*Optica Applicata, Vol. XLI, No. 2, 2011*

# Optoelectronic bacteria cells detection system

JACEK GRZELKA[1], RAFAŁ SZCZYPIŃSKI[1], DOROTA G. PIJANOWSKA[1, 2], REMIGIUSZ GRODECKI[2], JAN LESIŃSKI[2], PIOTR GRABIEC[2], JAN M. ŁYSKO[2*]

[1]Nałęcz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, ul. Ks. Trojdena 4, 02-109 Warszawa, Poland

[2]Institute of Electron Technology, al. Lotników 32/46, 02-668 Warszawa, Poland

[*]Corresponding author: jmlysko@ite.waw.pl

In general flow cytometry enables the characterization of individual cells/microparticles. When a sample is injected into a flow cytometer, the randomly distributed cells are ordered into a stream of single cells. Hence, they can be individually analyzed in the detection system. In this paper, an optoelectronic compact and portable system for bacteria cells detection is described. The system consists of PDMS (polydimethylsiloxane) microfluidic chip, a duralumin holder with microfluidic connectors – inlets/outlets, as well as optic fiber connectors and waveguides for laser diode and photodetectors (avalanche photodiode BPYP 59 or photomultiplier 9658B). The fluorochrome is excited by light beam of an appropriate wavelength coming from a light source, *e.g.*, the laser diode. The excitation light passes through hydrodynamically focused cells in the middle of the channel. After optical filtering, the light emitted by the fluorochrome is detected by a photodetector. Electrical signals from the selective nanovoltmeter are amplified and collected by the computer electronic system. Since in the real sample bacteria cells are selectively labeled with the fluorophores or antibodies conjugated with fluorochrome, in our experiments to evaluate lower detection limits of the developed detection system, exemplary fluorescent dye solutions of sodium fluorescein were used as testing solutions.

Keywords: microfluidics, optoelectronic detection, bacteria detection.

## 1. Introduction

Flow cytometry is a very popular method enabling the characterization of fluorescent--marked cells/microparticles. When a sample is injected into a flow cytometer and hydrodynamically focused by surrounding sheath flow, the randomly distributed cells are ordered into a stream of single cells. Hence, they can be individually analyzed in the detection system. The hydrodynamically focused cells are illuminated by the excitation light passing through the middle of the channel. After optical filtering, the light emitted by the fluorochrome is detected by the photodetector. Electrical signals from the voltmeter are amplified and collected by the computer electronic

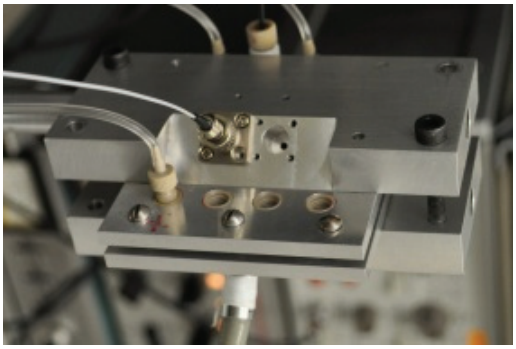

Fig. 1. Duralumin holder with microfluidic inlets/outlets and fiber optic connectors to the light source (diode or laser) and optical detectors (avalanche photodiode or photomultiplier). The holder was designed to fix a PDMS chip with two symmetrical microchannel layouts.

system. In our experiments, an exemplary fluorescent dye – sodium fluorescein (SF) solutions were used as testing solutions to evaluate lower detection limits of the developed detection system.

In this paper, an optoelectronic compact, portable and cheap system for bacteria cells detection is described. The system consists of PDMS (polydimethylsiloxane) microfluidic chip, a duralumin holder with microfluidic inlet/outlet connectors, as well as optic fiber connectors and waveguides linking the chip to the light sources (blue 460 LED diode or blue-violet 405 laser) and photodetectors (avalanche photodiode BPYP 59 or photomultiplier EMI 9658B), as presented in Fig. 1.

