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

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***Abstract*—In this paper, a fully BiCMOS integrated microfluidic cell sorting platform for cell properties study is introduced. Operating in the radiofrequency range, the presented device combined Lab-on-Chip and IC technologies to achieve label free cytometry using in flow cell selective dielectrophoresis deviation. Taking benefit of CMOS stack and integration capability, the first prototypes demonstrate the ability to separate cells with different intracellular dielectric properties. These results pave the way to new innovative high throughput integrated IC solutions for detection of rare cells in the frame of cancer researches and treatment**

***Index Terms*— High Frequency Dielectrophoresis, Glioblastoma, BiCMOS Chip, Biological cell manipulation and analysis.**

I. Introduction

Brain cancers are among the most difficult tumors to treat and remain a leading cause of death in US and Europe with 23,800 new cases and 16,700 deaths in 2017 in US estimated by the American Cancer Society, and 30,700 new cases and 24,500 deaths in Europe in 2012 [1]. Specifically, GBM (GlioBlastoma Multiform) is among the most common and deadliest malignant primary brain tumors in adults with respectively a median overall survival of about 15 months and 8 months for newly diagnosed and recurrent disease. GBM is actually classified as a Grade IV (most serious) astrocytoma and mostly develops itself from astrocytes cells that support nerve cells and can only be treated combining surgery, when a resection is possible, chemotherapies and radiation therapy. With a very low 5-Year Relative Survival Rate (less than 9% and 5% for 45-54 and 55-64 years old patient), the efficiency of available treatments is unfortunately low. Indeed, numerous tumor recurrence are attributed to subpopulation of malignant cells suspected to present strong therapeutic resistance and high aggressiveness [2]; leading to tumor survival, self-renewal and progression.

The large cell heterogeneity, occurring in GBM tumors, makes identification of such treatment resistant cells very complex. In addition, conventional cell analysis and sorting techniques based on antigen labelling appears to be inefficient, lacking for known real marker associated to cell aggressiveness.

For such purpose, development of alternative analysis methods and new cell sorting techniques is crucial, in particular label-free ones taking advantage of cell physical specificities. Dielectrophoresis (DEP) approaches are among them and have already demonstrated their ability to sort cells of different types [3]. Indeed, conventional DEP techniques typically work in the 10 kHz to 1 MHz range [4] mainly addressing cell plasma membrane properties or specificities (i.e. cell size, membrane polarizability…). On the other hand, there would be a great interest to extend such electromanipulation based analysis techniques to UHF frequencies, for which intracellular specificities can be now investigated [5-6] and difference between cell contents also exploited. This is the purpose of the work introduced in this paper to develop and experiment new types of cell sorter based on intracellular dielectric property difference with the objective to find target cells, as especially high aggressiveness cancer cells, and isolate them from heterogeneous populations.

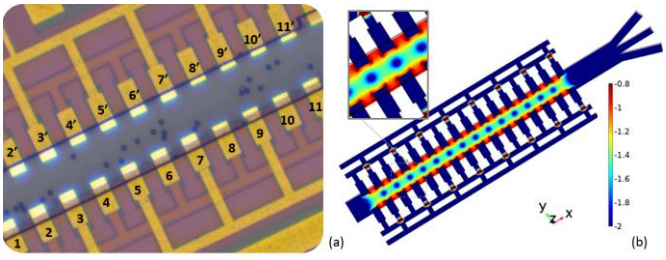

Fig. 1. Photograph of the proposed HF dielectrophoresis cytometer implemented on BiCMOS chip (a) and plot of the normalized electric field (log scale) once both side electrode pairs are biased with a 300MHz 3V magnitude signal.

II. Principle & Cytometer Design

The proposed biological cell-sorter microchip is shown in Fig 1.a. Cells are suspended in a survival medium and driven across the sensor through a microfluidic channel in which an array of 16 electrodes is implemented on both sides.

These electrodes are associated by pairs and biased accordingly in such way that odd-numbered electrodes are grounded whereas even-numbered ones are connected to a high frequency signal generator currently implemented outside the chip. As illustrated in Fig. 1.b, such configuration allows generating an electric field gradient inside the channel presenting high intensity areas close to the microchannel edges and very low intensity in the center of the channel.

