Creation of a device for detecting fluorescence from microfluidic chips for studying biosystems

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Abstract. The article substantiates the need to develop devices using real-time PCR reaction on microfluidic chips for the study of biological systems (objects) to determine environmental pollution and the consequences of these pollution. In situations of radioactive or chemical contamination, it is necessary to detect and eliminate them as soon as possible. For these purposes, PCR is ideally suited, which is currently the leading tool for chemical and biological research. However, most of the currently available PCR devices use tubes or microtiter plates and have some serious disadvantages. Disadvantages: uneven heating/cooling of volumetric systems, analysis speed does not meet the requirements of modern medicine, biology, environmental services, etc., namely, the requirement for rapid analysis. The solution to this problem is microfluidic chips since they are planar systems. Using microfluidic chips, it is possible to analyze more samples in less time. This article presents a layout of a fluorescence detection device using real-time PCR reaction on microfluidic chips. The results of experimental studies on the created layout and their analysis are presented.

1. Introduction
At the moment, there are many environmental problems [1-9]. The reasons why these problems have arisen are only getting worse. [3, 5, 8, 10-16]. This is due to the development of industry, energy, population growth, etc. [2-5, 10-12, 17-24]. Therefore, around the world, in many countries the ecological situation continues to change for the worse [1, 4, 5, 9, 10, 17, 18, 23, 25-29]. In conditions of deterioration of the ecological situation, large changes take place in the structure of biological objects [10-13, 28-33]. С другой стороны, in addition to dealing with the pollution, it is necessary to find out its consequences [19-21, 24, 26-32, 34-39]. Such environmental problems as pollution bodies of water, soil, air have a great impact on biological objects [26-32, 35-37, 40, 41].

Pathogenic changes in biological systems (objects) can be caused by environmental pollution, including chemical or radioactive pollution. Changes in the structure of biological systems resulting from environmental pollution can be very dangerous and should be detected and investigated as early as possible [28-32, 38-41].
Polymerase chain reaction (PCR) is one of the most demanded technologies in molecular biology [42-46]. Using PCR, specific sequences in a DNA or cDNA template can be copied or "amplified" a thousand or a million times using sequence-specific oligonucleotides, thermostable DNA polymerase, and thermal cycling techniques.

Detection and quantification of the amplified sequence in conventional PCR is performed after the last PCR cycle and includes an estimate of the resulting copy number, while in quantitative real-time PCR (qPCR), the PCR product is measured in each cycle. The initial amount of target is determined with great accuracy by observing the reactions during the exponential increase in copies. Measurements of the amount of DNA in real-time PCR are carried out using fluorescent dyes [42-48]. These dyes give a fluorescent signal that increases in direct proportion to the number of formed PCR product molecules (amplicons).

Thus, in order to study biological objects, a mock-up of a device for recording fluorescence from microfluidic chips during real-time PCR, that I created, can be used.

2. Structure and manufacturing of microfluidic chips

The transition to a microchip format when conducting analyzes based on real-time PCR reactions allows you to automate the analysis and reduce the influence of the human factor on its results. In recent years, polymers have taken the leading position as substrate materials for microfluidic devices [42-46]. They have excellent physical and chemical properties, enabling the creation of micro-sized structures with desired characteristics, which provide microscopic design features that cannot be realized in any other class of materials [49-56].

To create microchips, three types of plastics are most often used: polypropylene (PP), polycarbonate (PC), and polymethyl methacrylate (PMMA). Their main advantages: high heat resistance, good light transmission in the visible part of the spectrum. The design of the microchip (Fig. 1, dimensions are given in mm), which consists of three chambers with inlet channels, was obtained by thermal pressing in a hydraulic press MM-100 (MTDI, Korea) on a stainless-steel master mold made by laser micromachining. The microchip is 38 mm long, 25 mm wide and 1 mm thick. The distance between the loading ports of neighboring cameras is 11 mm. The width of the channels is 1 mm, and the depth of the chambers is ~ 0.3 mm with a bottom thickness of ~ 0.7 mm.

![Figure 1. Microfluidic chip design with numbering of reaction chambers.](image)

Microfluidic chips obtained by the above method are shown in Fig. 2.

![Figure 2. Microfluidic chips: left - made of polycarbonate; on the right - polypropylene.](image)
The chips are filled with water. The first chip is made of polycarbonate PK Novattro (Kazan), the second one is made of polypropylene PP 44455 (RF).

3. Mock-up of the device for registration of fluorescence
The light source 1 (Fig. 3) in this device is an SMD LED with a wavelength of emitted light of 480 nm, a power of 3 W, with a maximum control current of 700 mA and a luminous flux of up to 70 lm.

![Figure 3. Mock-up of the device for registration of fluorescence: 1 - source (LED); 2, 4, 6, 8 - lenses; 3 - excitation filter; 5, 7 - emission filters; 9 - photodetector; 10 - location of the chip; 11 - thermal cycler; 12 - optical fiber (optical fiber bundle).](image)

From the source, the light enters the system of plano-convex lenses 2 and 3 (Fig. 3) and excitation filter 3 (Fig. 3) with a wavelength of 490 nm. Further, the light enters the triple optical fiber 12 (Fig. 3, fiber-optic bundle of type O-BKh-1-3-250). One channel is excitation, the other two are registration of a fluorescence/emission signal. Light, passing through the fiber, falls into the solution located in the chip 10 (Fig. 3), and excites fluorescence. The chip is located in the thermal cycler 11 (Fig. 3), with which the PCR reaction is carried out. The thermal cycler also has a device for fixing the optical fiber, which allows not only to fix the lighting bundle in the thermal cycler, but also to control the distance from it to the chip. Fluorescence is detected using a photo application 9 (Fig. 3., Basler ace acA720-520um cameras with a quantum efficiency of 63.2%), on which light enters after passing through an emission filter 5 or 7 (Fig. 3) with a wavelength of 520 nm and plano-convex lenses 6 and 8 (Fig. 3).

4. Experimental investigations
For the experiment, the following PCR parameters were set:
• Primary denaturation: 264 K and duration 1 minute;
• Cycle parameters:
• Denaturation per cycle: 333 K and duration 20 seconds;
• Cycle synthesis: 248 K and duration 10 seconds;
• Annealing per cycle: 263 K and duration 10 seconds;
• Number of cycles: 30;
• Final incubation: 307 K and duration 2 minutes.

Studies have been carried out using Cy5 dye. A graph of the dependence of the signal level on the PCR time was obtained, shown in Fig. 4.

![Graph of the dependence of the signal level on the PCR time for the first channel.](image)

**Figure 4.** Graph of the dependence of the signal level on the PCR time for the first channel.

On the graph, we can observe the passage of the real-time PCR reaction, namely the real-time amplification process. Jumps in the signal level with reaching the peak at approximately equal intervals of time and with an increase in the signal level of the peak with each new jump, express cycles of the PCR reaction in the process of amplification. That is, after each cycle, the amount of product in the chip increases and, consequently, the fluorescence signal increases.

As a result of the experiments, graphs were obtained that adequately reflect the processes of thermal cycling, that is, the growth of the product in the chip. The task of recording the real-time amplification process was successfully completed.

5. Conclusion

As a result of experimental studies on the assembled layout, graphs were obtained reflecting the processes of thermal cycling, amplification and reaching a plateau in real time, that is, the real-time PCR process was recorded. Thus, the assembled device layout can be further used for biological and medical research on microfluidic chips during real-time PCR, which means it can be used to determine contamination and the consequences of contamination of biological objects.
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