Détection et la neutralisation de cellules souches cancéreuses sur lab on chip

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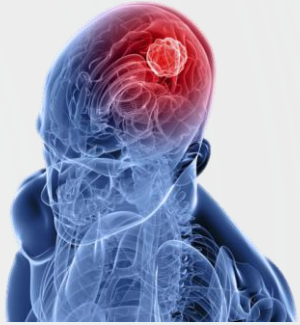
Motivation

Need for new therapeutic strategies dedicated to poor outcome diseases

Ex: Meduloblastoma , 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 ?

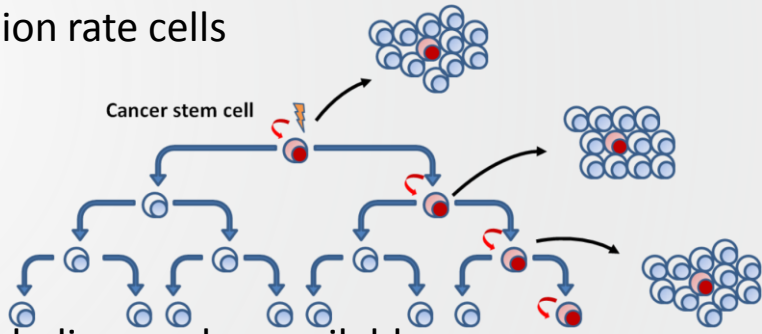
How fight them more efficiently?
What they look like?
How many are they?
Where are they?

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

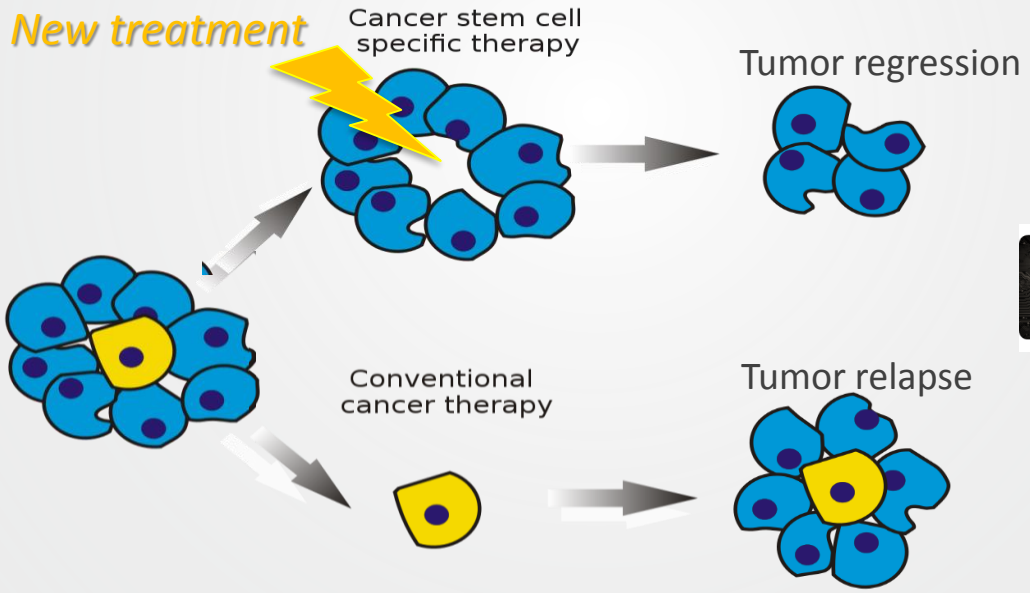


➡ Currently hypothesized to be the main cause of relapse and metastasis

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

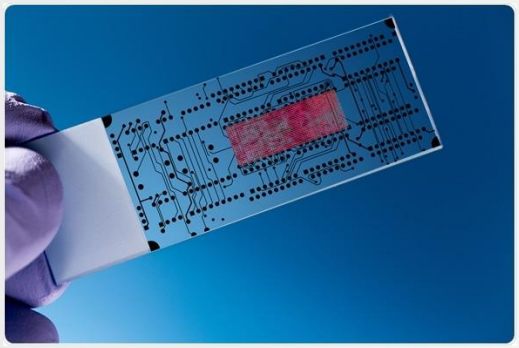


Sumcastec

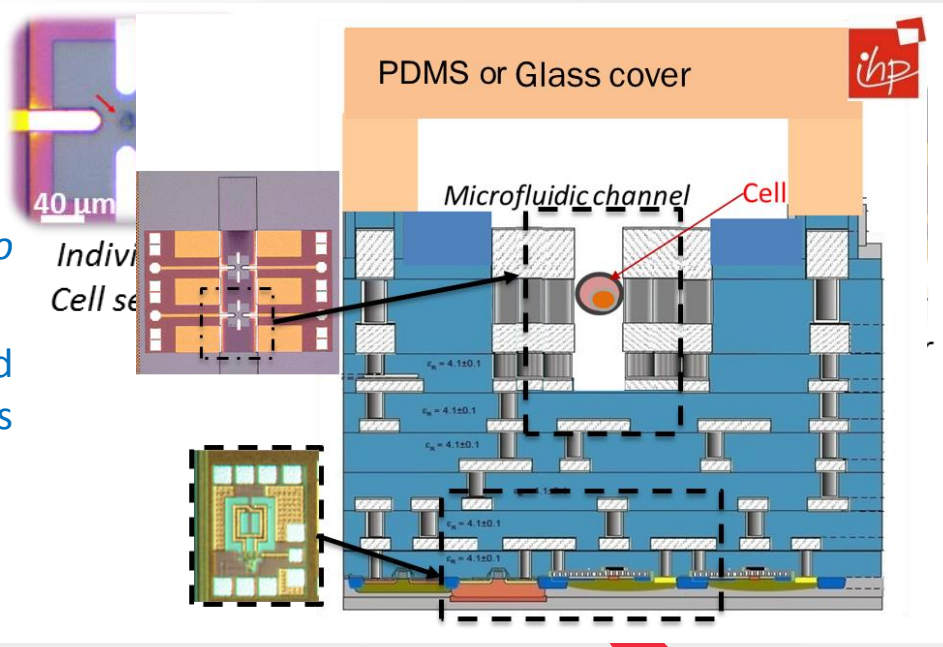
HORIZON 2020

New Lab-on Chip tools dedicated to cellular analysis

Example: New Generation of Microwave Lab-on-Chip for **Cancerous Stem Cells** Sensing & Neutralization using Electromagnetic Waves Stimulation



