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"Pulsar" Photon Counter in Electrogenerated Chemiluminescent Measurements

***Abstract**. The work is devoted to the development of the apparatus "Pulsar" that is a high-speed pulse counter with a dual interface. It is based on the modern ARM processor STM32F407. High peripheral modules integrations in the selected microcontroller and its productivity were used as a base to PMT integration in electrogenerated chemiluminescent (ECL) analytical systems. Apparatus testing with real ECL compositions showed its capability. ECL pulse response up to 84 MHz or 6 ns pulse duration is transformed to output counting data in two forms for digital and analog interfaces. High temporal resolution up to 10 µs supports measurement techniques based on fast electrode polarization. The built-in double interface simplifies system integration that well demonstrated, as an example, a combination of PMT module H 10682-210 (Hamamatsu Photonics, Japan) with potentiostat Methrohm Autolab 128N.*

***Streszczenie.** Praca poświęcona jest opracowaniu aparatu "Pulsar", czyli szybkiego licznika impulsów z podwójnym interfejsem. Oparty jest on na nowoczesnym procesorze ARM STM32F407. Integracja modułów peryferyjnych w wybranym mikrokontrolerze i jego produktywność posłużyły jako podstawa integracji PMT w systemach analitycznych elektrogenerowanych chemiluminescencyjnych (ECL). Testy aparatów z prawdziwymi kompozycjami ECL wykazały jego możliwości. Odpowiedź impulsowa ECL do 84 MHz lub 6 ns czasu trwania impulsu jest przekształcana na wyjściowe dane zliczające w dwóch postaciach dla interfejsów cyfrowych i analogowych. Wysoka rozdzielczość czasowa do 10 µs obsługuje techniki pomiarowe oparte na szybkiej polaryzacji elektrod. Wbudowany podwójny interfejs upraszcza integrację systemu, co dobrze zademonstrowało, jako przykład, połączenie modułu PMT H 10682-210 (Hamamatsu Photonics, Japonia) z potencjostatem Methrohm Autolab 128N. (**Licznik fotonów „Pulsar" w e pomiarach chemiluminescencji generowanej elektrycznie**)*

Keywords: Pulse counting, photomultiplier tube, ultra-weak light, electrogenerated chemiluminescence, ultramicroelectrode.
Słowa kluczowe: licznik pulsarowy, elektroluminescencja.

Introduction

Electrogenerated chemiluminescence is an attractive analytical method [1-3]. The phenomenon of light emission at the moment of solution electrolysis can be used to examine a medium content. Nanomaterials, biological molecules, fast kinetic electrochemical process investigations ask perfection in the temporal and special resolution of measurement instrumentation. The photon-counting mode of PMT operation has well-known possibilities to proof weak light measurements close to maximal possibilities, that actual in many analytical tasks. This problem is relevant in various fields of science and technology, for example, bioengineering, energy, particle physics, chemical analysis, and others. The possibility of detection of the light fluxes in a wide dynamic range with a high precision allows unprecedented accuracy in measurements. The integration of this light registration technique with correspondent electrochemical devices opens new opportunities like a single reaction recognition system. This method is based on use ultra-weak light registration in photon counting mode for the electrochemical reaction. The light emission is a result of generated ions interaction that a key to detect a single reaction act in solution. [4-7] Electrogenerated chemiluminescence has big potential to apply for single-molecule and single-particle registration tasks [8-10]. The design and availability of correspondent instrumentation are sufficient to progress in this modern analytical area.

At the market, photon counters are available, for example, SR400 photon counter by Stanford Research Systems [11] and The C8855-01 is a counting unit with a USB interface by Hamamatsu photonics, Inc. [12]. Both of these devices have advantages and weaknesses. SR400 support analog signal synthesis and operation with few measurement channels for specific operation modes when few PMT is used in tandem. C8855-01 supports the USB interface and PMT module powering. In the developed apparatus is a combination of features that needed for ECL measurement. Support is realized for the double interface with analog and digital output, precise operation without pulse lose or dead time. The proposed design has limited functionality relatively to SR400, it supports only one measurement channel, but it has extended features than C8855-01. At the same moment, the utilization of a modern ARM microcontroller efficiently solves photon-counting tasks at a lower price in compact dimensions. The apparatus functionality discussed below is well adjusted to conduct ECL measurements in different analytical systems.

