UHF-Dielectrophoresis Crossover Frequency Measurements do Allow Discriminating Cancerous Stem Cells From Differentiated Cells.

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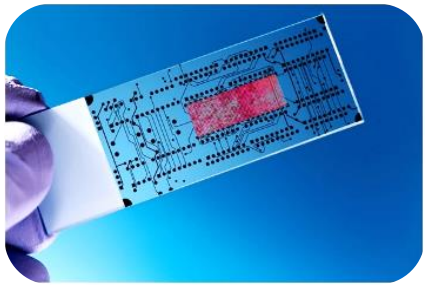
Outline

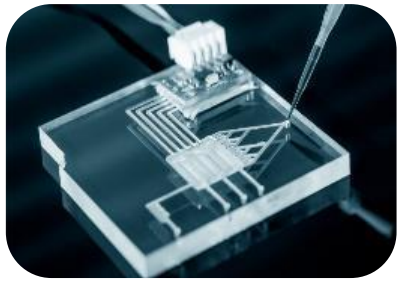
- SUMCASTEC project objectives
- Motivation: Targeting Cancerous Stem Cells
- Main challenges to identify CSC
- UHF dielectrophoresis as a new cell characterization approach
- Going to a novel UHF DEP cytometer for efficient CSC isolation?
- Conclusion and perspectives

The SUMCASTEC project

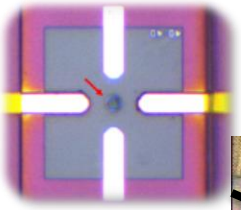
Sumcastec H2020 FET program supported by EU commission

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

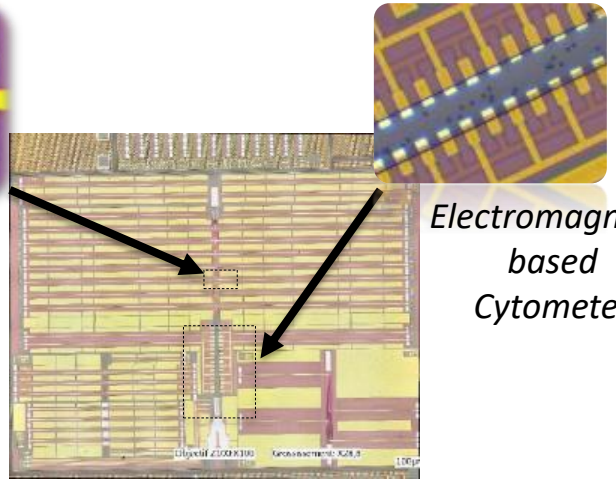




Concept: *Exploit the non-thermal effects of* ***EM radiations*** *on living organizes to* ***sense*** *and* ***stimulate*** *specifically targeted biological cells*



Individual Cell sensor



Electromagnetic based Cytometer

Methodology: *Take benefit of*

- ***Microsystem technologies*** *to individually treat cells on a dedicated Lab-on-Chip (LOC)*
- ***CMOS technology*** to implement required microwave sources, sensors, applicators and detectors on the same chip

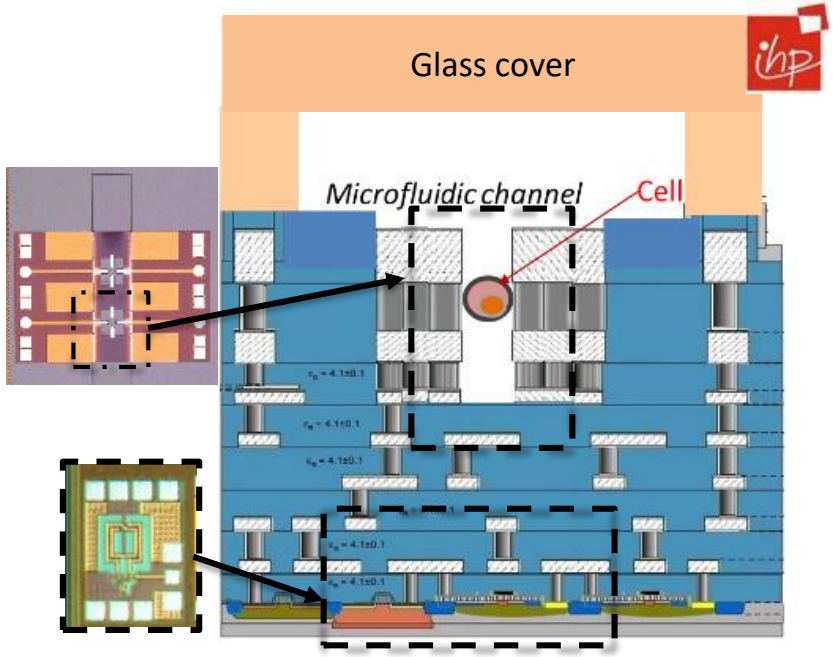
Prototype of microfluidic sensing platform on CMOS chip

Why CMOS technology?

Advantages of BiCMOS technology:

✓ Complete system integration with several electronic functions on the same chip

▶ ***Mature technology able to quickly address a large market***

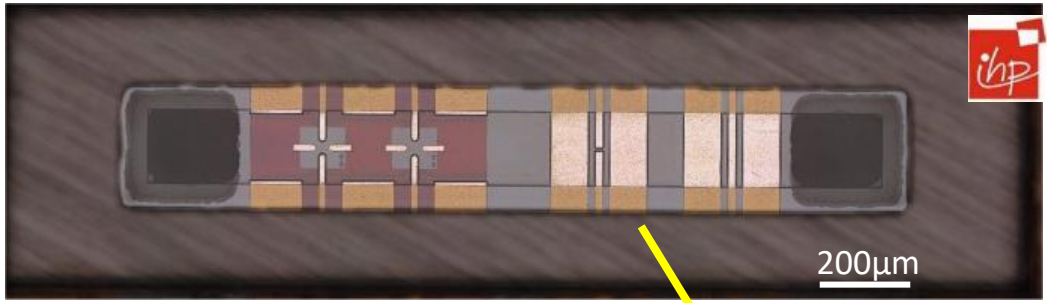


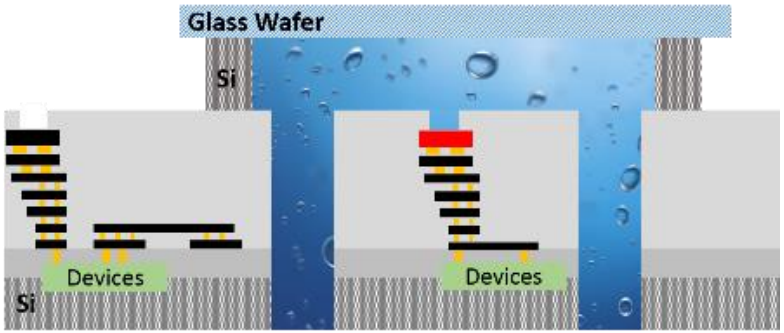
Instrumented CMOS lab-on chip

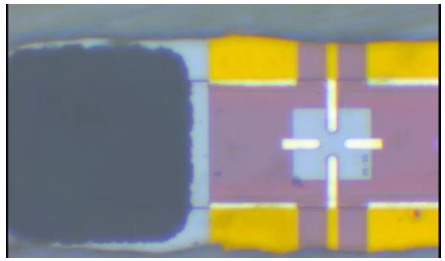
Advantages of BiCMOS technology:

