Tu2E- 1

Tracking Cancer Cells with Microfluidic High Frequency DEP Cytometer Implemented on BiCMOS Lab-on-Chip Platform

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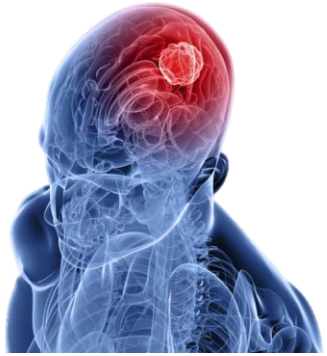
Motivation

- Need for new therapeutic strategies dedicated to poor outcome diseases

Ex: Glioblastoma:

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



Resulting efficiency from standard therapies is very low

- *Poor patient survival rate*
- *Frequent relapse*

Role of some hidden tumor-initiating cells ?

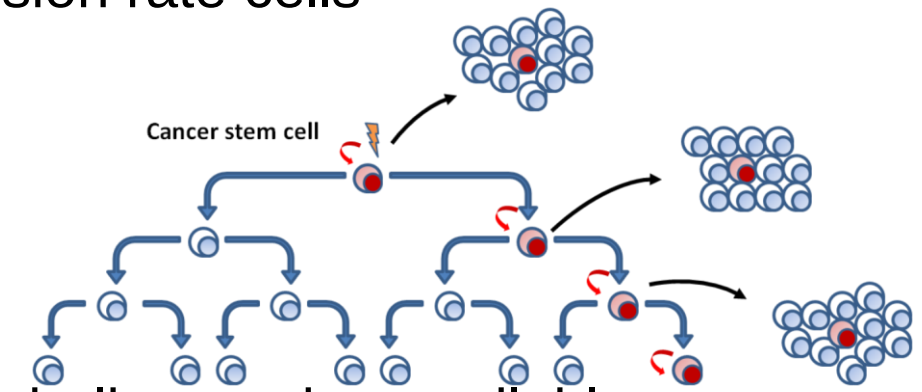
How fight them more efficiently?
What they look like?
How many are they?
Where are they?

Motivation

- Need for alternative tools able to track such specific and rare cells

Cancerous Stem Cells: *Tumorigenic cells with ability to give rise to all tumor cell type*

- Quiescent cells: escape from therapies targeting high division rate cells
- Differentiation into multiple cell types (progenitors…)
- Self-renewal capabilities
- Low number, Hidden in the tumor
- Undifferentiated cells: No specificity: lacking for specific labeling marker available

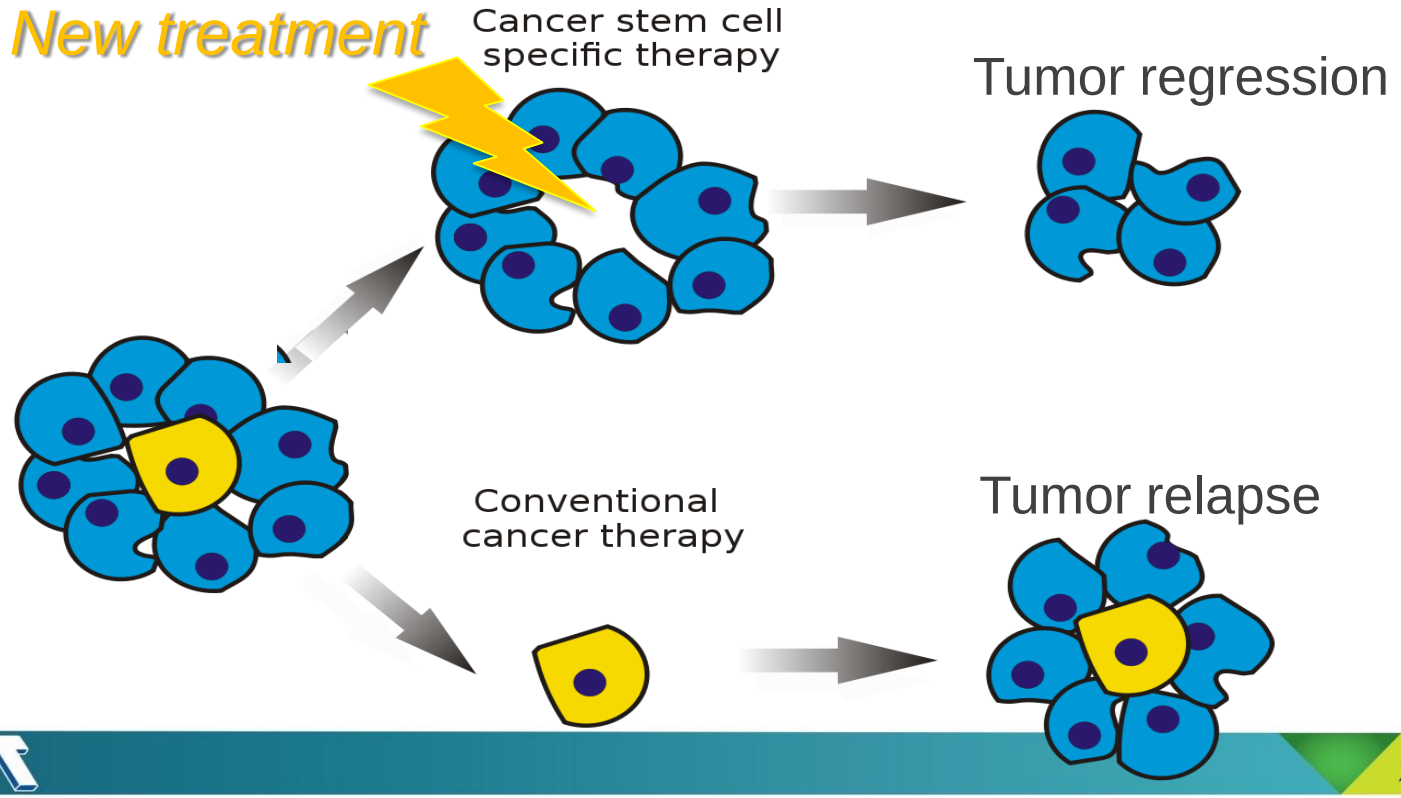


➡ Currently hypothesized to be the main cause of relapse and metastasis

Motivation

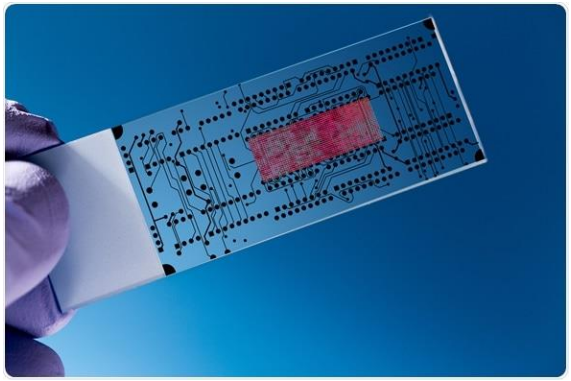
Tools able to identify CSC's in/outside the tumor might contribute to:

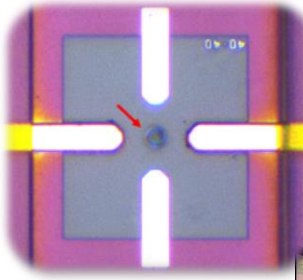
- help diagnosis and favor more appropriated treatment
- promote to the development of more efficient therapies



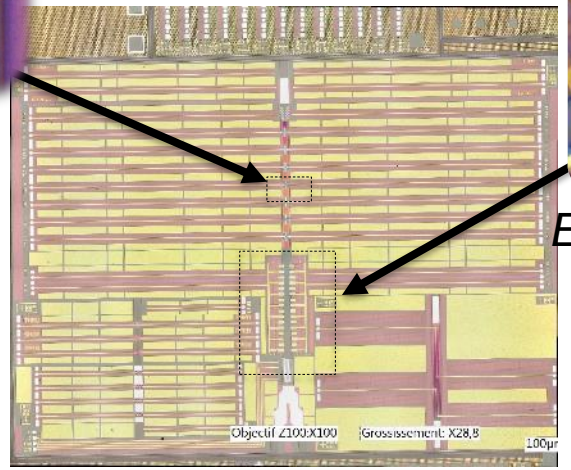
Our approach

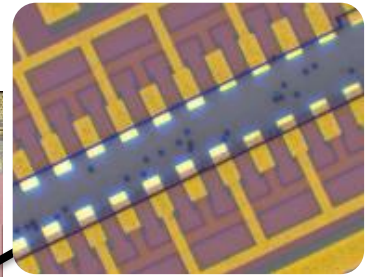
- Developing new Generation of Lab-on-Chip for Cancerous Stem Cells identification and sorting using **Electromagnetic Waves**





