On-chip Cytometric Detection of Single Biological Cells Using Integrated Photodiodes

S. Kostner, M. J. Vellekoop

Institute of Sensor and Actuator Systems
Vienna University of Technology, Gusshausstrasse 27-29
1040 Wien

In this contribution we present the principle and measurement results on cytometric detection of single biological cells using integrated photodiodes. The sensing element is built into a microfluidic flow cell that allows for positioning the cells directly over the sensor. Additionally to results with calibrated polystyrene beads we show sensor readings for yeast cells. Experiments show that those cells actually act like lenses, focusing the incoming light to the sensor.

Introduction

Cytometry is a measurement process in which physical or (bio-) chemical information about single cells is obtained [1]. Several methods can be found in literature like optical and impedance based techniques which are used to determine parameters like cell size/shape, protein content, electric conductivity and viability. Measuring single cells yields statistical information from a given sample that is usually hidden when the sample is measured in suspension. For obtaining this statistical information tens of thousands of particles have to be measured in a relatively short time period (for instance 10^6 events/second).

A typical optical setup for flow cytometry consists of a light source, a test volume, and one or more sensing elements. Usually the reflected, transmitted and/or absorbed light fraction is of interest and can be related to a physical parameter of the test substance. Very often the relation between wanted parameters and physical sensor readings is found in an empiric way by experiment.

Using microfabrication techniques the volume of the test channel can be made small enough so that suspended objects like small particles or biological cells can be detected as single events. The natural limitation to the miniaturization of these optical systems is diffraction: the size range of particles and photosensitive area cannot be much smaller than the wavelength used.

Compared to other systems our approach for gaining optical information about the cells is different in the sense that we place the photosensitive element directly into the analysis channel, thus sensing in the optical near field of the particle. This allows for obtaining information about the particle that is usually hidden for systems that pick up the far field only (like FACS systems).

Sensor Design and Technology

In order to produce repeatable sensor readings it is necessary not only to have a proper sensor layout but also to have means to control how the particles are being de-

livered to the sensor. Therefore a flow cell was designed that provides position control in 3D for the particles that move over the sensor [2]. This is achieved by generating a non-coaxial sheath flow that can be controlled by applying different flow rates to the fluidic ports of the device.

Figure 1 shows the principle of the sensor that consists of a strip photodiode (2 µm x 50 µm) that is arranged perpendicular to the flow channel which has a width of 150 µm. The device is a sandwich consisting of a bottom silicon wafer (with the integrated photodiodes; 1 µm standard bipolar process) and a top glass slide which are bonded together by an intermediate SU-8 layer (height: 70 µm) forming the fluidic structure [3]. The through-holes for the fluidic connection of the chip were etched in Si from the bottom.

When a particle moves over the sensor a change in the photocurrent or voltage can be measured that is proportional to the shadow area produced by the particle [4].

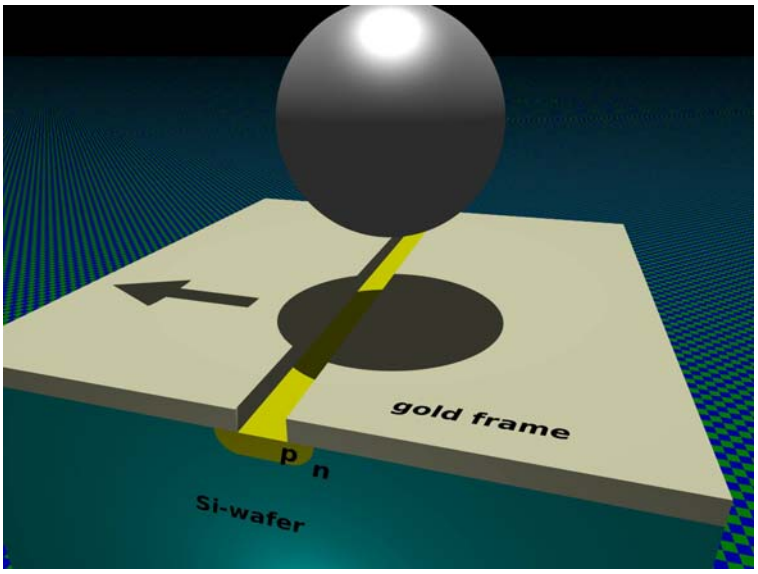

Fig. 1: Principle of the sensor with the integrated photodiodes. The arrow indicates the flow direction. The non-transparent particle produces a shadow on the light sensitive area which causes a signal drop.

