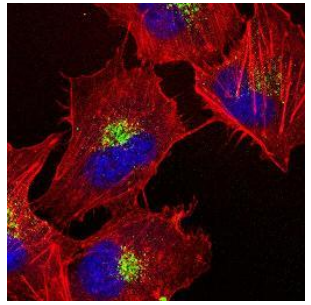


How biologists can study CSC's?

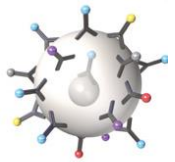
Optical microscopy



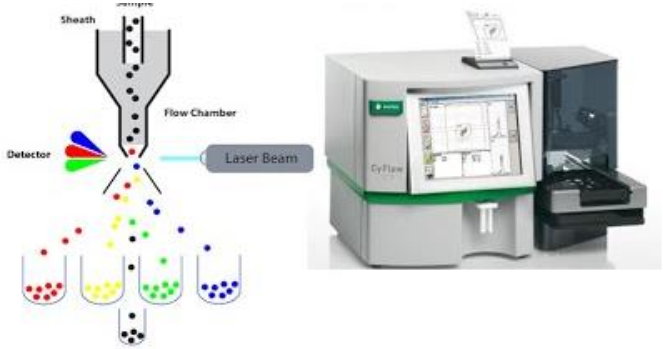
Staining



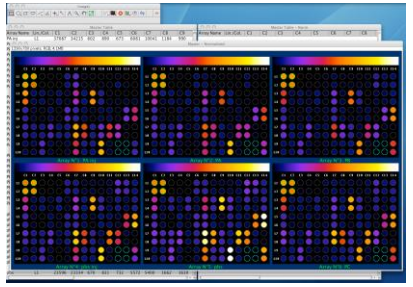
Fluorescence labeling

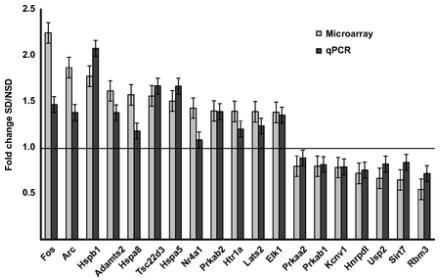


Flow cytometry



QPCR & Protein Array analysis





Drawback/ constrains:

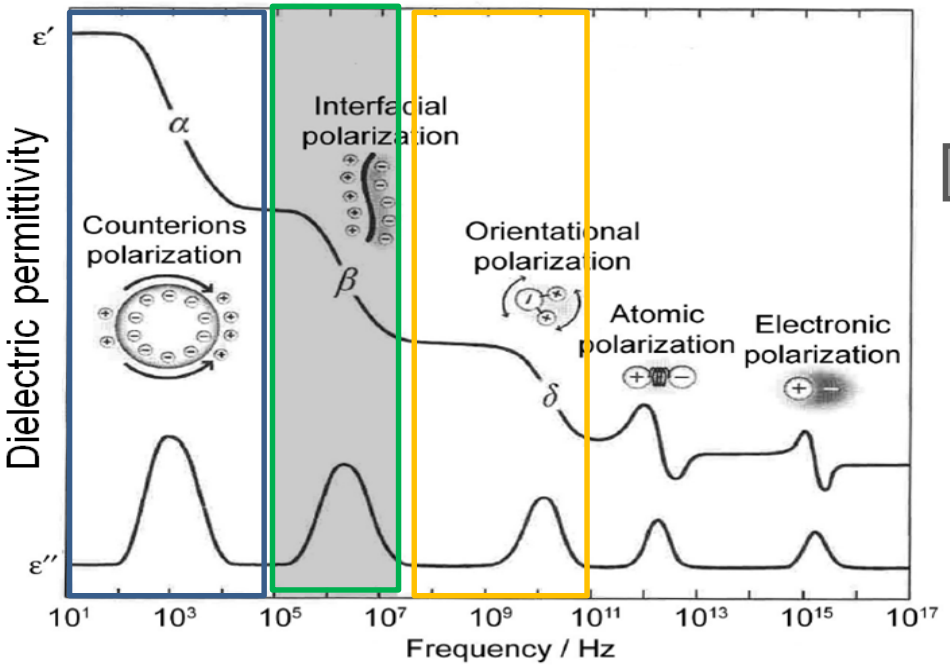
- ✓ *Specific label are lacking -> Cross coupling of generic markers*
- ✓ *CSC's are rare -> require amplification of the population*
- ✓ *Efficient functional tests exist (clonogecity, animal drafting) but results are very long*

➡ Others approaches investigating intracellular specificities?

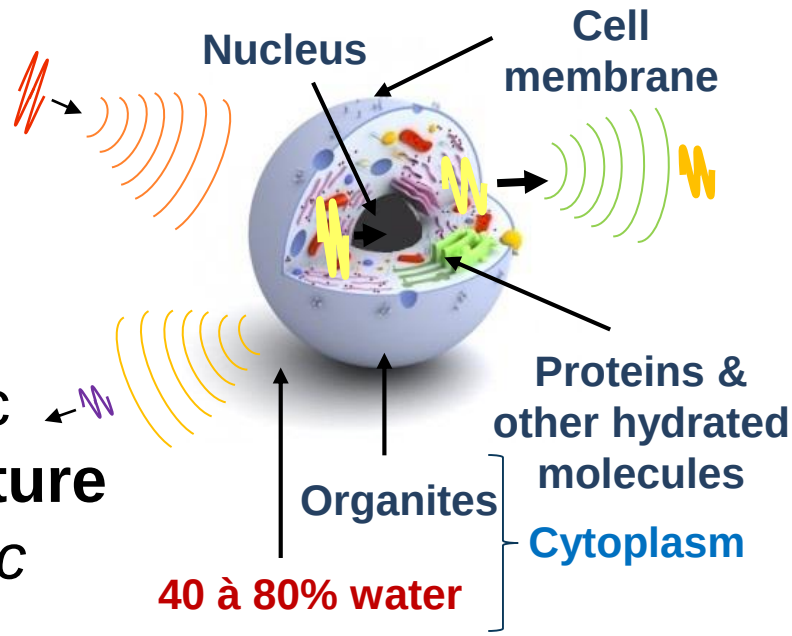
Why (not) using EM field to characterize cells?

