YEAST CELLS DETECTION IN A VERY FAST AND HIGHLY VERSATILE MICROFABRICATED CYTOMETER

R. Rodriguez-Trujillo[1,2] , O. Castillo-Fernandez[1,2] , M. Arundell[1,2,3] , J. Samitier[1,2,3] and G. Gomila[1,2,3] ,

[1]Institute for Bioengineering of Catalonia (IBEC), SPAIN, [2]University of Barcelona, SPAIN and [3]Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN),SPAIN

ABSTRACT

A novel microfluidic chip able to detect a wide range of different cell sizes at very high rates is reported. The device uses two-dimensional hydrodynamic focusing [1] of the sample (conducting) flow by three non-conducting flows and high-speed differential impedance detection electronics. High-speed counting of 15μm polystyrene particles and 5μm yeast cells with a rate of up to 1000 particles/s has been demonstrated. Using of two-dimensional focusing effect turn out to be essential in a device with very large cross-sectional area (100x43 μm^2) in which particles result undetectable in the absence of focusing.

KEYWORDS: Microfluidics, Polydimethylsiloxane, Impedance, Coulter-counter

INTRODUCTION

Micro-cytometers using electrical sensing techniques (known as micro-Coulter counters) usually work with a fixed aperture that cannot be changed once the device is fabricated [2,3]. In this type of devices, the smaller particle that can be detected is limited by the signal to noise ratio. New fabrication is required when objects with a smaller size want to be detected. The device proposed here allows enlarging the lower limit of particle sizes detectable using a single device. This is achieved by reducing the dimensions of the electrical sensing zone with the help of the 2D-hydrodynamic focusing.

A simpler device based on this concept was previously reported by our group [4] by just focusing samples in one dimension. The present device implies a great advance with respect to [4] as the electrical response is perfectly adjusted to the particle thus increasing dramatically the sensitivity and versatility of the device.

EXPERIMENTAL

A scheme of the structure of the microdevice is shown in Fig. 1. Channels were fabricated in Polydimethylsiloxane (PDMS) and sealed to a cover glass patterned with an array of six Ti–Au electrodes. The chip has three different inlets converging to a single outlet. Two-dimensional focusing is achieved by means of an additional orifice drilled in the central inlet channel (Fig. 1). All channels are 100 μm wide and 43 μm deep. Electrodes are 40 μm wide and 40 μm separated. Fabrication of the device followed the standard soft lithography protocol and has been described in detail elsewhere [4]. A photograph of the completed device is shown in Fig. 2a.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

A confocal microscopy image of a section transversal to the direction of the flux in the outlet channel is shown in Fig. 2b. The effect of the 2D-focusing of the sample flux (fluorescently marked) can be appreciated.

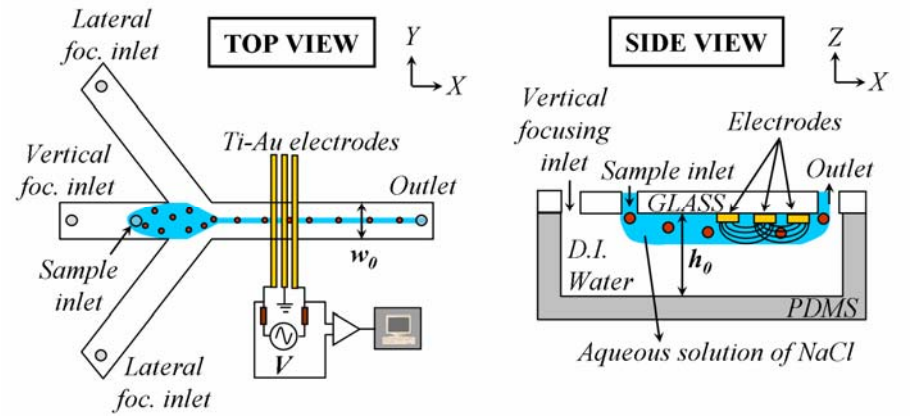

Figure 1. Structure of the fabricated micro-Coulter counter. Top and side views.