Concept: *Exploit the non-thermal effects of EM radiations on living organisms to **sense** and **stimulate** specifically targeted biological cells*



Investigation methodology: *Take benefit of*

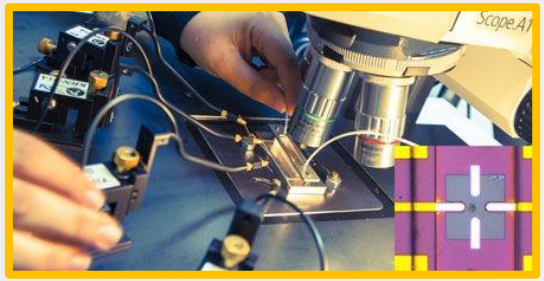
-***Microsystem & microfluidic technologies*** *to individually treat cells on a dedicated Lab-on-Chip*

-***CMOS technology*** to implement required microwave sources, sensors, applicators, detectors on the same chip

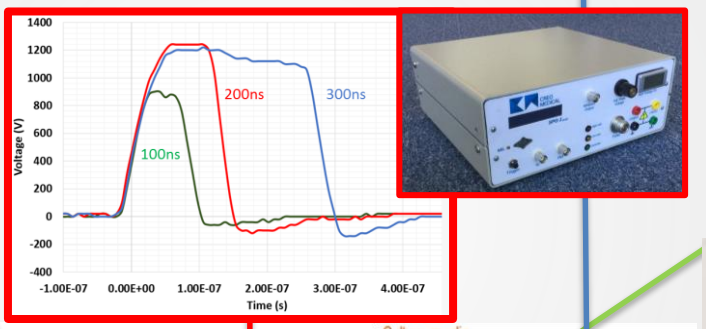
Challenges addressed by SUMCASTEC

Multidisciplinary expertise : Lab-on-Chip technology development, Electronic & RF design, Biophysics & BioEM, Off & On-chip experiments associated with CMOS foundry and Biologist teams including Clinicians & Surgeons

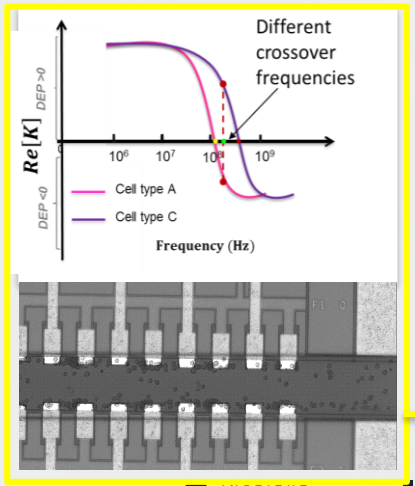
Lab-on chip experiments

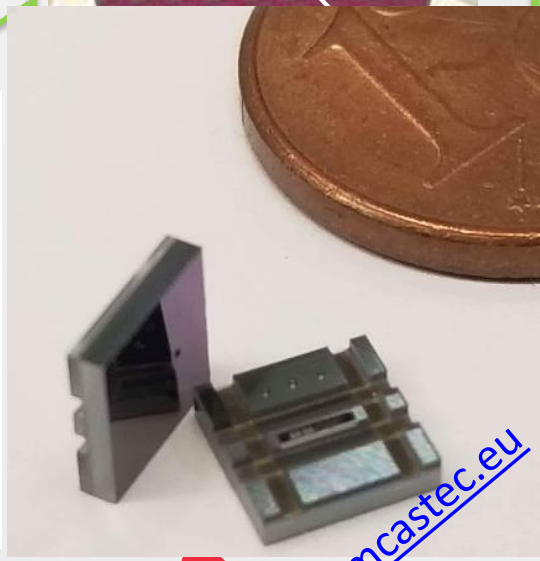


Specific EM stimulation generation



Cell electromanipulation & sensing

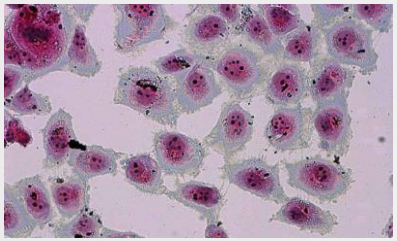




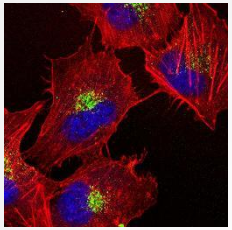
Though an fully instrumented lab on chip

How nowadays biologists can study CSC's?

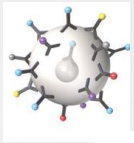
Optical microscopy



Staining

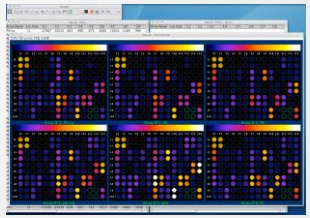


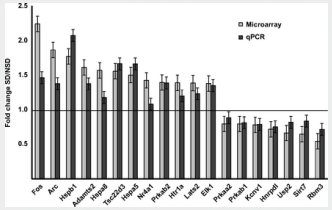
Fluorescence labeling



Flow cytometry

QPCR & Protein Array analysis





Drawback/ constrains:

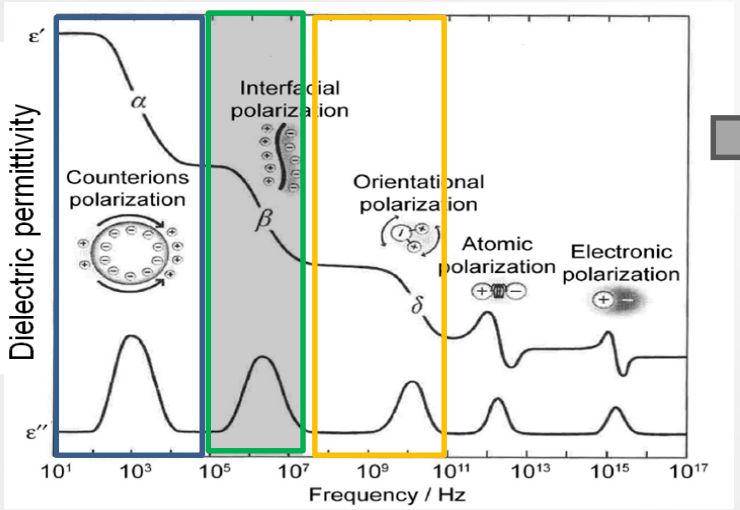
- ✓ *Specific label are lacking -> Cross coupling of generic markers*
- ✓ *CSC's are rare -> require amplification of the population*
- ✓ *Efficient functional tests exist (clonogecity, animal drafting) but results are very long*

➧ Others approaches investigating intracellular specificities?