Individual Cell sensor





Electromagnetic based Cytometer

Prototype of microfluidic sensing platform on CMOS chip

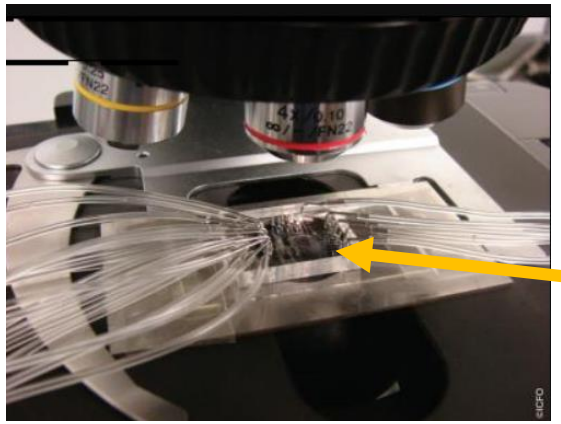
Proposed concept: *Exploit the penetration of* ***EM radiations*** *inside the cells to:*

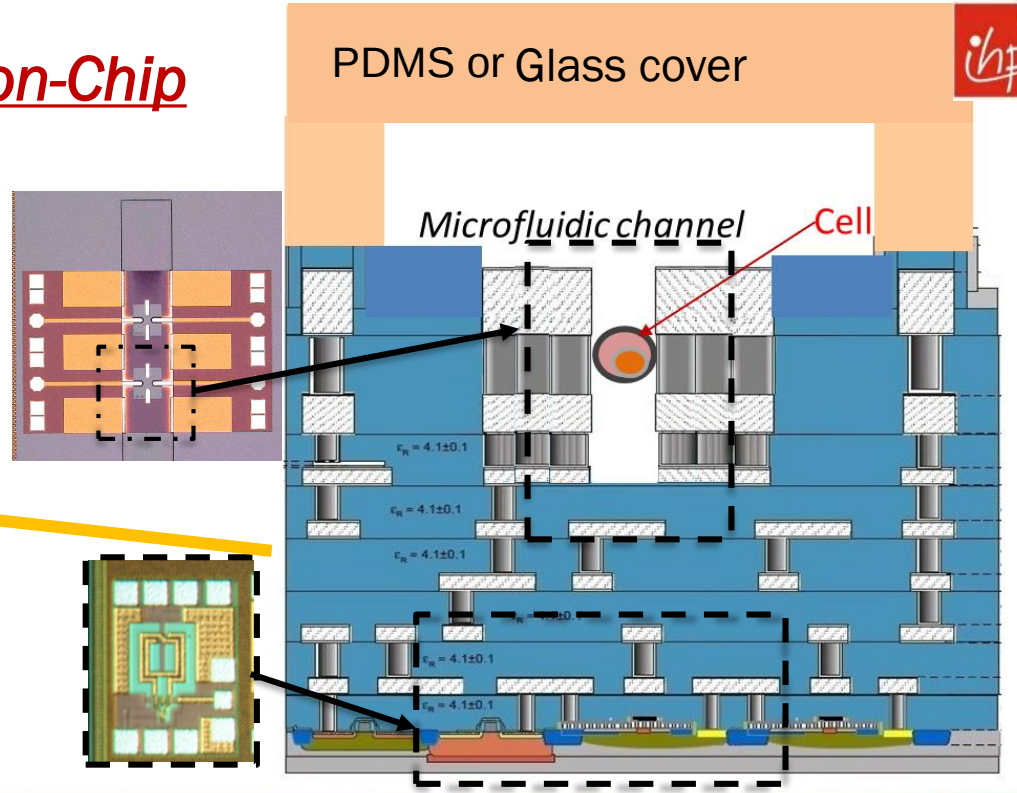
- ***sense*** *their content*
- ***electromanipulate*** *them accordingly to their own dielectric property specificities*

Taking advantage of BiCMOS technology

- ✓ Complete system integration with several electronic functions on the same chip
- ✓ Miniaturization of the complete device And coupling with microfluidics

- ▶ *Mature technology able to quickly address a large market*
- ▶ *On the way to fully instrumented Lab-on-Chip*



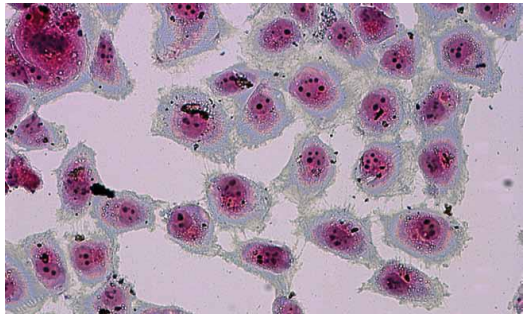


Outline

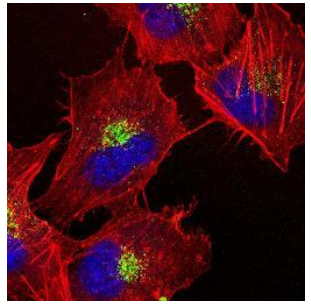
- Motivation and pursued approach
- How characterizing biological cells with high frequency DEP
- High frequency DEP Electromagnetic cytometer design
- First experimental results
- Conclusion and futures developments

How biologists can study CSC's?

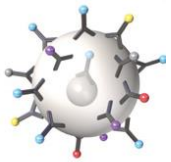
Optical microscopy



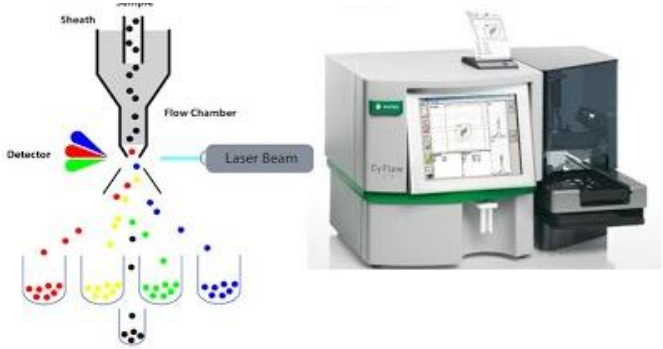
Staining



Fluorescence labeling



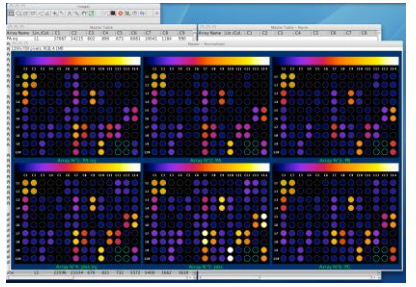
Flow cytometry

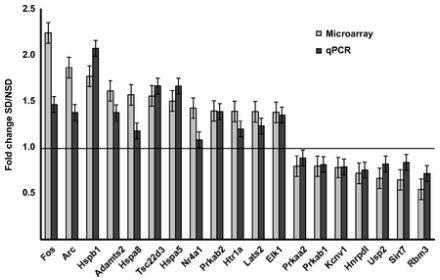


QPCR & Protein Array analysis

Drawback/ constrains:

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



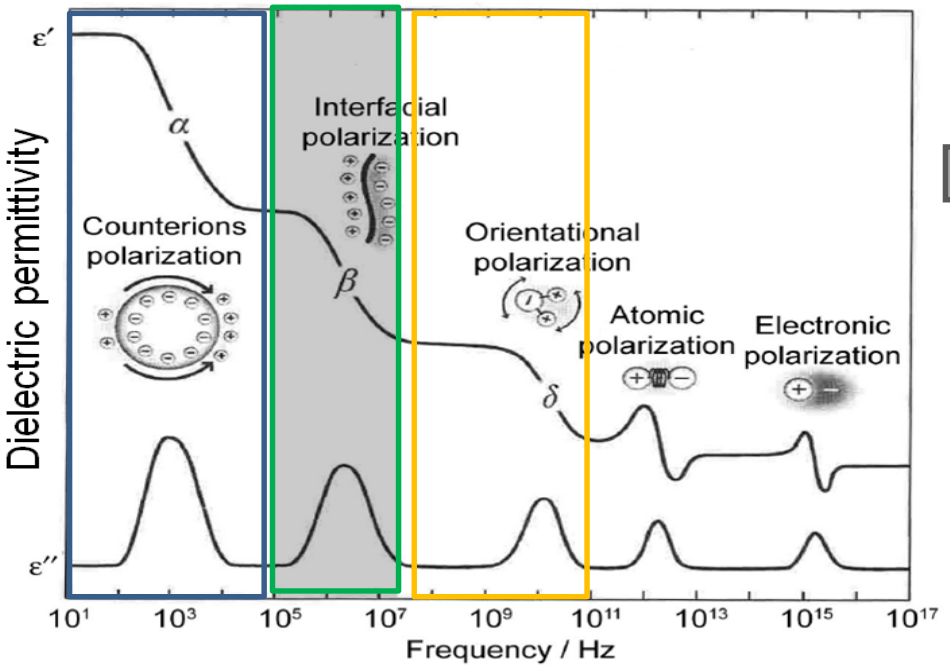


➡ Others approaches investigating intracellular specificities?

