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On-chip Flow Cell Sorting System Based on High frequency Dielectrophoresis implemented on CMOS technology

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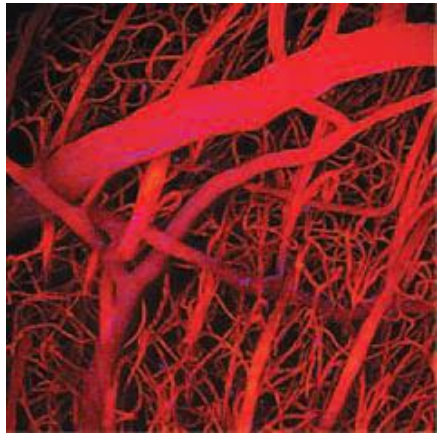
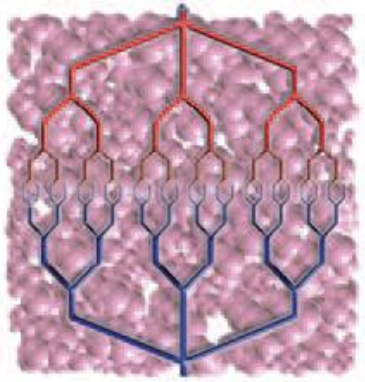
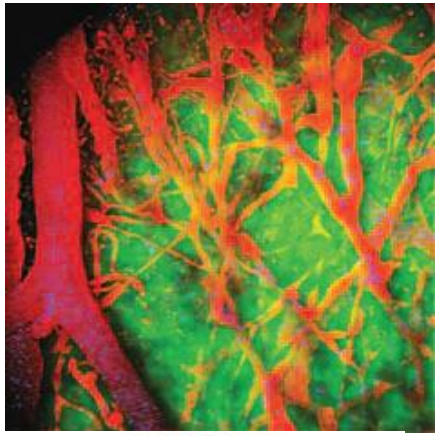
CSC location in tumor

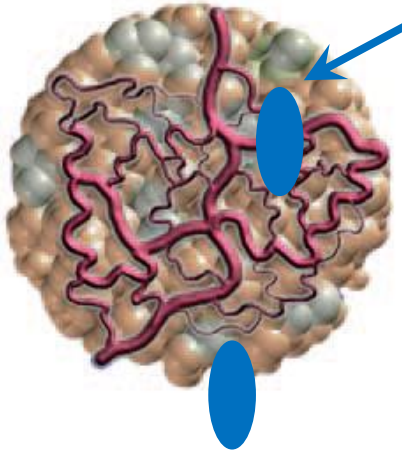
Normal brain tissue
Normal blood vessels