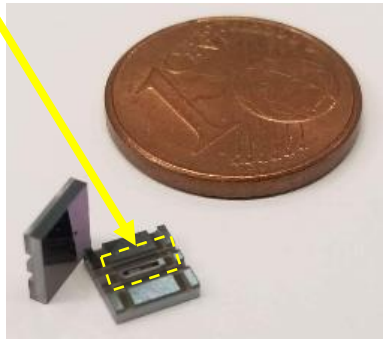
- ✓ Complete system integration with several electronic functions on the same chip
- ✓ Miniaturization of the complete device and Lab-On-Chip compatible

▶ ***Full and monolithic integration of microfluidic***







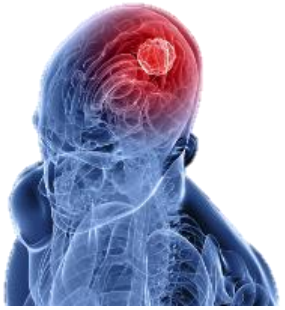


Motivation: Handling pathology with high recurrence

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*

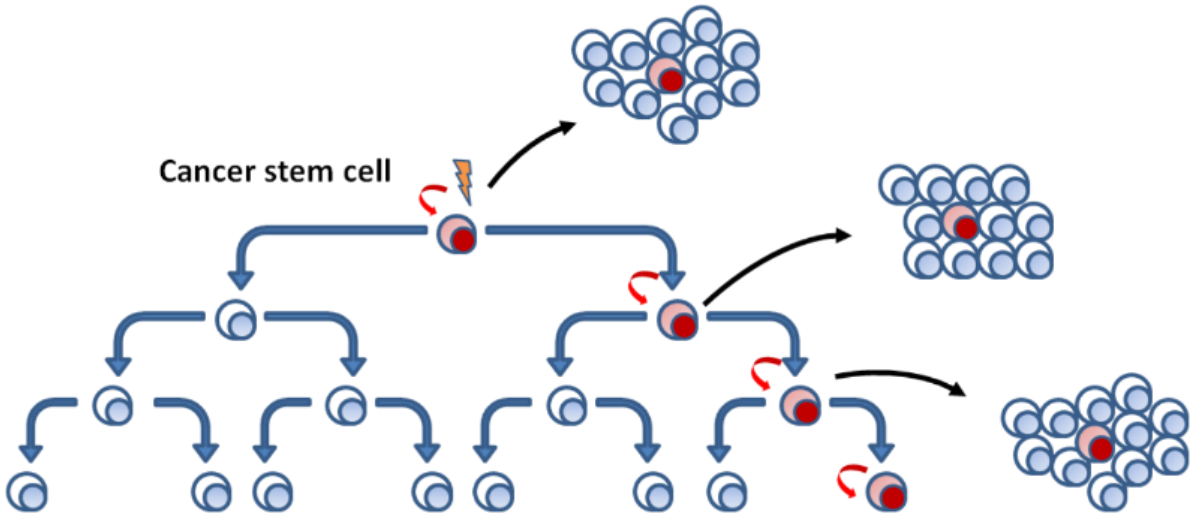
Role of some hidden tumor-initiating cells ?

How fight them more efficiently?
How many are they?
Where are they?

Cancerous Stem Cells

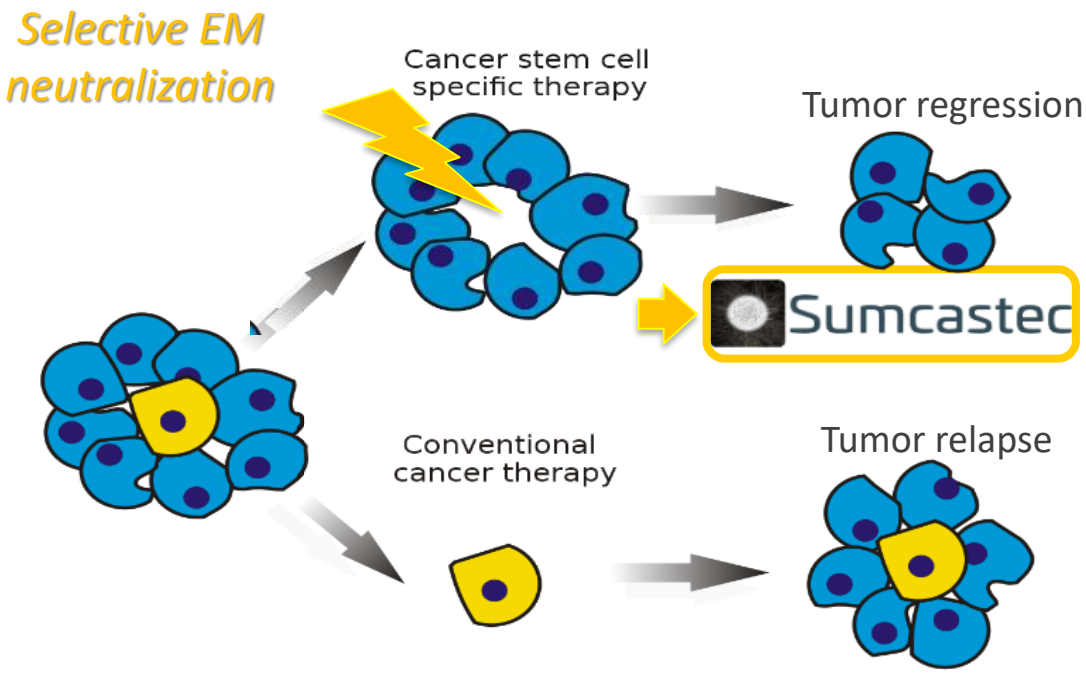
Tumorigenic cells with ability to give rise to all tumor cell types:

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



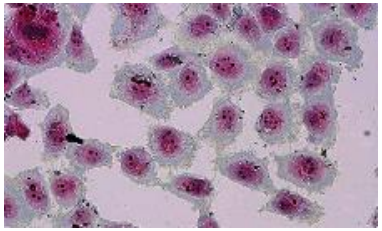
New therapies targeting CSCs

Quiescent properties -> Resistant to conventional chemo and ionizing treatments :

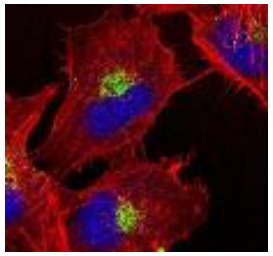


How biologists study CSC's currently?