When cells are flowing though this non-uniform electric field along the electrode array channel section, their trajectory is affected and modified due to lateral forces. Indeed, once polarizable particles are submitted to a non-uniform electric field, a dipole moment is engendered inducing a dielectrophoresis force F_{DEP} that can be strong enough to deflect particles. The dielectrophoresis theory basics [4] have demonstrated that, if we consider cells as a homogeneous spherical dielectric particles, the induced DEP force can be then computed using (1).

$$F_{DEP} = \left(4\pi r^3 \varepsilon_0 \, K(\omega) \, E(\omega,t).\nabla\right) E(\omega,t) \tag{1}$$

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

$$\varepsilon_a^* = \varepsilon_a - j\frac{\sigma_a}{\omega} \tag{3}$$

Where ε_0 is the vacuum permittivity, r is the particle radius, ω is the angular frequency of the applied electric field $E(\omega,t)$ and $K(\omega)$ the Claussius-Mossotti given by (2) in which ε_p^* and ε_m^* refer to the complex permittivity of the particle and the suspension medium, respectively.

In the present case, regarding the used electrode geometry, the dielectrophoresis theory [7] shows that we can assume that field induced phase changes have negligible effect. As a result (1) can be simplified in (4) as in most conventional DEP electrode system cases.

$$F_{DEP} = 2\pi\varepsilon_m r^3 \, \mathrm{Re}\left[K(\omega)\right] \, \nabla\left|E_{rms}\right|^2 \tag{4}$$

Where E_{rms} is the root mean square value of the electric field and *Re[K(ω)]* the real part of Claussius-Mossotti factor.

Hence, the sign of *Re[K(ω)]* defines the direction of the induced particle motion. When *Re[K(ω)]* is positive, the force is attractive and a particle in the fluidic channel moves toward the electrodes; and when *Re[K(ω)]*is negative, the particle is repealed away since the force is repulsive [7]. The sign of *Re[K(ω)]* depends on the difference of polarizability between the particle and its surrounding medium and the dissimilarity between both also depends on the E field frequency. It means that the choice of the frequency of the electrode array bias signal is determinant for achieving efficient particle deviation.

Generally, the complex permittivity of any material is given by (3) neglecting the dielectric loss contribution compared to ionic conduction, where ε and σ are conductivity and permittivity, respectively. Thus combining (2) & (3) results in (5) and illustrates how *Re[K(ω)]*can change sign as function of differences of conductivity and/or permittivity between the particle and its support medium. As a result, if a low conductivity support media is used, both positive & negative behavior can be obtained by adjusting the applied DEP signal frequency.

$$\mathrm{Re}\left[K(\omega)\right] = \left(\frac{\left(\varepsilon_p - \varepsilon_m\right)\left(\varepsilon_p + 2\varepsilon_m\right) + \frac{1}{\omega^2}\left(\sigma_p - \sigma_m\right)\left(\sigma_p + 2\sigma_m\right)}{\left(\varepsilon_p + 2\varepsilon_m\right)^2 + \frac{1}{\omega^2}\left(\sigma_p + 2\sigma_m\right)^2}\right) \tag{5}$$

As biological cells are considered as flowing particles, their intrinsic complex permittivity is strongly frequency dependent because of the influence of the thin plasma membrane that screens the dielectric contribution of intracellular content in low frequency regimes [8]. Whereas becoming transparent as the frequency increases above several tens of MHz, the membrane lets the electric field interact directly with the cell interior [4]. As a result, DEP forces generated at high frequency regimes may deviate differently some flowing cells (i.e. attract vs repeal or deviate more or less strongly) according to their own intracellular content dielectric specificities. Such specificities stem from their biological properties or physiological mechanisms linked to their nature, origin, differentiation state, pathological state, aggressiveness level [9]. It means that biasing an electrode array with appropriate high frequency signal, and especially using frequencies in the UHF domain as introduced in [5-6], it may be possible to selectively and efficiently sort cells that present more or less significant intracellular dielectric property difference.

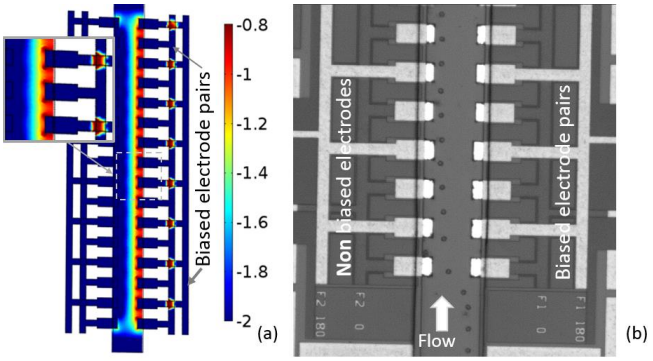

Fig. 2. Plot of the normalized electric field (log scale) once single side electrode pair's is biased with a 300MHz 3V magnitude signal (a), stacked images of a flowing single cell repealed by the E field gradient (b).

The proposed high Frequency DEP cytometer has been designed in this way. Based on COMSOL and ANSYS HFSS simulations, its electrode array geometry was optimized with the objective of having an E Field gradient (from high to low intensity region) gradually distributed within the microfluidic channel section (YZ plane) and homogenously distributed all along the channel length (along X axis, see Fig 1.b). Finally, optimal electrode width and step distance found were both 50µm. The

electrode thickness was set to 9µm in order to ensure a sufficient E field homogeneity and intensity in the channel thickness in order to act on cells that potentially flow at high altitude close to the upper limit of the microfluidic channel. In the end, a 1,5mm long, 190µm wide, and 35µm deep microchannel was implemented on these 16 electrode arrays.

To bias separately each electrode array, a microstrip line has been implemented on both sides of the cytometer with particular care for keeping a matched impedance to the point where electrodes penetrate inside the channel. Actually, impedance mismatch to the incident UHF biasing signal can result in highly reducing the electrode input voltage magnitude and consequently in a drop of E field intensity in the microchannel. Finally, all lines and electrodes were implemented on the back end of line (BEOL) stack of the SG25h4 BiCMOS process from IHP microelectronic.

Fig.2 illustrates how this cytometer works. When a cell flowing in the channel enters in the electrode array area, the present E field will deviate its trajectory accordingly to the biasing signal frequency. In the present case, this cell was submitted to a 300MHz CW signal that generates a negative DEP response. As a result, the particle is quickly repealed far the high intensity E field region to finally travel in low intensity one. Conversely, if a positive DEP signal had been applied, the cell would be attracted to high intensity regions and its trajectory kept close to biased electrodes.

III. Cytometer Tests: Materials And Methods

A. Cell preparation

Experiments were performed on LN18 cell line derived from malignant gliomas from adult patients, purchased from the ATCC. Cell pools were cultured at 37˚C in a humidified 5% CO2-95% air incubator in two conditions: (i) normal growing conditions in DMEM plus Glutamax complete medium (ii) stringent conditions in selective DMEM/F12 medium (Defined medium where main grow factors are removed). Actually, with such stringent conditions, mainly highest resistance cells with strong aggressiveness special features survive and can grow.

Finally, cells were resuspended in osmotic medium composed of water and sucrose titrated to a conductivity range of 20-22 mS/m, an osmolarity of 320 mOs/L and a pH of 7.4.

B. DEP properties Cell characterization

In order to find the appropriate DEP signal frequency to apply to the cytometer, both LN18 cell populations cultured with standard and Defined medium have been firstly characterized using same methodology introduced in [6]. The results of the characterization campaign led on more than 140 individual LN18 cells are discussed in [10]. Hence, the measured second cross-over frequencies (i.e. transition frequency between negative and positive DEP) showed really distinct values between both LN18 cell types. Especially the established difference between cross-over frequency median values (respectively 120MHz vs 62MHz for cells cultured in normal vs Defined medium) is relevant to exploit for achieving aggressive cell identification and separation from the others.

C. Cell sorting

Fig. 3 presents experiments done with LN18 cells grown in standard medium.

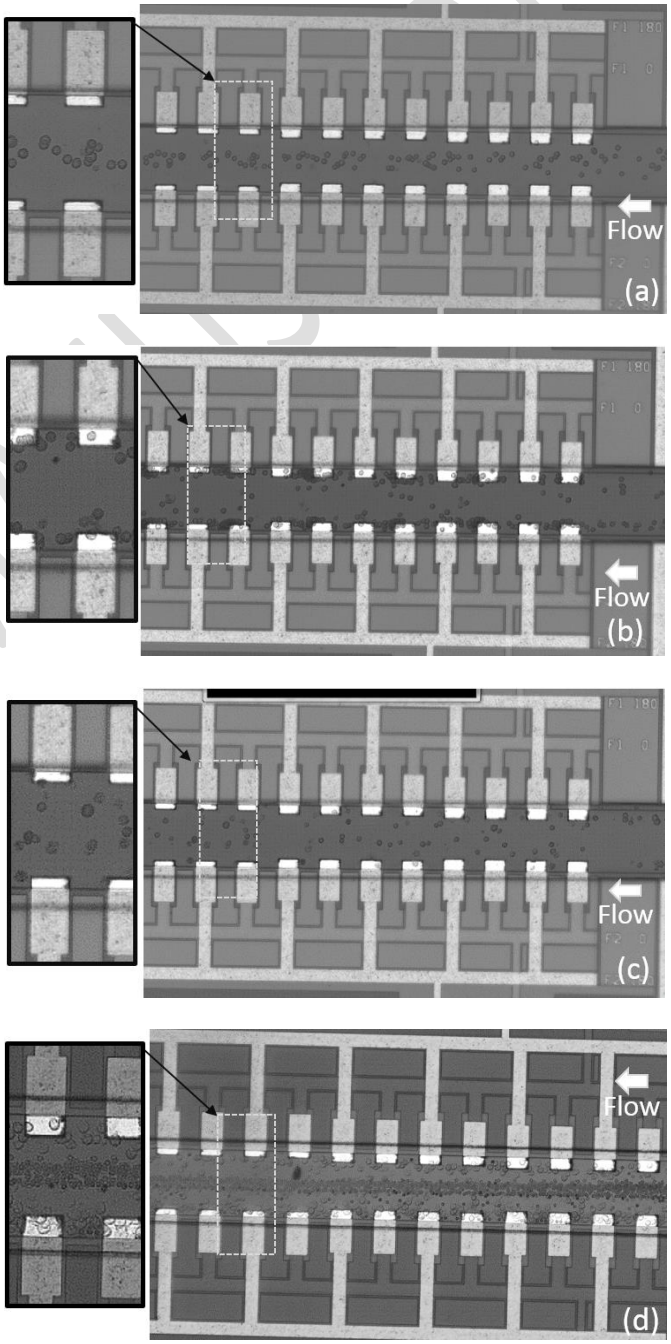

Fig. 3. Stacked images showing LN18 cell spatial distribution after flowing though the cytometer biased with a frequency higher (a), lower (b) and slightly lower (c) than the cross-over frequency median. Effective separation of a mixture of LN18 cells and polystyrene beads with a 50MHz DEP signal.

Suspended cells have been injected inside the High Frequency cytometer chip and submitted to different DEP signal frequencies, with an additional amplitude modulation to avoid permanent cell trapping on the electrodes. Fig.3.a shows that when the setup bias frequency is higher that the cross-over median (f_{CoM}) most cells are concentrated in microchannel center where the E field intensity should be the lowest. Conversely, in Fig 3.b, a large number of cells are distributed on the edge of the channel attracted and trapped by high intensity E field areas, when a frequency lower than f_{CoM} is set. When the DEP signal frequency becomes closer to f_{CoM}, as in Fig 3.c, cell spatial distribution is much disperse with a part of cell population more or less repealed in the center and another attracted to the channel edge. As a conclusion, to separate and extract targeted cells (the ones with potentially strongest aggressiveness features) from the whole LN18 cell population, it appears that the sorting conditions have then to be triggered between Fig.3.b & Fig.3.c cases. Nonetheless, Fig.3.d already shows the proposed cytometer chip ability to sort a mixture of particles. Here, all polystyrene beads (7µm particles) successfully gathered together in the channel center by negative DEP and separate from biological cells, isolated for their part by positive DEP.

IV. Conclusion

This paper introduces a novel type of high frequency dielectrophoresis lab-on-chip implemented on BiCMOS IC technology and shows its ability to efficiently separate and sort biological cells from their own intercellular dielectric specificity difference. These results might pave the way to new innovative IC Lab-on-chip sensors for detection and isolation of rare cells.

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