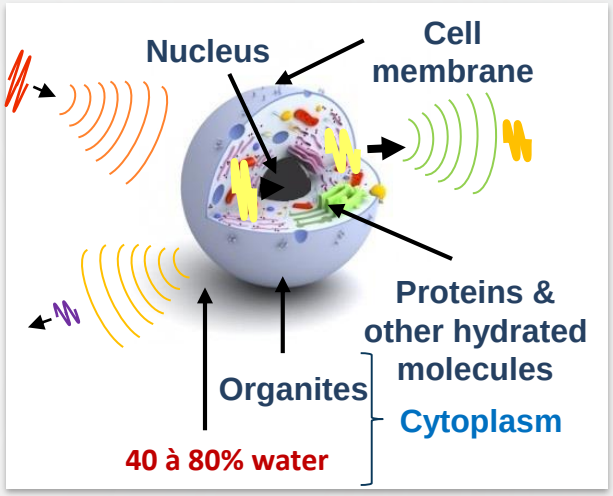
What about using EM field to characterize cells?

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

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

Related to the E field gradient intensity

-1<Re[K(ω)] <1

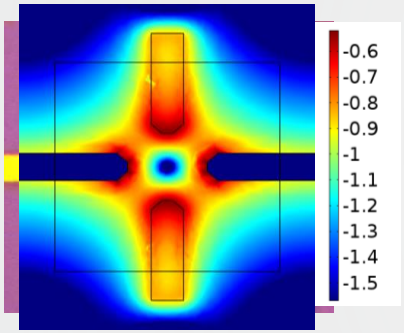
$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right)$$

Claussius-Mossotti factor

$$\varepsilon_p^* = \varepsilon_p - j\frac{\sigma_p}{\omega}$$

Complex permittivity of the particle

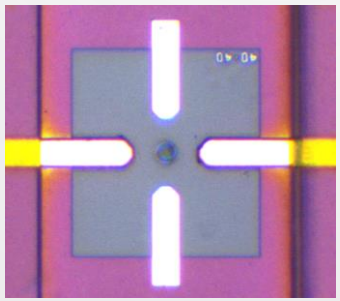
Electrical cage formed between electrodes



Quadrupole electrode system

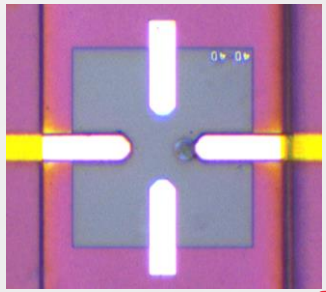
Re[K(ω)] <0

Repulsive force



Re[K(ω)] >0

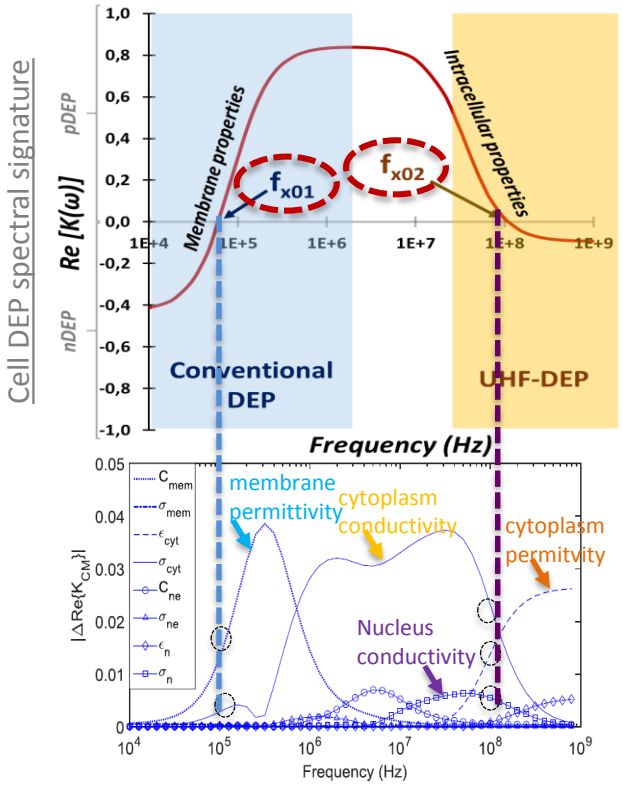
Attractive force



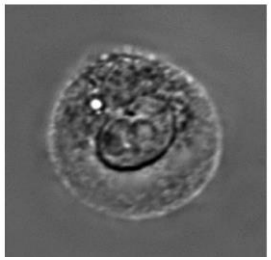
Cells can be individually electromanipulated accordingly their own dielectric properties

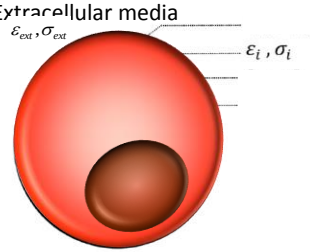
Specificities of cell DEP spectral signature

- Characterize cells to identify their 2nd DEP cross over frequencies as discriminant specificities



