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Creation of a device for detecting fluorescence from microfluidic chips

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Abstract. In this paper, we consider the creation and testing of a prototype for recording fluorescence from microfluidic chips during the polymerase chain reaction (PCR). The paper presents the characteristics of the main elements used to create the layout of the device for fluorescence detection. The results of experiments in testing the performance of mock-up elements and microfluidic chips are presented. The operability of the assembled layout was demonstrated during the real-time PCR reaction.

Keywords: polymerase chain reaction (PCR), microfluidic chip, DNA, fluorescence, dyes, thermal cycler, optical fiber, amplification.

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Создание устройства регистрации флуоресценции от микрофлюидных чипов

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Аннотация. В данной работе рассматривается создание и проверка на работоспособность макета для регистрации флуоресценции от микрофлюидных чипов при проведении полимеразной цепной реакции (ПЦР). В работе приведены характеристики основных элементов, использованных при создании макета устройства для детектирования флуоресценции. Представлены результаты экспериментов при проверке работоспособности элементов макета и микрофлюидных чипов. Продемонстрирована работоспособность собранного макета при проведении ПЦР реакции в реальном времени.

Ключевые слова: полимеразная цепная реакция (ПЦР), микрофлюидный чип, ДНК, флуоресценция, красители, термоциклер, оптоволкно, амплификация

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Introduction

Currently, the leading tool for chemical and biological research is the PCR polymerase chain reaction [1–5]. Other methods, mainly spectrometric, which are used for research in chemistry, biology and physics, based on the use of nuclear magnetic resonance, laser radiation, and others [6–15], cannot replace it. A small part of the PCR results can be obtained using refraction or magnetic resonance imaging (MRI) [16–23]. With PCR, specific sequences in a DNA template or to DNA can be copied or "amplified" a thousand or a million times using sequence-specific oligonucleotides, thermostable DNA polymerase, and thermal cycling techniques. Real-time PCR [2, 3, 24–26]. This is a type of PCR method that is commonly used to quantify DNA or RNA in a sample. Using sequence-specific primers, the copy number of a particular DNA or RNA sequence can be determined. Quantification is possible by measuring the amount of amplified product at each step of the PCR cycle. Quantification is possible by measuring the amount of amplified product at each step of the PCR cycle. Amplification will be observed in earlier cycles if a certain sequence (DNA or RNA) is present in the sample, and if the sequence is insufficient, amplification will be observed in later cycles or not recorded at all. Quantification of the amplified product is obtained using fluorescent probes or fluorescent DNA-binding dyes and real-time PCR tools that measure fluorescence while performing the thermal cycling required for the PCR reaction.

Most currently available devices for PCR analysis use test tubes or microtiter plates, and they have a number of serious drawbacks [24-26], which are also used in NMR and X-ray spectroscopy [27-30]. Disadvantages are 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, more samples can be analyzed in less time. Thus, the development and creation of devices for real-time PCR analysis using microfluidic technologies is essential for extremely important.

Design and manufacture 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. They have superior physical and chemical properties enabling the creation of micro-sized structures with desired characteristics that provide microscopic design features that cannot be realized in any other class of materials.

Three types of plastics are most commonly used to create microchips: polypropylene (PP), polycarbonate (PC), and polymethyl methacrylate (PMMA). Their main advantages are high heat resistance, good light transmission in the visible part of the spectrum. Polypropylene is more resistant to acids and solvents than polycarbonate and has lower water sorption (0.01–0.1% versus 0.23% for polycarbonate). The microchip design (Fig. 1, dimensions are given in mm), which consists of three chambers with supply channels, was obtained by thermal pressing in MM-100 hydraulic press (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.

Images of microfluidic chips obtained by the above method are shown in Fig. 2. 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).

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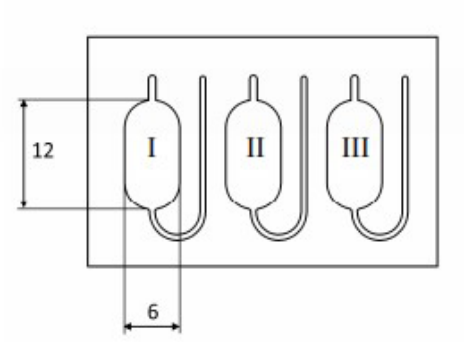

