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System for Low Intensity Fluorescence Light Measurement based on Silicon Photomultiplier.

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***Abstract* – In the paper a measurement system for low intensity light is presented. This system has been adapted for fluorescence light measurement, generated by fluorescein solution with pH equal 8.1. The whole system is capable of measuring the fluorescence light intensity of the solution deposited in a transparent cuvette, and positioned in a specially prepared optical system. A new semiconductor device, called Silicon Photomultiplier (SiPM) has been used as a light sensor, because of its sensitivity to single photons.**

I. INTRODUCTION.

Fluorescence is a kind of organic light emitted by fluorochromes exposed to the light of precisely defined wavelength, of which spectral response depends on chemical composition of the fluorescent dye. An emitted light spectrum depends as well on the pH factor of the solution under measurement. Peak of the emitted light intensity of the Fluorescein water solution appears at the 521nm wavelength. The maximum energy absorption however, is at the 494nm wavelength. Absorption and emission efficiency depends as well on the pH factor of the solution. The dependence is shown in Fig.1.

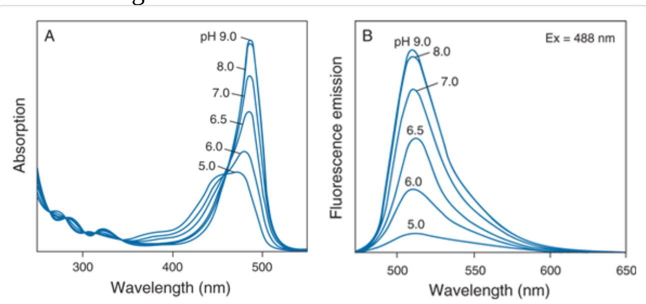


Fig. 1. Absorption and emission characteristics of fluorescein solution [5].

Fluorescence measurements are often used in biochemical and biotechnological analysis where a fluorescent dyes are used as a markers. Information about pigment concentration brings as well a knowledge about number of cells, which have the ability to absorb the pigment. Fluorescent light measurement issues were discussed in many articles recently [1, 2]. First publications about silicon photomultipliers (SiPM) were published over 10 years ago [3, 4]. They proved to be an effective tool for low flux photon detection. In this paper an application of a silicon photomultiplier in fluorescence light measurement is presented.

II. SYSTEM ARCHITECTURE

The measurement system combines two separated blocks: optical and electronic one. The optical module is a specifically-designed arrangement of chambers made of oxidized metal. The electronic unit includes transimpedance amplifier, integration capacitor, 16-bit analog to digital converter, a microcontroller and serial UART to USB converter.

A. Optical Block

In the optical block, we can distinguish five basic structural elements:

- blue LED,
- excitation light filter,
- cuvette with fluorescein specimen (pH=8.1),
- emission light filter
- silicon photomultiplier.

These elements are coupled together through the optical channel with a diameter of 5 mm. Figure 2 shows the design of the optical block.

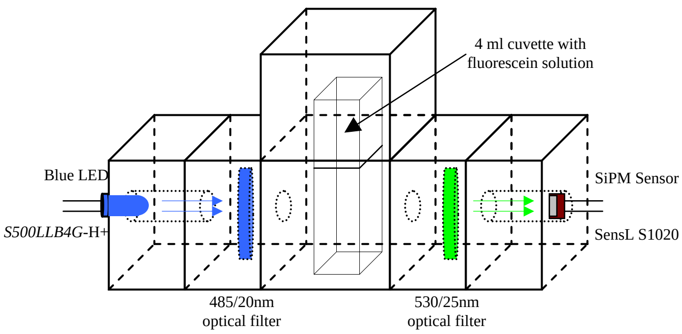


Fig. 2. Construction of the optical block.

The excitation light after passing through the filter of 485/20nm is filtered out to not to exceed a band 468-488nm (see Fig. 3) . Such a wavelength-defined pulses of the light cause an excitation of molecules of a dye (Fluorescein) in the solution, which while returning to equilibrium state emit a light within the spectrum with the maximum at 521nm. A generated in this process light is filtered afterwards by an emission, band-pass filter (530/25nm). Bio-Tek filters spectra (excitation and emission) are shown in Figure 3.

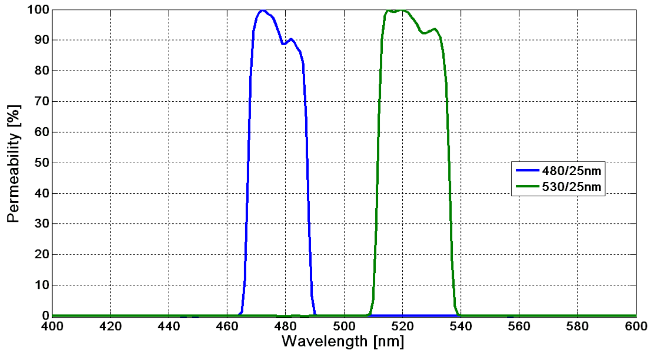


Fig. 3. Spectra of excitation and emission Bio-Tek filters.

