WFA

# Electromanipulate biological cells with high frequency signals: a new way to characterize cell aggressiveness in the frame of cancer treatment

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# Outline

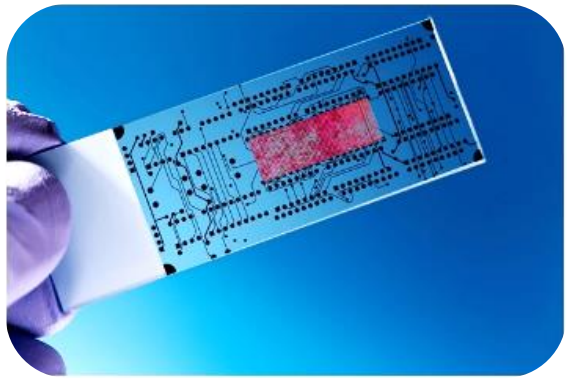
- SUMCASTEC project objectives
- Motivation: Cancerous Stem Cells issues
- Main challenges to identify CSC
- UHF dielectrophoresis as a new cell characterization approach
- Going to a novel UHF DEP cytometer for efficient CSC isolation?
- Conclusion and perspective

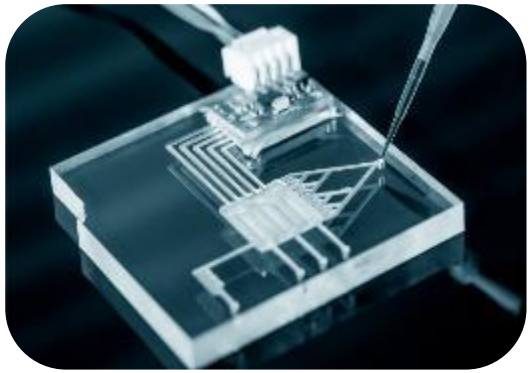
# SUMCASTEC project

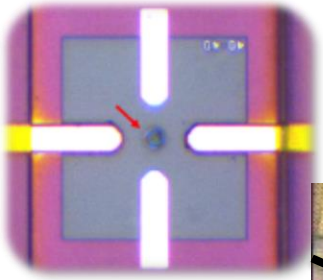
## Sumcastec: H2020 FET program supported by EU commission

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

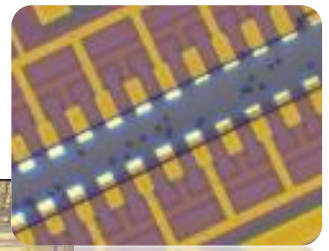
***Concept:*** *Exploit the non-thermal effects of **EM radiations** on living organizes to **sense** and **stimulate** specifically targeted biological cells*



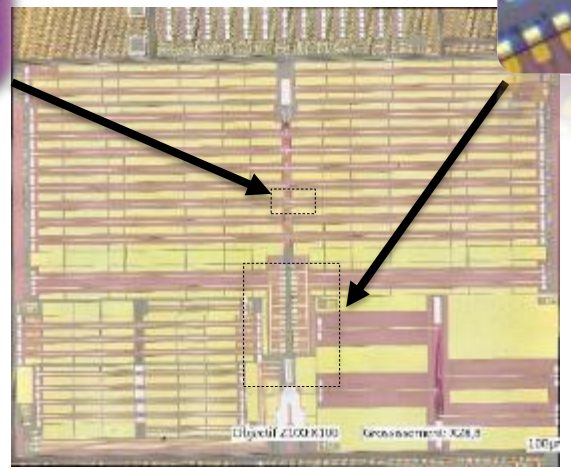




*Individual Cell sensor*



*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

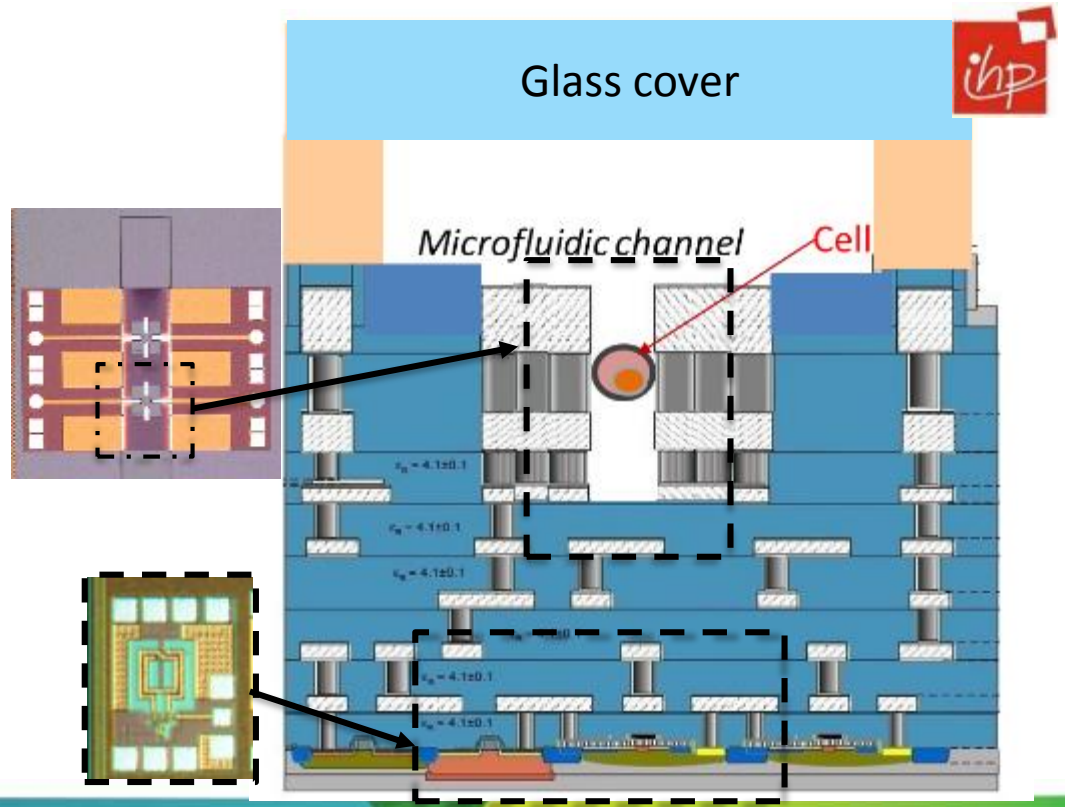
*Prototype of microfluidic sensing platform on CMOS chip*

# 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***

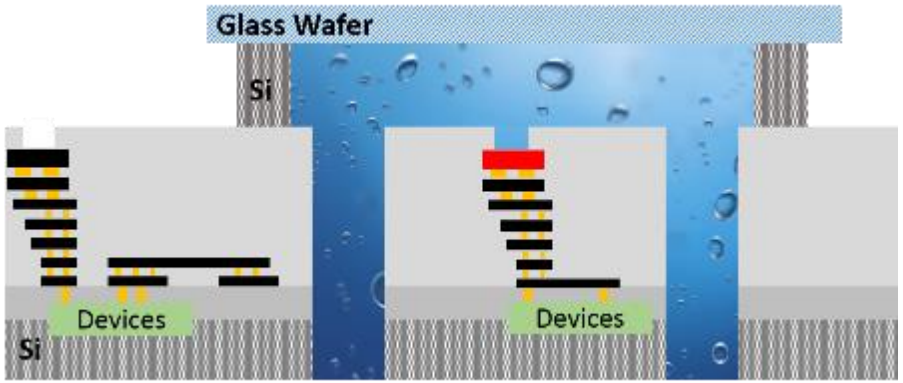


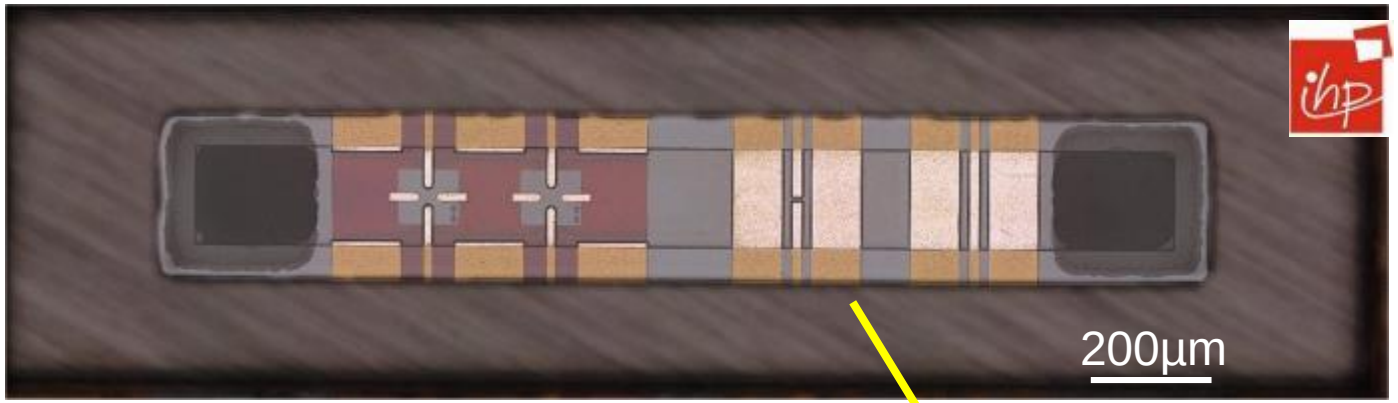
# Why CMOS technology?

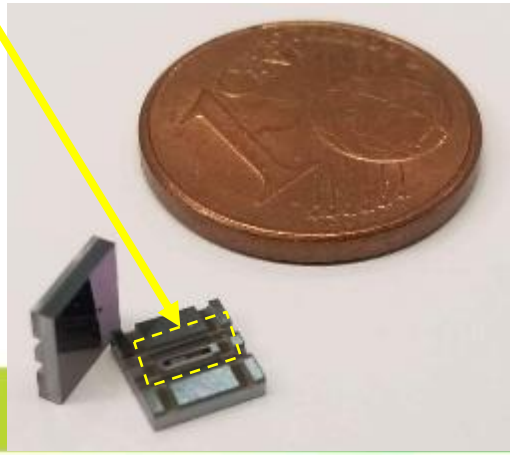
## Advantages of BiCMOS technology:

- ✓ 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***

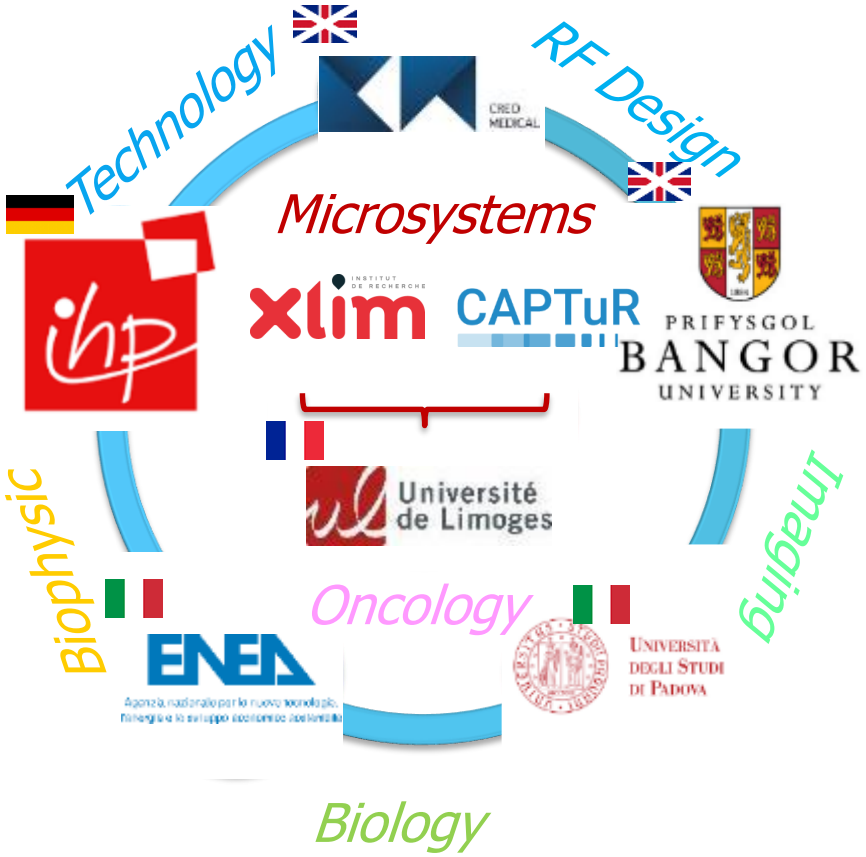


# SUMCASTEC team

*A multidisciplinary consortium to address a broad spectrum of research challenges*




10 teams from 6 institutions

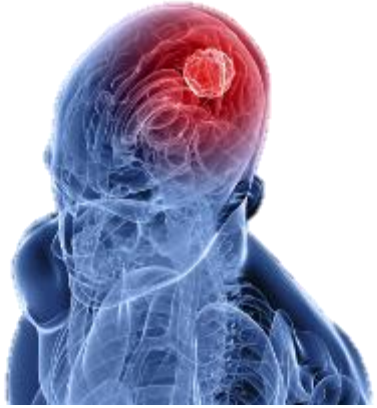
- ***RF &Microwave Engineering:***
  -> BANGOR, CREO, IHP, XLIM(UNILIM)
- ***Photonic & Imaging Engineering :***
  -> BANGOR
- ***Micro Technology Development:***
  -> IHP, XLIM(UNILIM)
- ***Biology & Oncology***
  -> ENEA, UNIPD, CAPTuR (UNILIM)
- ***Biophysics***
  -> ENEA, XLIM (UNILIM)

