LOW COST CYTOMETER BASED ON A DVD PICKUP HEAD

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ABSTRACT

Cytometry is a process that allows for measuring characteristics of single biological cells [1]. In this paper we report on a novel cytometric cell detector using a DVD pickup head. The system uses a microfluidic chip with a flow cell that allows for hydrodynamic focusing of the sample flow. The reflected optical signal intensity is influenced by particles that pass the beam and is measured directly in the pickup. Measurement results on a mixture containing erythrocytes and polystyrene particles are presented and the cytometric separation is shown in a two-parameter histogram.

Keywords: Cytometer, Single Cell Detection, Cell Counter, Digital Versatile Disk

1. INTRODUCTION

In optical flow cytometry characteristics of single biological cells are determined as light from a laser beam is scattered by the cells. Depending on the angle of the scattered light, the measured intensities can be related to biological or physical parameters of the cell. Often fluorescent dyes are used for labeling specific cells in a population. A common technique for cell counting is to add counting beads to a cell suspension that has different cytometric output. In a histogram the ratio between cells and counting beads can be used to calculate the density of the cell population. In this paper we present a low cost system that allows for optical detection of cells and particles.

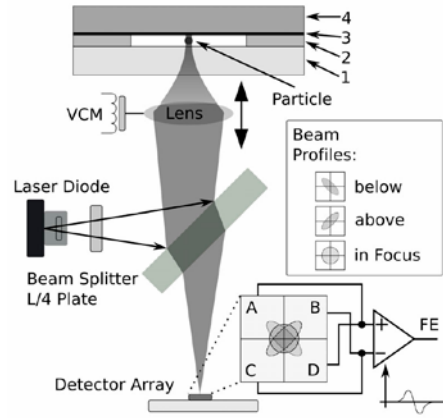

Figure 1: Principle of the cytometer: A laser beam is focused into a microchannel where it interferes with a particle and is reflected back to a detector array. A voice coil motor (VCM) is used to adjust the focus onto the reflective surface (3) utilizing the focus error (FE) signal which originates from the elliptic beam profile. The microfluidic chip consists of 1: transparent glass slide, 2: SU-8 layer, 3: mirror, 4: silicon wafer.

2. EXPERIMENTAL

A laser diode is used to generate an optical beam (650 nm) that passes a beam splitter and is focused to a small spot on a reflective surface inside a microchannel (Fig. 1). The position of the lens can be controlled using a voice coil motor (VCM). After reflection the beam passes the splitter again and propagates towards a four quadrant photodiode array that is used to measure the reflected intensity. An astigmatic distortion causes the beam profile at the detector to be circular only if the beam is reflected at its focal point. If not, the profile

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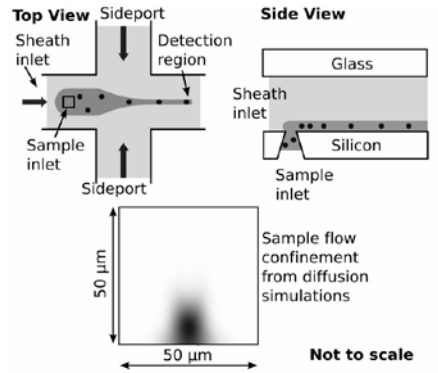

Figure 2: Flow cell for sample injection. Sheath liquid from back and side squeezes the sample closely over the mirror. The sample flow confinement for sample 5µl/min, sheath 40µl/min, and side 60µl/min is given in the cross section of the channel.

back and side squeeze the sample flow to a close distance to the reflective surface using syringe pumps. In FEM studies the optimum flow rates have been determined that result in a sample flow cross section given in Fig. 2.
Fig. 3 shows the fabricated microfluidic chip with channel dimensions of 50 µm x 50 µm at the detection region. It was fabricated using SU-8 wafer bonding technology.

3. RESULTS AND DISCUSSION

The principal operation of the device has been shown with measurements on polystyrene beads in flow and on cells that were adhered on a mirror surface [3]. Now we show for the first time measurements with biological cells (erythrocytes from cattle) in the flow. Fig. 4 shows the sorted sensor responses for a sample population containing both, red blood cells (left) and polystyrene particles (diameter 8 µm, right). Both types can be clearly distinguished by their signal shape. Moreover, a two-parameter histogram (minimum and maximum signal intensity) shows the clear separation between both subpopulations (Fig. 5).

becomes elliptic with the principal axis depending on the sign of the focus error (FE). This allows for generating an electric signal proportional to FE by summing up: A+D-B-C as shown in Fig. 1.

In the experiments the reflected intensity signal changes with each cell or particle that influences the optical path. Due to the high numerical aperture of the beam (NA=0.6) it is important to have control over the position at which the particles hit the beam. Therefore a flow cell (Fig. 2) has been designed that allows for precise positioning of the sample flow in the detection region [2]. Sheath liquid from the

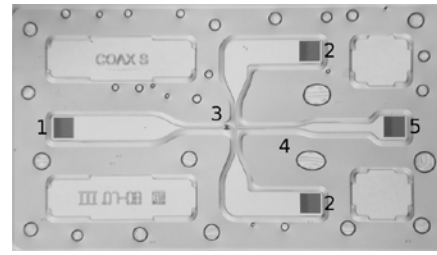

Figure 3: Image of the fabricated device (9 mm x 13 mm). 1: sheath inlet, 2: side inlets, 3: sample inlet, 4: detection region, 5: outlet.

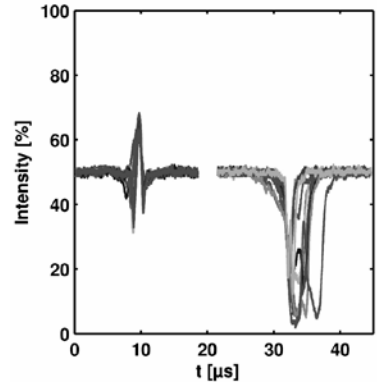

Figure 4: Sensor readings for erythrocytes (left) and polystyrene counting beads (right). The beads cause an intensity drop while erythrocytes cause a signal rise.

4. CONCLUSIONS

We have successfully shown the cytometric separation of erythrocytes from polystyrene counting beads in a two-parameter histogram utilizing a DVD pickup system. Our current research is focused on recognizing different cell types and automation of our experimental setup.

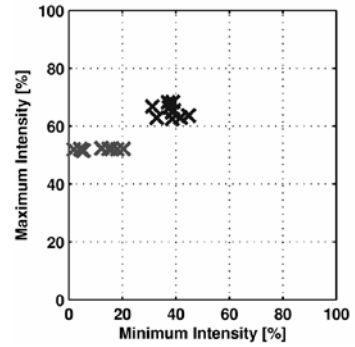

Figure 5: Histogram for the measurements from Fig. 4. The parameters are minimum intensity and maximum intensity of the peaks. The plot shows clearly two sub-populations in the sample.

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