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Biological Cell Discrimination Based on Their High Frequency Dielectropheretic Signatures at UHF Frequencies

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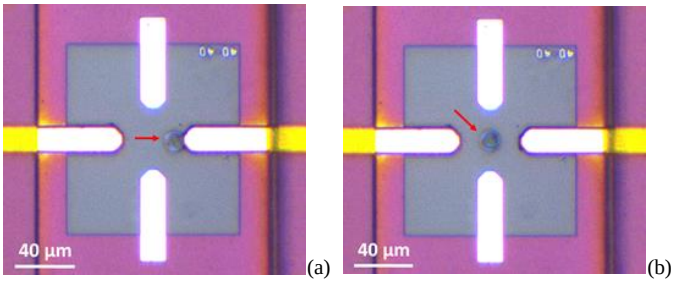
***Abstract*—This paper reports on the application of dielectrophoresis techniques in the radiofrequency range, in order to probe inner dielectric specificities and therefore characterize individual biological cells. The novelty of this work consists in exploring the capability of UHF signals to generate DEP-driven motion effects on flowing biological cells in a microfluidic micro-device. Additionally, with applied signals above 50MHz, distinct cross-over frequencies can be identified as function of both the cell type and the difference in the intracellular dielectric features, and between intracellular and extracellular media. Several experimental campaigns were led on three distinct cell lines by thoroughly scanning the UHF spectrum and specifically measuring the resulting second cross-over frequency for each cell type. The experimental results suggest that significant cross-over frequency differences can be observed from one cell line to the other and confirm that their high frequency DEP characteristics can be a relevant cell signature for discriminating them. This work is a first step towards the development of a UHF-DEP cytometer.**

I. Introduction

Currently the development of sensitive and label-free biosensors, able to handle biological cells for individual and accurate analysis, attracts many research efforts especially in the microwave community [1-5]. Labeling procedures are generally expensive, time-consuming and potentially cytotoxic, whereas label-free detection can preserve cell viability and enable post-analysis cell utilization. At RF and microwave frequencies, the interactions between electromagnetic fields and biological cells are particularly interesting; since the plasma membrane is no longer screening the incident signals, which can then be used for the characterization of intracellular properties, hence underpinning the development of cell analysis techniques. Nevertheless, measuring dielectric properties of such small volume samples (typical volume of single cell is in the picoliter range) immersed in a liquid medium, which is relatively lossy in such frequency range, is quite challenging [6] and requires highly sensitive detection techniques along with innovative sensors [5] combined with a robust measurement methodology [7].

An alternative approach to impedance spectroscopy is to take advantage of some specific effects induced on the cells under analysis by the incident high frequency signals. Notably, the DEP-driven trajectory followed by cells while flowing through a localized electric field area, can be a relevant indicator of their own electrical properties and specificities.



(a) (b)

Fig. 1. Individual biological cell handling and trapping at UHF frequencies respectively in (a) positive and (b) negative DEP configuration.

Actually, the DEP characterization principle uses the induced motion of cells, suspended in a liquid phase under a non-uniform AC electric field. Hence, the displacement analysis enables to determine the sample dielectric characteristics as function of the applied signal frequency. Conventional DEP techniques typically work in the 10 kHz to 1 MHz range [8] and provide information about cell plasma membrane specificities (cell size, membrane capacitance …).

Increasing frequencies above 50 MHz allows bypassing the cytoplasmic membrane and making the electric field interact directly with the cell interior [9]. By probing the cytoplasm content, UHF frequency signals can provide further insights on intracellular physical specificities. These can be in turn correlated to biological properties or physiological mechanisms such as cell apoptosis, cell differentiation, pathological state [10]… Therefore, extending DEP techniques to UHF frequencies could be very promising to develop novel flowing cell dielectric analysis methods and setup a new generation of high-throughput microwave cell cytometers as shown in [3].