# 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*

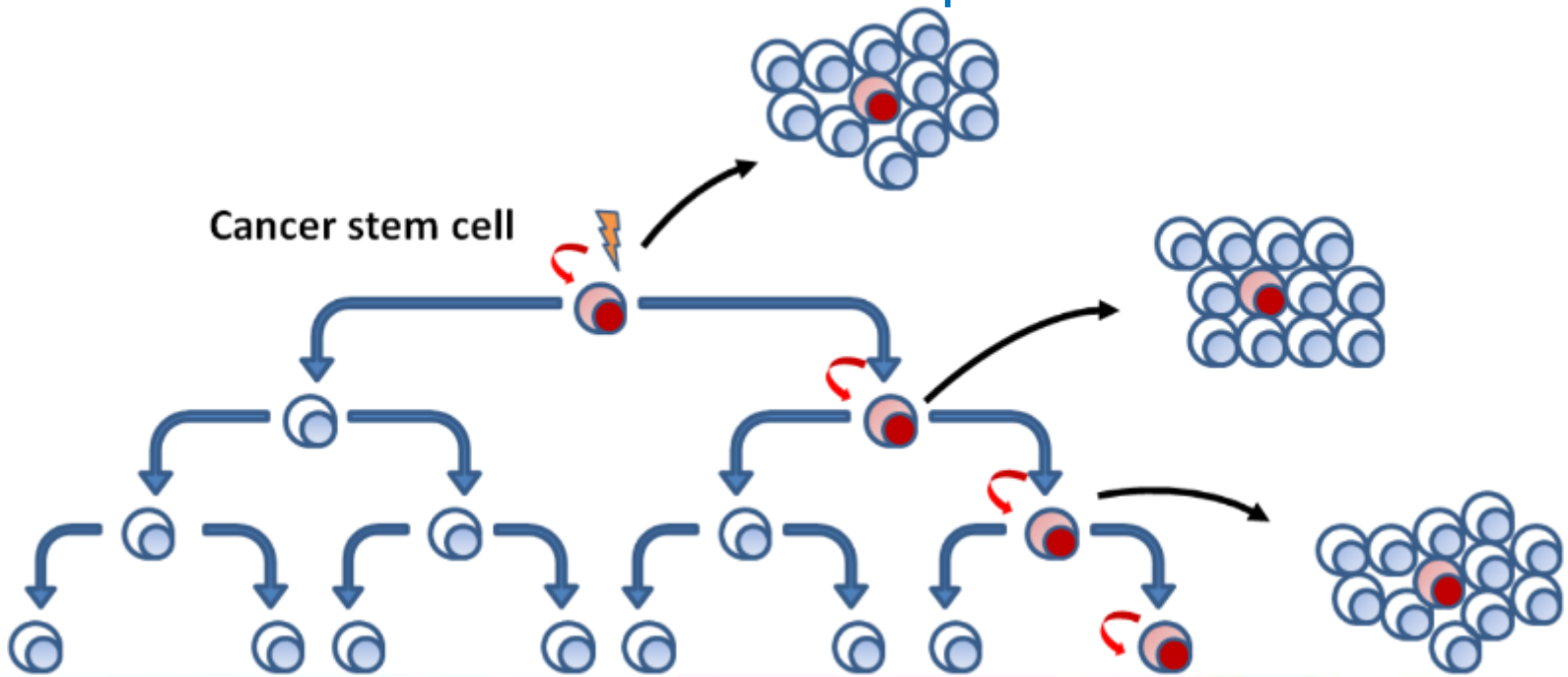
**Role of some hidden tumor-initiating cells ?**

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

# Cancerous Stem Cells

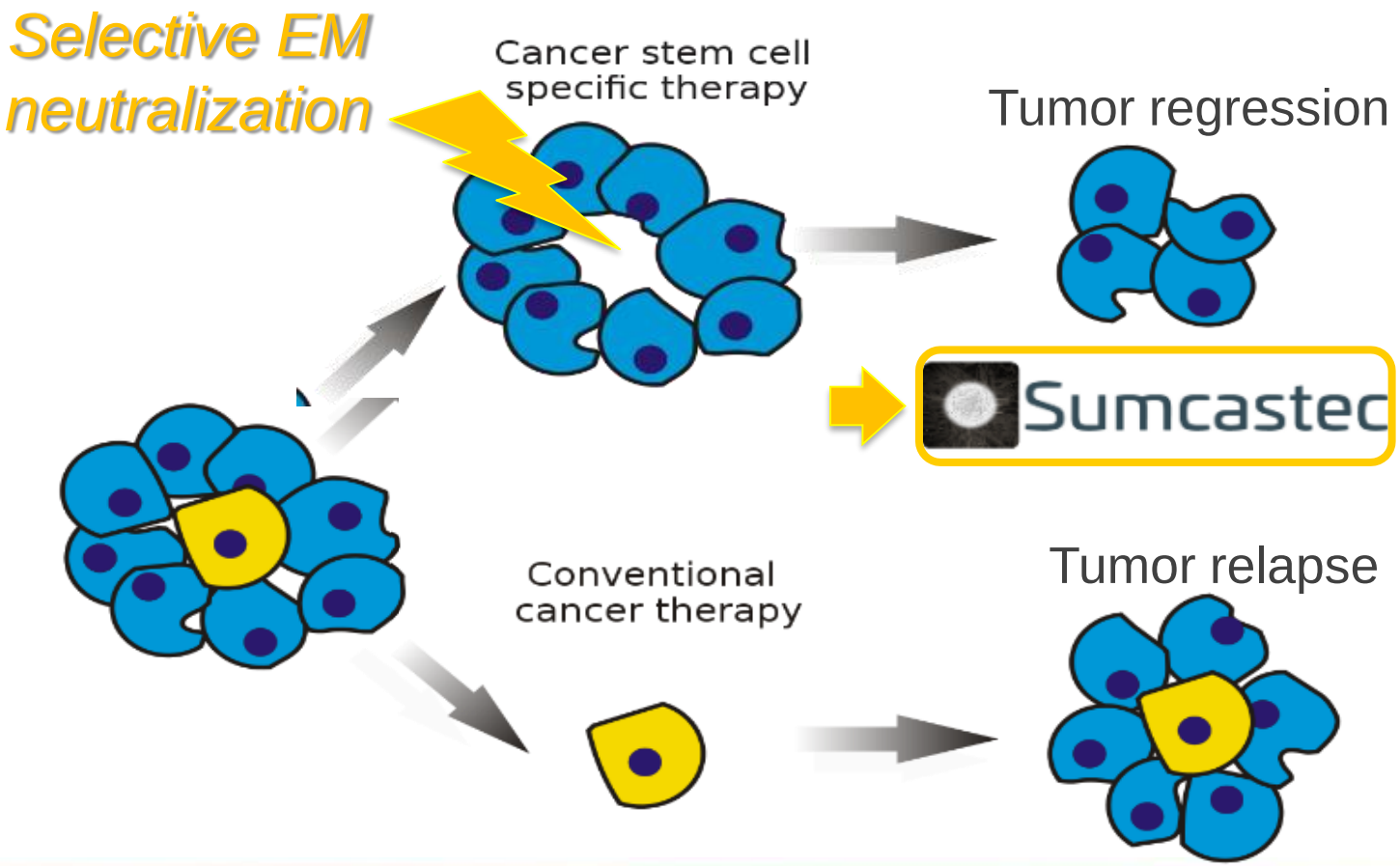
## Tumorigenic cells with ability to give rise to all tumor cell types:

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



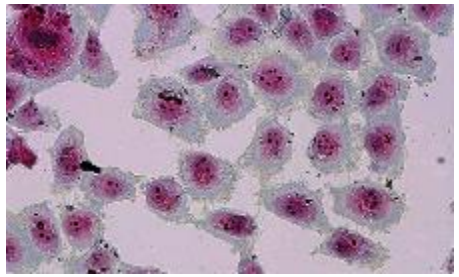
# New therapies targeting CSCs

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

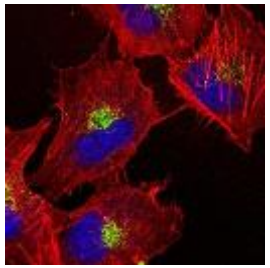


# How biologists study CSC's currently?

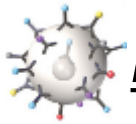
*Optical microscopy*



*Staining*



*Fluorescence labeling*

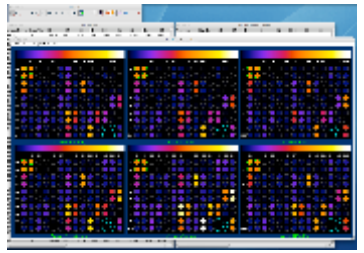


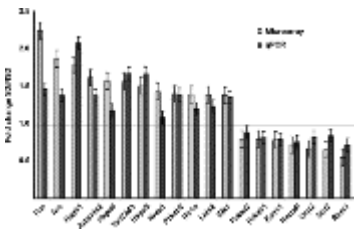
*Flow cytometry*


*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:

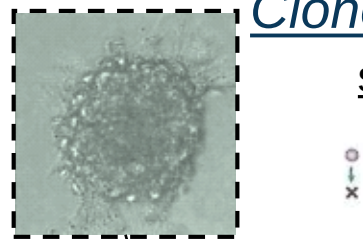
- *Undifferentiation & Anti proliferation markers* :Nanog, Sox2, OCT4, CD133…
- *Cross coupling* of makers gives evidence but without *100% absolute certainty*

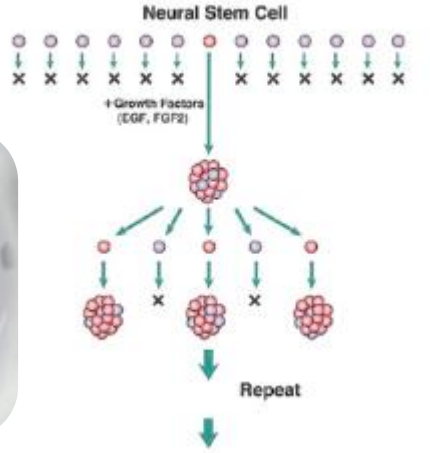
# Functional tests allow to identify CSC

## Functional tests prove ability to renew a tumor mass

*Clonogenicity tests*

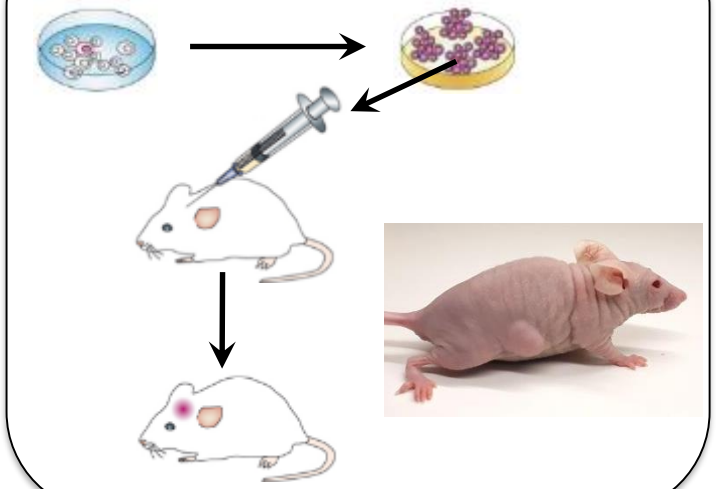
sphere-forming assays






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

*Tumorigenicity tests*

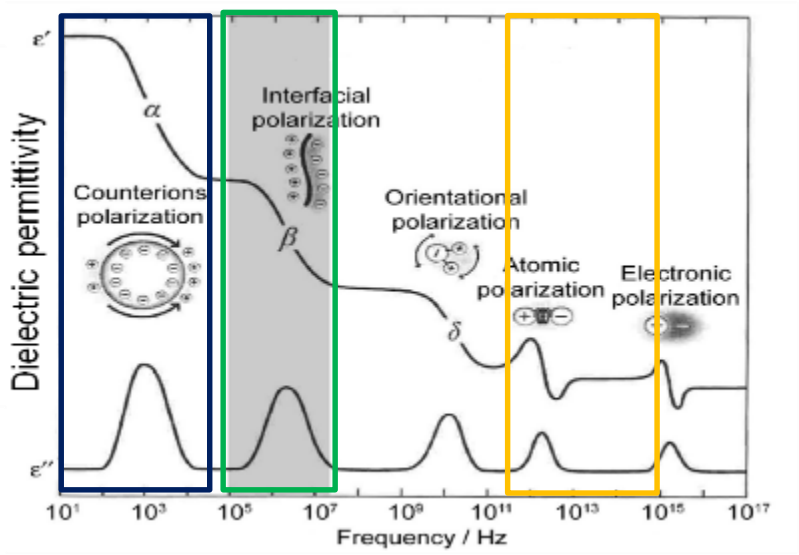


➡ Interest to develop others approaches investigating intracellular specificities

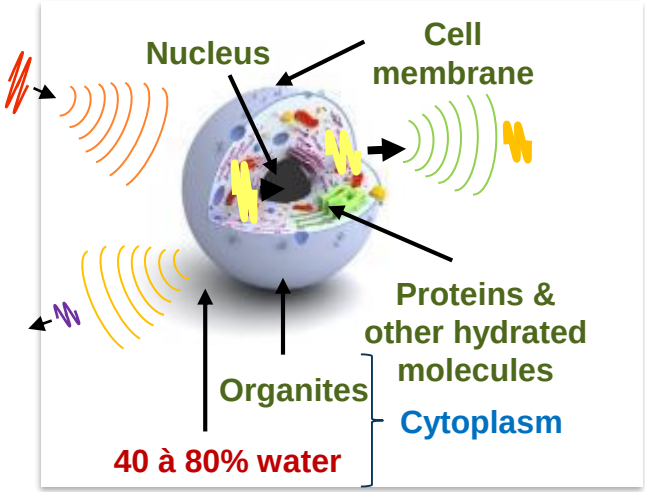
# What about using EM field to identify CSC's?

## 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



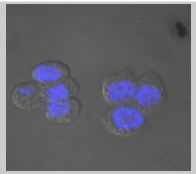
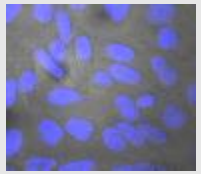
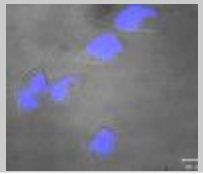
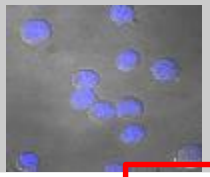

Own cell dielectric properties = **A signature** *that can be specific*



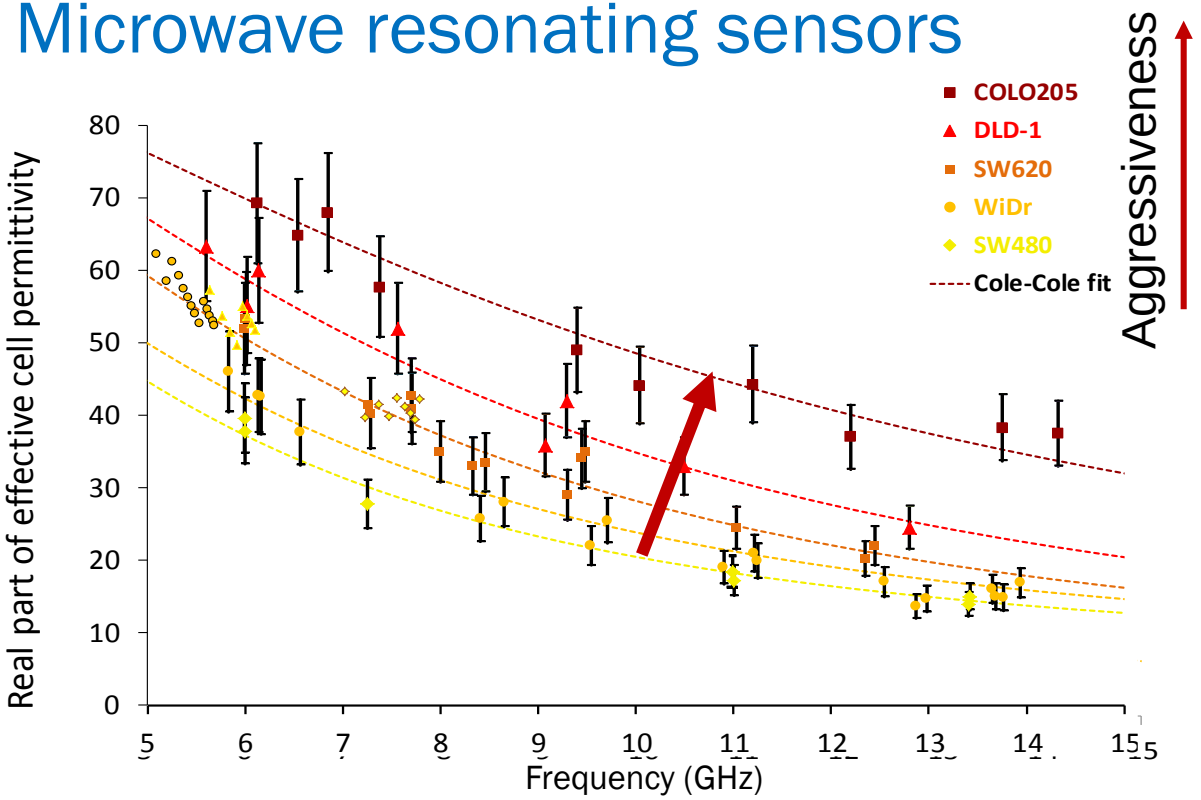

High frequency signal well suitable to access to cell interior properties and measure specificities

