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**Isolated gold micro singularities for high density cell trapping based on dielectrophoresis**

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Dielectrophoresis (DEP) is broadly used in microfluidic systems for the cell therapies or medical diagnostics [1] because of its capability to handle and sort biological cells [2,3]. In this paper, a new method to trap cells on-chip with high density arraying capabilities is proposed. The principle is based on the use of metallic singularities arrayed within the flowing channel of the biochip. These singularities, even at a floating potential, induce a non uniform electrical field within the structure, responsible of a strong DEP force applied to cells. Indeed, we will demonstrate in the paper that metallic singularities generate stronger DEP forces, compared to more conventional methods where micro-dots of insulating material are arrayed to produce the electrical field traps. [4]. To the best of our knowledge, we report here the first successful use of such floating potential metallic singularities to trap cells.

The main advantages of our method are: (i) the strength of the DEP forces that are generated by the metallic singularities, (ii) the capability to achieve high density trapping of cells as these singularities are not connected (iii) minimal perturbation of the cell flow when the polarization electrodes are not powered.

The structure of the biochip is shown in Fig.1. The polarization electrodes (4 $\mu$ m thick, electroplated gold) define the microchannel. A patterned SU8 layer is added to obtain the required channel height set to 25 $\mu$ m. the gold singularities (4 $\mu$ m thick), are patterned within the microfluidic channel, between the polarization electrodes. In this figure gold singularities are disposed as a line (but the principle is extendable to an array, as it will be shown in the final paper).

The chip was fabricated on a glass wafer (transparent and with good dielectric properties). A Cr/Au (50 Å /150Å) layer was evaporated on its surface and the gold layer was electroplated to reach 4 $\mu$ m (gold was chosen for its biocompatibility). Photolithography was then used to pattern successively the gold electrodes and the SU8 microfluidic channels. Finally, the biochip was packaged using a PDMS membrane bonded to the chip with a silanization process (Fig.2).

Fig 3 represents the DEP force simulation for a case where the singularities are arrayed. Cells are first extracted from the flow in the channel and trapped to the vicinity of the gold dots thanks to positive DEP (high field regions, strong DEP force). In a second step, the frequency is tuned so that cells are led to the center of gold dots thanks to negative DEP. In that low field region cells are maintained without electrical damage.

Biological experiments were performed with NIH3T3 fibroblast cells suspended in 20 mS/m medium. First a 25 V sinusoidal voltage was applied between the polarization electrodes with a frequency of 1 MHz. As expected, cells moved to the edges of the dots (positive DEP, Fig.4.b). Then the frequency was shifted to 100 kHz leading cells to be captured onto the gold dots (negative DEP, Fig.4c.).

These results demonstrate the efficiency of floating potential metallic singularities for the cell capture and arraying.

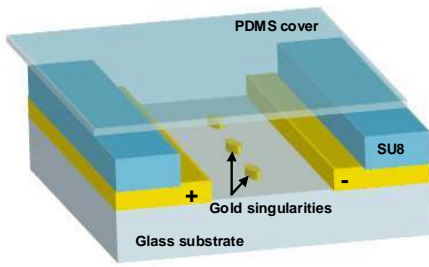


Figure 1. Schematic view of the developed microfluidic biochip (arbitrary scale).

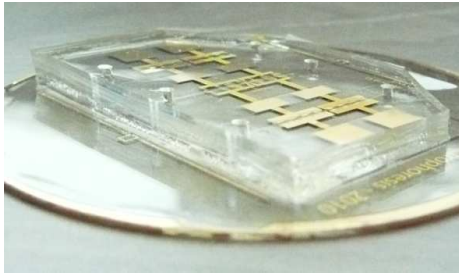


Figure 2. Photograph of the microfluidic biochip packaged with a PDMS cover.

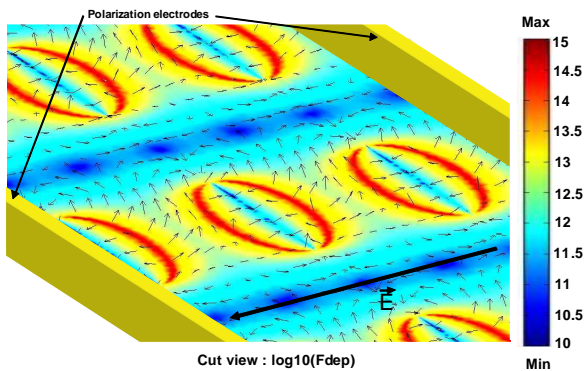


Figure 3. Finite element analysis of the DEP force topology (FDEP) in the structured microfluidic channel - Arrays: case of negative DEP.

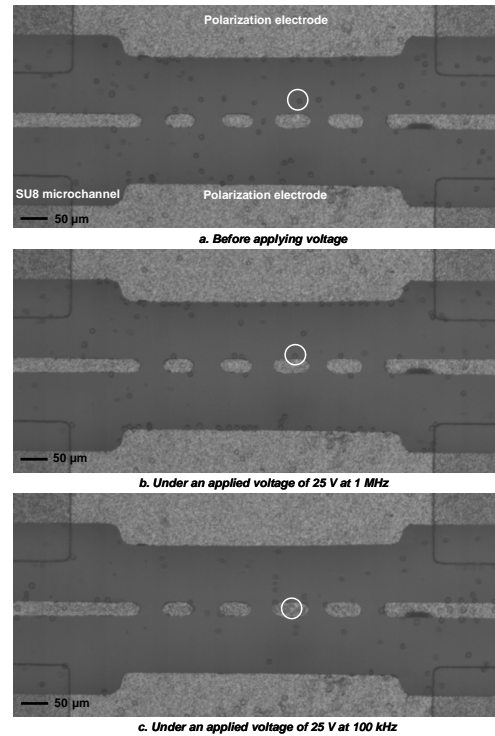


Figure 4. a) Gold singularities. NIH3T3 cells are flowing within the microfluidic channel b) cells are trapped at the vicinity of the gold dots ( $V=25\text{ V}$ ,  $f=1\text{ MHz}$  - positive DEP) and c) cells are led onto the gold plots ( $V=25\text{ V}$ ,  $f=100\text{ kHz}$  - negative DEP). On these images a single cell is circled to highlight the principle of the method.

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