In this work, we characterize different cell lines population by monitoring the cell DEP motion, submitting cells to an electric field with tunable frequency in the UHF range. As

shown in Fig 1, a quadruple electrode sensor has been exploited to electrically trap individual cell and determine the specificities of their DEP signatures. For each investigated cell, the specific frequency in the UHF spectrum for which its DEP behavior reverses from positive to negative DEP response has been found. We show that there are obvious differences between the measured high frequency cross-over frequencies for the investigated cell types. Such property could be exploited as a relevant discrimination factor especially in the frame of DEP based cell cytometer set-up.

II. Dielectrophoresis Force and Particles Manipulation Basics

DEP is a contact-less approach that allows manipulating, trapping and analyzing/characterizing suspended particles in a liquid. The principle is quite simple, once these dielectric particles are submitted to a non-uniform electric field, a dipole moment is generated inducing a dielectrophoresis force (F_{DEP}) that can be high enough to move particles. For a homogeneous spherical particle, the DEP force is expressed by (1) [8].

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_m r^3 Re[K(\omega)]\nabla {E_{rms}}^2 \quad (1)$$

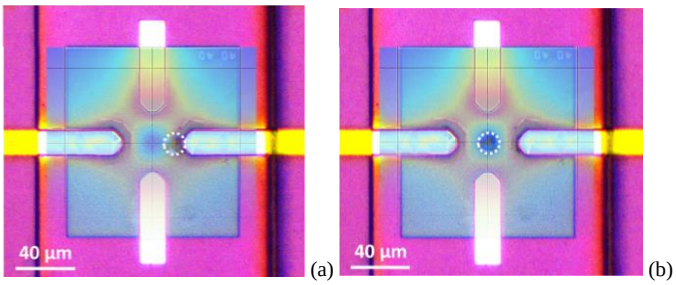
Where ε_0 is the vacuum permittivity, ε_m is the immersion medium permittivity, r is the particle radius, $Re[K(\omega)]$ is the real part of Claussius-Mossotti factor, ω is the angular frequency of the applied electric field and ∇E_{rms} is the electric field gradient.

The Claussius-Mossotti factor is given by (2):

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

Where ε_p^* and ε_m^* refer to the complex permittivity of the particle and the suspension medium, respectively.

Actually, $K(\omega)$ depends on the difference of polarizability between particles and their surrounding medium, and this dissimilarity varies as function as the frequency changes. Especially for biological cells, the intracellular content by itself may induce a tiny difference of dielectric characteristics between intra and extra cellular medium. Monitoring the motion of cells submitted to a DEP electric field gradient then allows gathering evidence of such dielectric properties difference.



(a) (b)

Fig. 2. Comparison of electric field intensity distribution on the quadrupole electrode structure (red high intensity, blue low intensity) and the optimal cell electrical trap region in (a) positive DEP and (b) negative DEP cases.

Considering (2), one can notice that $K(\omega)$ can be either a positive or a negative number. This implies that the generated DEP force can take one of two opposite directions and therefore induces an attractive or a repulsive particle motion with respect to the electric field direction. Thus, when a biological cell is subjected to a positive DEP force, it is attracted to areas of high electric field intensity (Fig2.a). While in the negative DEP force case, the particle is repelled towards regions of low field intensity (Fig2.b).

Hence, by sweeping the frequency, it is possible to observe the particle displacement that suddenly reverses going from positive to negative DEP behavior. The transition between the two DEP behaviors occurs at a frequency known as DEP cross-over frequency where the real part of Claussius-Mossotti factor becomes zero (Fig.3), hence canceling the DEP force that has no more influence on the particle at this specific frequency

