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Label-free Sorting and Counting of Yeast Cells for Viability Studies

G. Mernier[a,*,**], N. Piacentini[a,b,**], R. Tornay[a], N. Buffi[a] and P. Renaud[a]

[a] *STI-IMT-LMIS4, Ecole Polytechnique Fédérale de Lausanne, Station 17, CH-1015 Lausanne, Switzerland*

[b] *Dipartimento di Elettronica, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy*

Abstract

This paper reports the new combination of cell sorting and counting capabilities on a single device. Most state-of-the-art devices combining these technologies use optical techniques requiring complicate experimental setups and labeled samples. The use of a label-free, electrical device significantly decreases the system complexity and makes it more appropriate for use in point-of-care diagnostics.

Living and dead yeast cells are separated by dielectrophoretic forces and counted using coulter counters. The combination of these two methods allows the determination of the percentage of living and dead cells for viability studies of cell samples. It could further be used for sorting and counting of blood cells in applications such as diagnosis of insufficient cell concentrations, identification of cell deficiencies or bacterial contamination. The use of dielectrophoresis (DEP) as sorting principle allows to separate cells based on their dielectric properties in place of size-based separation, enabling sorting of large panels of cells and separation of infected and non-infected cells of the same type.

Keywords: Cell Sorting; Cell counting; Dielectrophoresis; Impedance Measurements; Label-free; Viability study

1. Introduction

Recent advances in microtechnology have enabled the fabrication of devices integrating fluidic, electrical and optical components for biological applications. These lab-on-chips make use of different techniques enabling sample preparation and analysis. In sample preparation, one important technique is the sorting of cells which can be performed optically or electrically, using among others dielectrophoresis[1-2], magnetophoresis[3-4], fluorescence-activated cell sorting[5] or optical tweezers[6]. On the other hand, on-chip analysis can be also integrated on chip, the most common example of which being cell counting. This counting can rely either on optical monitoring of a specific position on the chip[5,7], or on electrical impedance measurements. The latter technique is widely known in its coulter counter configuration, first miniaturised by Larsen et al.[8].

This paper presents a lab-on-chip integrating cell sorting and counting abilities, based on dielectrophoresis and coulter counting respectively. To the best of our knowledge, this is the first example of a fully electric system, which has the advantages of a label-free protocol and a relatively simple experimental setup. This device allows determining the concentration of cell subpopulations in a sample, with applications such as viability studies and differential cell counting for point-of-care diagnosis.

* Corresponding author. Tel.: +41-(0)21-693-6581; fax: +41-(0)21-693-5950. *E-mail address*: guillaume.mernier@epfl.ch

** Both authors contributed equally

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2. Materials and Methods

2.1. Chip design, fabrication and packaging

In order to integrate cell sorting and counting, a device is developed using the design shown in Figure 1, featuring microfluidic channels and electrodes for dielectrophoresis and impedance measurements. The fabrication of the device is described elsewhere[9] and briefly explained here. Platinum electrodes are deposited on a titanium adhesion layer by evaporation and patterned by lift-off. Microfluidic channels are then defined using SU8 photolithography. A silicone elastomer block placed on top seals the fluidic network and allows access to the inlets and outlets via a plastic holder. Pressure-driven flow is regulated by a pressure box, as described by Braschler et al.[10].

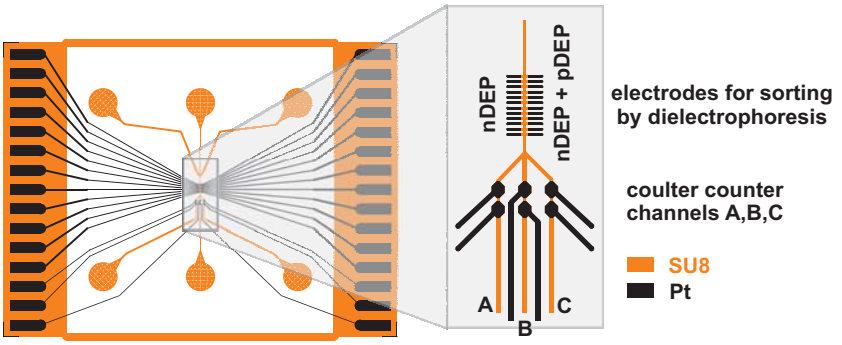

Fig. 1: Schematic of the chip design, including the microfluidic channels in SU8 and the platinum electrodes

2.2. Protocols

A mixed population of living and dead yeast cells (Baker's Yeast, *Saccharomyces cerevisiae*) is prepared in Phosphate Buffer Saline (PBS) solution with a conductivity of 55 mS/m. Dead yeast cells are obtained by heating a living sample for 30 min at 90 °C. A standard protocol for exclusion assay is used to stain dead cells with trypan blue for visual identification.

