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Fluorescence detection in microfluidics systems

***Abstract.** In this paper, two optical detection systems – first based on the tube and the second one on the silicon photomultiplier are described. The detection system was tested for fluorescent dyes - sodium fluoresceinate and resorufin excitated in two systems: static and dynamic i.e. in PMMA cuvettes and PDMS made microchannels, respectively. Sources of excitation light were 488 and 532 nm wavelength laser diodes and blue/green LEDs (light emitting diodes). In the experiment tube and silicon photomultipliers applied in the above-mentioned systems were compared.*

Streszczenie**. Jako fotodetektory w układach mikroprzepływowych stosuje się urządzenia takie jak matryce CMOS, fotodiody lawinowe czy fotopowielacze lampowe. W ostatnim czasie prowadzone są intensywne badania nad zastosowaniem fotopowielaczy krzemowych jako fotodetektorów. Jest to spowodowane między innymi względami ekonomicznymi oraz dużo łatwiejszą ich mobilnością. W naszym artykule zaprezentowane zostały porównawcze wyniki badań dla dwóch fotopowielaczy – lampowego oraz krzemowego w zastosowaniach w układach mikrofluidycznych.**(Detekcja fluorescencji w układach mikrofluidycznych).

Keywords: silicon photomultiplier, fluorescence light detection, PDMS, microfluidic chip.

Słowa kluczowe: fotopowielacz krzemowy, detekcja fluorescencji, PDMS, układ mikroprzepływowy.

Introduction

In microfluidic based analytical devices for cells/micro particles counting or identification, optical detection systems are the most frequently used. In cells analysis, fluorescence techniques are dominant. In this case, fluorescent dye is used as a label conjugated with the cell/particle markers e.g. antibodies. The simplified detection system coupled with microchannel is shown in figure 1. After fluorescence dye excitation by the laser light of adequate wavelength, next the emitted fluorescence light is detected by the photomultiplier.

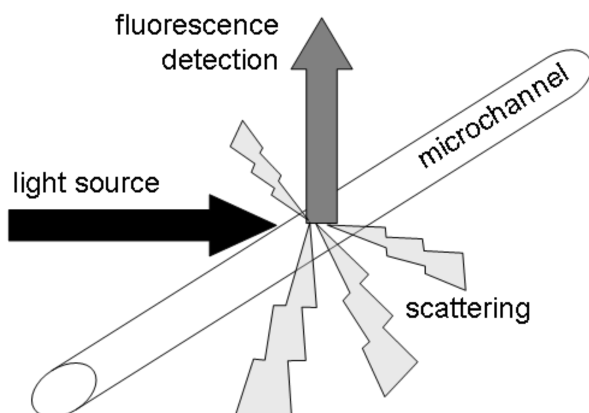

Fig. 1. Principle of the measurement of fluorescence light

Intensity of the emitted light is proportional to the concentration of the fluorescent dye on marked cells/microparticles [1, 2]. There are some fluorescent dyes that are commonly used. For example fluorescein isothiocyanate (FITC) has an absorption maximum at 494 nm and emission maximum of 518 nm, resorufin dye has two absorption peaks – first at 530 nm and its much lover than the second one which has maximum at 570 nm. The FITC can be excited by 488 nm laser while for resorufin 532 nm laser.

Over the years, with the progress of technology, systems have evolved to detect light of low intensity. A special role played by the progress in the development of optical sensors. For many years the leader in this field is photomultiplier tube, whose internal gain for single photon at the level 10^6–10^7 was unreachable by other types of photodetectors. Such gain is very important in the case of measuring the fluorescence in the microfluidic systems. Currently to detection of fluorescence light in these systems applies five types of photodetectors: photomultiplier tubes [3-5], avalanche photodiodes [3, 6], PIN photodiodes [7], image sensors (CCD, CMOS) [8, 9] and recently silicon photomultipliers [10, 11]. With the exception of tube and silicon photomultiplier, these elements require very complicated, low-noise amplifiers - only in this way the amplified signal could correspond to the number of detected photons. It has a significant impact on increasing costs of production equipment based on these sensors.

