

Detection And Isolation Of Circulating Tumor Cells With Single-Cell Resolution: A Successful Lab-on-a-chip Device

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Abstract This paper presents the unique features of DEPArray™ an automated system enabling image-based cell sorting with single-cell resolution and describes its potential application in the field of oncology.

Keywords: Dielectrophoresis, Microfluidics, Lab-on-a-chip, CTC, Tumor

1. Introduction

Molecular profiling of rare cells is important in a wide range of biological and medical fields. The isolation of rare cells from heterogeneous samples free of contaminations is challenging and in most cases impractical. At this purpose we developed an automated system which is capable to identify and isolate rare cells with unprecedented level of accuracy and purity. This high level of purity is necessary to enable the molecular analysis of single cells.

2. Device description and working principle

The core of the technology is a disposable microsystem device which integrates a silicon chip, microfluidic chambers and valves (Figure 1); microelectronics and microfluidics are combined synergically to provide unique single-cell manipulation and sorting capabilities.

The silicon chip implements a two dimensional array of about 300.000 micro-locations, each consisting of a surface electrode and embedded circuits [1][2]. The electrodes induce suitable closed dielectrophoretic (DEP) cages in the spatial region above selected microsities, within which single particles may be trapped and levitated

individually (Figure 1).

A gallery of high resolution images captured for each individual cell in the sample enables accurate cell analysis and selection based on fluorescence and morphology; target cells can thus be easily distinguished from spurious events (Figure 2).

Once identified each target cell can be isolated from the bulk population automatically: Step by step DEP cages are moved concurrently and independently along trajectories calculated by computer, grabbing and dragging each selected cell from the original location into a dedicated Parking chamber (Figure 3).

Once parked, each target cell can be moved from the Parking chamber into the Recovery chamber dedicated to the ejection of cells. At the end of the process the target cells are ejected from the device directly into a recovery support through an accurate microfluidic control. The recovery procedure can be repeated to obtain from the same device multiple recoveries of individual target cell or group of cells separately (Figure 4).

3. Applications and results

The system is ideally suited to isolate extremely rare cells from peripheral blood enriched samples. In the area of oncology for example DEPArray™ can be used to detect and isolate Circulating Tumor Cells (CTC) with 100% purity [3]. Recent results [4]

demonstrated how mutation sequencing on multiple individual CTCs is possible following single-cell sorting using DEPArray™.

4. Conclusions

Isolation of 100% pure Circulating Tumor Cells from enriched peripheral blood sample has been obtained successfully using a lab-on-a-chip which integrates microelectronics and microfluidics. The efficacy of the technology is proven by experimental results.

Bibliography

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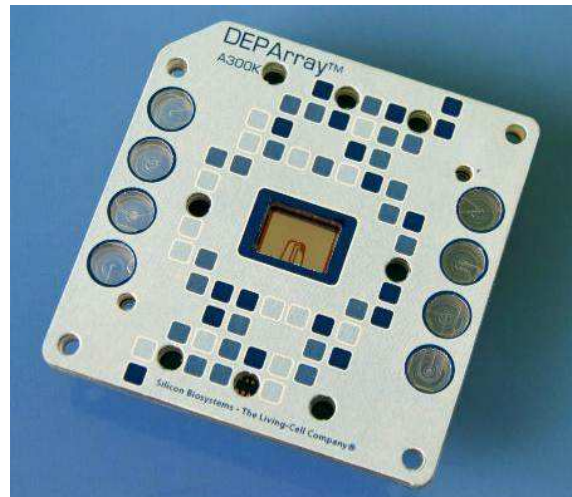


Figure 1: DEPArray™ chip. External size: 76mm x 76mm; active area: 12,8mm x 9,6mm (640x480 pixels; pitch 20 μm x20 μm).