Experimental Setup

The chip was clamped onto a custom made device holder that provides the electrical and fluidic connections. Syringe pumps with carefully chosen flow rates were used to apply a sheath flow to force the particles/cells closely over the photosensitive area. A modulated green laser source (592 nm, Roithner Lasertechnik GLMC1-10) was used to illuminate the chip from the top through the cover glass slice. The sensor signal (photodiodes in photovoltaic mode) was measured and demodulated with a lock in amplifier (Signal Recovery 7280). The demodulated signal was recorded with a digital storage oscilloscope. The whole setup was mounted on a solid construction in order to suppress vibrations in the optical path.

Results and Discussion

First experiments with polystyrene test particles of 20 µm and 24 µm diameter were carried out for the calibration of the sensor and published in [5]. In this contribution we show the use of the same system for the detection of yeast cells suspended in physiologic solution.

Figure 2 (left) shows sensor readings from experiments with polystyrene beads. The silver coated particles generate a negative pulse in voltage as expected since the shadow of the particle covers part of the photodiode. The transparent particles (plain polystyrene) show an increase in the sensor signal which can be explained by a “lens effect” where the particle actually focuses the light to a bright spot on the sensor area (Fig. 2 right).

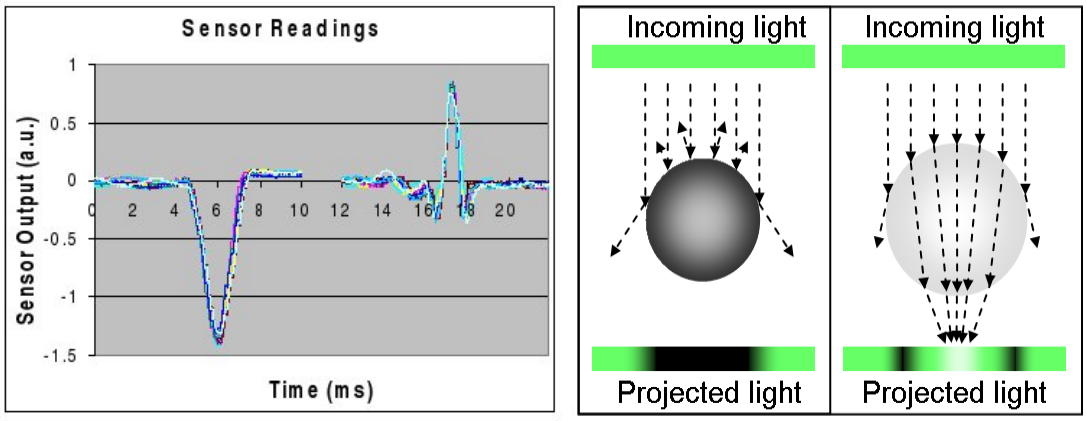

Fig. 2: Sensor readings from experiments with polystyrene beads (left). Silver coated particles cause a signal drop, while transparent plain particles show a signal increase. This behavior can be explained by a lens effect where a transparent particle focuses the incoming light to a bright spot, thus increasing the light intensity in the center of the projection (right) [5].

Because of this lens effect the intensity of the projected light depends on the height at which the particle passes the sensor. For repeatable results it is very important to keep this height constant.