3. Experimental results

3.1. Cell sorting

After flowing between the sorting electrodes, the cells are separated with respect to their different dielectric properties at specific frequencies, as discussed by Demierre et al.[2]. Low-frequency signals on both sides of the channel focus the cell stream in an equilibrium position while a high-frequency signal is added on one side to separate the different cells (see Table 1). The sorted populations are separated in different channels, as shown in Figure 2.

Table 1: Amplitude and frequency of the voltages used for cell sorting and counting.

Signals	Amplitude	Frequency
nDEP (left)	1 V_{pp}	200 kHz
nDEP (right)	2 V_{pp}	200 kHz
pDEP (right)	2 V_{pp}	5.05 MHz
Counting signal	1 V_{pp}	105 kHz

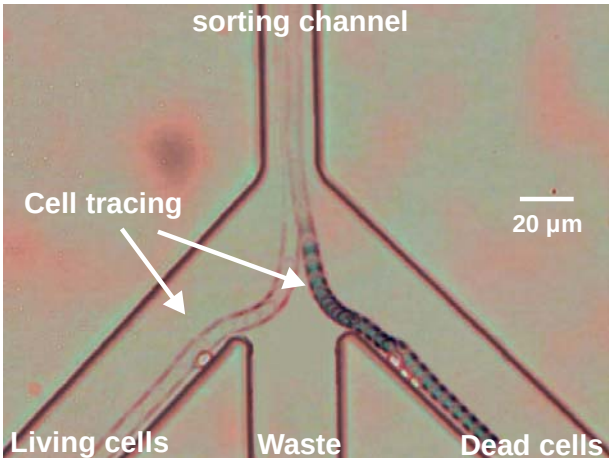

Fig. 2: Superposition of consecutive video frames, showing the trajectory of a non-colored living cell (left) and a colored dead cell (right) sorted by dielectrophoresis

3.2. Cell counting

After sorting, the cells flow through microelectrodes arranged in coulter counter configuration, where their respective number is determined by impedance measurements. A voltage is applied at the counting electrodes and the measured current is converted into voltage, amplified and differentiated between the left and right channels. As the final measurement is the difference between the two channel signals, a cell passing through one channel causes an increase in the voltage whereas a cell passing through the other channel decreases it, as shown in Figure 3.

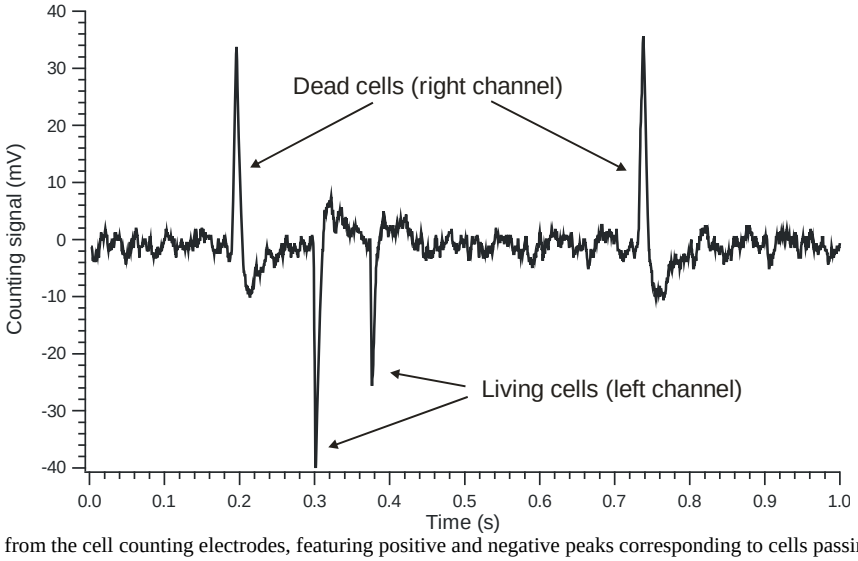

Fig. 3: Signal recorded from the cell counting electrodes, featuring positive and negative peaks corresponding to cells passing in the right and left channels

3.3.Viability determination

Proper signal analysis allows automatic counting of the population of living and dead cells, providing a measurement of sample viability. In our experiment 399 cells were counted of which 133 were alive and 165 were dead, giving a viability of 45%.

4. Conclusions

This paper described the design and fabrication of a chip capable of both electrical cell sorting and counting using the combination of a dielectrophoretic separation and coulter counting. This device was used to evaluate the viability of a mixed sample of living and dead yeast cells. The advantages of such a system are the use of a label-free realtime analysis which is not modifying the sample, and the relatively simple experimental setup without optical components. Moreover, the sorting technique used allows a more subtle separation than the traditional size-based methods, enabling differentiation of cells depending on their dielectric properties. As the sorting and counting can be performed in a fast and automatic way, this lab-on-chip is particularly adapted for point-of-care applications such as diagnosis of insufficient cell concentrations, identification of cell deficiencies or bacterial contamination.

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