RESULTS AND DISCUSSION

First, we have validated our device with the high-speed counting of polystyrene particles 15 μm in diameter (Fig. 3a). Very short transition times of about 1 ms give to this system the possibility to count up to 1000 particles/s. The excellent signal to noise ratio is due to the confinement of the resistive response of the liquid to the sample flow of very small dimensions (~20x20 μm^2). Secondly, the exceptional versatility of our microcytometer has been shown by the high-speed detection of yeast cells as small as 5 μm in a channel with a large cross-sectional area of 100x43 μm^2 (Fig. 3b).

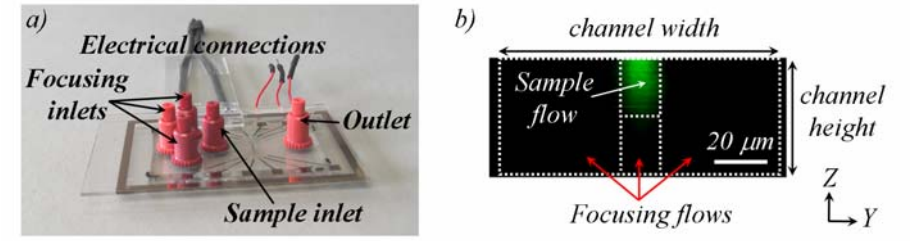

Figure 2. a) Photograph of the completed device. b) Confocal microscopy image of a section in the measurement channel showing the 2D focusing effect.

Further increase of the focalization stability conditions, together with an improvement in the electronic noise screening could increase these limits. This would imply an incredibly high versatility for our system being able to detect from big mammalian cells 20-30 μm in diameter to some types of bacteria using a single chip.

This property of our devices simplifies the design and fabrication process of microcytometers for on-chip applications, and the realization of the corresponding experiments, as one can use always the same design and experimental set up to address an enormous variety of cell counting measurements.

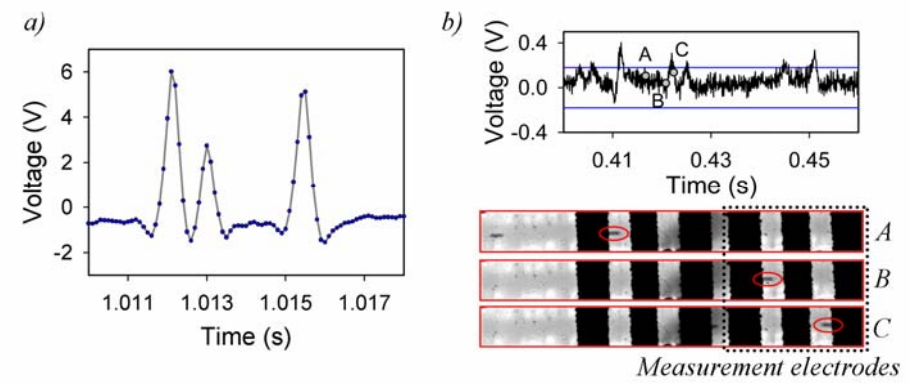

Figure 3. a) Eight milliseconds of signal acquisition corresponding to the transitions of three 15 μm polystyrene beads. b) Synchronized electrical (top) and optical (bottom) measurements of the passage of yeast cells (S.Cervisiae).

CONCLUSIONS

An aperture-adjustable micro-Coulter counter has been fabricated and characterized. The small sample flow defining the aperture concentrates the resistive response of the device thus largely increasing its sensitivity and versatility. Very high-speed counting of 15 μm polystyrene particles and 5μm yeast cells has been performed within a channel cross-sectional area as large as 100x43 μm^2.

ACKNOWLEDGEMENTS

This research was supported by the Spanish Ministry of Science under project TEC2004-06514-C03-02. We thank M.C. Jaramillo from the group of Prof. A. Juarez at the IBEC for yeast cell sample preparation. We thank M. Garrido from Univ. Barcelona for development of the electronic instrumentation used in this work.

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