Recently, many research groups work on miniaturized analytical devices – for example micro flow cytometers [7, 12-20]. These kind of devices have many advantages – they are portable, cheaper and low power. Moreover, it can be combined with other microfluidic devices for multitask applications [21, 22]. Usually used material for fabrication of microfluidic devices is poly(dimethylsiloxane) – PDMS, that is easy for handling and great for optical measurements. It has some advantages – biocompatibility, transparency and it can be easily combined with other materials like glass, silicon or another PDMS plate. Detection lower limits achieved with particular measurements systems are presented in table 1.

As we can see in table 1, detection level of every system is different and depends on kind of photodetector, microchannel dimensions and investigated sample. However all results of investigation are similar. In general, the fluorescence light emitted by fluorescent dyes can be measured in two different measurement systems. In the first system, as the fluorescence detector was used photomultiplier tube – successfully used for many years inter alia in flow cytometry [20-22] and in the second one – silicon photomultiplier. Its application for the measurement of fluorescence light intensity has been presented in several publications in recent years [10, 23, 24].

In this paper, results concerning detection of fluorescence light in micro-flow system. To compare capabilities of two photodetectors for application in microfluidic systems, the fluorescence light emitted by model fluorescent dyes has been measured in cuvettes (static conditions) and flow-through microchannel (dynamic conditions) with two different measurement systems.

Table 1. Comparison of optical detection systems based on different kinds of photodetectors

Photodetector	Light source	Microchannel dimensions	Sample	Detection level	Reference
Photomultiplier tube	635 nm laser	50x200 µm	10.2 µm diameter labeled cells	$5x10^5$/mL	[6]
Avalanche photodiode	635 nm laser	50x200 µm	10,2 µm diameter labeled cells	$5x10^5$/mL	[6]
PIN photodiode	440 or 635 nm laser	100x300 µm	Yeast cells	$1.9x10^4$/mL	[7]
CCD image sensor	532 nm laser	30x300 µm	200 nm diameter beads	$18.5x10^3$/mL	[8]
CMOS camera image	532 nm laser	25x25 µm	2 µm diameter beads	0.1% weight concentration	[9]
Silicon photomultiplier	405 nm laser	30x300 µm	Leukocytes	$4x10^5$/mL	[10]
Avalanche photodiode	532 nm laser	150x200 µm	20 µm Leukemia cells	10^6/mL – cells 10 ng/mL – resorufin	[13]

Experimental

Measurement system based on tube photomultiplier

A PDMS microfluidic device for optical measurements was fabricated according to procedure described in [25]. Other methods of PDMS microchips fabrication were described elsewhere [26-29]. Depth and width of the microfluidic channel were 60 µm. Flow rate of fluorescent dye solution samples of different concentrations in range: 0.1 pg/mL - 1 µg/mL in the microchannel was 15 µL/min.

Two kinds of light sources were used for excitation of sodium fluoresceinate solutions – blue LED diode with 485 nm band pass filter and 450 nm laser diode, while a 532 nm laser diode as excitation light source for resorufin solutions was used. A tube photomultiplier was used as a photodetector. Data were collected in LabView System (National Instruments). Scheme of the optical detection system based on tube photomultiplier presents figure 2.

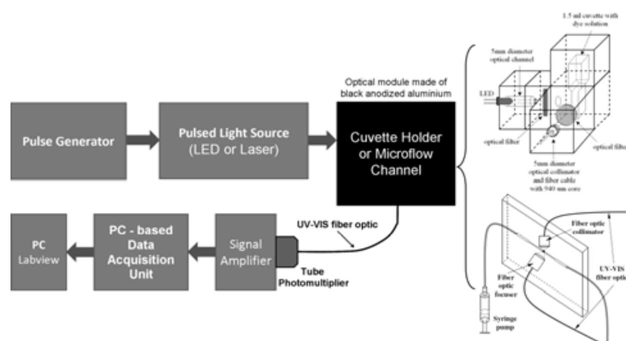

Fig. 2. Simplified system architecture

First system based on the PMMA cuvettes filled by sodium fluoresceinate and resorufin solutions was tested. Fluorescent dye was excited by blue or green LED diode. Results of this experiment are shown in figure 3.

On the chart in figure 3, a linear dependence of fluorescence intensity on fluorescent dye concentrations can be observed. For sodium fluoresceinate a linear range is from about 50 pg/ml to 1 µg/ml whereas for resorufin detection limit was about 7 ng/ml.

Next we investigated fluorescence of sodium fluoresceinate and resorufin solutions in the microchannel of PDMS based micro cytometer structure. Figure 4 presents results of these measurements with using of 450 nm laser diode as a light source to excitate sodium fluoresceinate and 532 nm laser light for excitation of resorufin.

