Silicon Photomultipliers Applied to Fluorescence Detection of Biomarkers

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Introduction

The Silicon Photomultiplier (SiPM) has advantages that allows it to detect low light intensity in medical, chemical, biological applications but also to build integrated, portable microfluidic systems. This paper presents an application based on the SiPM designed for the detection of fluorescence of biomarkers.

Data acquisition system

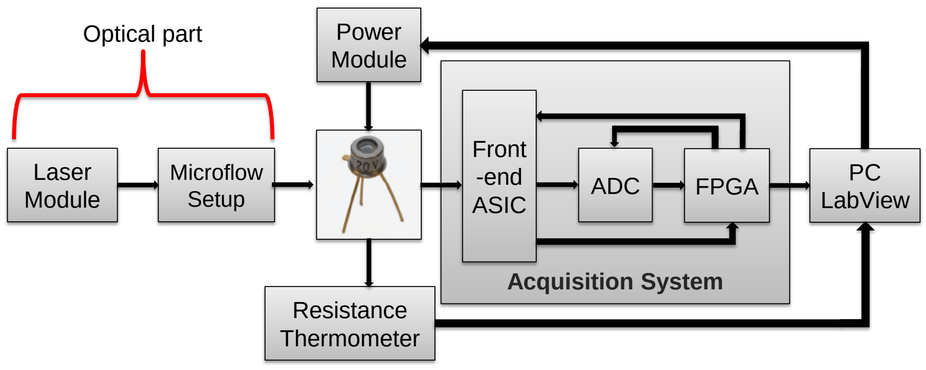

Figure 1. Flowchart of the data acquisition system

At first the measurements were performed in stationary photometric cuvettes. In order to minimize the volume of tested sample the measurements have been transferred to a microfluidic system. The data acquisition system (Fig. 1) consists of a dedicated application specific integrated circuit (ASIC) required to amplify and shape signals from the photodetector. The application has been designed with the aim of transforming it into a portable device. Detection method chosen in the research is based on the flow cytometry and single molecule detection. Although, only single photodetector has been used, presented applications can be adopted in multi-detector measurements.

Optical setup

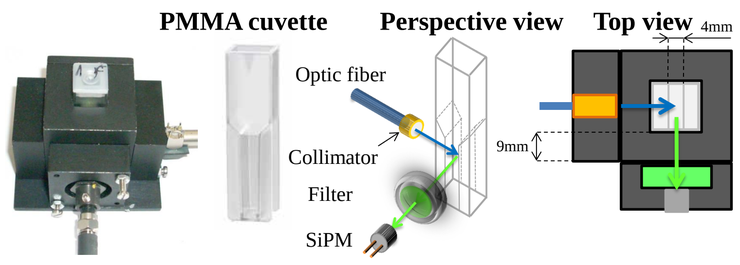

Figure 2. Stationary optical system with photometric PMMA cuvettes

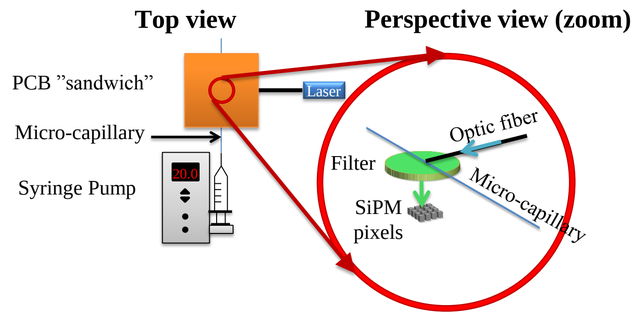

Figure 3. Microflow optical system with glass micro-capillary

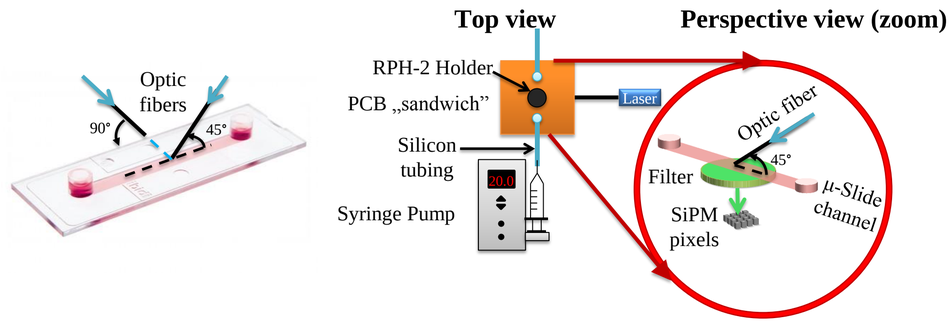

Figure 4. Microflow optical system with μ-Slide plate

Three version of the optical system for fluorescence measurements have been designed (Fig. 2-4). The first, is a stationary detection system with PMMA photometric cuvettes. The remaining two, are microfluidic systems with the μ–Slide plates or the glass micro-capillary. The source of excitation is 488 nm Phoxx laser with regulated pulse length. Blue light is delivered to the biomarker by a 200μm diameter UV-VIS optic fiber. Syringe pump transfers the biomarker with 20 μl/min rate. The inner diameter of the capillary hence the size of the flow channel is 100μm x 100μm. The volume of excited biomarker is equal to **2 nl.** The sizes of microchannels in μ-Slide plates are: 50mm x 5mm x 0.1mm and 50mm x 5mm x 0.6mm. The estimated volume of excited biomarker is equal to **26.7 nl.**

Fluorescence detection

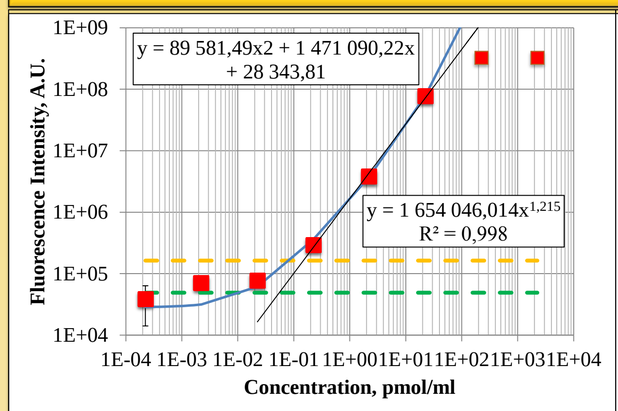

Figure 5. CF488 fluorophor in stationary system with PMMA cuvettes. Optical setup no. 1

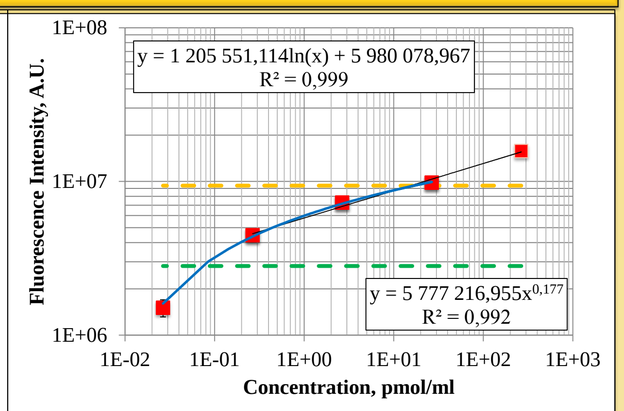

Figure 6. Fluorescein in microflow system with glass micro-capillary. Optical system no. 2

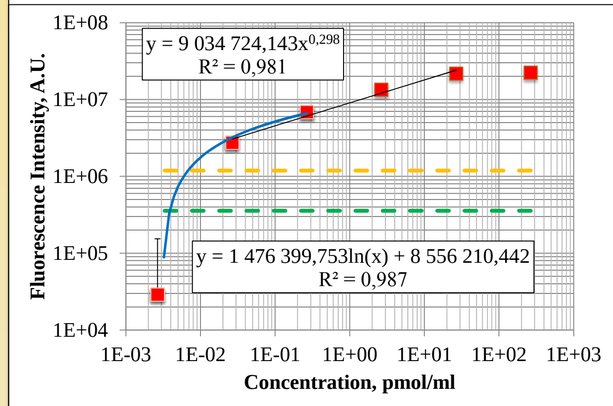

Figure 7. Fluorescein in microflow system with μ-Slide. Optical system no. 3

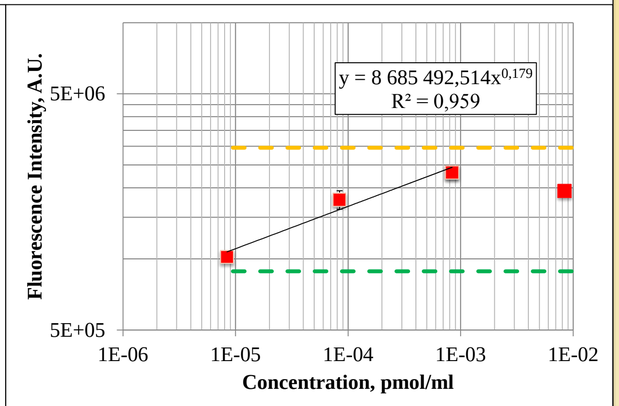

Figure 8. Antibody-fluorophor compound in microflow system with μ-Slide. Optical system no. 3

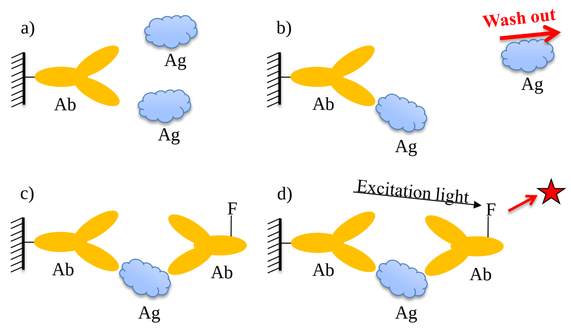

Figure 9. An illustration of the FLISA principle of operation with an antibody (Ab), an antigen (Ag) and a fluorophor (F). After the fluorophor is excited it produces fluorescence radiation.

Flurophor/ Biomarker	Optic System	Sample Volume	LOD		Sensitivity
			pmol/ml	pg/ml	*A.U.* / pmol/ml
CF488	PMMA cuvettes	78.5 μl	0.0140	12.70	1.215
Fluorescein	Glass micro-capillary	2 nl	0.0040	**1.51**	0.091
	ibidi μ-Slide	4.4 nl	0.0039	**1.47**	0.298
		26.7 nl	0.0071	**2.67**	0.161
Anti-NPR1 with CF488	ibidi μ-Slide	26.7 nl	0.000005	**0.55**	0.179

Table 1. Measurement results performed in all three optical setups.

The samples for both stationary and microfluidic detection were prepared from the base concentration which has been diluted in 10 mM, 8.5 pH Tris buffer (Tris-buffered saline). Next sample has always 10 times smaller concentration than the previous one.
Fluorescence detection results are presented in Fig. 5-8. Fig. 8 presents detection of biomarker where the sample was prepared based on FLISA protocol (fluorescence-linked immunosorbent assay Fig. 9).
The lower detection limit for the measurement series is determined by the Limit of Detection parameter (LOD):

$$\mathrm{LOD} = S_{buf} + 3 \times \sigma_{buf}$$

where: S_{buf} – the averaged signal from the SiPM measured for the buffer, σ_{buf} – standard deviation for buffer measurement. The LOD defines what is the minimum concentration that can be detected with given confidence limit.

Conclusions

- It has been verified that it is possible to detect the CF488 fluorophor with the concentrations not lower than 12.7 pg/ml (0.014 pmol/ml) in the stationary system.
- For the fluorescein the lowest detectable concentration is 1.47 pg/ml (0.0039 pmol/ml) in the microfluidic system.
- For the Anti-NPR1 biomarker used in detecting the small-cell carcinoma, the lowest concentration measured with the system is 0.55 pg/ml (0.000005 pmol/ml).

Acknowledgment

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Reference

[1] J. Emory et al., „Design and development of a field-deployable single-molecule detector (SMD) for the analysis of molecular markers" Analyst, vol. 137, pp. 87-97, 2012.
[2] K. Mathwig et al., „A nanofluidic mixing device for high-throughput fluorescence sensing of single molecules", Procedia Technology, vol. 27, pp. 141-142, 2016