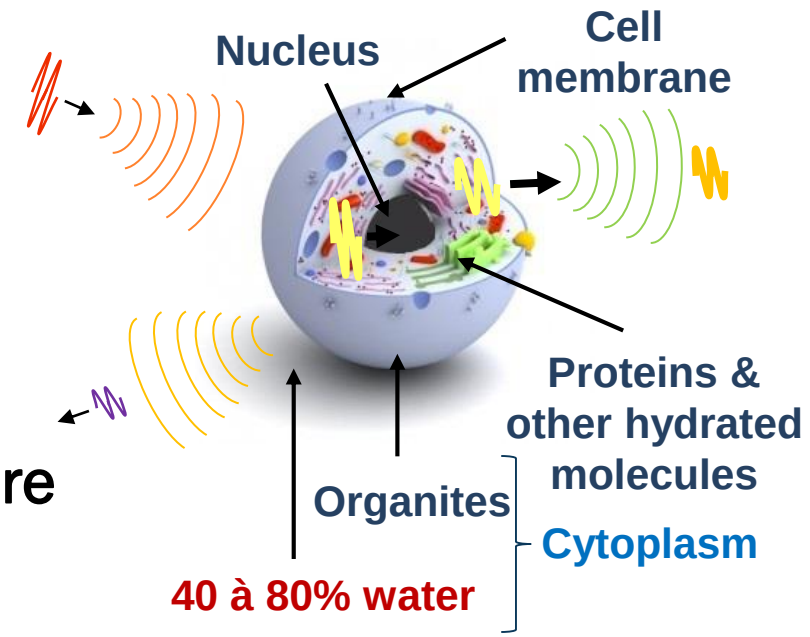
What about using EM field to characterize cells?

Depending the frequency EM field could interact with different cell constituents

- Low frequency -> Cell shape/ morphology/size influence
  - Mid frequency -> Plasma Membrane specificities
    - High frequency -> Intracellular content properties



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



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

Dielectric spectroscopy allows non destructive & label free characterization

Dielectrophoresis vs Dielectric Spectroscopy approach

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

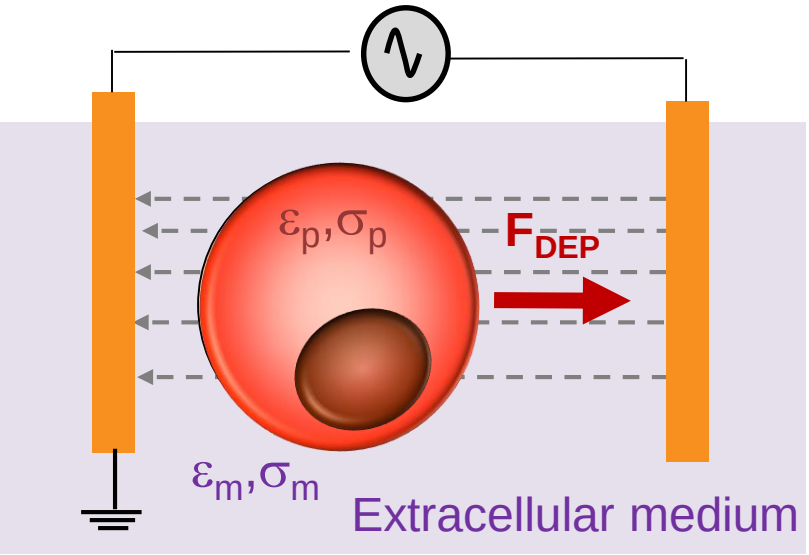
Basic DEP theory $F_{DEP} = 2\pi\varepsilon_m r^3 \mathrm{Re}[K(\omega)] \nabla|E_{rms}|^2$ ← Related to the E field gradient intensity

-1<Re[K(ω)] <1

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

Claussius-Mossotti factor

Complex permittivity of the particle



Re[K(ω)] <0 → **Repulsive force**

Re[K(ω)] >0 → **Attractive force**

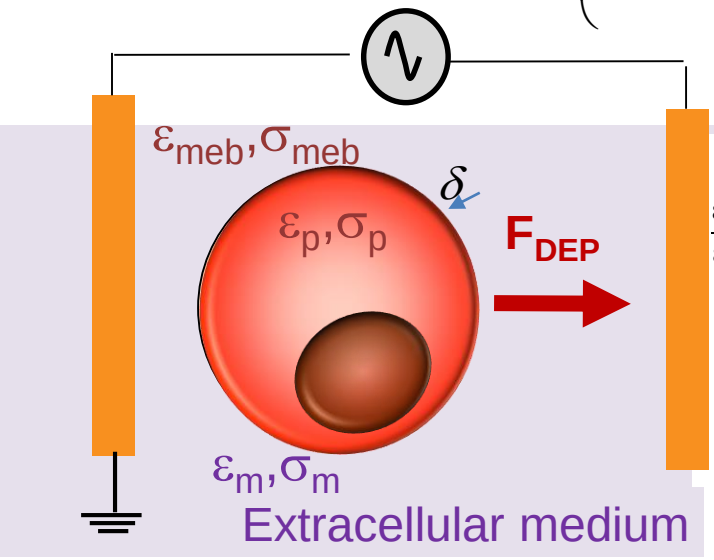
Cells can be individually electromanipulated accordingly their own dielectric properties

Dielectrophoresis vs Dielectric Spectroscopy approach

Claussius-Mossotti factor also depends on the DEP signal frequency

Few basics

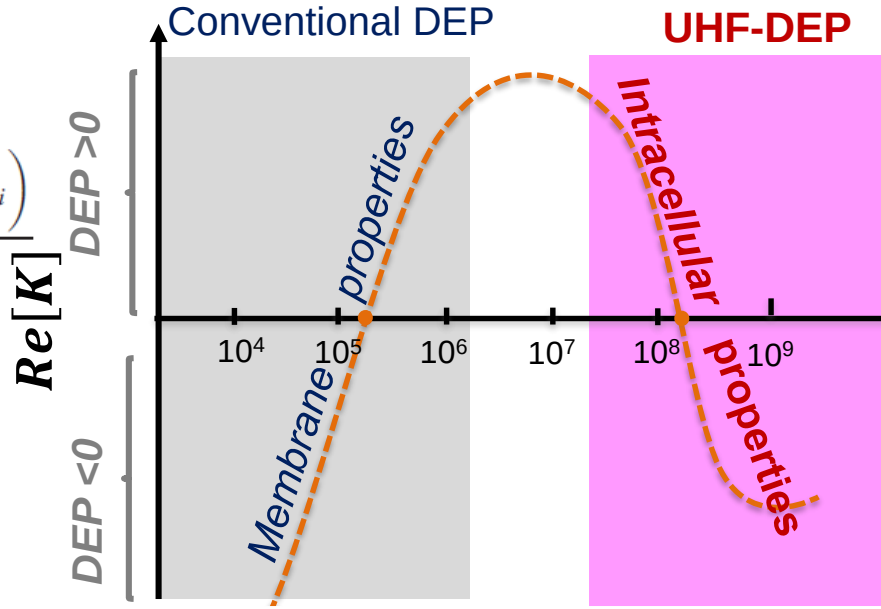
$$\mathrm{Re}[K(\omega)] = \left(\frac{(\varepsilon_p - \varepsilon_m)(\varepsilon_p + 2\varepsilon_m) + \frac{1}{\omega^2}(\sigma_p - \sigma_m)(\sigma_p + 2\sigma_m)}{(\varepsilon_p + 2\varepsilon_m)^2 + \frac{1}{\omega^2}(\sigma_p + 2\sigma_m)^2} \right)$$



$$\frac{\varepsilon_p}{\varepsilon_0} = \frac{\left(\frac{\varepsilon_m}{\varepsilon_0}\frac{\varepsilon_i}{\varepsilon_0} - \frac{\sigma_m\sigma_i}{\omega^2\varepsilon_0^2}\right)\left(\frac{\varepsilon_m}{\varepsilon_0} + \frac{\delta}{R}\frac{\varepsilon_i}{\varepsilon_0}\right) + \frac{1}{\omega^2\varepsilon_0^2}\left(\sigma_m\frac{\varepsilon_i}{\varepsilon_0} + \frac{\varepsilon_m}{\varepsilon_0}\sigma_i\right)\left(\sigma_m + \frac{\delta}{R}\sigma_i\right)}{\left(\frac{\varepsilon_m}{\varepsilon_0} + \frac{\delta}{R}\frac{\varepsilon_i}{\varepsilon_0}\right)^2 + \frac{1}{\omega^2\varepsilon_0^2}\left(\sigma_m + \frac{\delta}{R}\sigma_i\right)^2}$$

$$\sigma_p = \frac{-\left(\frac{\varepsilon_m}{\varepsilon_0}\frac{\varepsilon_i}{\varepsilon_0} - \frac{\sigma_m\sigma_i}{\omega^2\varepsilon_0^2}\right)\left(\sigma_m + \frac{\delta}{R}\sigma_i\right) + \left(\sigma_m\frac{\varepsilon_i}{\varepsilon_0} + \frac{\varepsilon_m}{\varepsilon_0}\sigma_i\right)\left(\frac{\varepsilon_m}{\varepsilon_0} + \frac{\delta}{R}\frac{\varepsilon_i}{\varepsilon_0}\right)}{\left(\frac{\varepsilon_m}{\varepsilon_0} + \frac{\delta}{R}\frac{\varepsilon_i}{\varepsilon_0}\right)^2 + \frac{1}{\omega^2\varepsilon_0^2}\left(\sigma_m + \frac{\delta}{R}\sigma_i\right)^2},$$

