High frequency Electromagnetic Fields sensing based on lab-on-chip technology for Cancer Stem Cells Isolation and Analysis

Claire Dalmay[1], Barbara Bessette[2], Fabrice Lalloué[2], Arnaud Pothier[1]

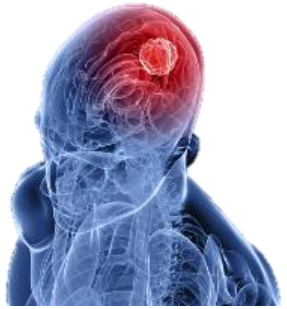
[1]XLIM – University of Limoges – CNRS UMR n°7252

[2]CAPTuR, University of Limoges – EA3842

Glioblastoma / Cancerous Stem Cells

Glioblastoma

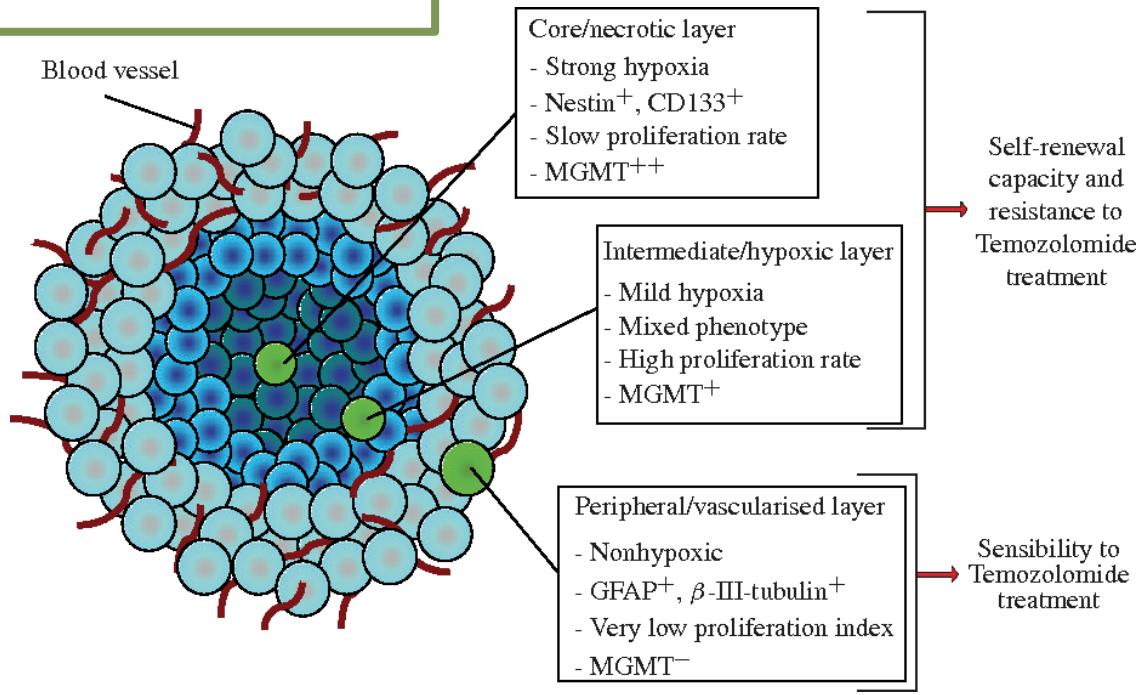
- *Tumor with high recurrence*
- *Strong resistance to existing treatments*
- *Highly heterogeneous brain tumors*



Role of Cancerous Stem cells ?

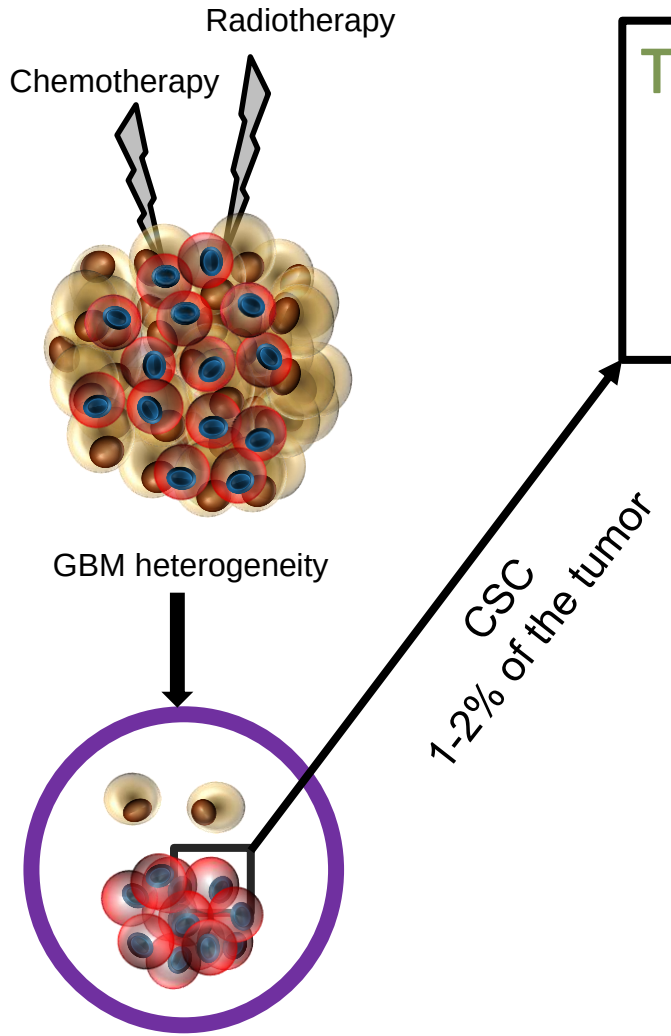
How many are they?

Where are they?



Persano L et al., ScientificWorldJournal. 2011

Cancerous Stem Cells / Glioblastoma



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

Therapeutic issues

How to identify CSC ?

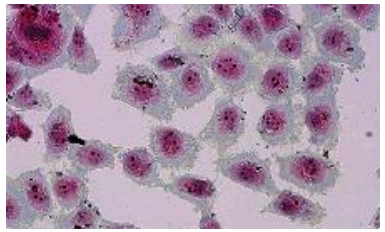
Can they be targeted ?

Conventional biological methods (1/2)

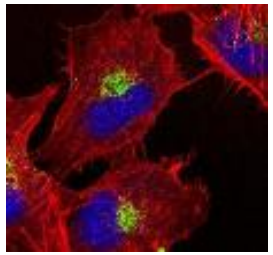
⇨ Generic markers of normal stem cells like u*ndifferentiation & Anti proliferation markers* :Nanog, Sox2, OCT4, CD133… are used but not very efficient

⇨ *Cross coupling* of makers can increase the détection

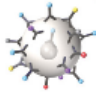
Optical microscopy



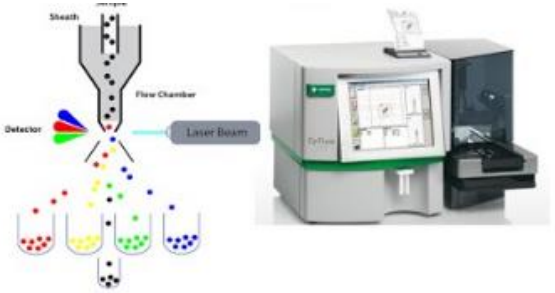
Staining



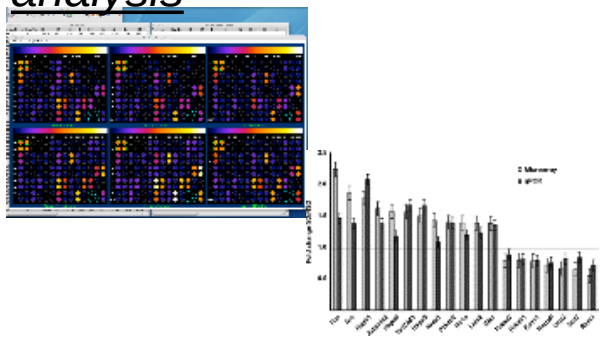
Fluorescence labeling



Flow cytometry



QPCR & Protein Array analysis



Main difficulties :

- CSC are rare and require amplification of population

***- Specific* immunostaining markers are lacking**

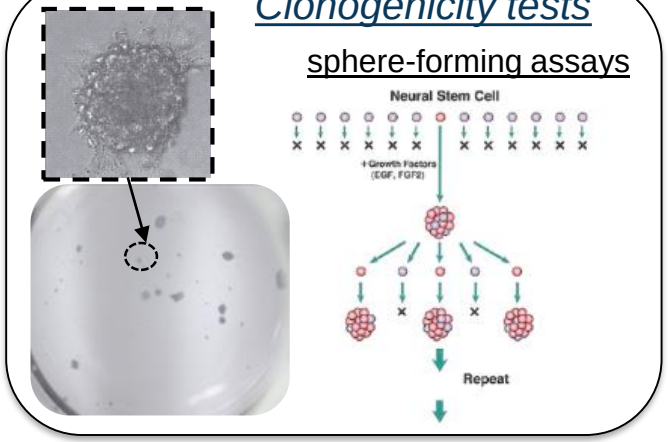
Conventional biological methods (2/2)

Functional tests are nowadays the most reliable test :