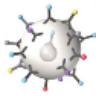
Optical microscopy



Staining



Fluorescence labeling

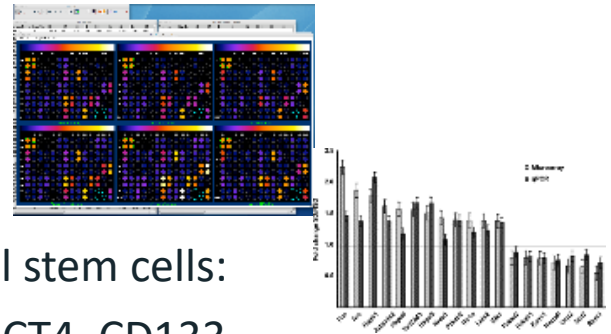


Flow cytometry

QPCR & Protein Array analysis

Main difficulties :

- *CSC's are rare and require amplification of population*
- *Specific* immunostaining markers are lacking



Stemness lineament are accessed using *generic markers* of normal stem cells:

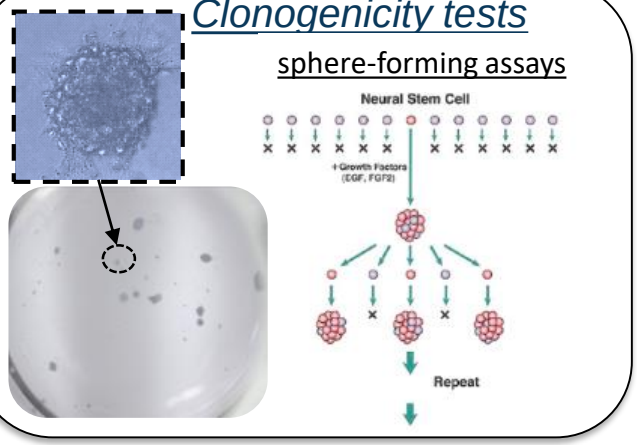
- *Undifferentiation & Anti proliferation markers* :Nanog, Sox2, OCT4, CD133…
- *Cross coupling* of makers gives evidence but without *100% absolute certainty*

Functional tests allow to identify CSC

Functional tests prove ability to renew a tumor mass

Clonogenicity tests

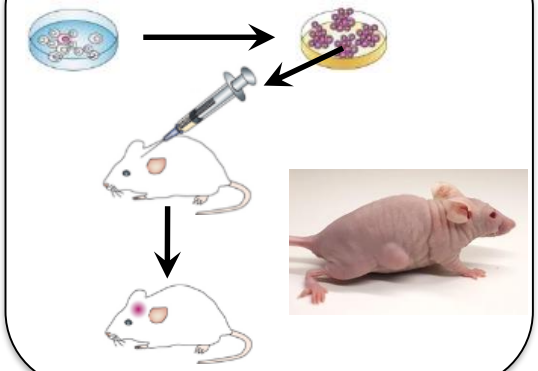
sphere-forming assays



But….
long (~20-40 days), costly and complex tests to implement

-> Never used in clinic..

Tumorigenicity tests

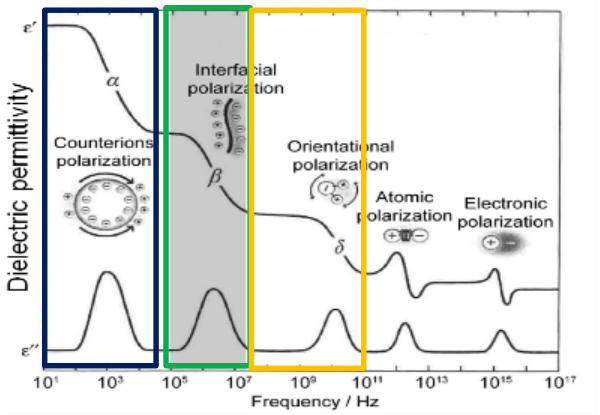


Interest to develop others approaches investigating intracellular specificities

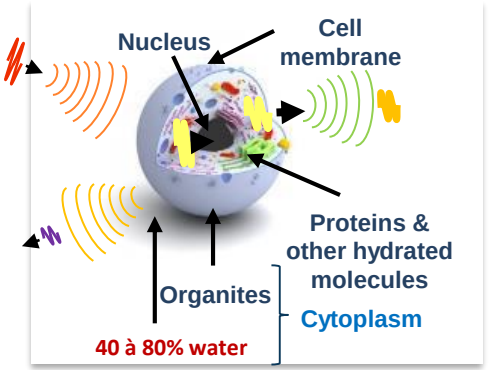
What about using EM field to identify CSC's?

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 basics

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

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

Related to the E field gradient intensity

-1<Re[K(ω)] <1

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

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

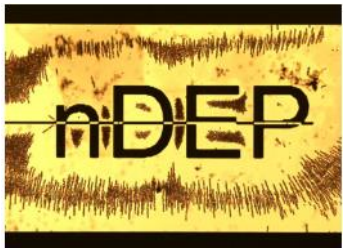
Complex permittivity of the particle

Clausius-Mossotti factor

Cells can be individually electromanipulated accordingly their own dielectric properties

Re[K(ω)] <0

Repulsive force

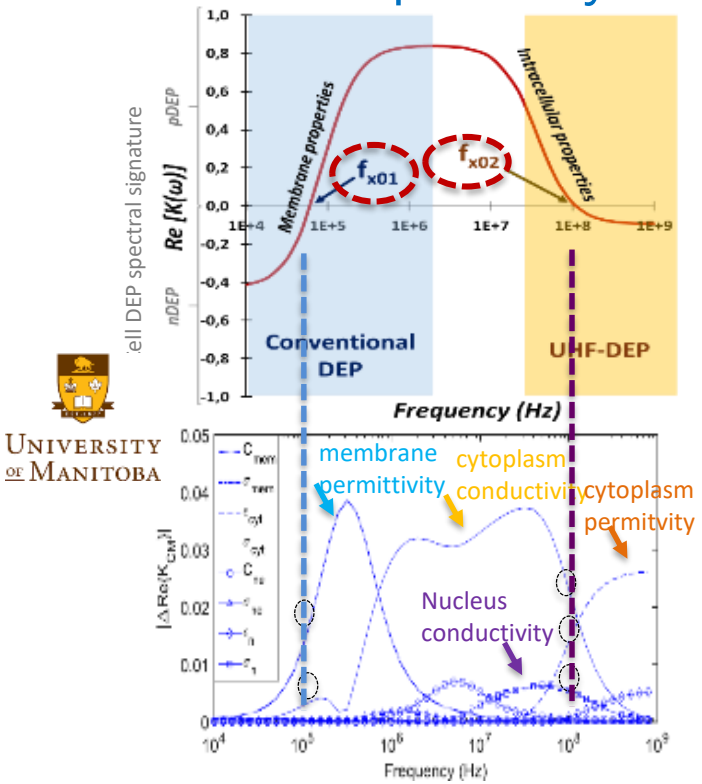


Re[K(ω)] >0

Attractive force

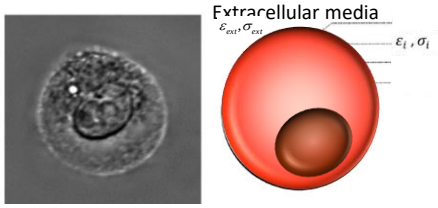
Specificities of cell DEP spectral signature

Characterize cells to identify their 2nd DEP cross over frequencies as a discriminant specificity