The first part of this paper explains the process behind the creating of cheap PDMS microstructures, which is a soft lithography. The second part presents the preliminary results concerning hydrodynamic focusing inside a 60 μm-wide microchannel.

## 2. Soft lithography

### 2.1. Introduction to soft lithography

Templates applied in soft lithography can be fabricated in different technologies (*e.g.*, UV, e-beam or X-ray photolithography, and DRIE – Bosch process) from various materials like: silicon and polymers. A very popular ones are SU-8 templates on rigid substrates, *e.g.*, silicon [1], or without rigid substrates (soft template). Soft templates are fabricated by dissolving a sacrificial polymeric layer which is located between substrate and crosslinked master of SU-8 [2].

The SU-8 negative photoresist based on EPON resin was patented in 1989 by IBM company and in the 1990s applied in MEMS and lab-on-a-chip technology. Since 1996 SU-8 is produced by MicroChem in three main ranges: SU-8, SU-8 2000, SU-8 3000 and XP Micro Spray (MicroChem) for special purposes. All of them contain epoxy resin monomer, photoacid generator (PGA): triarylsulfonium hexafluoroantimonate salt and solvent $\gamma$-butyrolactone (GBL) or cyclopentanone (CP). After UV irradiation,

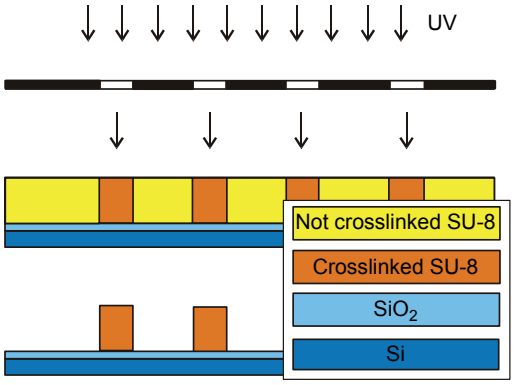


Fig. 2. The general principle of SU-8 UV photolithography.

a photoacid generator decomposes to generate Lewis acid which initiates cationic polymerization of monomer by opening epoxy groups. The reaction is stimulated by post-exposure baking (PEB). GBL has higher boiling point (204 °C) in comparison to CP (131 °C). That gives the possibility of longer planarization after spin-coating.

## 2.2. Technology

Materials: negative epoxy photoresist SU-8 (25, 50) (MicroChem), SU-8 developer (MicroChem), isopropanol, $H_2SO_4$, $H_2O_2$ (POCh), $N_2$.

SU-8 templates for soft lithography were fabricated from epoxy negative photoresist SU-8 (MicroChem) on silicon substrates (Fig. 2). Silicon 4" wafers were cleaned in piranha solution ($H_2SO_4$, $H_2O_2$) and to improve photoresist adhesion to the substrate, thermally oxidized ($xSiO_2$ = 500–1200 Å). First, a thin 15–20 µm polymeric SU-8/25 layer was spin-coated on wafers using Convac spin-coater 500(5 s)/2600–2620(30 s), then planarized, soft-backed, exposed to UV light (365 nm, 200 mJ/cm$^2$) and post-exposure backed (PEB). Next, a constructive layer was fabricated from SU 8/50, spin-coated with different velocity in the case of expected thickness. To obtain 70 µm structures, the photoresist was spin-coated on the wafers with parameters 500(10 s)/1500(30 s) using Convac, planarized, soft-backed, exposed to UV light – 365 nm and power density 350 mJ/cm$^2$, and finally post-exposure backed. Before use, SU-8 was degassed at room temperature and under atmospheric pressure for over 24 hours. All heating procedures were performed on a hot plate with