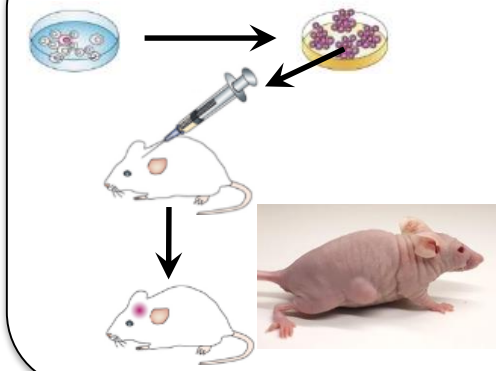
⇨ to prove the ability to renew a tumor mass

Clonogenicity tests

sphere-forming assays



Tumorigenicity tests



Main difficulties :

It requires very long time (≈ 40 days)

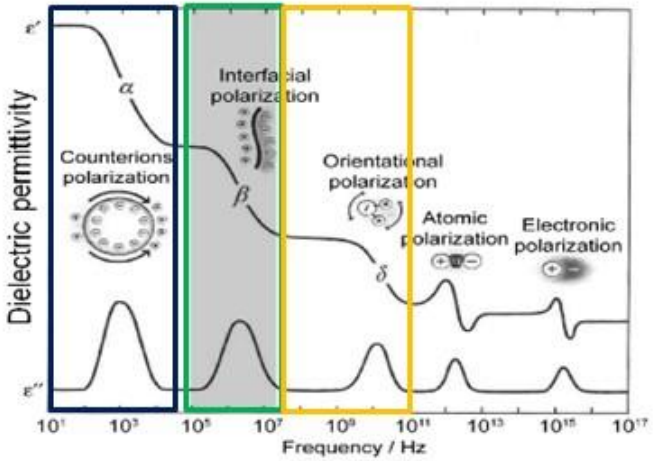
It is costly and complex tests to implement

Interest to develop others approaches investigating intracellular specificities (without labeling)

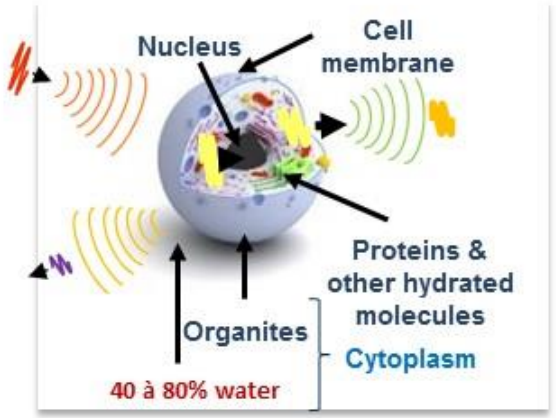
What about using EM field to identify CSC ?

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



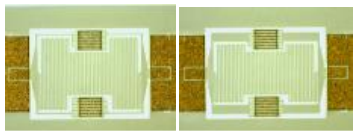
Own cell dielectric properties = **A signature** *that can be specific*



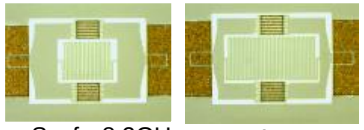
High frequency signal well suitable to access to cell interior properties and measure specificities

Dielectric spectroscopy allows **non destructive** & **label free** characterization

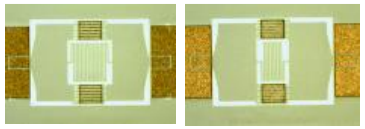
Discriminating cells with dielectric spectroscopy



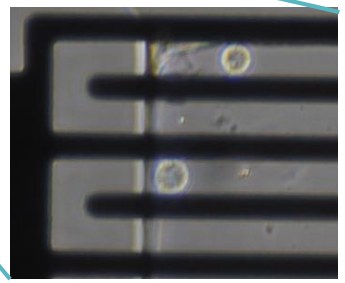
S_A: f_0=6.2GHz S_B: f_0=7,5GHz

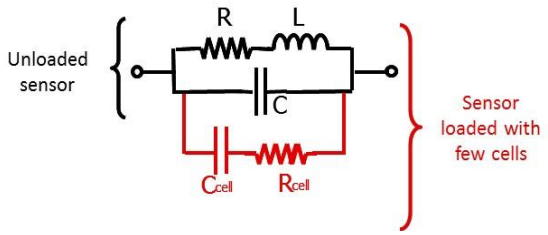


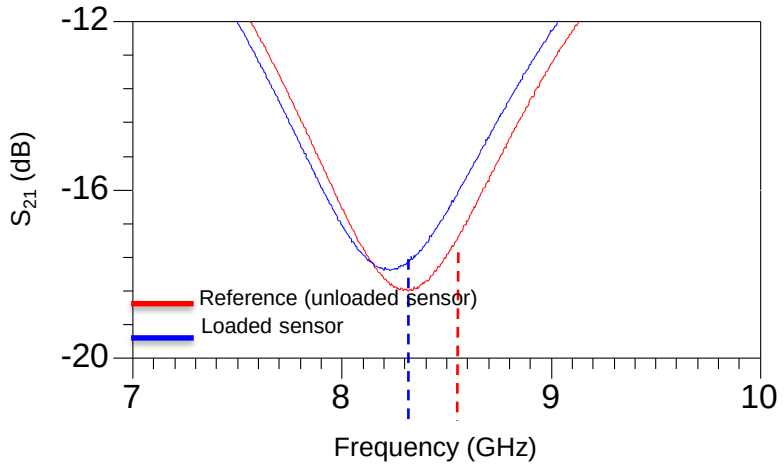
S_C: f_0=8.2GHz S_D: f_0=9.9GHz



S_E: f_0=12GHz S_F: f_0=16GHz







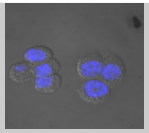
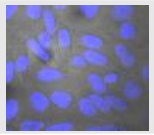
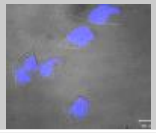
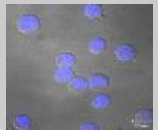
V_{cell} represents the intacellular volume
G is a geometric factor depending on the sensitive area design

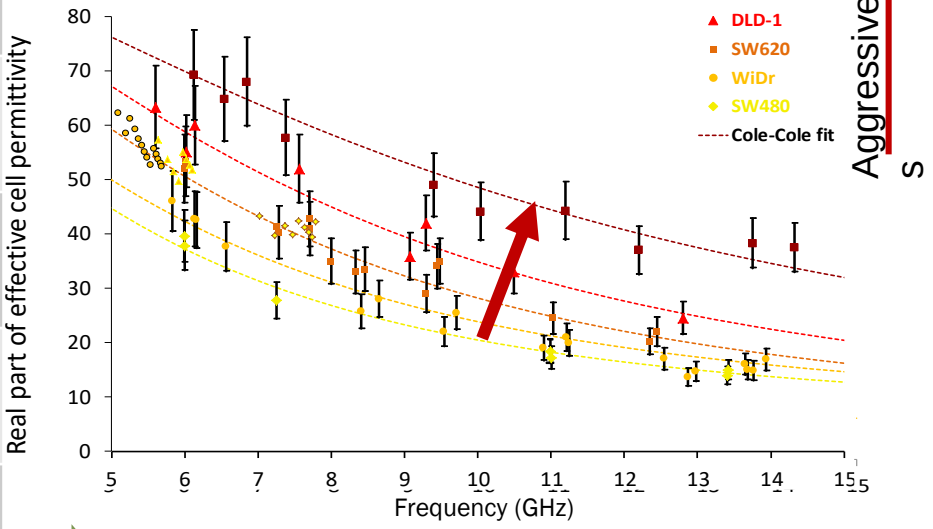
$$e_{cell} \mu \frac{C_{cell}}{V_{cell}}.G$$

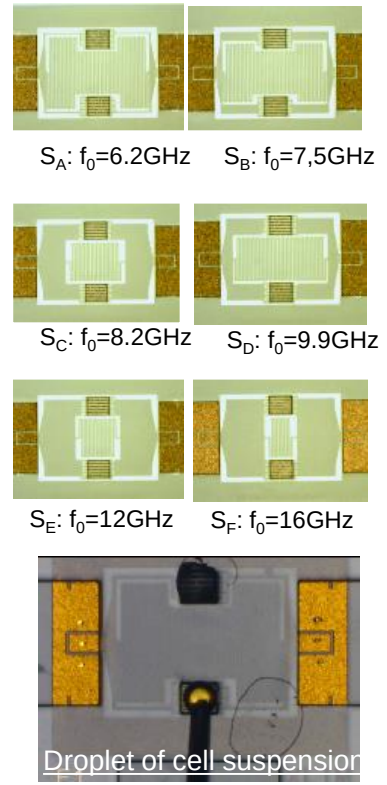
$$\begin{cases} F_0 = \dfrac{1}{2p\sqrt{L(C_0)}} \\ F_1 = \dfrac{1}{2p\sqrt{L(C_0 + C_{cell})}} \end{cases}$$

Discriminating cells with dielectric spectroscopy

⇨ diagnostic and prognostic evaluation in the therapeutic management of cancer patients