As ω↗, plasma membrane influence ↘
& cytoplasm influence ↗



DEP also allows characterizing cell dielectric properties

Cell DEP spectral signature

How taking advantage of Cell DEP spectral signature

Once cells are suspended in low σ medium

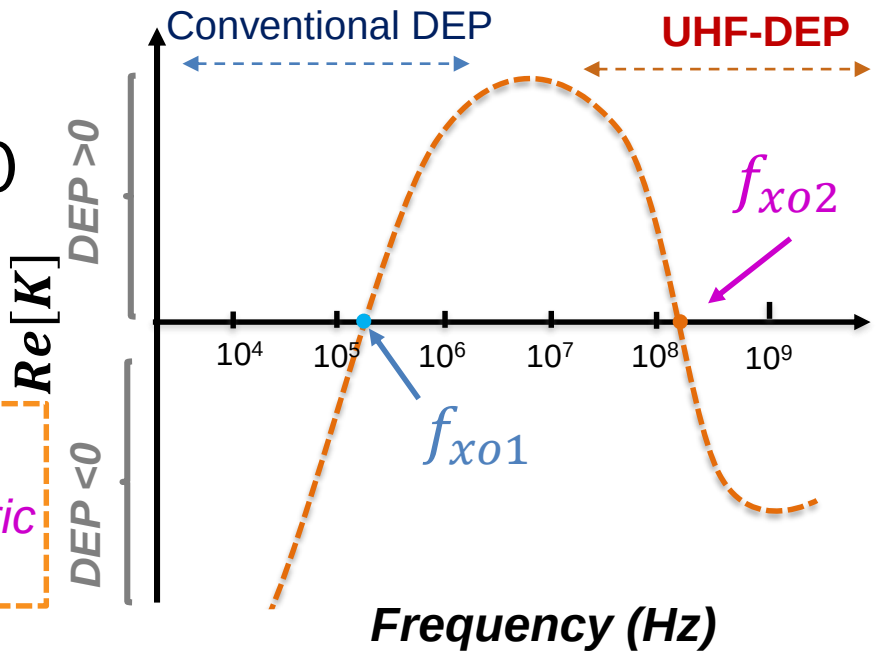
Two *crossover frequencies* appears -> Re[K]=0

$$f_{x01} = \frac{\sqrt{2}}{2\pi\, r C_{mem}}\sigma_m$$

Dominated by membrane dielectric properties

$$f_{x02} = \frac{1}{2\pi}\sqrt{\frac{\sigma_{int}^2 - \sigma_{int}\sigma_m - 2\sigma_m^2}{2\varepsilon_m^2 - \varepsilon_{int}\varepsilon_m - \varepsilon_{int}^2}}$$

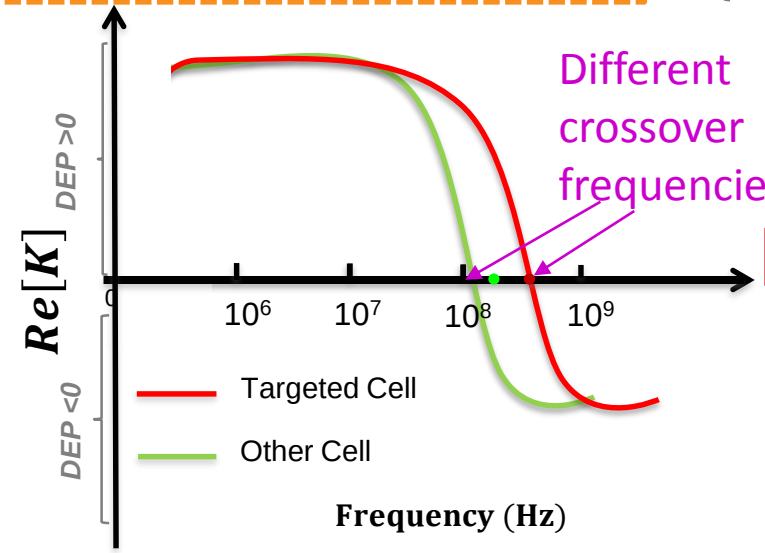
Depend on intra vs extra cellular dielectric properties



Different cells

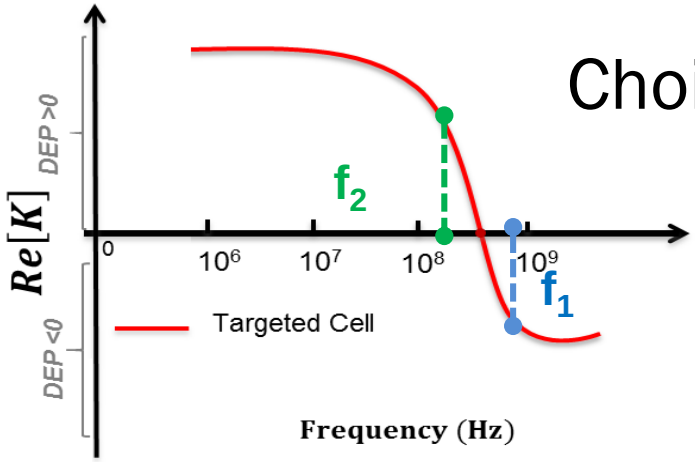
Vs

Different spectral signatures



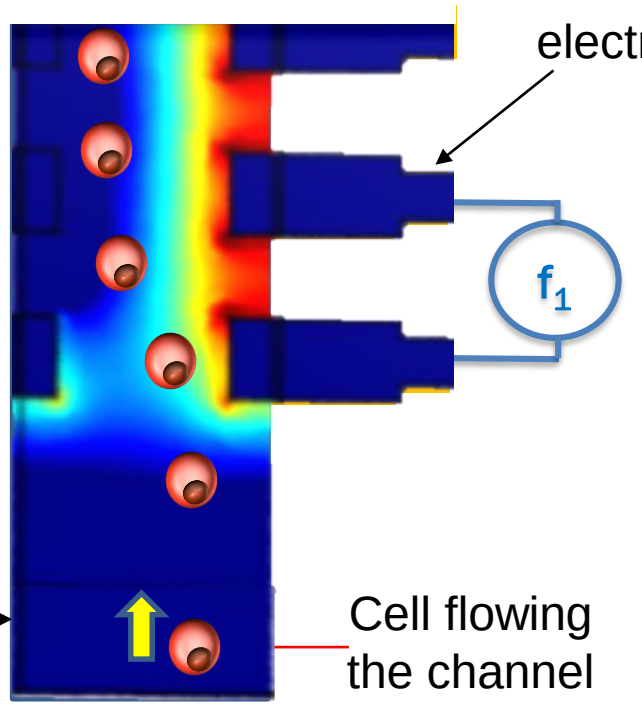
Characterize cells to identify their DEP cross over frequencies as discriminant specificities

Combined electromanipulation & characterization



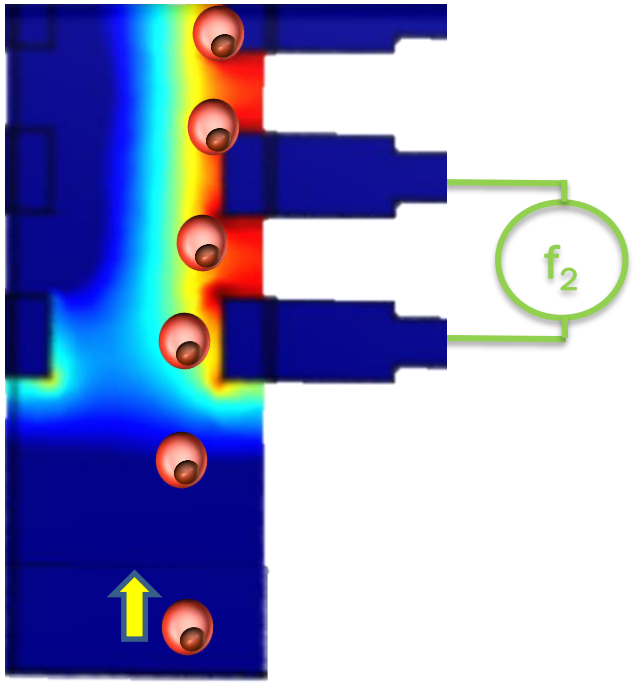
Choice of appropriated DEP signal frequency is important