Dielectrophoresis theory basics

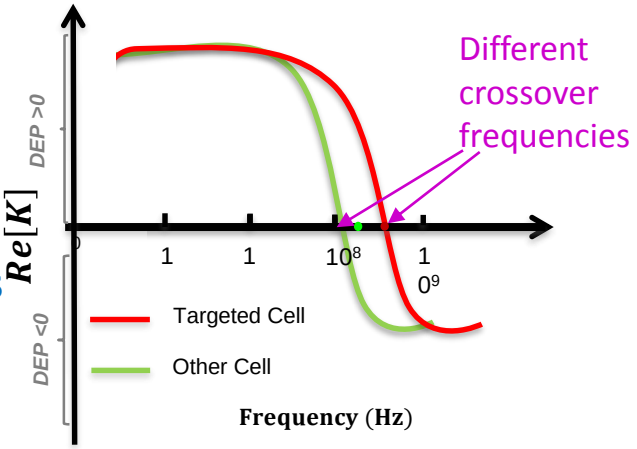




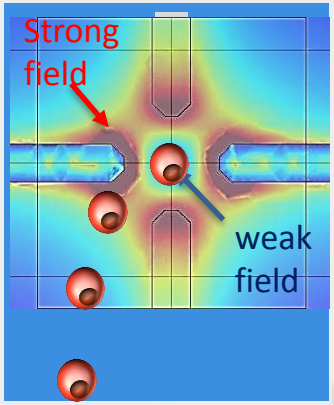
Different cells

Vs

Different spectral signatures



Methodology for cell crossover frequency measurement



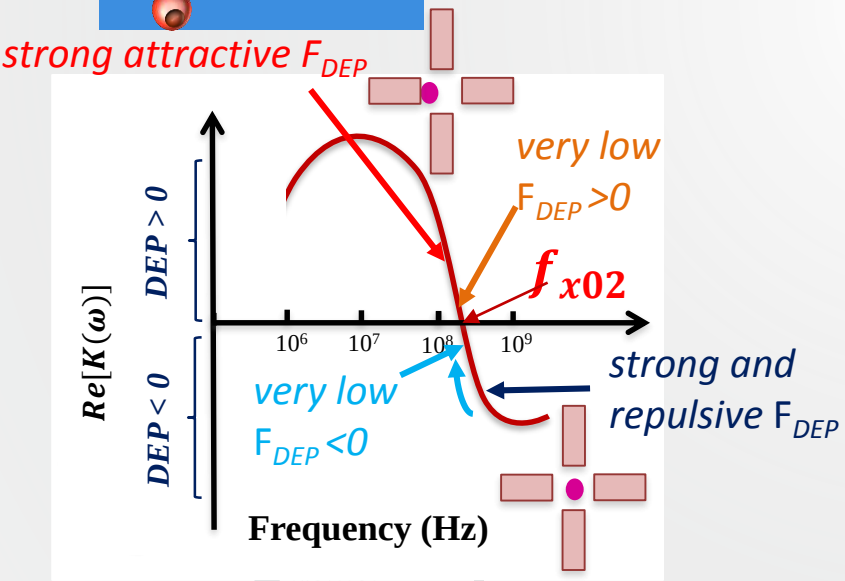
$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[K(\omega)\right] \nabla\left|E_{rms}\right|^2$$

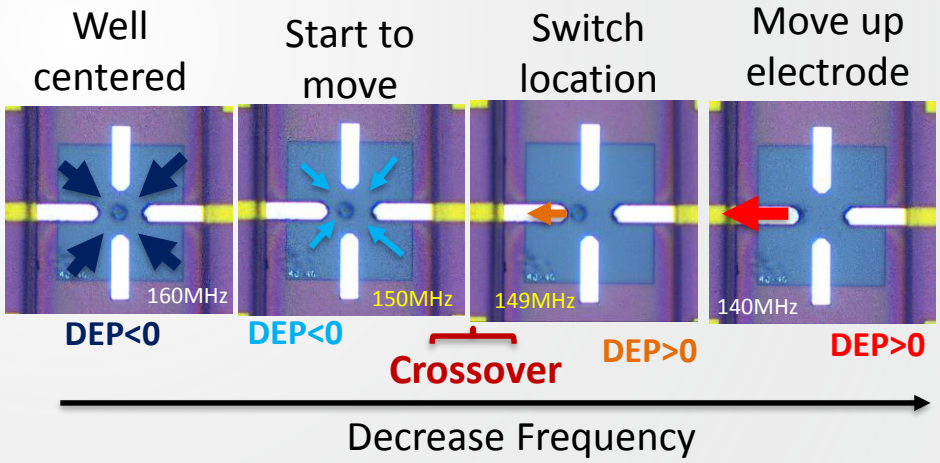
-> F_{DEP} will be high in strong field areas

-> low in weak field areas

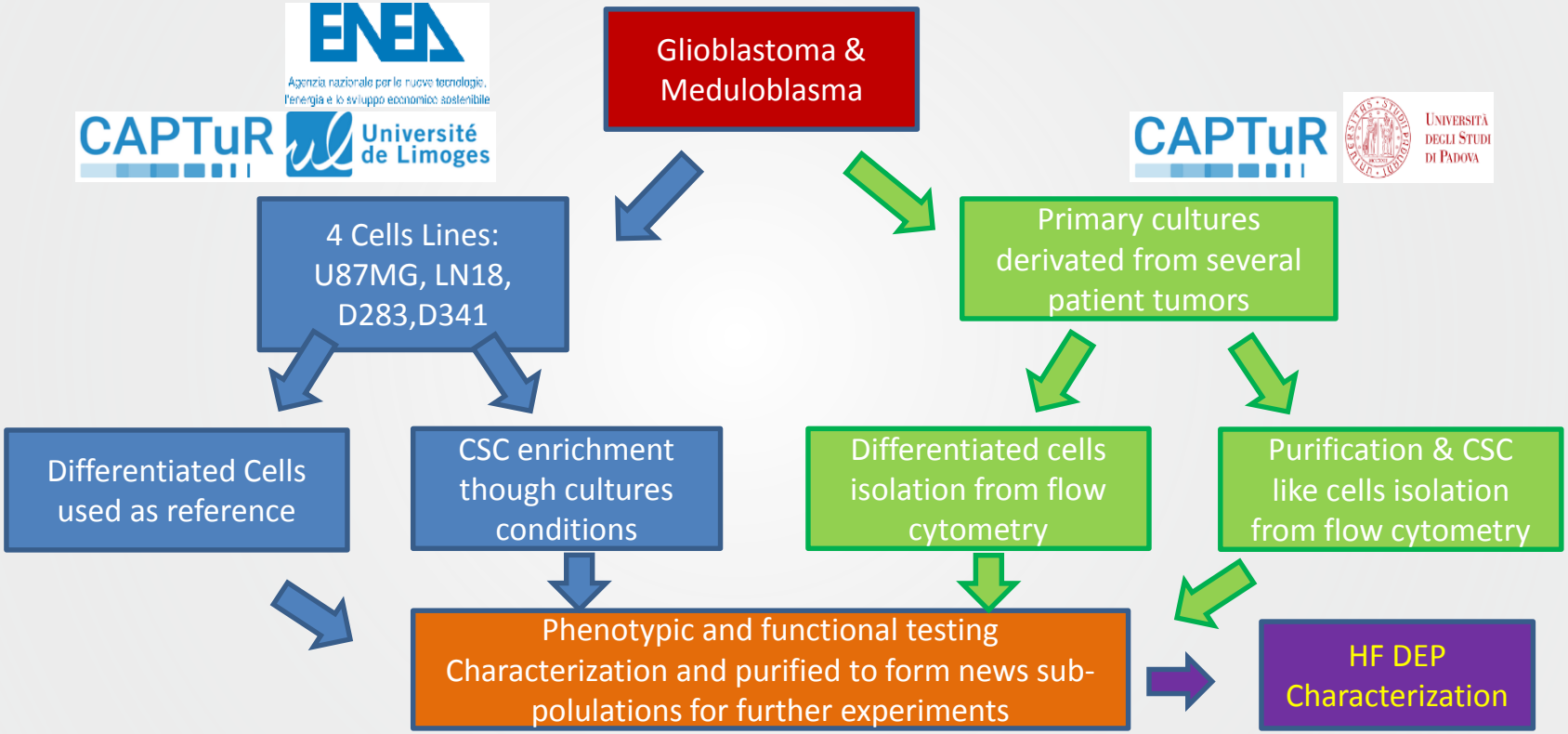
Methodology:

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



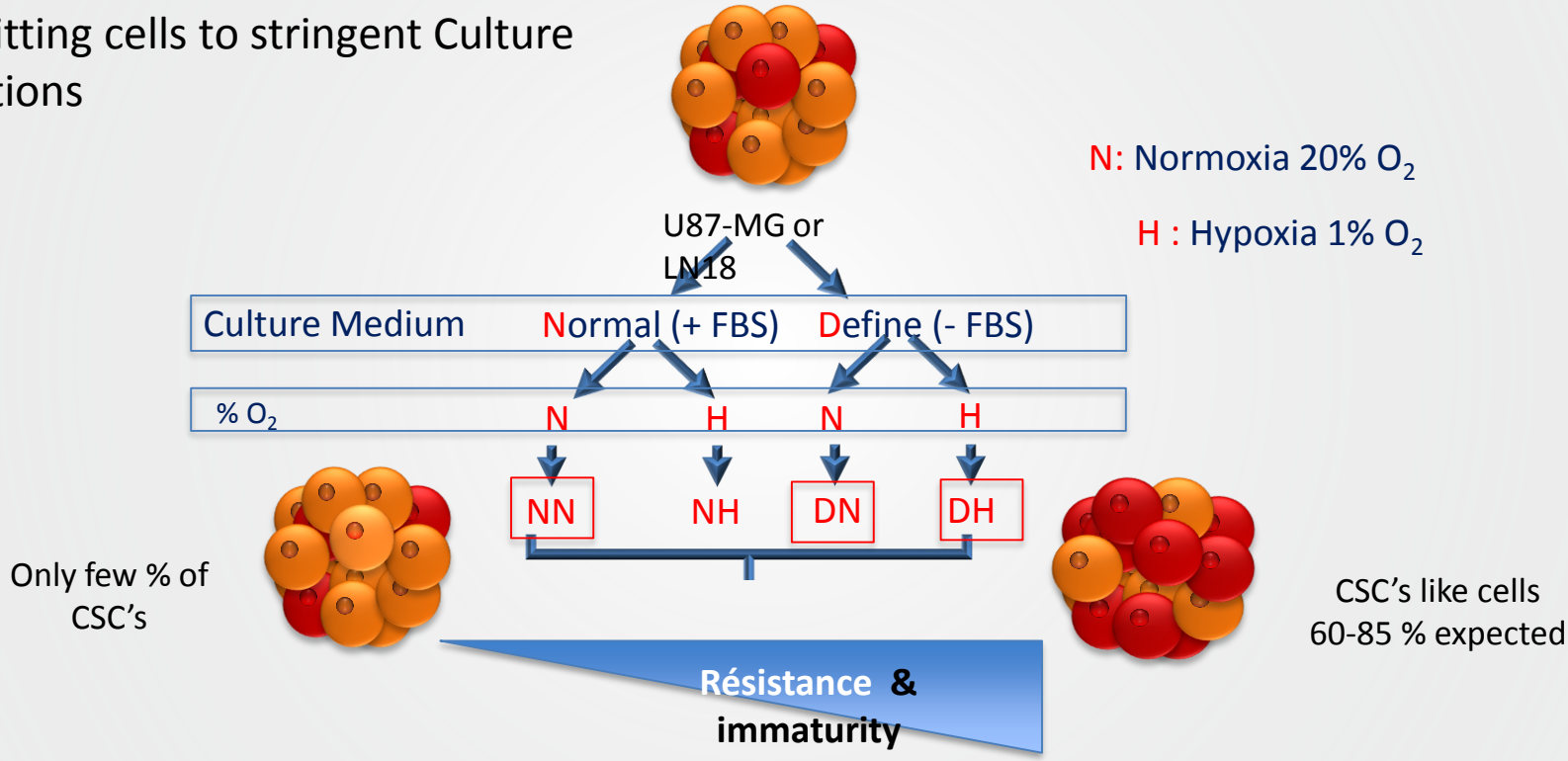


Followed Methodology for cell preparation



Enrichment in CSC's starting from cell lines

- Submitting cells to stringent Culture conditions

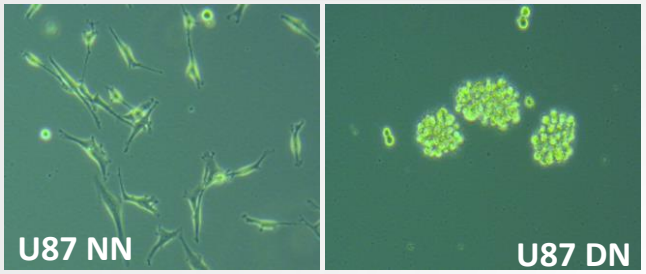