Depending the frequency EM field could interact with different cell constituents

- Low frequency -> Cell shape/ morphology/size influence
  - Mid frequency -> Plasma Membrane specificities
    - High frequency -> Intracellular content properties



Own cell dielectric properties = **A signature** *that can be specific*



High frequency signal well suitable to access to cell interior properties and measure specificities

Dielectric spectroscopy allows non destructive & label free characterization

Dielectrophoresis vs Dielectric Spectroscopy

DEP relies on the fact that EM fields generate forces that can move cells

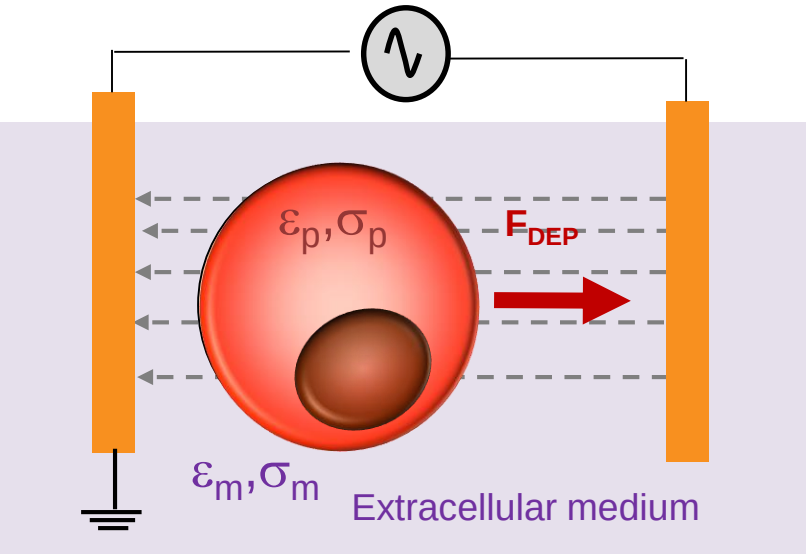
Basic DEP theory $F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}[K(\omega)]\,\nabla|E_{rms}|^2$ ← Related to the E field gradient intensity

-1<Re[K(ω)] <1

$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right) \leftarrow \varepsilon_p^* = \varepsilon_p - j\frac{\sigma_p}{\omega}$$

Claussius-Mossotti factor

Complex permittivity of the particle



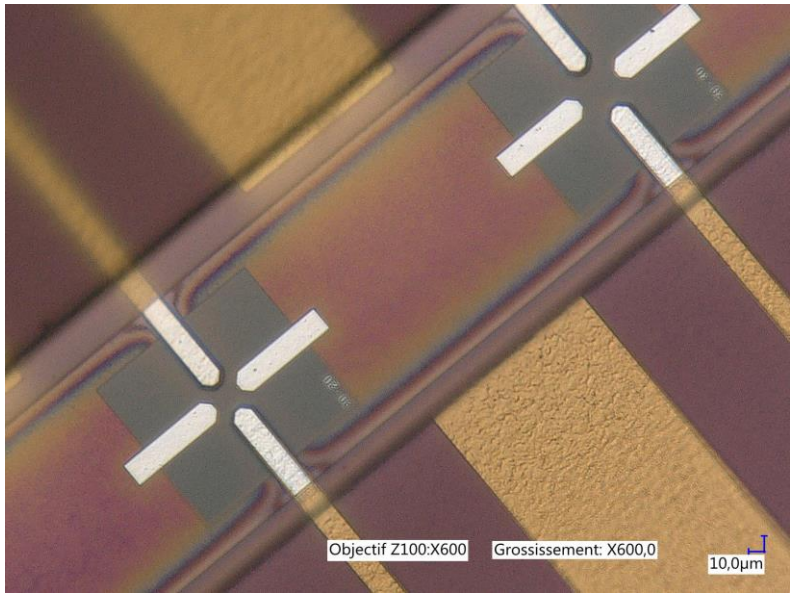
Re[K(ω)] <0 → **Repulsive force**

Re[K(ω)] >0 → **Attractive force**

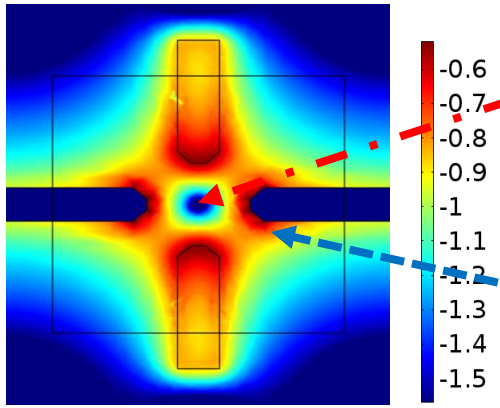
Cells can be individually electromanipulated accordingly their own dielectric properties

How taking advantage of DEP force?

