



Des ondes électromagnétiques peuvent-elles être utilisées pour identifier et neutraliser des cellules souches cancéreuses?

Vers de nouveaux laboratoires sur puces instrumentées en technologies CMOS

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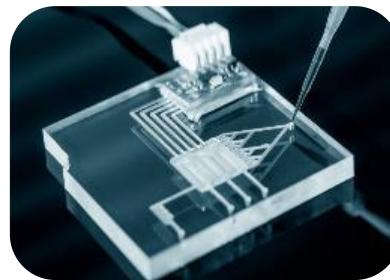
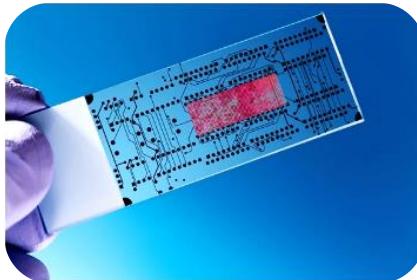
⁶CREO Medical, Bath, UK

The SUMCASTEC project

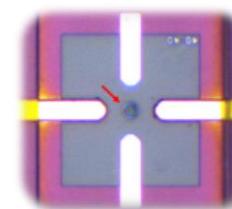


Sumcastec H2020 FET program supported by EU commission

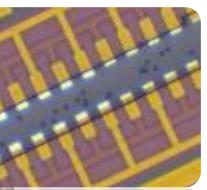
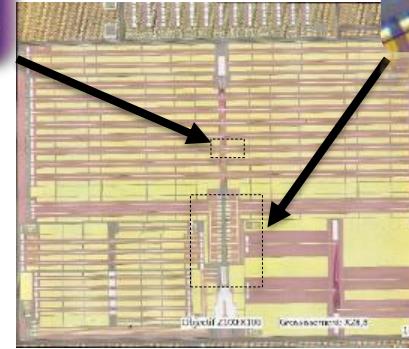
New Generation of Microwave Lab-on-Chip for **Cancerous Stem Cells** Neutralization using Electromagnetic Waves Stimulation



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



Individual
Cell sensor



Electromagnetic
based
Cytometer

Prototype of microfluidic sensing platform on CMOS chip

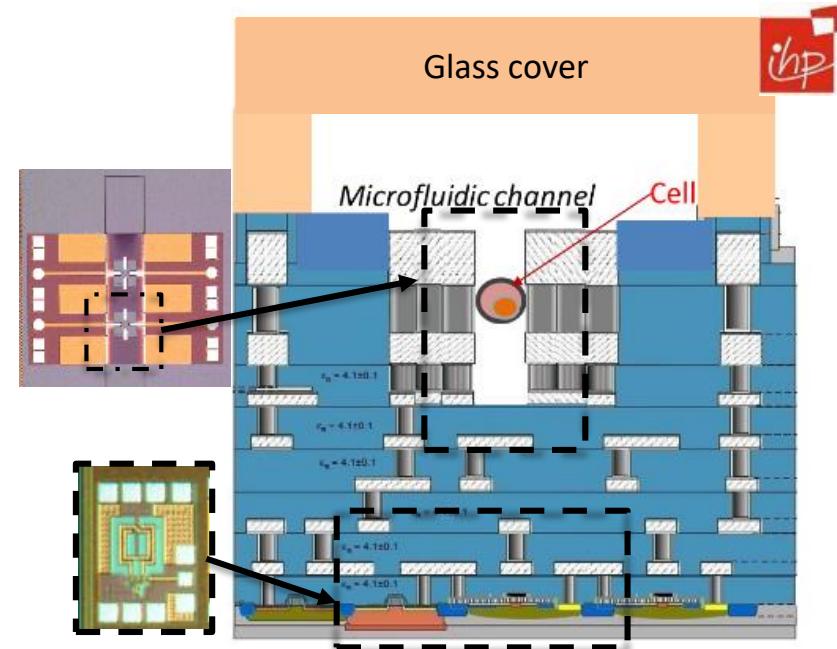
Why CMOS technology?

Advantages of BiCMOS technology:

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



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

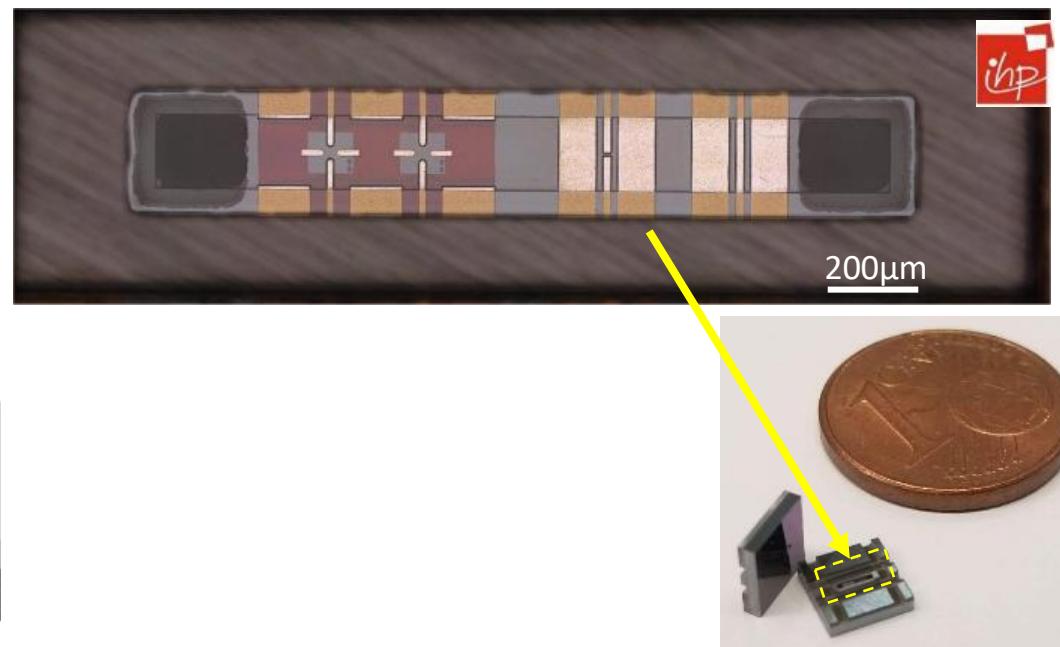
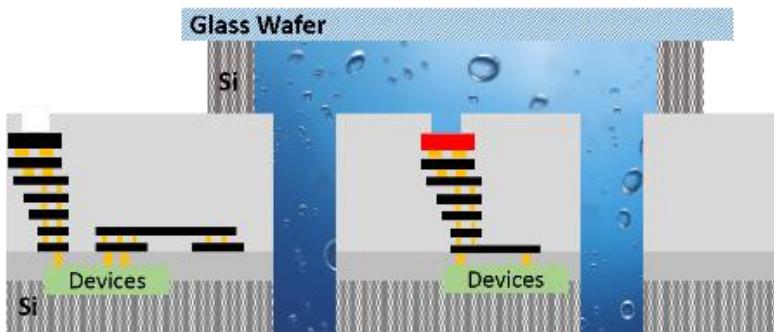


Why CMOS technology?

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

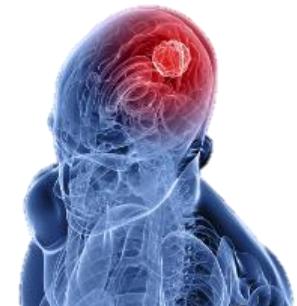


Motivation: Handling pathology with high recurrence

Need for new therapeutic strategies dedicated to poor outcome diseases

Ex: Medulloblastoma ,
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

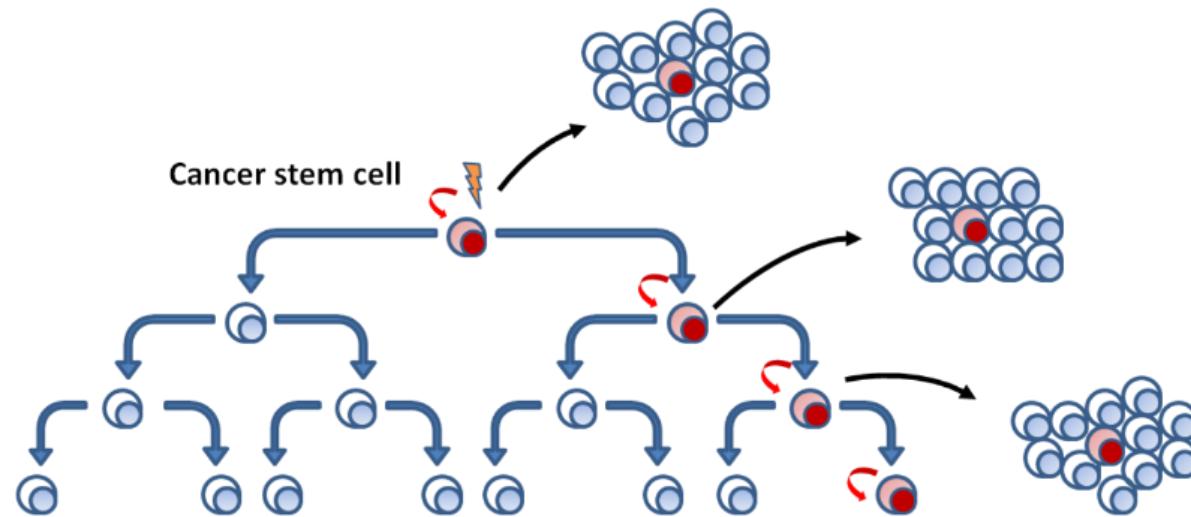
} **Role of some hidden tumor-initiating cells ?**

*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:

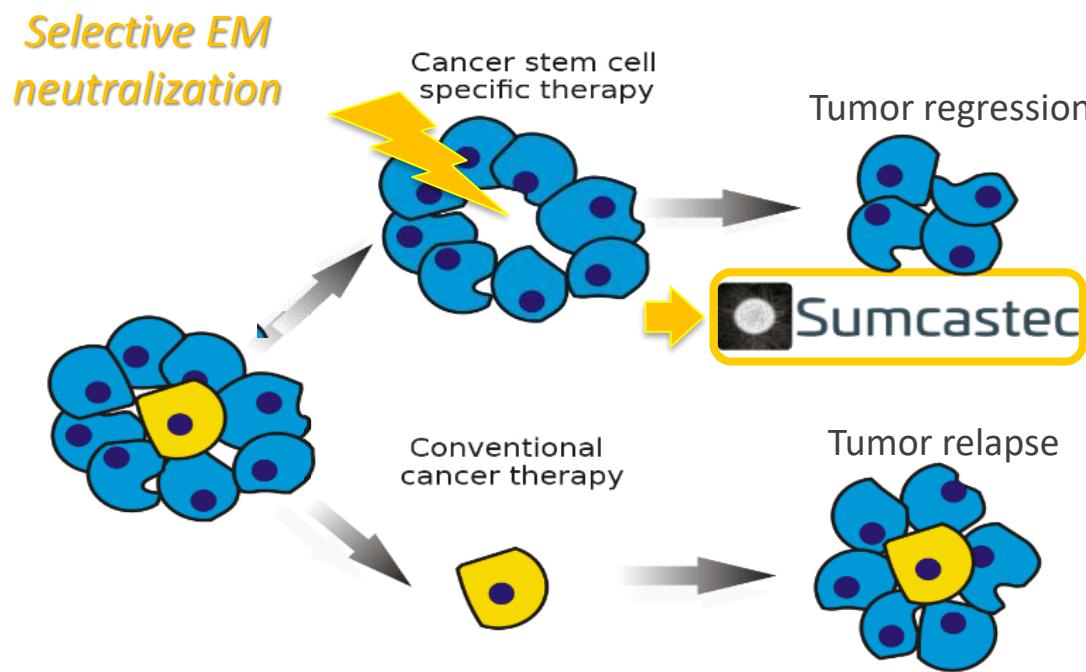
- ▶ with self-renewal capabilities
- ▶ differentiation into multiple cell types (progenitors...)
- ▶ hypothesized to be the main cause of **relapse** and **metastasis**





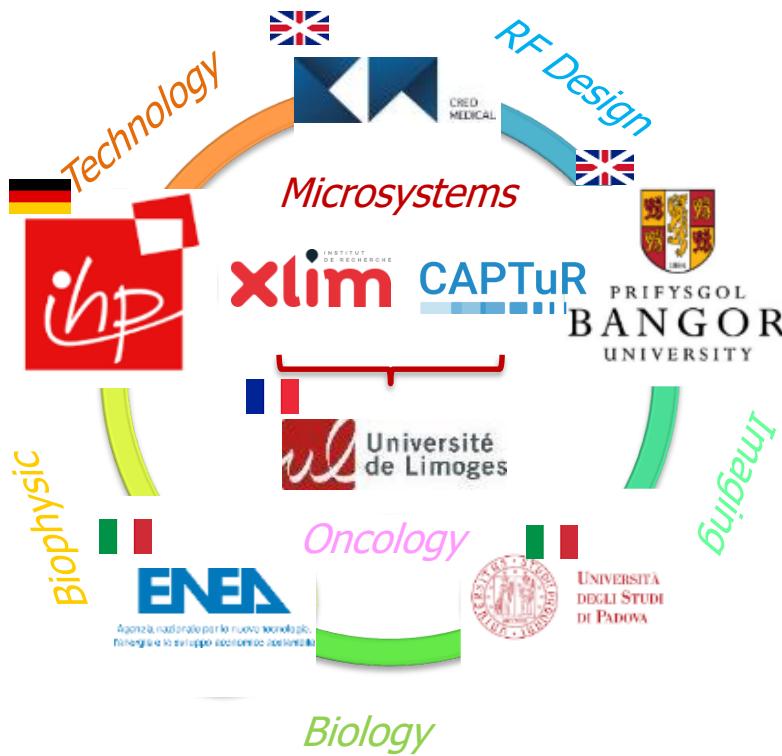
New therapies targeting CSCs

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



SUMCASTEC team

A multidisciplinary consortium to address a broad spectrum of research challenges



10 teams from 6 institutions

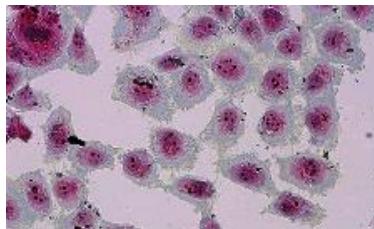
- **RF & Microwave Engineering:**
-> BANGOR, CREO, IHP, XLIM
- **Photonic & Imaging Engineering :**
-> BANGOR
- **Micro Technology Development:**
-> IHP, XLIM
- **Biology & Oncology**
-> ENEA, UNIPD, CAPTuR
- **Biophysics**
-> ENEA, XLIM



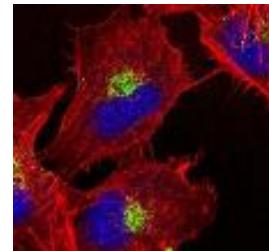
How biologists study CSC's currently?