Fig. 1. Microchip design with numbering of reaction chambers

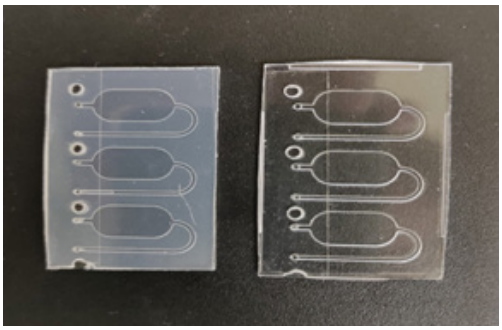

Fig. 2. Microfluidic chips made from PC (left) and PP (right)

On Fig. 3 shows the developed layout of the device for detecting fluorescence from microfluidic chips.

The main elements of the device layout are: a microfluidic chip, a LED, a photodetector (camera), a fiber optic bundle, a thermal cycler, optical lenses and filters. The light source in the device is an SMD type 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. From the source, light enters a system of plano-convex lenses and an excitation filter with a wavelength of 490 nm. Next, the light enters the triple optical fiber (fiber optic bundle). One channel is for excitation, the other two are for registration of the fluorescence/emission signal. Light passing through the optical fiber enters the solution in the microfluidic chip and excites fluorescence. The chip is located in a thermal cycler 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 detection occurs with the help of a photodetector (camera), on which light enters after passing through an emission filter with a wavelength of 520 nm and plano-convex lenses.

The operability of the mock-up device was tested by performing a PCR reaction on it with specially set parameters and using a microfluidic chip filled with a reagent, under which the parameters of the PCR reaction were selected. The fluorescence signal from the chip was recorded using a camera and then processed by a computer program to obtain a PCR reaction graph. After that, it was possible to analyze the resulting graph.

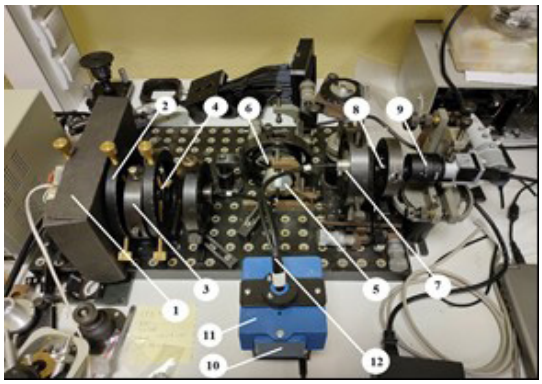

Fig. 3. Mock-up of the device for registration of fluorescence:
1 – source (LED);
2, 4, 6, 8 – lenses;
3 – excitation filter;
5, 7 – emission filters;
9 – photodetector;
10 – location of the chip;
11 – thermal cycler;
12 – optical fiber (optical fiber bundle)

The results of experimental studies of the assembled layout

For the experiment, the following PCR parameters were set:
- Primary denaturation: 91 °C and duration 1 minute;
- Cycle parameters:
  - Denaturation per cycle: 60 °C and duration 20 seconds;
  - Cycle synthesis: 75 °C and duration 10 seconds;
  - Annealing per cycle: 90 °C and duration 10 seconds;
- Number of cycles: 30;
- Final incubation: 36 °C and duration 2 minutes.

To fill the microfluidic chip, we used a set of reagents for the detection of plant DNA in food products, food raw materials, seeds and feed by real-time polymerase chain reaction "Plant Universal". Cy5 dye was used.

A graph of the dependence of the signal level on the PCR time was obtained, shown in Fig. 4.

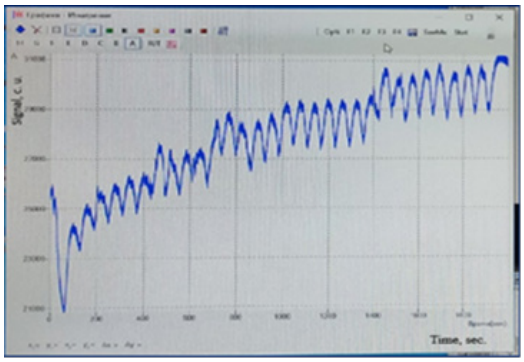

Fig. 4. Graph of the dependence of the signal level on the PCR time

On the graph (Fig. 4) one 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 during the amplification process. That is, after each cycle, the amount of product in the chip increases and, consequently, the fluorescence signal increases.

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. The device model assembled in this way can be further used for biological and chemical studies on microfluidic chips during real-time PCR.

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