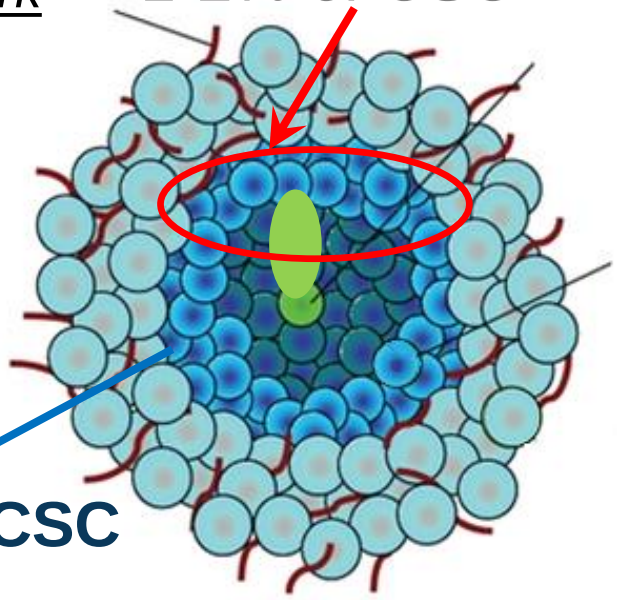


Brain Tumor
Anarchical vessel network





Hypoxic niche:
1-2% of CSC



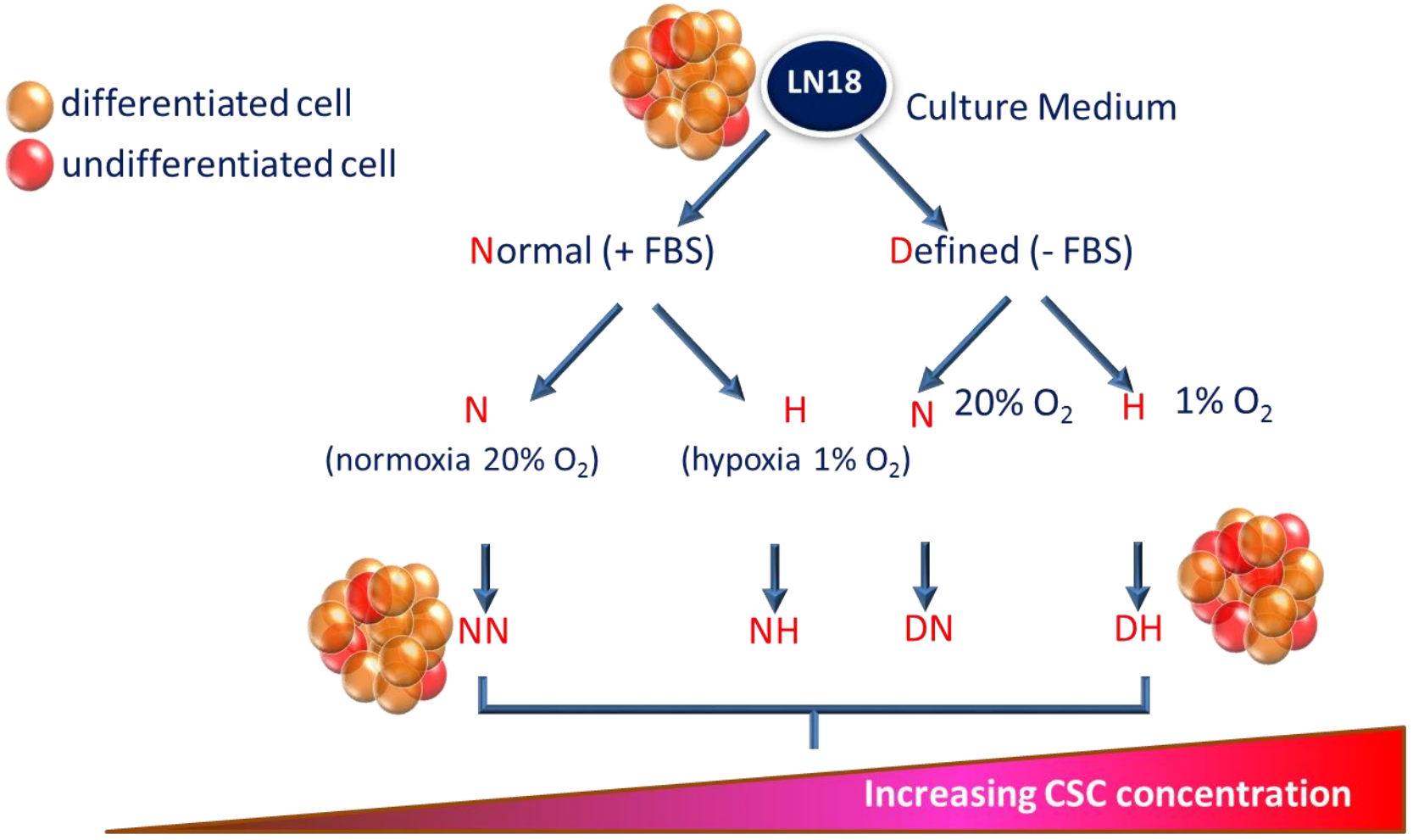
CSC

Specific environment

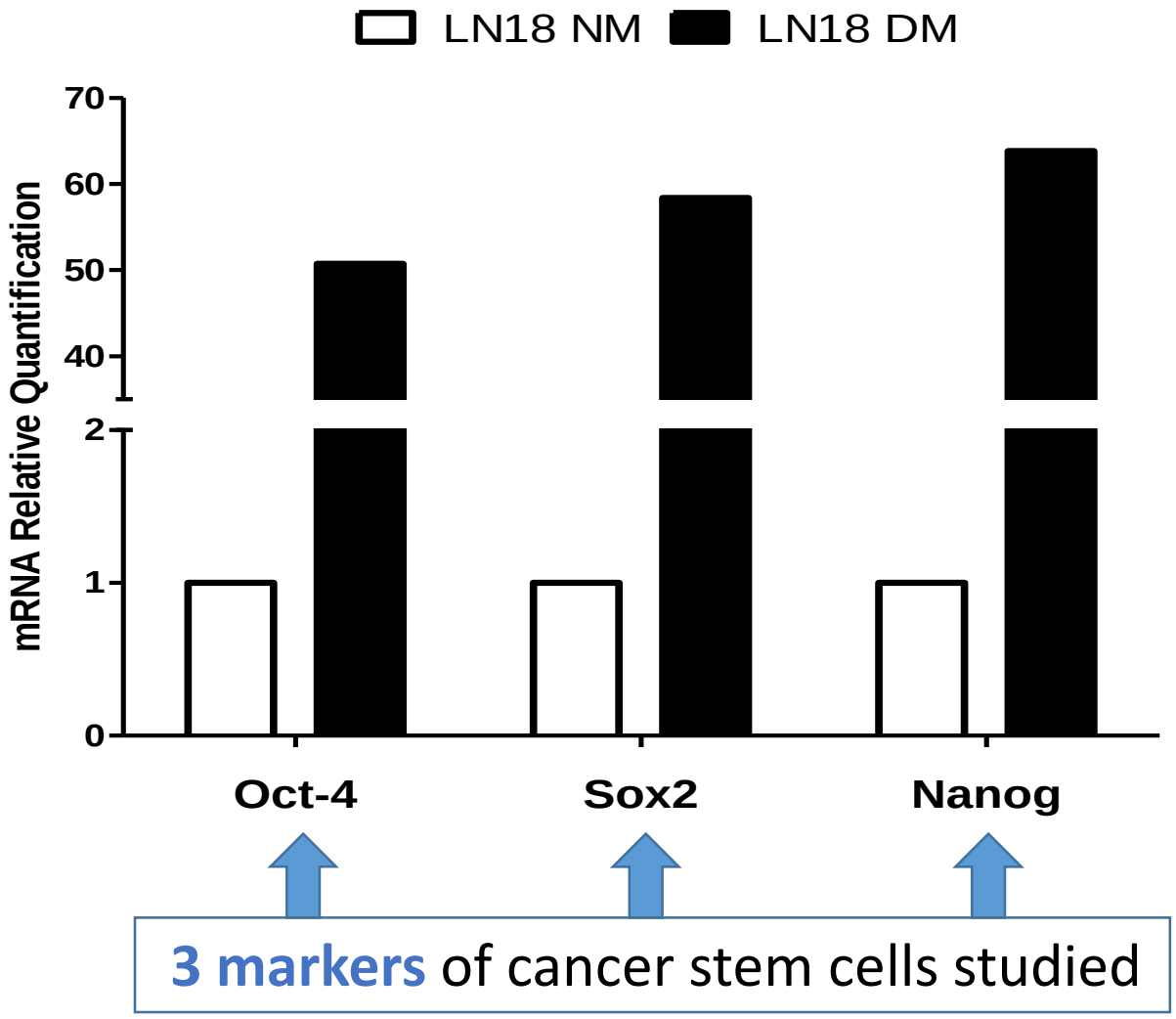
- *0_2<1%*
- *high growth factor concentration*

How create CSC grow conditions in vitro ?

Adjusting culture media and removing 0_2. Stringent grow conditions



Phenotypical characterization of cell-pools



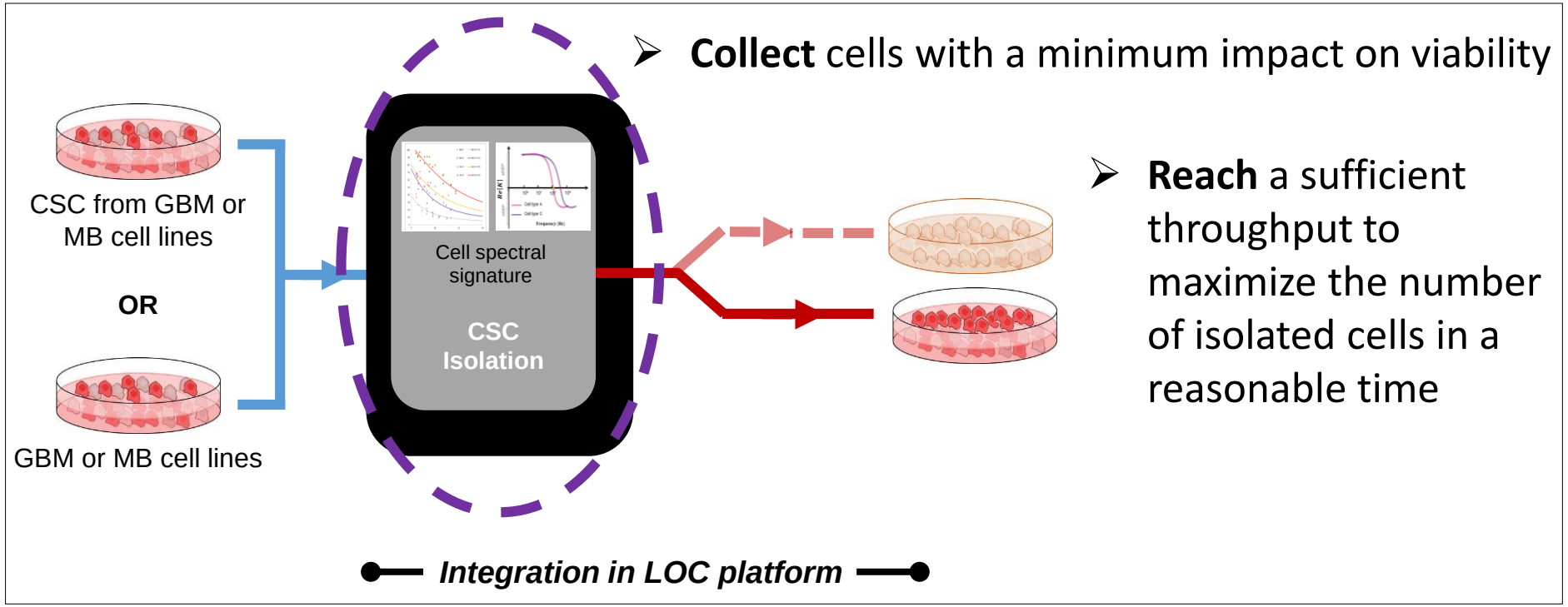
CSCs enrichment in Defined medium

Sensing module objectives

- **Recognize** (distinguish from others) targeted cells

  *Characterizing **physical properties** (dielectrical, mechanical, densitity, deformability…) of **dissociated cells set in suspension***

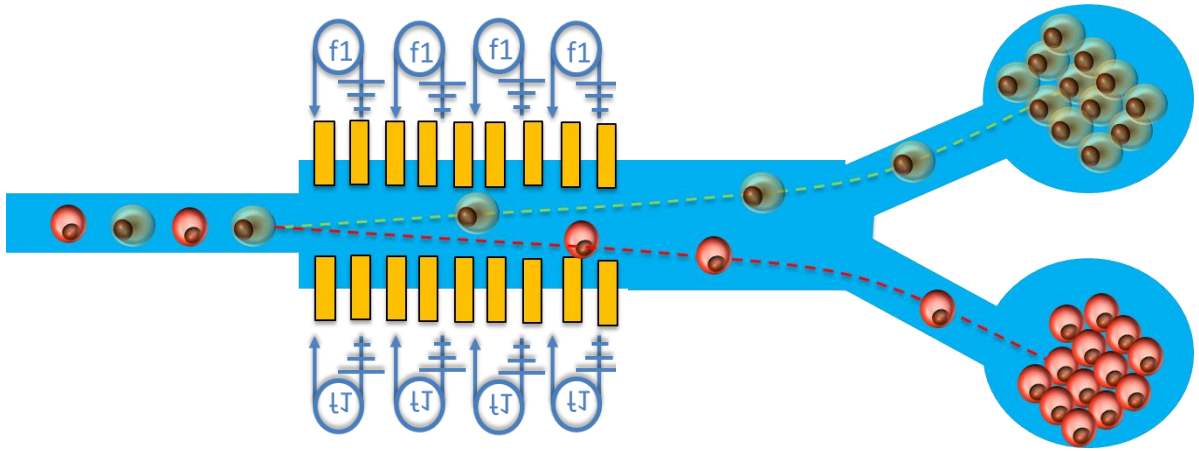
- **Isolate** targeted cells (at least remove a maximum of unwanted cells)