Proposed sensor: Quadrupole electrode system with specific biasing

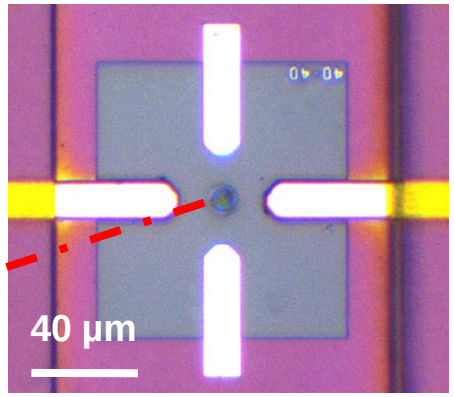


Electrical cage formed between electrodes

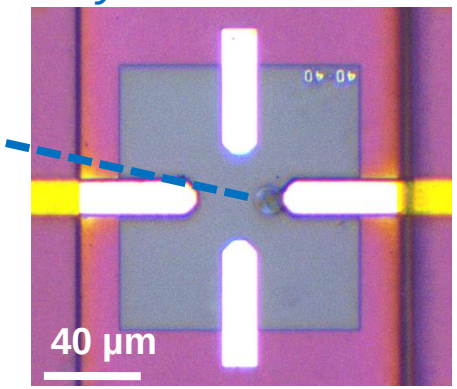


Normalised Electric Field gradient intensity

Cell repeals by DEP<0 moves to center



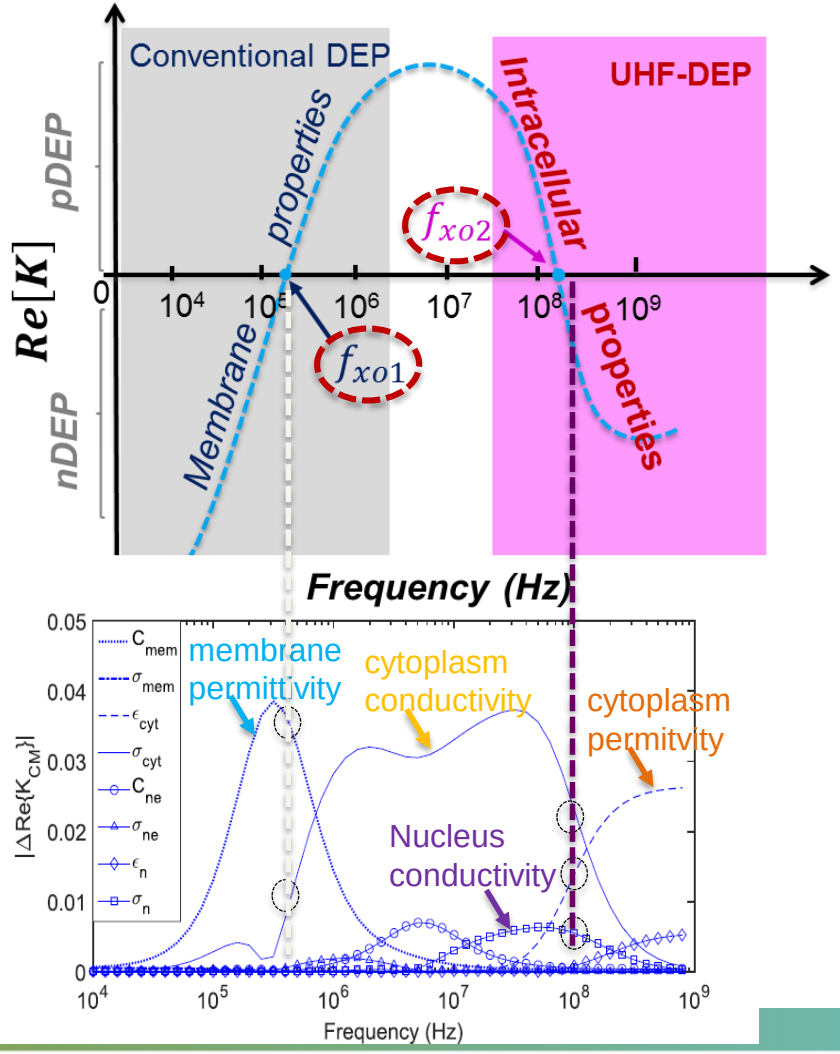
Cell attracted by DEP>0 moves to electrode



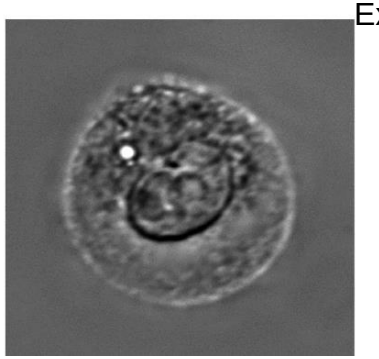
Specificities of cell DEP spectral signature

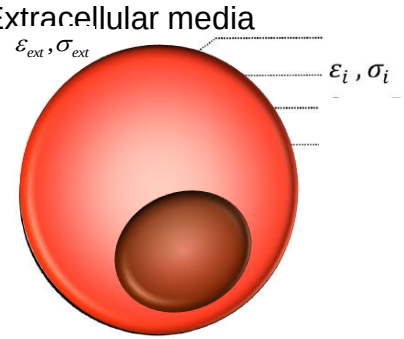
- Characterize cells to identify their DEP cross over frequencies as discriminant specificities