N: Normoxia 20% O_2

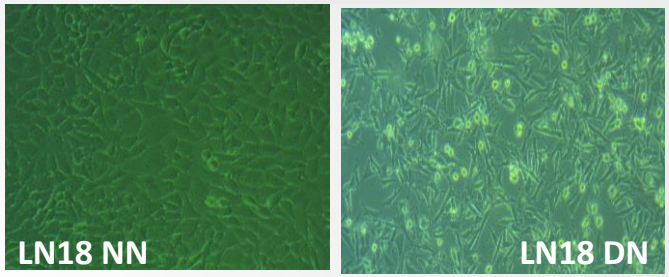
H : Hypoxia 1% O_2

Effect of culture conditions cell phenotype

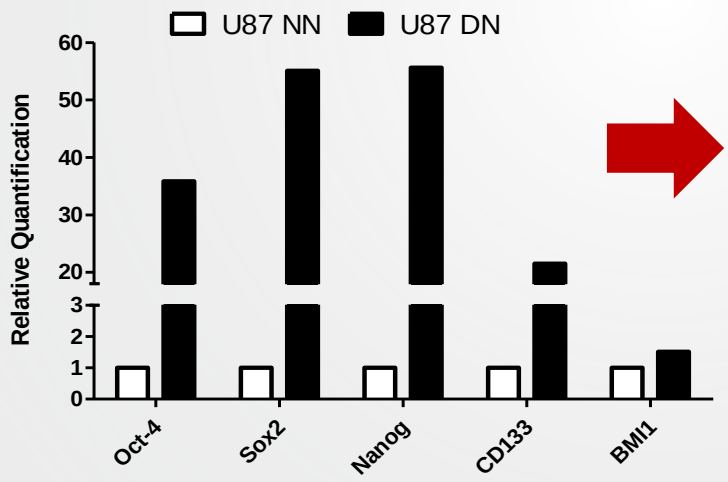
U87 NN vs DN

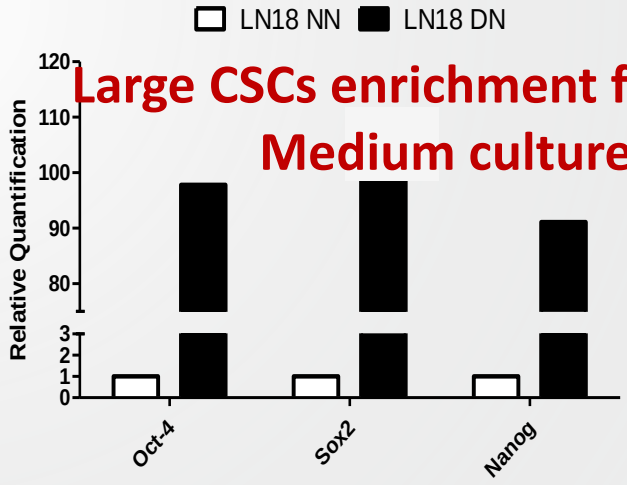


LN18 NN vs DN



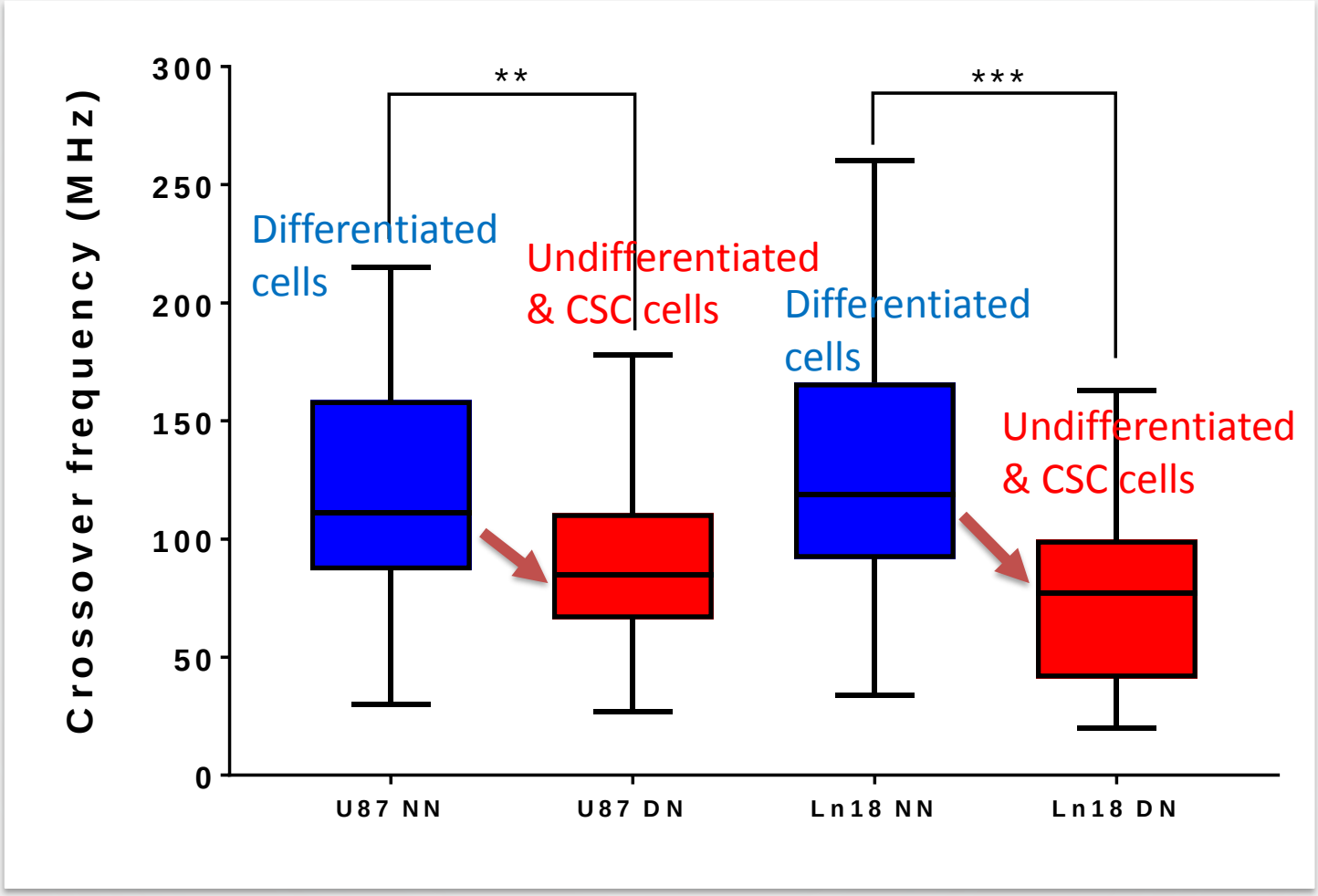