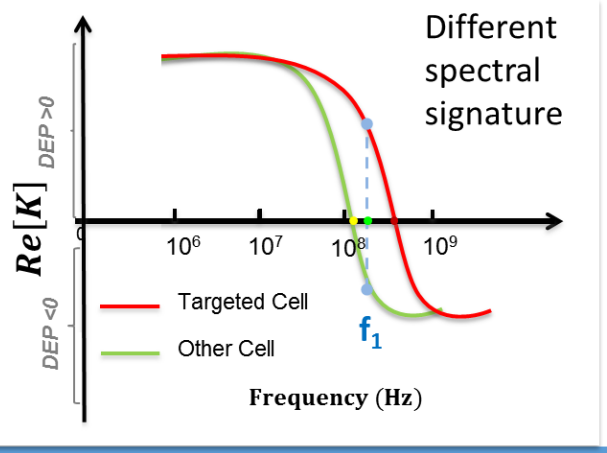
- **Collect** cells with a minimum impact on viability
- **Reach** a sufficient throughput to maximize the number of isolated cells in a reasonable time

Approach investigated

Combined hydro-fluidic & ElectroMagnetic (EM) manipulation



- Cell are **dynamically** sorted depending their "susceptibility" to **specific** EM signal

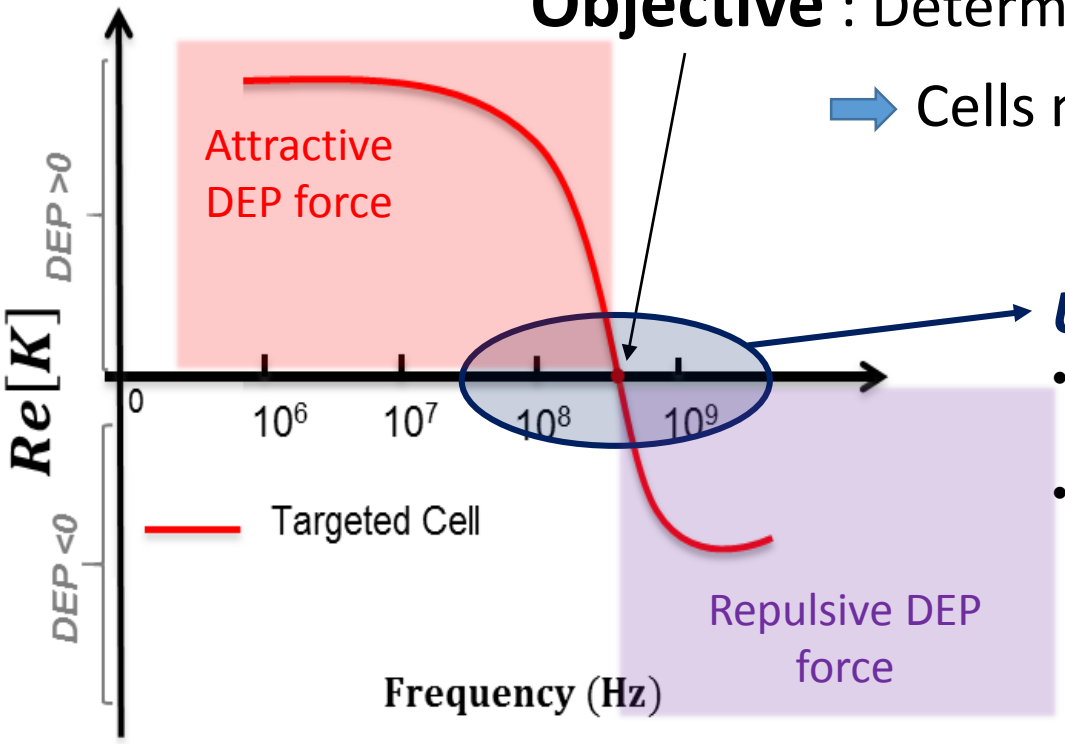


Dielectrophoresis ***(DEP)*** *approach*

DEP - Methodology and measurement system

Objective : Determine the crossover frequency of each cell population

➡ Cells mix sorting

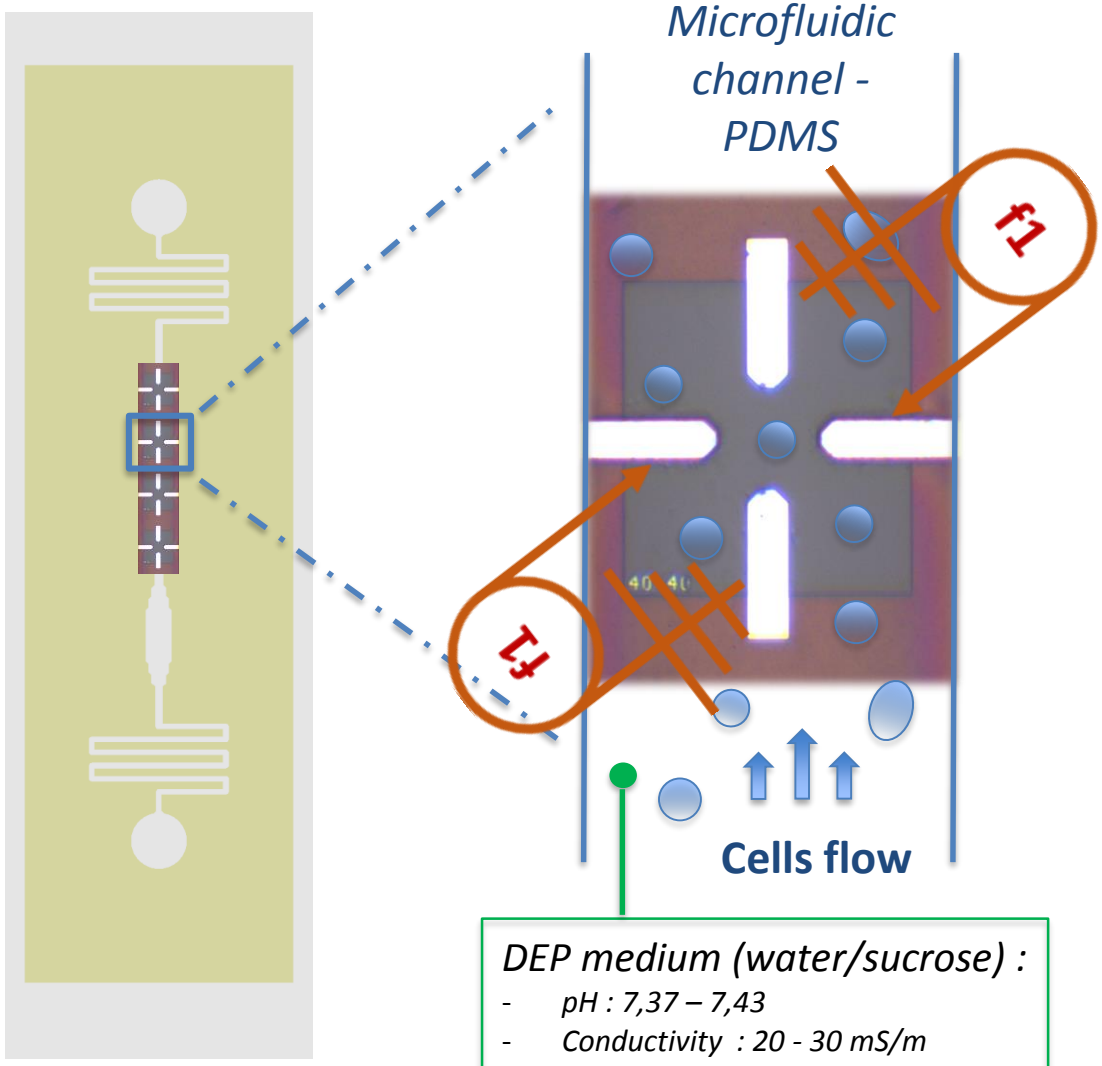


UHF → Intracellular properties dominate the cell response :