B. Electronic Block.

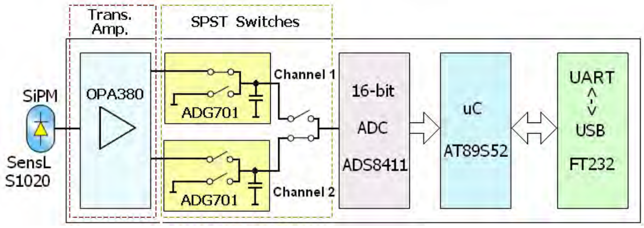
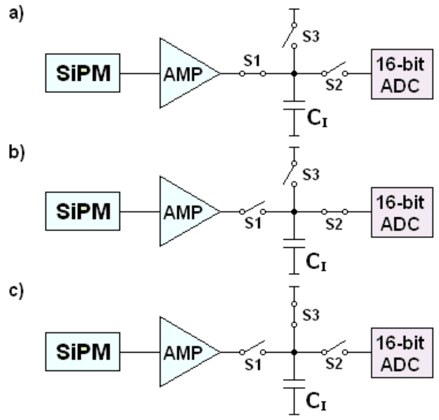


Fig. 4. Block diagram of electronic part.

A block diagram of the electronic part (Fig. 4.) provides a general idea of the device structure.

At the input of the electronic system, impinging fluorescent light photons generate a current signal in the silicon photomultiplier. The signal generated by SiPM is amplified by the transimpedance amplifier before integration. The main feature of this amplifier is conversion of the current signal to a proportional voltage. After the amplifier, a signal is being splitted between two channels, which work synchronously and in a complementary way. By the time when the first channel integrates a charge, a second one converts the charge stored in the previous cycle (as a voltage in a capacitor) to its digital representation (ADC). Each channel is also equipped with an SPST (Single Pole Single Throw) switches, which resistance is ~4-8 ohms during ON state. A principle of operation of the acquisition unit will be described on the basis of a single channel, since they are identical. Operation phases of the system are presented in Fig. 5.



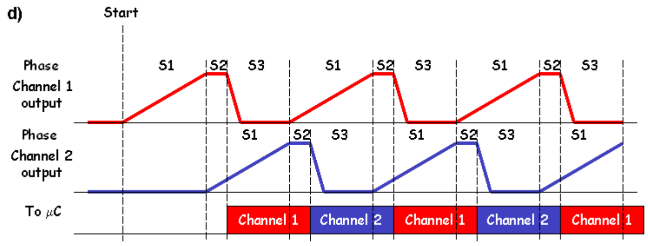


Fig. 5. Phases of acquisition: a) S1 - charging, b) S2 - conversion, c) (S3 - discharging), d) channels outputs at the time of acquisition.

In the first phase (integration), the capacitor is being charged by the current delivered by the amplifier output during defined period of S1. When the charging phase is finished in one channel, the microcontroller swaps the channels and in the same time goes to the second phase: ADC conversion. Now the capacitor voltage, proportional to the integrated charge is being converted to its digital form. Responsible for this is the 16-bit ADC converter with 2 MSPS sampling rate. Digital value is then handed over to the microcontroller, which sends out the data to the equipment computer through the USB interface. As a last phase, the integrating capacitor gets discharged to be ready for the next conversion. In the same time the charge in the second channel is still being integrated, and once this process is finished, the microcontroller swaps again the channels. The algorithm of operation of the microcontroller, steering an integration devices is shown in Figure 6.

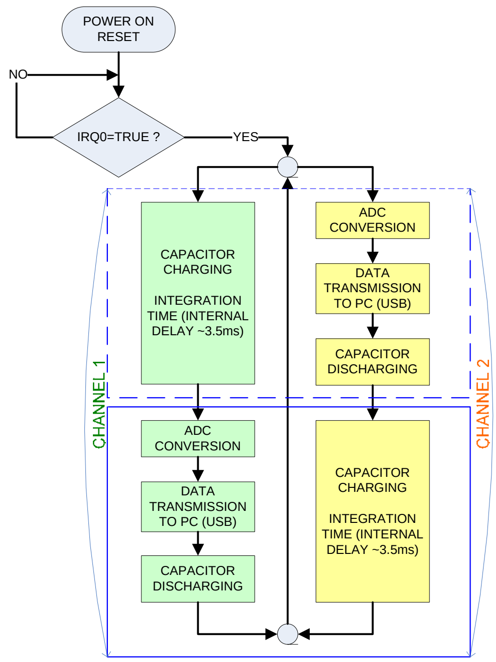


Fig. 6. Microcontroller program algorithm.