Cell DEP spectral signature



Dielectrophoresis theory basics

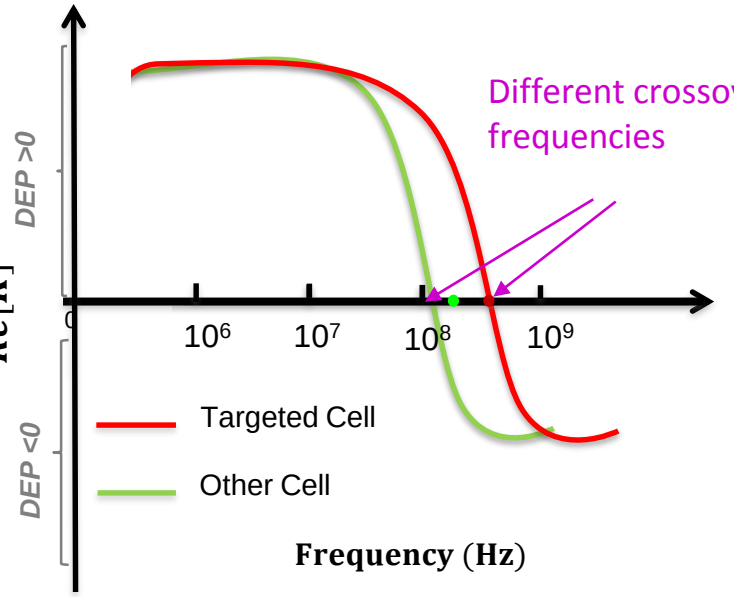




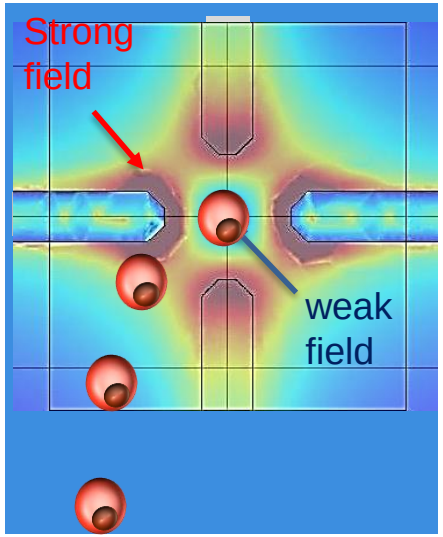
Different cells

Vs

Different spectral signatures



Methodology for cell crossover frequency measurement



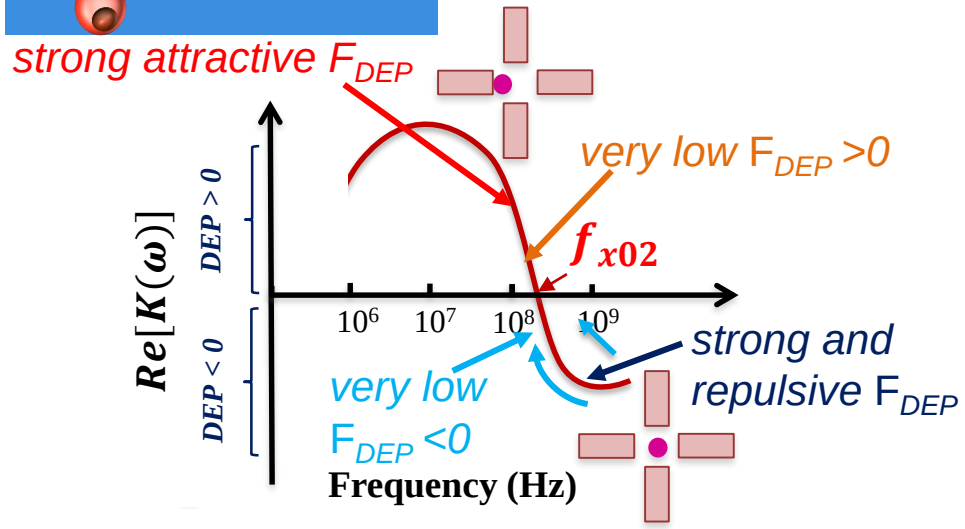
$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[K(\omega)\right] \nabla\left|E_{rms}\right|^2$$

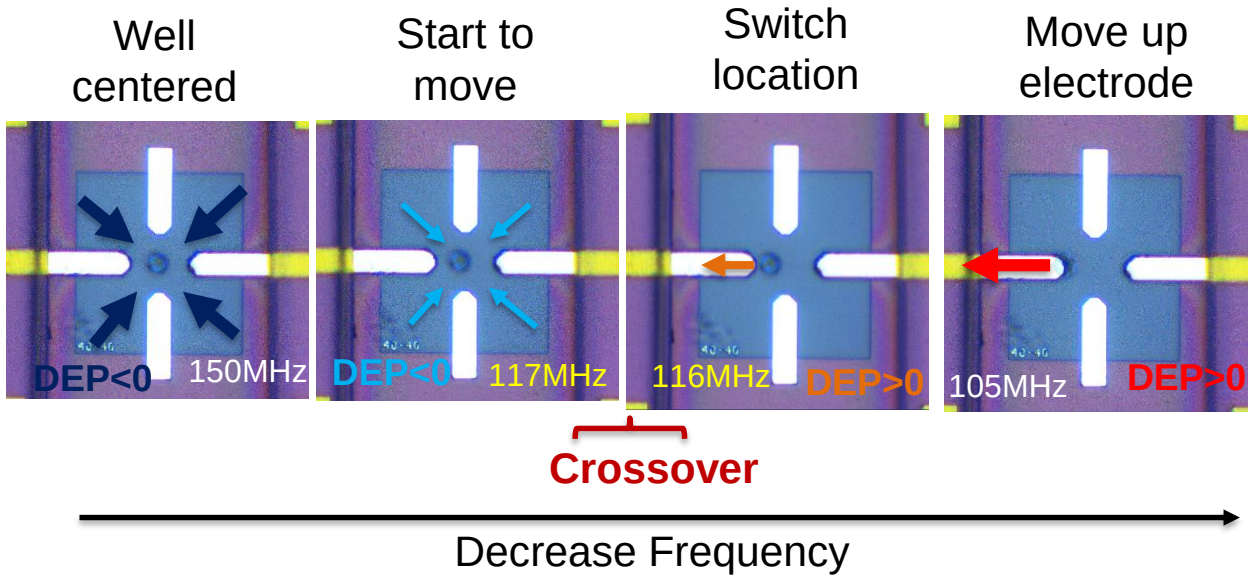
-> F_{DEP} will be high in strong field areas