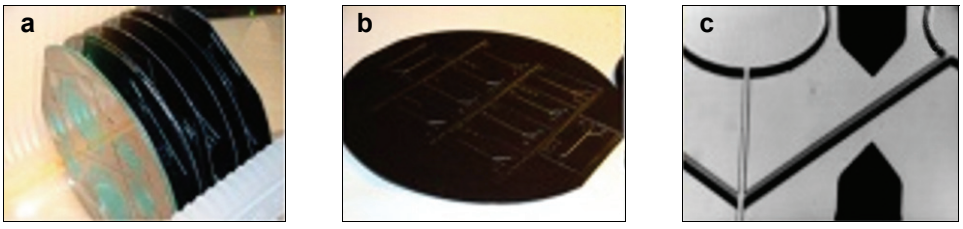


Fig. 3. SU-8 templates for PDMS soft lithography fabricated by UV photolithography: **a**, **b** – general view, **c** – different shapes of microchannels inflows.

ramping. The templates were developed in SU-8 developer (MicroChem), soaked in isopropanol and hard backed (HB) at 150 °C. The hard baking allowed to avoid cracking of microfluidic structures. After post-processing thermal treatment, templates with low stress polymeric structures were obtained. Figure 3 presents the examples of SU-8 templates made on 4" Si wafers.

The SU-8 templates were used as substrates in the process of soft lithography to create cheap PDMS modules of a flow microcytometer [3].

## 3. Hydrodynamic focusing

The hydrodynamic focusing in the 60 μm-wide microchannel of a square cross-section was investigated. The central fluid with fluorescent dye was pumped through the central inlet using a syringe pump CMA 102, while the transparent sheath fluid (water) was fed through two outer inlets using a syringe pump Gemini 88 KD Scientific. The sheath flow rate was ranging from 0 to 80 μl/min, while the central stream flow rate was constant – 10 μl/min. The width of the focused central stream was determined by means of an optical microscope Olympus SZX 16 with a digital camera Olympus e520 attached, as well as by fluorescence measurement, assuming that the intensity of the light emitted from the illuminated microchannel area was proportional to the central fluid stream width. The example photograph of hydrodynamic focusing is shown in Fig. 4. The observed focused stream area and the microchannel junction were 7 mm apart.

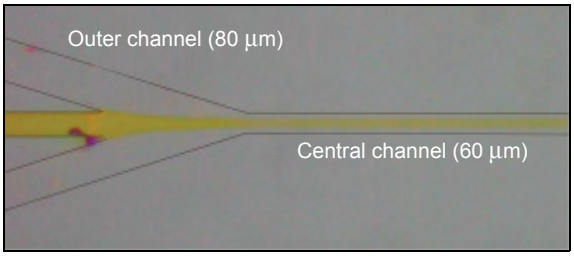


Fig. 4. A photograph of hydrodynamic focusing of sodium fluorescein solution in the 60 μm wide microchannel. The microchannels are outlined.

The used fluorescent dye was sodium fluorescein (SF), which absorption maximum is ~490 nm and emission maximum is ~520 nm [4]. The light sources used for illumination were a 460 nm blue LED diode and a blue-violet 405 nm laser. In the case of diode excitation, two Carl–Zeiss bandwidth optical filters were used: 475 nm filter for excitation and 550 nm filter for emission. In the case of laser excitation, only 500 nm Carl–Zeiss bandwidth emission filter was used.

The results of the fluorescence measurement and the geometrical analysis of the central fluid width are presented in Figs. 5 and 6, respectively.

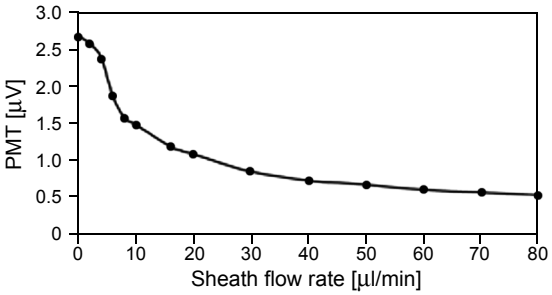


Fig. 5. Dependence of measured fluorescence intensity on the sheath flow rate. The sodium fluorescein concentration was 450 µg/ml, the central fluid flow rate was 10 µl/min. Light source – blue LED diode.