Negative DEP



*Cell is **repealed** from electrode*

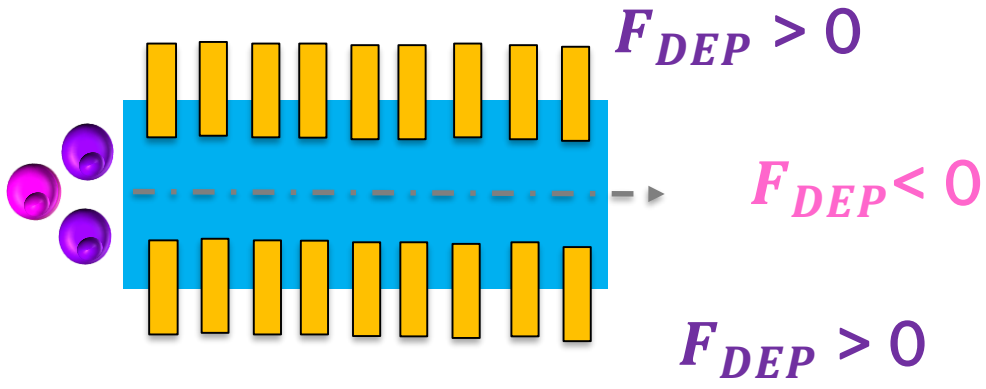
Positive DEP



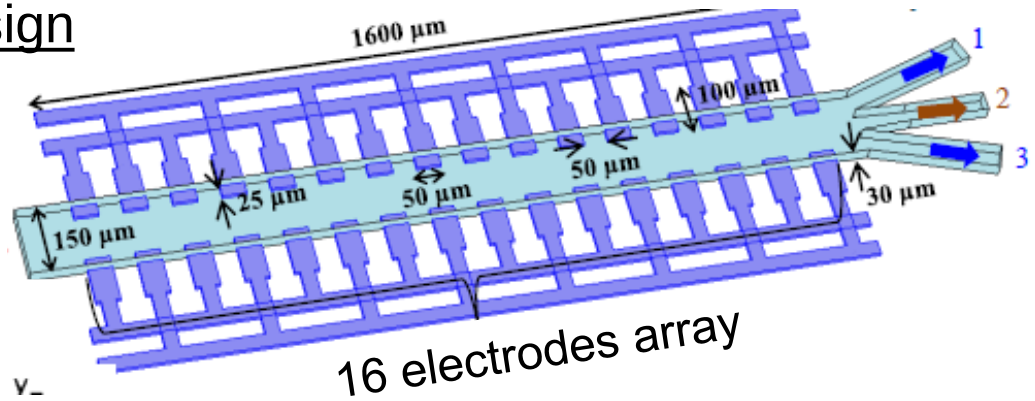
*Cell is **attracted by** electrodes*

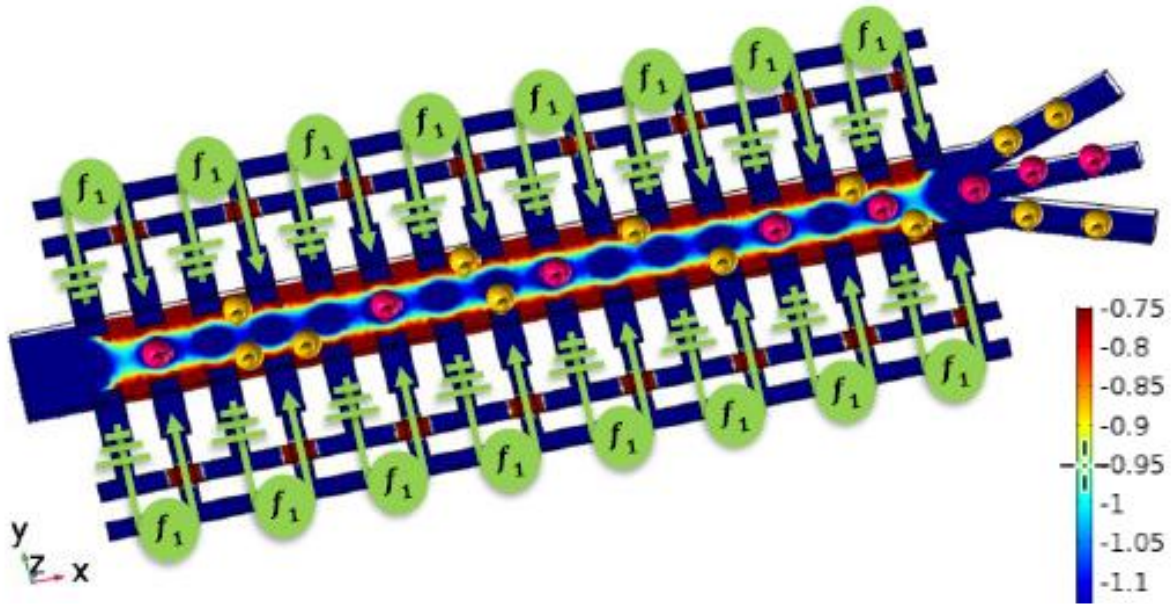
Proposed High Frequency DEP cytometer design

- Double Array of electrode allowing progressive cell trajectory deviation
  - Optimization of electrode penetration inside channel and periodicity