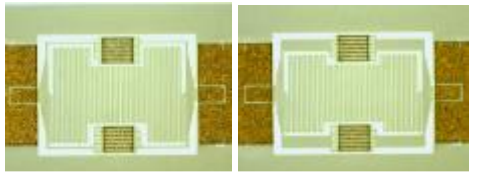
Dielectric spectroscopy allows **non destructive** & **label free** characterization

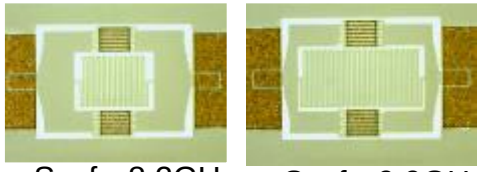
# Discriminating cells with dielectric spectroscopy

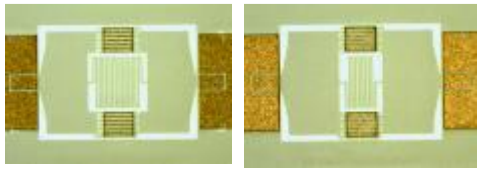
| Cell Line | Stage | Morphology |
|---|---|---|
| WiDr | II |  |
| SW480 | II |  |
| SW620 | III |  |
| DLD-1 | III |  |
| Colo 205 | V |  |

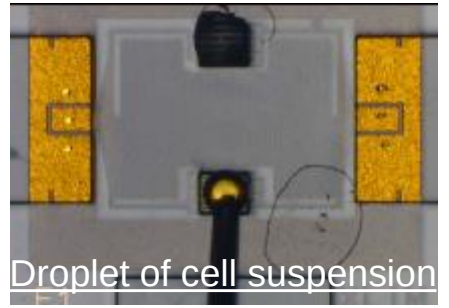
## Dielectric signature established using Microwave resonating sensors



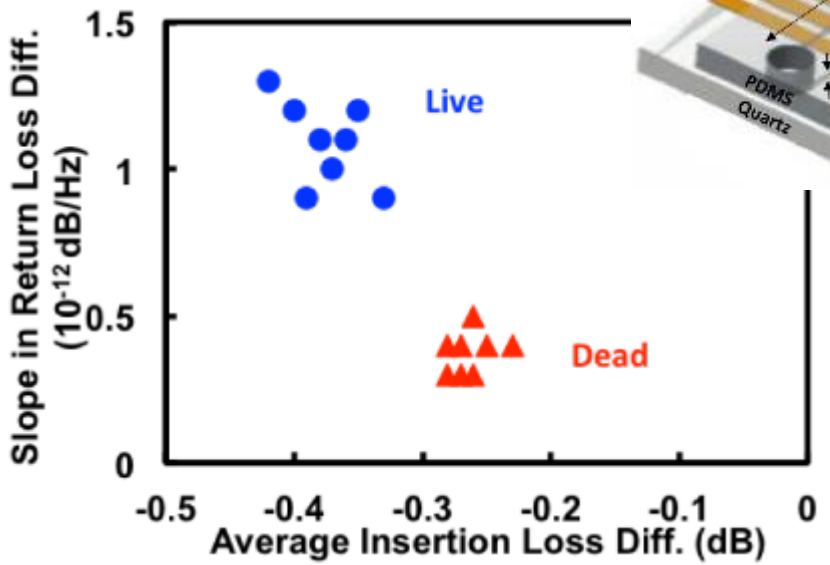

***Correlation* with the cell tumor grade**

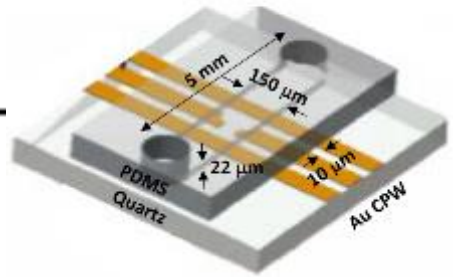
*L.Y. Zhang et all Discrimination of Colorectal Cancer Cell Lines using Microwave Biosensors Sensors & Actuators: A. Physical, Vol 216, Sept 2014.*

# Dielectric spectroscopy on living cells

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

LEHIGH UNIVERSITY



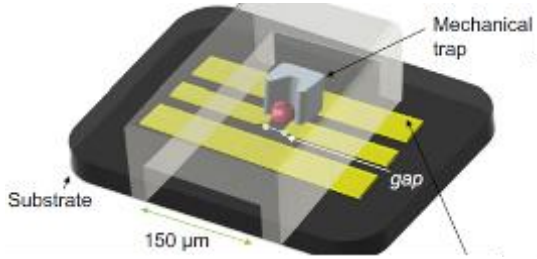



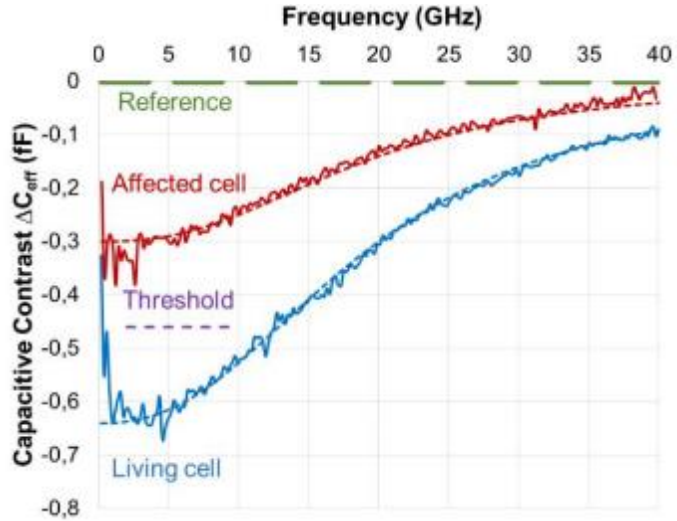

H. Li et al, DOI: 10.1109/TMTT.2017.2659736

Challenges:

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

LAAS CNRS

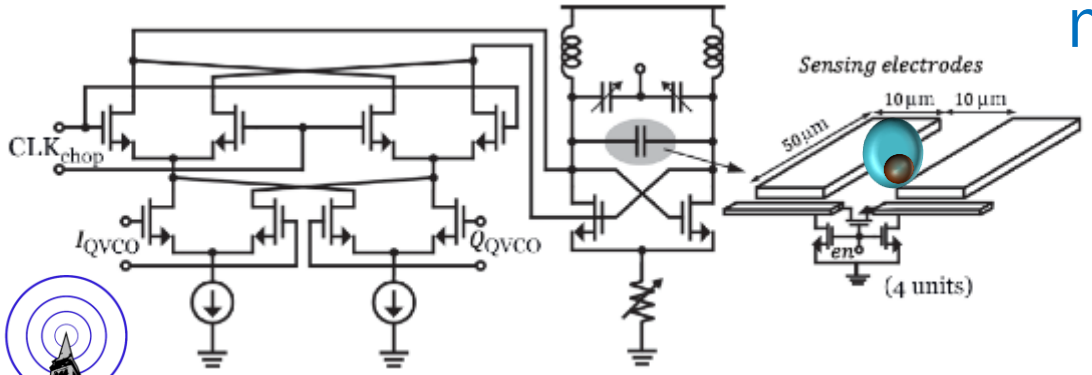


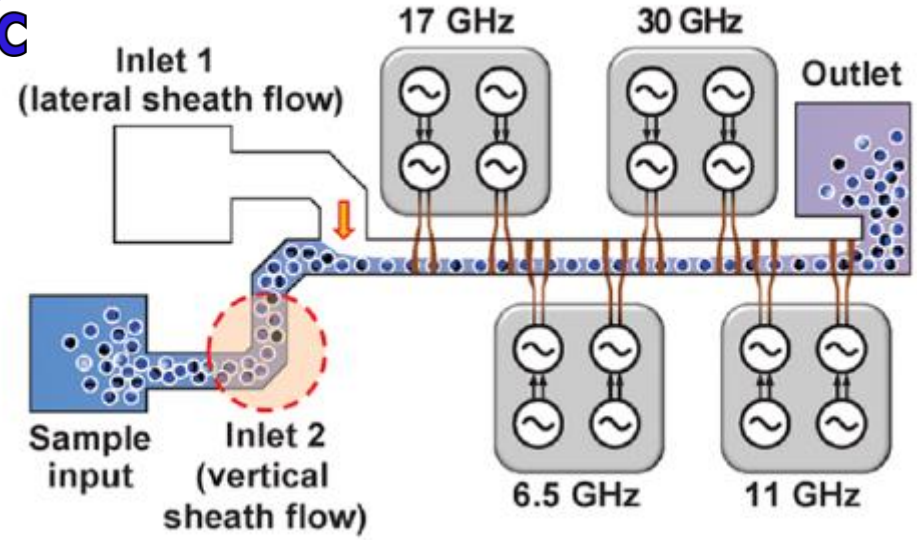




A. Tamra et al, D0I: 10.1109/TMTT.2017.2653776

# To dielectric spectroscopy cytometer concept

## Potentially High-Throughput flowing cells microwave characterization

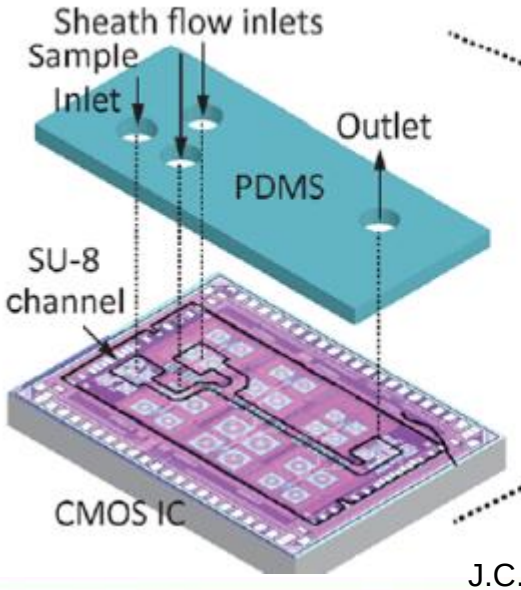


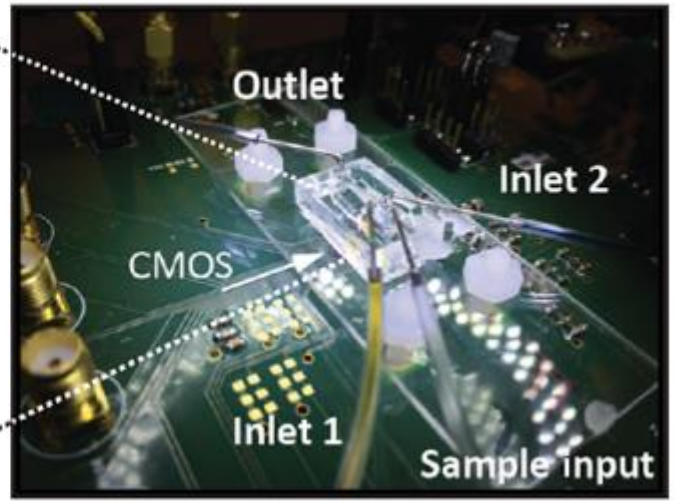




Challenges:

-Require strong sensitivity (ppm range!) sensor design with attoF resolution

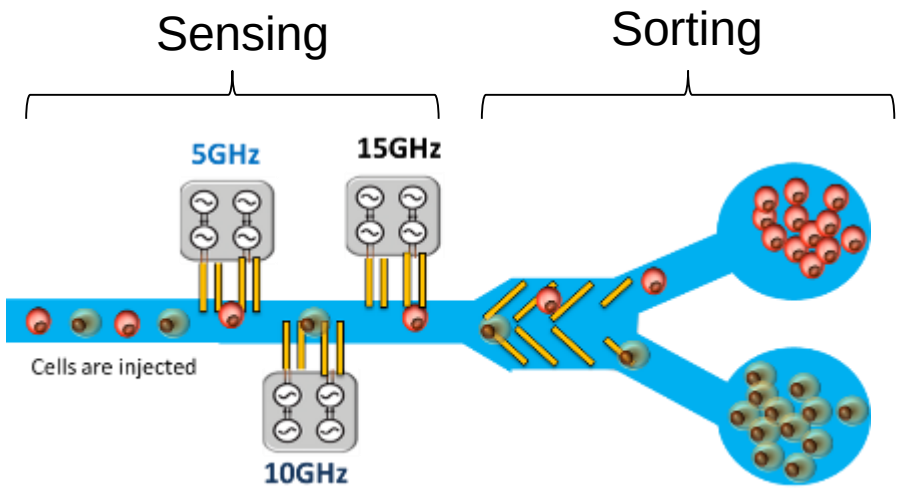
- Need to be associated with cell sorting system

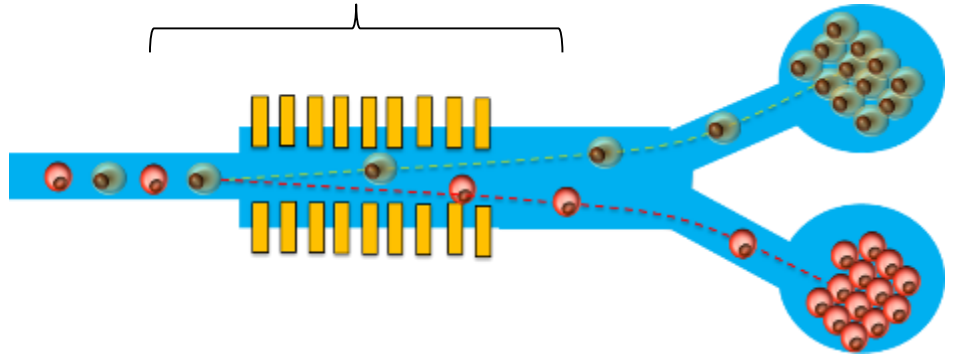


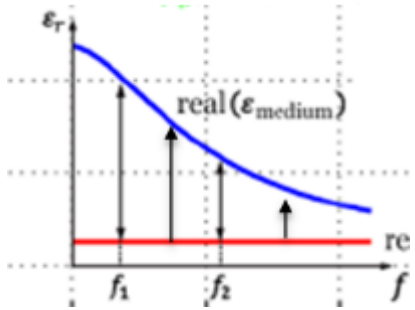

J.C.Chien et al, D0I: 10.1109/JSSC.2015.2500362

# Another approach: Dielectrophoresis




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




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

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

Related to the E field gradient intensity

-1<Re[K(ω)] <1

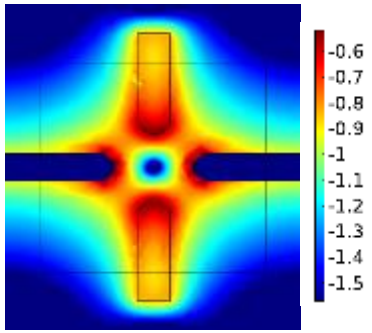
$$K(\omega) = \left(\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right)$$