A linear dependence of fluorescence intensity on fluorescent dye concentrations can be observed on the chart in figure 4. For sodium fluoresceinate a linear range was from about 40 ng/ml to 100 µg/ml whereas lower detection limit was for resorufin about 3 ng/ml.

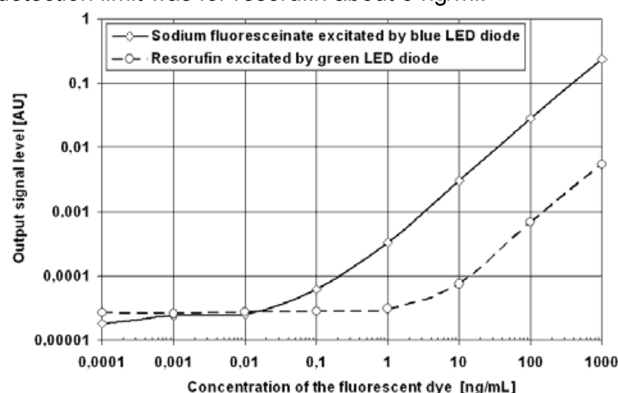

Fig. 3. Investigation of fluorescence intensity of sodium fluoresceinate and resorufin in PMMA cuvettes

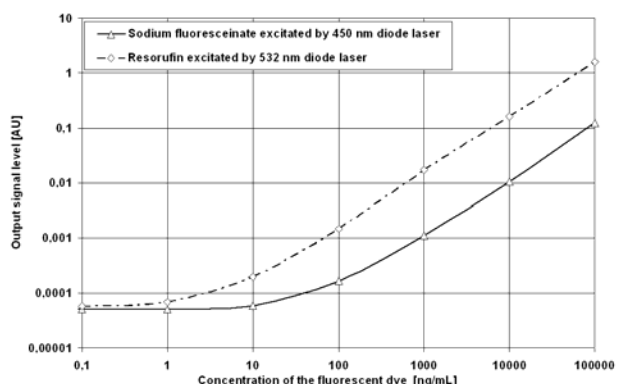

Figure 4. Investigation of fluorescence intensity of sodium fluoresceinate and resorufin in 60 µm x 60 µm microchannel

Measurement system based on silicon photomultiplier

Next experiments were performed with silicon photomultiplier (SiPM). As a fluorescence detector model S10362-11-100C developed by Hamamatsu has been chosen. This silicon photomultiplier consists of 100 avalanche photodiodes called pixels. Photons falling on the pixels cause generation of electric charge, which in turn cause flow of current between the electrodes of photomultiplier. This current is proportional to number of fired pixels – registered photons. Amplified signal from

SiPM is given to one of the inputs of QDC (charge to digital converter) device. Conversion of charge to digital form is splitted into two phases: integration of charge in the capacitor for the time specified via duration of gate pulse (conversion into voltage level) voltage conversion to 12-bit digital form by fast analog to digital converter (ADC).

Gate input signals for QDC are generated by Agilent 33250 function generator. These pulses must be delayed relative to the nanosecond pulses from the Picosecond 2600 generator. Pulses with duration of few nanoseconds trigger a light source (LED or laser) which generates an excitation light pulses for fluorescent dyes. Configuration of optical part of the system was shown earlier in Figure 2. Converted signal from QDC is transmitted to PC application via USB. Application in LabView environment can collect any length of data vector. This length is equivalent to a number of events registered by QDC. For all tested samples of fluorescent dyes, the vector length was set at the value of 100 000. Collected data can be represented by histogram, where x-axis represents all ADC digital values and y-axis is ADC counts. In the figure 5, a connection between number of photons in each of registered events and separated levels of amplified signal from SiPM is presented. Method of calculating accumulated signal from silicon photomultiplier for registered 100 000 events was described in [24].

Measurements have been carried out with samples of sodium fluoresceinate and resorufin dyes prepared in TRIS buffer with pH 8. In the first step investigations of configuration of the system with cuvette holder was performed. In figure 6 are presented the results of measurements, performed for solutions of fluorescent dyes in 1.5 volume PMMA cuvettes, positioned in optical block. In this case as a source of excitation light was used LED diode.