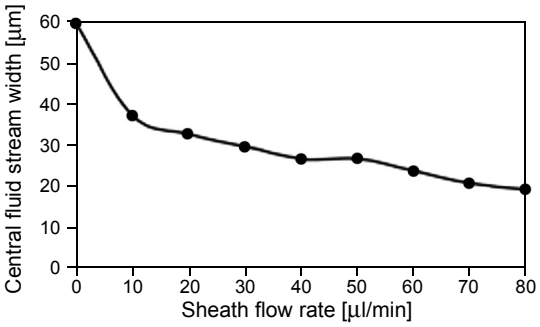


Fig. 6. Dependence of the central fluid stream width on the sheath flow rate. The central fluid flow rate was 10 µl/min. The width was measured based on the photographs obtained by the digital camera (see Fig. 4).

The effectiveness of two light sources was evaluated. The high concentration sodium fluorescein solutions (~500 µg/ml) were hydrodynamically focused and illuminated with a blue-violet laser and a blue LED diode. The central fluid flow rate was set at 10 µl/min. Additionally, with no fluorescent dye present in the channel, the background noise was measured. The results are presented in the Table. The LED diode is characterized by lower signal but higher signal-to-noise ratio.

T a b l e. Comparison of hydrodynamically focused high concentration fluorescent solution brightness, with two different light sources (405 nm laser and 460 nm diode).

| | PMT voltage [µV] | |
|---|---|---|
| Sheath flow rate [µl/min] | Blue-violet laser (667 µg/ml SF) | Blue LED diode (453 µg/ml SF) |
| 0 | 84 | 2.7 |
| 40 | 37 | 0.74 |
| 80 | 25 | 0.54 |
| Sheath fluid filling the whole channel | 28 | 0.5 |

## 4. Summary

We examined the hydrodynamical focusing of the fluorescent dye solution in a 60 μm wide PDMS microchannel. The first method was based on geometrical analysis of the central stream width in the photographs of the microchannel. The second method was based on the emitted light intensity measurements. In both cases, the obtained results were similar, however the first method is burdened with errors due to the blurred border between the central and sheath fluids. Below central to sheath fluid flow rates 1:8, the fluorescence intensity of focused 450 μg/ml sodium fluorescein solution became undistinguishable from the background noise (0.54 μV signal compared to ~0.5 μV background). It was stated that the blue LED diode was a better choice than the blue-violet laser for sodium fluorescein excitation, although not without drawbacks, such as for example broad light emission spectrum of the LED requiring the employment of an excitation optical filter.

*Acknowledgements* – Project financed by the Ministry of Science and Higher Education, part entitled *Development and Optimization of Demonstrator for the Biological Pollution Detection Technology Based on the Flow Cytometry Method*, (Project No.: PBZ/28-208-4.3.2/2007/WAT).

### References

[1] YU-JEN PAN, RUEY-JEN YANG, *Fabrication of UV epoxy resin masters for the replication of PDMS-based microchips*, Biomed Microdevices **9**(4), 2007, pp. 555–563.

[2] LUCAS N., SICHER P., BUTTGENBACH S., PENACHE C., BARTELS V., KLAGES C.-P., *Flexible master structures of SU-8 for double-sided moulding of PDMS*, Proceedings of the 5th Euspen International Conference, May 9–12, 2005, Montpellier, France, pp. 665–668.

[3] SZCZYPIŃSKI R., PIJANOWSKA D.G., *Technologia i zastosowanie poli(dimetylosiloksanu) – PDMS w mikroukładach analitycznych*, Elektronika **11**, 2008, pp. 249–253 (in Polish).

[4] SHAPIRO H.M., *Practical Flow Cytometry*, 4th Edition, Wiley–Liss, 2003, p. 45.

*Received September 25, 2010*