mRNA expression of Stem cell markers





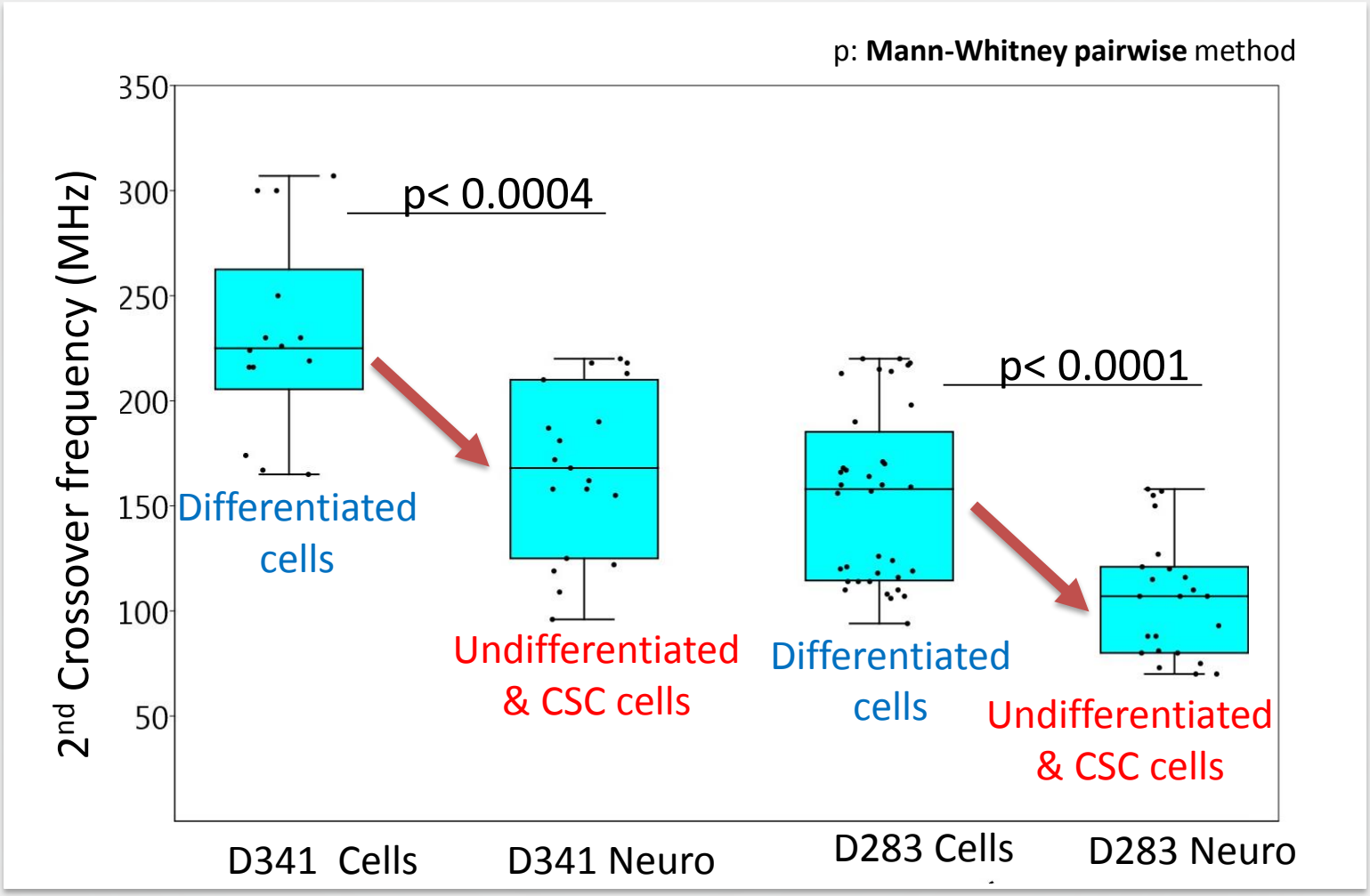
Large CSCs enrichment for Define Medium cultures

Measured DEP signatures on GBM lines



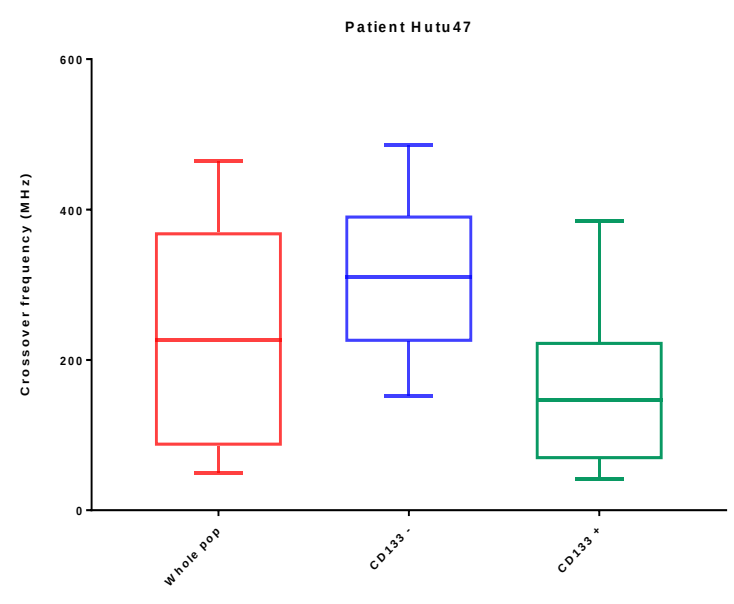
Difference of phenotype -> difference of DEP signature