E. Salimi et al, DOI: 10.1063/1.4940432

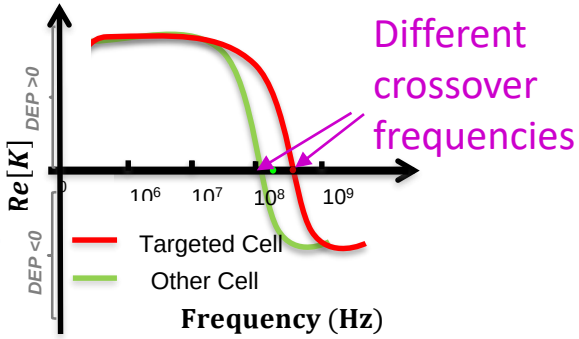
Dielectrophoresis theory basics



Different cells

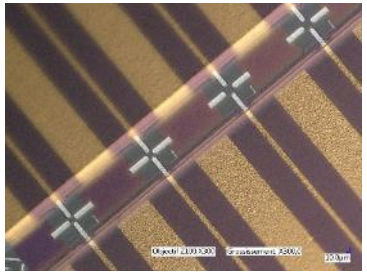
Vs

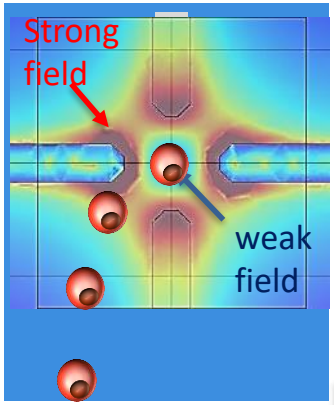
Different spectral signatures



f_{x02} is an intracellular marker!

Methodology for crossover frequency measurement





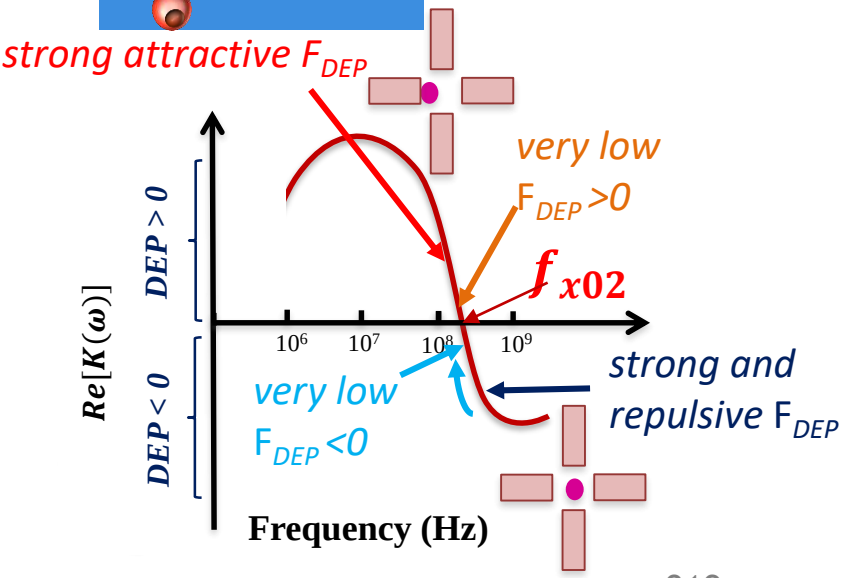
$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[K(\omega)\right] \nabla\left|E_{rms}\right|^2$$

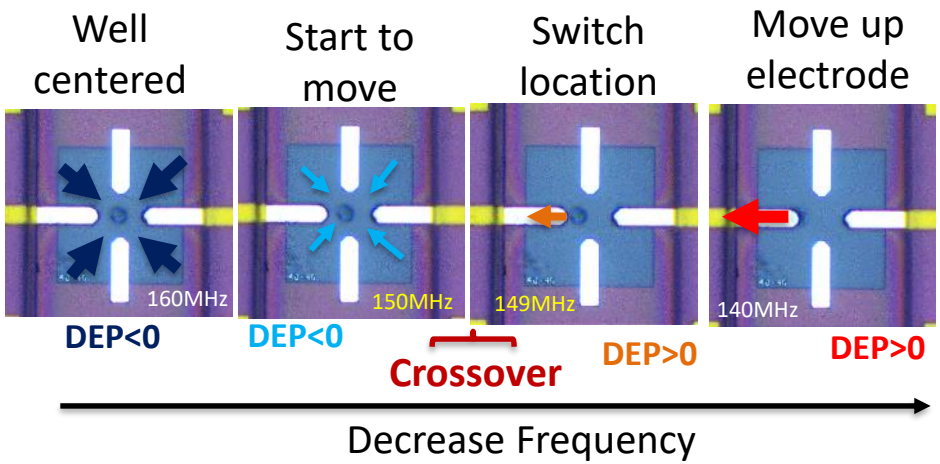
-> F_{DEP} *will be high in strong field areas*

-> *low in weak field areas*

<u>Methodology:</u>

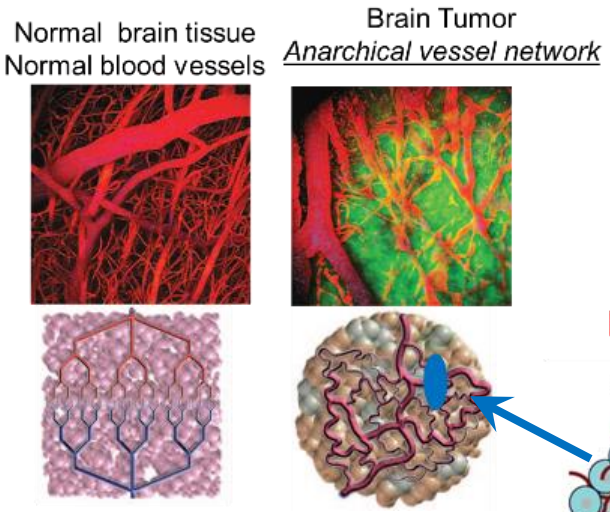
1) Cells are trapped in DEP<0
2) Flow is stopped
3) Frequency is tuned <u>every MHz</u> until finding positive DEP