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





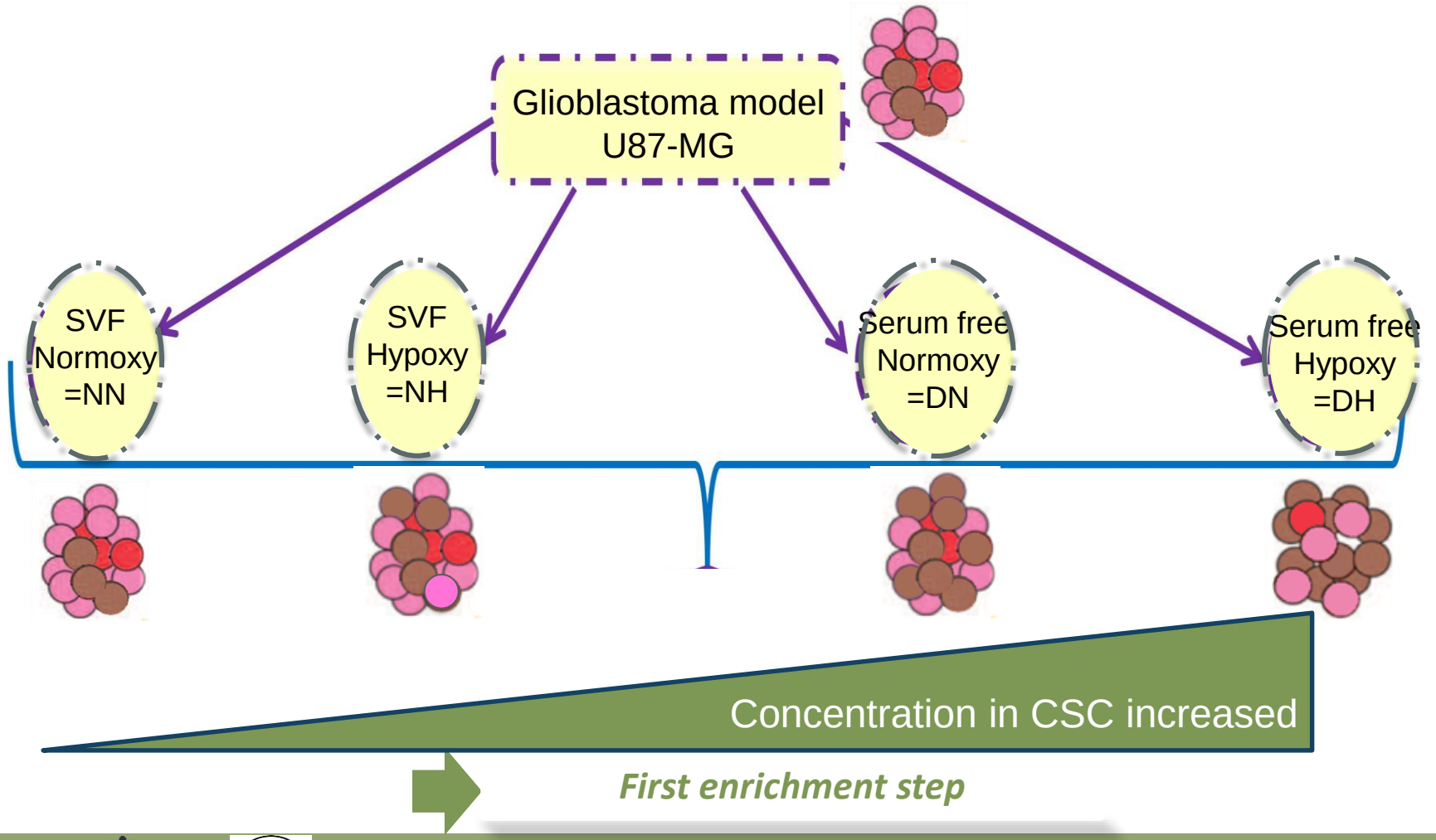
⇨ *Correlation with the cell tumor grade*

Oncomedics

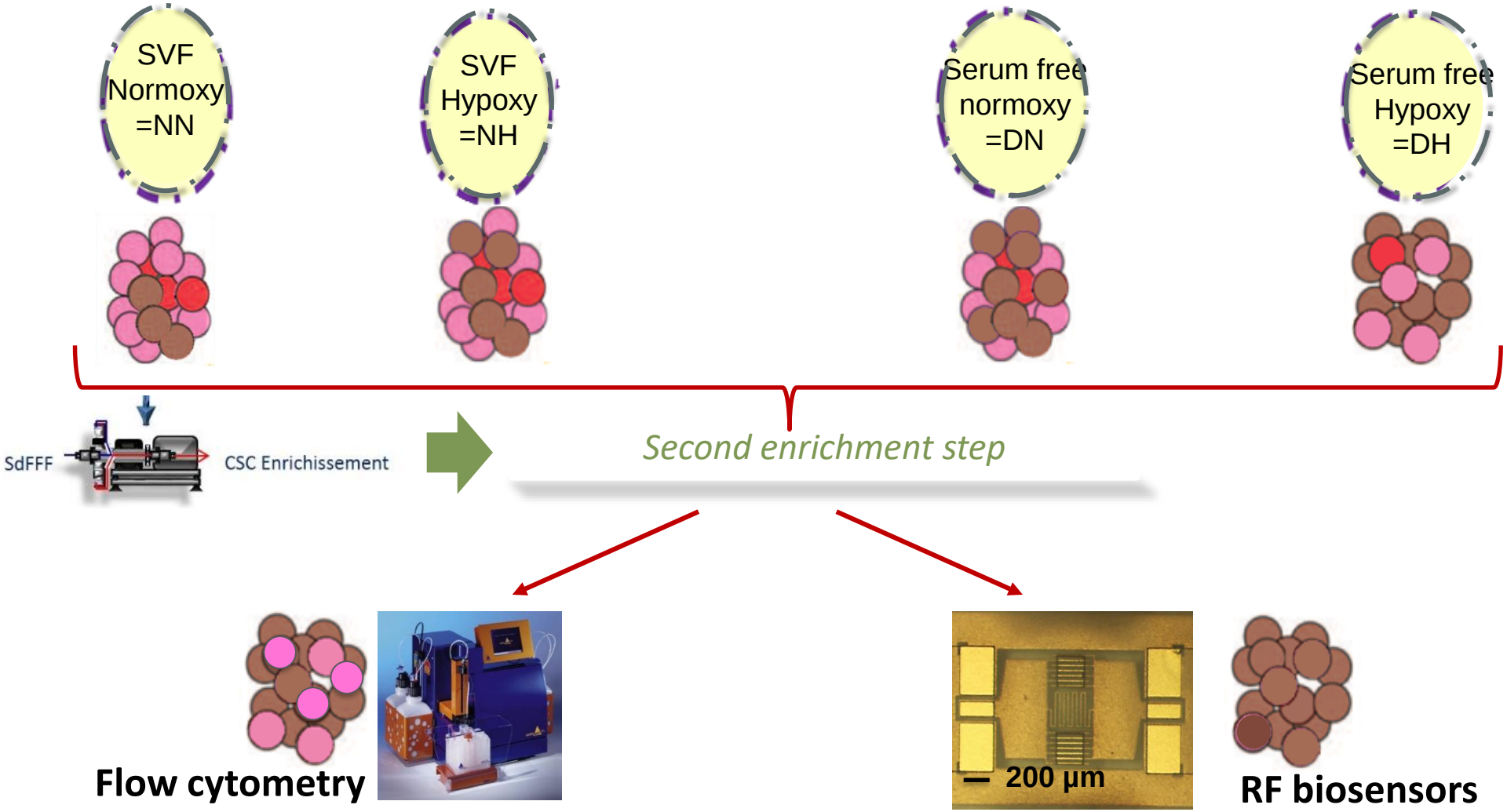
L.Y. Zhang et al, Discrimination of Colorectal Cancer Cell Lines using Microwave Biosensors Sensors & Actuators: A. Physical, Vol 216, Sept 2014.

What about using EM field to identify CSC ?

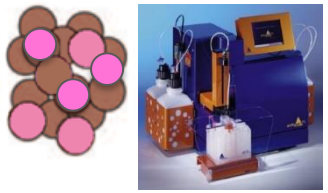
⇨Biological model : enrichment in CSC with culture conditions



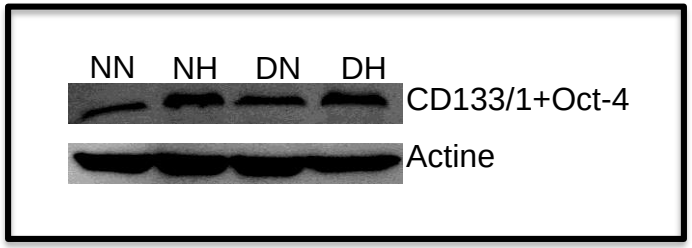
What about using EM field to identify CSC ?