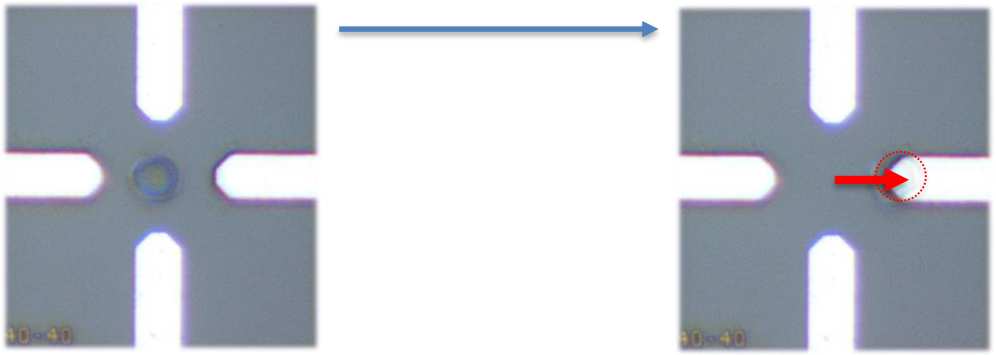
- plasma membrane lets the electric field penetrate the cell and interact directly with the cell interior
- Dielectric specificities / Physiological properties (stem cells versus differentiated ones):
  - nature
  - origin
  - differentiation state
  - pathological state
  - aggressiveness level

➡ Different effects of DEP forces generated (Attract vs. Repeal)

DEP - Methodology and measurement system

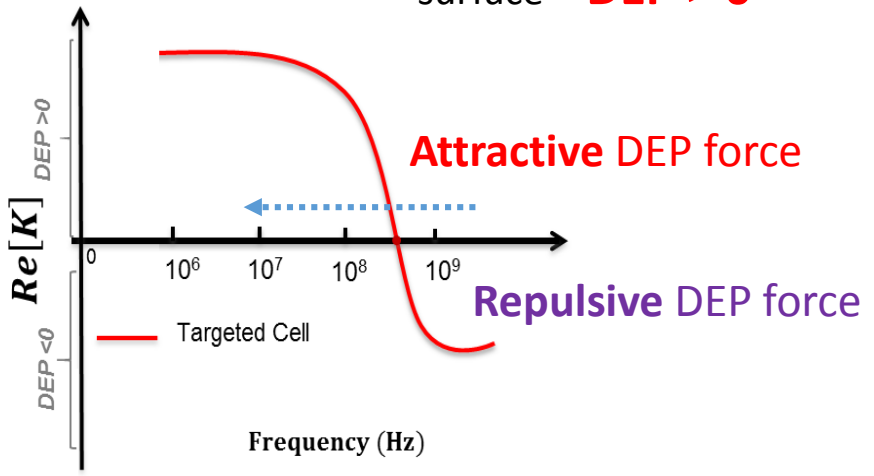


Frequency range variation – **UHF frequencies**



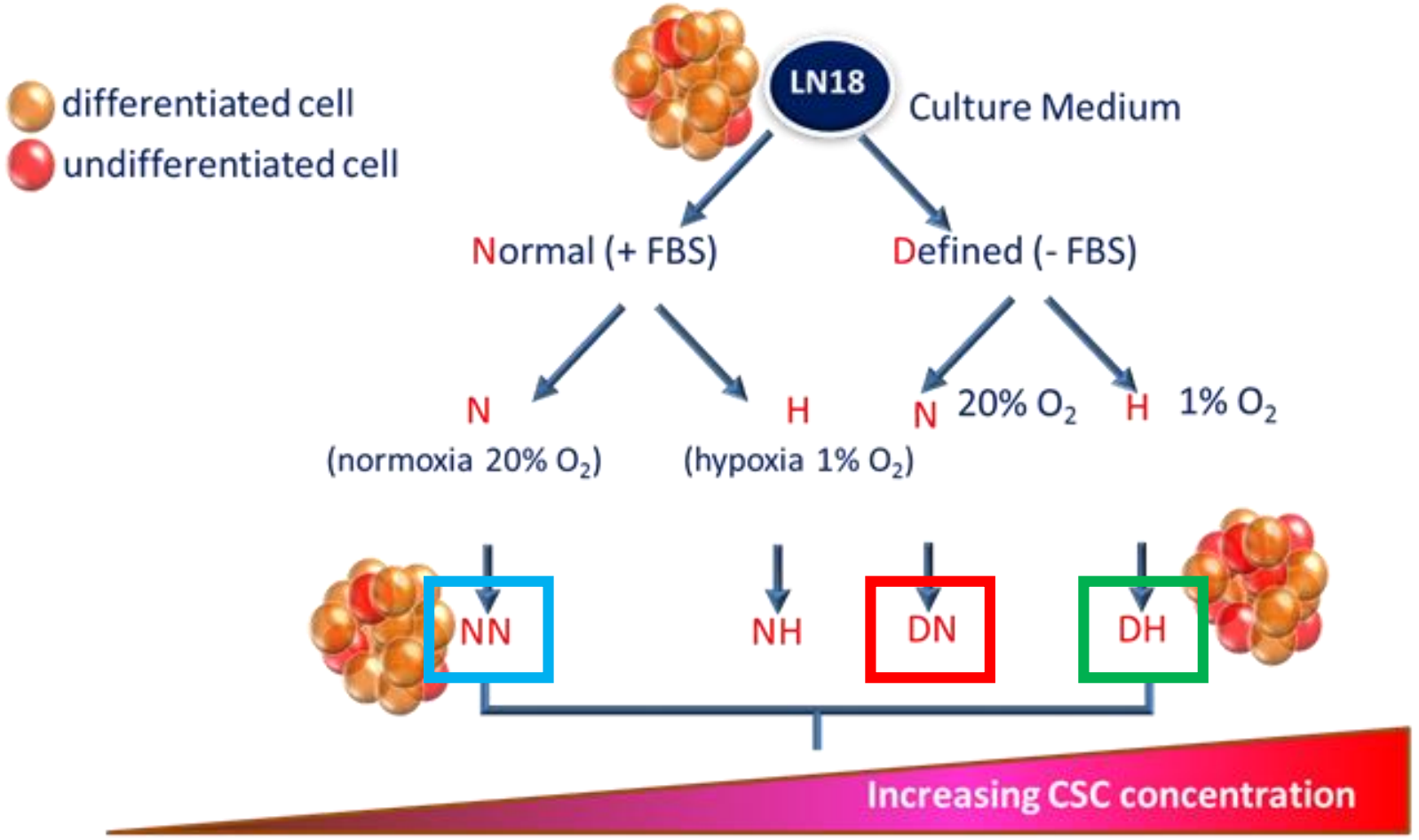
Cell trapping– Repulsion at system center - **DEP < 0**

Crossover frequency – Attraction to electrode surface - **DEP > 0**



Crossover frequencies study

Cell line : Glioblastoma **Ln18** (derived from malignant gliomas adult patients)



➡ 3 culture conditions tested

Crossover frequencies study

- **Standard** medium

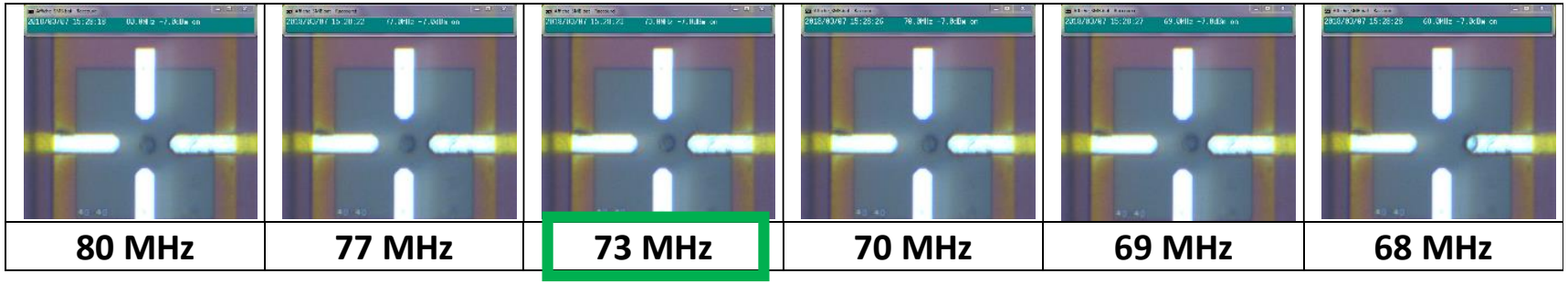
95 cells analysed

- **Defined** medium

81 cells analysed

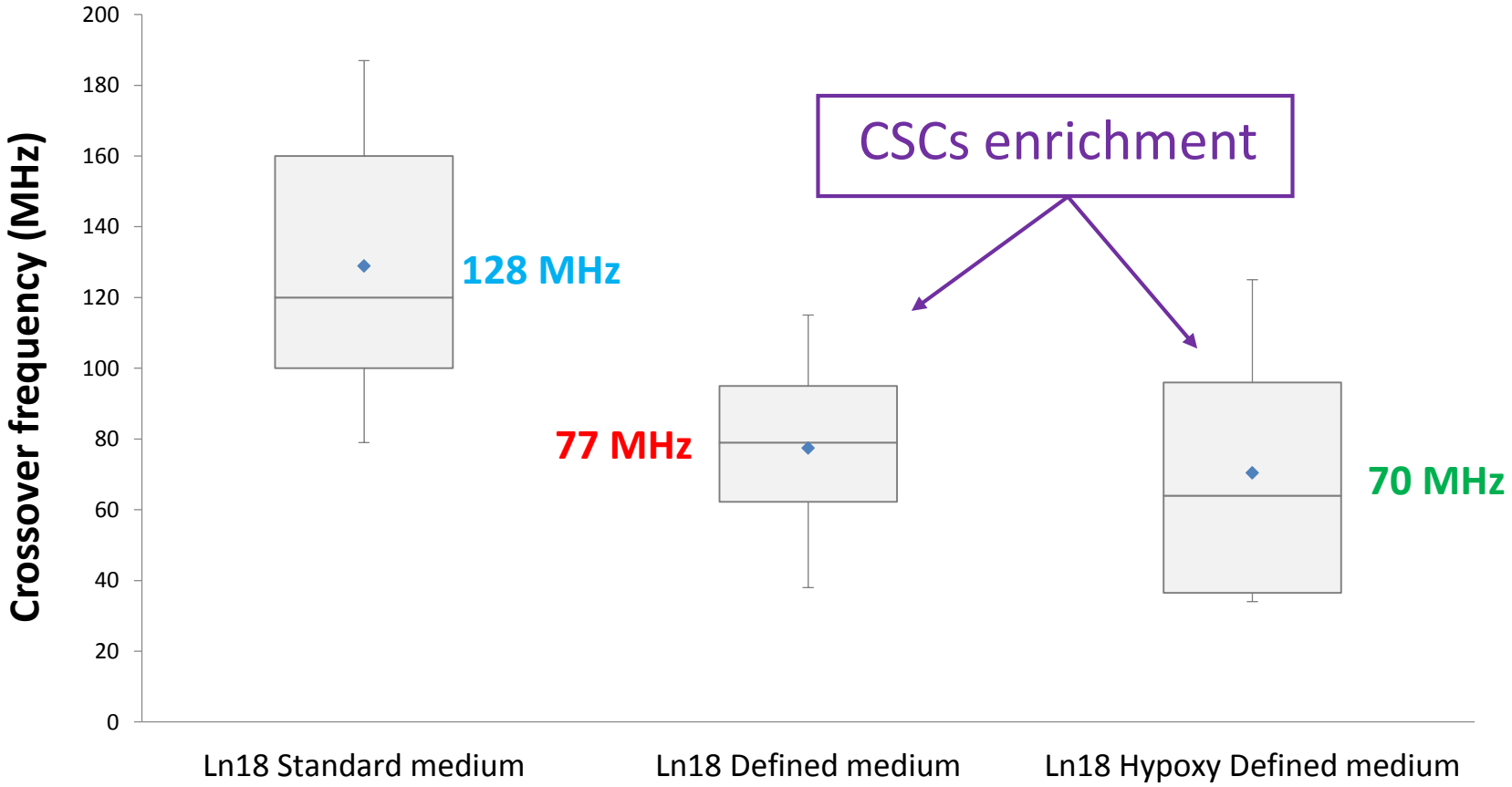
- **Hypoxy Defined** medium

31 cells analysed



Crossover frequencies study

UHF → Intracellular properties dominate the cell response

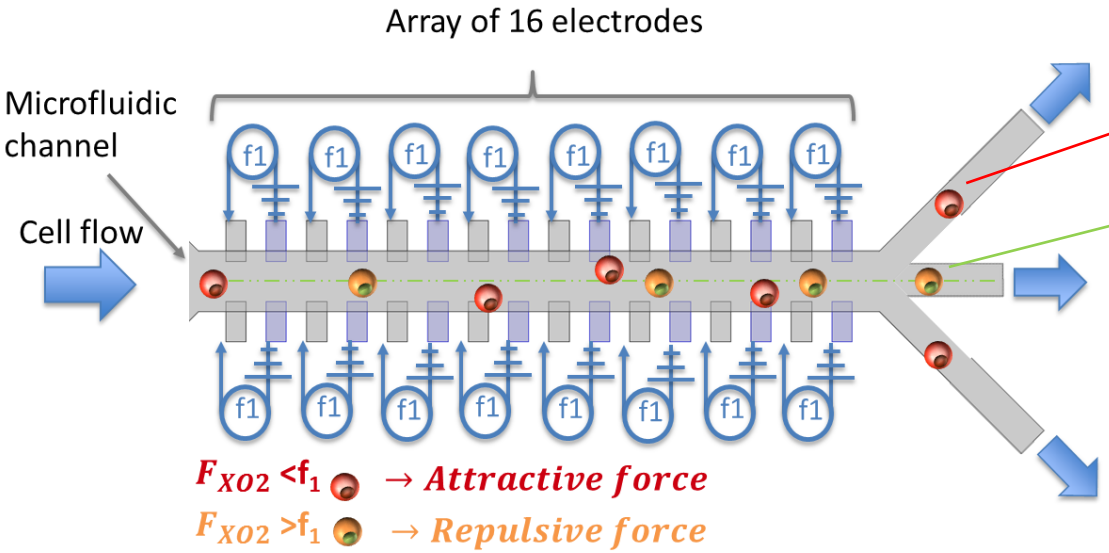


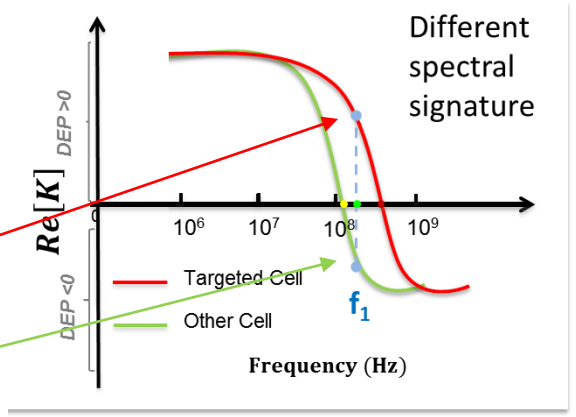
- Crossover Frequency signature for each cell population
- Mesures variations – Cell populations **heterogeneity**

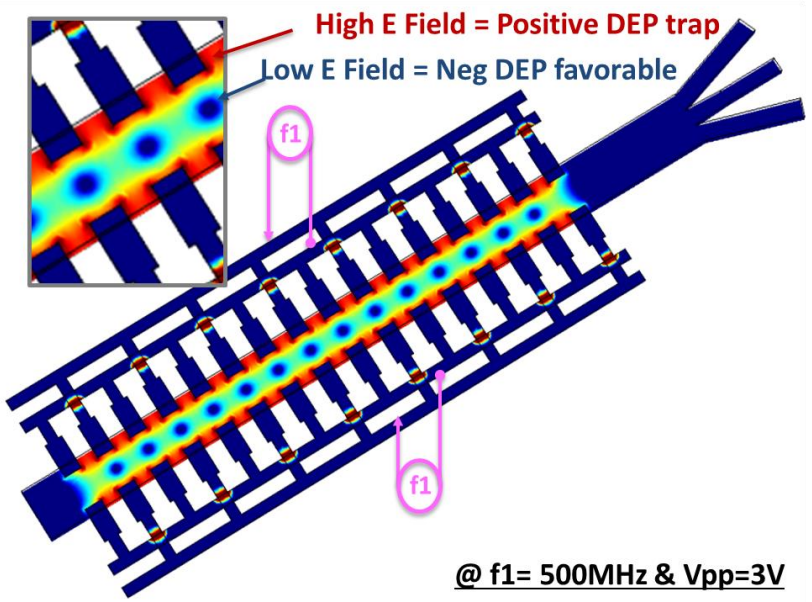
CSCs Sorting

Combined hydro-fluidic & ElectroMagnetic DEP cell manipulation

First design based on a distributed electrode array biased with a DEP signal with appropriately chosen frequency



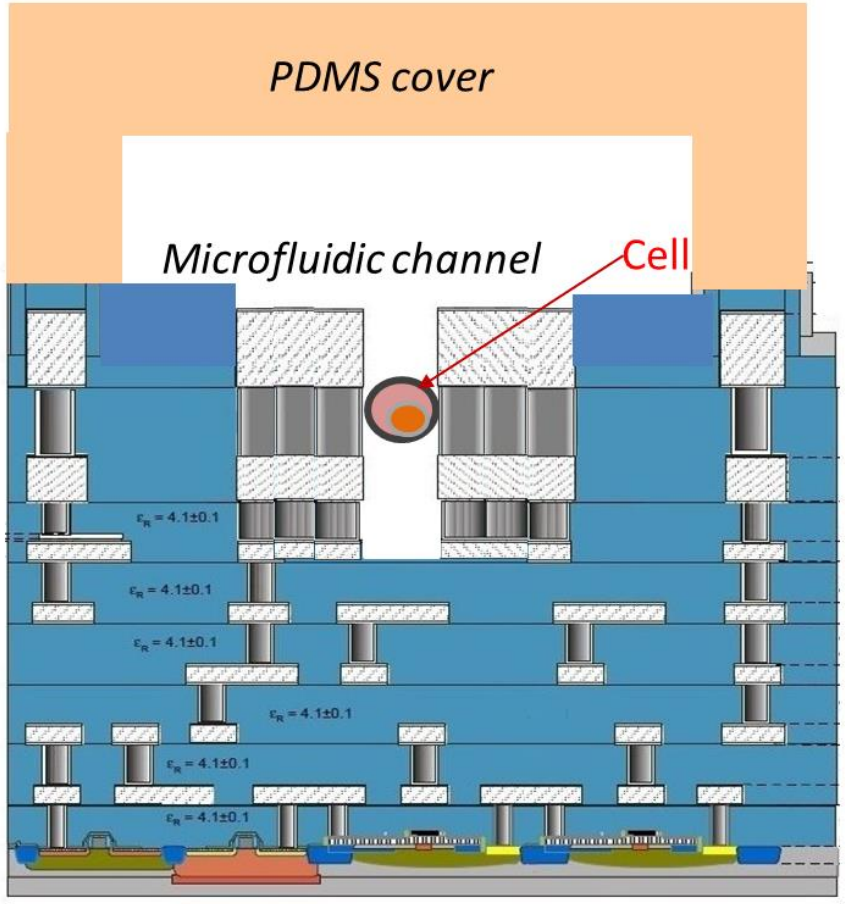




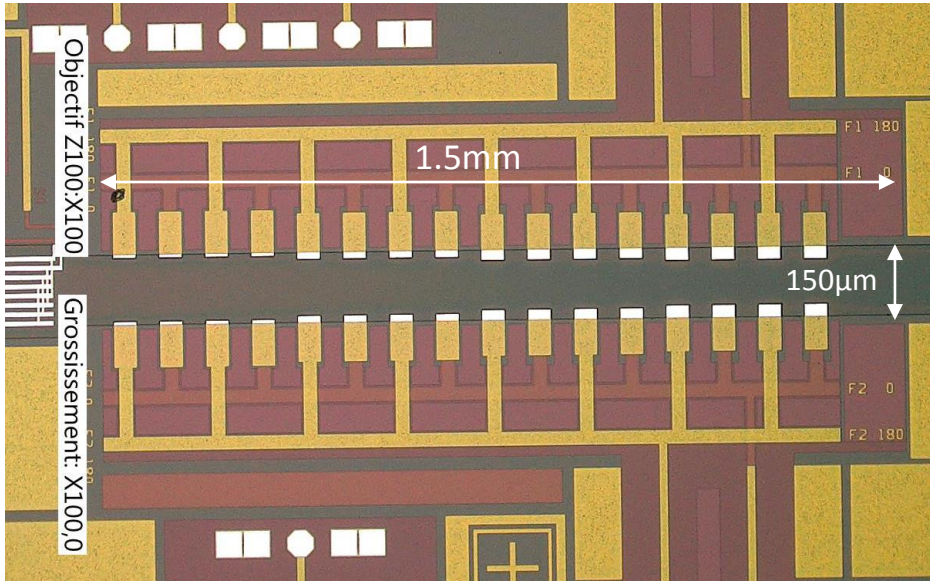
EM simulations predict a suitable E field gradient distributed along the channel section

Combined hydro-fluidic & ElectroMagnetic DEP cell manipulation

First prototypes based on IHP technology with PDMS cap for microfluidic



~ 9µm thick electrodes
embedded in the µfluidic channel

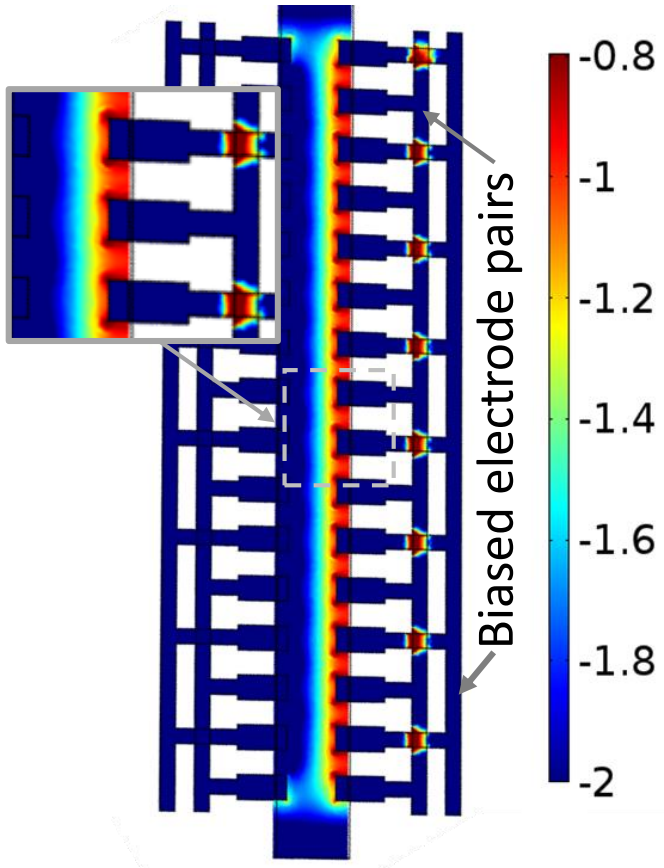


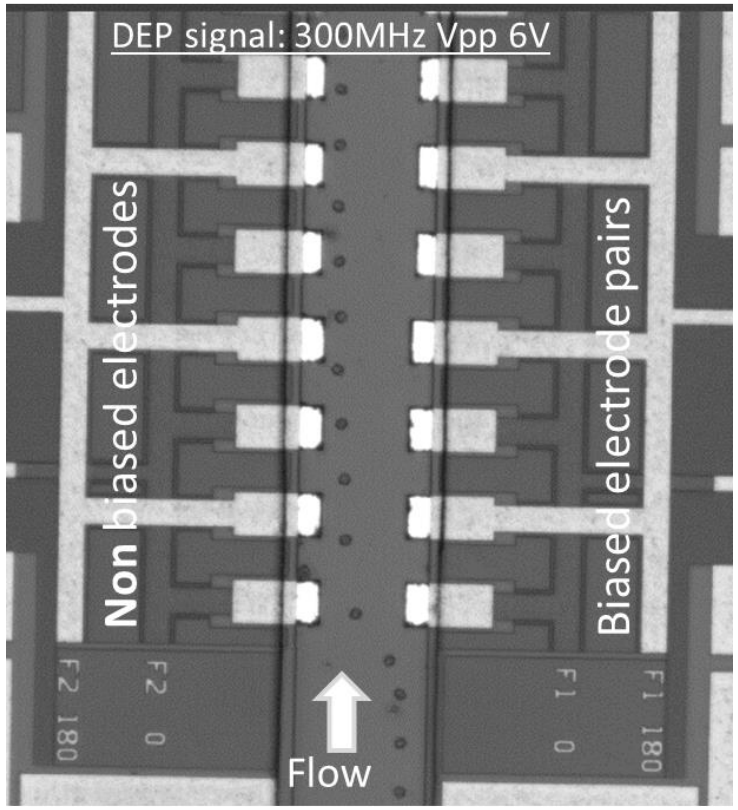
Fabricated in IHP clean room

First trials with flowing cells

Effect of applied DEP E field on particle trajectory

High efficiency on low cell velocity motion (< 0.1mm/s)



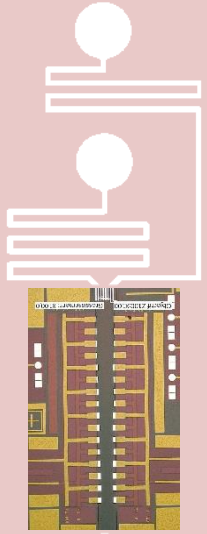


Stacked image of cell trajectory deviation

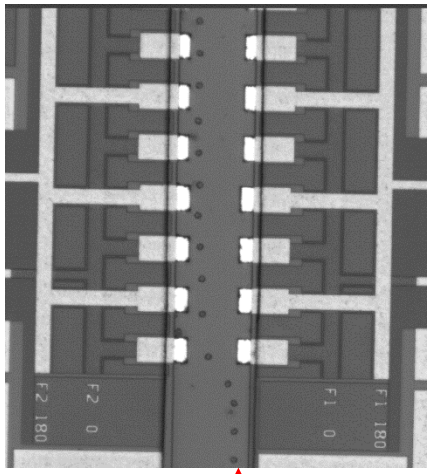
Particle is quickly repealed far the high intensity E field region to finally travel in low intensity one

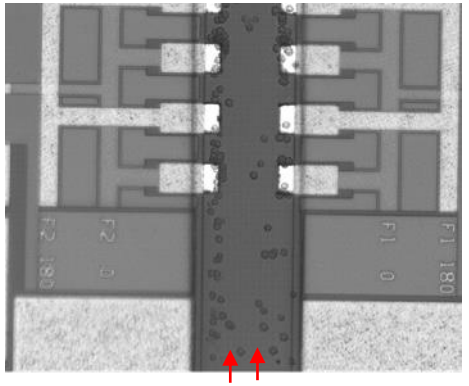
Optimization of cell injection inside the cytometer

Microfluidic channel - PDMS

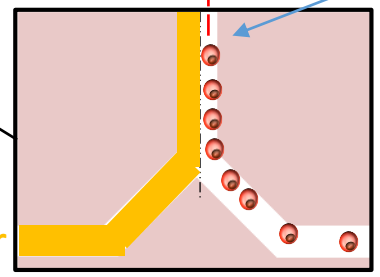


New micro channel design to ensure optimal cell location thanks to natural cell flow focusing



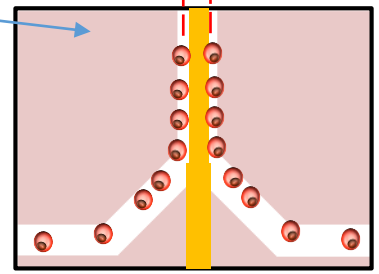


Cell well focused on the channel edge



Buffer

Cell suspension

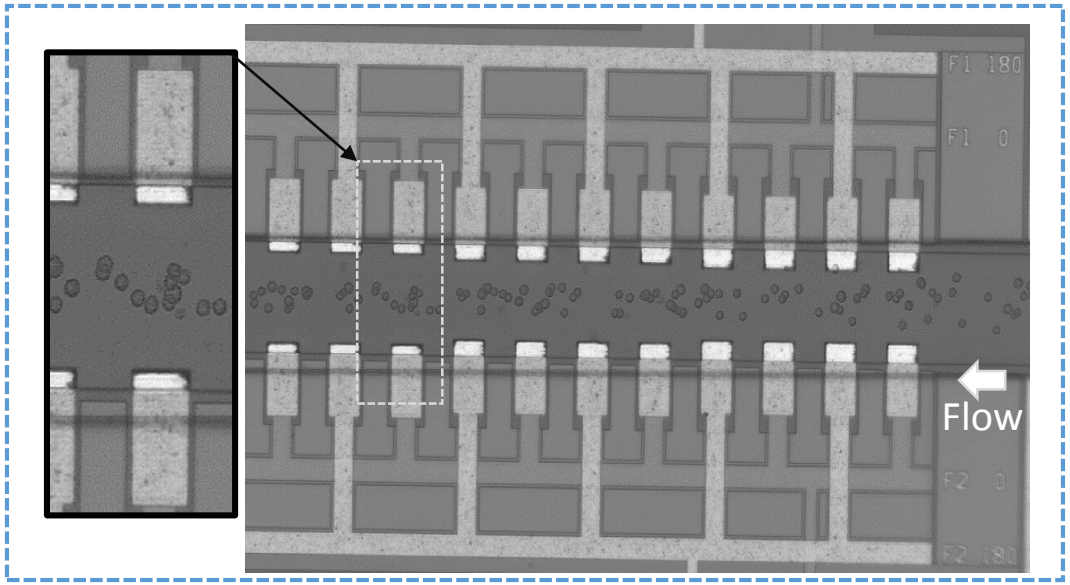


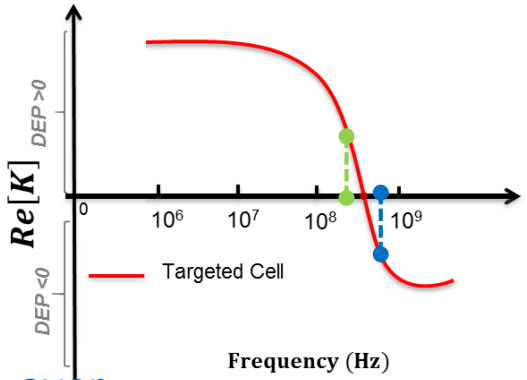
Cell suspension

Buffer

Cell suspension

Preliminary Experiments on LN18 cells (standard medium)