-> low in weak field areas

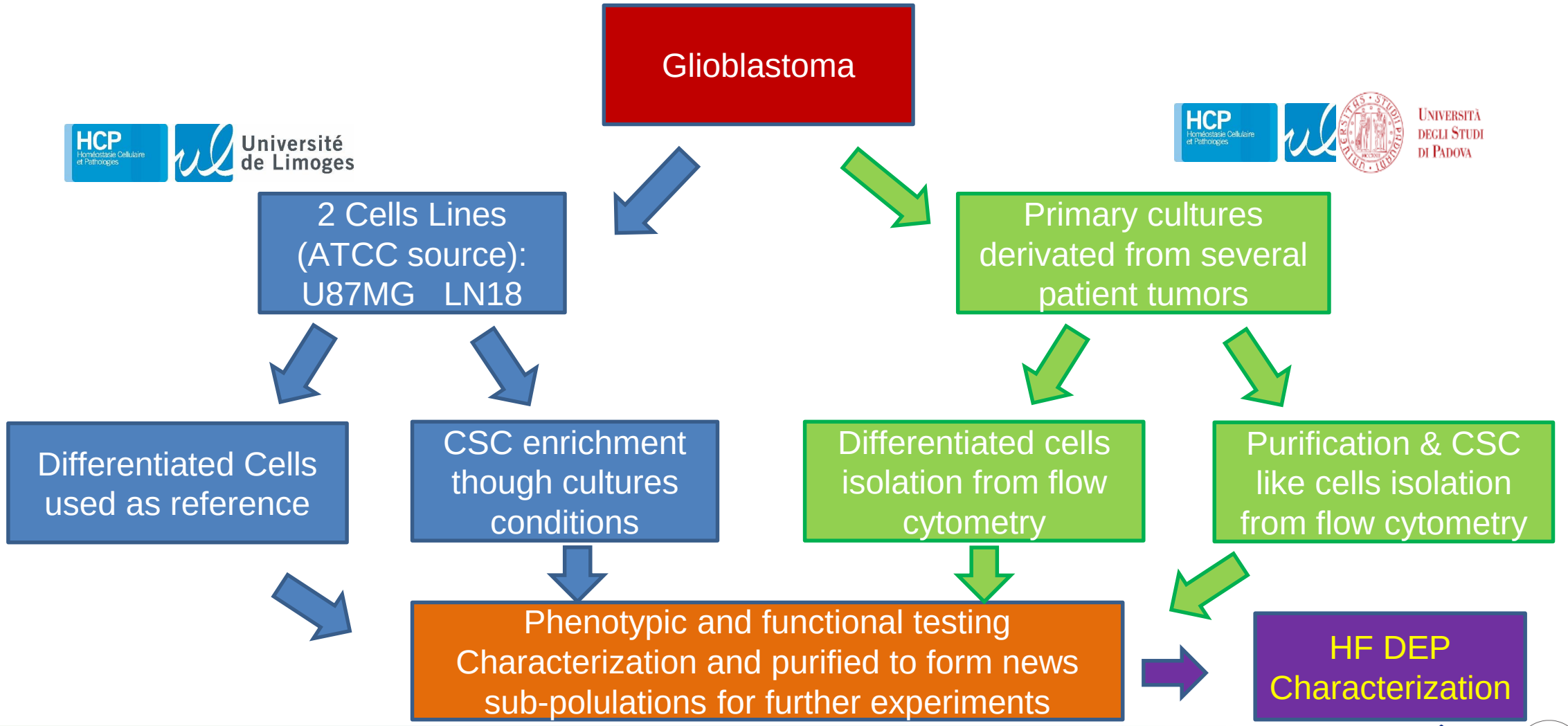
Methodology:

1) Cells are trapped in DEP<0
2) Flow is stopped
3) Frequency is tuned every MHz until finding positive DEP



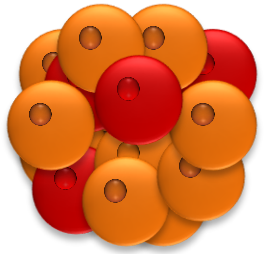


Followed Methodology for cell preparation



Enrichment in CSC's starting from cell lines

- Submitting cells to stringent Culture conditions



U87-MG or LN18

N: Normoxia 20% O_2

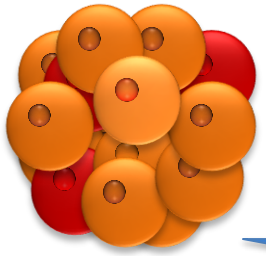
H : Hypoxia 1% O_2

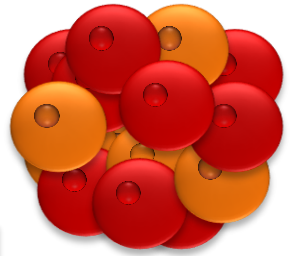
Culture Medium: Normal (+ FBS) / Defined (- FBS)