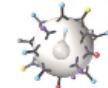
Optical microscopy



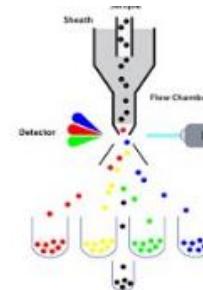
Staining



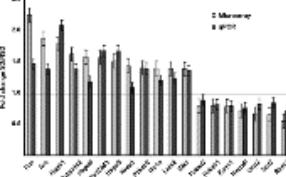
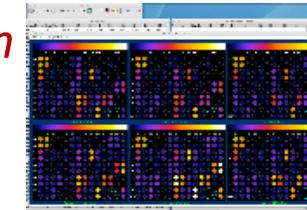
Fluorescence labeling



Flow cytometry



QPCR & Protein Array analysis



Main difficulties :

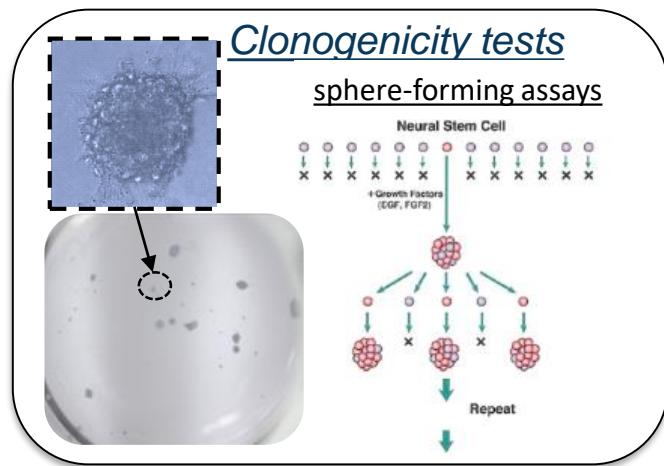
- CSC's are rare and require amplification of population
- Specific immunostaining markers are lacking

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

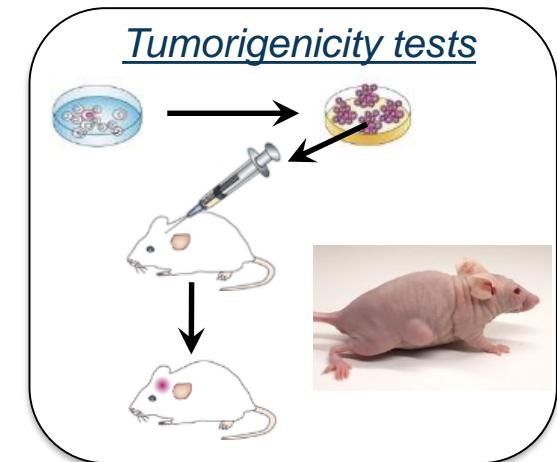
Functional tests allow to identify CSC

Functional tests prove ability to renew a tumor mass



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

*-> Never used in
clinic..*

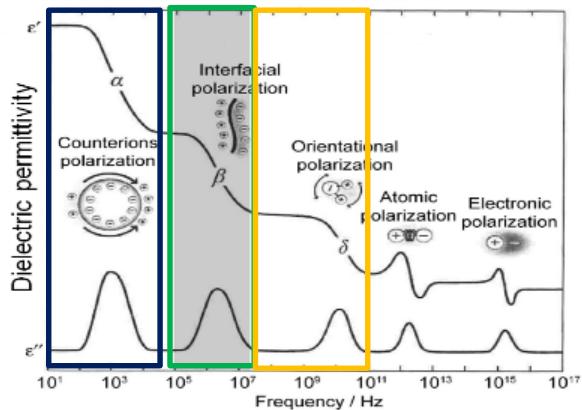


Interest to develop others approaches investigating intracellular specificities

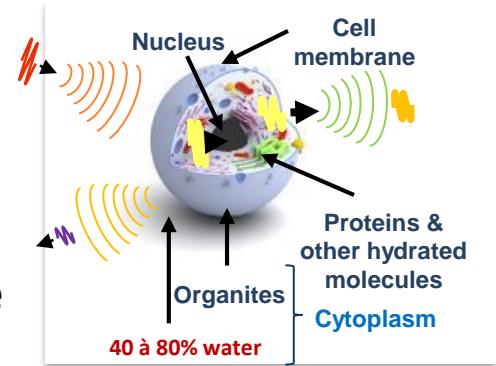
What about using EM field to identify CSC's?

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

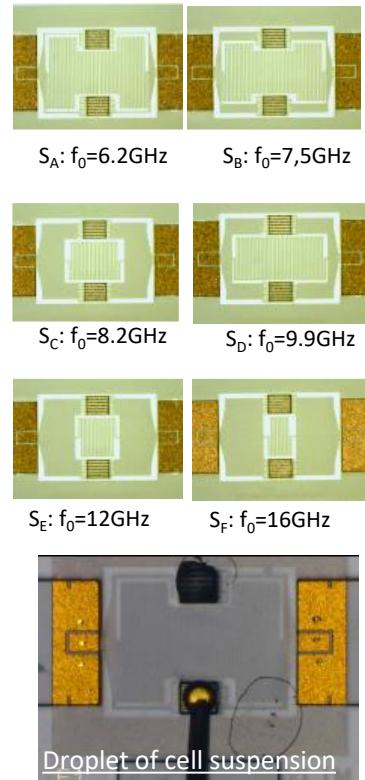
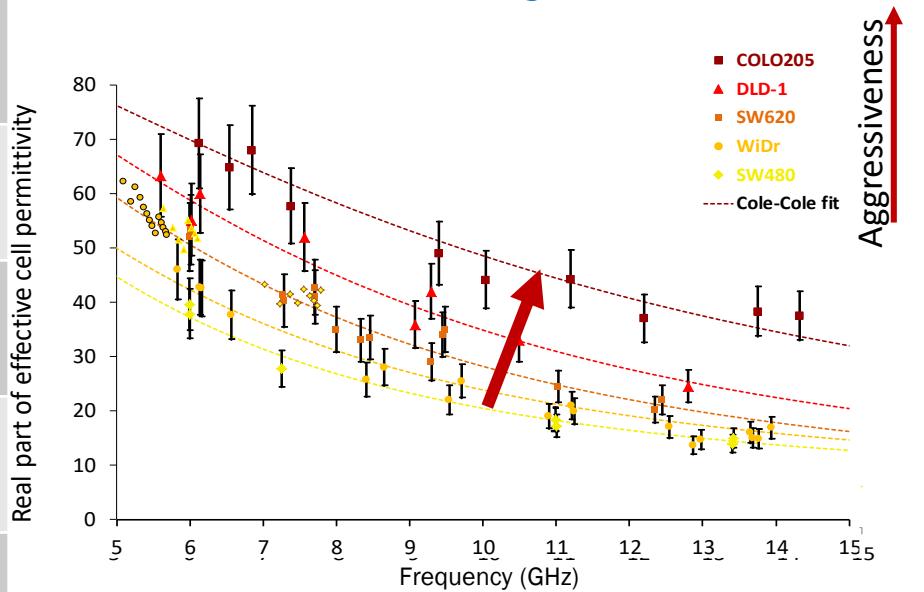




Discriminating cells with dielectric spectroscopy

Dielectric signature established using
Microwave resonating sensors

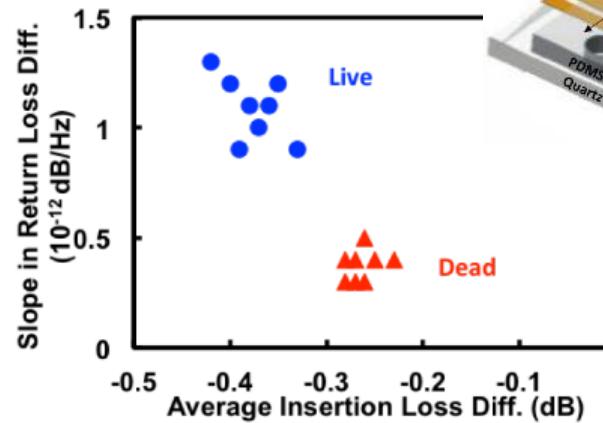
Cell Line	Stage	Morphology
WiDr	II	
SW480	II	
SW620	III	
DLD-1	III	
Colo 205	V	



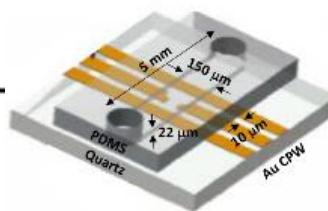
L.Y. Zhang et al, Discrimination of Colorectal Cancer Cell Lines using Microwave Biosensors Sensors & Actuators: A. Physical, Vol 216, Sept 2014.

Dielectric spectroscopy on living cells

Probing flowing cells (microfluidic channel) with microwave sensors allows to measure own dielectric specificities of cell cytoplasm

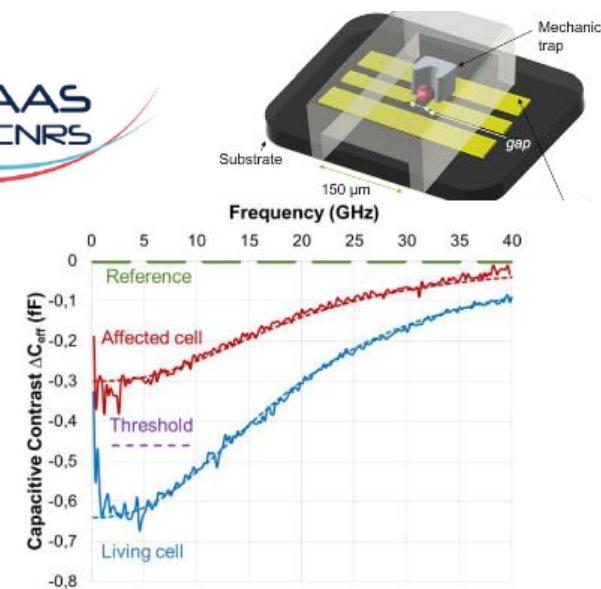


H. Li et al, DOI: 10.1109/TMTT.2017.2659736



Challenges:

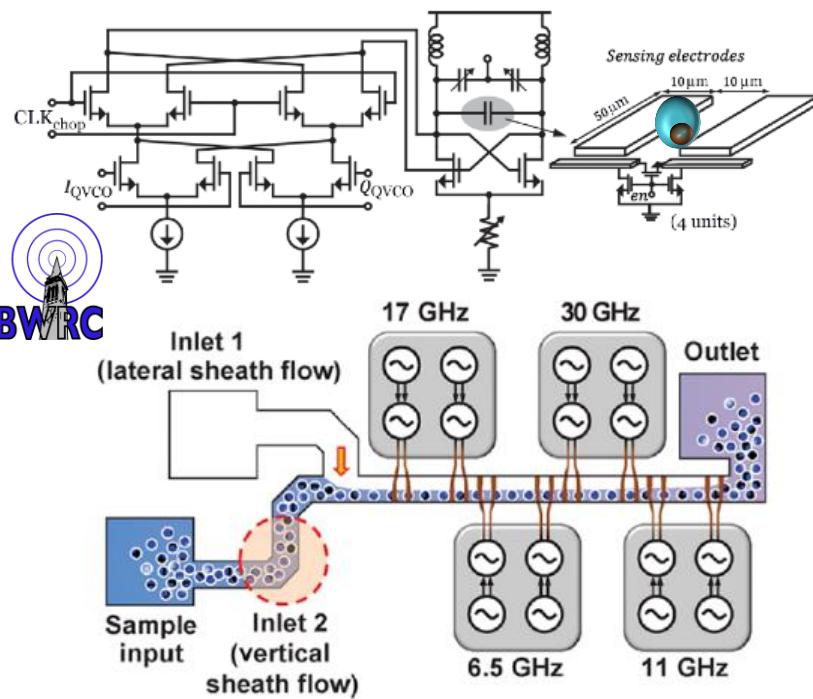
- Measurement accuracy and stability
- Choice of probing frequency
- Single cell measurement
- Need to be associated with cell trapping



A. Tamra et al, DOI: 10.1109/TMTT.2017.2653776

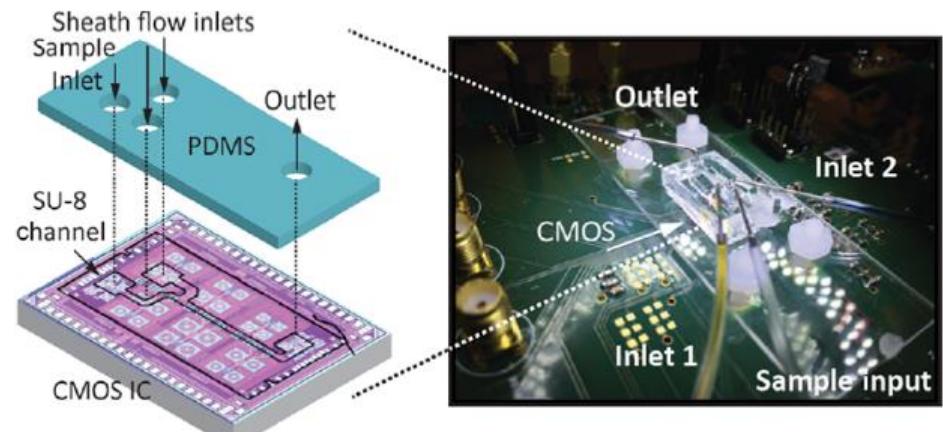
To dielectric spectroscopy cytometer concept

Potentially High-Throughput flowing cells microwave characterization



Challenges:

- Require strong sensitivity (ppm range!) sensor design with attoF resolution
- Need to be associated with cell sorting system

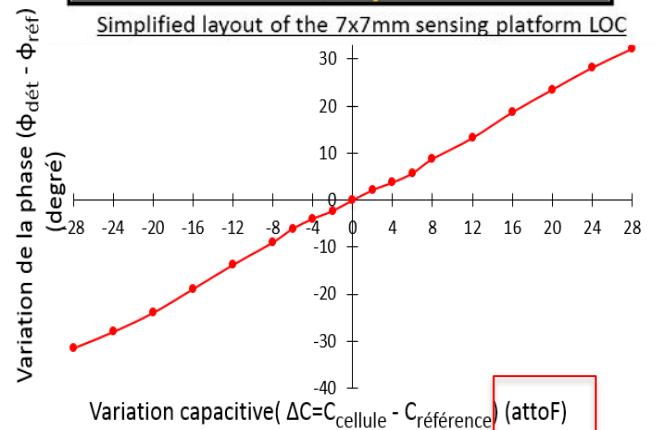
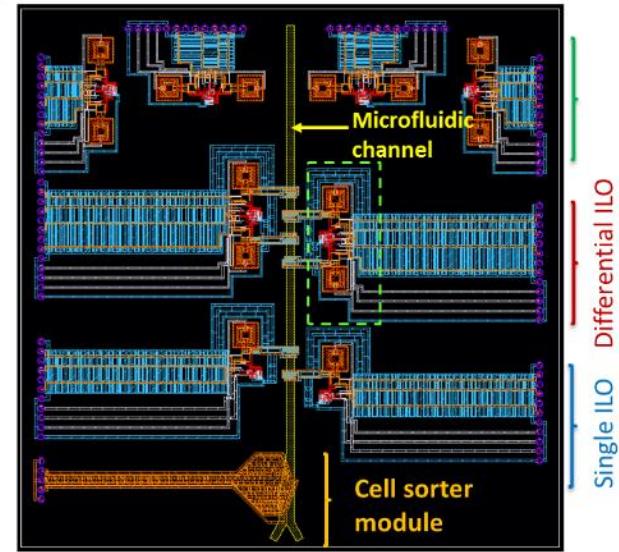
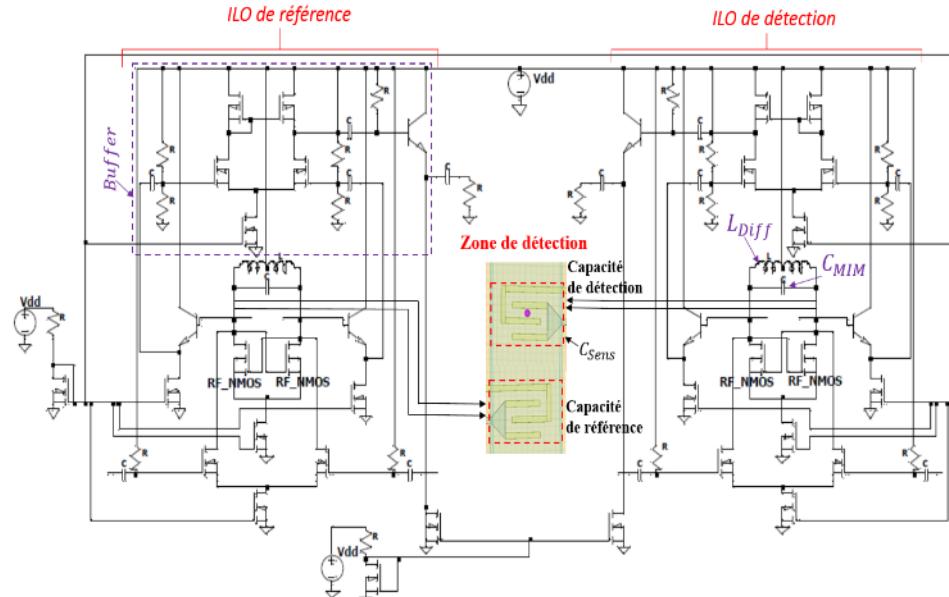


J.C.Chien et al, DOI: 10.1109/JSSC.2015.2500362

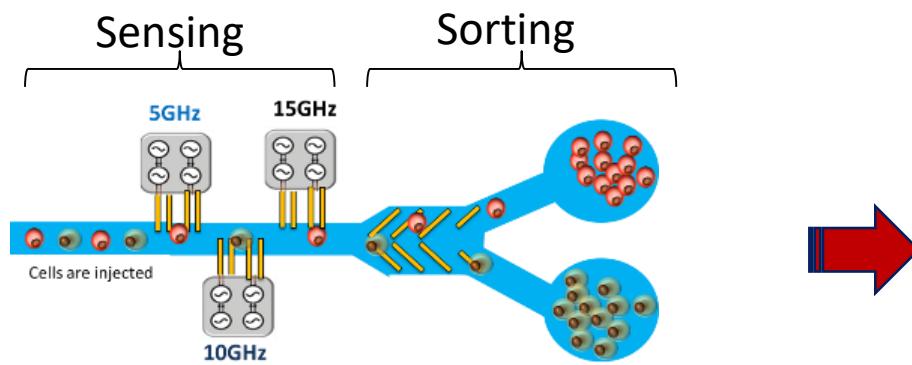
Ultra sensitive sensors based on Injection locked oscillator

BiCMOS integrated microfluidic cell sensor

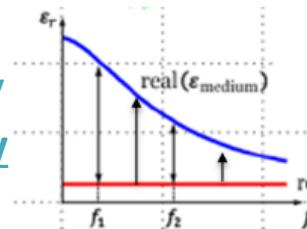
- ▶ Loss compensation
- ▶ Differential measurement
- ▶ ILO architecture for frequency and low noise stability



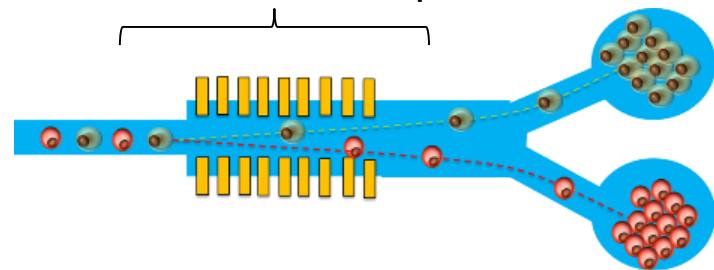
Another approach: Dielectrophoresis



Based on measured EM signatures of each cell, they have then to be individually sorted / isolated



Selective electromanipulation



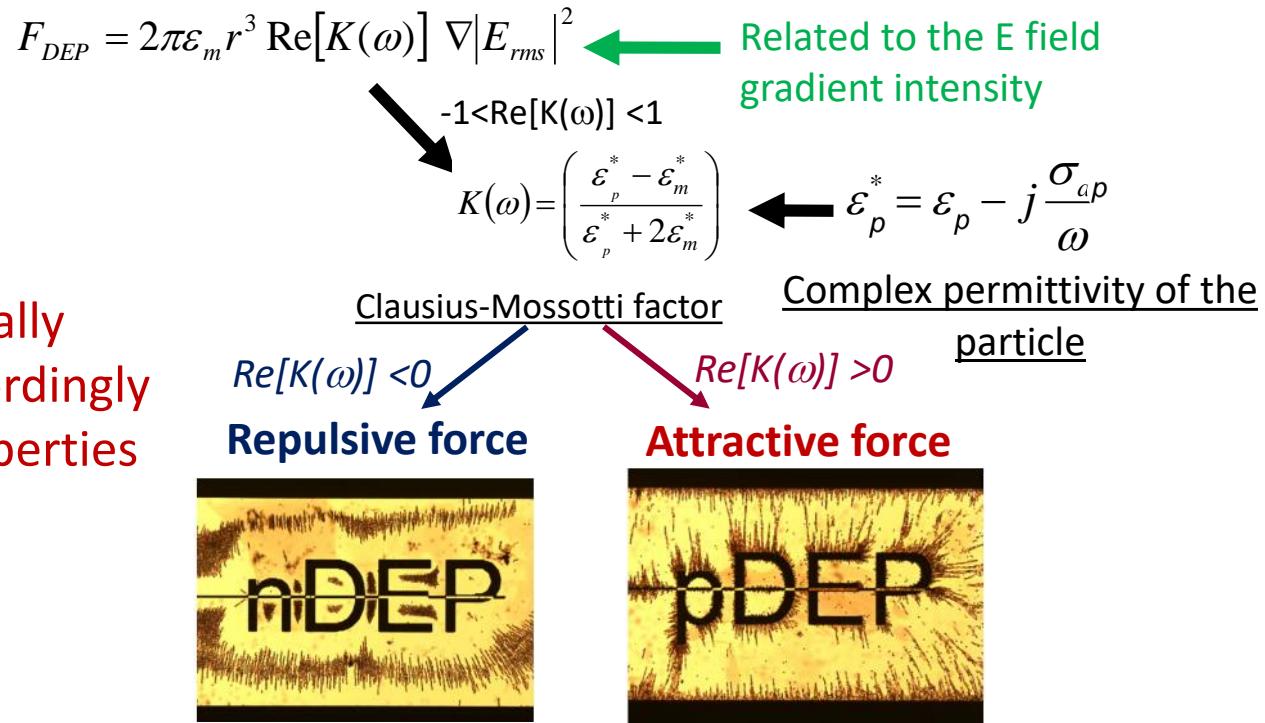
Cell can be **dynamically** sorted depending their “susceptibility” to specific EM signal

→ Require perfect synchronization between sensors and sorting module

→ Require combined hydro-fluidic & electromagnetic dielectrophoresis manipulation

Dielectrophoresis basics

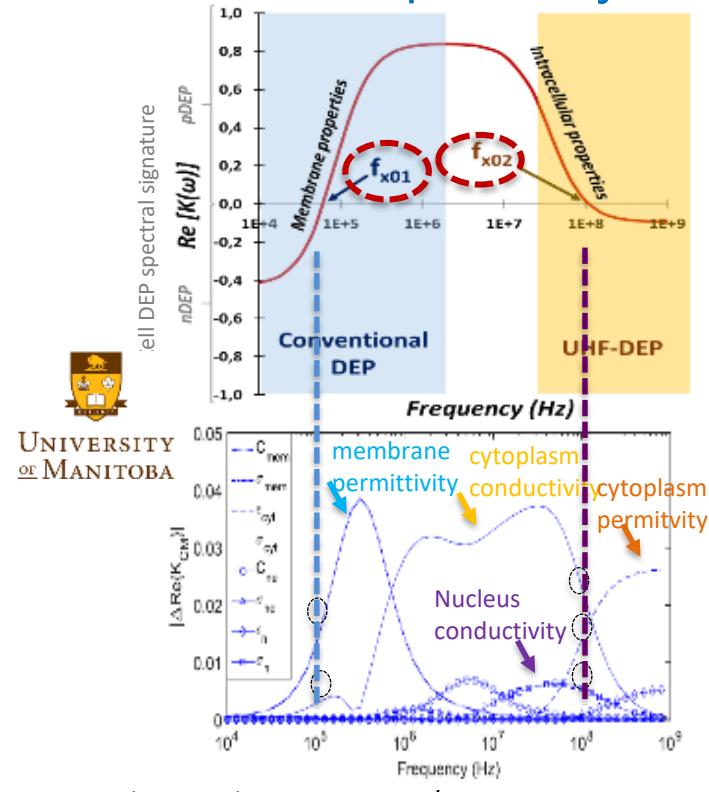
DEP relies on the fact that EM fields generate forces that can move cells



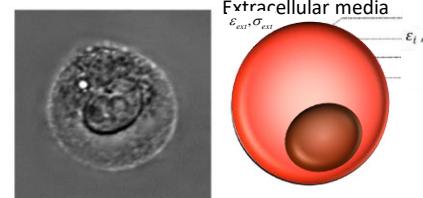
Cells can be individually electromanipulated accordingly their own dielectric properties

Specificities of cell DEP spectral signature

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



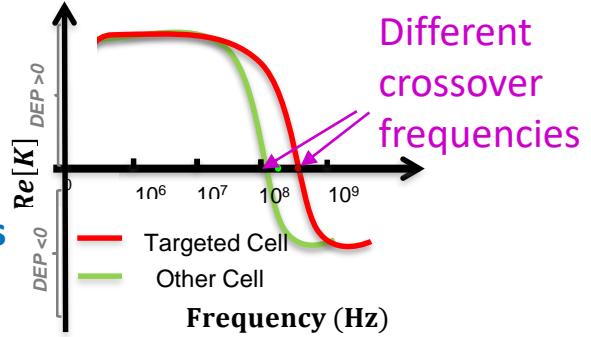
Dielectrophoresis theory basics



Different cells

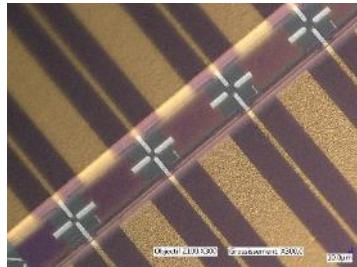
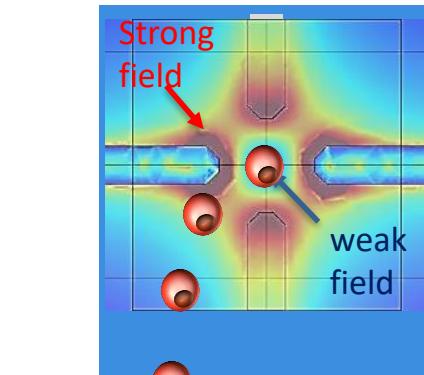


Different spectral signatures



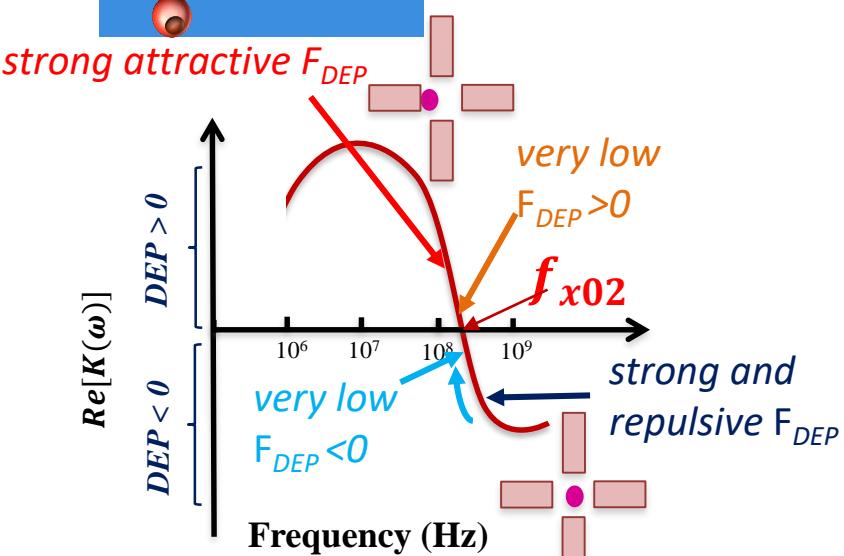
f_{x02} is an intracellular marker!

Methodology for crossover frequency measurement

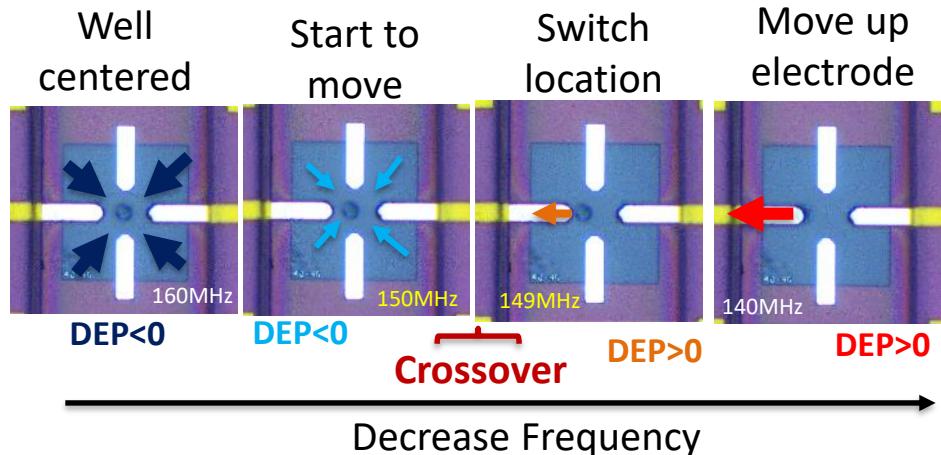


$$F_{DEP} = 2\pi\epsilon_m r^3 \operatorname{Re}[K(\omega)] \nabla |E_{rms}|^2$$

$\rightarrow F_{DEP}$ will be high in strong field areas
 \rightarrow low in weak field areas



17/05/2019



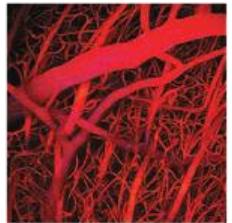
Methodology:

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

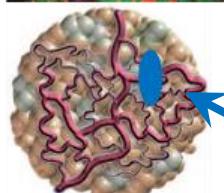
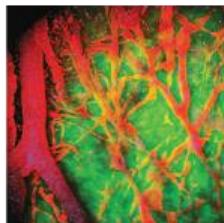
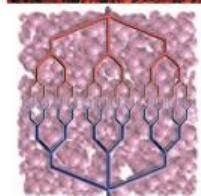
Obtaining CSC population starting from cell line

Mimic CSC micro environment conditions to enrich population

Normal brain tissue
Normal blood vessels



Brain Tumor
Anarchical vessel network

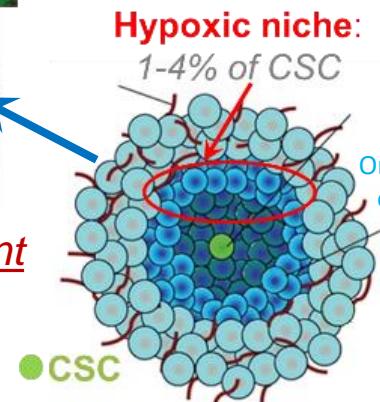


Hypoxic niche:
1-4% of CSC

Only few %
of CSC's

Specific micro environment

- $O_2 < 1\%$
- low growth factor concentration



➢ Submitting cells to stringent Culture conditions



Basal cell line

Culture Medium Normal (+ FBS) Define (- FBS)

% O₂ N H N H



NN

N

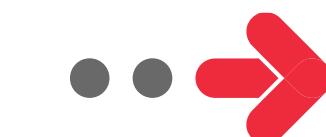
NH

H

DN

DH

Résistance &
immaturity

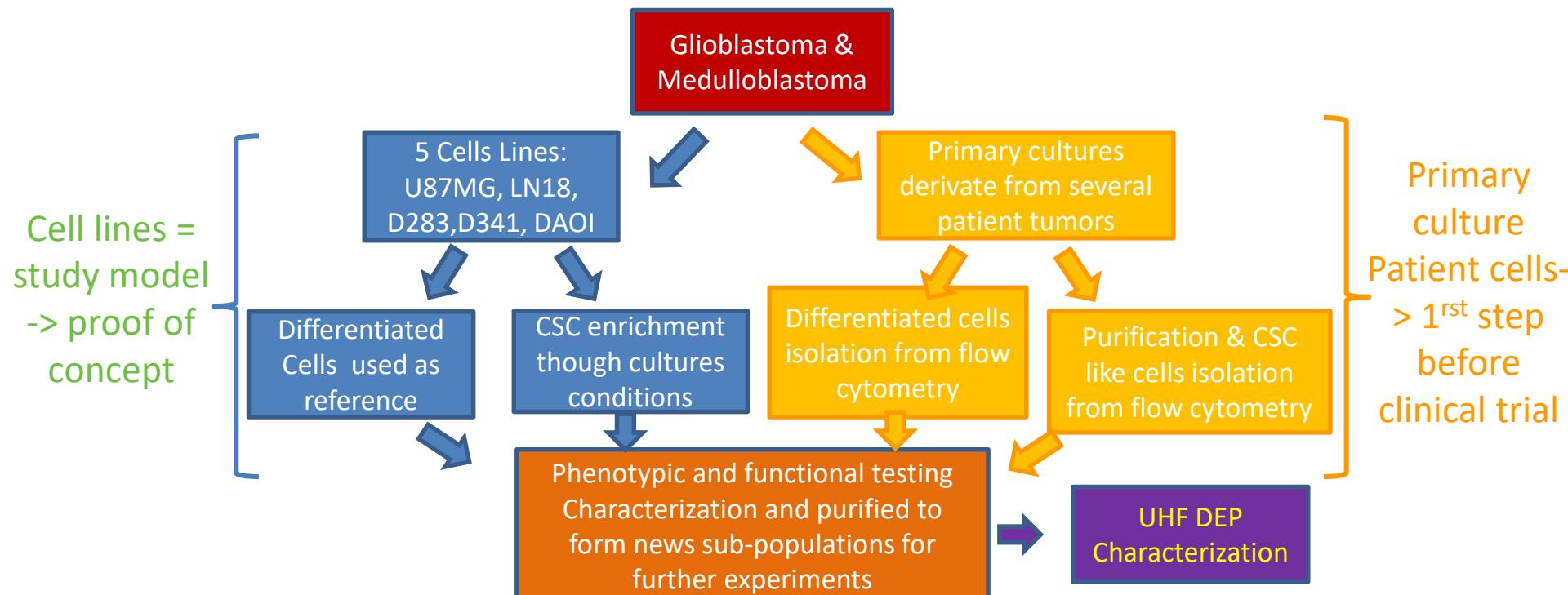


20

N: Normoxia 20% O₂
H : Hypoxia 1% O₂

CSC's like
cells
60-85 %
expected

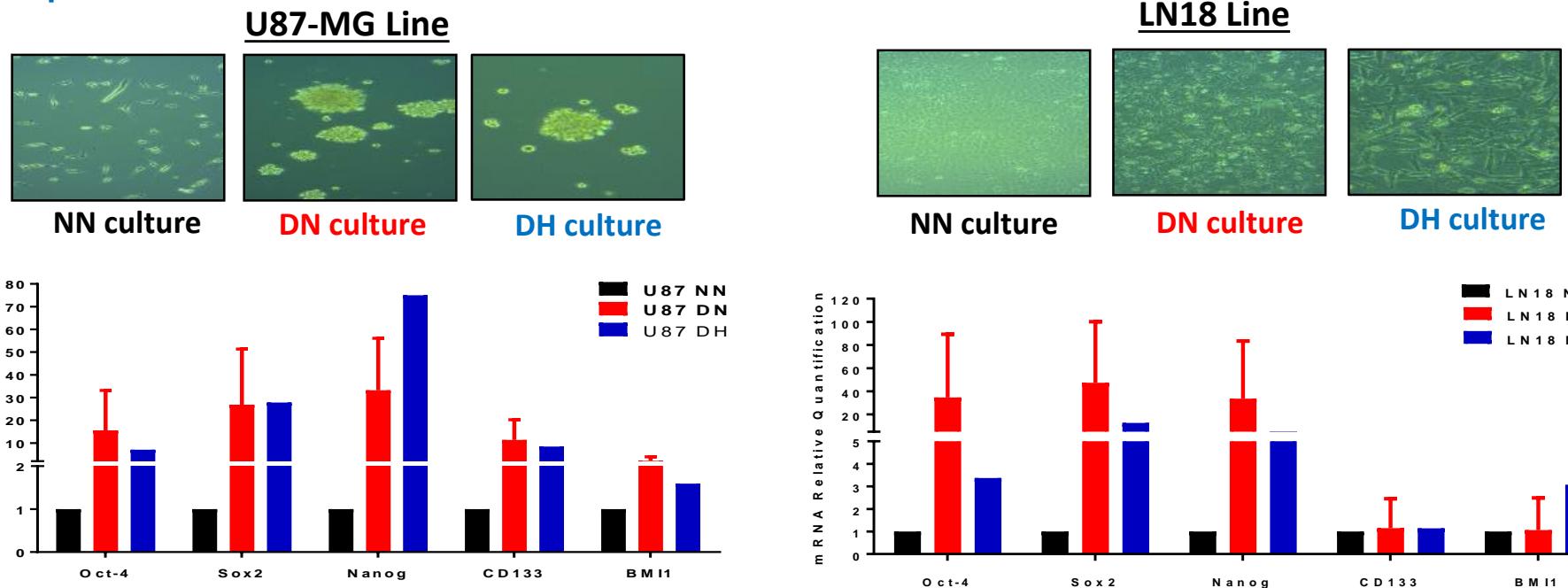
Followed methodology for cell characterization





Confirmation of culture conditions influence on cell phenotype

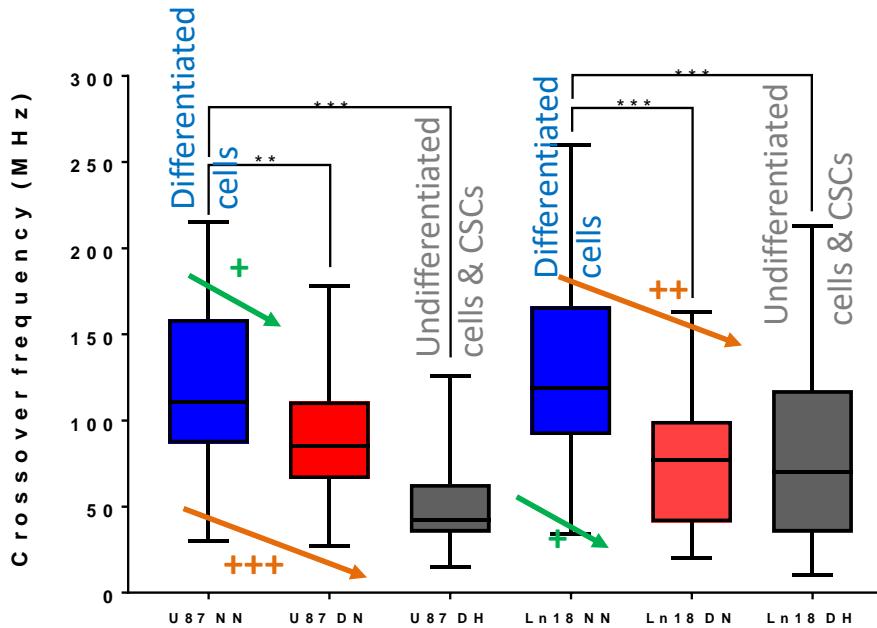
Glioblastoma human cell lines: Analysis of CSC markers at transcriptional and protein level



➡ 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

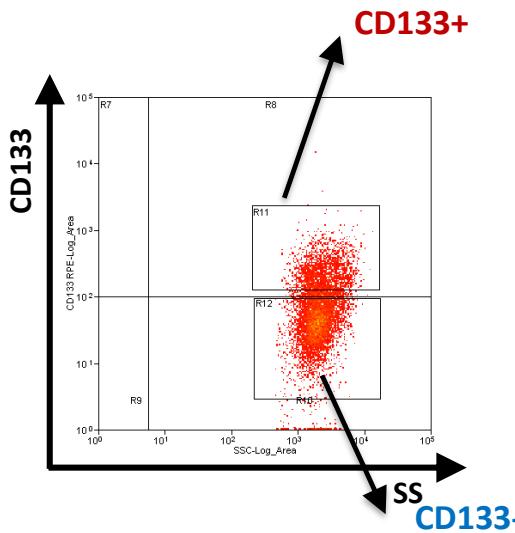
→ Difference of phenotype -> difference of DEP signature

What about primary culture?

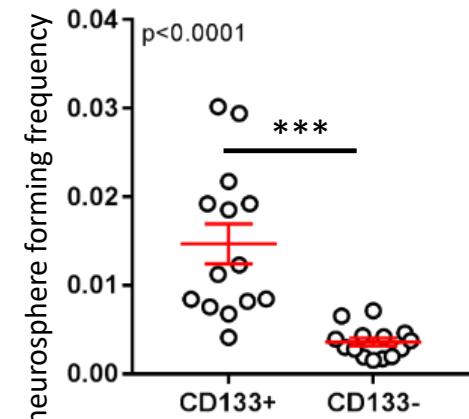
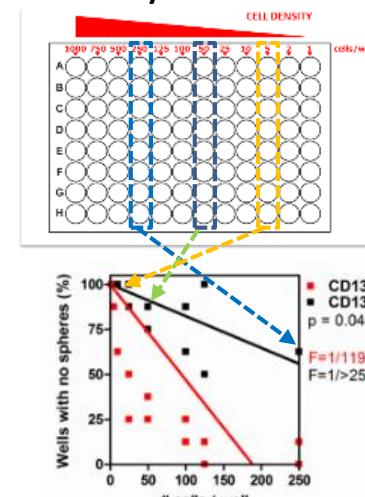
17 patient glioblastoma tumors investigated



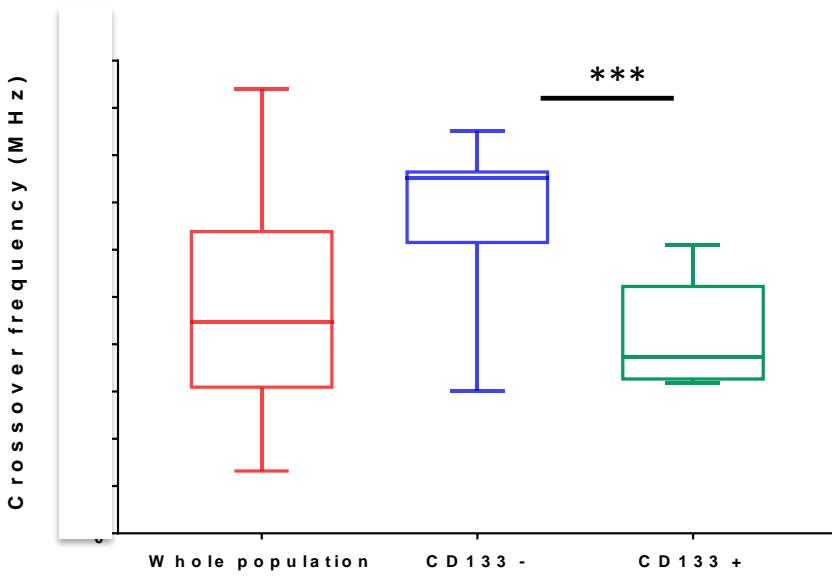
- Cells expressing CSC protein membrane markers are isolated by fluorescence flow cytometry
- Sub population phenotype and functional features are tested



- Ability to renew tumor evaluated by LDA



Crossover frequency of GBM primary culture cells



Clear difference of signature

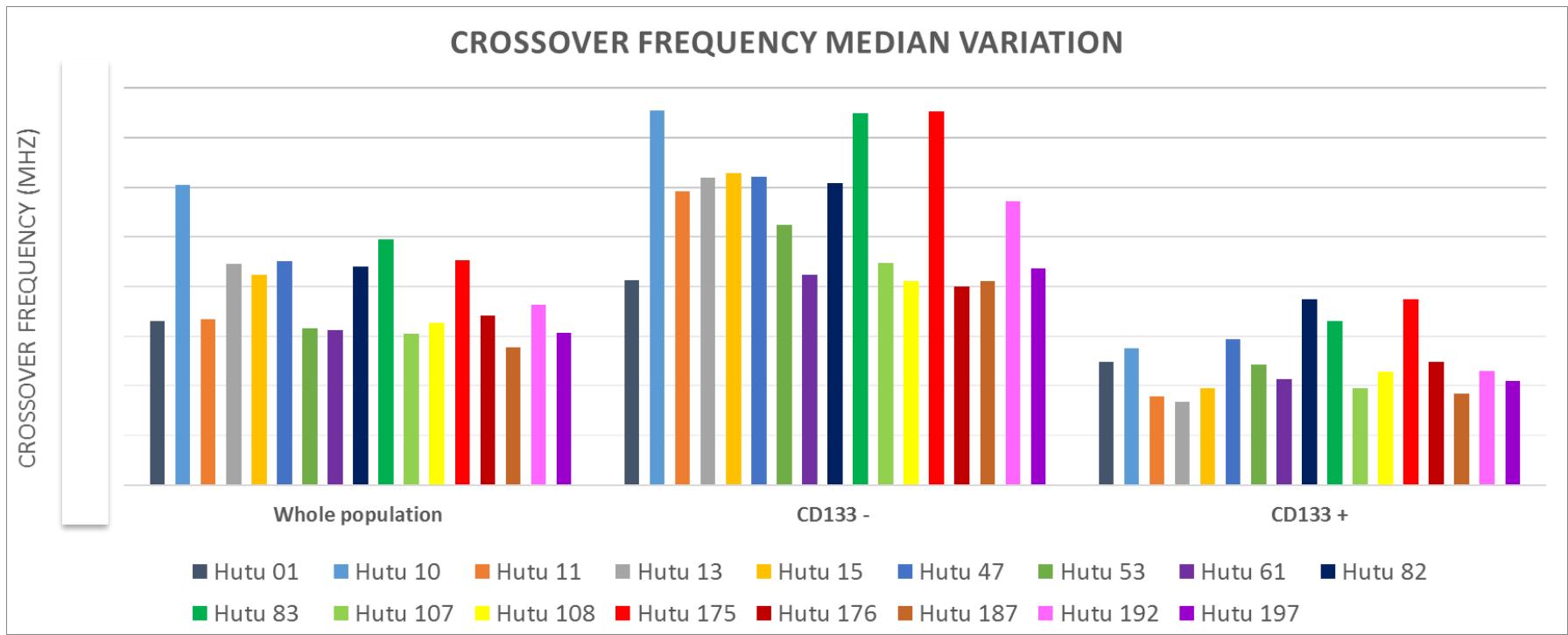


Correlation between difference of crossover frequency and expression level of CD133 and so CSC occurrence



CD133⁺ cells also show lower DEP signatures

Crossover frequency of GBM primary culture cells



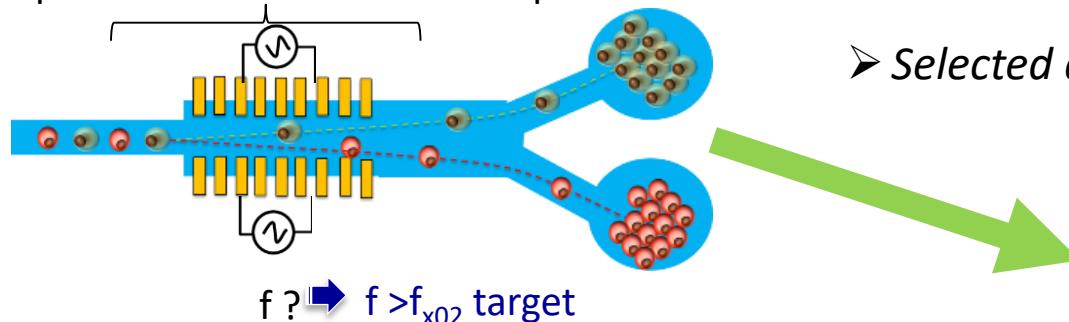
Whatever patient considered $CD133^+$ cells always show lower DEP signatures

→ UHF-DEP crossover frequency appears as relevant CSC marker!

How exploiting cell crossover frequency specificities

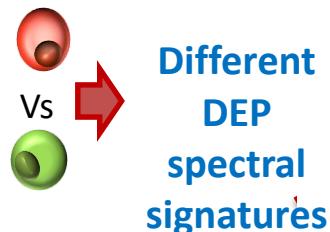
Prior cell population characterization will help to select the more selective sorting UHF-DEP frequency

Expected selective electromanipulation

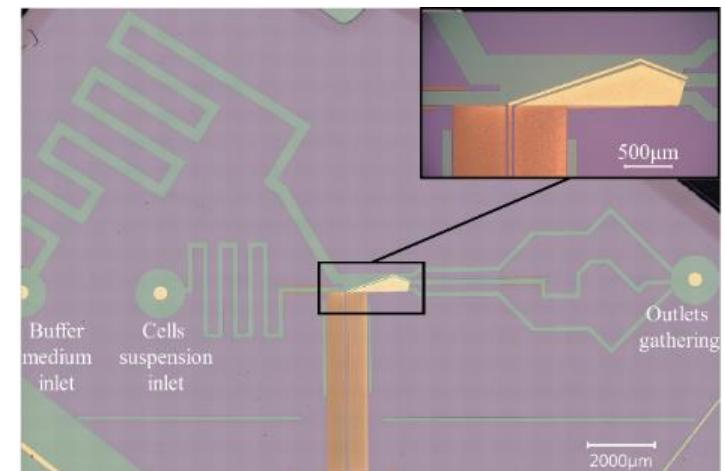
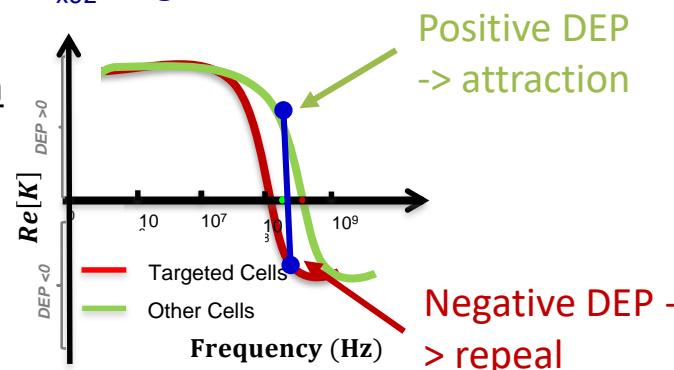


➤ Selected approach: gradual cell deviation using single frequency biasing

Mixed cell population



Different
DEP
spectral
signatures

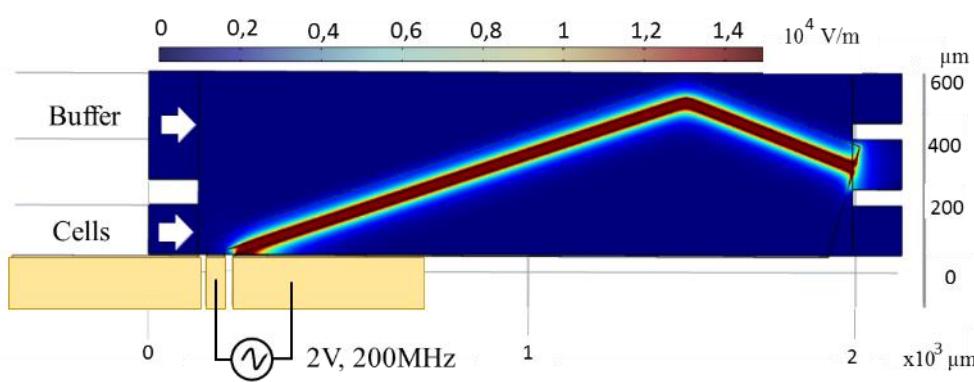
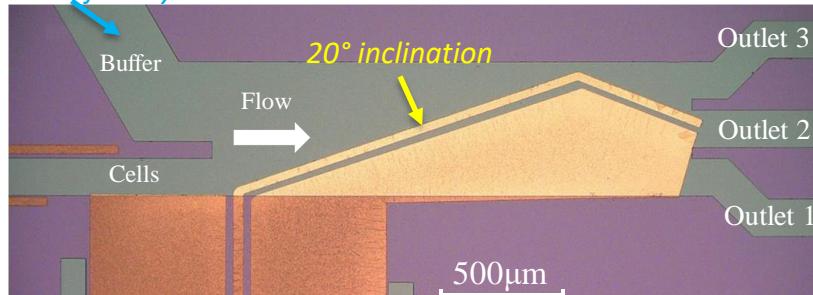


T. Provent et al, JNM 2019

Proposed cytometer design

→ Coupling of DEP & hydrofluidic forces to dynamically sort cell

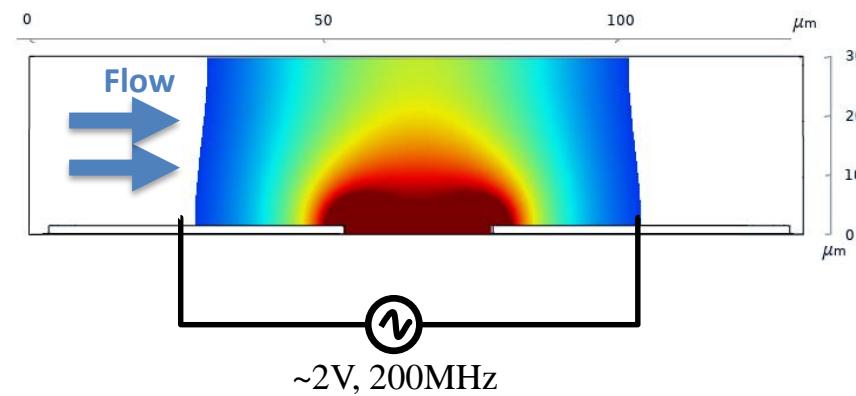
Control the initial cell trajectory



Working principle:

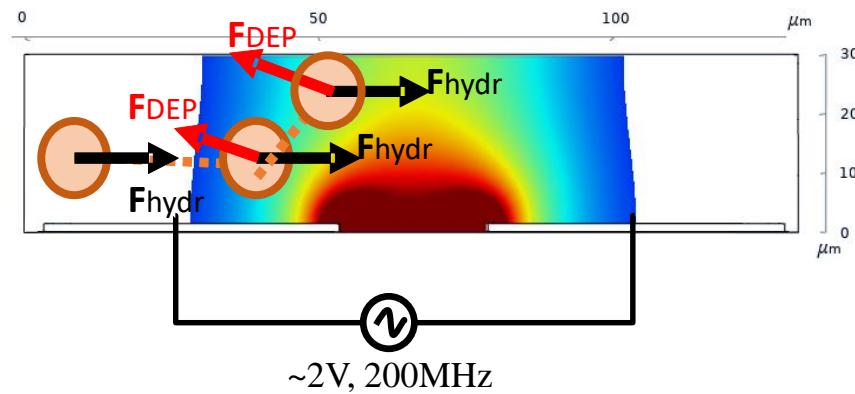
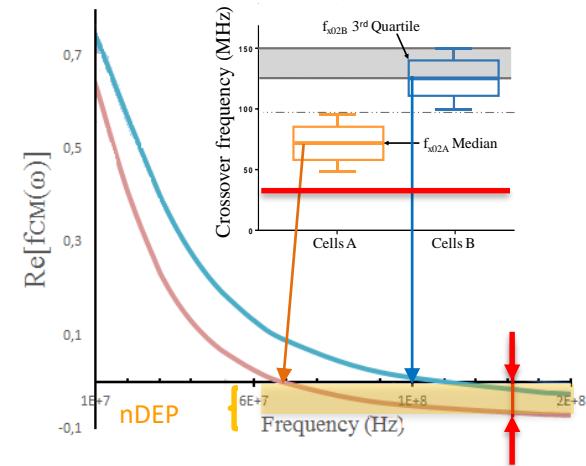
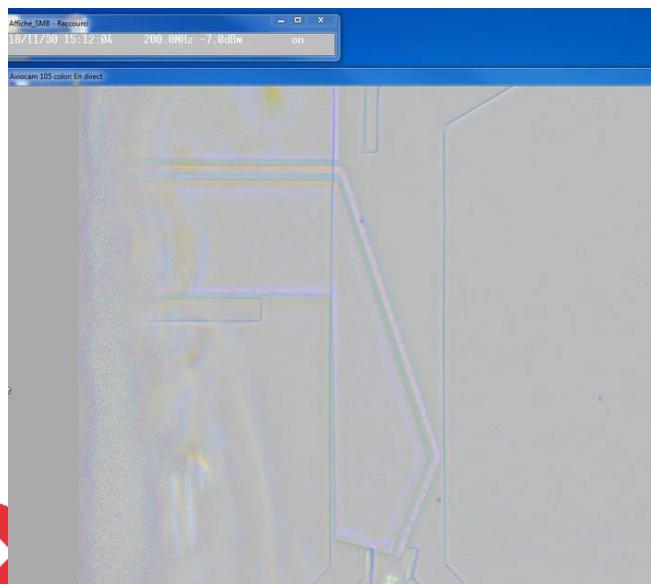
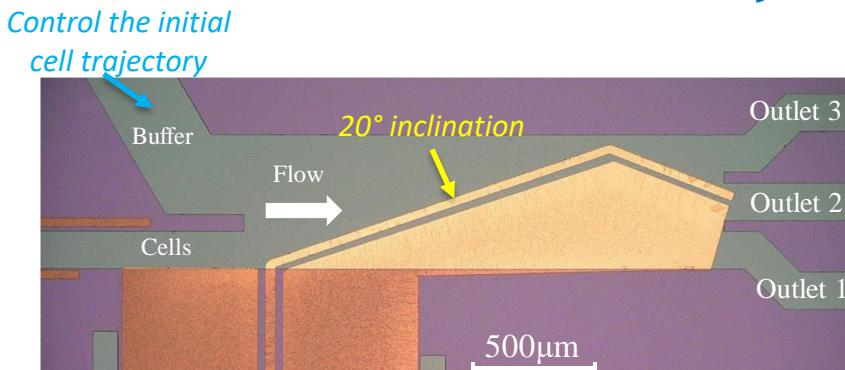
Tune the DEP force at constant fluidic force to act on cell trajectory

- > by the electrode design (angle related to cell flow)
- > by the DEP signal magnitude
- > by the choice of DEP frequency related to targeted cell crossover



Proposed cytometer design

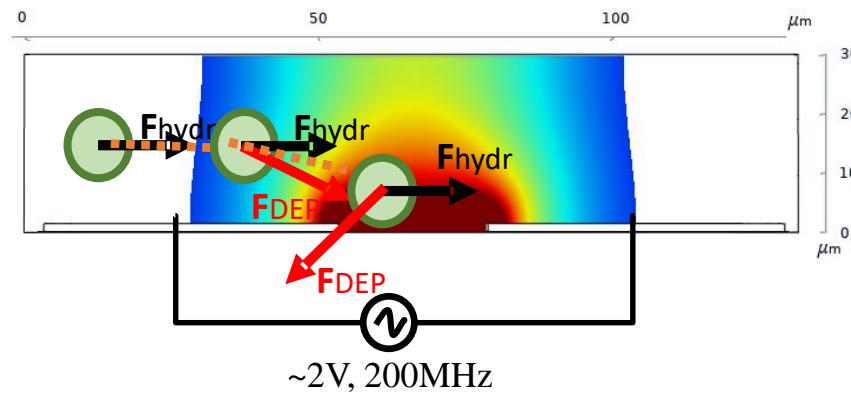
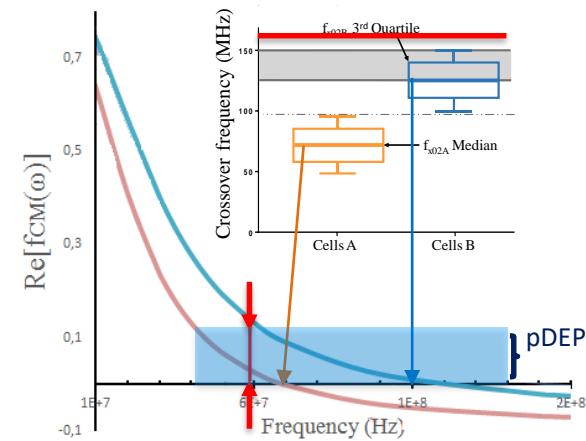
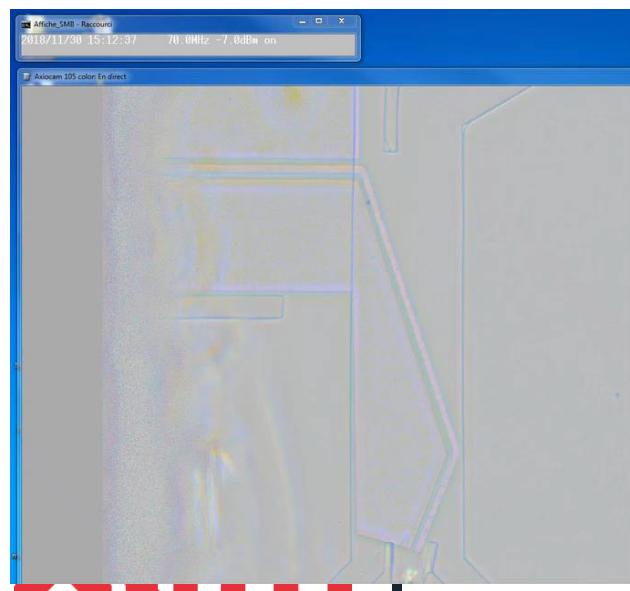
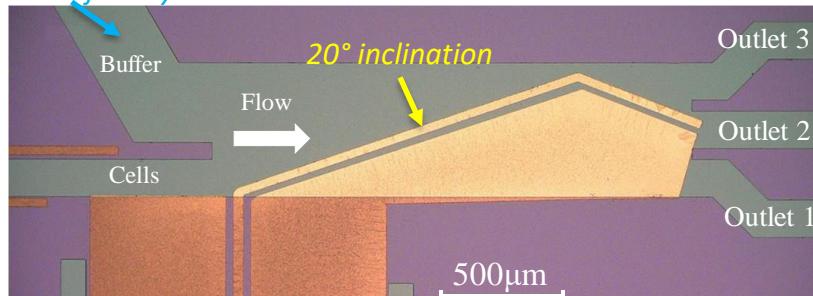
→ Coupling of DEP & hydrofluidic forces to dynamically sort cell



Proposed cytometer design

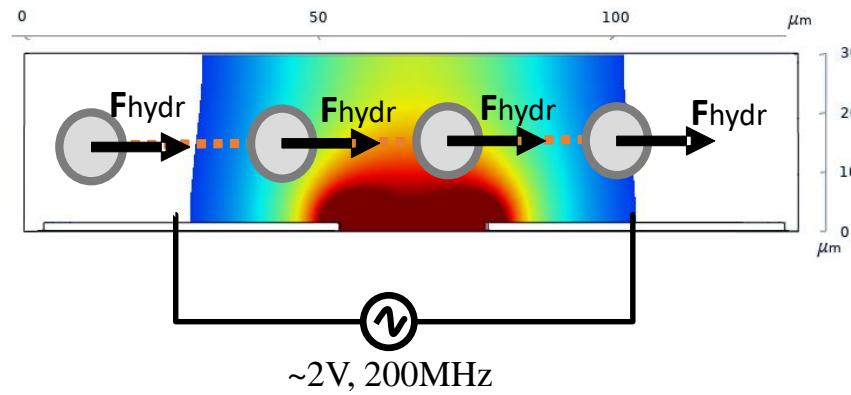
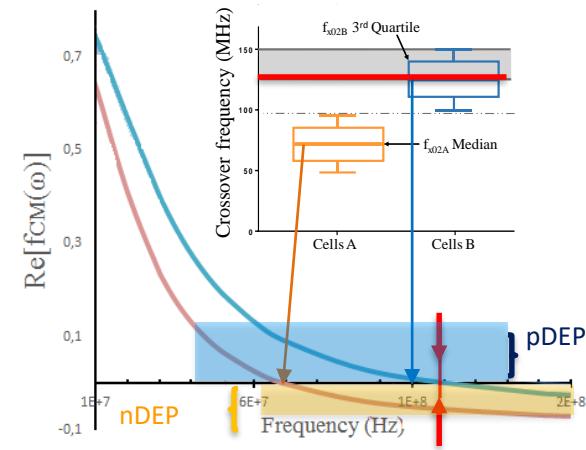
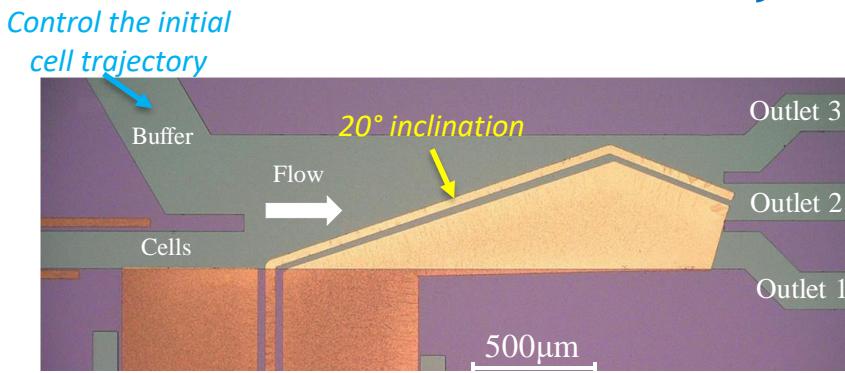
→ Coupling of DEP & hydrofluidic forces to dynamically sort cell

Control the initial cell trajectory

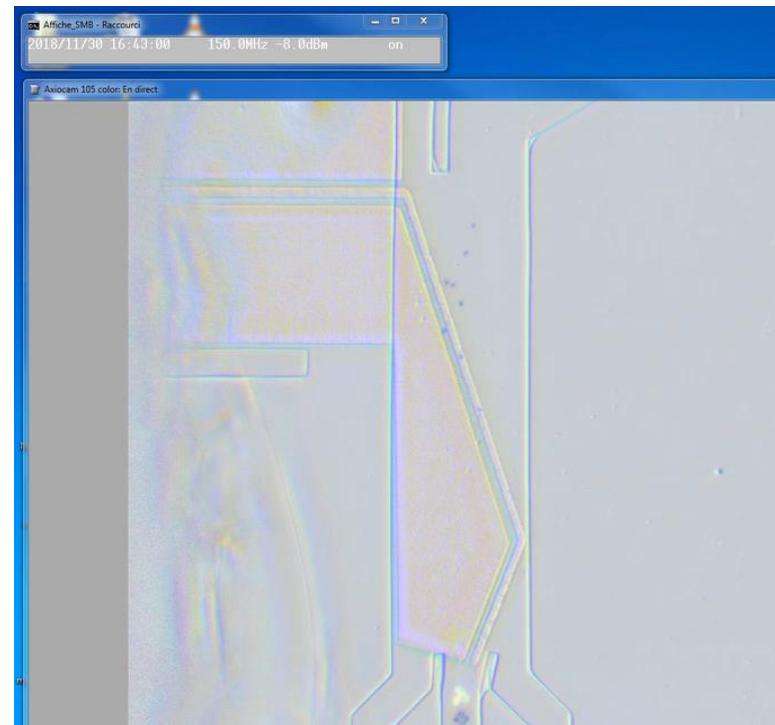
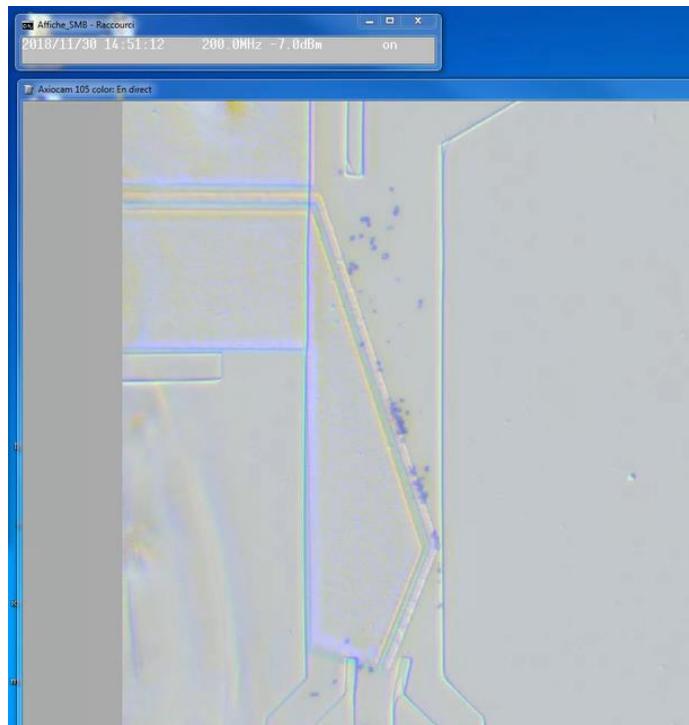


Proposed cytometer design

→ Coupling of DEP & hydrofluidic forces to dynamically sort cell



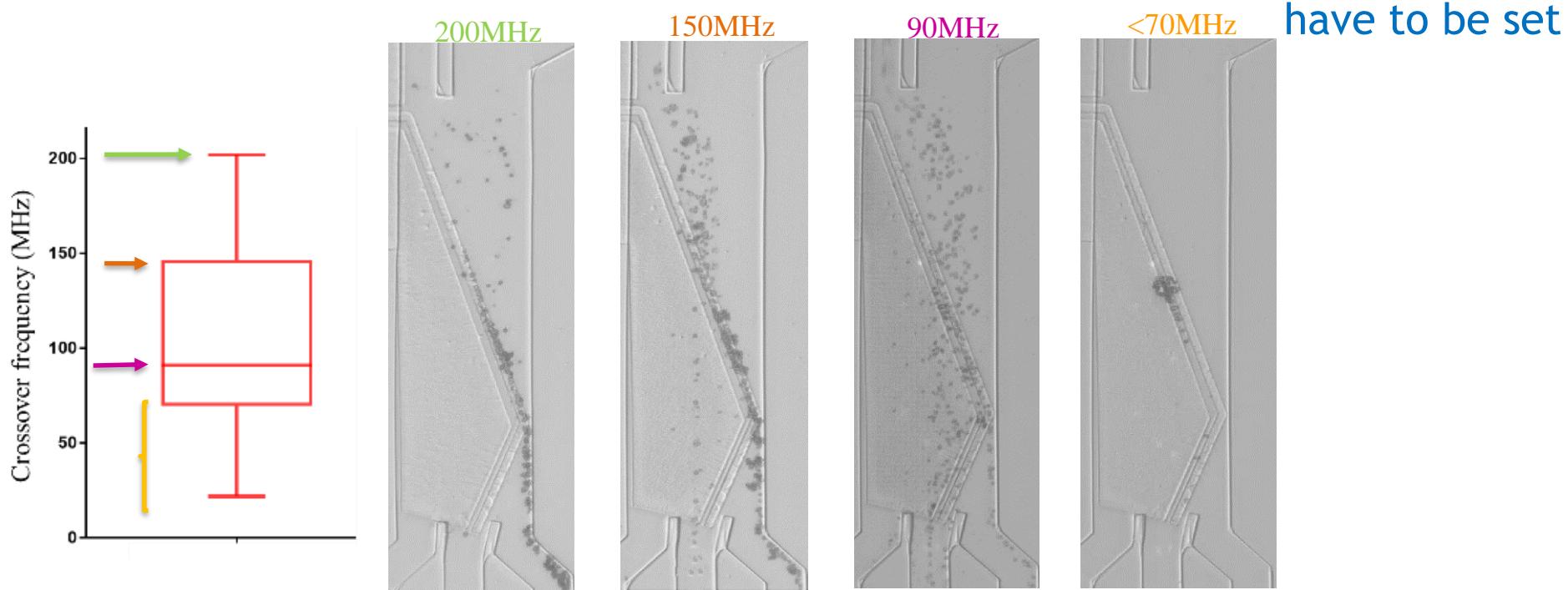
Tuning the DEP signal frequency





Handling a dispersive property cell population

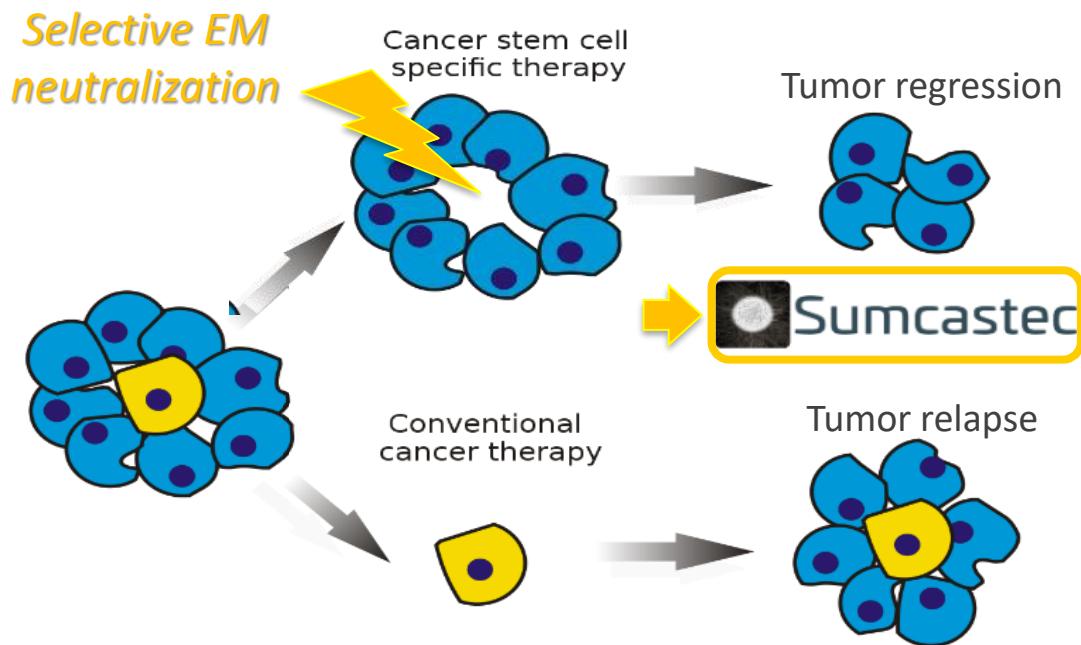
For targeted cell: optimal DEP signal frequency and magnitude vs flow speed



First design : Still working on improving cell sorting efficiency and implementation on CMOS technology



Neutralizing CSCs with EM fields

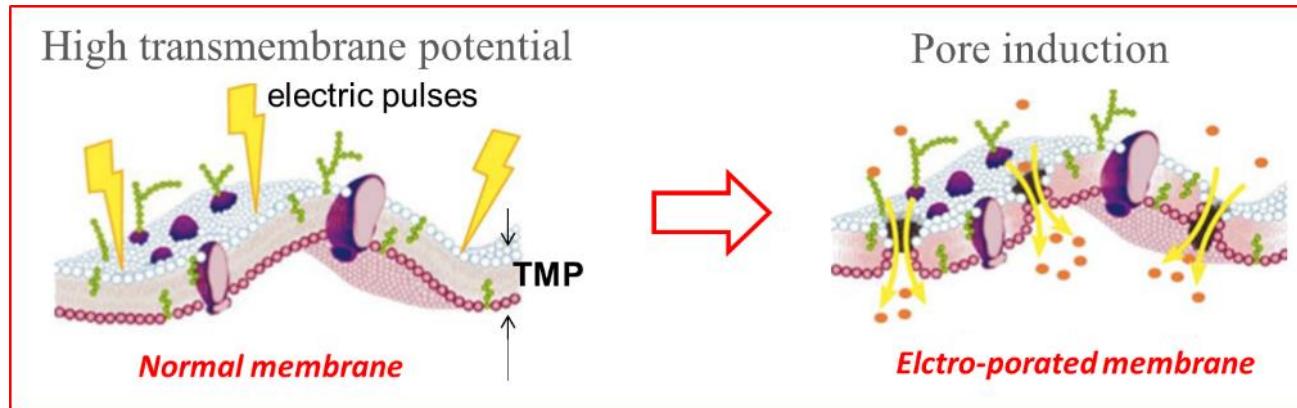


Which type of EM wave for neutralization?

Ultra short pulsed (**kV/m**) electric fields: $\mu\text{sPEF} \rightarrow 100\mu\text{s} \rightarrow 100\text{ns}$

⇒ main target : plasma membrane

- ▶ Charge displacement -> TMP disturbance -> *pore opening*
- ▶ Numerous mechanisms induced and disturbed -> *apoptosis , activation of stress pathways...*
- ▶ Non thermal effects on cells



Which type of EM wave for neutralization?

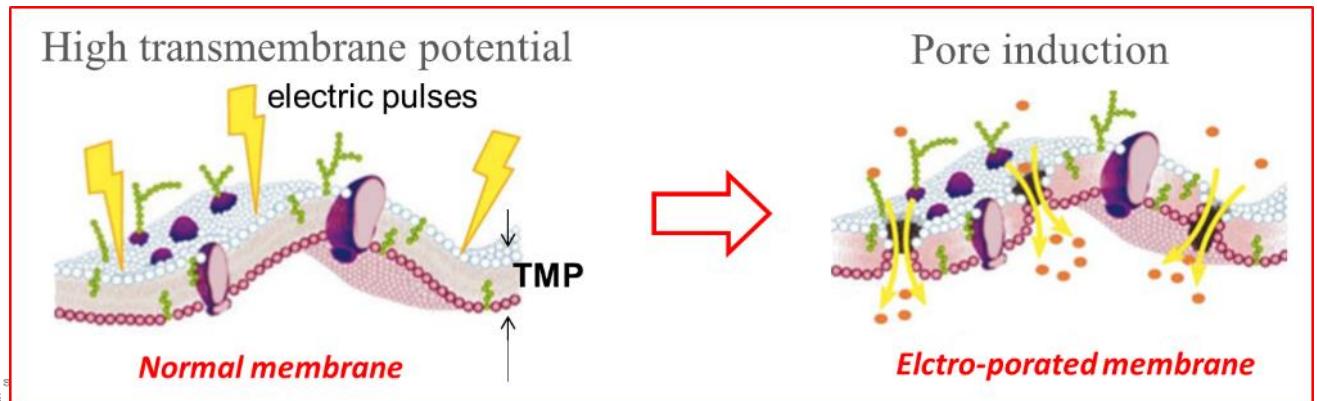
Ultra short pulsed (kV/m) electric fields: $\mu\text{sPEF} \rightarrow 100\mu\text{s} \rightarrow 100\text{ns}$

⇒ main target : plasma membrane

Extremely short pulsed (MV/m) electric fields: $\text{nsPEF} \rightarrow 0.1\text{ns} \rightarrow 50\text{ns}$

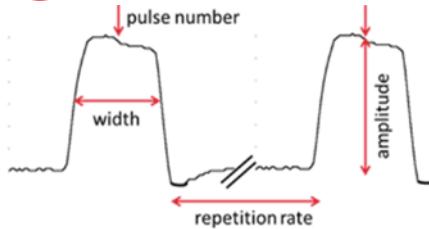
⇒ main target : cell interior (organelle membrane, mitochondria, nucleus...)

- ▶ Limited displacement but dipole orientation-> TMP disturbance \rightarrow pore opening
- ▶ Numerous mechanisms induced and disturbed \rightarrow apoptosis , differentiation, effect on gene expression
- ▶ Non thermal effects on cells

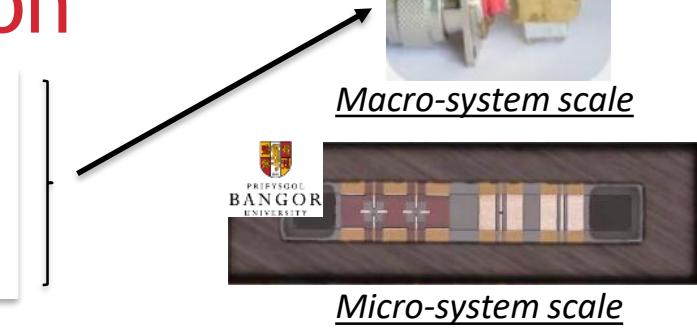




Effect of Ultrashort pulses on CSC enriched population

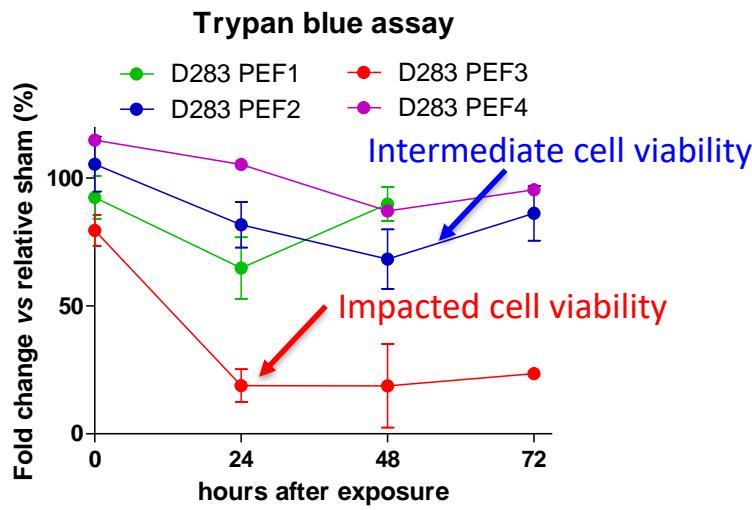


PEF 1
PEF 1
PEF 3
PEF 4

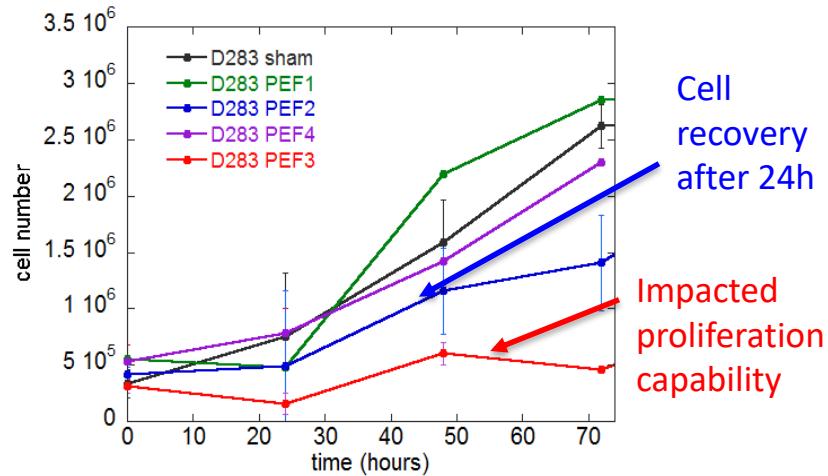


➤ D283 Medulloblastoma cell line

Cell viability monitoring:



Cell proliferation:



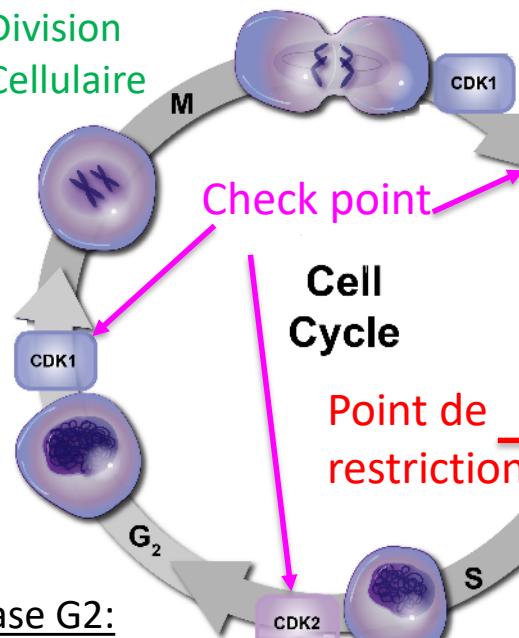
Impact of Ultrashort pulses on cell cycle

The cell cycle is the series of events in which cellular components are doubled, and then accurately segregated into daughter cells

Phase M: Mitose

Division

Cellulaire



Phase G₀: Sortie du Cycle

Phase G₁: Preparation à la duplication du génome

Phase G₂:
Preparation à la division Cellulaire

Phase S: Duplication du génome

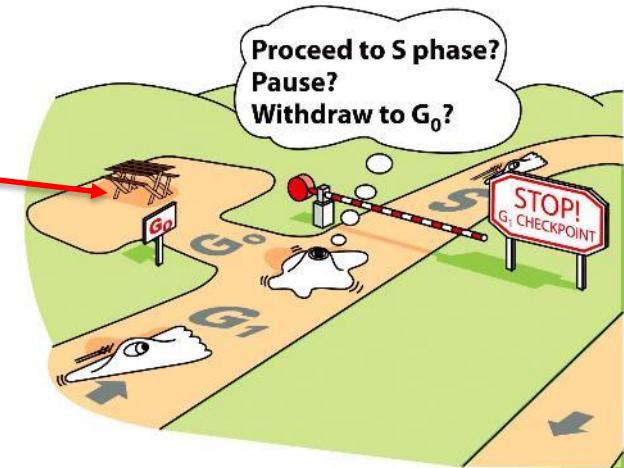
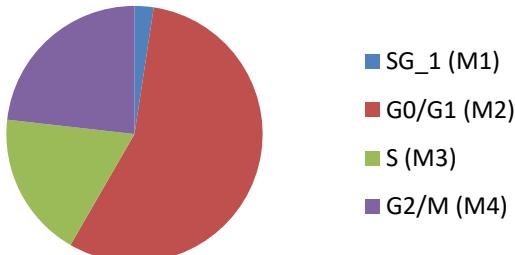


Figure 18-12 Essential Cell Biology 3/e (© Garland Science 2010)

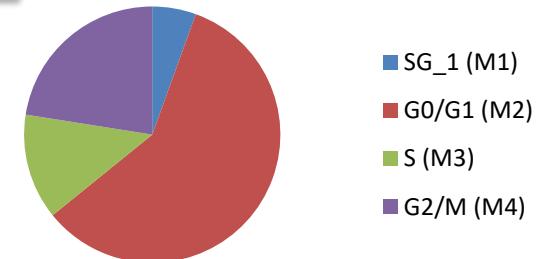
24H after Exposure

D283 Sham 24h



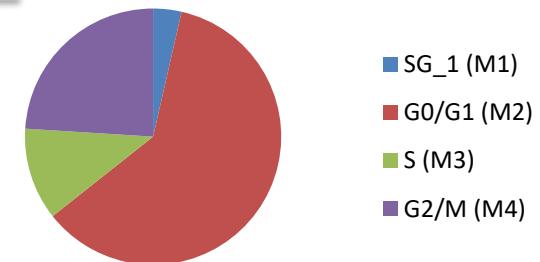
PEF 1= 1Hz

D283 PEF-1 24h



PEF 2= 3Hz 1Hz

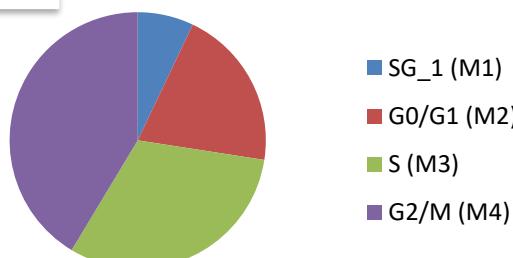
D283 PEF-2 24h



PEF 3 exposure conditions significantly impact the cell cycle:
->Lower cell number in G0/G1

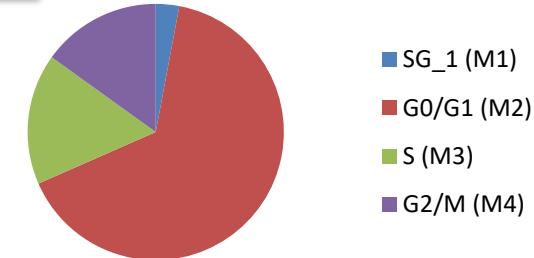
PEF 3= 1Hz

D283 PEF-3 24h



PEF 4= 1Hz

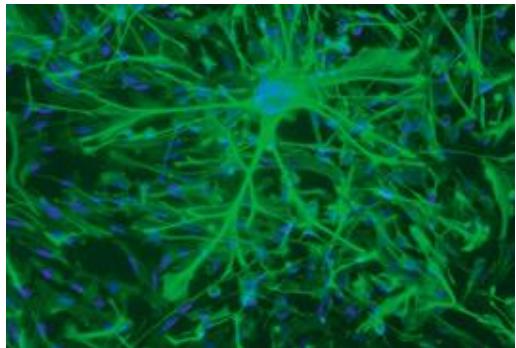
D283 PEF-4 24h



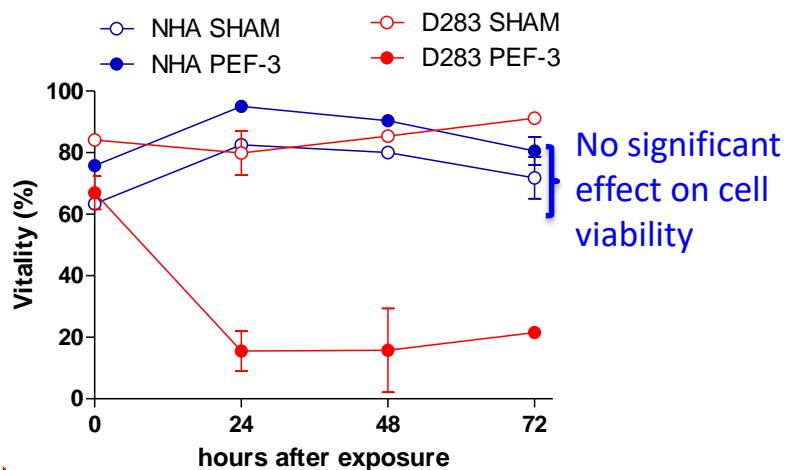
→ Numerous cells seems stopped in S and G2 phase

What impact on normal cells?

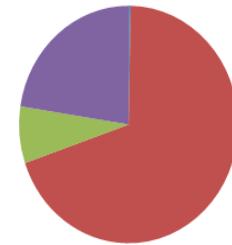
Normal Human Astrocytes (LONZA CC-3186)



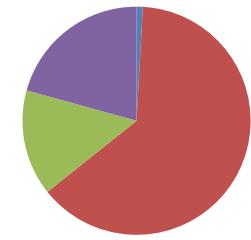
Cell viability monitoring:



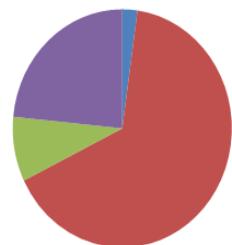
NHA sham 24h



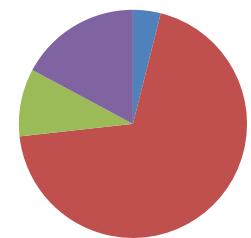
NHA PEF 3 24h



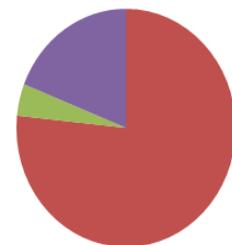
NHA sham 48h



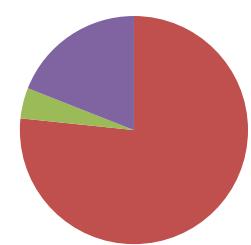
NHA PEF 3 48h



NHA sham 72h



NHA PEF 3 72h



→ Exposure to PEF-3 seems to have a selective impact on CSCs viability and cycle



Next step... In vivo validation



EM Neutralization

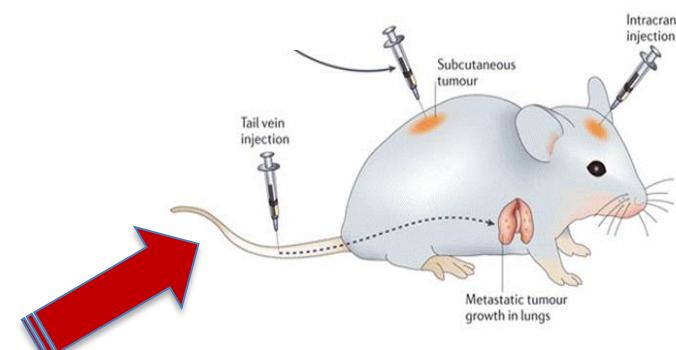


+



X-Ray

2, 5 or 8 Gy





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 through 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

Merci pour votre attention



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Un grand merci à tous ces chercheurs