Final design



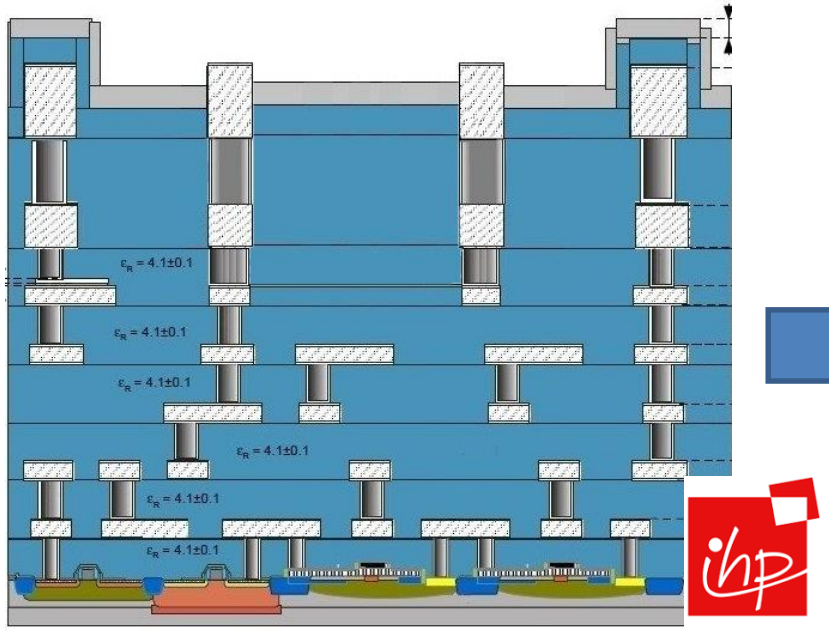


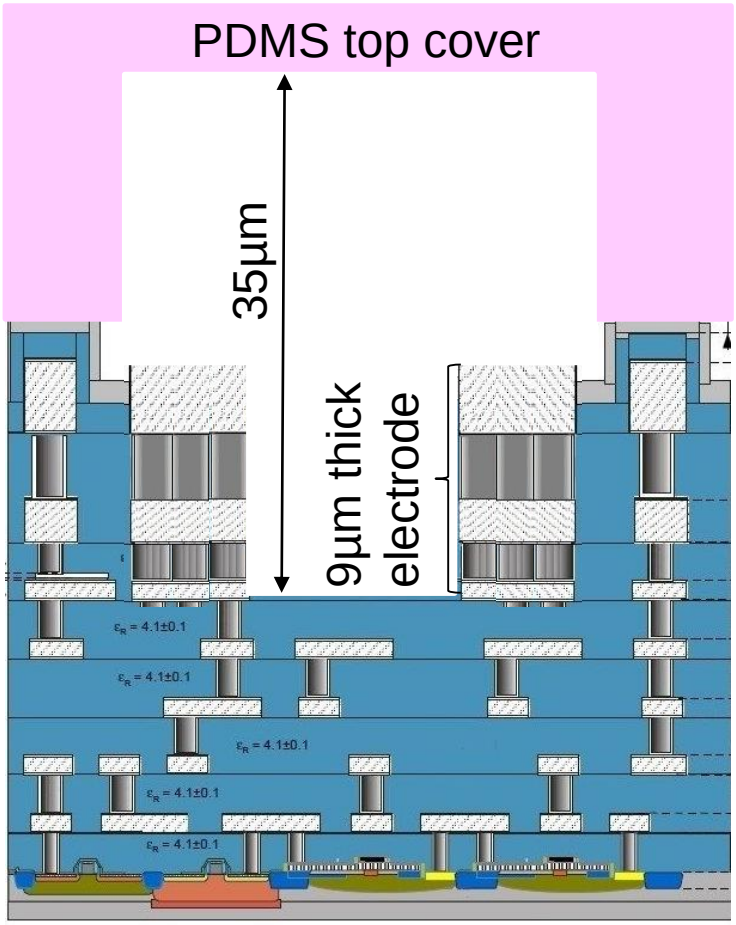
Implementation on BiCMOS technology

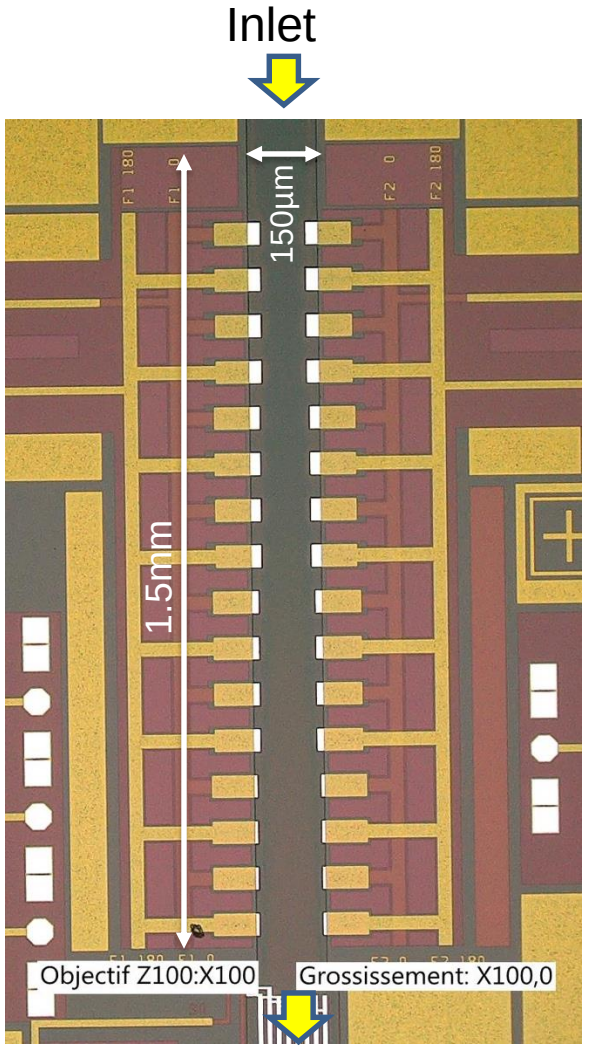
Starting with the BEOL stack IHP SG25H4 process

+ Post process µchannel etch

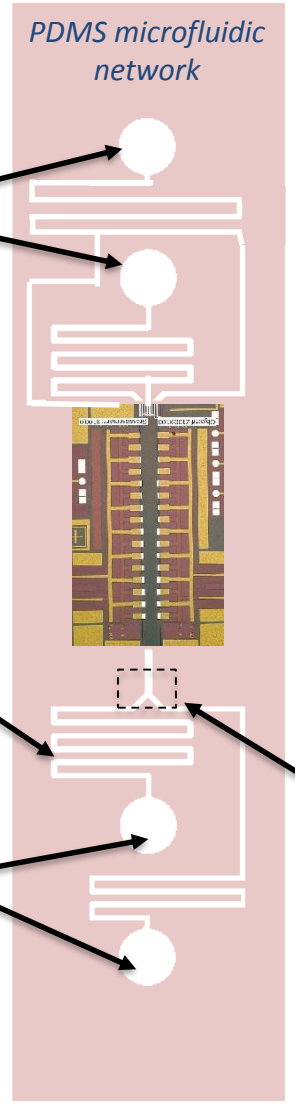
+ PDMS packaging



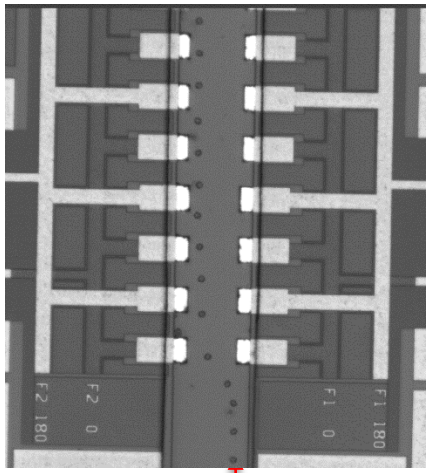


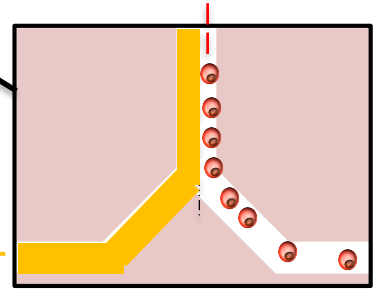


Microfluidic network implementation

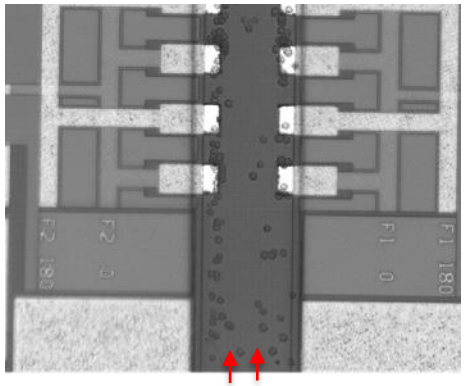


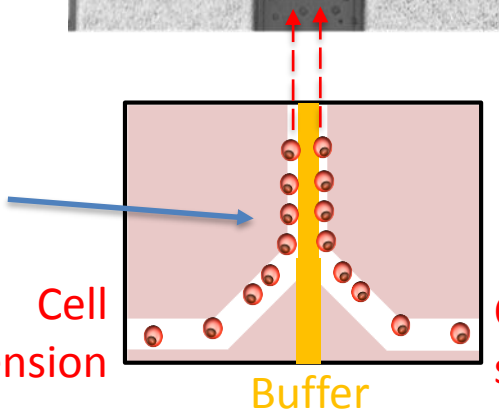
- Outside chip fluidic network for optimal cell injection/collect and velocity control
- Flow focusing techniques to improve DEP effect





Cell well focused on the channel edge

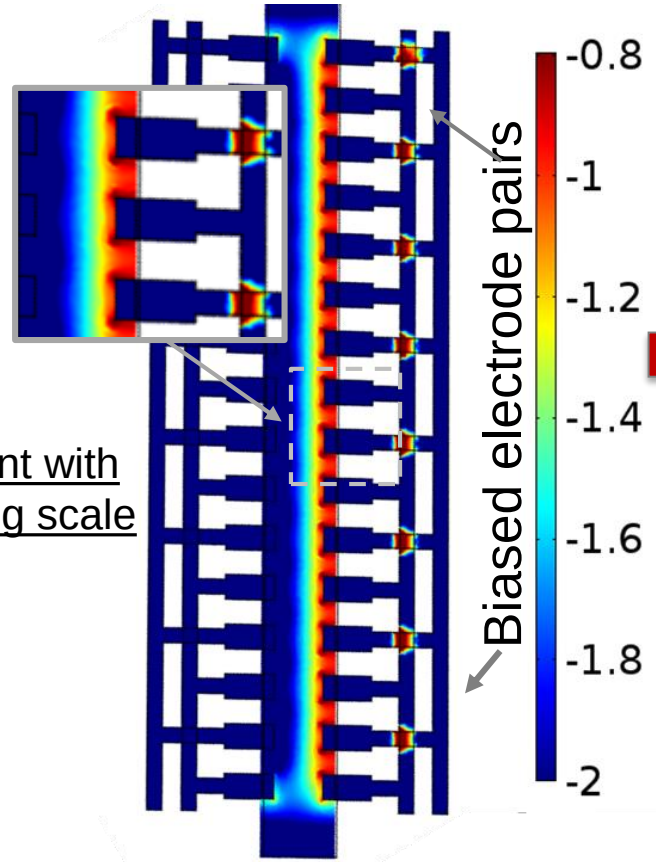




Working principle experimental validation

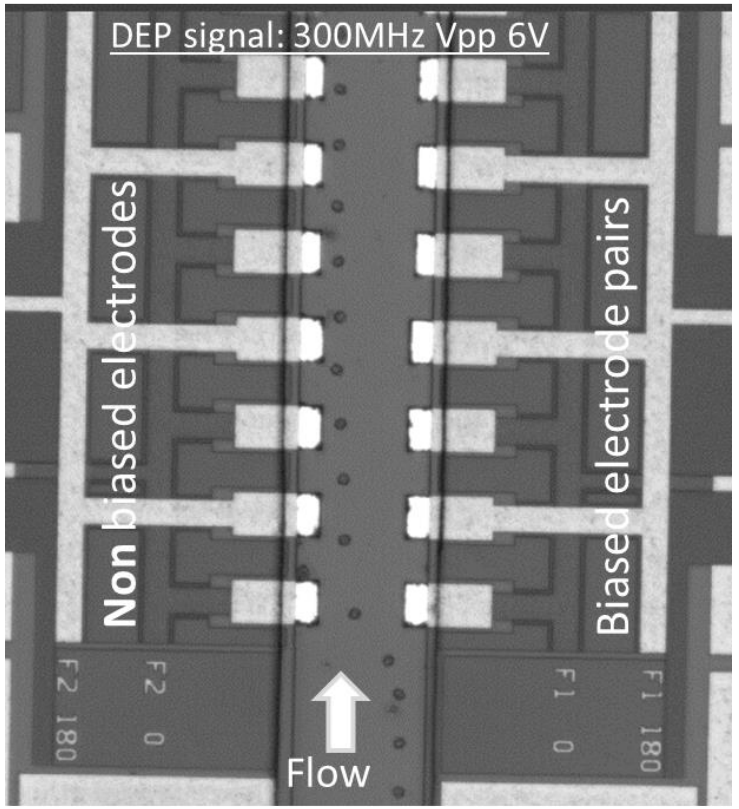
Test on LN 18 cells with single biased electrode array

- ✓ *DEP signal frequency set to DEP<0*
- ✓ *Cell velocity slow to 0.1mm/s*



E field gradient with normalized log scale

Cell is well quickly repealed far the high intensity E field region to finally travel in low intensity one