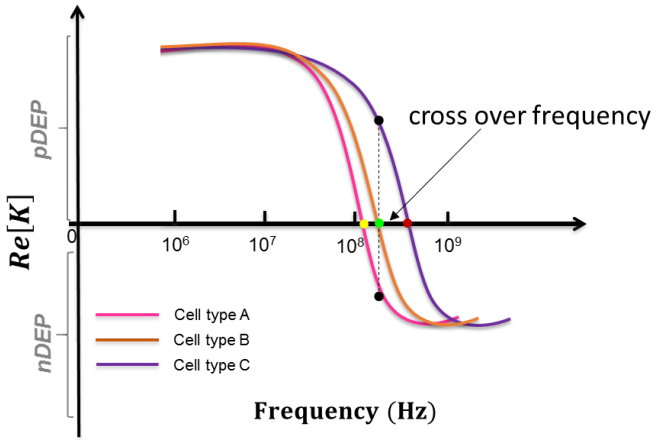


Fig. 3. Potential effect of intracellular dielectric specificities on the Claussius-Mossotti factor for various biological cell types (schematic view).

This DEP force cancelation phenomenon is well known at low frequencies where the cell plasma membrane properties are predominant and has been successfully exploited to demonstrate cell-sorting capability [11]. Above 50 MHz, another second cross-over frequency can appear, relying this time on the difference between the dielectric properties of the intracellular content and extracellular media.

This second cross-over frequency has been poorly explored until now, but its promising use for cell analysis and discrimination is starting to be recognized [3][9][12].In this context, we have led several experimental campaigns on three distinct cell lines by thoroughly scanning the UHF spectrum and specifically measuring the resulting second cross-over frequency for each cell type. The aim of this study was to evaluate if some significant cross-over frequency difference can be observed from one cell line to the other; confirming that their high frequency DEP characteristics could be a relevant signature for cell discriminating (Fig.3).

TABLE I: SUMMARY OF CROSS-OVER FREQUENCY MEASUREMENT (MHZ)

Cell Lines	Number of cells	Avg	Median	Dev Std	Error Std	Min	Max
SW620	16	253	239	30.4	7.6	220	297
BL-41	14	172	171	67.6	18.0	70	370
HCT 116	6	112	97	27.3	11.1	90	160

III. HIGH FREQUENCY DEP EXPERIMENTS ON BIOLOGICAL CELLS

Three cell lines from ATCC source have been selected for this study. SW620 and HCT 116 cells, which are both, are human colon cancer cells but with different aggressiveness features (SW620 are derived from metastatic tumor whereas HCT 116 come from precancerous tissue), and a lymphocyte cell: BL-41 line. The objective was to run experiments on cells flowing in microfluidic channels, having similar size and morphology but with distinct biological characteristics and origin.

All cell lines were grown in complete medium composed of Minimum Essential Medium supplemented with 10 % fetal bovine serum, 500 U/ml penicillin and 500 µg/ml streptomycin (Invitrogen). Cultures were maintained in a humidified atmosphere with 5 % CO2 at 37 °C. One hour before measurements, cells were collected. After centrifugation, the cell pellet was suspended in an osmotic medium conventionally used for DEP experiments and mainly composed of water and sucrose (conductivity range between 19-22 mS/m for a 7.42 PH). This low conductivity medium allows maintaining cell viability for couple of hours while handling them under both positive and negative DEP signals.

Each cell suspension was characterized using the same quadrupole electrode sensor design (Fig. 1). A frequency adjustable DEP signal has been applied to the left and right electrodes whereas top and bottom ones were grounded. Cells flowed to the sensing area in a microchannel built on top of the sensor and their speed was controlled with a micro-fluidic flow controller system.

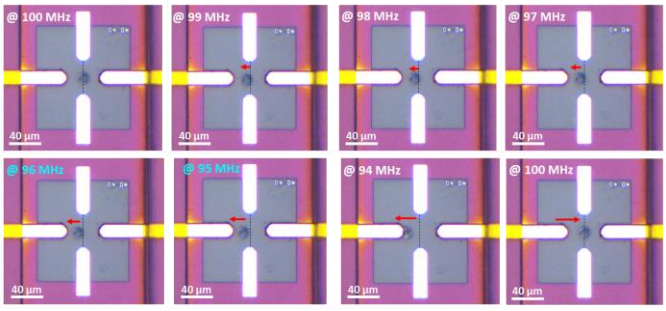


Fig. 4. Microscope imaging of HCT 166 trapped cell movement as the applied DEP signal frequency is swept with a 1MHz step: Measured cross-over frequency =96-95MHz.