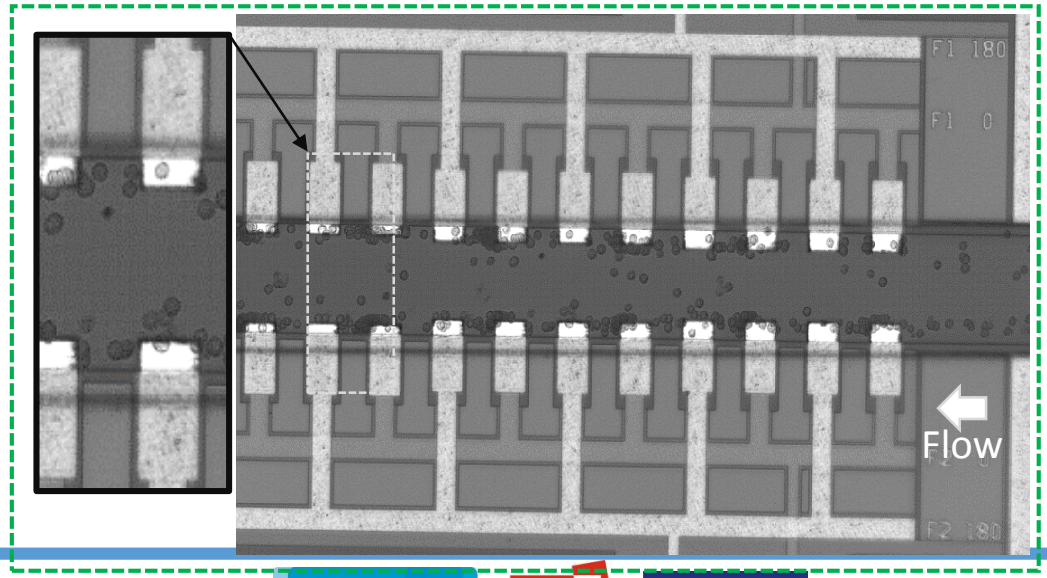


F_{DEP}> Fcross-over

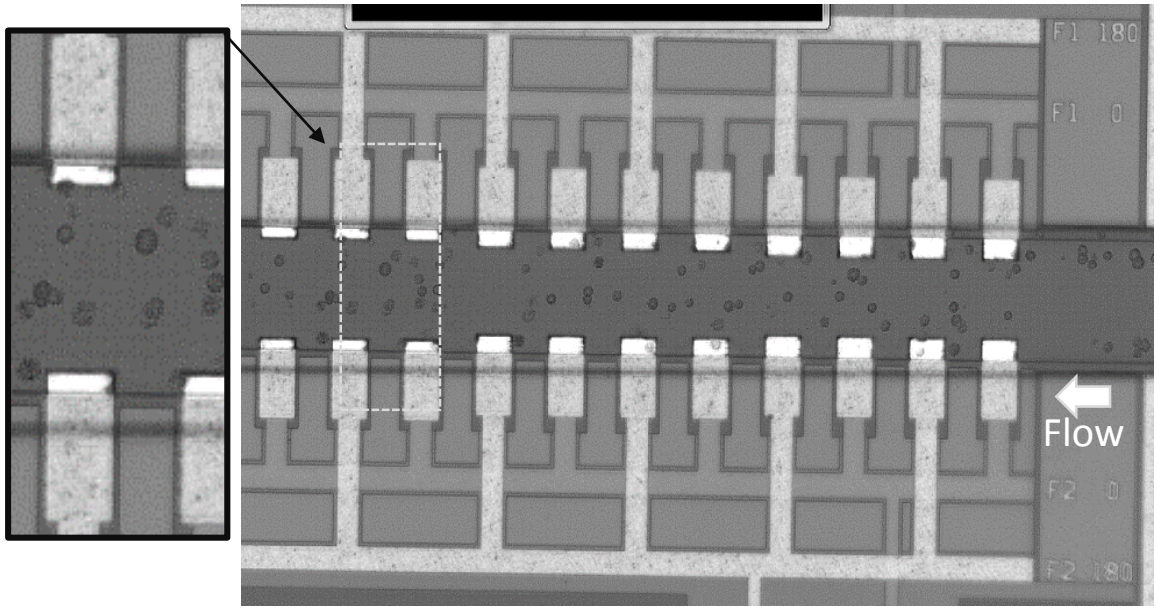
As expected cells are concentrated in microchannel center where E field intensity should be the lowest

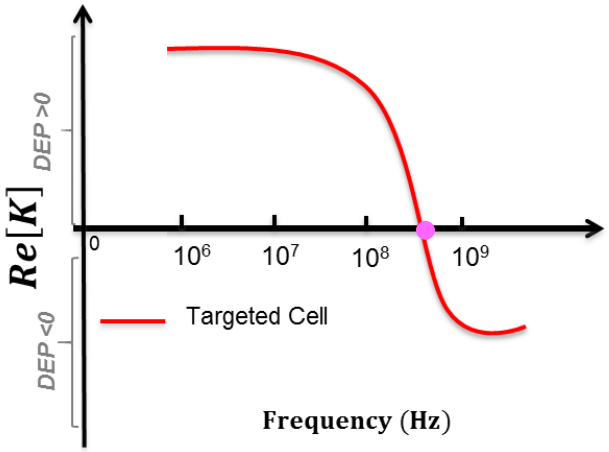
F_{DEP}< Fcross-over

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



Preliminary Experiments on LN18 cells (standard medium)

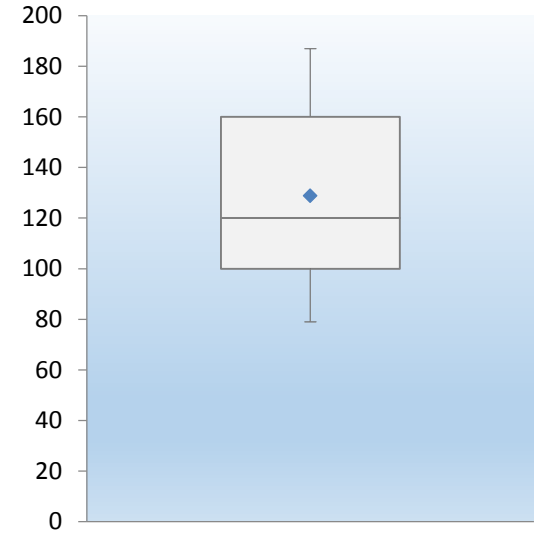




F_{DEP}~ Fcross-over (median value for characterized population)

Cell spatial distribution is much disperse (repealed in the center / attracted to the channel edge)

Mainly caused by cell line heterogeneity to whom some potential CSC presence



Ln18 Standard medium

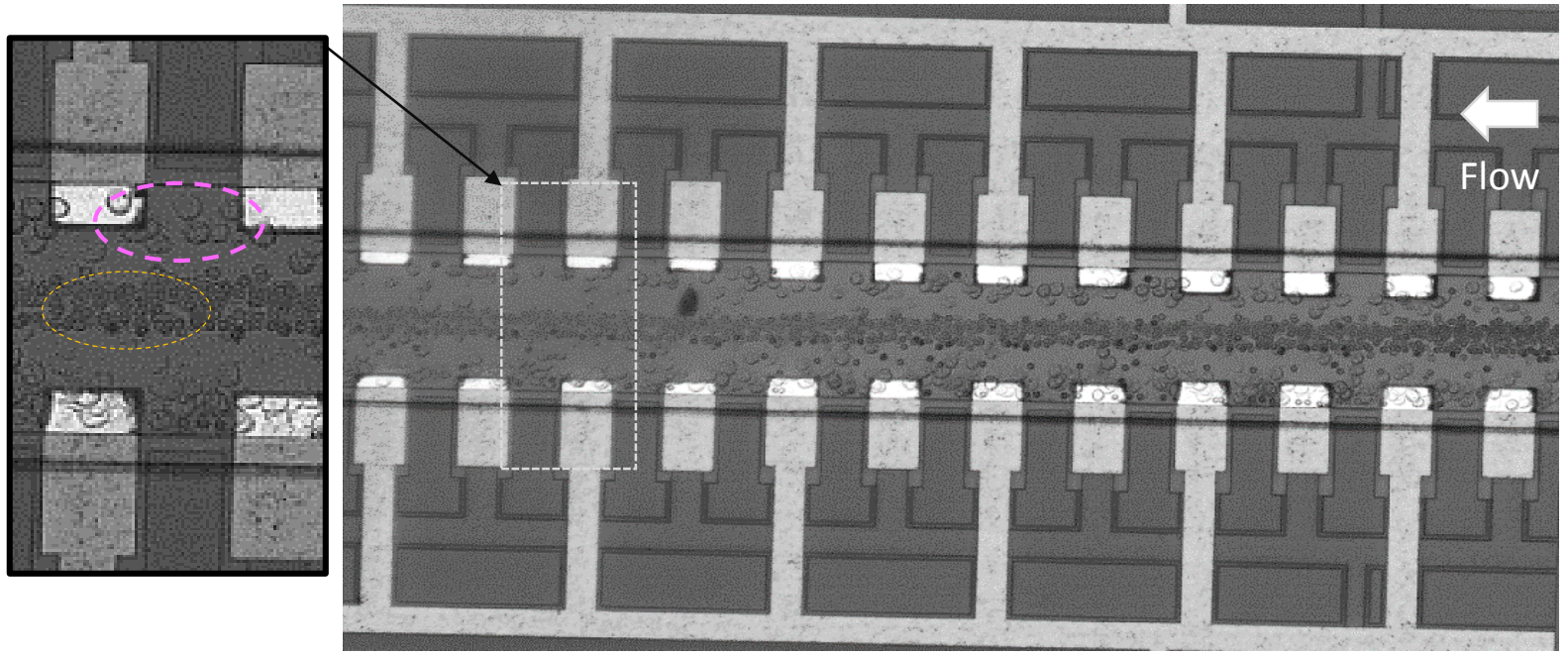
Particle sorting capability

Evaluation of sensor sorting capability to separate different type of particles

With a 50MHz DEP signal:
- all polystyrene beads react in Neg DEP
- most of LN18 cells react in Pos DEP
- very few cells seem not be deviated (dead or damaged cells?)

LN18 Cells

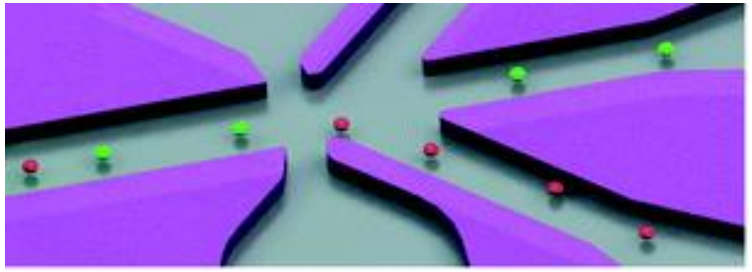
7µm polystyrene beads



Stacked image of LN18 cells and polystyrene beads with a 50MHz DEP signal

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

Conclusion



- Sorting principle validated
- Cell injection condition and suitable cell speed still under optimization
- Future tests will imply new high speed particle tracking monitoring system
- Future Implementation of fluorescence imaging capability to follow labelled cells with die
- Sorting of cell mixtures according to their crossover frequencies

Acknowledgments

We would like to thank ours collaborators and especially SUMCASTEC project partners (FET OPEN Research and innovation project) grant agreement N° 737164.