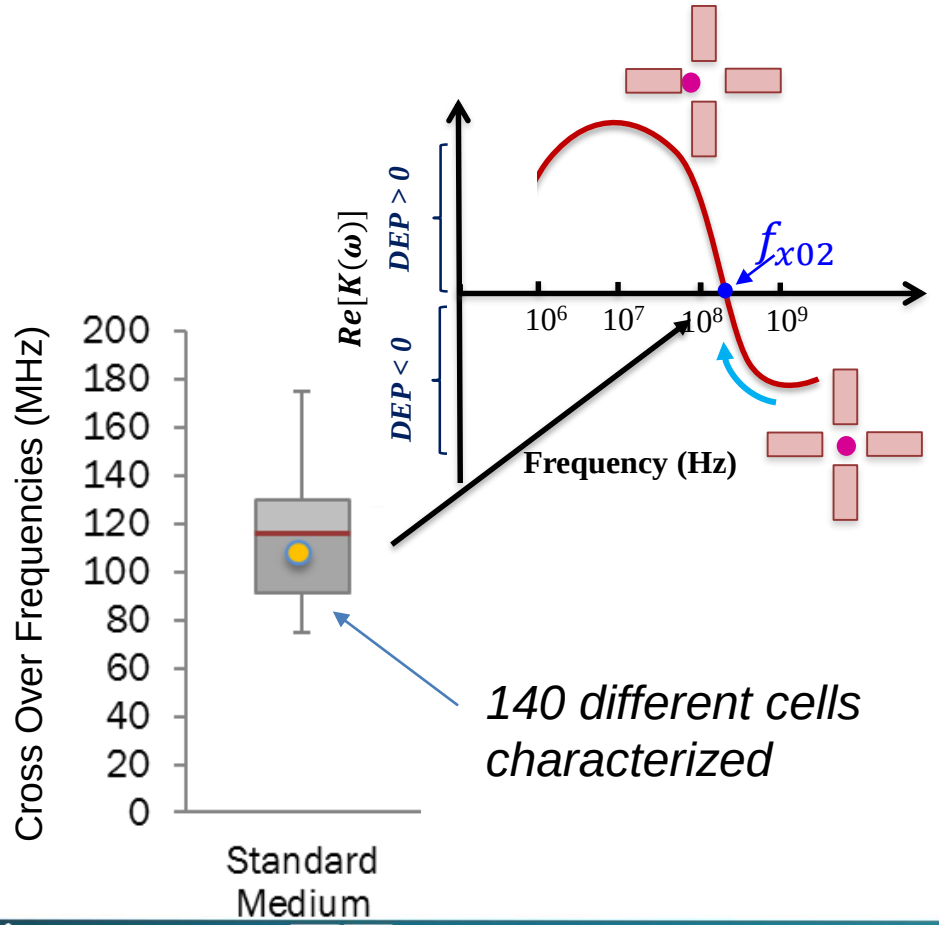


Stacked image of LN 18cell trajectory deviation

DEP characterization of GBM cell line

LN 18 cells cultured in conventional culture medium

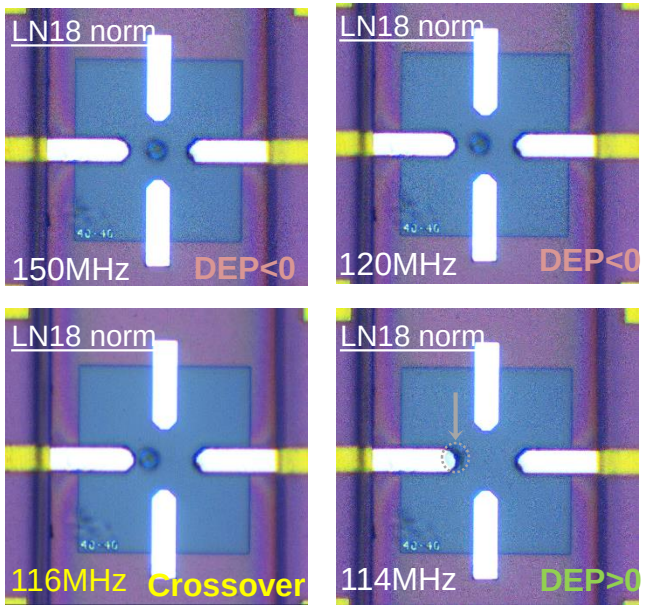
- *Specific quadrupole sensor for measuring crossover frequency*



140 different cells characterized

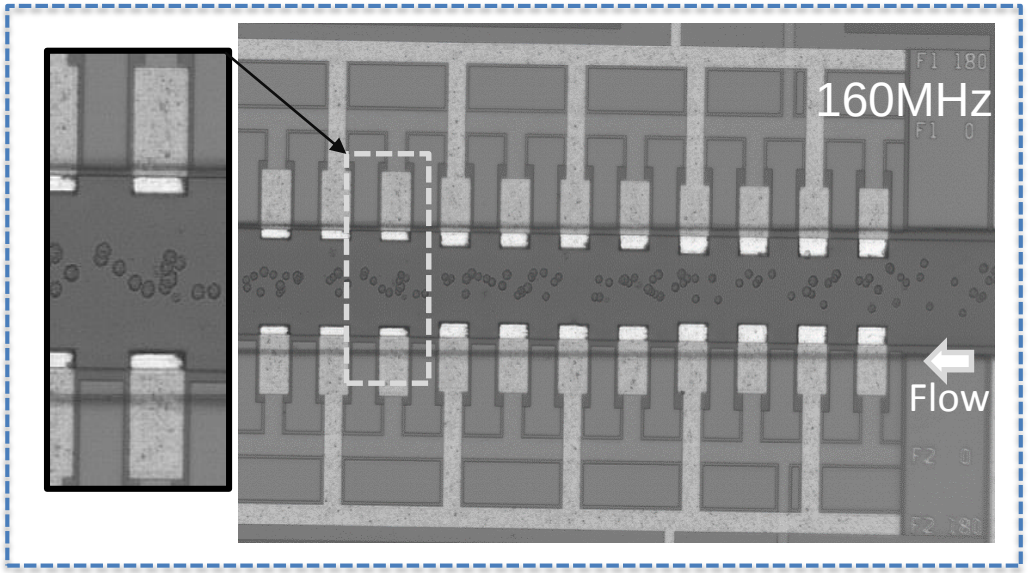
Methodology:

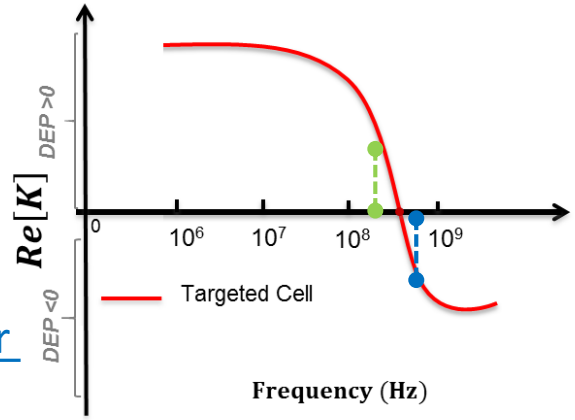
1) Cells are trapped in DEP<0
2) Frequency is tuned until finding positive DEP



Discrimination of Glioblastoma Cancer Stem Cells by Measuring Their UHF-Dielectrophoresis Crossover Frequency, IMBIOC 2018

Sorting test on LN18 cells



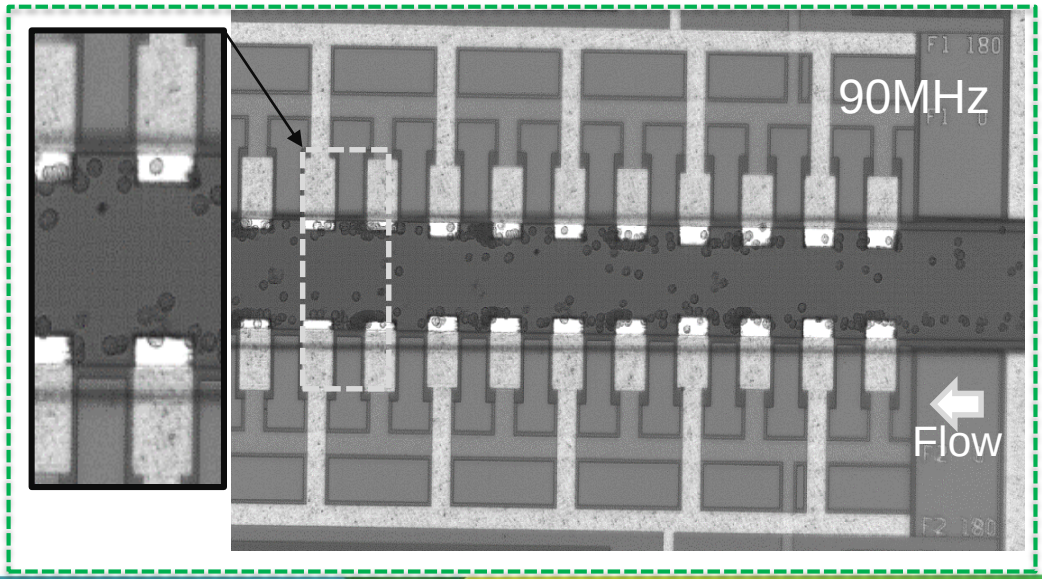


For F_{DEP}> Fcross-over

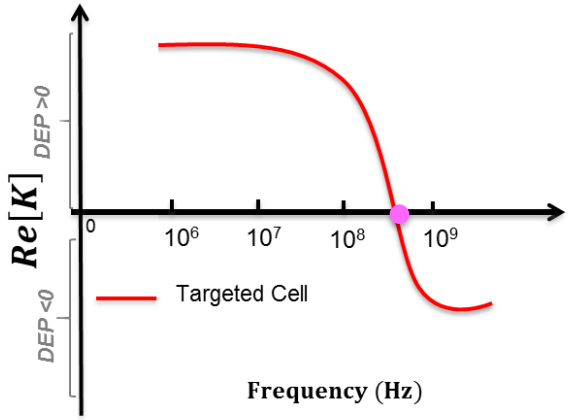
All cells are concentrated in microchannel center where E field intensity is the lowest