Claussius-Mossotti factor

$$\varepsilon_{cp}^* = \varepsilon_{(p} - j\frac{\sigma_{cp}}{\omega}$$

Complex permittivity of the particle

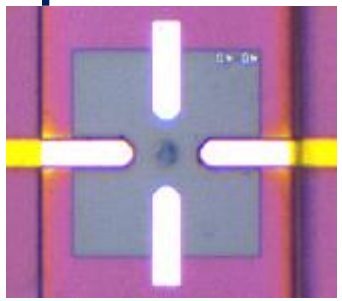
Electrical cage formed between electrodes




*Quadrupole electrode system*

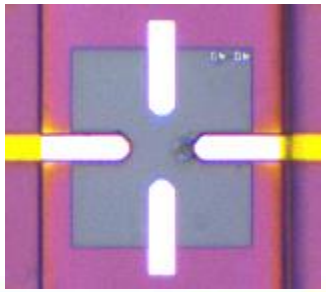
*Re[K(ω)] <0*

**Repulsive force**



*Re[K(ω)] >0*

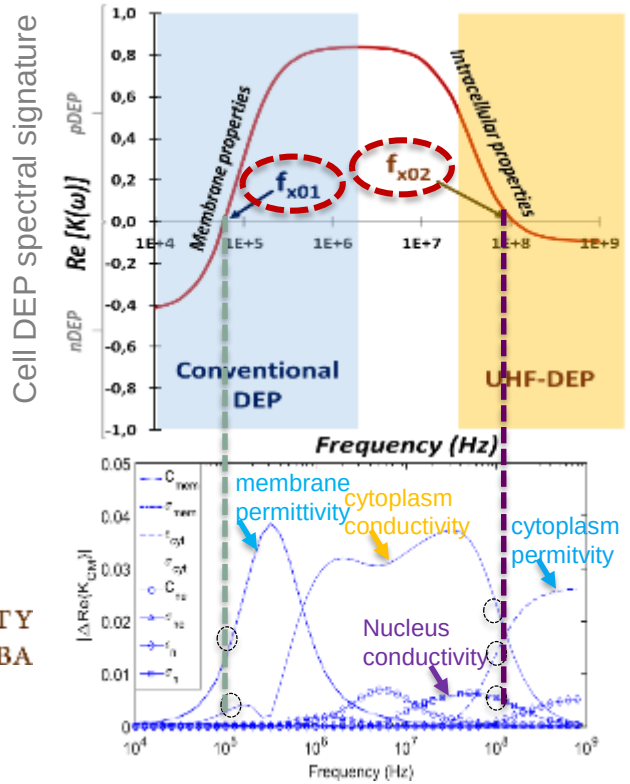
**Attractive force**



Cells can be individually electromanipulated accordingly their own dielectric properties

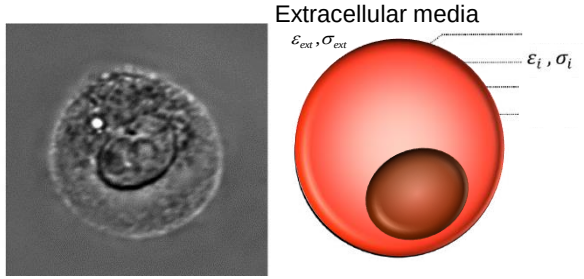
# Specificities of cell DEP spectral signature

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




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

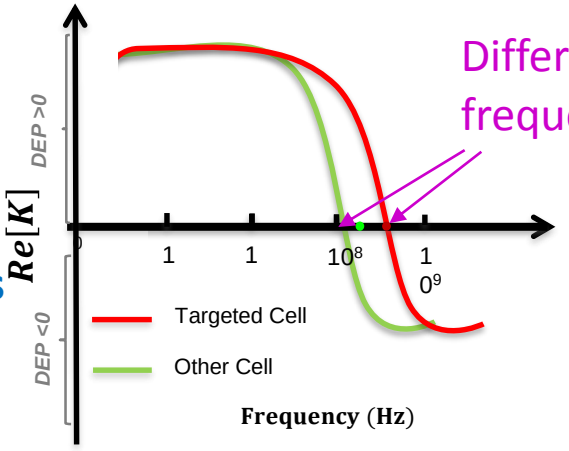
Dielectrophoresis theory basics




Different cells

Vs

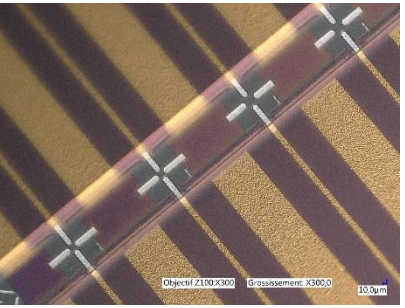
Different spectral signatures

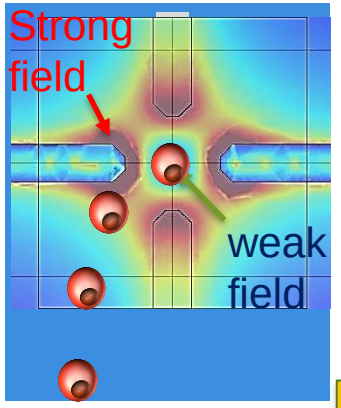



Different crossover frequencies

***$f_{x02}$ is an intracellular marker!***

# Methodology for crossover frequency measurement





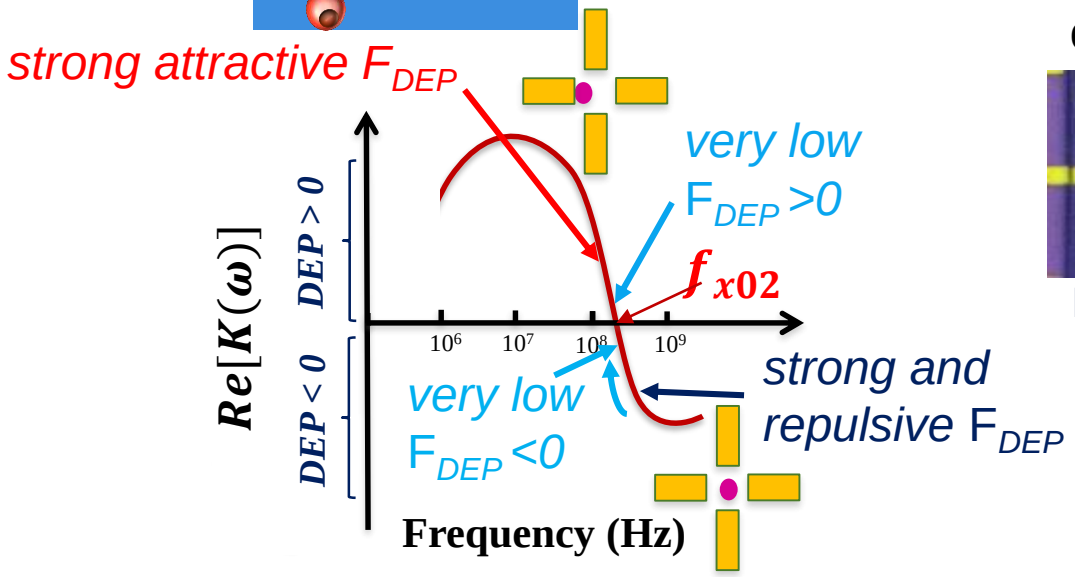

$$F_{DEP} = 2\pi\varepsilon_m r^3 \,\mathrm{Re}\left[K(\omega)\right] \nabla\left|E_{rms}\right|^2$$

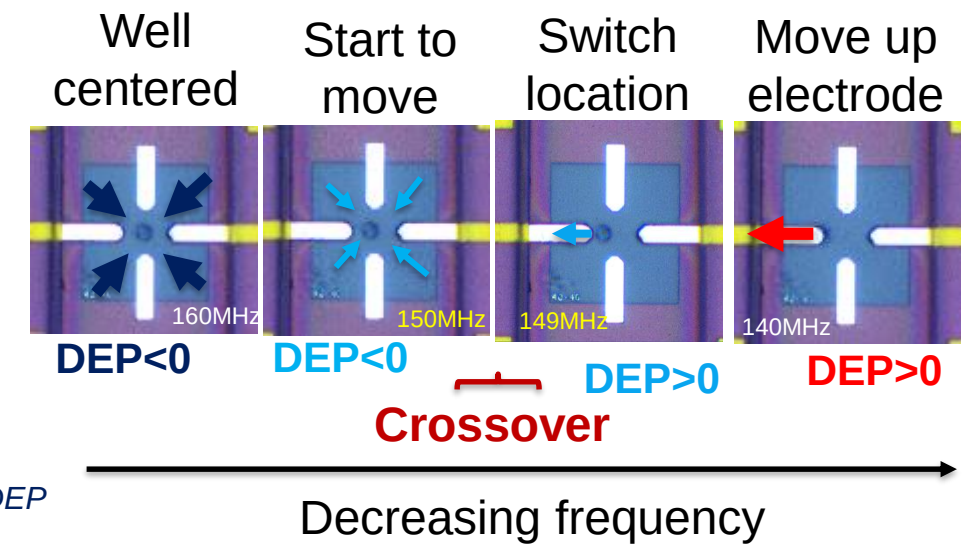
-> $F_{DEP}$ will be high in strong field areas

-> low in weak field areas

Methodology:

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



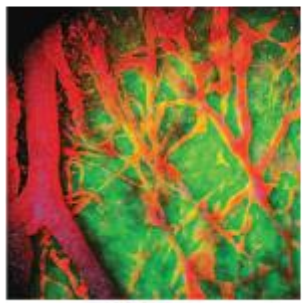



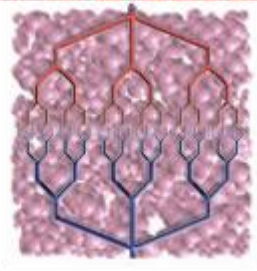
# Obtaining CSC population starting from cell line

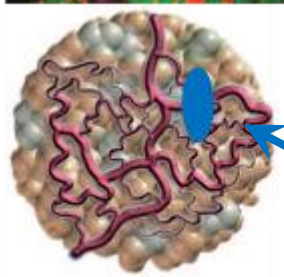
## Mimic CSC micro environment conditions to enrich population

Normal brain tissue
Normal blood vessels

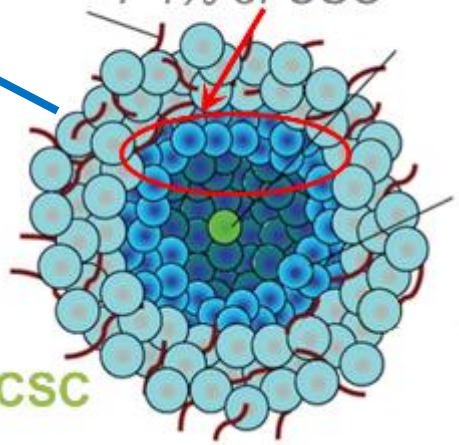
Brain Tumor
*Anarchical vessel network*







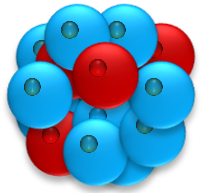
**Hypoxic niche**:
*1-4% of CSC*




***Specific micro environment***

- *$0_2$<1%*
- *low growth factor concentration*

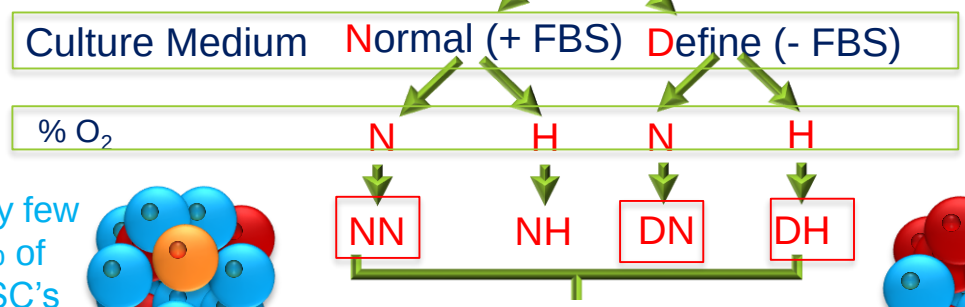
➢ *Submitting cells to stringent Culture conditions*