Measured DEP signatures MB lines

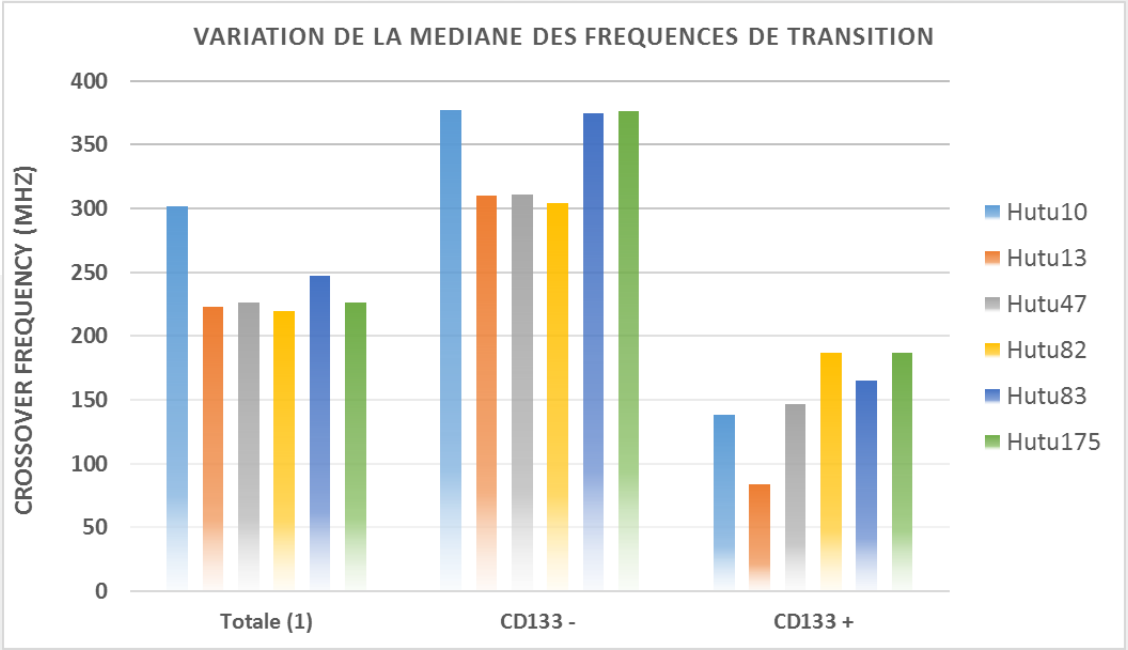


Difference of phenotype -> difference of DEP signature

Same Trend observed on primary culture



- 6 primary cultures coming from 6 different patients investigated
  - Cells sorted by FACS based on CD133 protein membrane expression



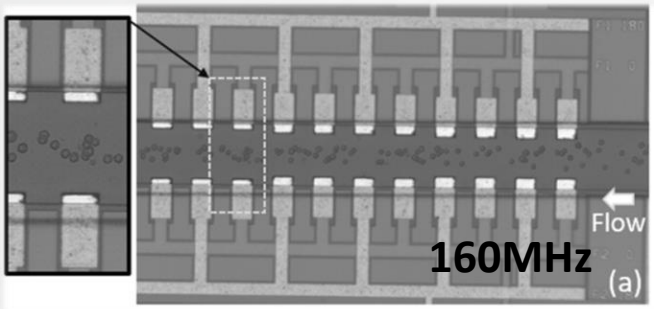
CD133⁺ cells show lower DEP signatures

Lab-on-chip approach to go to on-chip cell sorting

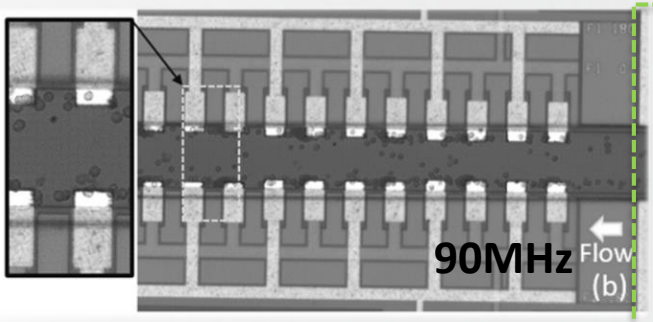
- Exploiting DEP signature difference to sort cells on a silicon chip

A. F_{DEP}> Fcross-over median

Cells are concentrated in microchannel center (where E field intensity is the lowest)



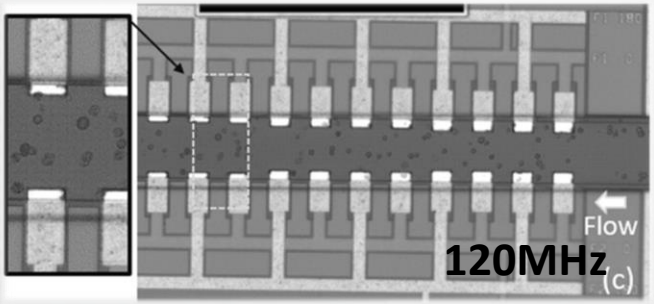
B. F_{DEP}< Fcross-over median

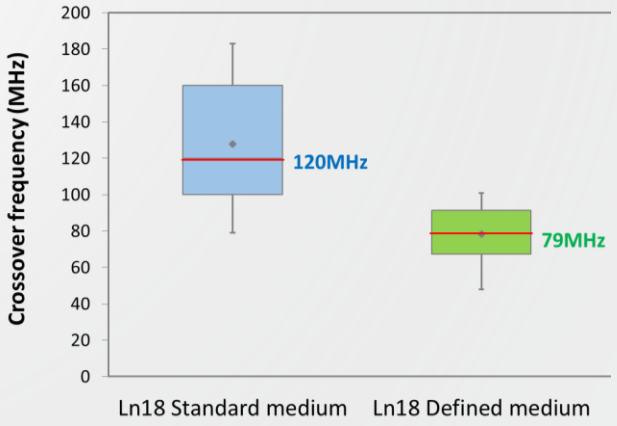


Cells are distributed on the edge of the channel

C. F_{DEP} ~ Fcross-over median

Cell spatial distribution is much disperse (repealed in the center / attracted to the channel edge)





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