% O_2: N / H / N / H

NN / NH / DN / DH

Only few % of CSC's



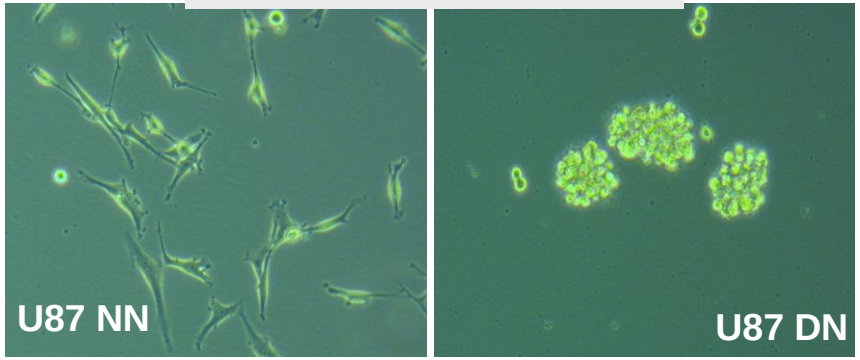


CSC's like cells
60-85 % expected

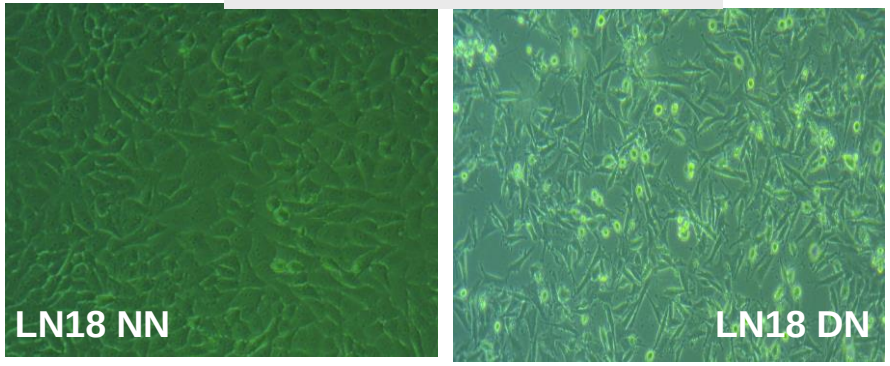
Résistance & immaturity

Effect of culture conditions cell phenotype

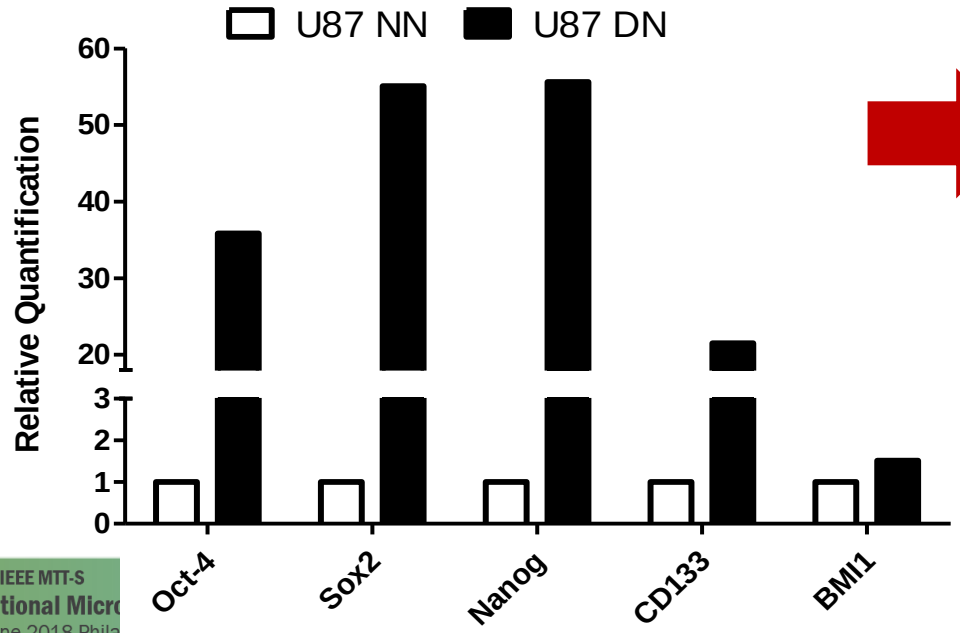
U87 NN vs DN

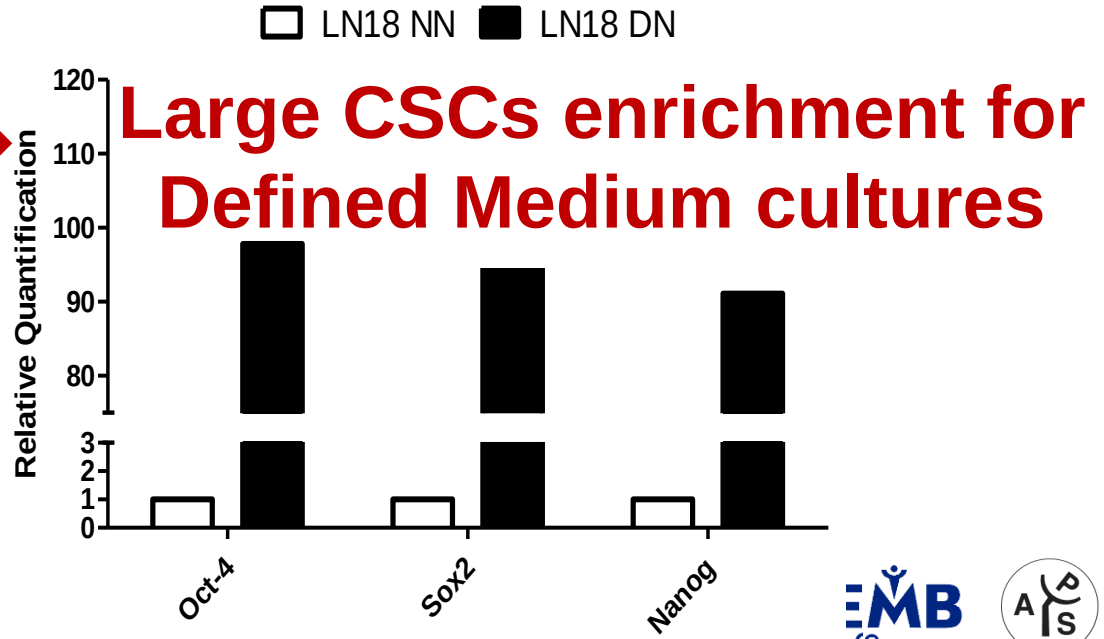


LN18 NN vs DN



mRNA expression of Stem cell markers

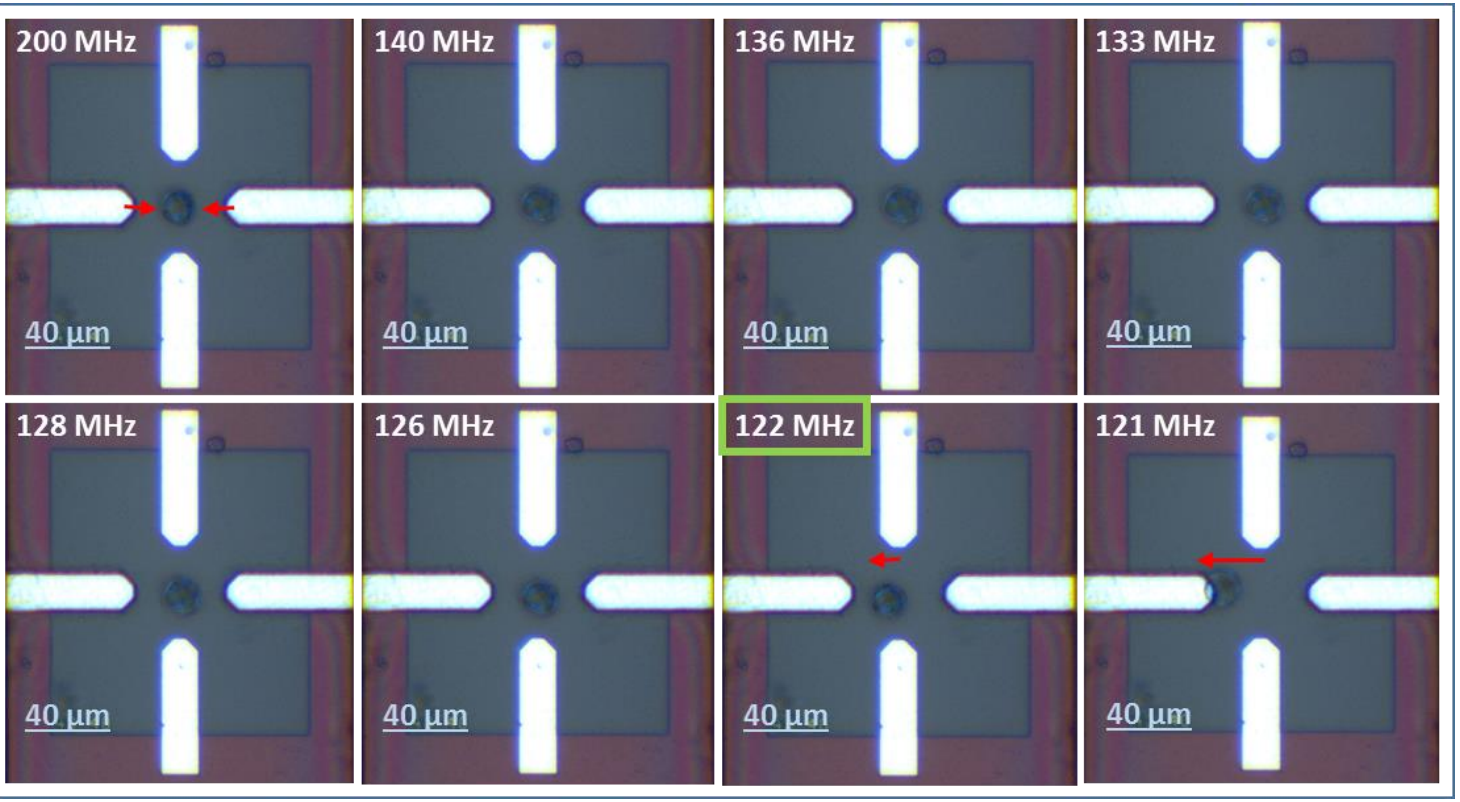




Large CSCs enrichment for Defined Medium cultures

DEP signature characterization of NN LN18 cells

Cross over frequencies measured on more 100 cells from standard population



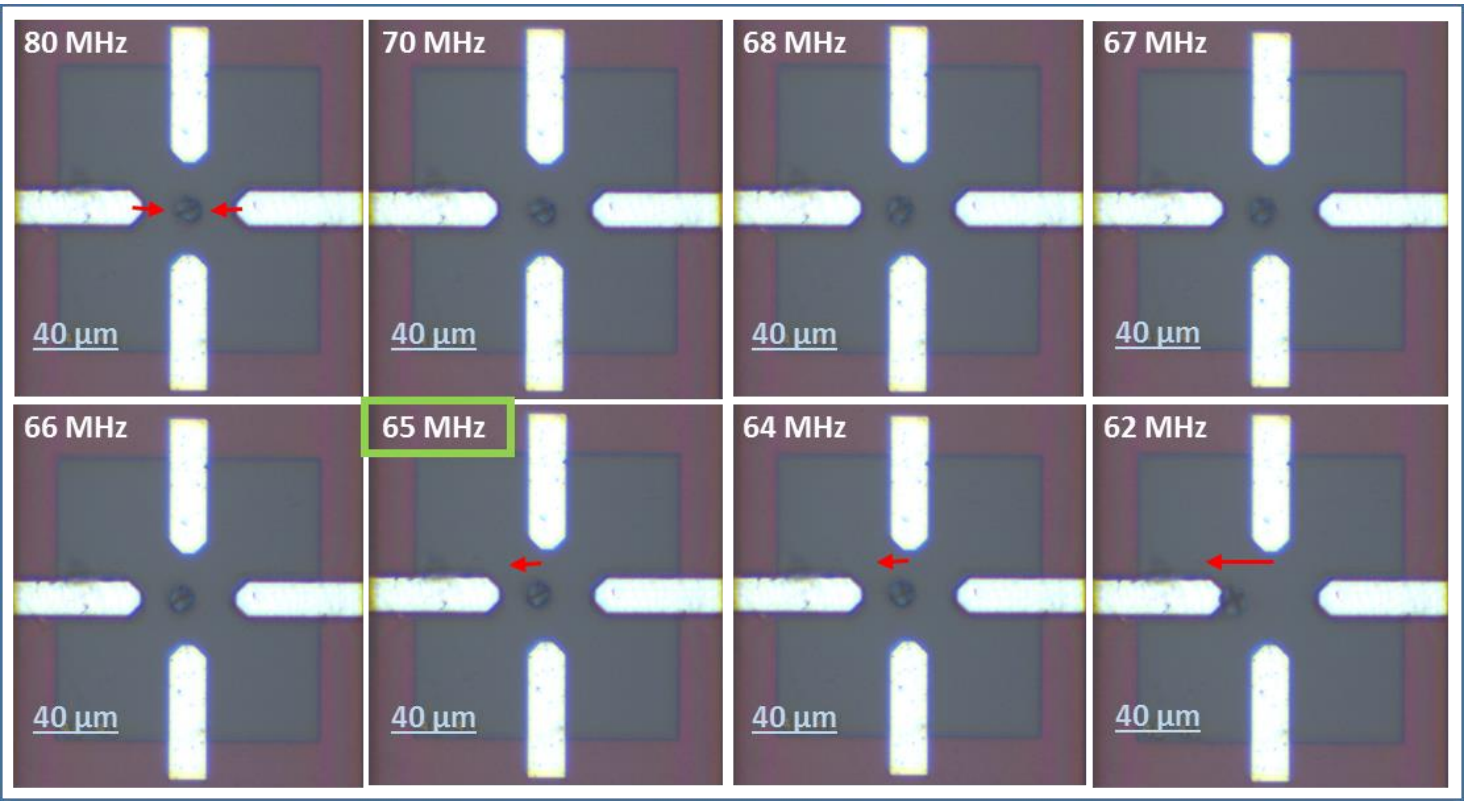
Sum up of collected data