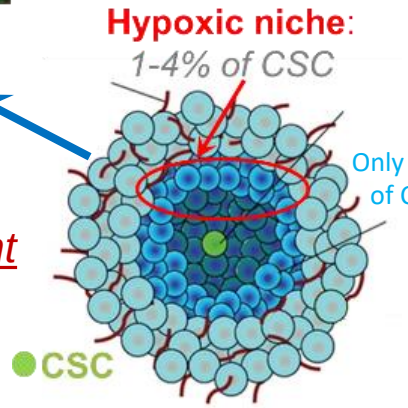




Obtaining CSC population starting from cell line

Mimic CSC micro environment conditions to enrich population

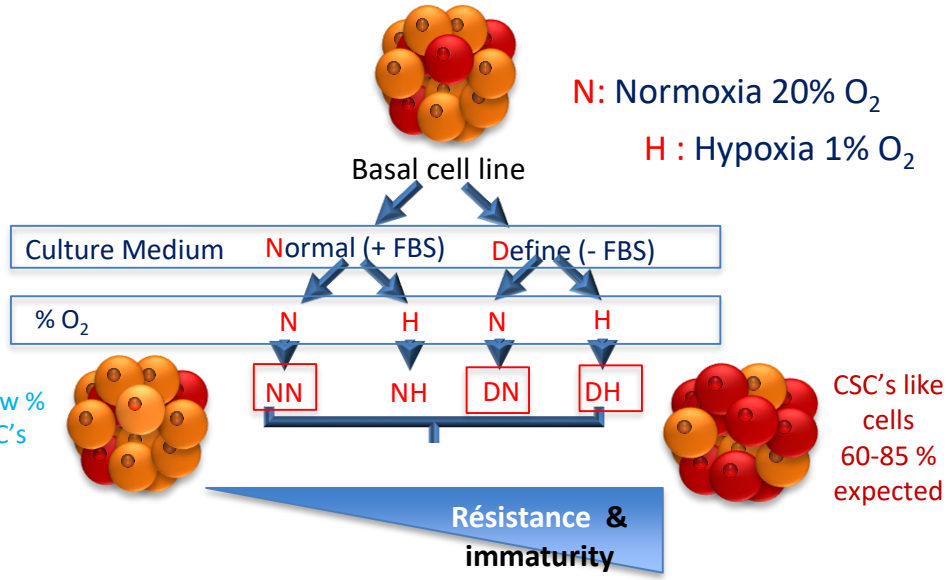




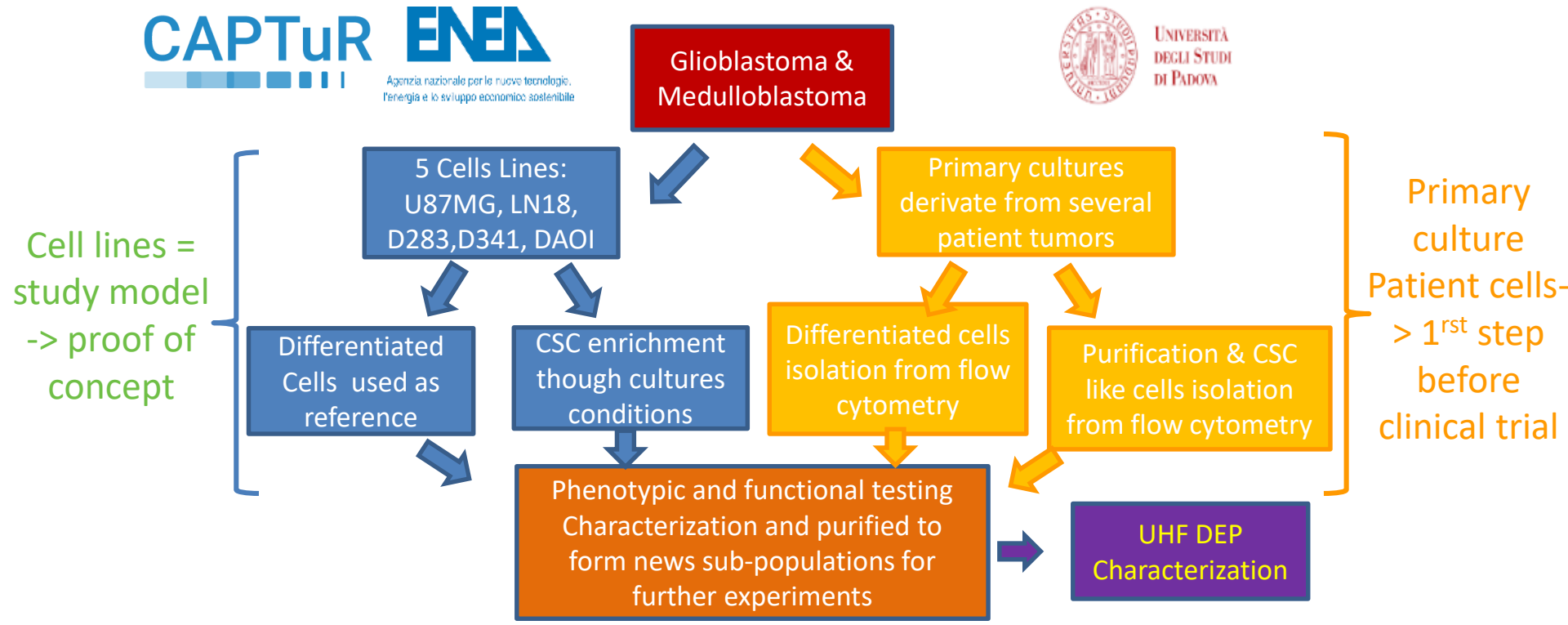
Specific micro environment

- 0_2<1%
- low growth factor concentration

➢ *Submitting cells to stringent Culture conditions*



Followed methodology for cell characterization



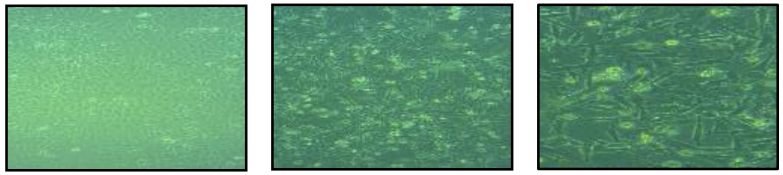
Confirmation of culture conditions influence on cell phenotype

Glioblastoma human cell lines: Analysis of CSC markers at transcriptional and protein level