Figure 7 shows a PCB of designed system.

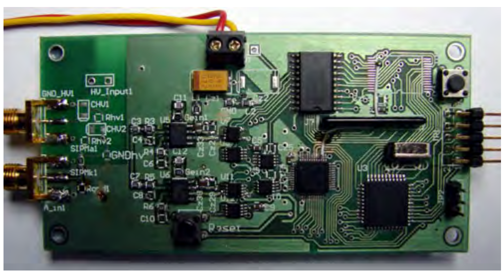


Fig. 7. Electronic Block PCB

III. Detail Description of Analog Track of the System

As already mentioned in the analog part of the system are three basic elements:

- S1020 – silicon photomultiplier (SensL)
- OPA380 – transimpedance amplifier (Texas Instruments)
- ADG701L – analog switch (Analog Devices)

Selection of these items was preceded by a thorough analysis of end-use of the system. As a light sensor a model S1020 has been chosen, manufactured by SensL company. It has a superior Signal to Noise Ratio than standard APDs. The sensor operates in standard scheme from manufacturer's application note. Typically breakdown voltage for this SensL SiPMs is equal 28.4V but it's possible to overrun voltage about 2V. In the final device, breakdown voltage is equal 30.2V. Before the analog track is splitted between 2 channels, the signal from SiPM is converted and amplified by transimpedance amplifier. Scheme of amplifier's section is shown in figure 8.

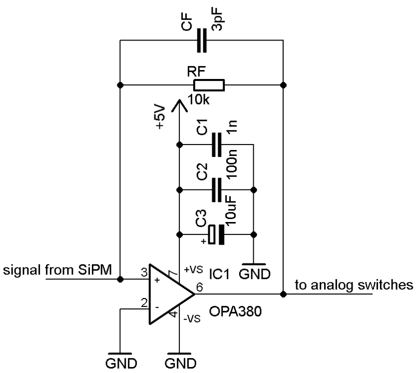


Fig. 8. Transimpedance amplifier section.

Gain of the amplifier is settable by C_F capacitor and R_F resistor in feedback. In the system, the gain has been set about 80dB. The frequency response of the amplifier is shown in figure 9. Selected gain is marked with red dots.

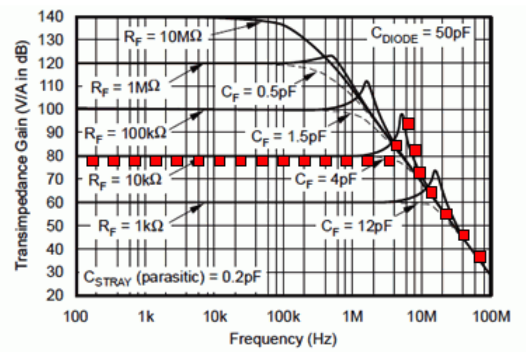


Fig. 9. Transimpedance amp characteristic.

Each channel has a set of three analog switches. Integrating capacitor is charged from the output of the amplifier via a switch one. With the knowledge of the resistance in the ON state, can be done charging and discharging capacitor simulation. Simulations made in PSpice allowed to define approximately the capacitance of integrating capacitor. Figure 10 shows a circuit diagram of analog switches and integrating capacitor for the one channel.

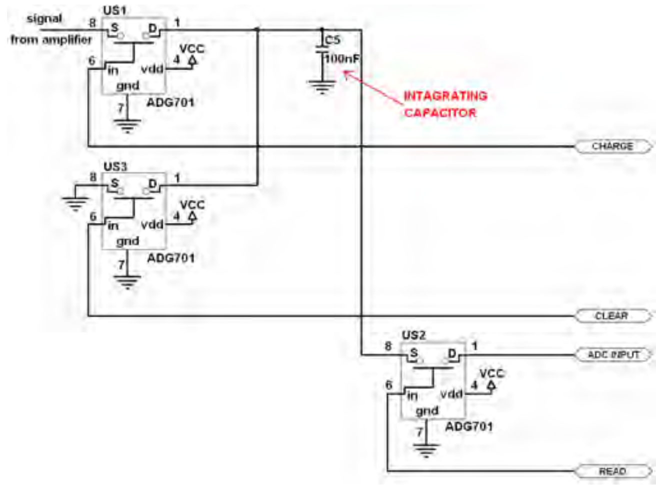


Fig. 10. Analog switches and integrating capacitor section.

The capacitance is equal 100nF that ensures the correct operation of the whole system.

As the analog to digital converter was used ADS8411 device manufactured by Analog Devices. This 16-bit converter has a voltage reference output (4096mV). It provides excellent resolution and sensitivity at the level of 625uV.

IV. Measured Results.

Described in the paper the measurement system scheme, is shown in Figure 11. A data acquisition software with GUI for the PC is written in Labview environment. It provides visualization of incoming data in on-line mode.

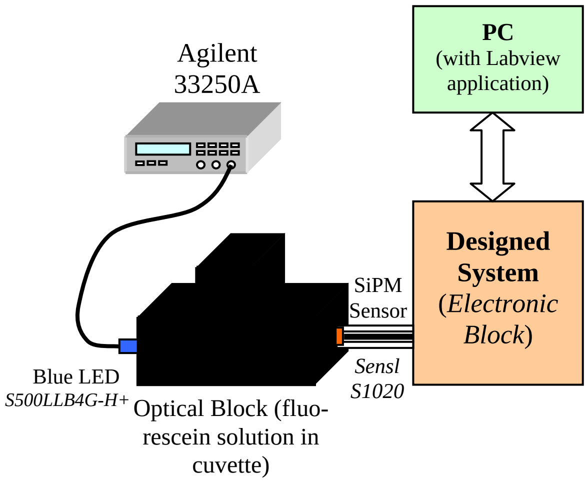


Fig. 11. Measurement system.

A waveform generator does provide voltage pulses to the blue LED (S500LLB4G-H+) of 5V amplitude and 20ns duration. The frequency of these pulses is set to 10MHz. Described measurements has been performed with six samples with the following Fluorescein concentrations:

1) 1562.5 [ng/ml]
2) 781.2 [ng/ml]
3) 390.6 [ng/ml]
4) 195.3 [ng/ml]
5) 97.66 [ng/ml]
6) 48.83 [ng/ml]

For each concentration 10000 measurements (conversions) has been done, to obtain required statistics. As a result, the mean values for each channel have been calculated and are presented in the chart in Fig. 12.

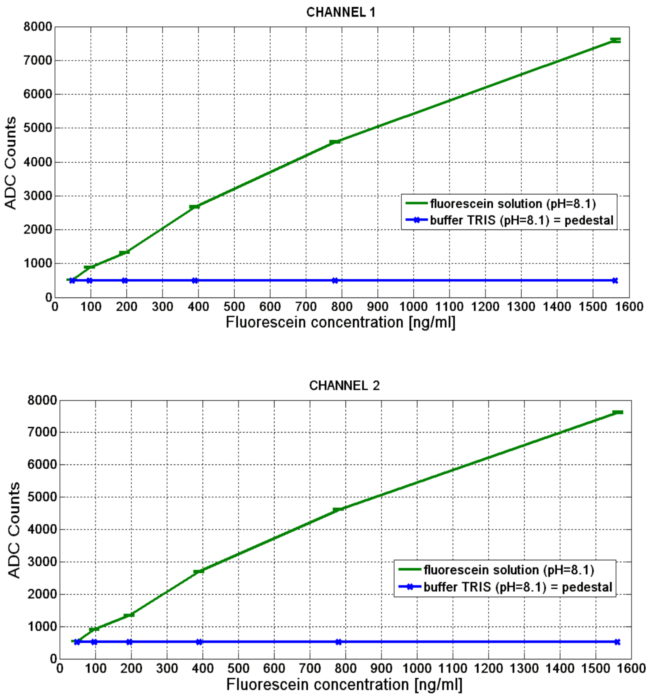


Fig. 12. Measurements results.

On the charts in Fig. 12 a linear dependence of the light intensity from fluorescein concentration can be observed.

TABLE I

MEAN VALUE AND RELATIVE ERROR OF MEASURMENTS.

Fluorescein concentration [ng/ml]	Mean Value [ADC]		Relative Error [%]	
	CH 1	CH 2	CH 1	CH 2
1562.5	7584.8	7619.9	0.61	0.34
781.2	4585.9	4615.4	0.53	0.27
390.6	2672	2699.4	0.51	0.37
195.3	1317.2	1342.1	0.77	0.69
97.66	885.4	908.4	0.71	0.6
48.83	510	532.7	0.29	0.24
Buffer	490.2	512.7	0.26	0.24

Calculated results, which are presented in table 1 prove the effectiveness of the system. Relative error for all measurements is lower than 1%.

V. CONCLUSIONS

An effective system for fluorescence light measurement has been presented in the paper. The system is based on a silicon photomultiplier detectors which are very low light field sensitive detectors. The designed device is handy, offers good sensitivity, which can be used to measure light intensity of the concentrations of Fluorescein at the level of 50ng/ml up to 1.6 μg/ml. A measurement precision is of concern as well and right values are presented in the Table I. Moreover the system does not have a dead time, hence the measurement can be done in a continuous way. In the near future improvement of the sensitivity of the device is planned. Investigation of use of the system for measuring the fluorescein concentration on the basis of measurements of the light intensity is a main objective.

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