Design description

"Pulsar" apparatus (fig.1) is designed to control PMT operation in photon counting mode to record ultra-weak photon flux. This apparatus is fully compatible with the products of Hamamatsu Photonics as well as other sensors from different manufacturers with the pulse output. High integrated ARM 7 microcontroller STM32F407 is used to construct the apparatus. This controller is high productivity as a zero-wait state of data is for program execution from a flash memory up to 168 MHz CPU frequency.

Fig.1. The external view of «Pulsar».

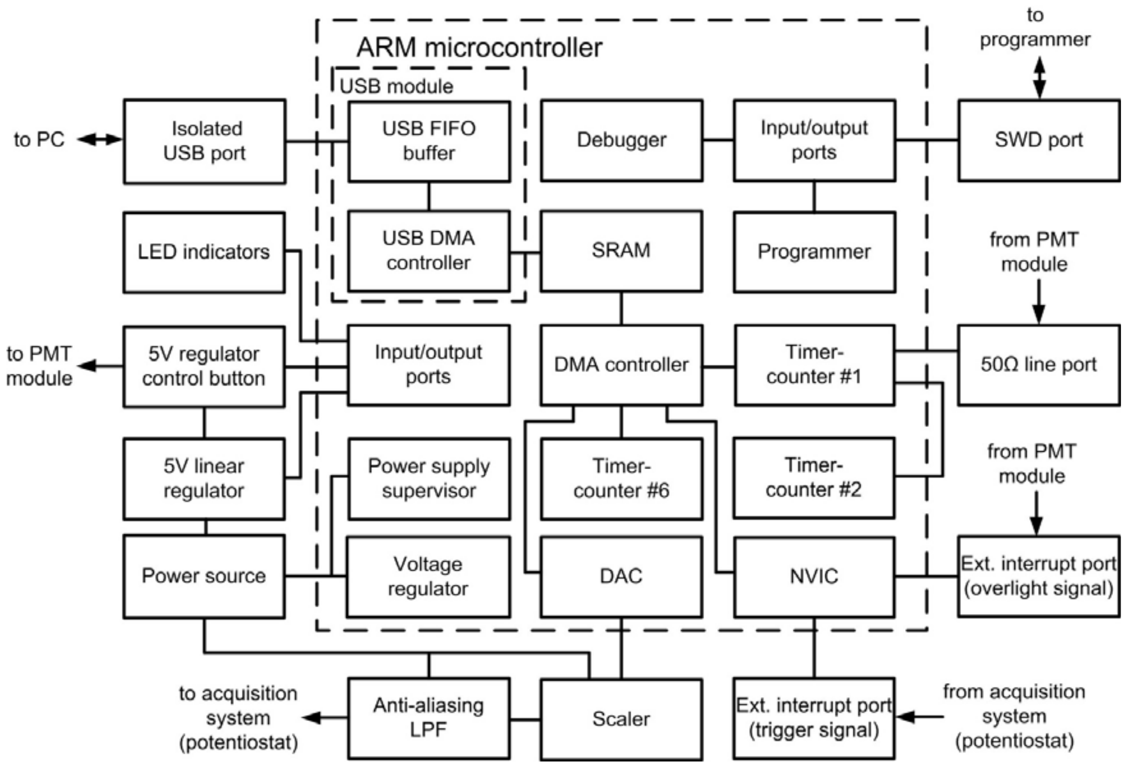

Fig.1. Block diagram of "Pulsar" apparatus.

The adaptive real-time memory accelerator implements an instruction reading to a prefetch queue cache, which increases program execution speed from the flash memory. It minimizes execution delay for a flash program execution and performs productivity. The presence of a high-speed counter, DMA controller, USB modules, and DAC provides a high capability to the apparatus design with a minimum of external electronic parts.

The main function of the device is a pulse signal acquisition. Each pulse with a minimum duration of 6 ns can be counted. It means the apparatus accumulates the number of pulses at a fixed time called a gate period. Data about counting are transformed into adequate form. In the proposed device, two possibilities are realized. The result of counting is transferred as a digital data stream, and, synchronously, continue analog signal is produced by the direct digital synthesis (DDS).

To realize the proposed functionality, the next inner units of microcontrollers that are shown in the block diagram (fig.1) are involved. The distinctive feature of ARM timers is operation as a counter with a capture channel for an external signal. Timer TIM1 was used to capture pulse signal, the timer pin was configured as input, and 50 Ohm terminator resistor was added on a signal line from the PMT module. Timer TIM1 has the operation timer clock capability up to the system clock 168 MHz that supports 84MHz signal capturing, it equals to 6 ns pulse duration with 6 ns pause. This is enough to work with most PMT modules.

TIM 1 is a 16-bit timer with an overflow flag. The last one is used to control data that is read from the counter TIM1 register without the timer stop. If overflow is detected, it marks the recalculation of data that transferred from the TIM1 buffer.

To minimize a time jitter, the DMA controller manages data in the realized apparatus. The timer TIM6 synchronizes the gate period to read counts. DMA controller executes data transfer from the timer TIM1 register to SRAM buffer. After data transfer, the DMA controller activates interrupt for the data processing function. Multiple buses in the system and high priority DMA request prevents a time delay in the data transfer. The core operation time is reduced by DMA operation that enlarges the time for data processing. In the handler function for the DMA data transfer interrupt, the program includes a control for the counter overflow. After timer TIM1 reading, the data transfer is divided into two threads. One is a data stream of counting transferred as the digital information to PC, another is the data transformation to the analog signal. The voltage amplitude of the analog signal is proportional instantaneous photon flow rate calculated for the gate period.

Transfer of digital data to PC is realized by USB with double buffering. The finish of DMA transfer between the timer TIM1 register and SRAM activates an interrupt its handler function transfers the timer data from SRAM to a FIFO buffer placed in SRAM. This FIFO buffer is software controllable. The built-in USB module in selected MC has an own DMA controller and 1.25 kbytes FIFO buffer for data transfer, so data is accumulated in SRAM and transferred to USB module buffer. The USB data transactions are controlled by the host PC that frees the USB buffer. The software fills only the software FIFO buffer. Data transaction between the USB module and SRAM is controlled by the USB DMA controller.

The data transformation to the analog signal includes signal processing. Each data processing step is optional and dependent on the experiment parameter set. In the beginning, according to needs, the linearity correction of sensor counting characteristic is used. It is actual for strong photon flows when one pulse can satisfy registration pairs of photons with high probability. This phenomenon is common and corrected according to the counting possibilities of PMT electronics and the output pulse parameters.

The next step is digital filtration. In the realized low-pass filter, the analog signal agrees to the acquisition system connected to Pulsar. An additional channel for data collection in a potentiostat, a device for electrochemical measurements, is used for this purpose. After the digital filter, data for DDS get to the built-in DAC. It is a 12-bit resolution only, but the correct selection of the transformation coefficient is enough to use DAC possibilities efficiently. The analog signal sampling rate is 10 kHz, for maximal count rate 84 MHz is 8400 counts per gate period, and ADC can propose 4096 levels resolution. Respectively, as a bright signal is not frequently, so a signal transformation is possible without losing.

DAC operation does not as strong synchronized as the gate period because DDS is in the data processing function. The DDS signal sampling rate is 10kHz, and time jitter in calling of this function is negligible. Capabilities of built-in DAC of selected MC are enough for precision DDS in the developed apparatus.

The output of DAC is buffered by an operational amplifier that scales signal to the 10V dynamic range, thus a signal resolution of a connected data acquisition system is used totally.

The apparatus has an output hardware filter to suppress the effect of sampling at the output. The output low-pass filter eliminates the effect of data sampling in DAC with a fixed data rate in 10 kHz. As the sampling rate for DDS is constant, so the output filter is optimized to operate with cut-off frequency at 1kHz. Sixth order Butterworth filter has suppression in -120 dB at frequency 10 kHz. It is enough for many measurements with signal disturbance even lower than the noise level in 16-bit resolution systems. The AC transfer characteristic of the output filter (figure 3) is measured with arbitrary form generator DG3121A by Rigol, Co., and digital oscilloscope Rigol DS1204B.

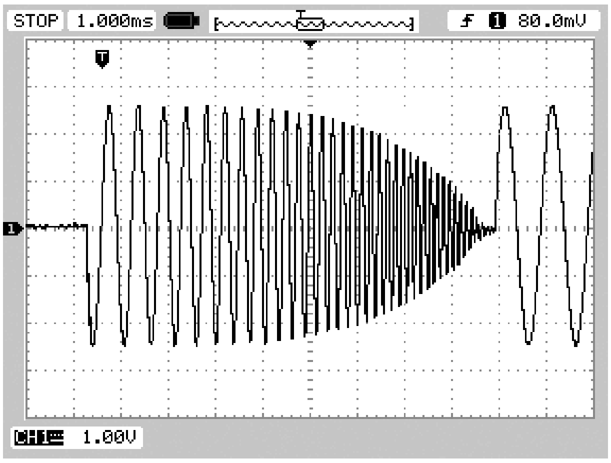

Fig.3. The oscillogram of “Pulsar” anti-aliasing LPF as response on the input sinusoidal signal with frequency sweep from 1 to 10 kHz range (exponential sweep of frequency).

Potentiostats have different possibilities as the bandpass, the sampling rate, and the presence of the anti-aliasing filter. This asks the DAC output signal to agree to the acquisition system. Signal integrity in the system is provided by agreement between the signal spectrum and transfer possibilities of a measurement channel. High-frequency components beyond data processing of the measurement system should be suppressed. This task is solved by low-pass anti-aliasing filters. In Pulsar, filtering is done into two places. The hardware filter is optimized to 1 kHz bandpass, the additional limitation in the signal spectrum provides the software filter. The software filter is adaptable to the data acquisition system and can change the depth of filtration up to 4000 samples, which is 0,4 s.

Pulse counting is free from a pulse loss or a "dead" time due to continues operation of the timer TIM1, a double buffering technology, direct memory access, a vectorized multilevel interrupt controller. The counting process accomplished with the accurate hardware control of the gate period allows to conduct precise measurements.

Electromagnetic interference is a big problem in sensitive electrical measurements. To avoid noise penetration to a measurement system, Pulsar and PC communicate by isolated USB interface. IC ADuM4160 provides high electromagnetic isolation of connected equipment.

Virtual COM port (VCP) protocol implementation simplifies apparatus integration to measurement systems. Therefore, potentiostat software can control Pulsar operation via VCP, for example, Nova to control Autolab potentiostats. Pulsar operation parameters and measurement result displaying are done in potentiostat control software that removes needs in additional software.

"Pulsar" counter is fully compatible with USB 2.0 and is connected in a "Full-Speed" mode via the electrically isolated interface to the PC. The presence of galvanic isolation between counter and computer can improve the electromagnetic compatibility of integrated equipment by protecting against electromagnetic interference with the host computer. "Pulsar" counter is fully compatible with USB 2.0 and is connected in a "Full-Speed" mode via the electrically isolated interface to the PC. The presence of galvanic isolation between counter and computer can improve the electromagnetic compatibility of integrated equipment by protecting against electromagnetic interference with the host computer.

“Pulsar” supports the options of software and hardware triggers. Software control activates data collection by a command via USB. Another possibility is a hardware trigger. An external signal is used as a trigger signal to activate the counter, which stays in a waiting condition after parameter setting. The handler function of this interrupt signal has a suppressed stack operation, so the delay of activation extremely small. 6 clocks period is a maximum guaranty time delay to interrupt a process in ARM and 3 activation commands (3 clocks) at the beginning of the interrupt handler function that is 53.6 ns delay. Initial disabling of any interrupts guarantees synchronization for two timers’ activation in the next two sequential commands. After the timers start, the microcontroller renews an interruption processing.

```
__disable_irq();
TIM1->CR1 |= TIM_CR1_CEN;
TIM6->CR1 |= TIM_CR1_CEN;
__enable_irq();
```

Additionally, the self-testing function is realized in Pulsar. The timer TIM2 is configured to produce a pulse sequence with a specific rate. The timer TIM1 configuration allows a change of a capture signal source from one pin to another that connected to the output signal from timer TIM2. Counting performance is examined by satisfying the number of counted pulses by the timer TIM1 to the number of generated pulses by TIM2. Both timers are system clock dependent, so to verify performance, it is better to use external pulse generator as arbitrary form generator DG3121A by Rigol, Co. The last one was utilized to examine the developed apparatus performance to count pulses as well as to generate the analog signal. Engaging of precise voltmeter Rigol DM 3068, digital oscilloscope Rigol DS1204B, and measurement in the real analytical system provided testing static parameters as well as dynamic characteristics. It proved the absence of a pulse lose at different pulse rates, including variable rates, and the analog signal satisfies a pulse rate in each gate period as a signal shape reproduces a variation law for pulse generation rate that verifies a dead time absence also.

The additional feature of the “Pulsar” apparatus is a power delivery for a connected PMT module. For example, modules H-7828, H-10682 by Hamamatsu have a powering voltage at 5V. The power source is based on a linear regulator. To control the 5V power source for PMT in apparatus, the power button with a built-in LED indicator is. It provides hand control of PMT powering. The powering is activated only together with a software command as the button connects in series with the voltage source controllable by MC.

In the apparatus, 4 LEDs indicate apparatus statuses. Controller initialization is finished by light on the orange LED "On". The connection and correct enumeration at USB are indicated by blinked blue LED “USB”. Red LED “Source” indicates a received command to switch on PMT power, a command to switch off PMT turn off the LED also. The green LED “Run” begins to light after receiving a complete parameter set for the apparatus, and its fast blinking activates together with data collection. After a measurement, the “Run” indicator is switched off.

Some PMT modules can generate an overlight signal on an additional signal line. This happens at bright light illumination when PMT counting mode possibilities is disappeared. The overlight signal monitoring prevents errors of data reading in the apparatus. The raise of this signal is detected by the port of the external interrupt and forces the apparatus to transfer information about an overlight condition in the data stream.

Testing with classical ECL composition

The apparatus “Pulsar” was tested as part of the analytical setup for electrogenerated chemiluminescent assays. PMT module H 10682-210 (Hamamatsu Photonics, Japan) assembled into a home designed lightproof chamber was connected to Methrohm Autolab 128N potentiostat. “Pulsar” transforms photon counting data to an analog signal with a sampling rate of 10^4 samples/s. The digital filtration was by averaging of 100 samples. The polarization potential scan of the working electrode was from 0 V to 1.4V and backward to 0 V (relatively to Ag/AgCl reference electrode) with the sweep rate of 100 mV/s. The digital data collection was with the gate period 10 µs.

The ECL experiment was conducted in a light-proof box. Measurements were done in a cylindrical 6 ml cell from borosilicate glass with a tetrafluoroethylene cap that fixed an electrode system. The reference electrode was a miniature silver/silver chloride electrode. The counter electrode was a foil platinum electrode (the surface area is 150 mm^2). The working electrode was a glassy carbon disk electrode, the disk diameter was 3 mm. Its cylindrical tetrafluoroethylene insulation shield had a 6.5 mm outer diameter. The solution for ECL experiment was 10 µM tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate (purchased from Merck) in 2 ml of phosphate buffer solution (0.1 M concentration, pH = 7.0) and 1 mM tripropylamine co-reactant (purchased from Merck).

The results both of data collections are represented in figure 4. Analog signal recorded by potentiostat corresponds to ECL response obtained in similar conditions [13] shows an equivalent behavior. Adequate scaling of signals can compensate for the difference in sensitivities of used PMT in these experiments. The small variation of the signal at the peak is a result of the stochastic nature of the chemical reaction. The digital pulse collection gives a higher temporal resolution. To see equivalence with the analog signal, the data processing equivalent to analog channel is needed for the digital data of counting. It includes averaging signal and transformation count/s to volts. The pulse response (the grey area) looks like a bar graph in figure 4 which is a result of stochastic signal variation for short-time resolution. The pulse response (the grey area) looks like a bar graph in figure 4 which is a result of stochastic signal variation for short-time resolution. Forward and backward curves fill the area. Averaging of the pulse signal shows identity to the analog signal registered by the potentiostat.

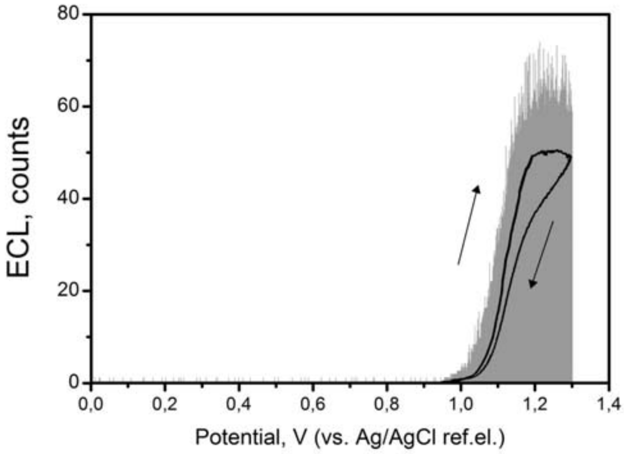

Fig.4. ECL response for $Ru(bpy)_3^{2+}$ + TPA composition. The black curve is the analog signal representation with 1 ms resolution (arrows show direction of signal with a potential scan). The grey curve is the digital signal representation with 10 µs resolution.

Testing in ECL experiment on ultramicroelectrode

A change in the electrode area proportionally reflects in light emission. Utilization of ultramicroelectrode is a way to improve electrochemical measurement possibilities. Special limitations of the electrode system change the rate of a mass-transport. Linear diffusion to a planar electrode is transformed into more efficient hemispherical diffusion to a miniature electrode. A faster mass-transport to ultramicroelectrodes assists to achieve a steady-state regime over a shorter time. A fast electrode polarization can additionally minimize an involved volume of a sample to electrode reactions. The spatial and temporal limitation of electrochemical processes by ultramicroelecrodes is used in electrochemical probing [14]. Advantages of PMT as high sensitivity and fast response in photon counting mode is perspective to use in modern analytical tasks that aimed to detect single molecules, single particles, and biological structures by electrochemical methods [4,8-10].

The experiment with ultramicroelectrode was conducted in the previous electrochemical cell, but the working electrode was replaced by glassy carbon utramicroelectrode with a working disk diameter of 25 µm. The insulator shield was a glass tube with an outer diameter 1.5 mm stretched to a cone shape on a working electrode side. A test solution was 0.1 mM 9,10-diphenylanthracene (DPA) in a mixture of acetonitrile and benzene (v./v. 4:1) with 0.5M tetrabutylammonium perchlorate the supporting electrolyte. Chemicals including solvents were purchased from Merck and used as were. DPA is a known ECL luminophore [15] and remains interesting for the approbation of new ideas and techniques [16]. Deoxygenation of the prepared solution was done by argon barbotage over 10 minutes. Pulsar collected counts from PMT and transferred data to a connected PC. The electrochemical cell polarization was done with an oxidation pulse 1.5 V, and a reduction pulse is -1.5 V to Ag/AgCl reference electrode. The polarization program was generated by the arbitrary form generator DG3121A connected to the developed ultra-fast potentiostat [14].

The obtained ECL response to show the kinetic evolution at the long-time observation period (figure 5.a), and the stochastic nature of emission at the short-time resolution (figure 5.b,c). Over the first 50 ms, few counts for some cycles are observed (figure 5.b). A little bit later, almost every anodic pulse produces ECL emission (figure 5.c).

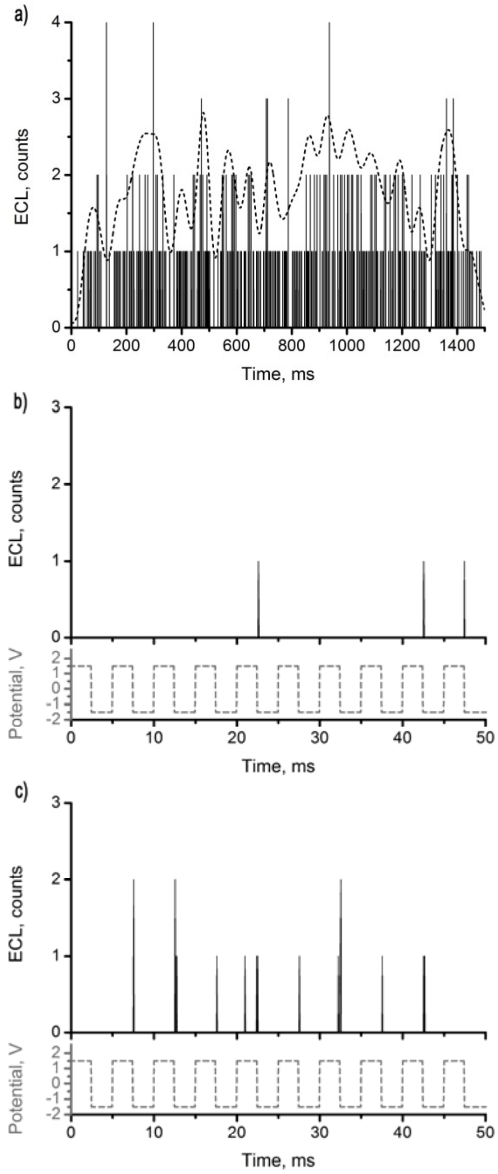

Fig.5. ECL response of ultramicroelectrode for 5ms pulse cyclic polarization: a) general ECL kinetics in millisecond time domain (dotted line is smoothed and ×100 times magnified ECL counting response), b) first 50 ms of ECL experiment, c) period between 300 ms and 350 ms. The solid line bar is ECL response counts, the dash line is the electrode polarization potential.

The rise of the ECL reaction is associated with the cation radical accumulation near the working electrode during electrolysis. The flash of ECL is observed at the anodic period of a cycle at the beginning. That moment, the concentration of cations is biggest in the volume near the electrode, they ready to recombine with newly generated anions. The cathodic period in the electrode polarization cycle does not have ECL emission, it claims the short lifetime of anions, so anions disappear to the moment of new cations appearance. Cations cannot find a pair for annihilation, and they are accumulated near the electrode. Diffusion processes increase the involved solution volume in the ECL reaction during electrolysis. The lifetime of ions limits this region and determines the saturation behavior in the ECL signal rise.

Developed apparatus characteristics verified in tests are summarized in Table 1.

Table 1 – “Pulsar” technical characteristics

Parameter	Value
Input	
Number of input signals	1 channel
Logical signal level	CMOS positive logic (high level >2V)
Pulse duration	6 ns and bigger
Input impedance	50 Ohm
Counter	
Counting method	continuous
Maximum pulse rate	84 MHz
Maximum counter capacity	216 (for gate period)
Counter data sampling	
Sampling signal	Internal
Sampling period	10 μs – 5s with 10 μs step
Triggering	
Triggering method	software or external hardware
External trigger signal	Positive TTL
Connectivity	
Interface	USB 2.0 FS, isolated
Protocol	virtual COM port
Capability	up to 255 devices on the USB bus
Output analog signal	
Voltage range	0 - 10 V
DAC resolution	12 bits
Analog filter bandwidth	1 kHz
Capability of digital filtering	bandwidth from 0.25 Hz and above
General	
Dimensions (H × D × W)	75 × 185 × 175 mm
Mass	1.0 kg
Power supply	220 V ± 20 %, 50 Hz

Conclusion

Developed instrumentation is usable in the realization of new analytical methods that demonstrated on the example of ECL measurements at ultramicroelectrodes. The capability to represent photon emission in two forms assists easy integration photon counting PMT to ECL analytical systems. Analog signal output is useful as a traditional way of photodetector integration with a potentiostat. On the other hand, the parallel data collection in digital form is a way to more precise ECL analysis. Higher temporal resolution and unprecedented sensitivity are helpful to reach the possibilities of single-particle and single-molecule detection.

Apparatus “Pulsar” can join with most photon counting modules by Hamamatsu Photonics that expand possibilities to choose an appropriate detector for analytical tasks. The demonstrated example on flexibility of the proposed apparatus in integration to analytical system demonstrates the usefulness application of photon counting units in ECL measurements.

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REFERENCES

[1] Miao W. Electrogenerated chemiluminescence and its biorelated applications, *Chem. Rev.*, 108 (2008), 2506–2553. DOI: 10.1021/cr068083a

[2] Liu Z., Qi W., Xu G., Recent advances in electrochemiluminescence, *Chem. Soc. Rev.*, 44 (2015), 10, 3117 –3142. DOI: 10.1039/c5cs00086f

[3] Muzyka K., Saqib M., Liu Z., Zhang W., Xu G., Progress and challenges in electrochemiluminescent aptasensors, *Biosensors and Bioelectronics*, 92 (2017), 241-258. DOI: 10.1016/j.bios.2017.01.015.

[4] Collinson M.M., Wightman R.M., Observation of Individual Chemical Reactions in Solution, *Science*, 268 (1995), 1883-1885. DOI: 10.1126/science.268.5219.1883

[5] Collinson M.M., Wightman R.M., High-Frequency Generation of Electrochemiluminescence at Microelectrodes, *Anal. Chem.*, 65 (1993), 2576-2582.

[6] Collinson M. M., Pastore P., Maness K.M., Wightman R.M., Electrochemiluminescence Interferometry at Microelectrodes, *J. Am. Chem. Soc*., 116 (1994), 4095-4096.

[7] Collinson M.M., Wightman R.M., Evaluation of Ion-Annihilation Reaction Kinetics Using High-Frequency Generation of Electrochemiluminescence, *J. Phys. Chem.*, 98 (1994), 11942-11947.

[8] Bard A.J., Fan F.-R.F., Electrochemical Detection of Single Molecules, *Acc. Chem. Res.,* 29 (1996), 572-578. DOI: S0001-4842(95)00244-5.

[9] Fan F.-R.F., Bard A.J., Observing Single Nanoparticle Collisions by Electrogenerated Chemiluminescence Amplification, *Nano Letters*, 8 (2008), 1746-1749. DOI: 10.1021/nl8009236.

[10] Peng Y.-Y., Qian R.-C., Hafez M. E., Long Y.-T., Stochastic Collision Nanoelectrochemistry: A Review of Recent Developments, *ChemElectroChem*, 4 (2017), 1–10. DOI:10.1002/celc.201600673

[11] SR400 — Gated photon counter (2-channel). Datasheet. https://www.thinksrs.com/downloads/pdfs/catalog/SR400c.pdf

[12] USB interface compatible counting unit C8855-01. Datasheet. https://www.hamamatsu.com/resources/pdf/etd/C8855-01_TACC1078E.pdf

[13] D. Snizhko, G. Bani-Khaled, K. Muzyka, G. Xu, Apparatus "Spark" for luminescent and electrochemiluminescent measurements, *Przegląd Elektrotechniczny*, Vol. 94, No 6, (2018). DOI:10.15199/48.2018.06.07

[14] D. Snizhko, A. Kukoba, Ultrafast Potentiostat as Compromise between Current Sensitivity vs. Response Time, *Przegląd Elektrotechniczny*, 95 (2019), 102-107.
DOI:10.15199/48.2019.08.24

[15] K.S.V. Santhanam , A.J. Bard, Chemiluminescence of Electrogenerated 9.10- Diphenylanthracene Anion Radical, *J. Am. Chem. Soc.*, 87 (1965), 139-140.

[16] K.M. Omer, A.J. Bard, Electrogenerated Chemiluminescence of Aromatic Hydrocarbon Nanoparticles in an Aqueous Solution, *J. Phys. Chem. C*, 2009, 113 (27), 11575-11578. DOI: 10.1021/jp901038h.