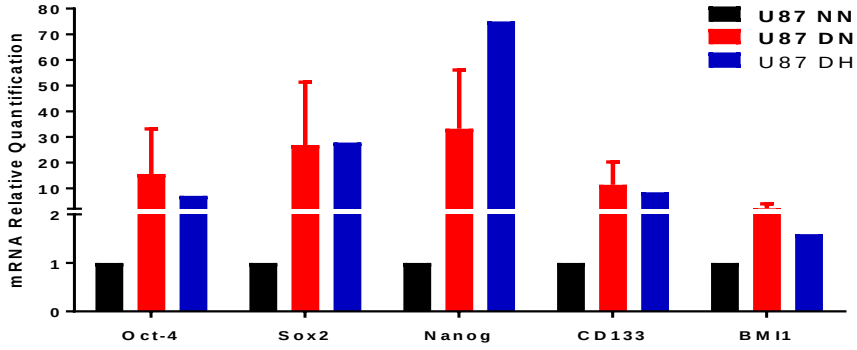
U87-MG Line

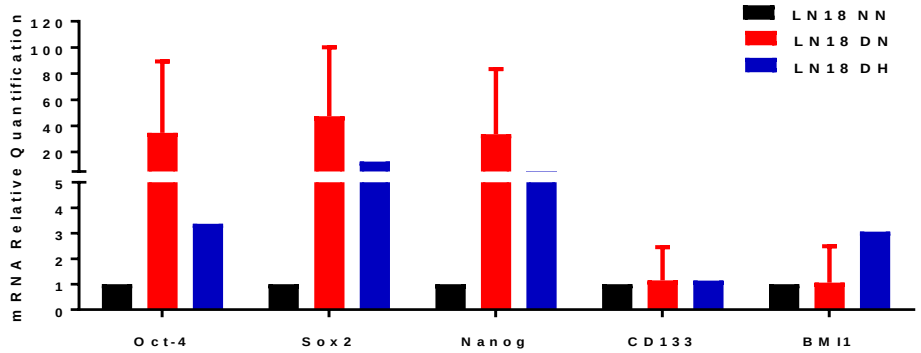
NN culture | DN culture | DH culture

LN18 Line



NN culture | DN culture | DH culture

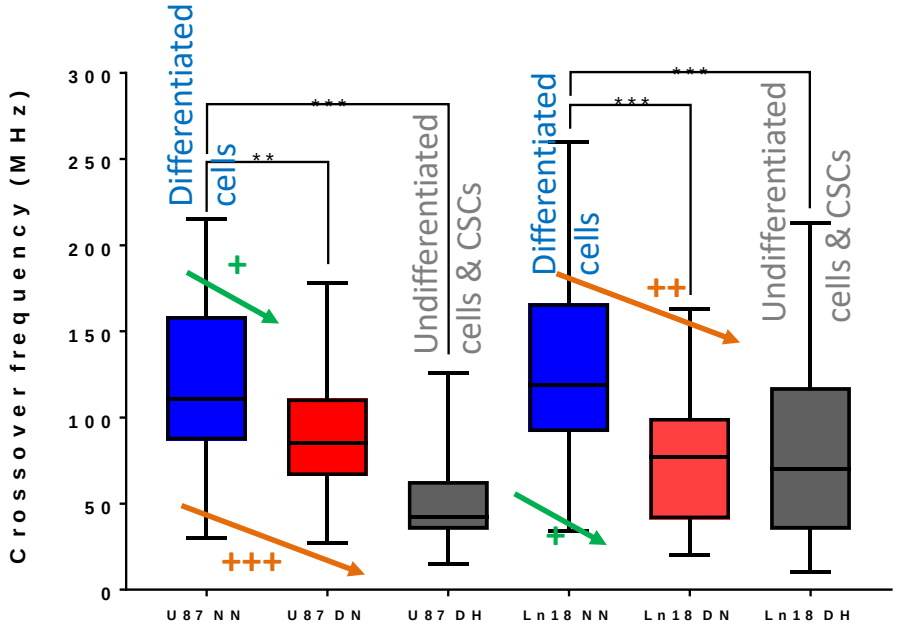




Large CSC enrichment for Define Medium cultures

Crossover frequency characterization of GBM cell lines

More than 500 cells measured



CSCs enriched populations show **lower** crossover frequencies

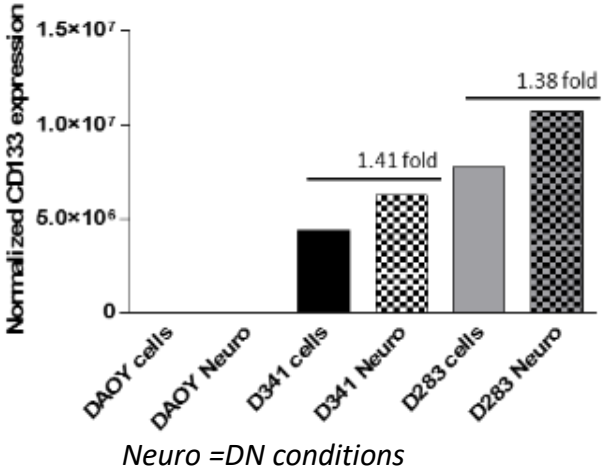
Negative correlation *between crossover frequency and CSC occurrence*

R. Manczak et al, DOI: 10.1109/JERM.2019.2895539

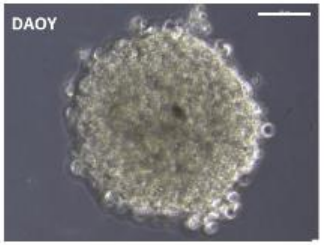
Difference of phenotype -> difference of DEP signature

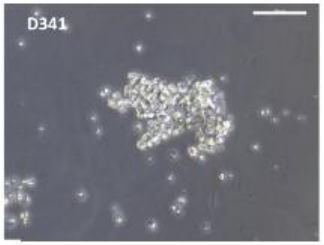
Culture conditions influence on cell phenotype & functional properties

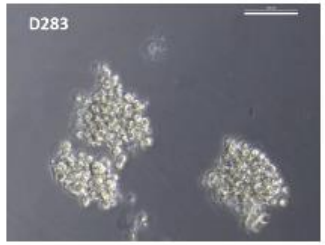
Medulloblastoma human cell lines:



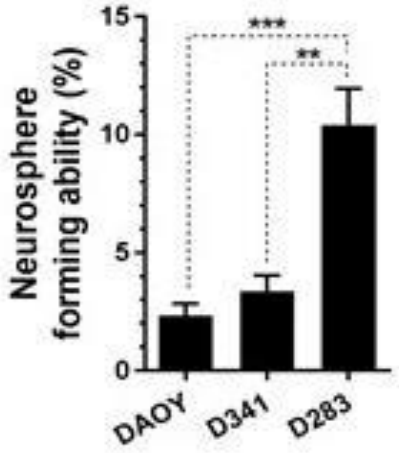
Neuro =DN conditions







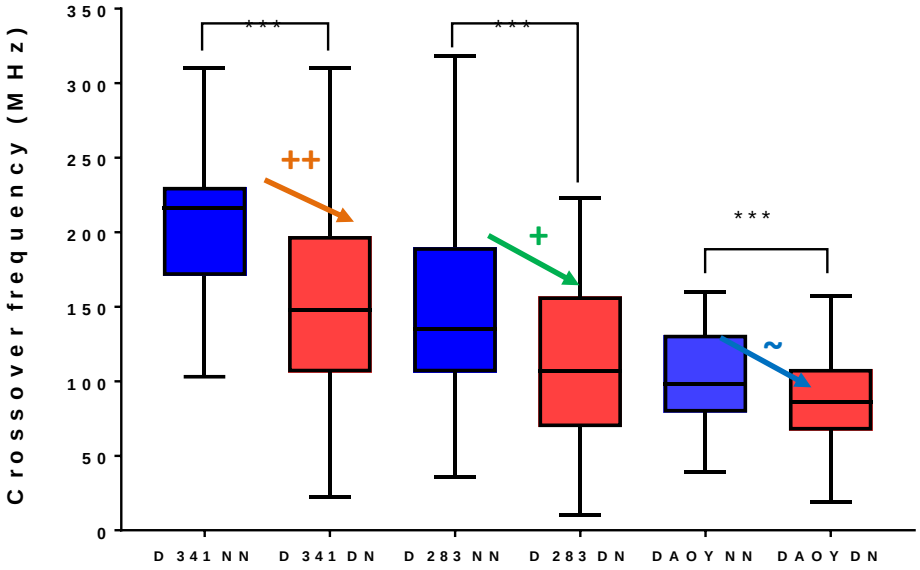
- ✓ D283 cells naturally express **high level** of CD133 and others CSC markers
- ✓ DAOY line shows **poor** CSC features



For D341 & D283, evidence of CSC enrichment in Define Medium cultures

Crossover frequency characterization of MB cell lines

More than 400 cells measured



Phenotypic analysis showed **highest** CSC number for:

- D283: **NN**$^{+}$ or **DN**$^{++}$ culture
- D341: **DN**$^{++}$ culture

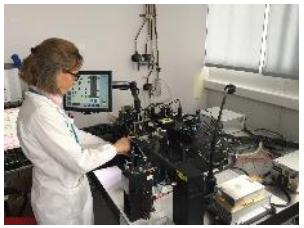
➢ *DAOY: **NN**$^{-}$ or **DN**$^{-}$ culture = poor/ no CSC-> similar signature expected*