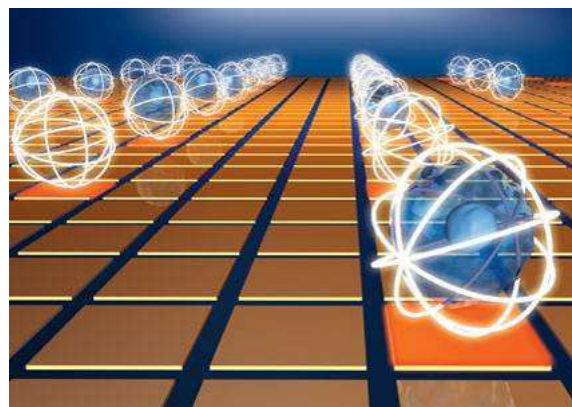


Figure 2: Sketch representing dielectrophoretic (DEP) cages entrapping cells in stable levitation. According to the theory of Moving DEP Cages (described in [1]) the electrodes (bright orange) hosting the cages are energized using AC electrical signals in-phase with the lid which is transparent and conductive; the electrodes surrounding the cages are energized using counter-phase signals.

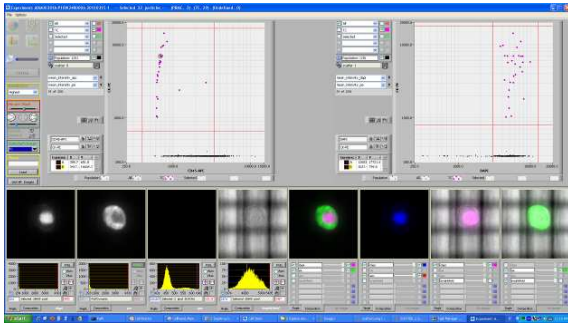


Figure 3: Image of software GUI showing two scatter plots: CK vs CD45 on the left and CK vs DAPI on the right. For each event in the scatter plot a gallery of images is available enabling morphological analysis. Tumor cells are DAPI and CK positive but CD45 negative. A tumor cells is shown in the image gallery. The image sequence is the following: (1) DAPI, (2) CK, (3) CD45, (4) Bright-field, (5) DAPI + CK, (6) DAPI + CD45, (7) Bright-field + DAPI, (8) Bright-field + CK.

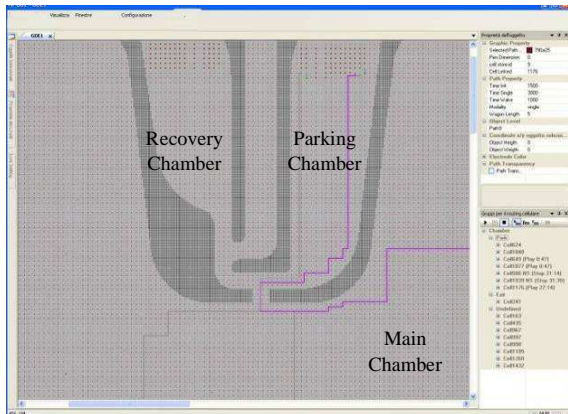


Figure 4: Image of software GUI dedicated to control the routing phase. During the routing cells moves from the main chamber towards the Parking chamber; trajectories are shown in figure.

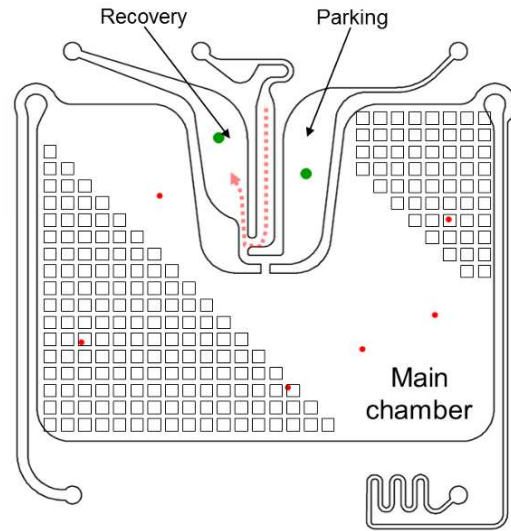


Figure 5: Microfluidic structures integrated within the chip are sketched in figure. The Parking and Recovery chambers are filled with clean buffer while the Main chamber is filled with sample. Once in the Recovery chamber target cells are ejected directly from the device into the recovery support (multi-well, tube, slide, etc.) by flowing clean buffer in the Recovery chamber as shown in the figure (dotted red path).