Once cells arrive above the quadrupole sensor, the liquid flow is reduced and a negative DEP signal is applied in order to stop and trap one of these cells. This allows positioning it accurately in the middle of the electrode system (Fig.1 b).

The microchannel liquid flow is then stopped, balancing both input and output pressure at each microchannel end. Actually, before starting the analysis, the DEP signal is first turned off to check if the cell remains stable and well centered between the electrodes, as an evidence that the investigated cell is no longer subject to other motion forces.

Now to determine the cell cross-over frequency, the DEP trapping signal is turned on again (negative DEP arrangement) and its frequency is decreased while monitoring the induced cell motion. As illustrated in Fig. 4, once the cell starts to leave the sensor's center, the frequency sweep is progressively reduced. The frequency-tuning step is adjusted in order to finely detect the moment when the cell will suddenly switch position and become trapped on the lateral electrode edge (positive DEP arrangement). The sought cross-over frequency is therefore determined. This value is then confirmed by alternatively sweeping the DEP signal frequency around the cross-over region and seeing the cell respectively moving from center to positive electrode edge, coming back to center and so forth... At the end, the cell is then released before a new one is trapped and characterized following the same principle.

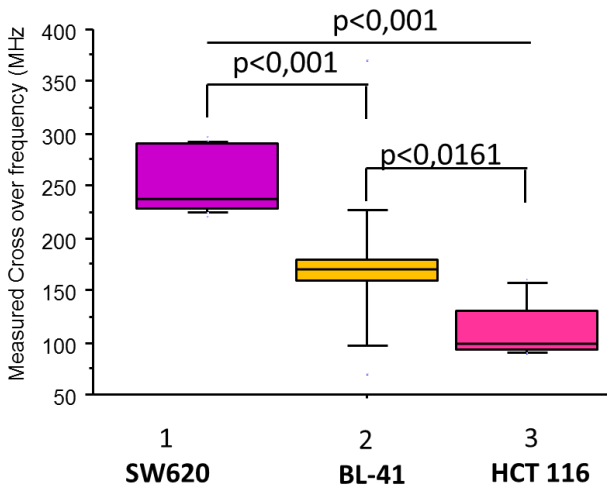


Fig. 5. Box plot of measured cross-over frequencies for SW620, BL-41 and HCT116 cell lines.

Table I summarizes the results of measurement campaign that has been led respectively on SW620, BL-41 and HCT 116 cell lines. For the two first ones, more than 14 measurements on different cells have been achieved so far, and the collected data begin to be representative. Additional characterizations of HCT 116 cell population are still required and are currently underway. Nevertheless, despite the fact that for few characterized cells a more or less large dispersion can be observed owing to the natural heterogeneity within any cell population, Fig. 5 shows that the measured cross-over frequencies of each cell line are finally relatively homogeneous (limited thickness of box plot). Remarkably, we noticed that a significant difference (p< 5%) exists

between median cross-over frequencies for these three cell lines. These preliminary results show that in the high frequency spectrum, the DEP characteristics of the investigated cells present some clear differences. Hence, the specificity of such signatures could be well used as a relevant discrimination factor between cell types.

IV. Conclusion

Characterization of individual cells at UHF frequencies was demonstrated using microfluidic based quadrupole electrode sensors and particle dielectrophoresis motion tracking approaches. The proposed characterization method allows fine assessment of the DEP cross-over frequency for the investigated cells relying on indirect measurements of intracellular dielectric properties specificities versus extracellular medium. These results strengthen our conviction that the use of UHF-DEP cell signatures can open the way to the development of novel cell sorter/cytometer generation, for which the presented cell characterization approach will be suitable to select the most efficient and selective DEP signal frequency to apply.

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