***Negative correlation** between crossover frequency and CSC number*

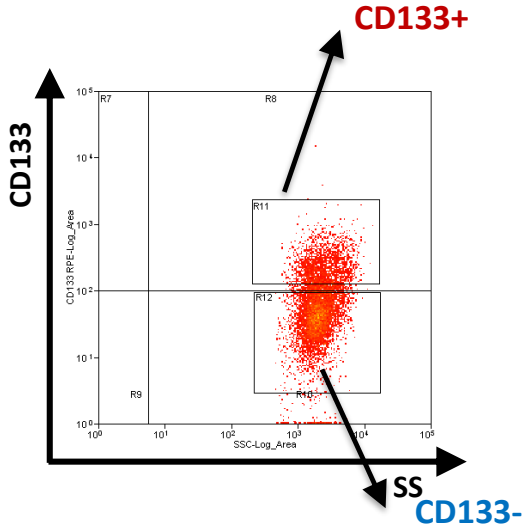
Difference of phenotype -> difference of DEP signature

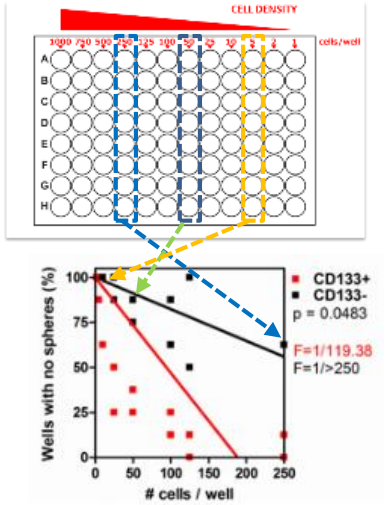
What about primary culture?

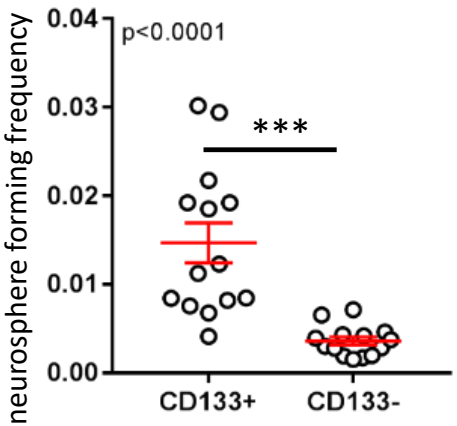
17 patient glioblastoma tumors investigated



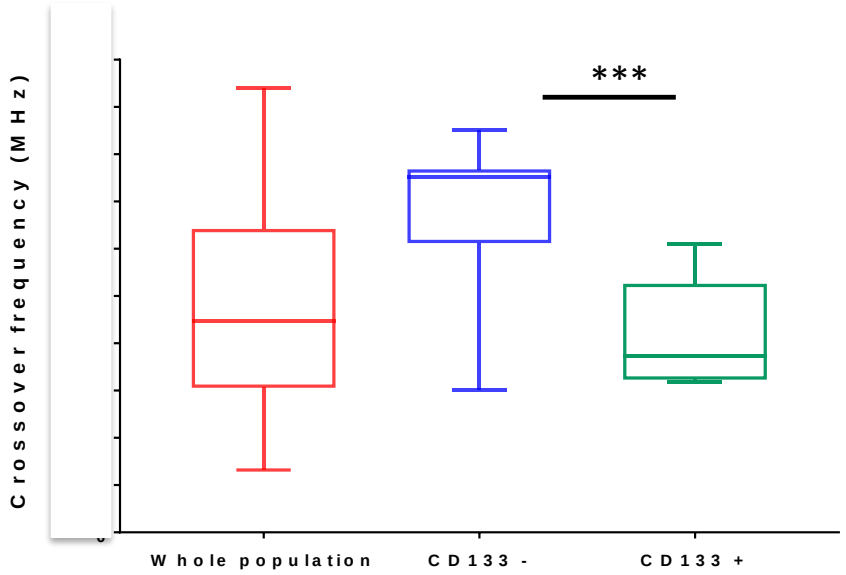
- Cells expressing CSC protein membrane markers are isolated by fluorescence flow cytometry
  - Sub population phenotype and functional features are tested
    - Ability to renew tumor evaluated by LDA







Crossover frequency of GBM primary culture cells

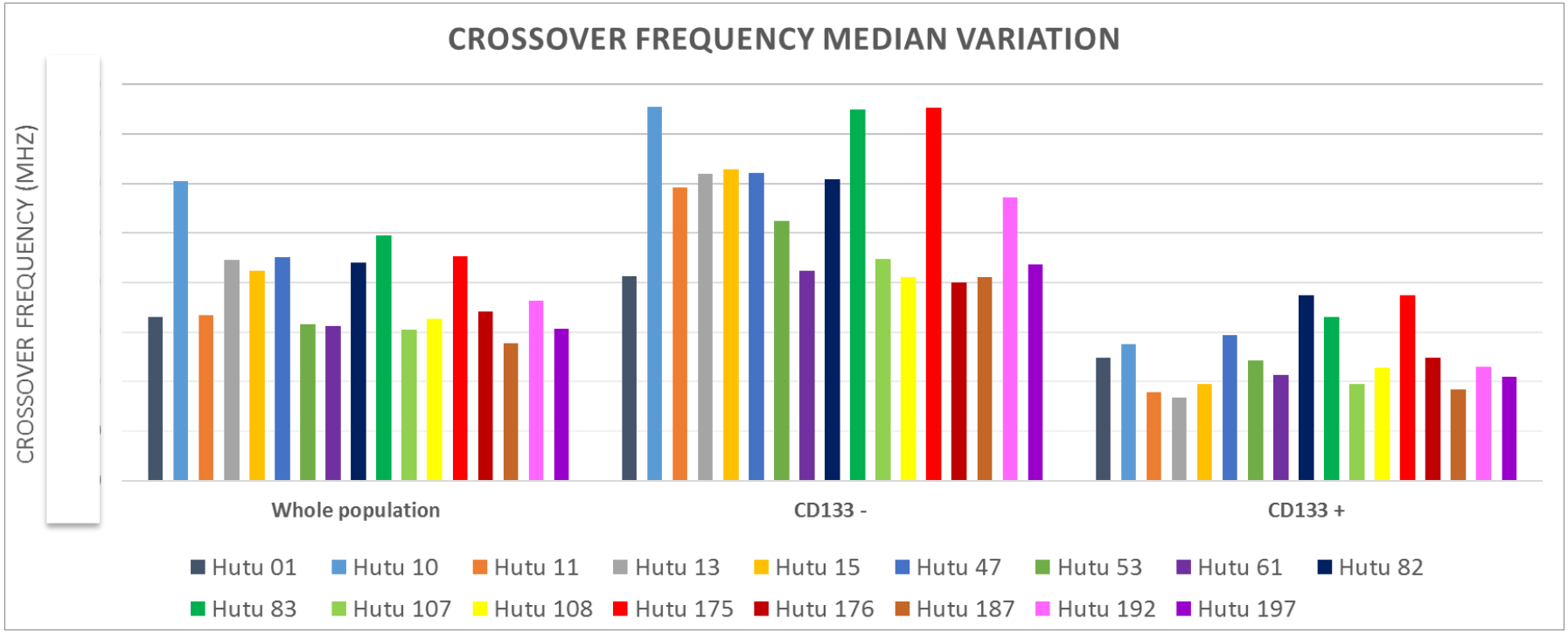


Clear difference of signature

*Correlation between difference of crossover frequency and expression level of CD133 and so **CSC occurrence***

CD133⁺ cells also show lower DEP signatures

Crossover frequency of GBM primary culture cells



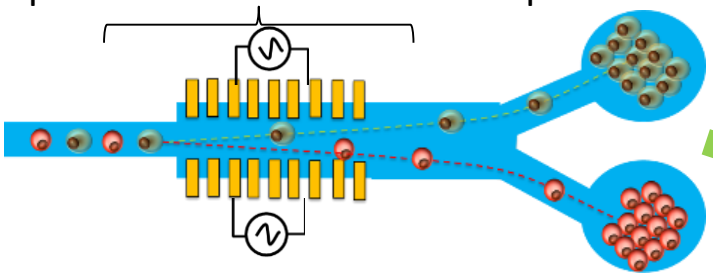
Whatever patient considered CD133⁺ cells always show lower DEP signatures

UHF-DEP crossover frequency appears as relevant CSC marker!