Dependence of fluorescence intensity on fluorescent dyes concentration is shown in figure 6. For sodium fluoresceinate a linear range is from about 60 pg/ml to 1μg/ml whereas for resorufin a linear range is from about 4 ng/ml.

The results obtained for sodium fluoresceinate for a flow rate equal to 80 μL/min in a flow-through channel with cross-section 300 μm x 100 μm are presented in figure 7. As a source of excitation light, a blue laser of 488 nm wavelength was used.

It can be seen, that there is a very good sensitivity of measurement system was obtained for sodium fluoresceinate excited by blue laser. In this case, a linear range is from about 1 ng/ml to 1μg/ml.

That lower detection limits are comparable with systems presented in [13, 30].

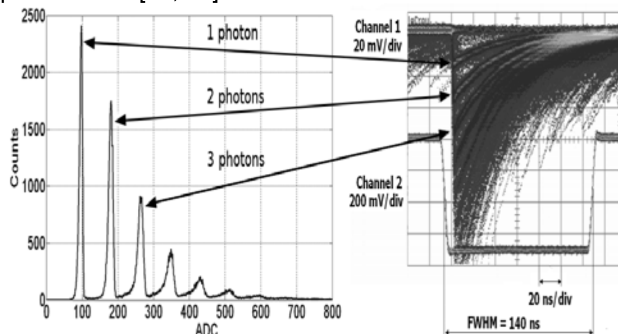

Figure 5. Oscillogram of amplified signal from SiPM and its equivalent in form of calculated histogram

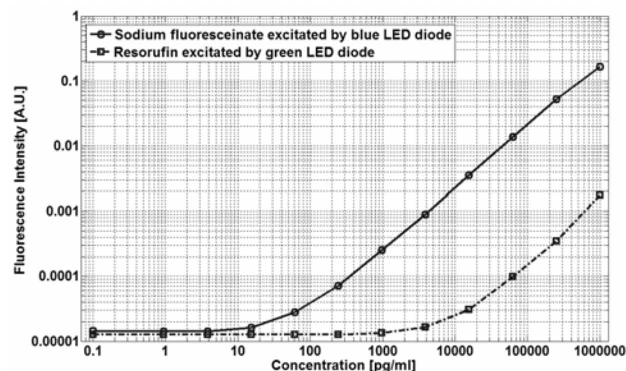

Figure 6. Measurements results obtained with using PMMA cuvettes and LEDs

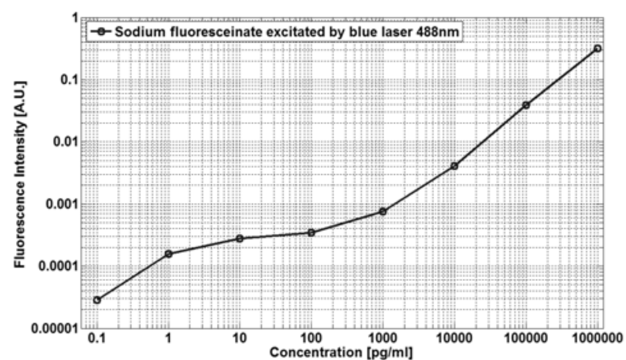

Figure 7. Measurements results obtained with using PDMS micro-channel and blue laser

Table 2. Comparison of measurements results between tube and silicon photomultipliers

Fluorochrome	Photodetector	Detection level		
		PMMA cuvette/ light source	PDMS Microchannel (60 μm x 60 μm)	PDMS Microchannel (300 μm x 100 μm)
Resorufin	Tube photomultiplier	7 ng/ml green LED diode	3 ng/ml 532 nm laser diode	N/A
	Silicon photomultiplier	4 ng/ml green LED diode	N/A	N/A
Sodium fluoresceinate	Tube photomultiplier	50 pg/ml blue LED diode	40 ng/ml 450 nm laser diode	N/A
	Silicon photomultiplier	60 pg/ml blue LED diode	N/A	1 ng/ml 488 nm laser diode

Conclusions

The developed detection systems based on tube and silicon photomultipliers have similar fluorescence light detection levels (measurements with using of PMMA cuvettes). The lower detection limit for resorufin measured in the microchannel (60 μm deep and wide) was ca. 3 ng/mL. For sodium fluoresceinate detection limit was higher than for resorufin and was established about 1 ng/mL for measurements carried out in microchannel with cross-section 300 μm x 100 μm (tab. 2).

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