For F_{DEP}< Fcross-over

As expected cells are distributed **on the edge** of the channel attracted and trapped by high intensity E field areas

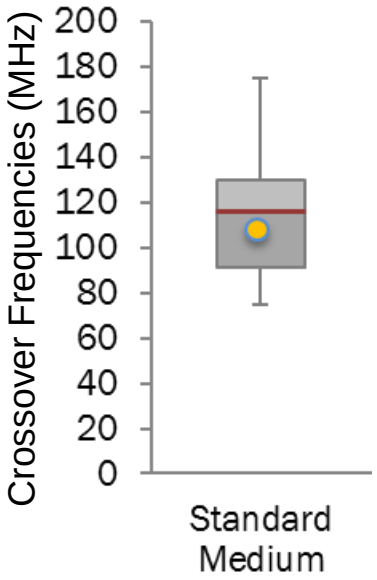


Sorting test on LN18 cells

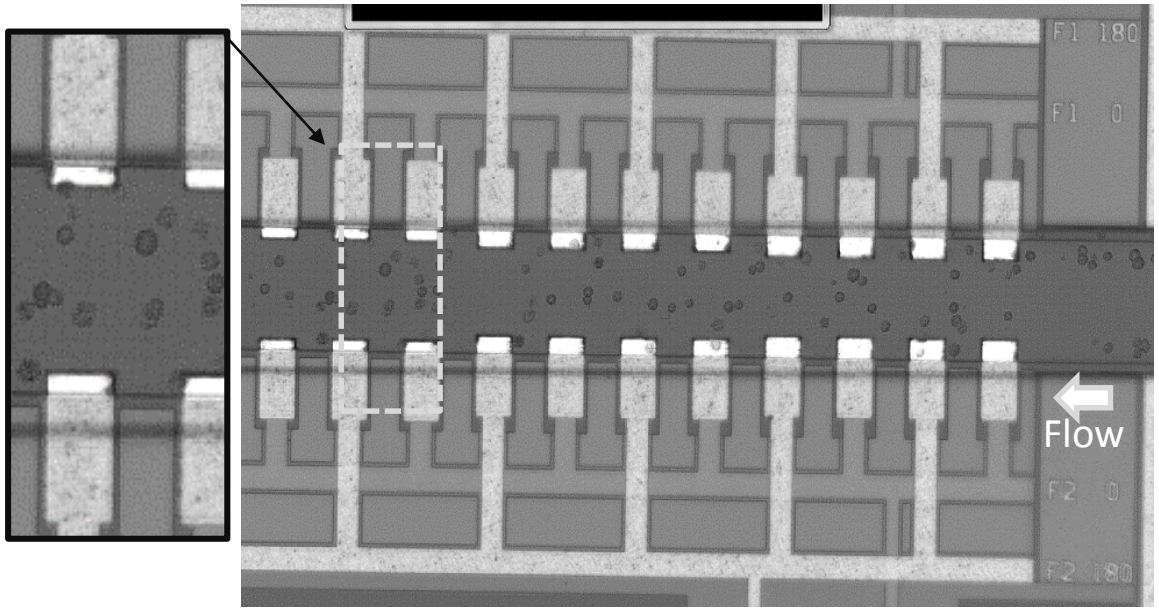


For F_{DEP}~ Fcross-over (median value for LN18 population)

Cell spatial distribution is much disperse (some repealed in the center , others attracted to the channel edge



Effect of the cell heterogeneity in the cell line



Operating too close Fcross-over might not allow efficient cell sorting

Mixing Particle solution sorting capability

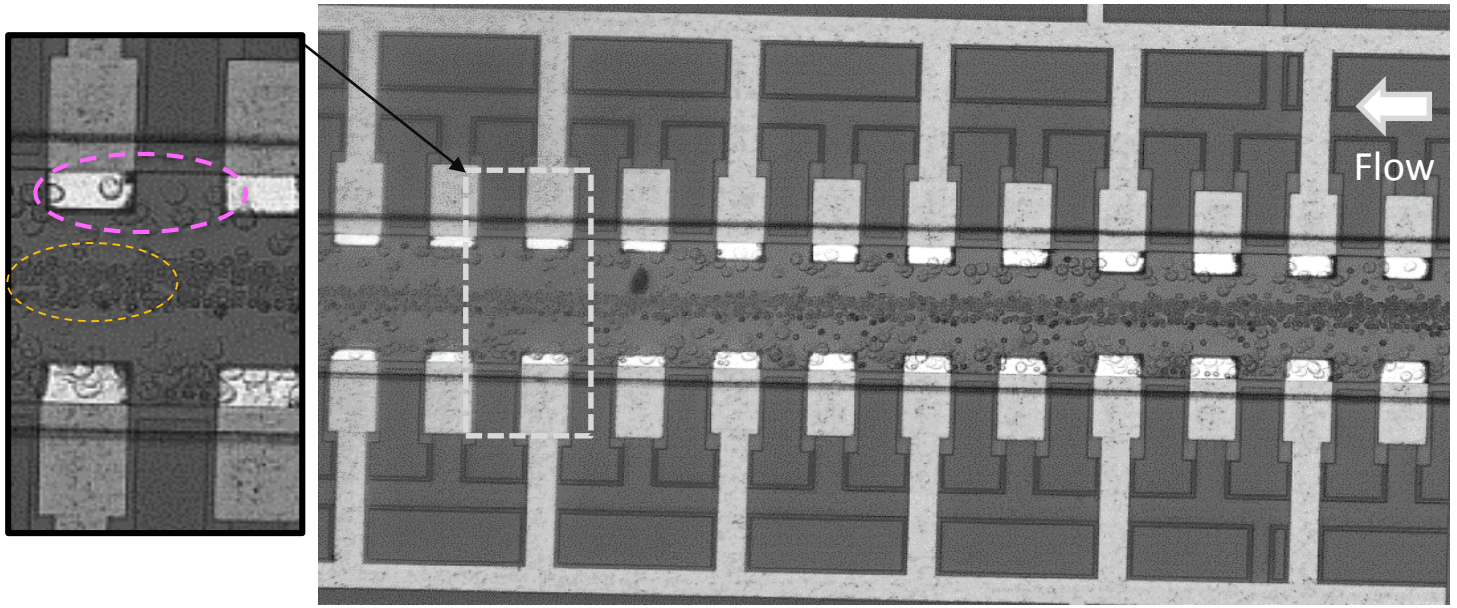
Mixing of LN 18 cells and polystyrene beads

50MHz DEP signal: - all polystyrene beads react in DEP<0

- most of LN18 cells react in DEP>0
- very few cells seem not be deviated (dead or damaged cells?)

LN18 Cells

7µm polystyrene beads



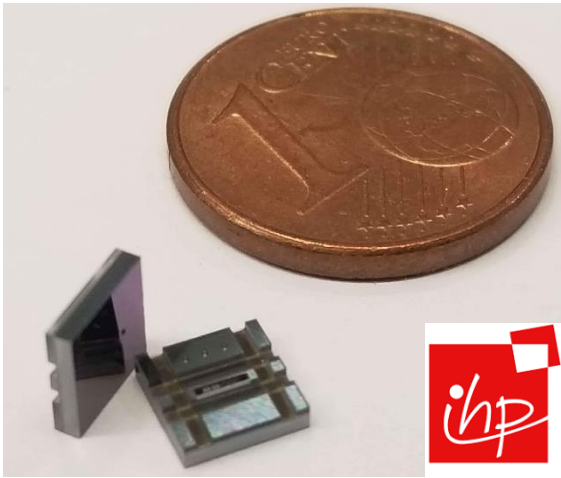
➡ At the sensor output particle distribution testifies of its good sorting ability

Conclusions & prospect

- High frequency DEP could be a powerful tool for cell analysis
- A first prototype has been designed and fabricated based on the coupling of hydrofluidic and selective DEP forces
- HF DEP cytometer working principle validated
- Successful sorting of particles mixtures

  -> cell mixtures in coming weeks
- Technological development still on going improving ....

  ...integration



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ihp

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ENEA Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile

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