How exploiting cell crossover frequency specificities

Prior cell population characterization will help to select the more selective sorting UHF-DEP frequency

Expected selective electromanipulation



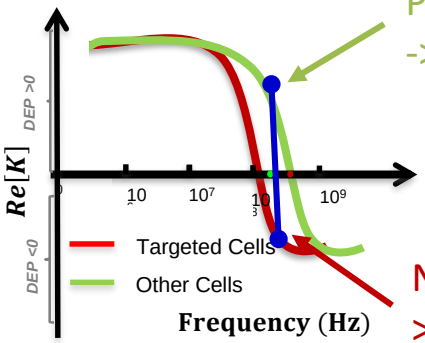
f ? ➡ f >f_{x02} target

➢ *Selected approach: gradual cell deviation using single frequency biasing*

Mixed cell population

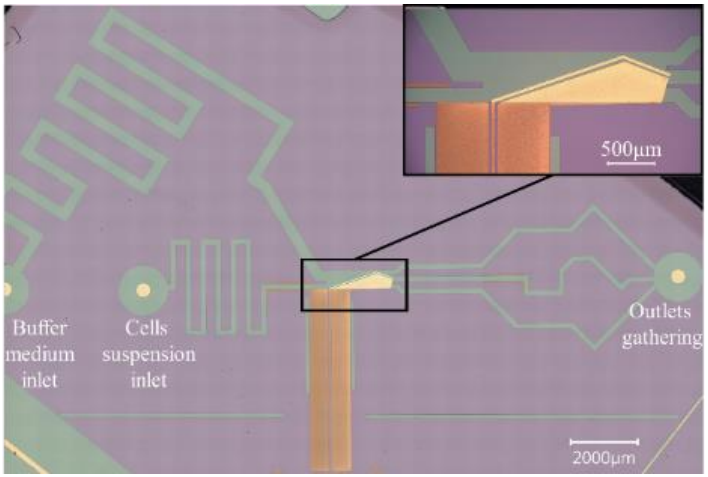
Vs

Different DEP spectral signatures



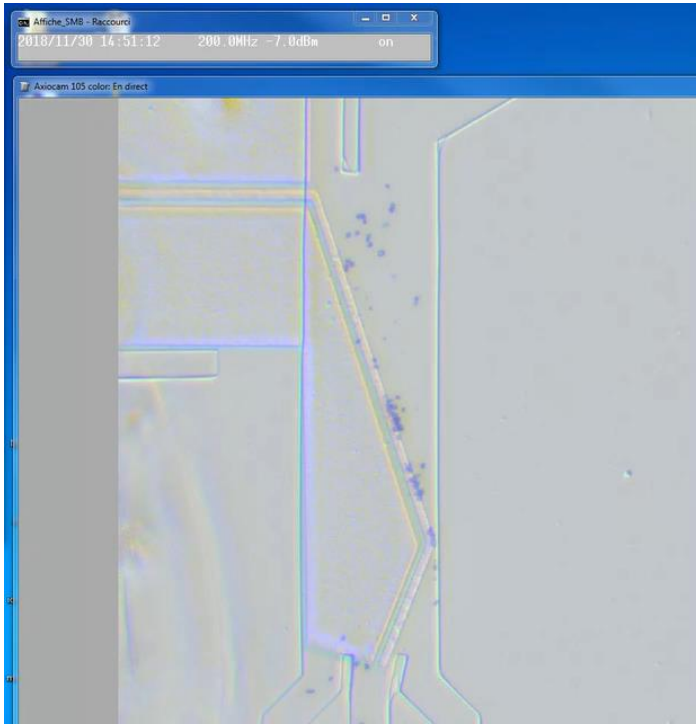
Positive DEP -> attraction

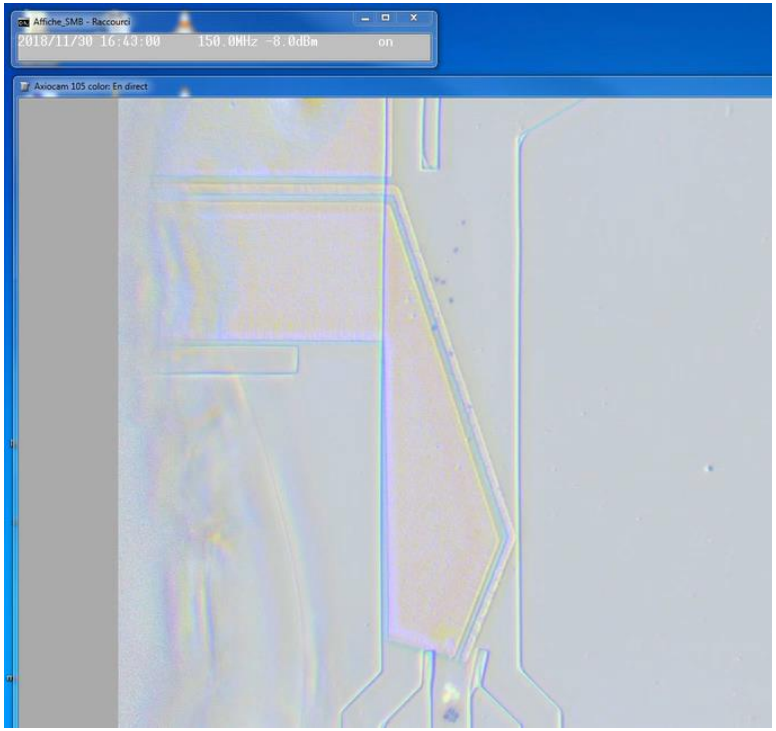
Negative DEP -> repeal



T. Provent et all, IMS 2019

Cell sorting on a lab-on chip





Conclusion

- Wide Potential of EM waves use for oncology purposes: Diagnostic therapeutic
- Example of actual need for better Cancerous Stem Cells study and handling offer possibility to develop and work on new & original approaches though very interesting and fruitful transdisciplinary research
- Collaborative work between different community is the key to reply to such complex societal challenges
- Regarding SUMCASTEC targeted objectives: a lot of work is still required
  - Proof of concept still need to be push away and fully demonstrated
  - Pre clinical trials might be set
  - Extension to other diseases envisioned

Acknowledgement

Sumcastec : *This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement N° 737164*

Project partners: