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A High Frequency Dielectrophorosis Cytometer for Continuous Flow Biological Cells Refinement

Thomas Provent[#1], Audrey Mauvy[*2], Rémi Manczak[#3], Sofiane Saada[*4], Claire Dalmay[#5], Barbara Bessette[*6], Fabrice Lalloué[*7], Arnaud Pothier[#8]

[#] XLIM, University of Limoges, UMR CNRS 7252, F-87000, France

[*] CAPTuR, University of Limoges, EA3842, F-87000, France

[1]thomas.provent@xlim.fr, [3]remi.manczak@xlim.fr, [5]claire.dalmay@xlim.fr, [8]arnaud.pothier@xlim.fr

[2]audrey.mauvy@unilim.fr, [4]sofiane.saada@unilim.fr, [6]barbara.bessette@unilim.fr, [7]fabrice.lalloue@unilim.fr

Abstract*— This paper presents a microfluidic radiofrequency device operating at a few hundred MHz, which is able to sort biological cells. It uses a non-invasive and label-free technic based on intracellular dielectric specificities of biological cells. The sorting principle relies on a dynamic dielectrophoresis (DEP) deviation resulting from the interaction between a high frequency electric signal and the cell cytoplasm content. Driven in a microfluidic channel by a continuous flow, cells are individually deflected from their primary trajectories after having entered a non-uniform electric field generated by a microelectrode system. Designed with different slopes, these electrodes allow a selective guiding of cells to different outlets depending on the dielectrophoresis deviation efficiency. To allow a successful cell sorting, the intensity of deviation forces acting on cells is modulated according to the particle speed, the dielectrophoresis signal frequency and the electrode slope angles related to the Clausius-Mossotti factor of each cell. As proof of concept, experiments with cells from glioblastoma line were carried out, using different DEP signal frequencies to highlight system ability to sort cells from heterogeneous basal population into less disparate sub-populations.

Keywords—UHF-Dielectrophoresis, microfluidics, biological cells, label-free sorting, crossover frequency

I. Introduction

Past years, dielectrophoresis (DEP) technics have proved their great efficiency in sorting biological cells [1], based on their ability to measure the dielectric properties of each cells. Characterizing and sorting cells is a key point in cellular biology research as well as in the biomedical field to achieve therapeutic assessment.

A great advantage of the DEP principle is that it is a contactless and label-free method. When cells, set in suspension, are subjected to a non-uniform electric field (E field), a dielectrophoresis force is induced, resulting in cell's motion. This motion can be an attractive, achieved via a positive DEP force (pDEP), or repulsive though a negative DEP force (nDEP). Applications of such forces to sort cells based on their size or their dielectric property differences were hence largely demonstrated in the literature [2]-[3]. However, most of the previous works implies DEP electromanipulation in the range of 10 kHz to 10 MHz for which the difference of membrane dielectric property between cells is mainly exploited.

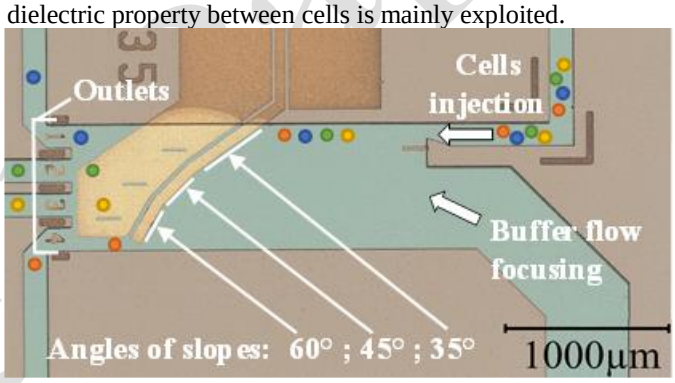

Fig. 1. Photograph of the main DEP cytometer sorting area. The implemented microelectrode system includes 3 sections, each presenting a different slope to separate and guide the cells to 4 different outlets.

In this paper, we use Ultra-High Frequencies (UHF), i.e. several hundreds of MHz, as DEP signal frequency. High frequency signals are able to bypass the cell membrane and penetrate inside the cells to probe the intracellular dielectric properties of their cytoplasm [2]. In the present study, a continuous flow cell cytometer has been developed for exploiting such DEP signal sorting capabilities (Fig.1). Using a flow-focusing principle, this microfluidic device aims to focus flowing cells suspended in a low conductive osmotic medium to a sorting area where a specifically designed electrode system has been implemented and is biased with a UHF-DEP signal. Polarized by the generated E field, cells can be deflected to different outlets related to the intensity of the resulting DEP force (FDEP) applied to them. This FDEP is modulated as a function of the particle initial speed, the DEP signal frequency and the electrode slope angles are related to the investigated cell intracellular dielectric properties.

Along the section II of this paper, the DEP phenomenon and the cytometer sorting principle are presented. We will see how cell trajectories can be adjusted as a function of the intensity of the FDEP they are submitted. The section III presents sorting experiments where heterogeneous population of glioblastoma cells was successfully refined in four subpopulations,

highlighting the importance of setting properly the DEP frequency choice during the sorting process.

II. Principle of UHF-DEP Sorting and Design of Microelectrode

A. How Exploiting Cell Dielectric Specificities

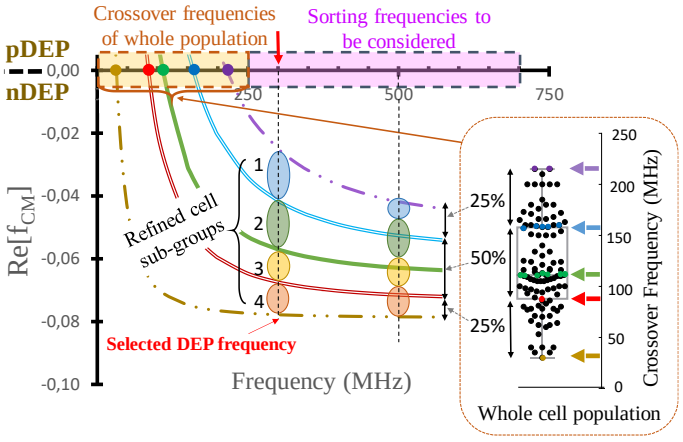

Fig. 2. Typical expected range of CMF for a heterogenous cell population as function as the working frequency. Box plot representation of the measured crossover frequencies dispersion for gliobastoma U87-MG cells demonstates the heterogenity of dielectric properties for such cell culture. Setting properly the sorting frequency may allow to achieve an efficient whole population refinement in sub-groups of cells with more homogenous properties.

As previously mentioned, cells exposed to a non-uniform electric field become polarized and are subject to dielectrophoresis forces which can induce a course change during their travel in the cytometer main microchannel. This phenomenon is exploited here and we will see that it can be modulated as function as the dispersion of dielectric properties appearing in a cell population.

Actually, the intensity of this F_{DEP} force can be estimated using equation (1) [3]. Where ε_m is the relative permittivity of the cell suspension medium, f_{CM} and r are respectively, the Clausius-Mossotti factor and the radius of the cell, and $\nabla|E_{rms}|$ is the gradient of the electric field, which relies both on the applied DEP signal potential and the electrode geometry.

$$F_{DEP} = 2\pi\, \varepsilon_m\, Re[f_{CM}]\, r^3\, \nabla|E_{rms}|^2 \qquad (1)$$

The Clausius-Mossotti factor (CMF) relies on the difference of complex dielectric permittivity between the particle (ε_p^*) and the medium in which it is immerged (ε_m^*) as shows (2):

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

This factor modulates the DEP force intensity and imposes its repulsive (nDEP) or attractive (pDEP) behaviour. It can have different values as function of the dielectric properties of investigated cells. It depends also on the selected frequency of the DEP signal applied on the microelectrode system. A frequency that can be easily adjusted in order to look for appropriate sorting conditions to achieve the targeted cell separation.

Working with UHF frequencies is actually relevant since such signals are able to probe the internal dielectric properties of the cell cytoplasm whereas conventionally used kHz frequency range allows electromanipulation mostly based on the difference of cell size and membrane dielectric properties. With hundreds of MHz, the sorting selectivity is hence expected to be more efficient and may reflect faithfully the biological heterogeneity occurring in a cell population.

Usually, derived cells form patients present a large heterogeneity reflecting the natural cell diversity occurring in lining tissues or organs. Such disparities are sometime difficult to handle, especially to interpret properly experiment's results. Such heterogeneity related issue also occurs for commonly used patient derived cell lines as the one (U87-MG) which was used in this study. Actually, it was previously shown [4]-[5] that glioblastoma cultures result in a wide diversity of cell crossover frequencies (CF). This diversity is illustrated for U87-MG line by the Fig.2 box plot. Each dot on the box plot figure represents a measured CF for an individual cell. The measured range of CF is indeed a consequence of the cell heterogeneity occurring for such cell line. One may notice that half of the U87-MG cell population presents CF ranging between 90MHz and 160MHz (red and blue arrows of the box plot quartiles) with a median value of 110MHz (green arrow). The two others quarters of the population show CF that spread from 30MHz to 90MHz and from 160MHz to 215MHz. Such dispersive characteristic trend is consistent and has been reported for other cell types [6]-[7].

One may understand than the mentioned crossover frequency in fact matches with the conditions for which the cell passes from a positive to a negative DEP behavior. This specific frequency is hence characteristic of the cell own dielectric properties since it implies that its CMF is inevitably null at the crossover. The heterogeneity of the investigated cell population also translates in a dispersion of CMF values at frequency higher than the range of CF. The Fig.2 plot this expected diversity in CMF value varying with the frequency.

Hence, as function as the chosen frequency to be set for the cytometer, some CMF sub-ranges can match with several refined cell sub-fractions of the whole population. Sub-populations of separated cells will show much more homogeneous dielectric properties.

The proposed cytometer system aims to sort cells based on their CMF values, using as discrimination factor the diversity of intensity of DEP force acting on each cell during the sorting process. Consequently, it can be used to separate and refine the whole cell population into four sub-populations expecting to regroup cells with similar dielectric specificities. As in the fictitious example given in the Fig.2, considering a 300MHz sorting frequency, it can be envisioned to potentially split at equal share (~25%) the glioblastoma cell suspension into four distinct sub-populations. To collect such refined sub-populations, appropriated electrode system geometry should be

used, in order to selectively drive separated cell groups to a different device outlet. We will introduce now how it was designed.

B. Cytometer Sorting Principle - Microelectrode Design

The implemented microfluidic system consists of two inlets (on the right in Fig.1): one is used to inject the cell suspension to be sorted whereas the wider one has been implemented to inject an osmotic buffer solution alone. Tuning the flow rate difference between these two inlets allows mastering the cell suspension initial trajectory and focussing them along the microfluidic channel edge in order to focus cells above the upper part of the microelectrode. At the other device end, four outlet channels have been implemented to collect sorted cells. In between, the sorting zone consists of a 780µm large microfluidic channel in which a three sections gold electrode system is patterned at the bottom of the channel. Each section has a different angle, respectively 35°, 45° and 60°, related to the initial trajectory of the flowing cells.

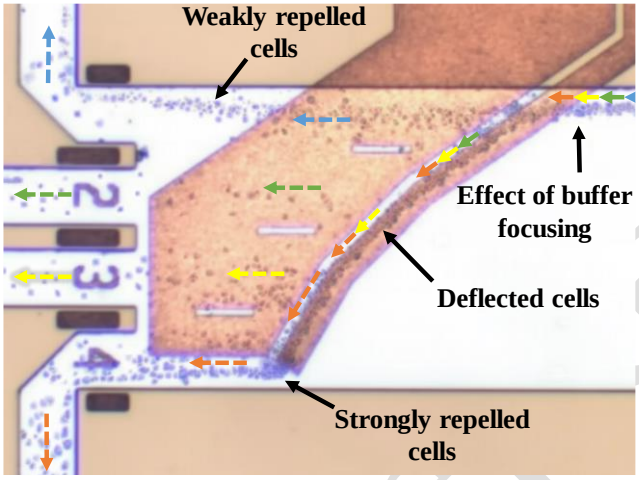

Fig. 3. Stack of images extracted from a 240 seconds sequence of cell sorting for which the applied DEP frequency has been tuned from 250MHz to 500MHz: Illustration of the microelectrodes influence on cell trajectories and resulting cell spread at the cytometer outlets.

The cell population to be refined is injected in the device from the top inlet. The cell flow is guided in a narrow strip of the microchannel until the 35° slope electrode zone. Once reaching the vicinity of the electrode, the cells enter under the E field influence. Only the cells that are subject to low F_{DEP} intensity will continue their way to end up in the outlet n°1 (blue trajectory in Fig. 3); other cells will be repelled and deflected laterally. Due to the continuous flow taking place in the microchannel, their new trajectory follows the electrode until reaching the second electrode slope part. Because of the angle change, i.e. 45° vs 35°, efficiency of DEP deflection suddenly decreases. Consequently, part of the cells previously deflected, now can cross the repellent E field area and end up their way in outlet n°2 (green trajectory in Fig. 3), others will be deflected again. The same phenomenon occurs with the following change of angle (45° to 60°) where, cells with insufficient DEP repulsion will pass through the E field and continue to the outlet n°3 (yellow trajectory in Fig. 3) whereas those subject to the strongest F_{DEP} influence will be driven alongside the electrode to the outlet n°4 (orange trajectory in Fig. 3).

Hence, thanks to this successive angle change approach, a proper cell separation on the base of their difference of CMF can be achieved. For the proposed electrode system design, the selection of implemented electrode slopes has been done based on multiphysics fluidic and electrostatic simulations and should be suitable for a wide range of sorting requirements.

III. Experience Set Up and Results

A. Material Preparation

In order to demonstrate the capabilities of the developed sorting system, experiments on U87-MG glioblastoma cell line culture have been led. This choice was mainly motivated by the substantial biological heterogeneity presented in such cell population [8]. Associated to this high biological heterogeneity, various dielectric properties [5] can be expressed, making this cell population a good candidate to demonstrate the refinement capabilities of the developed system. The human glioblastoma cell line U87-MG was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). They were grown during 5 days in DMEM medium, supplemented by 10% SVF, 1% PEN / STREP antibiotic, 1% MEM Non-essential amino acids (Gibco) and 1% Soduim Bicarbonate (Gibco) at 37°C in a humidified 5% CO2-95% air incubator before been seeded in appropriate flasks at 5×10^5 viable cells/ml . Cells are detached with 3mL of trypsin during 5min at 37°C. Then, after two successive centrifuge washes, cells were suspended in an ion free osmotic medium osmotic sucrose based medium (pH: 7.4, conductivity: 38 mS/m) conventionally used for DEP experiments. To maintain cells alive during experiment, the medium osmolality has been set at 304mOsm by adjusting the sucrose concentration in pH buffered deionised water. Finally, the cell concentration in the suspension to be injected in the cytometer has been adjusted with an appropriate dilution factor to limit cell-clustering phenomenon inside the cytometer and decrease risk of errors during cell counting sequences.

Used cytometer prototype was fabricated on high resistivity silicon wafer, using a 2µm thick gold layer to define the microelectrode geometry. The microfluidic channel has been patterned using 50µm thick negative epoxy based dry film: DF-1050 from EMS, laminated on the top of micro-electrodes and defined by standard UV photolithography process. A removable PDMS cover is stacked and mechanically maintained on the top of DF film, hence forming at the end a 35-45µm high microchannel network modulated as function the pressure applied on the PDMS piece.

B. Experimental Setup

For the present experiments, the cell suspension was injected at constant flow rate into the micro-channels and the cytometer microelectrodes was biased with a constant magnitude DEP signal. Only the signal frequency was tuned.

Resulting effects on cell trajectories of such DEP signal have been monitored and recorded using an advanced high-speed camera system. Used image capture system (CV-X vision sensor from Keyence) has been mounted on a non-inverted microscope. It allows an automatic tracking and a real time counting of particles during experiments. It has been configured to count the number of cells going out the cytometer from each outlet during a defined acquisition time.

To initiate the experiments, the fluidics conditions are set up to ensure a cell speed flow of 1.5mm/s in the sorting area. The flow focussing ensures that cells are well directed at the beginning of the 35° angle section of the microelectrode (Fig.3). Then, a high frequency DEP signal is applied to the electrode system, for which most of the cell population is expected to be strongly repelled by the three slope sections. The magnitude of the signal is chosen to ensure that a large proportion of the cell population is fully deflected and goes out though the outlet n°4. The effective cell separation is recorded and the number of cells coming out though each outlet counted.

Next, same type of experiments is done decreasing the DEP signal frequency gradually. As presented in Fig.2 for an increasing number of cells, decreasing frequency should imply a decrease of their CMF. Hence, it also leads to a decrease of the resulting intensity of the applied deviation force on each cell passing though the cytometer. Therefore, as function of the frequency set, the whole cell population can be split in different way between the four outlets and spread in fractions with a related cell proportion that can be adjusted.

C. Results

As illustration of the cytometer sorting capability, the results of three cell counting performed at respectively 700, 350 and 250MHz are summarized in Fig.4. As expected, the choice of sorting frequency affects the proportion of cells counted at each outlet. When a high frequency is set, cells are well submitted to a strong repulsion from the E field, as one see in Fig.4, where 83% of U87 cells are directed to the outlets n°3 and n°4 at 700 MHz (with 55% exclusively in outlet n°4). Only few cells (less than 7%) are escaping though the outlet 1. To the contrary, at 250MHz, the number of cells going out from outlet 1 strongly increases reaching 40%, which testifies that for a representative part of the population the CMF decreases significantly. In the meantime, the number of cells driven to outlet 4 drops to less 15%, which is consistent. The relative fractions of cells that coming out though outlet 2 or 3 are more complex to predict in term of relative number of cells, since they really rely on the effective distribution of dielectric properties present in the investigated cell population. Objectively, there is no biologic or statistically supported reason that such population present a linear continuum of CMF values instead of a Gaussian or even a more randomized distribution.

The cell spread observed for 300MHz sorting conditions is quite interesting, since it illustrates well that some configuration can be found for which an almost balanced split of the population between the four outlets is achievable. Above the three mentioned examples, others performed experiments have demonstrated us the capability of this cytometer to split a heterogeneous population in four more or less cell concentrated subpopulations with a stronger dielectric properties similarities. Hence, appropriately adjusting the working frequency might also allow separating and isolating some particular cells with some specific features: for example cells with stronger aggressiveness potential. Future work will aim to increase the sample collection efficiency requires to perform dedicated biological analyses for each subpopulation collected at the outlets; in order to see if some correlations between specific CMF ranges and biological specificities can be established.

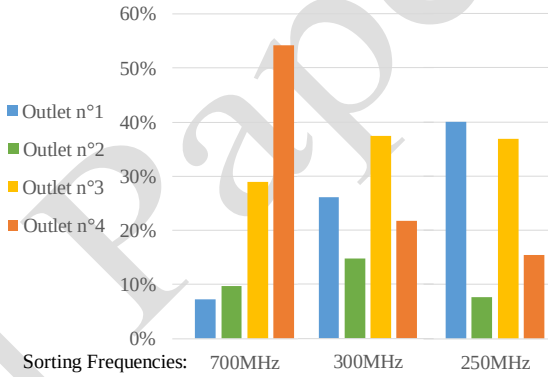

Fig. 4. Distribution of collected cells at each sorting system outlets as function the selected sorting DEP signal frequency.

IV. CONCLUSION

This article presents a continuous flow UHF DEP lab-on-chip cytometer concept that exploits the intracellular dielectric specificities of biological cells to refine them into subpopulations with more homogenous dielectric properties. Indeed, the proposed design exploits a modulation of the repulsive DEP force intensity acting on cells during their flow through the system. It results in a discriminating trajectory change as function as difference between their cytoplasm dielectric properties. Hence, the presented work illustrates the radio frequency capabilities to perform in a full label-free way a proper population refinement and purification starting from a heterogeneous native cell culture. Led experiments especially highlight the importance of the chosen RF signal frequency to perform such cell sorting, which requires to be properly selected in order to achieve the targeted cell refinement.

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