Cells lines	Avg	Median	Dev Std	Error Std	Min	Max
LN18 NN	126	120	31,43	3,6	79	183

f_{XO2}~120 MHz -> Reference signature for differentiated cells

DEP signature characterization of DN LN18 cells

Cross over frequencies measured on more than 75 cells from CSC enriched population



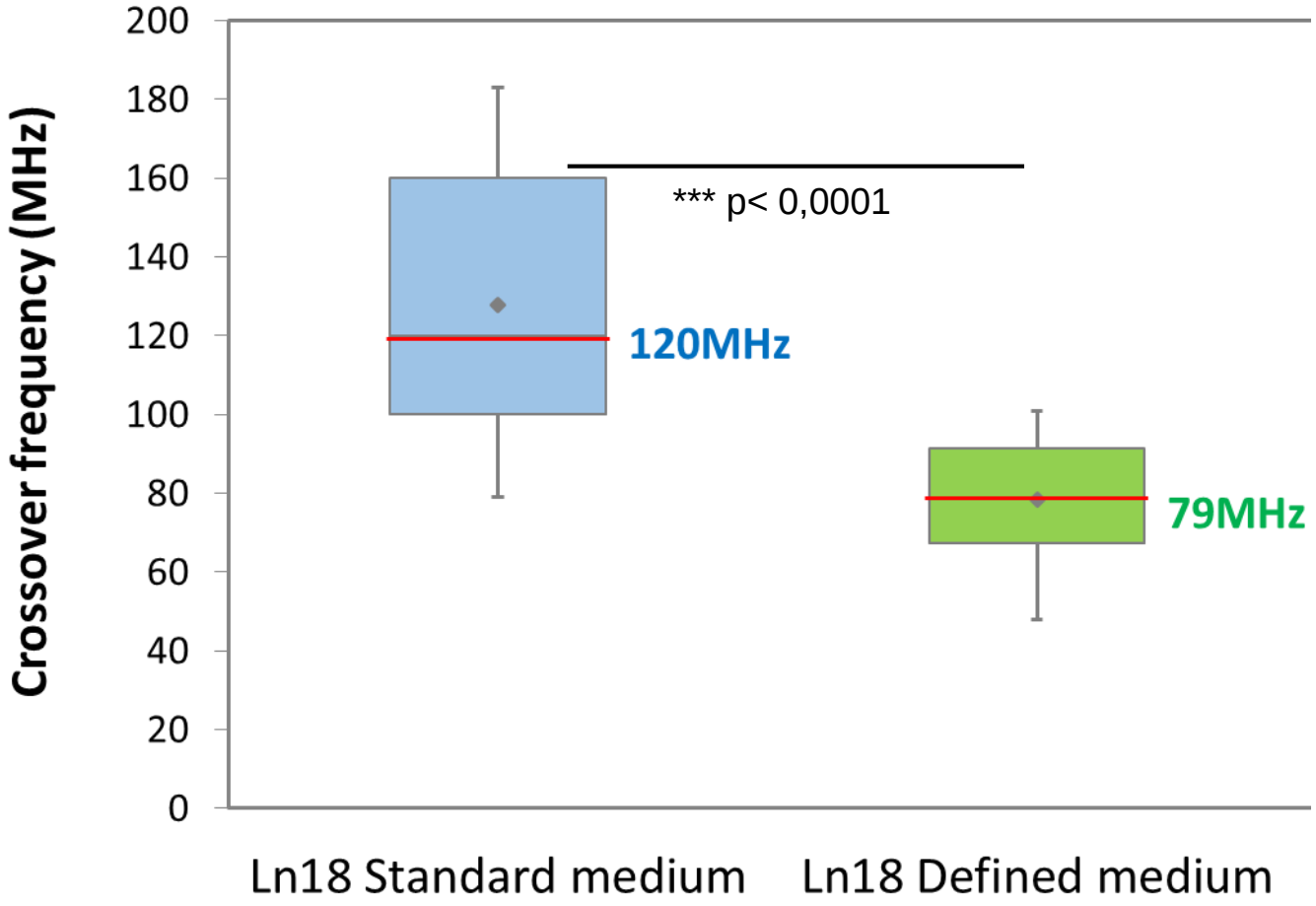
Sum up of collected data

Cells lines	Avg	Median	Dev Std	Error Std	Min	Max
LN18 DN	78	79	15,11	2,23	48	101

f_{XO2}~79 MHz -> Reference signature for undifferentiated cells

Collected data done on LN18 line

p: **Mann-Whitney pairwise** method



Summary & conclusion

Cells cultured in normal medium vs cells cultured in stringent conditions present some clear different intracellular dielectric properties

- Good correlation with the result of phenotypical & functional tests

Signatures of “Normaly cultured” U87 & LN18 seems close, some differences appear between enriched CSC population from both lines with still an overlap of spectral signature

To be confirmed with GBM primary culture cell characterization (on going)

Acknowledgement

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Project partners:

HCP Homéostasie Cellulaire et Pathologies

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ihp

Università degli Studi di Padova

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

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