Basal cell line

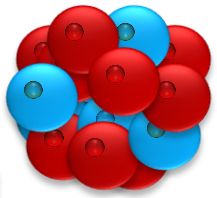
N: Normoxia 20% $O_2$

H : Hypoxia 1% $O_2$



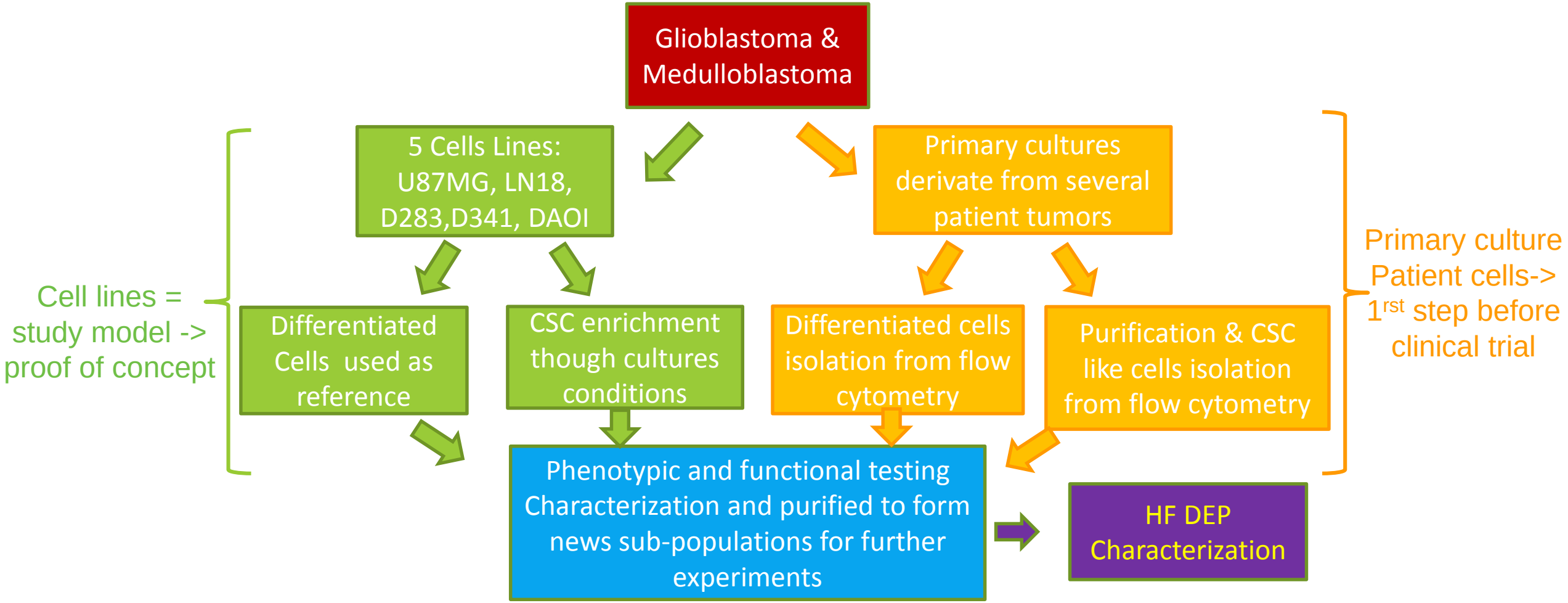

Only few % of CSC's





CSC's like cells 60-85 % expected

# Followed methodology for cell characterization

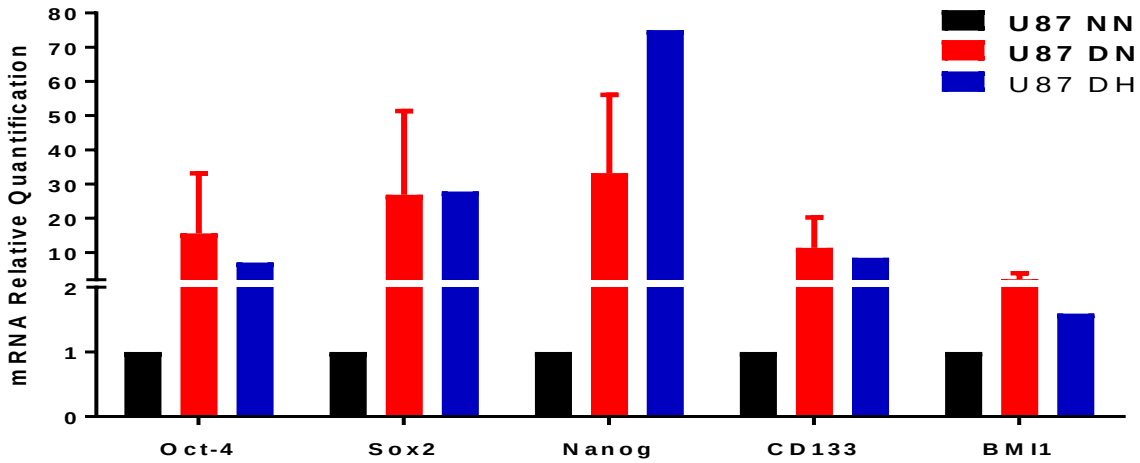


# Confirmation of culture conditions influence on cell phenotype

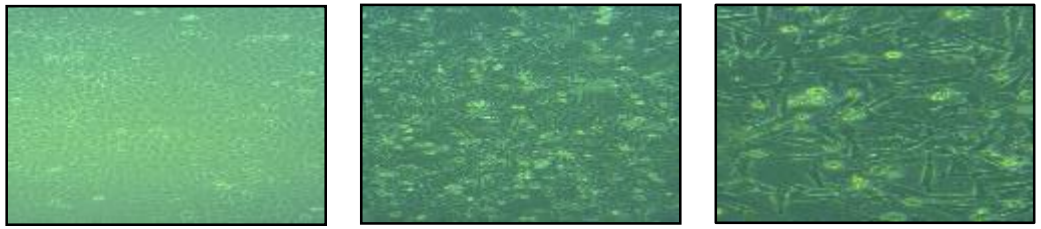
## Glioblastoma human cell lines: Analysis of CSC markers at transcriptional and protein level

**U87-MG Line**

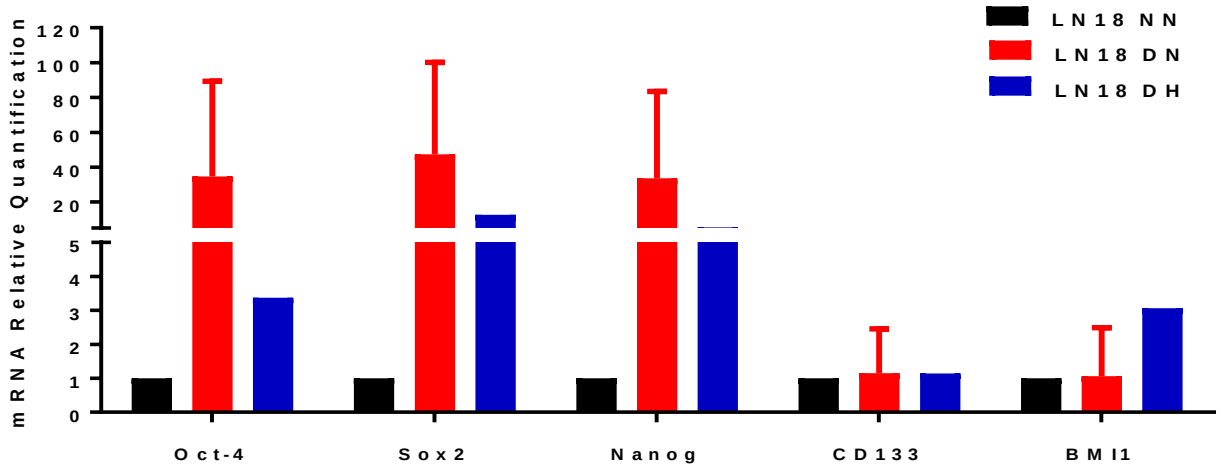
NN culture | DN culture | DH culture




**LN18 Line**



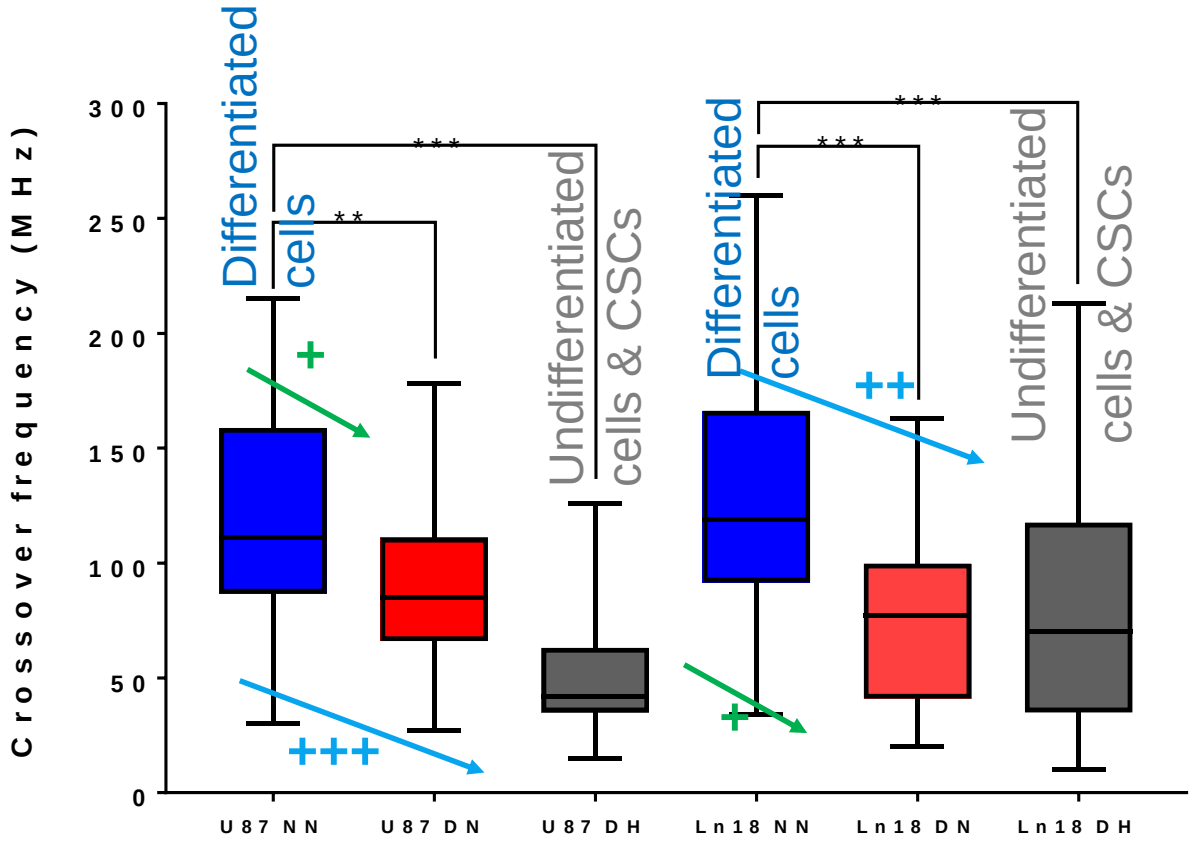
NN culture | DN culture | DH culture




**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

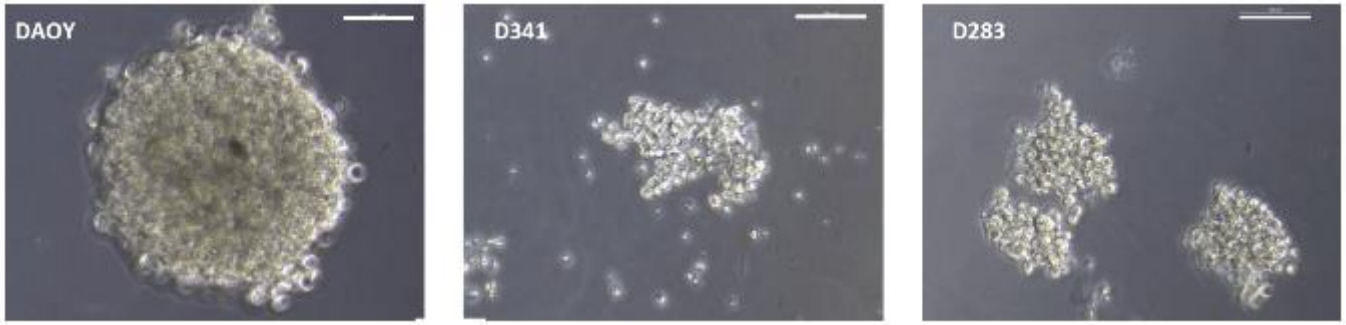
***Negative correlation*** *between crossover frequency and CSC occurrence*

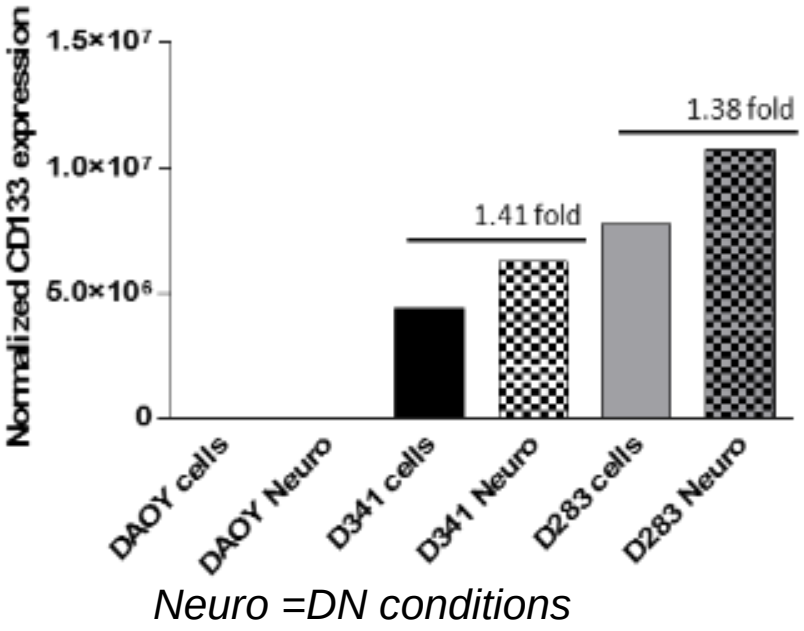
**Difference of phenotype -> difference of DEP signature**

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

# Culture conditions influence on cell phenotype & functional properties

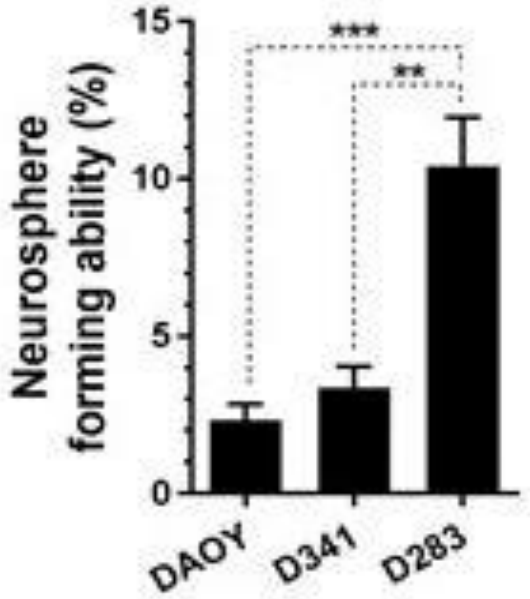
## Medulloblastoma human cell lines:




*Neuro =DN conditions*

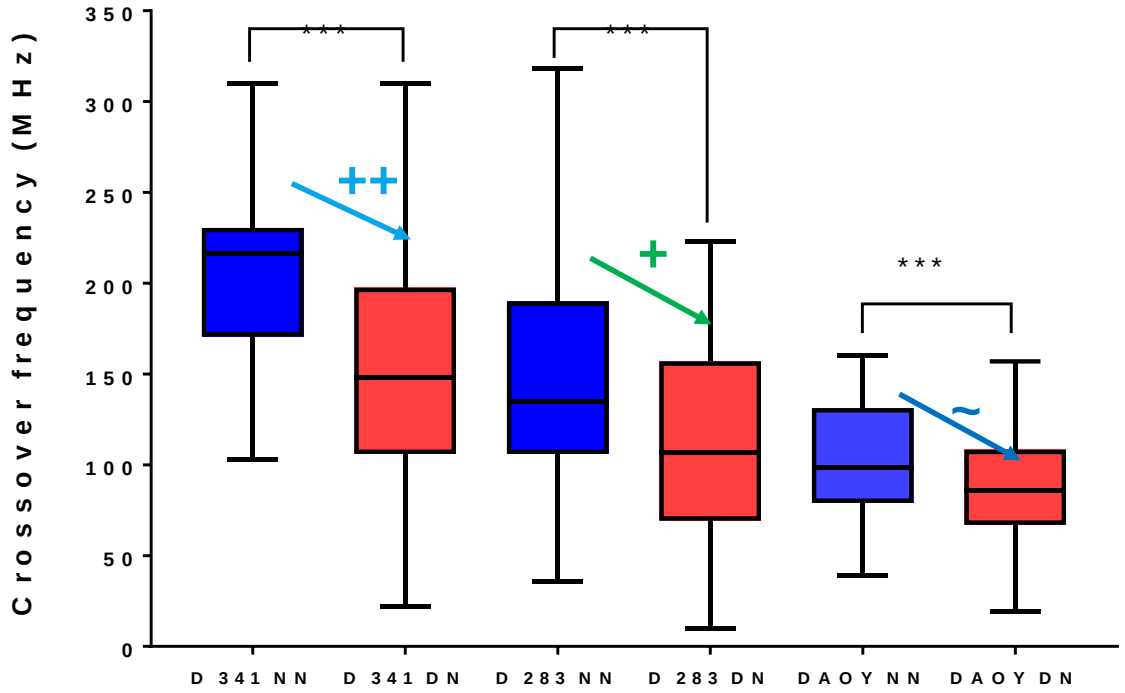
- ✓ D283 cells naturally express **high level** of CD133 and others CSC markers
- ✓ DAOY line shows **poor** CSC features




**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

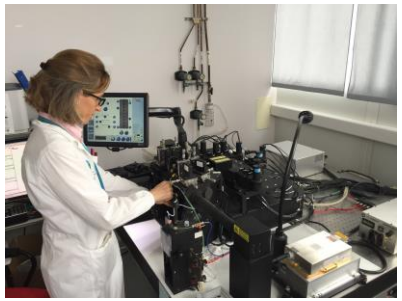
➢ *DAOY: **NN** $^{-}$ or **DN** $^{-}$ culture = poor/ no CSC-> similar signature expected*

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

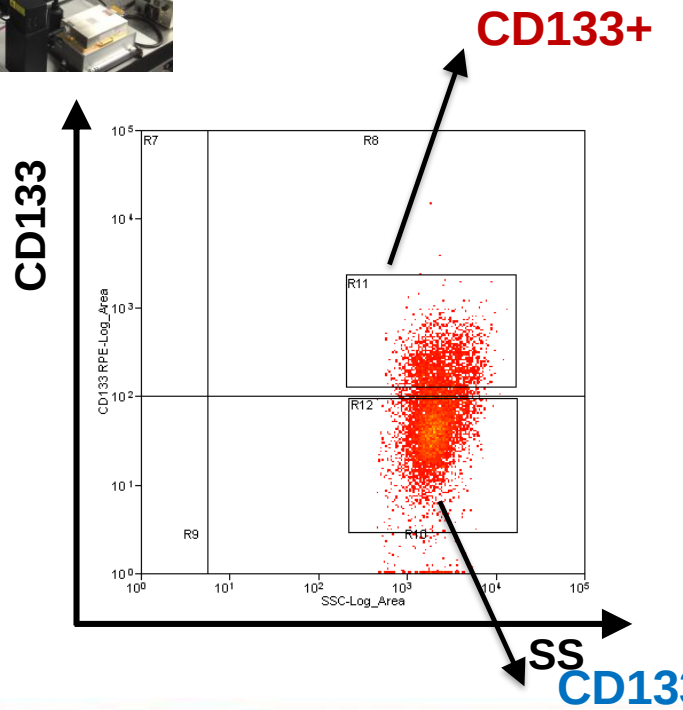
**Difference of phenotype -> difference of DEP signature**

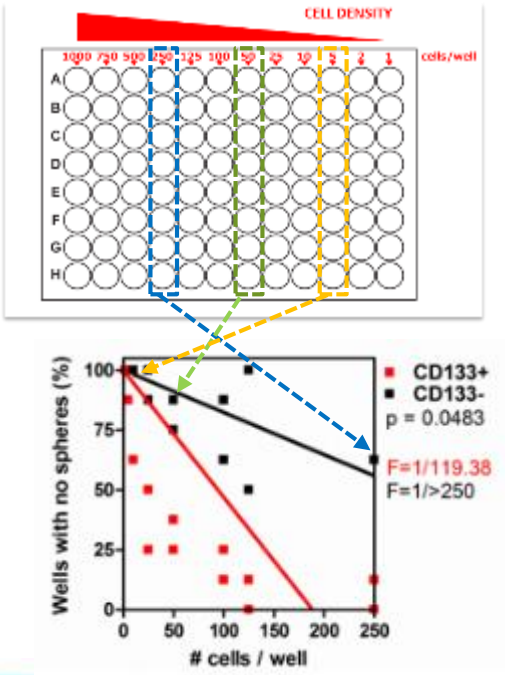
# What about primary culture?

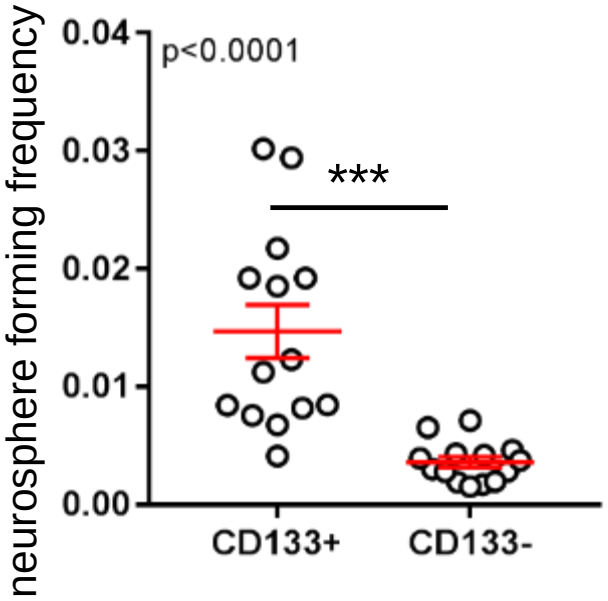
## 17 patient glioblastoma tumors investigated



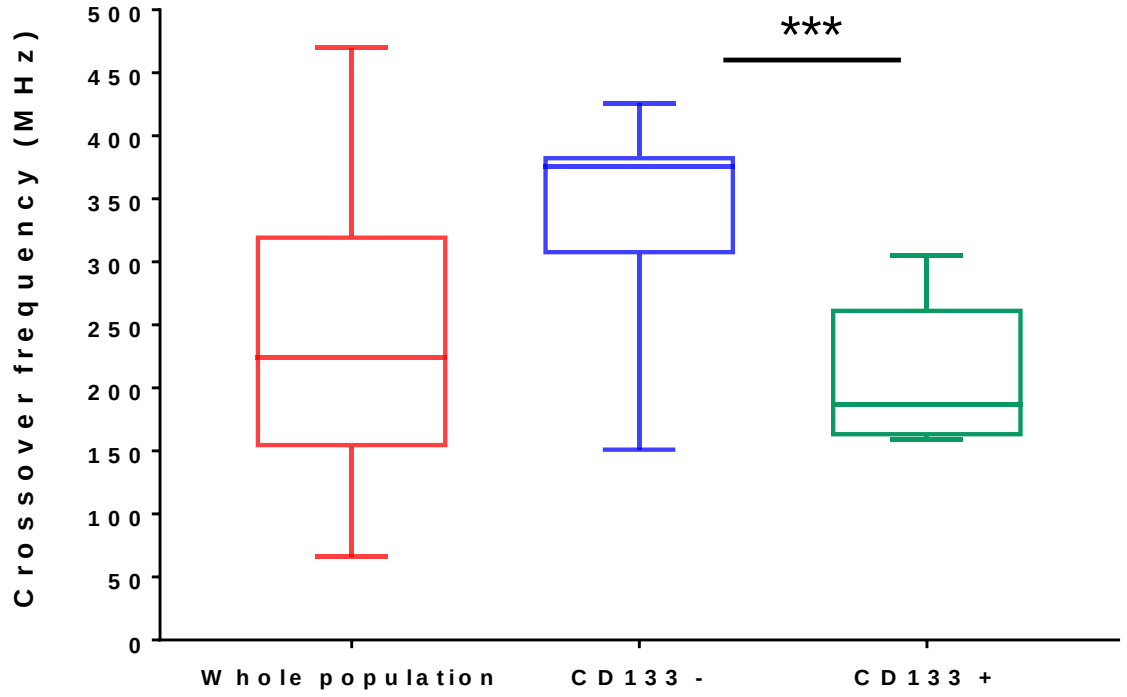
- Cells expressing CSC protein membrane markers are isolated by fluorescence flow cytometry
  - Sub population phenotype and functional features are tested
    - Ability to renew tumor evaluated by LDA method



# Crossover frequency of GBM primary culture cells




*Clear difference of signature*

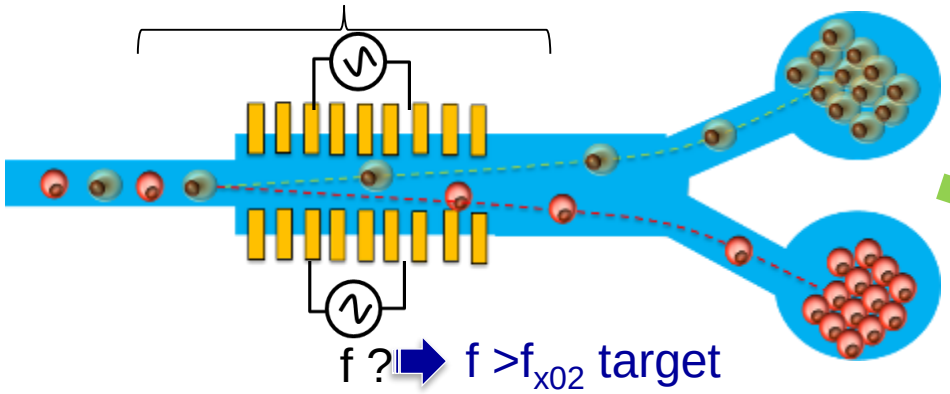
*Correlation between difference of crossover frequency and expression level of CD133 and so* ***CSC occurrence***

**CD133⁺ cells always show lower DEP signatures**

# How exploiting cell crossover frequency specificities

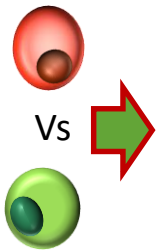
## Prior cell population characterization will help to select the more selective sorting UHF-DEP frequency

Expected selective electromanipulation



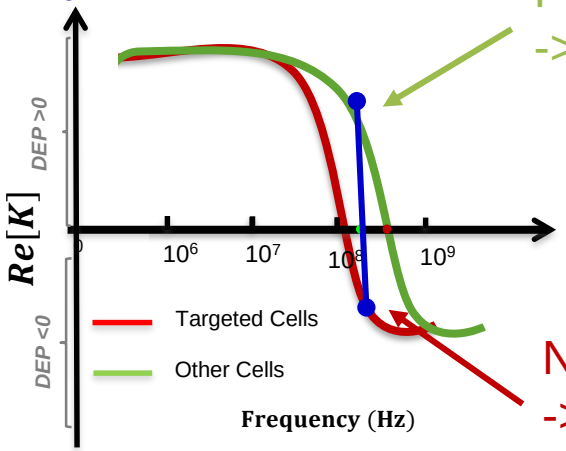
f ? ➡ f >$f_{x02}$ target

Mixed cell population



Vs

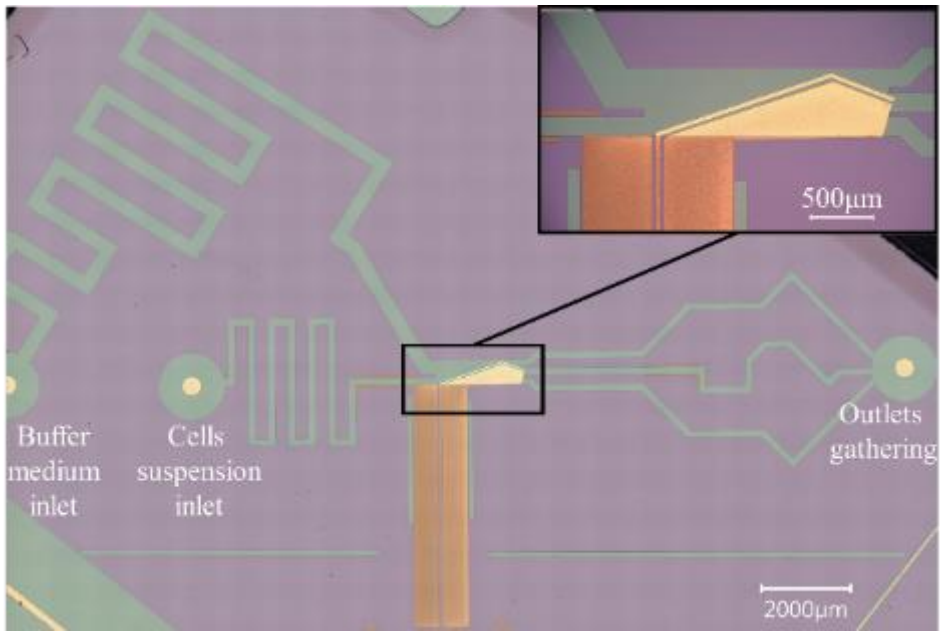
**Different DEP spectral signatures**




Positive DEP
-> attraction

Negative DEP
-> repeal

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

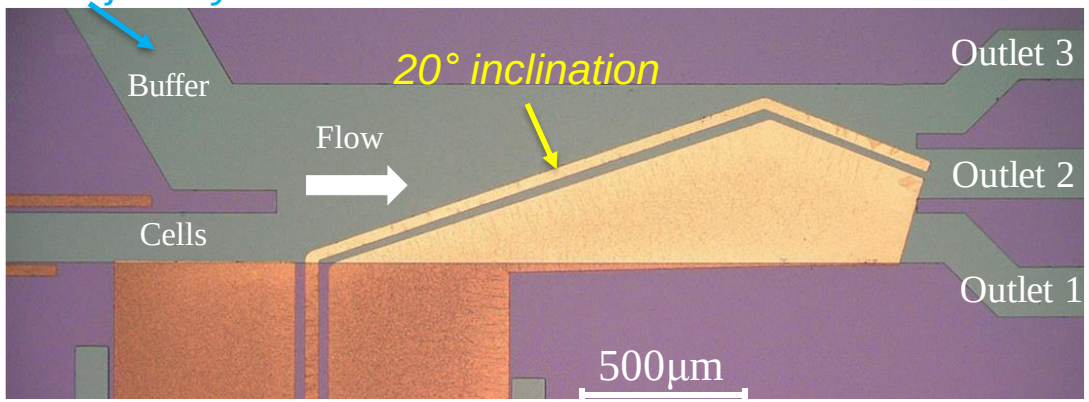



T. Provent et all, IMS 2019

# Proposed cytometer design

## Coupling of DEP & hydrofluidic forces to dynamically sort cell

*Control the initial cell trajectory*



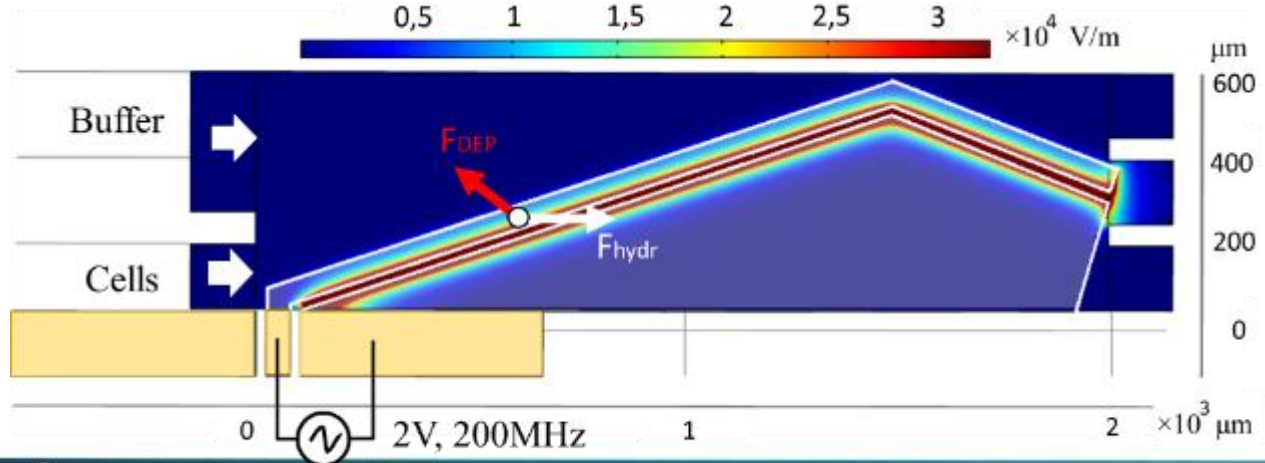

Working principle:

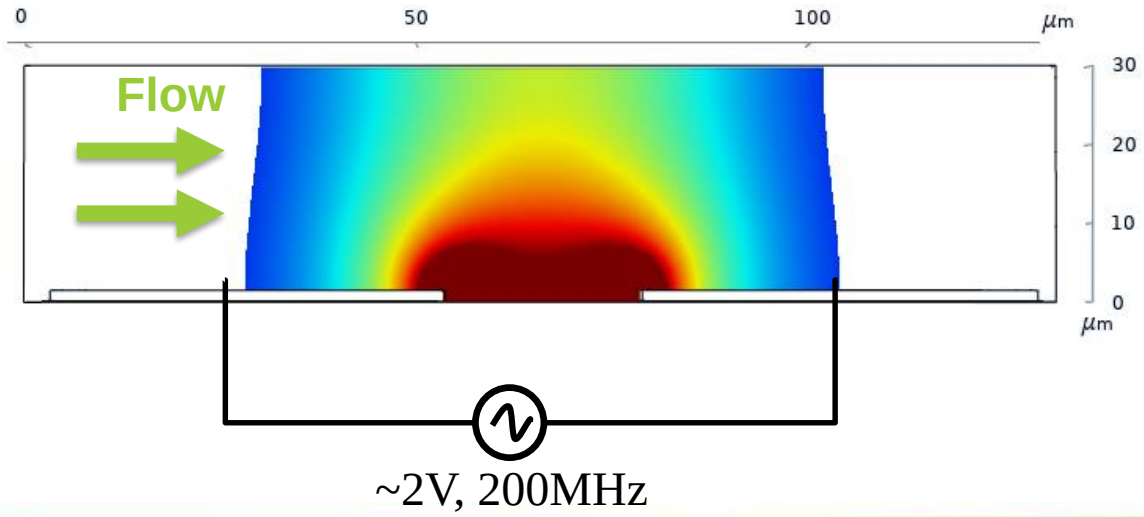
Tune the DEP force at constant fluidic force to act on cell trajectory

-> 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



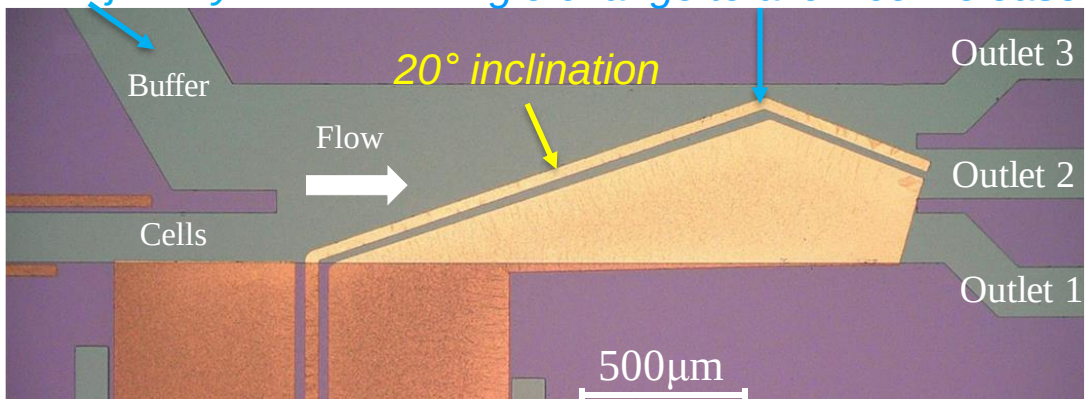



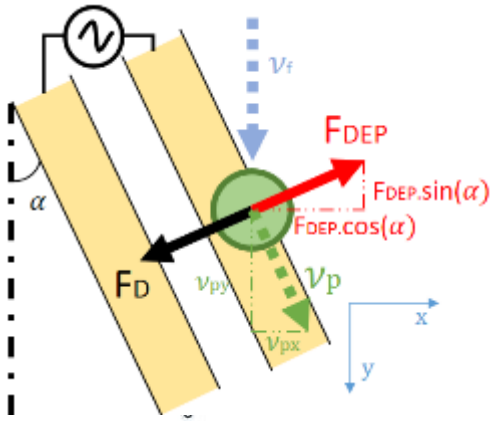
# Proposed cytometer design

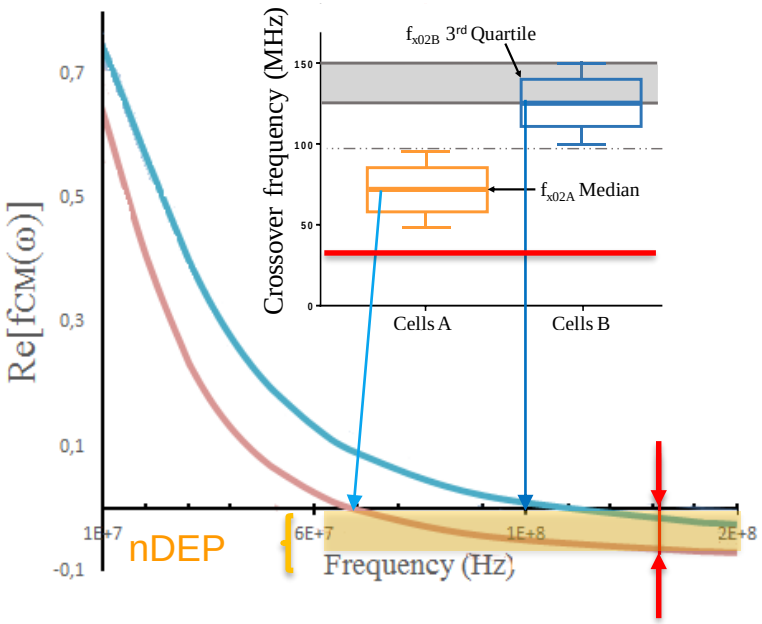
## Coupling of DEP & hydrofluidic forces to dynamically sort cell

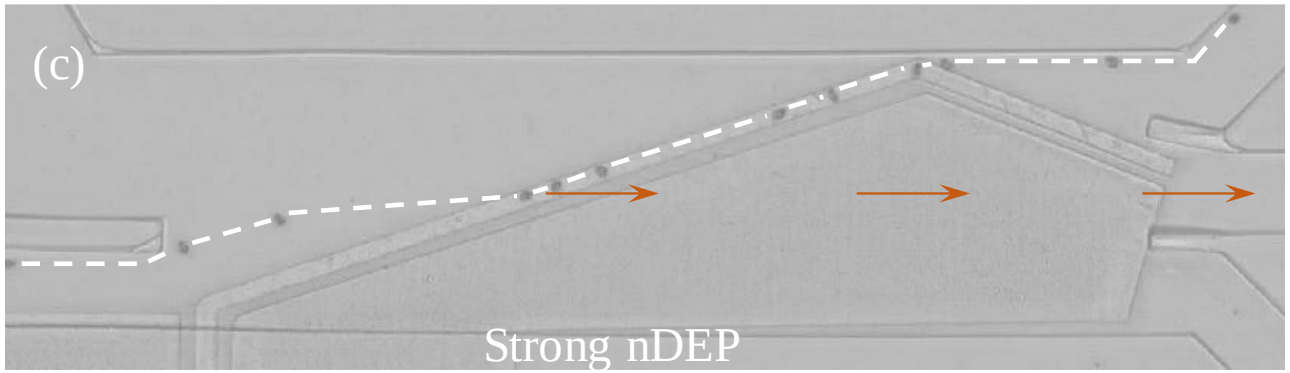
*Control the initial cell trajectory*

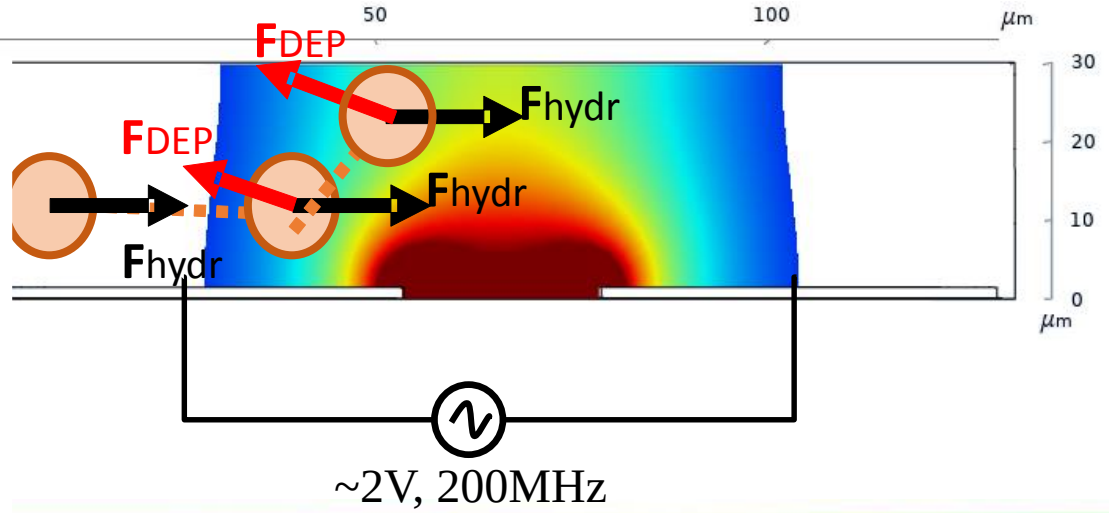
*Angle change to allow cell release*



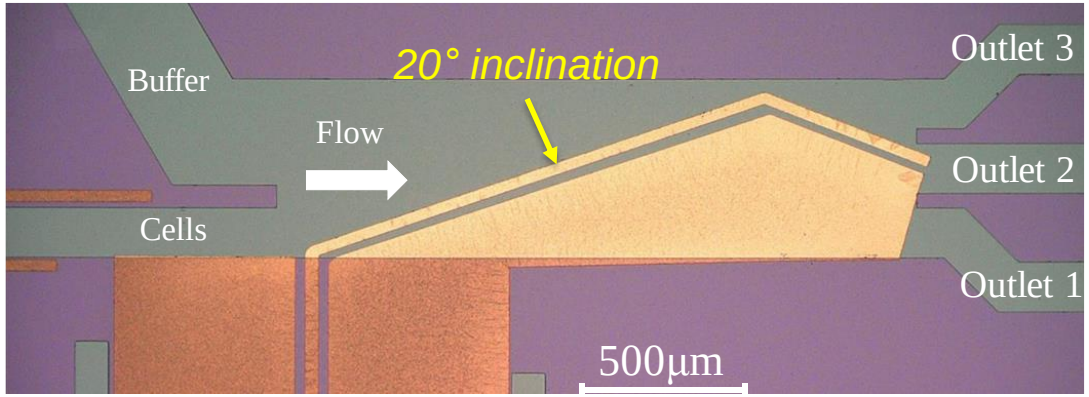



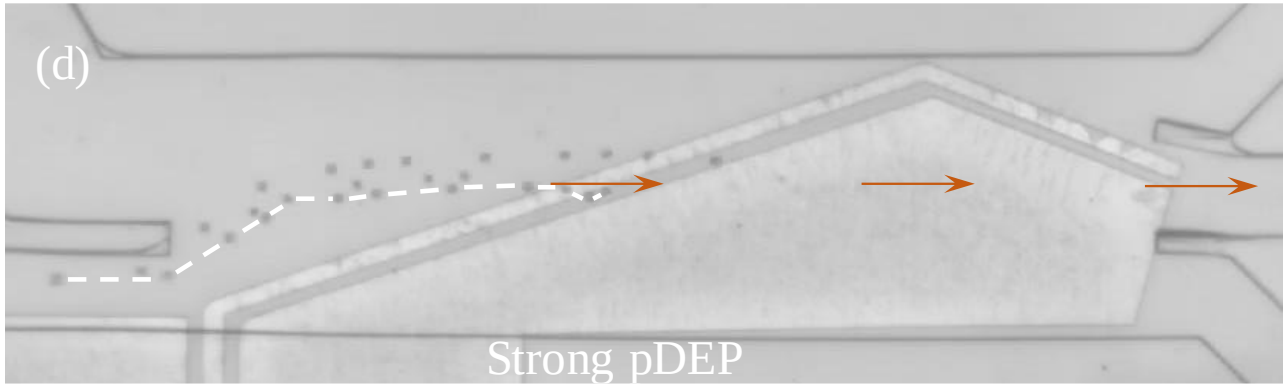



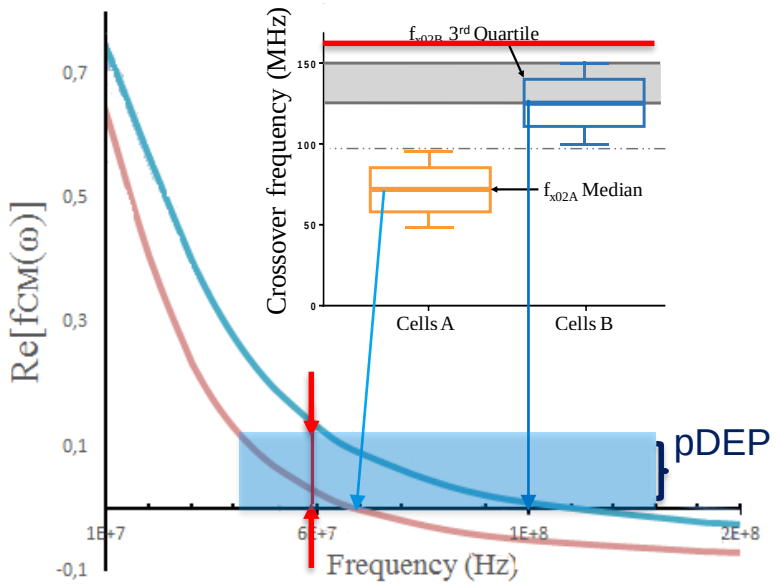



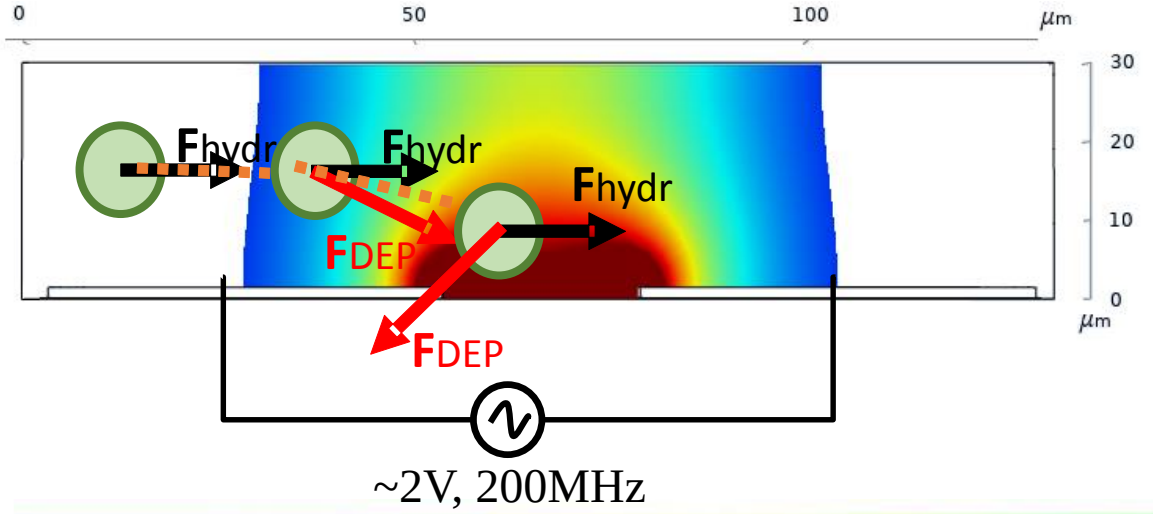
# Proposed cytometer design

## Coupling of DEP & hydrofluidic forces to dynamically sort cell



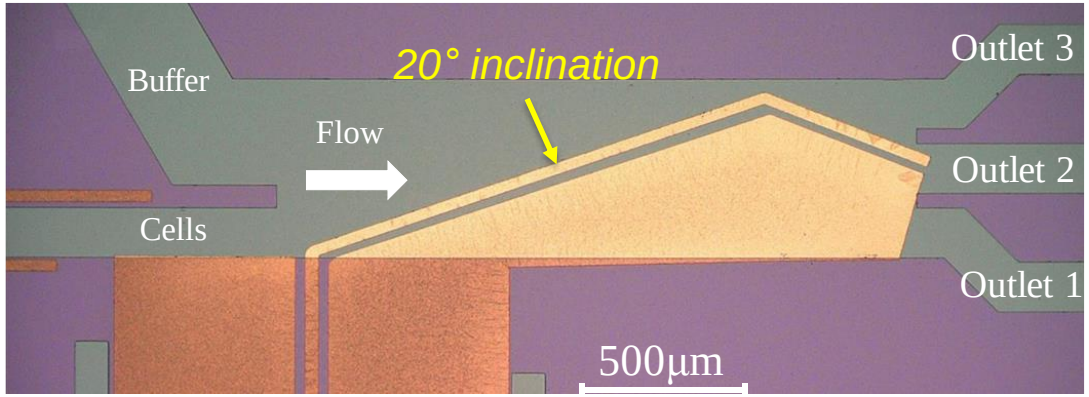



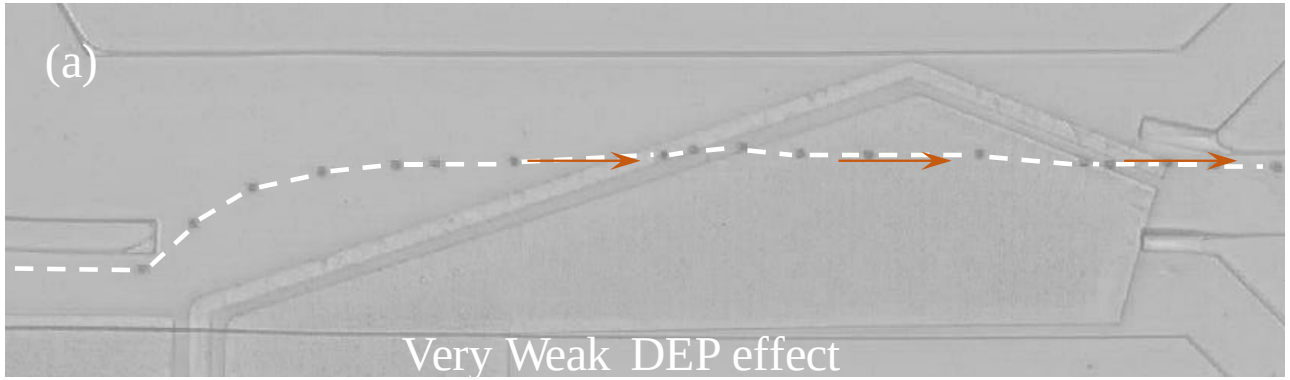



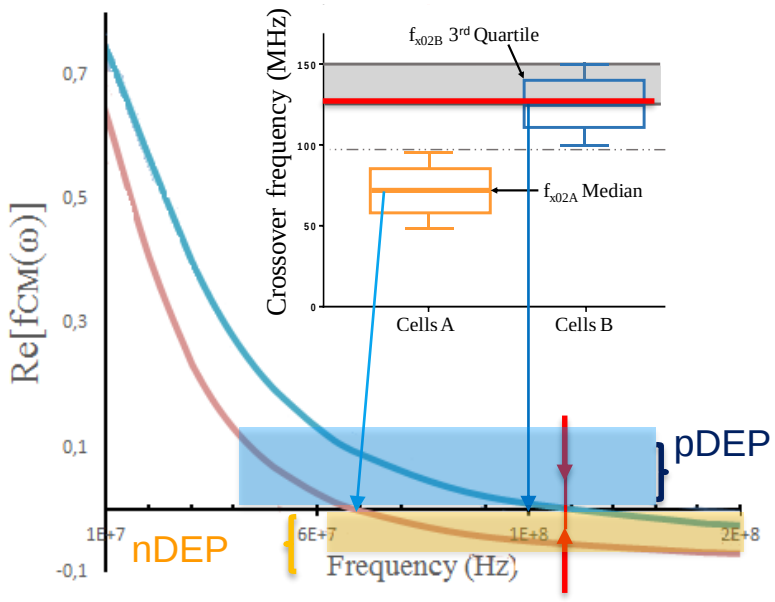



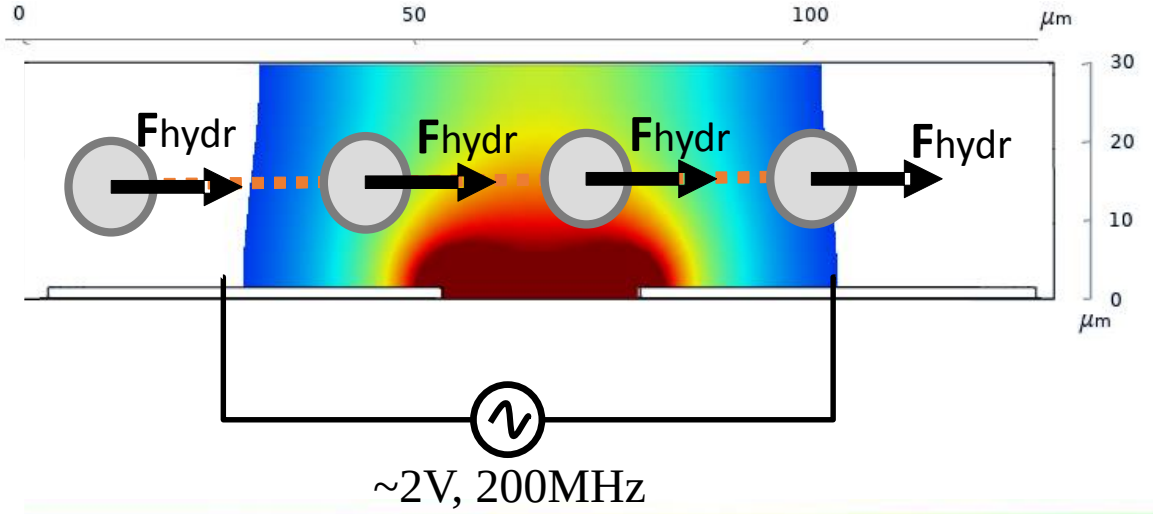
# Proposed cytometer design

## Coupling of DEP & hydrofluidic forces to dynamically sort cell



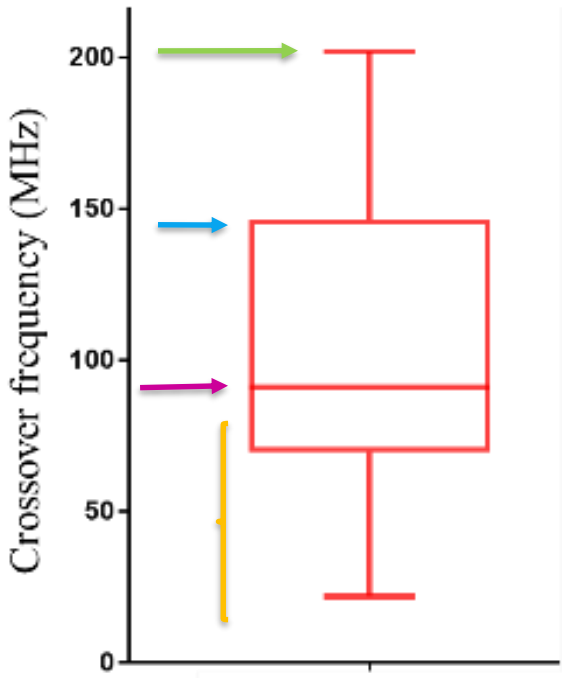



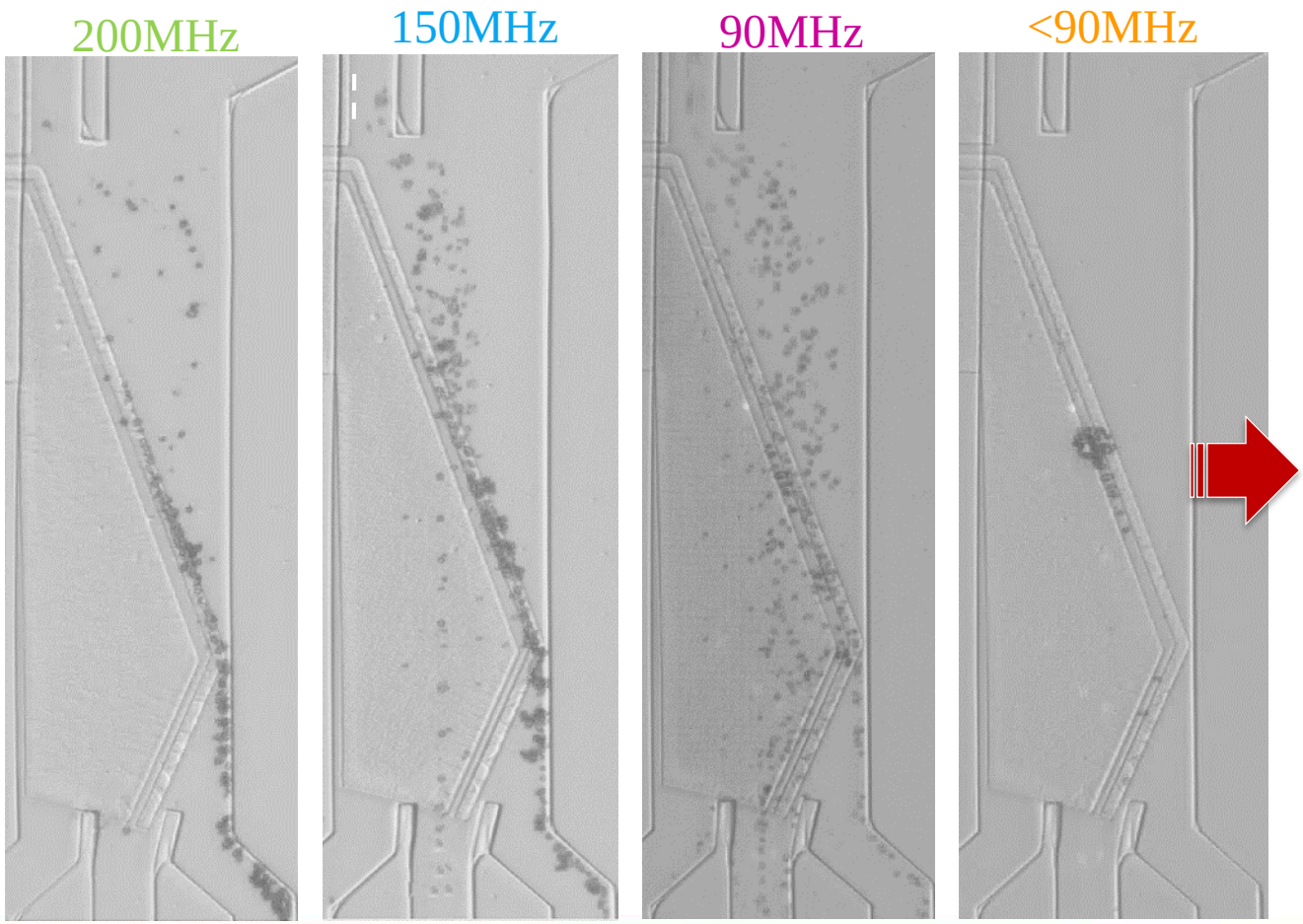



# Handling a dispersive property cell population

For targeted cell: optimal DEP signal frequency and magnitude vs flow speed have to be set




*Improvement to limit cell clogging are still required: Electrodes* ***surface treatment***

# Conclusion

- Exploiting DEP 2nd Crossover frequencies appears very promising for label free cell discrimination applications
- Especially for CSC case exploiting intracellular specificity vs differentiated cells
- An novel UHF DEP cytometer has been prototyped and validated
- Application for validation of CSC isolation from enriched or basal cell population is currently on going…
- Application to others concerns/pathologies might be considered too

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