Discrimination of CSC by their dielectric signatures

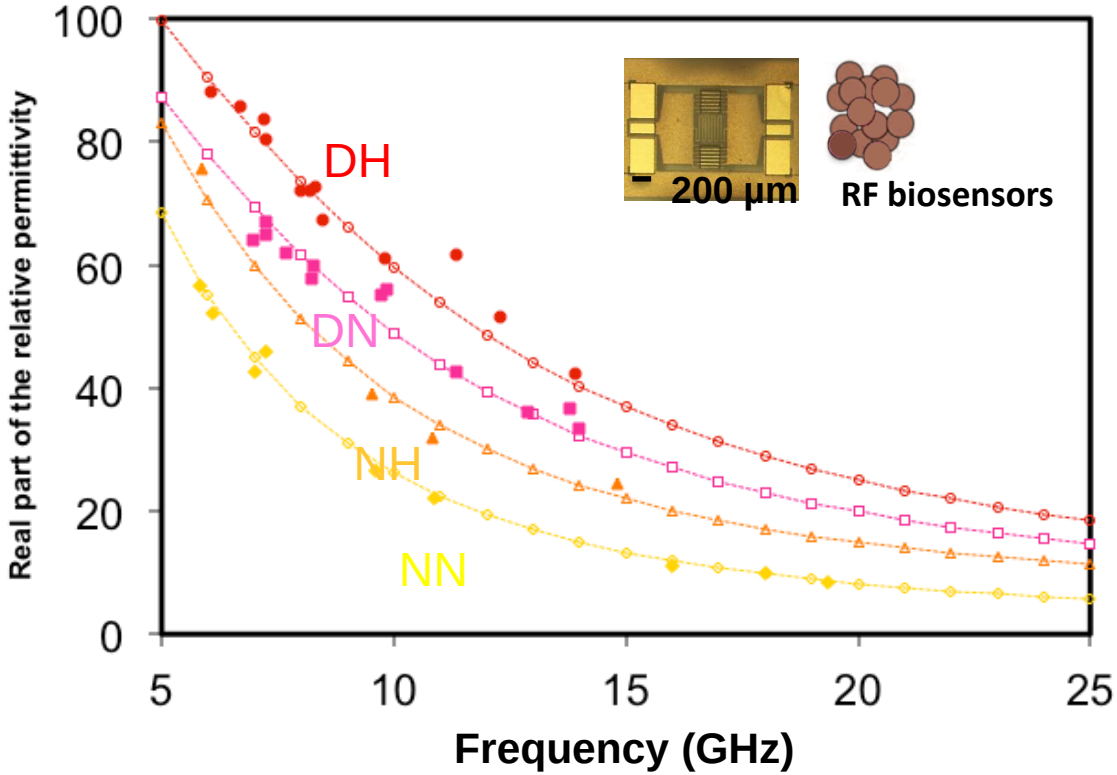


Gold standard



Tiny differences between the 4 conditions

⇒ Inefficient to sort CSC



Distinct electromagnetic characteristics depending on the culture conditions

⇒ High potential for accurate CSC sorting

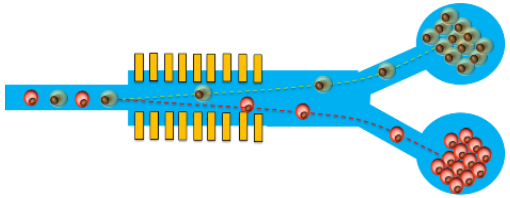
To dielectric spectroscopy cytometer concept

Main challenges :

⇨ Development of a cell sorter based on their specific EM signature
⇨ Selective electromanipulation by EM fieds **DEP**

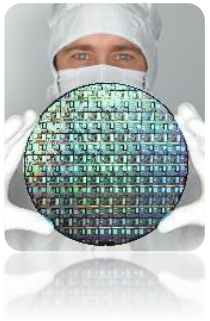
⇨ Sensitivity to intracellular properties **Ultra High Frequencies (UHF)**

UHF-DEP

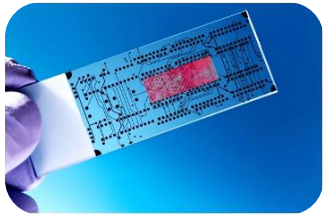


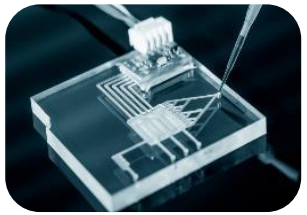
⇨ Mature technology able to quickly address a large market
⇨ Miniaturization of the complete device and Lab-On-Chip compatible, full and monolithic integration of microfluidic

CMOS technology



SUMCASTEC Project



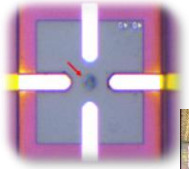


New Generation of Microwave Lab-on-Chip for **Cancerous Stem Cells** Neutralization using Electromagnetic Waves Stimulation

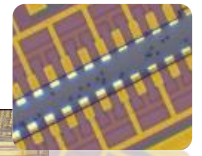
> Concept: *Exploit the non-thermal effects of **EM radiations** on living organizes to **sense** and **stimulate** specifically targeted biological cells*

> 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



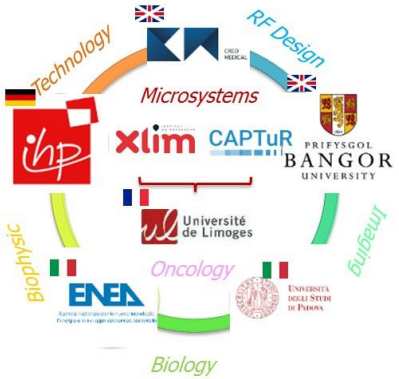
Individual Cell sensor



Electromagnetic based Cytometer

Prototype of microfluidic sensing platform on CMOS chip

> The team : **A multidisciplinary consortium to address a broad spectrum of research challenges**



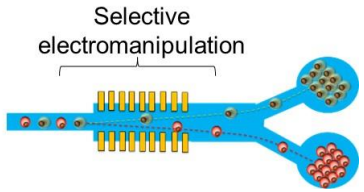
10 teams from 6 institutions

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

Sumcastec

H2020 FET program supported by EU commission

Dielectrophoresis basis



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

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

Clausius-Mossotti factor

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

Complex permittivity of the particle

-1<Re[K(ω)] <1

➡ Cells can be individually electromanipulated accordingly their own dielectric properties

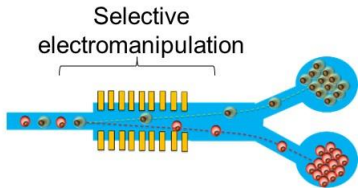
Re[K(ω)] <0

Repulsive force

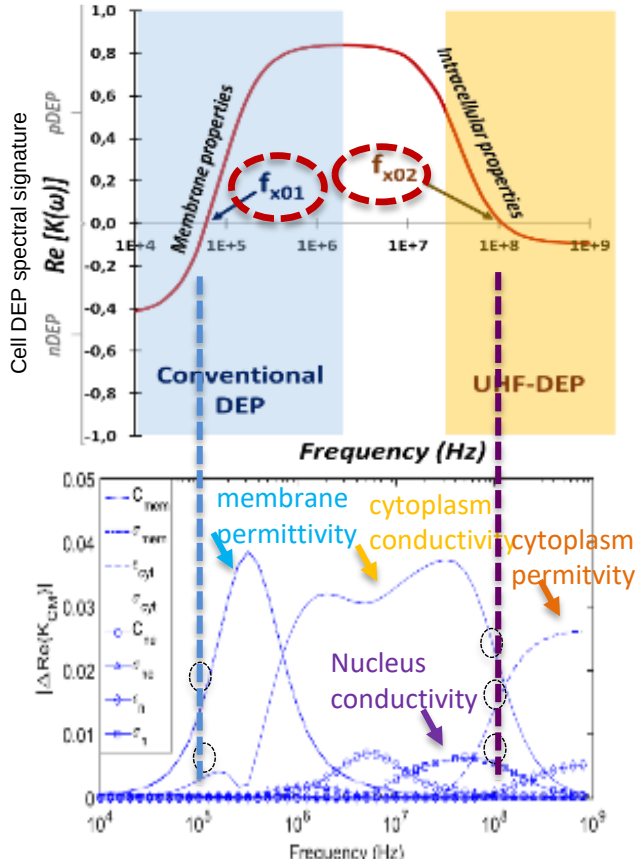
Re[K(ω)] >0

Attractive force

Specificities of cell DEP spectral signature

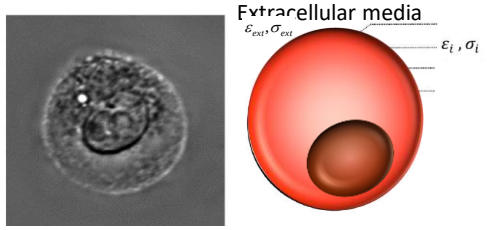


Cell caracterization to thier cross over frenquencies (HF-f_{x02}) as discriminant factor