With the polystyrene particle measurements as a reference, the sensor was now operated with suspended yeast cells. These measurements are more difficult because the cells are much smaller (about 3 – 5 µm diameter) and there is variation in some properties compared to calibration beads: the variation in size causes uncertainties, also the shape is not perfectly spherical and the surface is not well defined. As a result the focused beam has uncertainties in the focal length (caused by shape and size variation) and the intensity (absorption and diffraction at the irregular surface).

Measurement results are shown in Fig. 3. The cells are very well detectable but the SNR in the system is lower compared to measurements with the large polystyrene particles. Very interesting is the fact that those cells cause a positive peak which indicates their transparent character.

Conclusions

We have successfully demonstrated the optical detection of yeast cells in a projection cytometer. In our current setup we were able to detect particles down to the size of

yeast cells with the sensor (photodiode) placed in the near field of the particle. Non-transparent particles produce a drop in the sensor signal as expected due to their shadow. In the case of (semi-) transparent particles an increased sensor signal was observed which is explained by a lens effect. Measurements with yeast cells showed a similar sensor output (positive peaks) which indicates their transparent character.

We conclude that this analysis method can be used to distinguish different types of cells by their optical transmission and absorption properties.

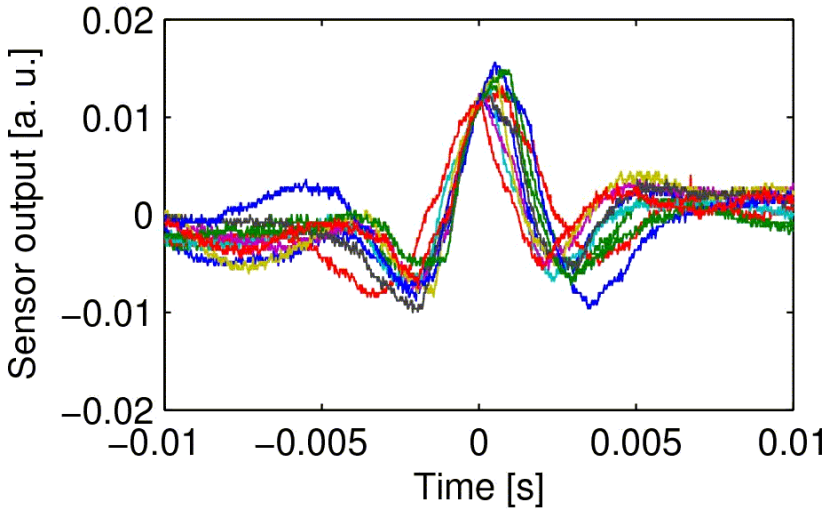

Fig. 3: Measurement results from experiments with yeast cells. The positive peak indicates the transparent character of the cells.

Acknowledgement

The chips were made in cooperation with the Delft University of Technology and co-funded by the Technologiestichting STW (project DPC.5521).

References

[1] H. M. Shapiro, “Practical Flow Cytometry”, 4th ed., 2003, Wiley & Sons

[2] J.H. Nieuwenhuis, J. Bastemeijer, P.M. Sarro, and M.J. Vellekoop, “Integrated Flow-Cells for Novel Adjustable Sheath flows”, Lab on a Chip, vol. 3, pp.56-61, 2003

[3] P. Svasek, E. Svasek, B. Lendl, and M. Vellekoop, “Fabrication of miniaturized fluidic devices using SU-8 based lithography and low temperature wafer bonding”, Sensors and Actuators A, vol. 115, pp. 591-599, 2004

[4] J.H. Nieuwenhuis, J. Bastemeijer, A. Bossche, and M.J. Vellekoop, “Near-Field Optical Sensors for Particle Shape Measurements”, IEEE Sensors Journal, vol. 3, pp. 646-651, 2003

[5] J.H. Nieuwenhuis, P. Svasek, P.M. Sarro, and M.J. Vellekoop, “Particle Discrimination with an Improved Projection Cytometer”, Proc. of μTAS 2004, Malmö, Sweden