A finite element method approach to a microfluidic impedance cytometer design

Gabriella Marcucci
Supervisor Prof. Federica Caselli
Engineering Sciences
University of Rome Tor Vergata

Purpose and sources

The following work has been focused on two points:

1) state of the art of lab-on-chip devices with references to:

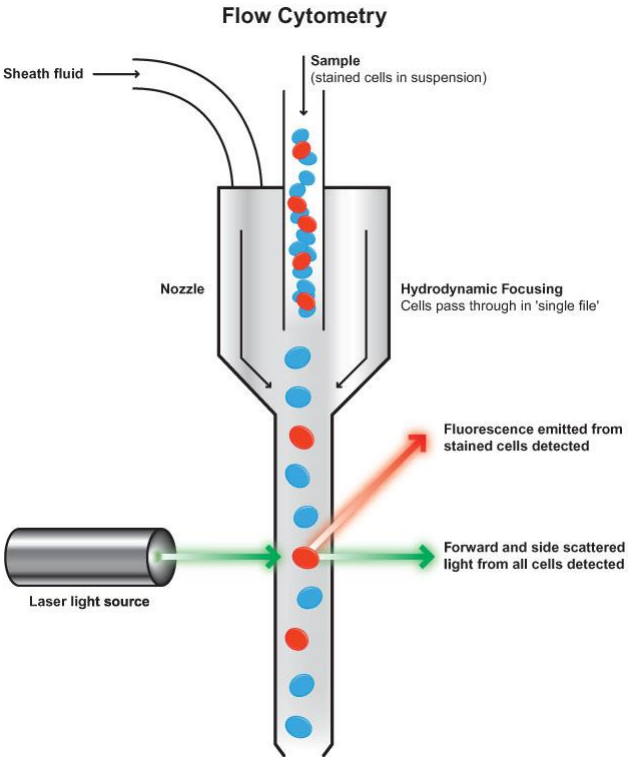
- K. Cheung, S. Gawad, and P. Renaud, "Impedance spectroscopy flow cytometry: On-chip label-free cell differentiation," 2005;
- T. Sun and H. Morgan, "Single-cell microfluidic impedance cytometry: A review," *Microfluidics Nanofluidics*, vol. 8, Apr. 2010;
- S. Gawad, K. Cheung, U. Seger, A. Bertsch, and P. Renaud, "Dielectric spectroscopy in a micromachined flow cytometer: Theoretical and practical considerations," *Lab Chip*, vol. 4, 2004.

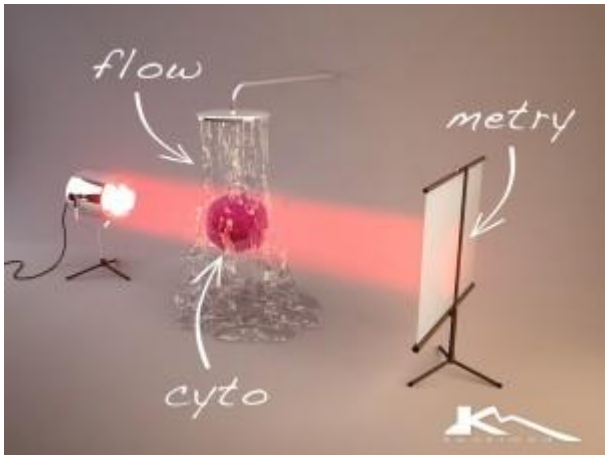
2) development of a toolbox of numerical simulation with the FEM in order to study already existing technologies and design new arrangements to improve the performances.

Flow cytometry

It is an **optical technique** which allows the analysis of the cells of a suspension in a buffer solution; in particular:

- counting;
- identification;
- sorting;
- detection of cell cycle progression and possible blood disorders.

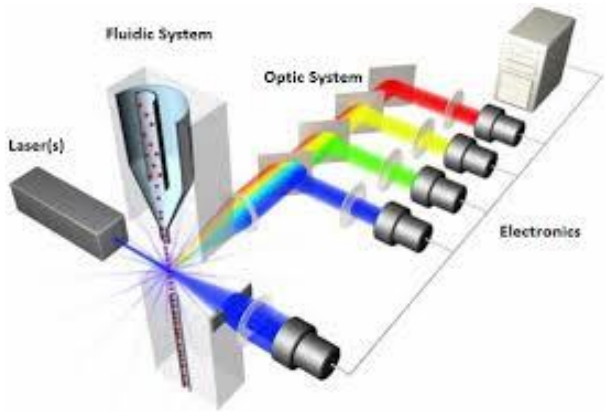




Strengths and weaknesses

Advantages:

- high throughput;
- low analysis times;
- detection of rare events;
- acquisition of several parameters per cell.



Disadvantages:

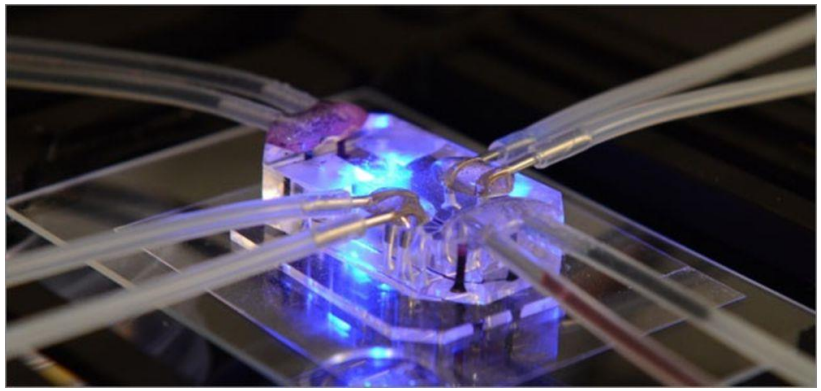
- costly;
- huge bulky size;
- vast mechanical complexity;
- need for highly trained personnel;
- need for labeling.

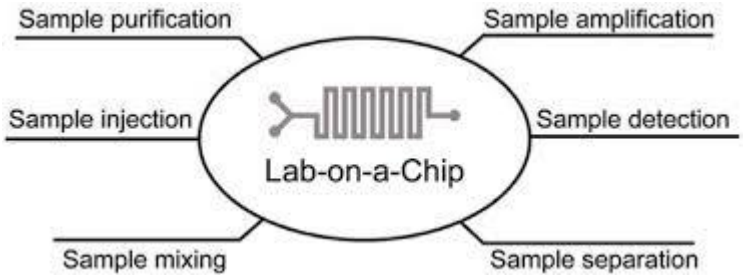
MEMS and lab-on-chip (LOC)

More accessible microfluidics-based flow cytometers are now being designed, with important implications in both immunological and hematological diagnosis fields.

They are part of a subset of the so called **MEMS** and they are based on the concept of **lab-on-chip** which integrates one or several laboratory functions on a single chip to achieve:

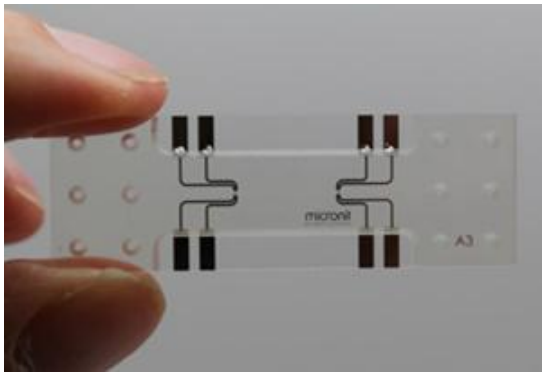
- automation;
- high-throughput screening when handling extremely small fluid volumes (sheat flows occur on length scale of the order of µl-fl).





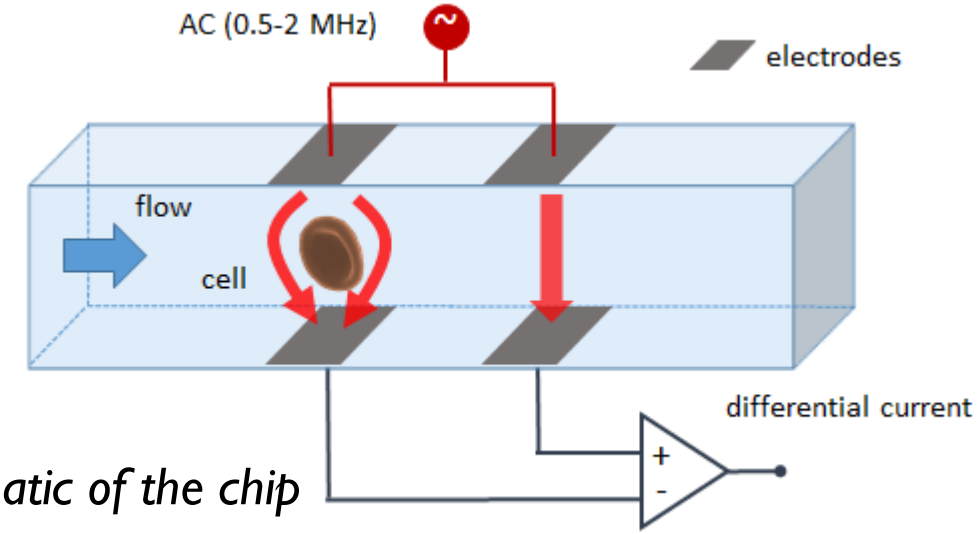
Microfluidic impedance cytometer

Cells are dispersed in an **electrolyte** such as phosphate-buffered saline solution (PBS) and are forced through a microfluidic channel with two pairs of planar electrodes patterned at the top and bottom.



An AC voltage is applied to the upper electrodes and the current change upon passage of a particle is measured differentially between the two lower electrodes.

The core of the cytometer is a chip which can be schematically represented as a channel with integrated **electrodes**.

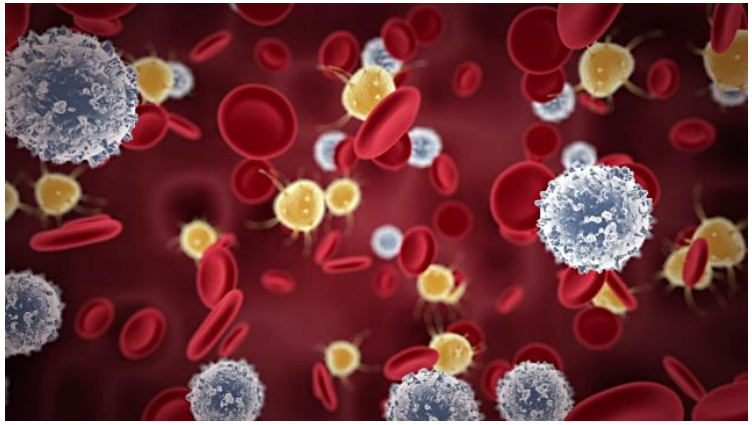


Schematic of the chip

Strengths and weaknesses

Advantages:

- complete miniaturization on a single device;
- good integration with other analysis methods (i.e. optical detection);
- multiple parameter analysis;
- label free;
- fast throughput (~1000 cells/s);
- hand-handling and cheap.



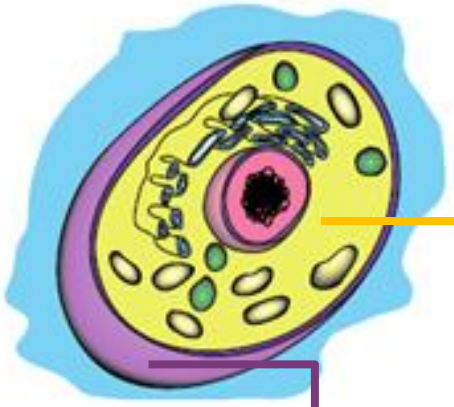
Disadvantages:

- good analysis for erythrocytes and plasters, but some limitations for the differential leukocyte count (an optical method is generally chosen in this case).

Cell analysis

Which is the link between the registered signal and the properties of the cell?

The cell consists of a **cytoplasm** layer covered by a plasmatic **membrane** made of insulating lipids which oppose a resistance to the current passage so that a variation in the flow of current (**spike**) and thus in the conductance will be registered. The cytoplasm typically contains a high concentration of ions (conducting).



cytoplasm

- high concentration of ions
- conducting (σ)

membrane

- double lipidic layer
- insulating

Cell discrimination

- It is possible to represent both the **membrane** and the **surrounding environment** as a capacitor and a resistor in parallel and the **cytoplasm** as a single resistor.
- Using Electrochemical Impedance Spectroscopy (EIS), the frequency response of the cell can be measured and fitted to the circuit model.

A) If a bigger cell is passing through the channel, the conductance will be bigger and the MEMS will measure the **electrical impulse** (proportional to the dimensions of the cell).

B) If the cell is insulating, then it will show a higher impedance with respect to a conducting particle, so that also the measured voltage will be bigger.

As a consequence, different signals can be registered according to either **geometrical** or **dielectric properties** of the cells, thus allowing a discrimination among them.

The impedance data for frequencies lower than 100 kHz, between 100 kHz–1 MHz, 2–5 MHz and 10–100 MHz reflect the electrical double layer, cellular size, membrane capacitance and cytoplasm resistance, respectively.

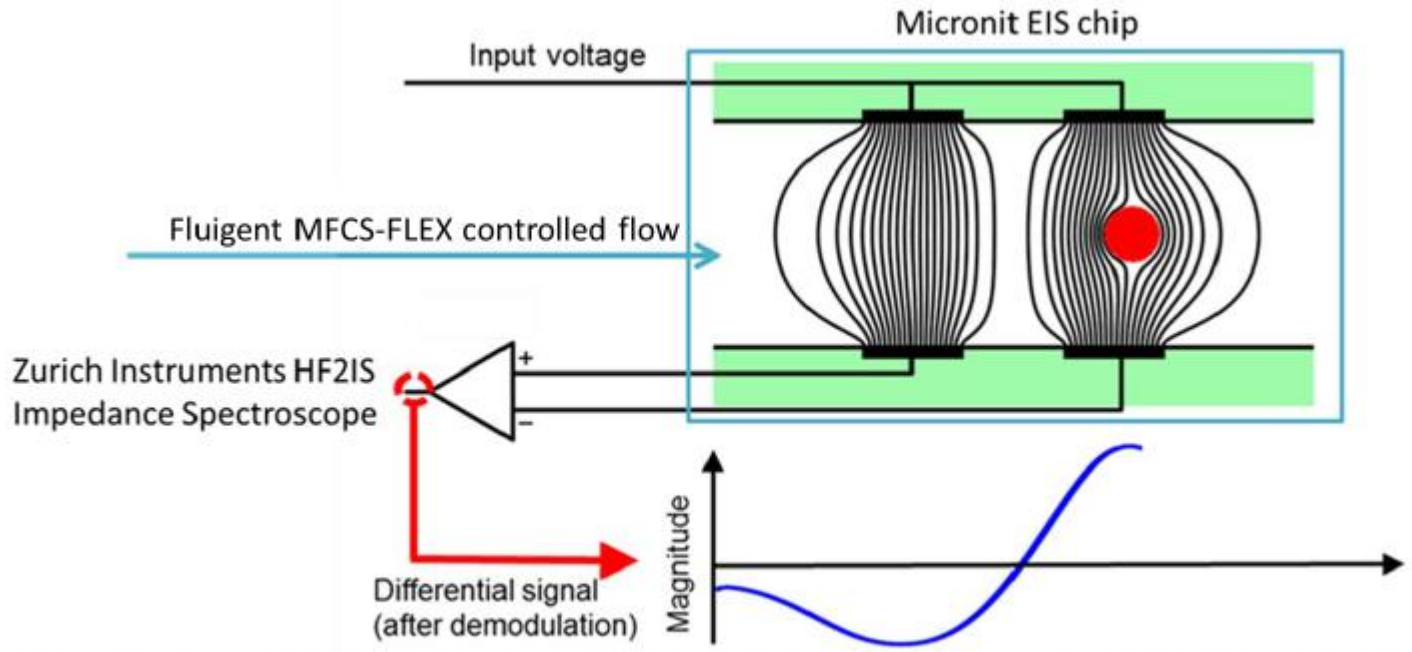


Figure 4: Schematic of cell detection within an EIS-chip. Based on image courtesy of Zurich Instruments

Simulations: tools

The following *in-silico* simulations have been obtained by using the ACDC Module provided by **COMSOL Multiphysics 5.2** software and adopting quadratic Lagrangian tetrahedral elements for the FEM modelling.

Finite Element Method (FEM) allows the user to overcome typical limitations of other techniques:

- Coulter counter models fail to account for the frequency dependence of the measured impedance;
- Mixture equations are based on a complex dielectric cell model and account only for homogeneous electric fields of given frequency.

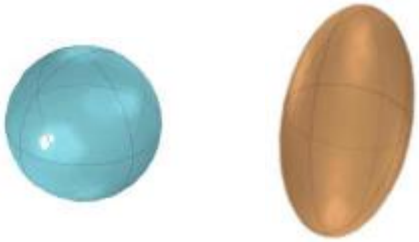
COMSOL

Advantages of FEM:

- determines the theoretical cell-induced impedance spectrum change in a non-homogeneous electric field;
- predicts the sensitivity of a modeled microfabricated flow cytometer;
- underlines the relative influence of several cell parameters.

Assumptions:

- the electrodes are made of platinum (σ=8.9e6, εr=0);
- the fluid is a phosphate-buffered saline solution (PBS);
- the cell is made of polystyrene (almost insulating);
- the working frequency is equal to 2MHz;
- the applied voltage is equal to 1V.



Geometries:

- sphere and prolate ellipsoid (aspect ratio of 2) are considered; dimensions are compatible with real cells case.

Simulations: equations

The cytometer is modeled as the union of two homogenous conducting regions Ω1 (the cytoplasm) and Ω2 (the fluid).

In the **Fourier domain**, the problem of determining the electric potential *v* in the cytometer is solved with the use of the Electric Currents Interface of the ACDC Module (current conservation, insulating boundary condition, continuity of the current flux density) and it is stated as:

$$-\mathrm{div}(\sigma * \nabla v) = 0 \qquad \text{in } \Omega 1 \cup \Omega$$

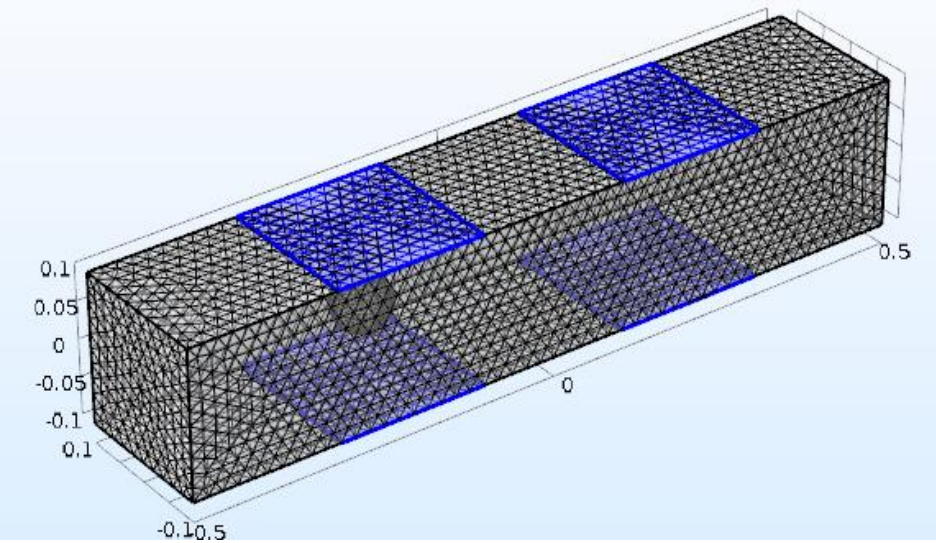
$$[[\ \sigma * \nabla v \cdot n]] = 0 \qquad \text{on } \Gamma$$

$$Y\ [[v]] = \sigma * \nabla v \cdot n \qquad \text{on } \Gamma$$

where Γ is thetwo-dimensional interface of the cell and n is the normal unit vector to Γ pointing into Ω2

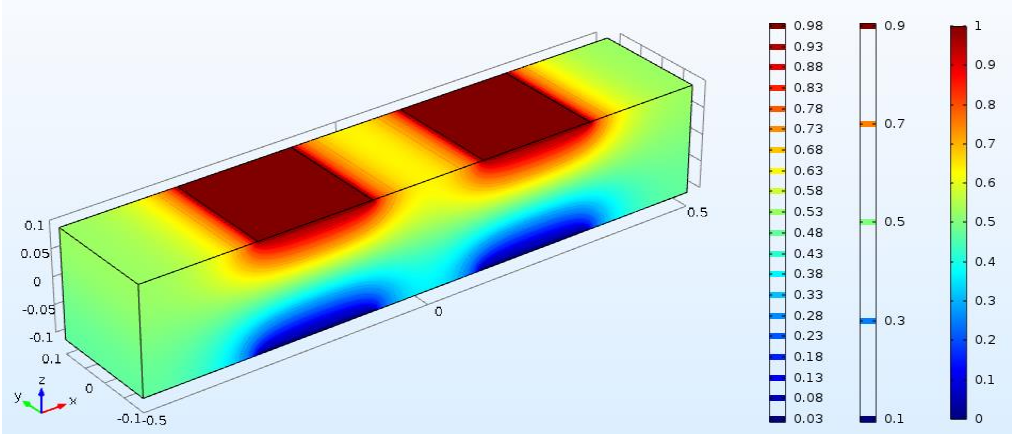
Simulations: configurations

1) Two electrodes on upper and lower surface of the chip

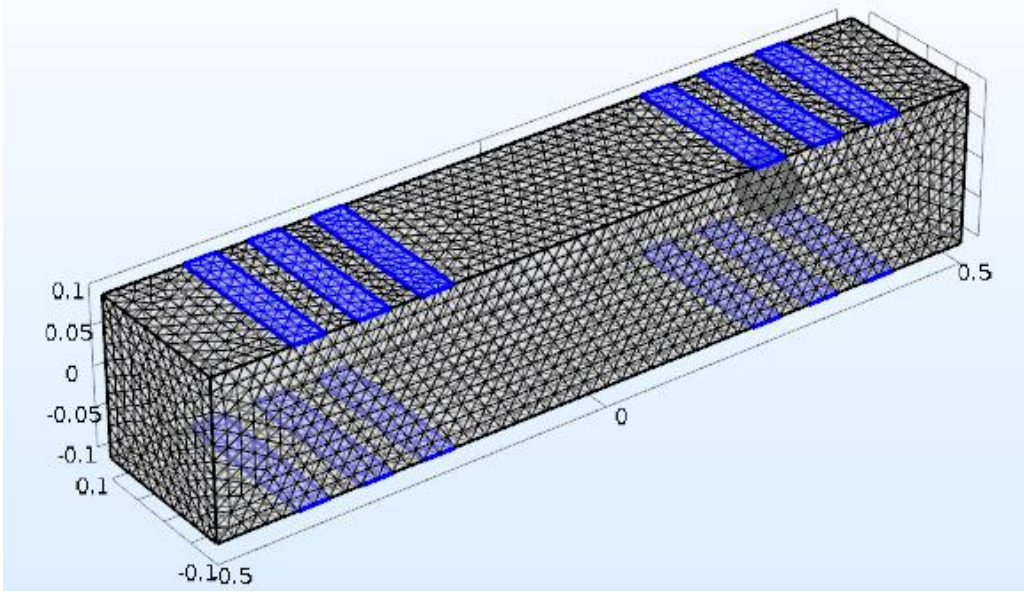


a - This is the traditional configuration; the electrodes have an area of 20µm^2; the cytometer has a length of 100µm; the sphere has radius R=0.35 µm (1:10 for erythrocytes).

b - The parallel electrodes must be perfectly aligned in order not to have disturbances in the signal registration.

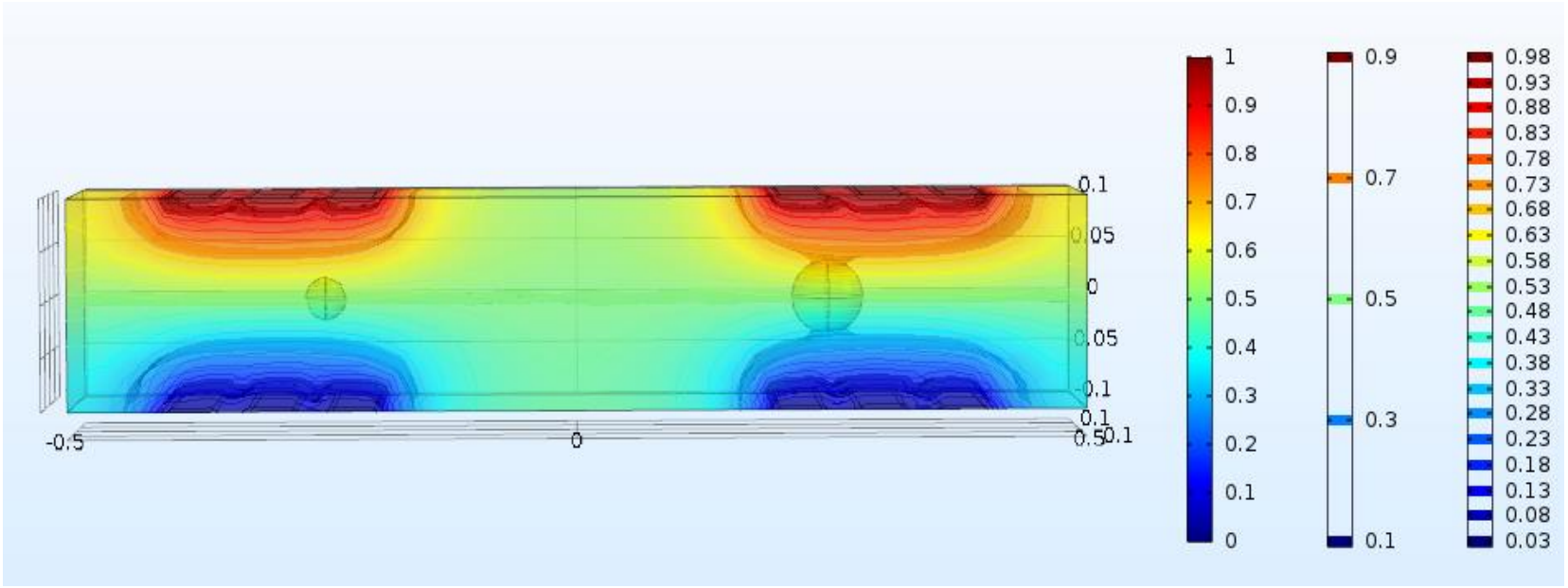


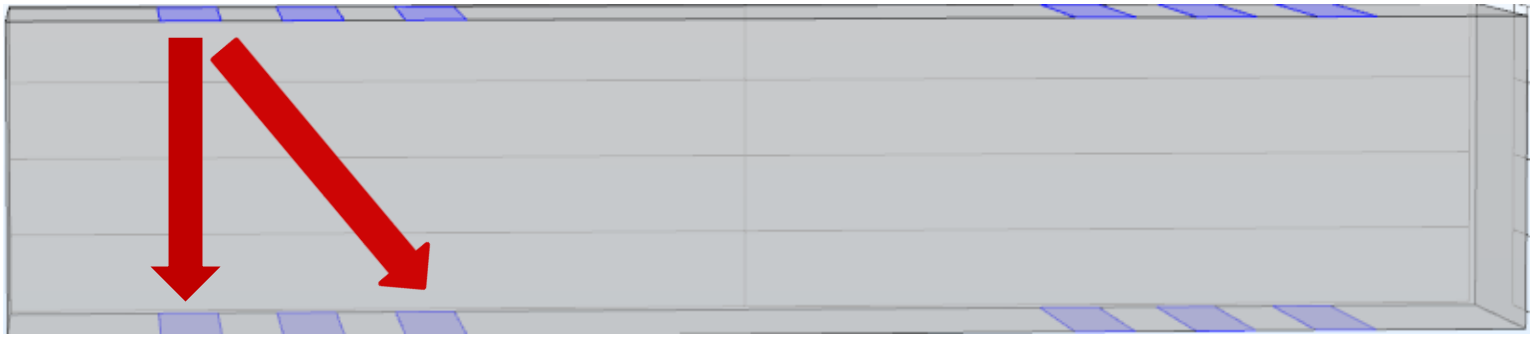
2) Two couples of three electrodes on upper and lower surface of the chip



a -The electrodes are put in evidence in **blue**.

b - Comparison between the case of a sphere of radius R and a cell of radius 2*R.

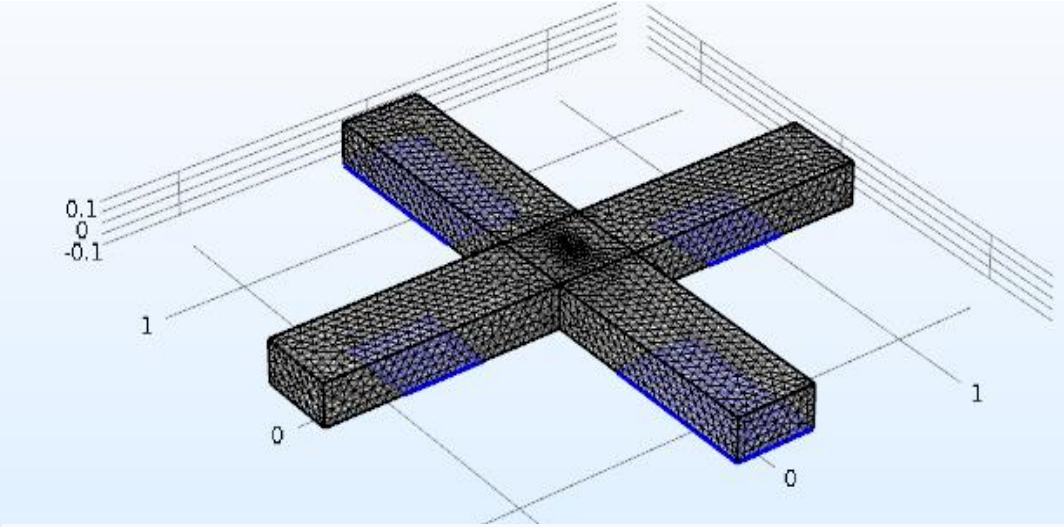




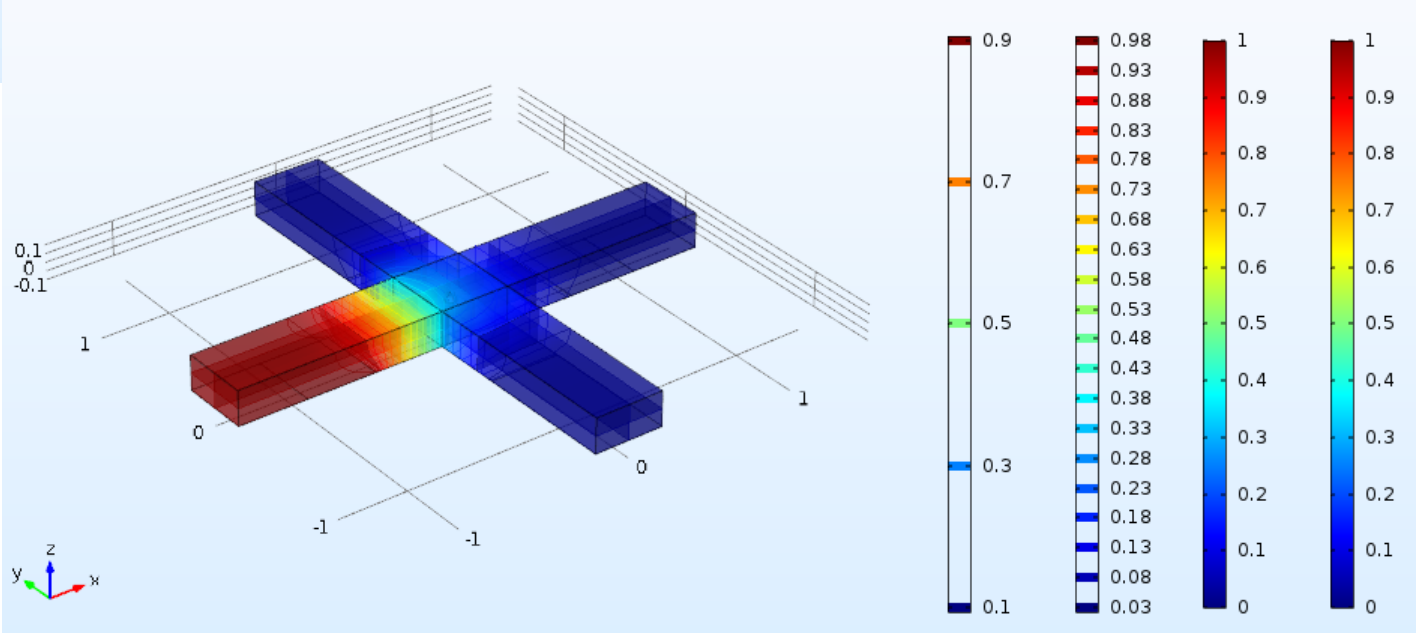
The electrodes of the first configuration are splitted into three smaller electrodes: this allows us to analyse current paths in different spatial orientations as shown by the **arrows**.

In both cases the electrodes are integrated in both upper and lower surface, while the following configurations (cross-shaped and H-shaped chips) will offer a coplanar design, with all the electrodes on the bottom surface, and both vertical and horizontal paths will be taken into account.

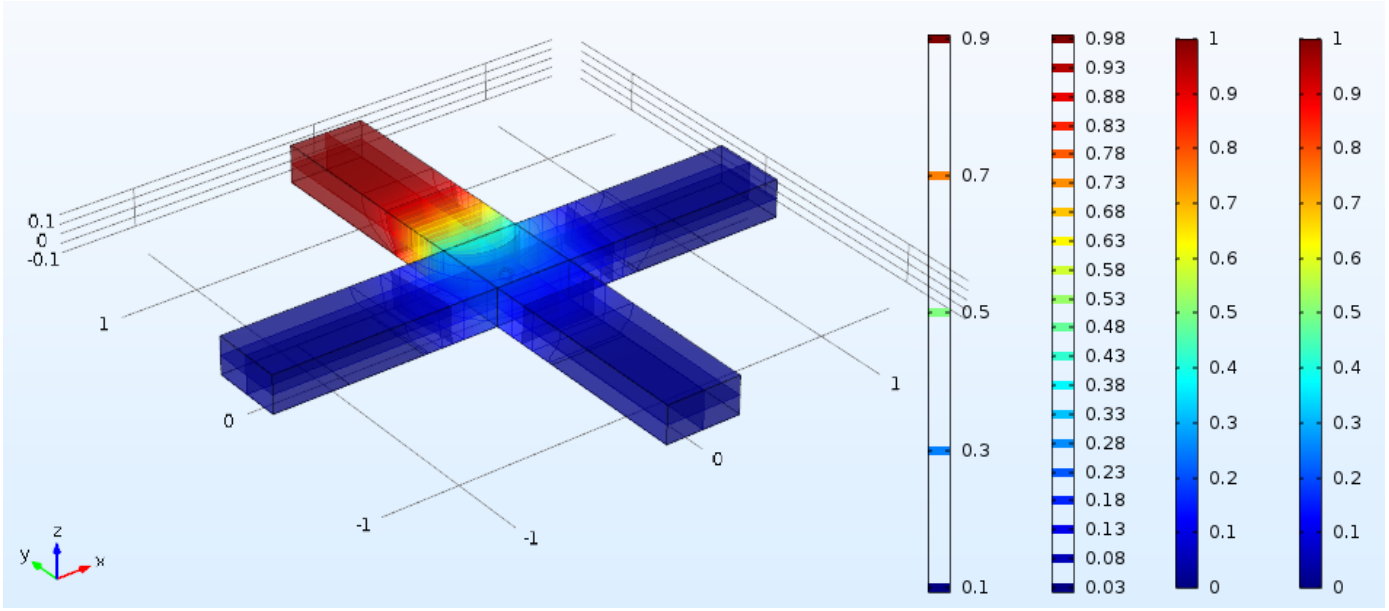
3) Cross-shaped chip with four electrodes



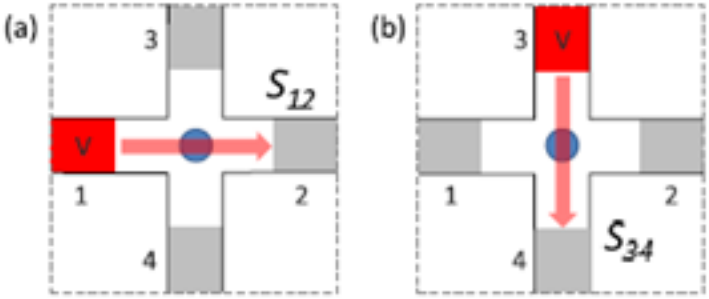
a - Both the main and the lateral channel are 40 µm wide, 20 µm high and 280 µm long; the **electrodes** in the main channel are 40 µm long and 120 µm apart, the ones in the lateral channel are 80 µm long.



b - *Longitudinal applied signal* S12

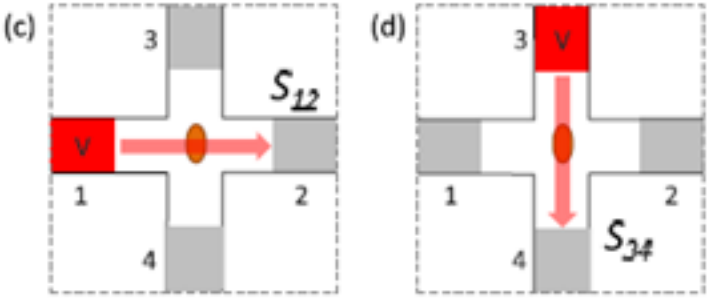


c - *Transverse applied signal* S34

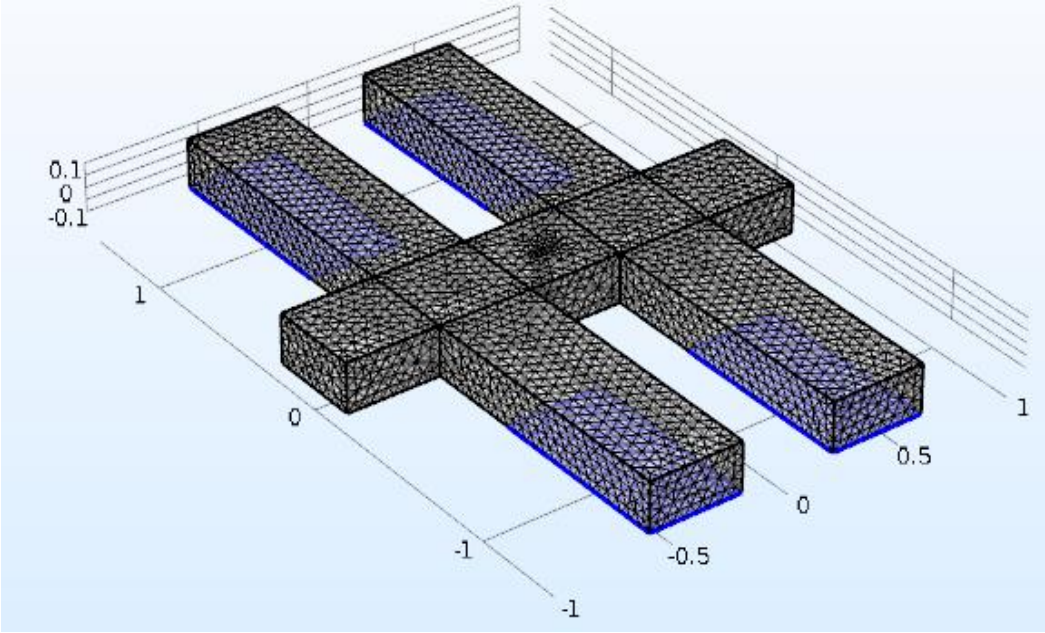


S12 and S34 are coincident in the centre of the cross for isotropic objects (sphere); they are different for anisotropic objects (prolate ellipsoid).

Anisotropy indices are independent from cell volume and rather insensitive to small imperfections in the focusing system.

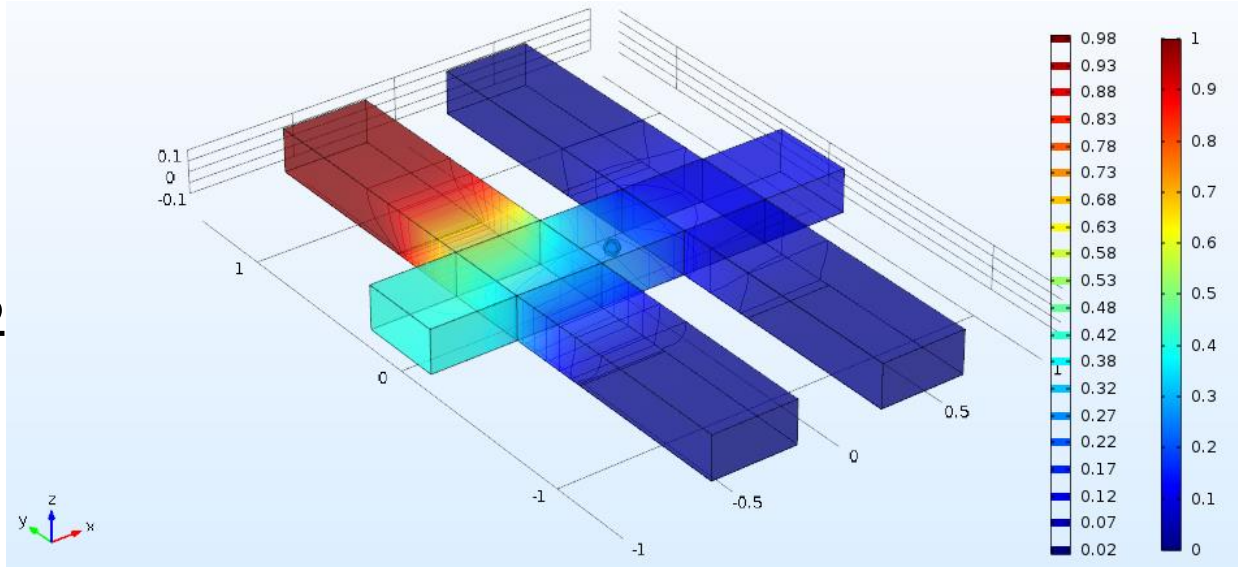


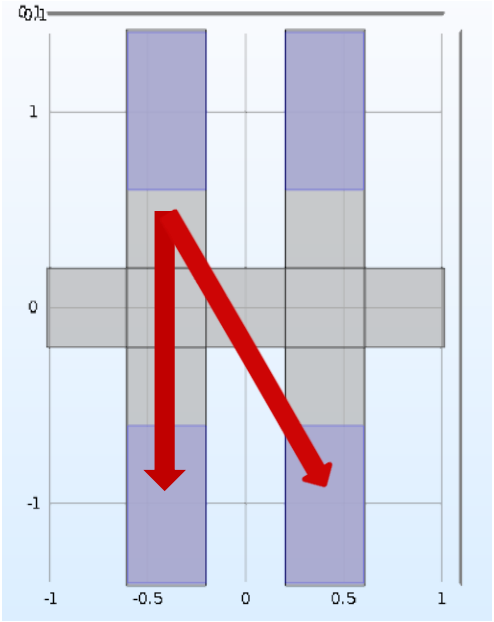
4) H-shaped chip with four electrodes



a - The main channel is 40 µm wide, 20 µm high and 200 µm long; the lateral channels, 40 µm apart from each other, are identical to the one of the cross-shaped chip.

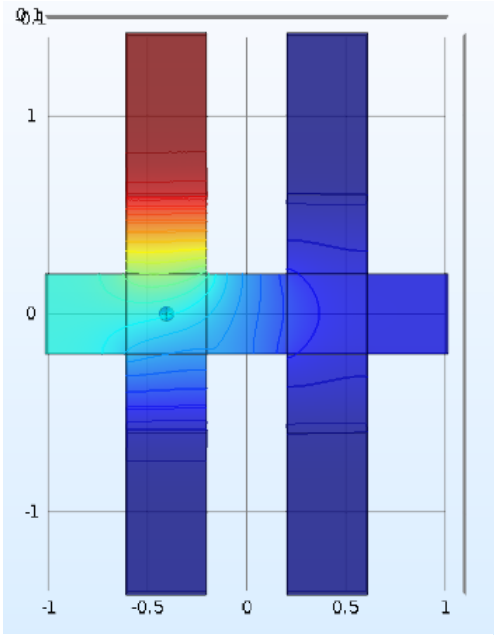
b - Voltage is applied to **electrode 1** and two signals are acquired: S12 (longitudinal) and S14 (transverse).

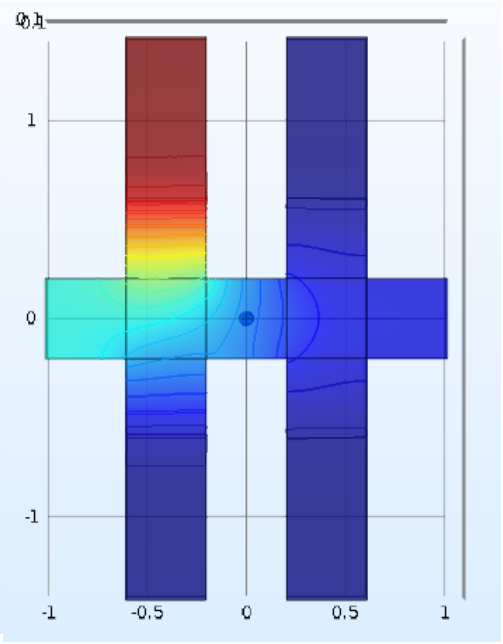


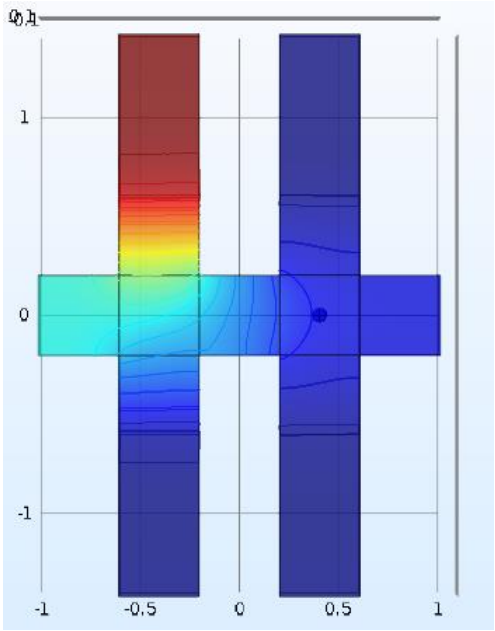


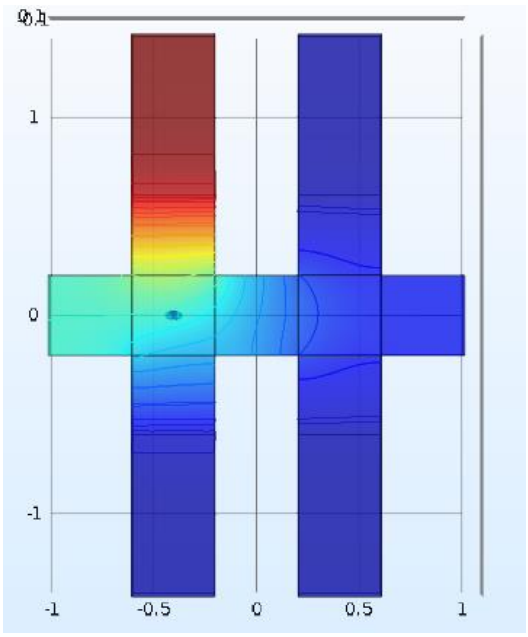
c - An H-shaped chip gives information about the transversal position of the cell in the channel provided that good wiring is present to establish the right current paths.

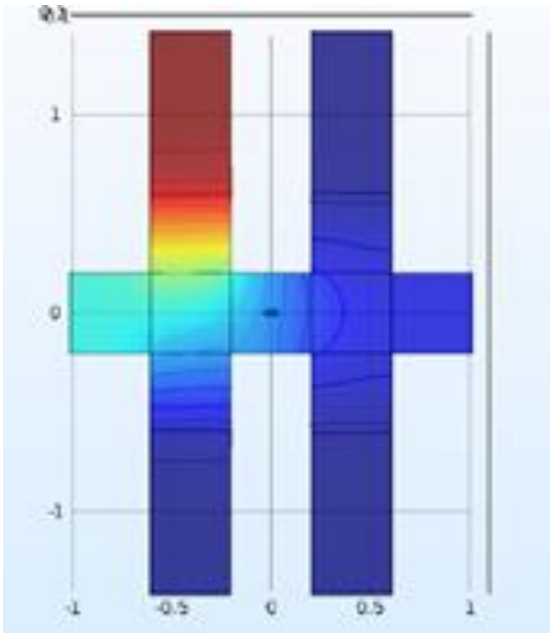
d - *Isotropic case (sphere)*
The two signals S12 and S14 are identical (the anisotropy index is equal to 1)

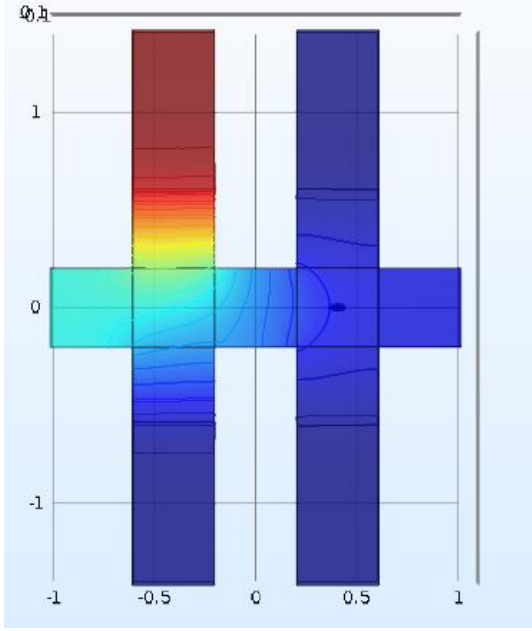












e - *Anisotropic case (prolate ellipsoid)*

The two signals S12 and S14 are different (AI is greater than 1); more precisely, it is given by the ratio of S12min to S14min, with the whole factor multiplied by **c** (chip-dependent calibration coefficient aimed at equalizing the two signals for spherical objects).

Conclusions

This work has been focused on the validation of a FEM approach to the study and optimization of microfluidic impedance cytometers and the design of novel devices with the aim of using them for an increasing number of applications.

Even though some limitations are still present in the available technology, the impedance cytometer is widely used in several fields:

- protein engineering;
- blood test;
- biomarker detection;
- quality control in food industry.

Thank you for your attention!