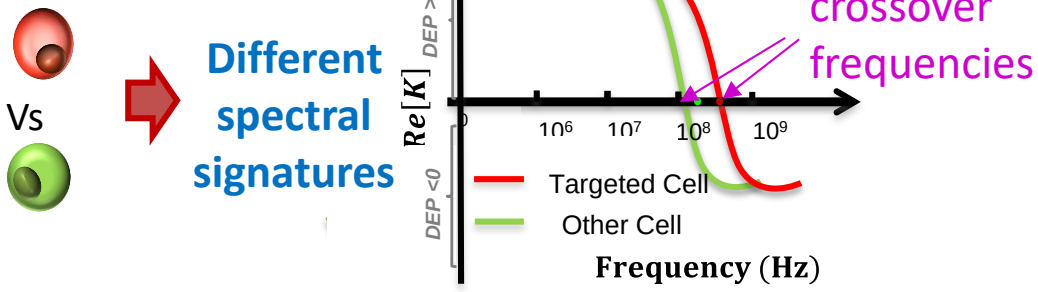


E. Salimo et al., DOI: 10.1063/1.4940432

Dielectrophoresis theory basics

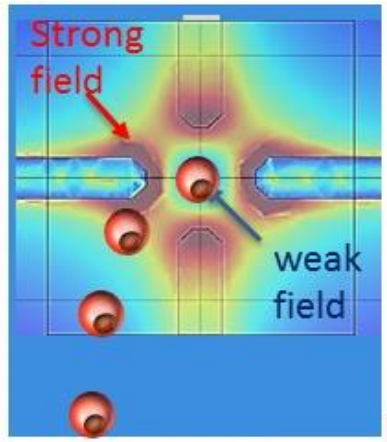


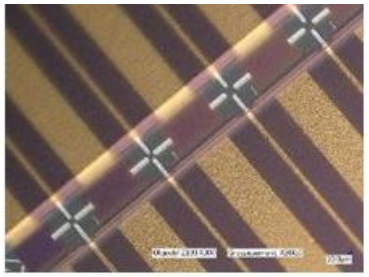
Different cells



f_{x02} is an intracellular marker

Crossover frequency measurements





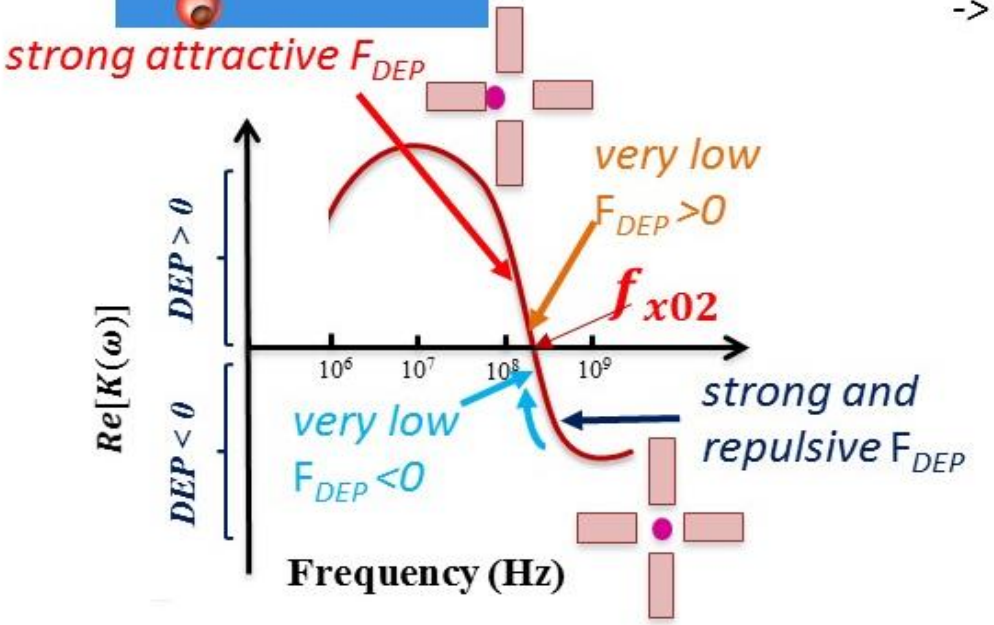
$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[K(\omega)\right] \nabla\left|E_{rms}\right|^2$$

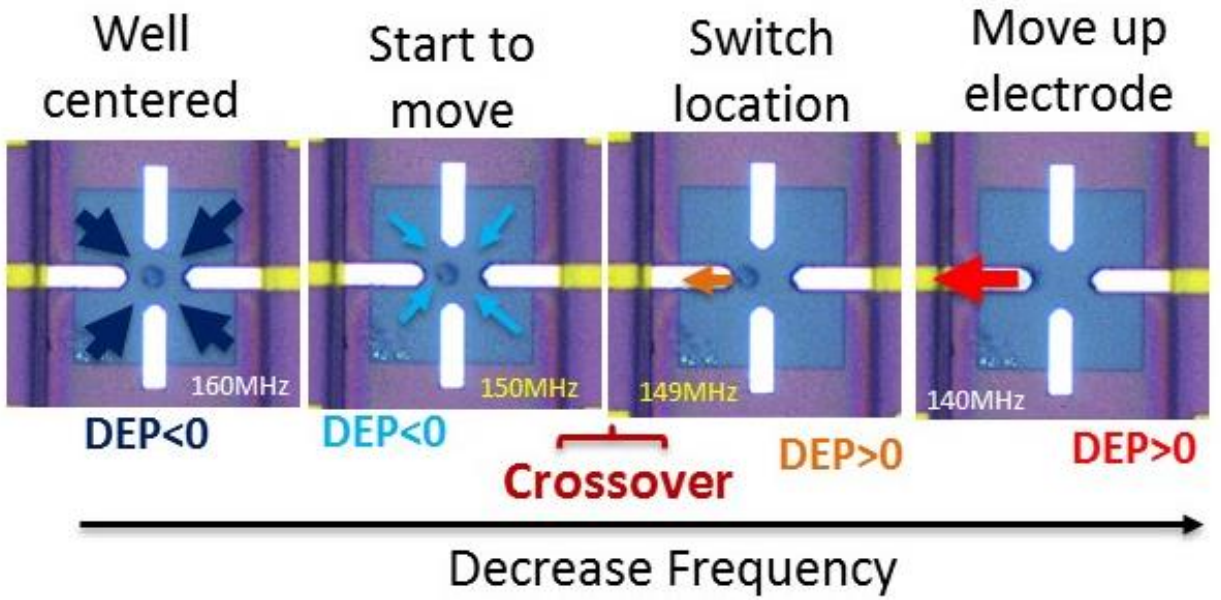
-> F_{DEP} will be high in strong field areas

-> low in weak field areas

Methodology:

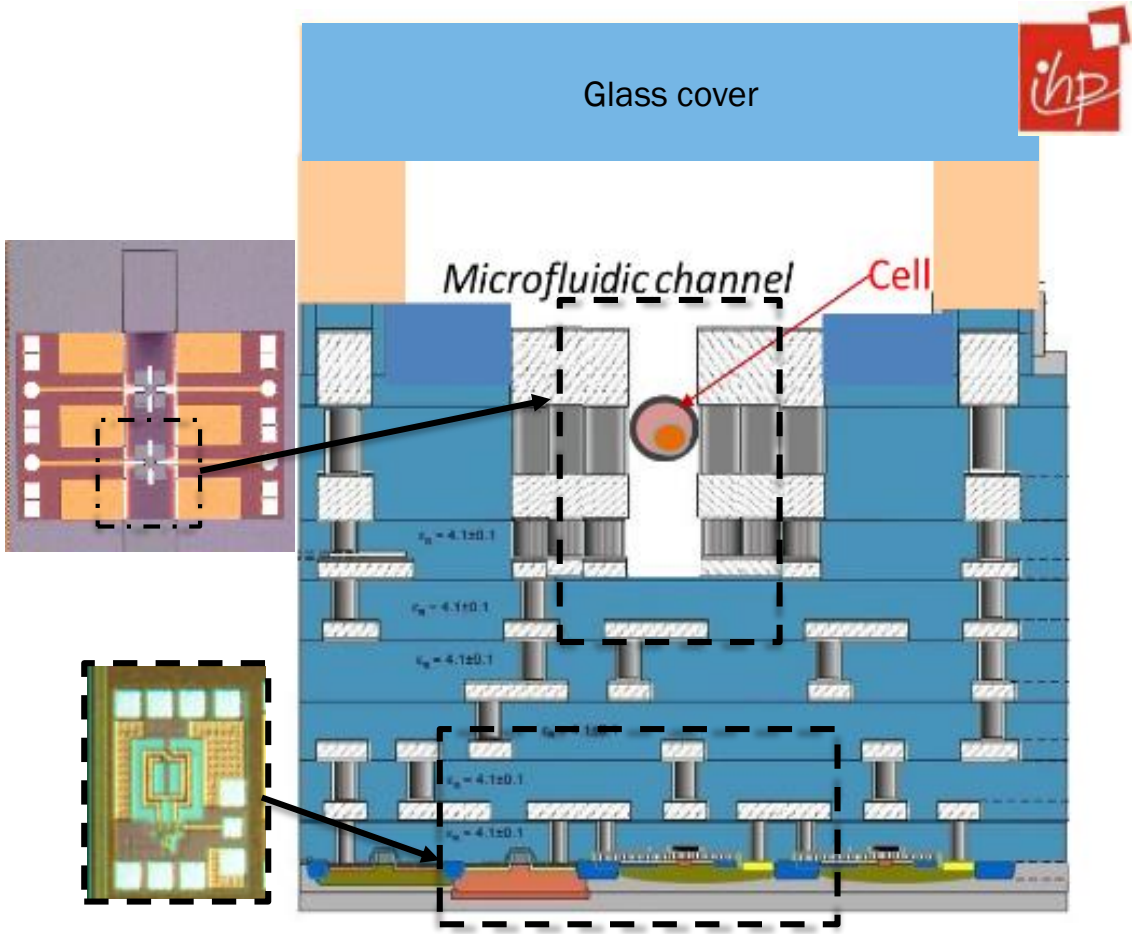
1) Cells are trapped in DEP<0
2) Flow is stopped
3) Frequency is tuned every MHz until finding positive DEP

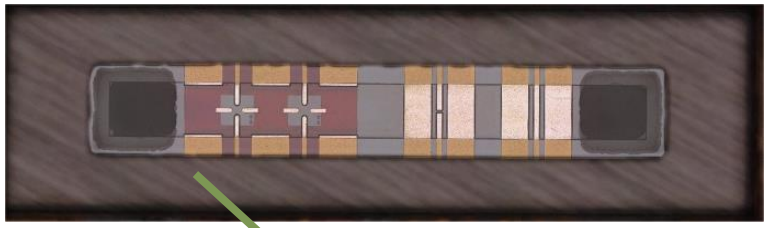


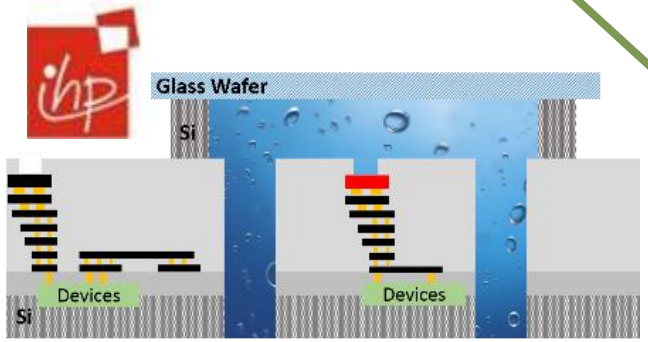


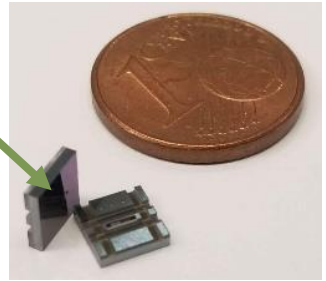
BiCMOS technology

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



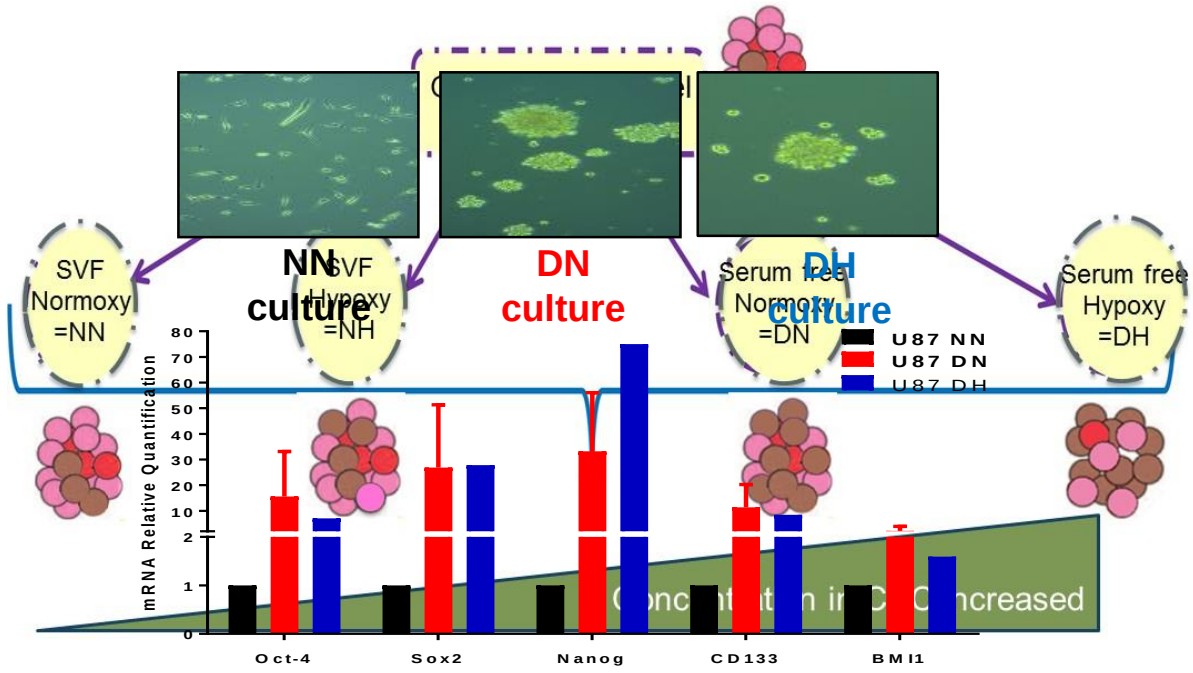




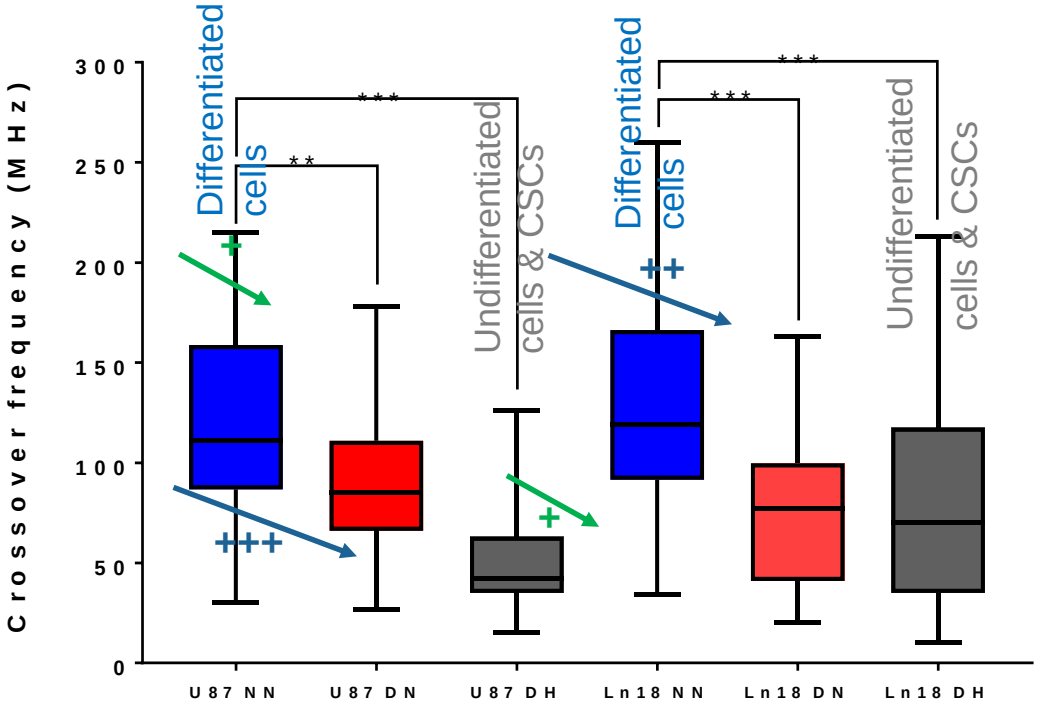


Crossover frequencies - GBM cell lines

Analysis of CSC markers at transcriptional and protein level



More than 500 cells measured



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

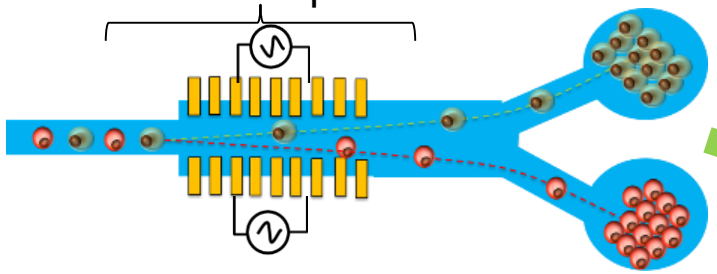
Difference of phenotype
⇨ difference of DEP signature

CSCs enriched populations show lower crossover frequencies

How exploiting crossover frequency specificities

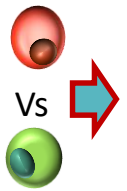
Cell population characterization ➡ selection of the more selective sorting UHF-DEP frequency

Expected selective electromanipulation



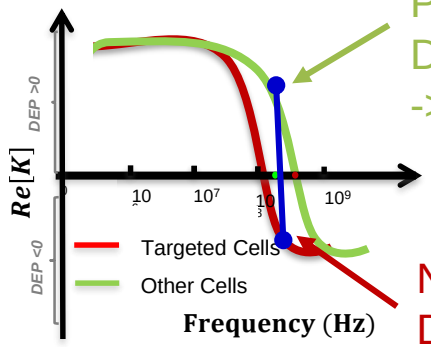
f ? ➡ $f > f_{x02}$ target

Mixed cell population



Vs

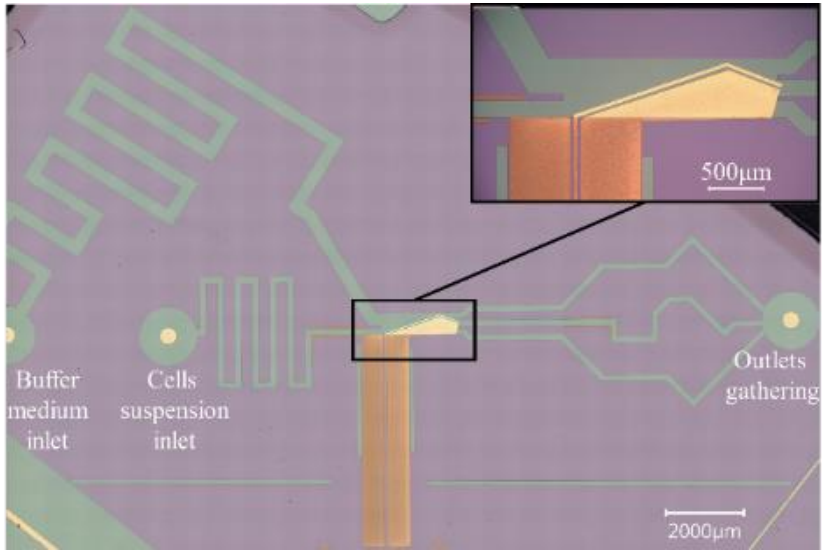
Different DEP spectral signatures



Positive DEP -> attraction

Negative DEP -> repeal

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

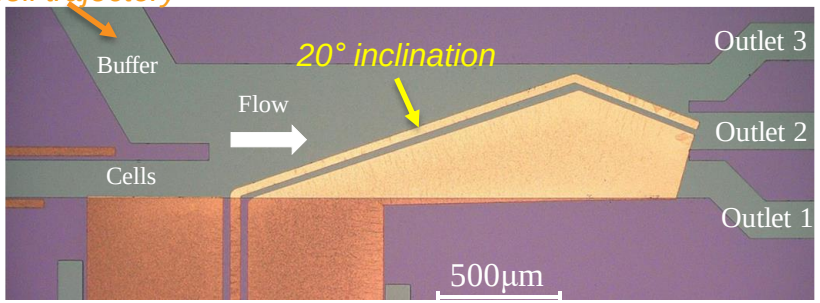


T. Provent et all, JNM 2019

UHF-DEP cytometer design

➡ Coupling of DEP & hydrofluidic forces to dynamically sort cell

Control the initial cell trajectory



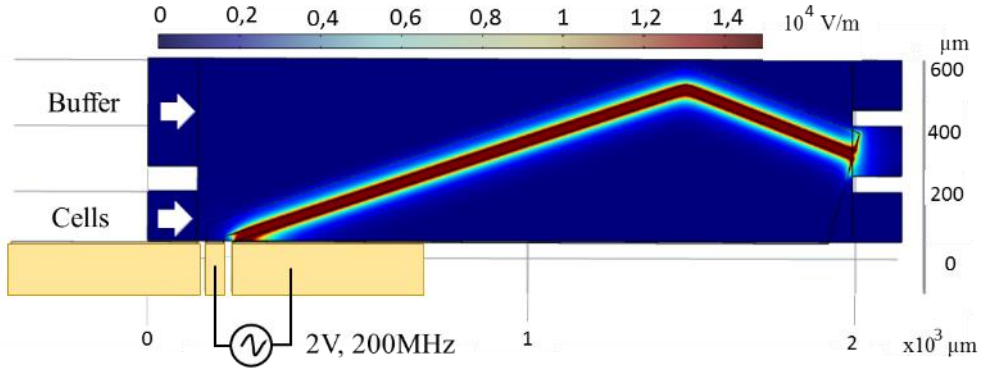
Working principle:

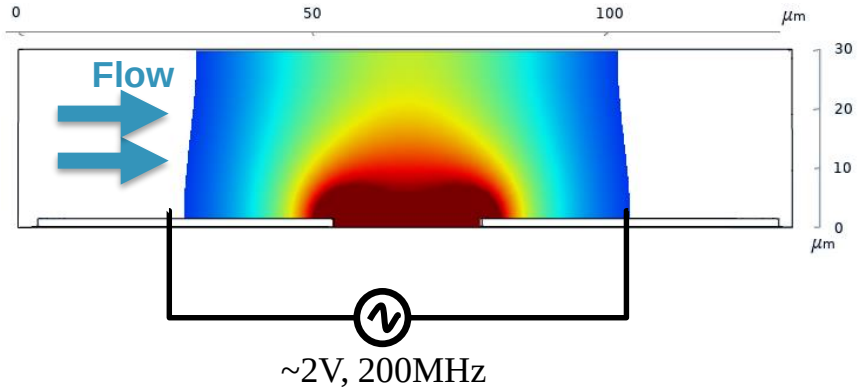
Tune the DEP force at constant fluidic force to act on cell trajectory

-> 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





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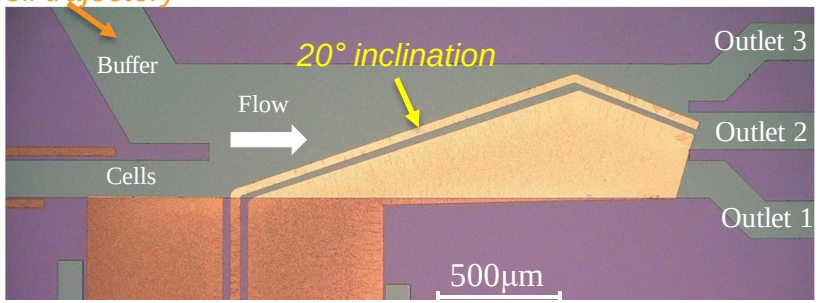
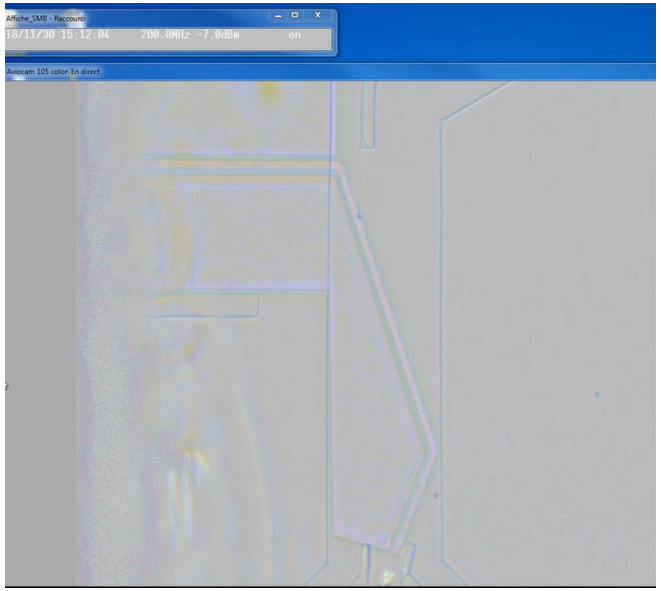
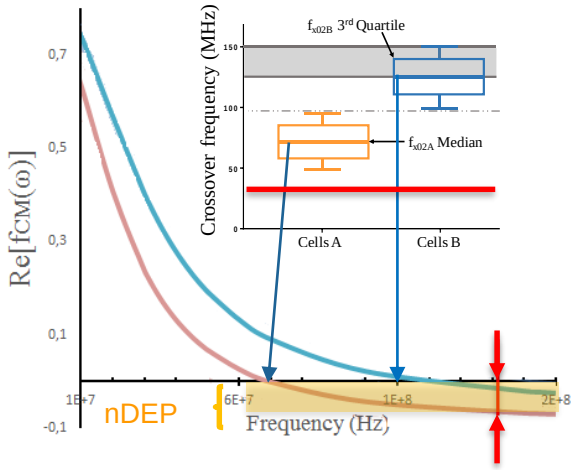
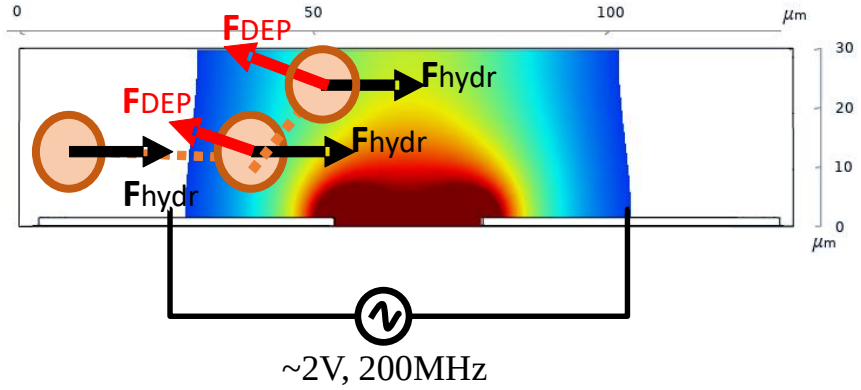




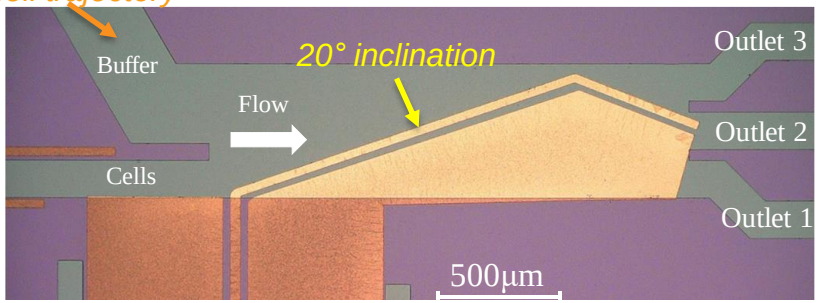


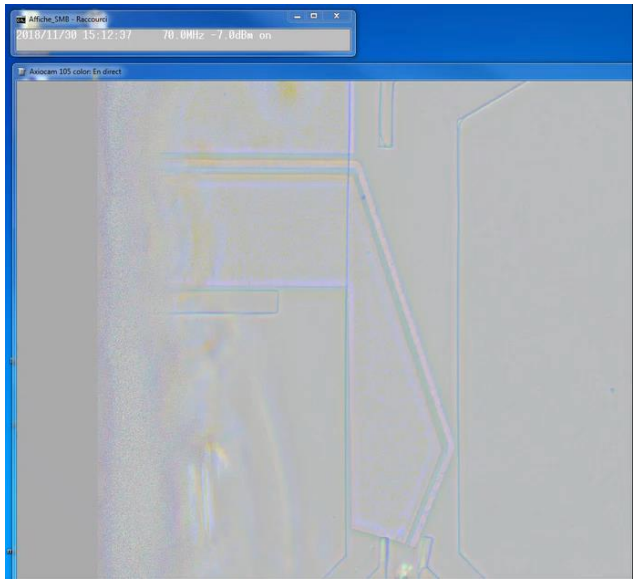


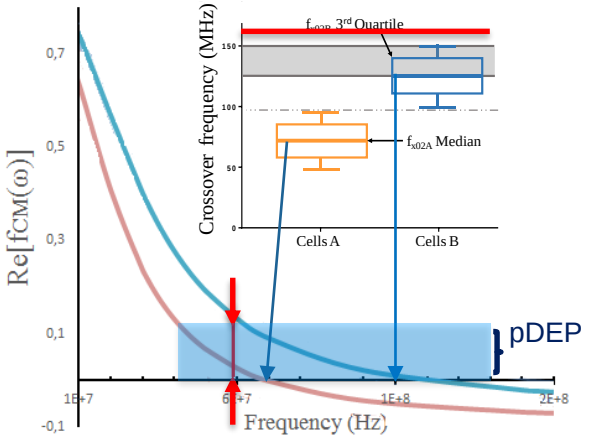
UHF-DEP cytometer design

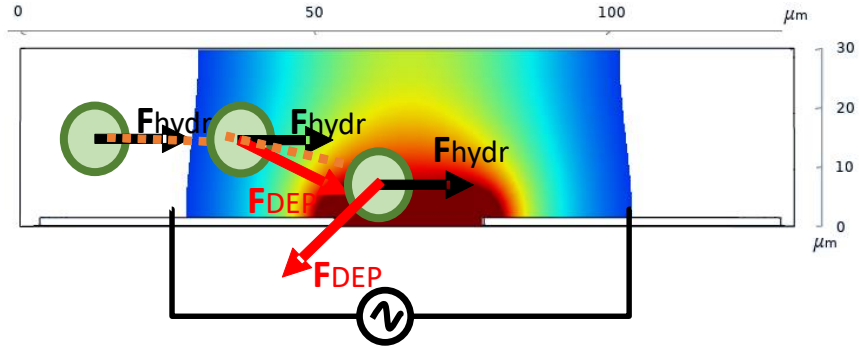
➡ Coupling of DEP & hydrofluidic forces to dynamically sort cell

Control the initial cell trajectory





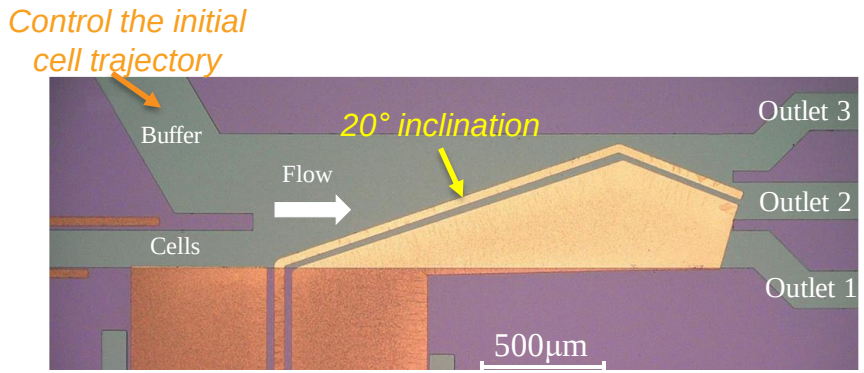


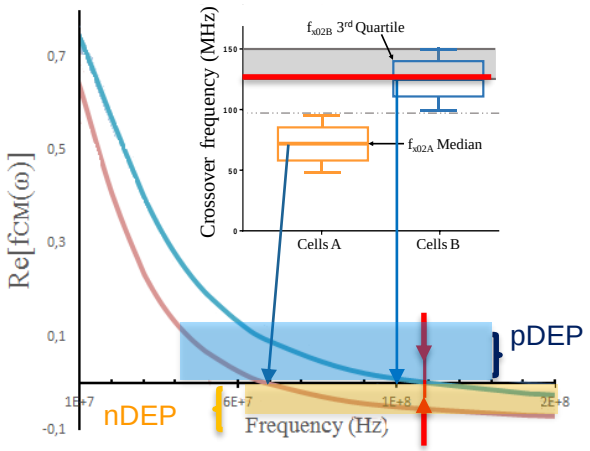


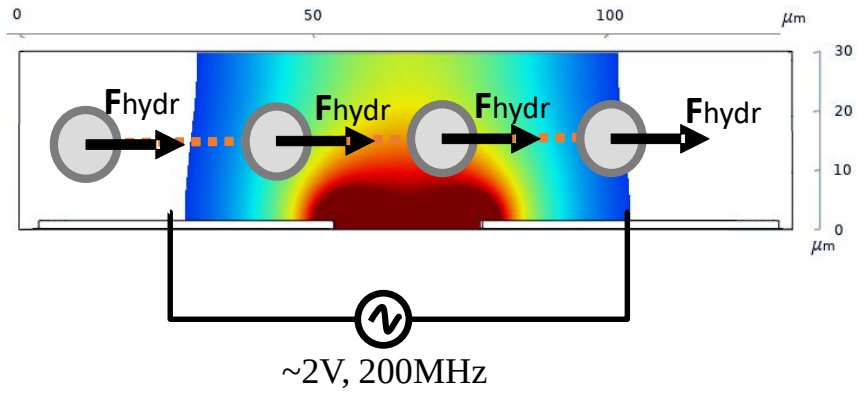
~2V, 200MHz

UHF-DEP cytometer design

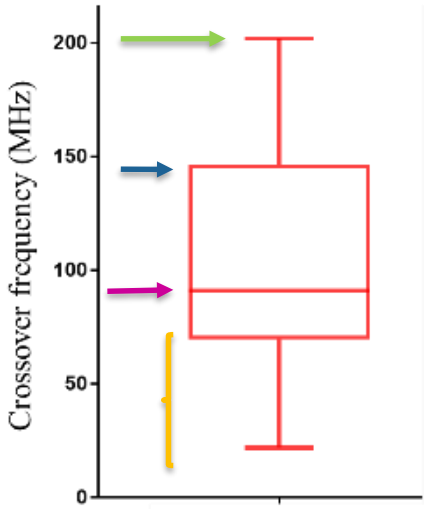
➡ Coupling of DEP & hydrofluidic forces to dynamically sort cell

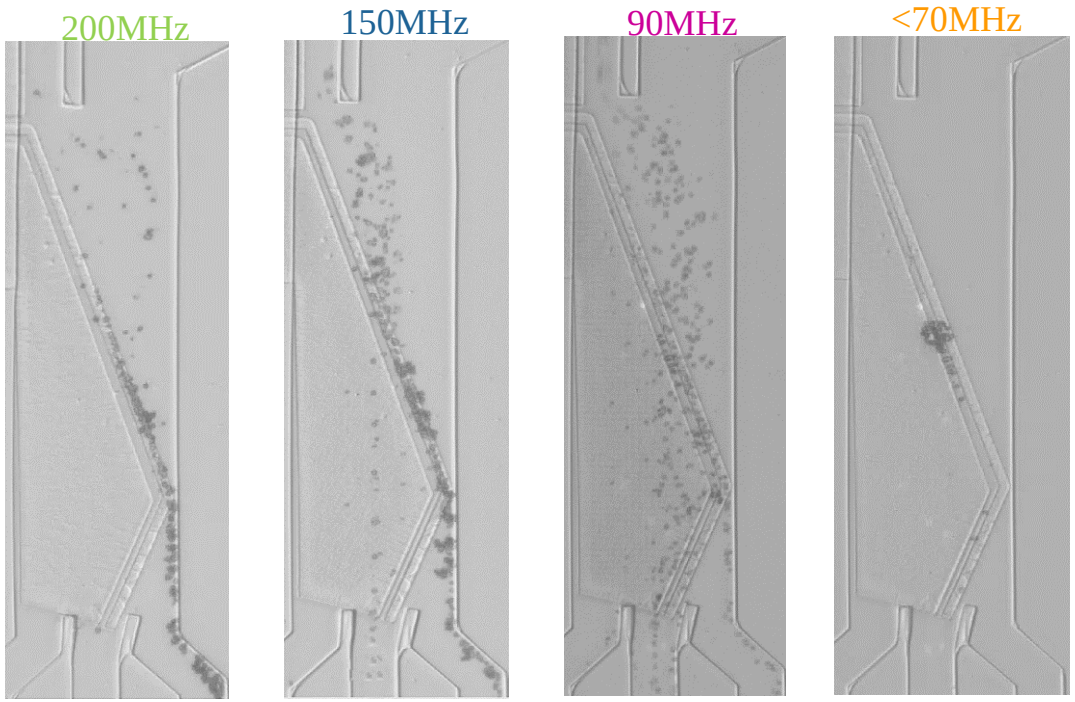






Handling a dispersive property cell population





Next steps : ⇨ optimal DEP signal frequency and magnitude vs flow speed have to be set
⇨ improving cell sorting efficiency
⇨implementation on CMOS technology

Thanks for your attention

Contact Information : claire.dalmay@xlim.fr

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Project partners: