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Development of a sample preparation unit

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Abstract. The necessity of using a single-molecule sequencer in biology and medicine is substantiated. The role of sample preparation in conducting these studies is noted. It was noted that with increasing requirements for the results of genetic studies, it is necessary to modernize and develop new designs of sample preparation units. The study performs the sample preparation unit, which can be applied for experiments with biological materials, particularly for genetic research. The main components of the system were introduced, such as position module, sample loader and temperature control module. Test trials of the water dozing and thermal stabilization were carried out.

Keywords: sample preparation, automatic dosing, temperature control, dispenser, thermostat, biological materials

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Разработка блока пробоподготовки

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Аннотация. Обоснована необходимость использования в биологии и медицине одномолекулярного секвенатора. Отмечена роль пробоподготовки при проведении данных исследований. Отмечено, что с повышением требований к результатам генетических исследований необходима модернизация и разработка новых конструкции блоков пробоподготовки. Представлен блок пробоподготовки одномолекулярного секвенатора, предназначенный для экспериментов с биологическими материалами, в частности для генетических исследований. Были рассмотрены основные компоненты системы: позиционер, модуль загрузки образца и модуль контроля температуры. Также были проведены тестовые испытания дозирования воды и термостатирования.

Ключевые слова: подготовка пробы, автоматическое дозирование, системы дозирования, контроль температуры, дозатор, термостат, биологический материал, генетические исследования

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Introduction

Nowadays, a lot of research is carried out using laser radiation or related effects [1–6]. One of the large sections in these studies relates to biology and medicine [7–11]. Temperature control and precision dosing of biological samples are important steps in biological and medical research [12, 13]. Temperature control makes it possible to maintain the temperature stability of the samples, which in turn ensures the safety of their state and properties. Automatic dosing of liquid samples improves the accuracy and speed of analysis. Manual dosing can lead to errors due to human error, is time-consuming and can cause operator fatigue [13, 14].

Automation of the sample preparation process will reduce the risk of contamination of samples and the operator during work with biological materials. This is especially important when dealing with dangerous viruses or bacteria.

Thus, the development of a sample preparation unit that performs temperature control of liquid biological samples, as well as their automatic dosing, may be necessary to improve the accuracy, speed, and safety of the process of studying biological materials, as well as to optimize the work of the laboratory.

Sample preparation unit structure

The development of the block diagram of the sample preparation unit was carried out according to the principle of functional completeness of each individual module. The functions of the Sample preparation unit were decomposed, then the electrical modules were isolated, presented in the block diagram (Fig. 1).

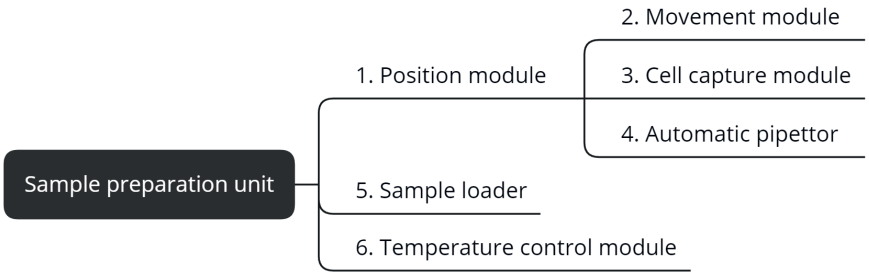

Fig. 1. Block diagram of the sample preparation unit

Position module is a complex system for 3D position control of liquid reagents and reactive cells, consists of several modules:

- two-coordinate movement module for horizontal XY plane;
- cell capture module designed for transfer the cell from the storage position to the fill, detect, and reset positions;

- automatic pipettor designed for automatic reagents input from containers and output into designated areas.

Due to the focus on working with biological materials, the additional functional module was performed to adopt the sample preparation unit for using in DNA sequencing. Sample loader is a module aimed to investigate the immobilization of the DNA-polymerase complex at nanoholes. There are three ways of loading a sample: passive, magnetic and electrophoretic [15]. Magnetic loading implies control parameters such as: duration, speed and direction of rotation, loading cycle algorithm type.

Temperature control module

Temperature control module provides conditions for storing reagents on the desktop. Maintaining the set temperature of the tablets with reagents is carried out by changing the current flowing through the Peltier element. Automatic adjustment is performed by proportional integral derivative (PID) controller, implemented in the program of temperature control board`s microcontroller STM32G071CBT6. Control action is calculated as:

$$u(n) = P \cdot e(n) + I \cdot T \cdot \sum_{0}^{n} e(n) + D \cdot \left[e(n) - e(n-1) \right], \quad (1)$$

where P is proportion coefficient, e is error calculated as difference between current temperature and set temperature, I is integral coefficient, T is discretization period, D is derivative coefficient.

Digital temperature sensor TPM117 is used to get data about tablet temperature. Sensor has got the following features:

- 16 bits data width;
- 7.8125 m°C resolution;
- 0.25 seconds conversation period;
- 32 averages per conversation.

There is sensor`s data correction function implemented in the program of microcontroller to increase the accuracy of set temperature maintaining. Corrected temperature is calculated as:

$$T_{cor} = A + B \cdot T_{sens}, \quad (2)$$

where A is offset, B is slope factor, T_{sens} is sensor`s data.

The electrical boards and software were designed for each module. The sample preparation control board is the master for all other boards: stepper boards of the position module, the sample loader boards, and the temperature control module boards. The RS-485 was chosen as the data interface and Modbus-RTU as communication protocol. Connecting devices of the same type to one communication line will avoid conflicts on the line and simplify further management organization.

Three software levels were identified: the upper user level – UI, the middle level – the unit control system, and the lower level – the microprocessor firmware of the module boards and their configurations.

The upper level is based on a drug-and-drop architecture to improve UX comparing to console-based interface, the technology stack is HTML+CSS, JS (bootstrap, j-query). The middle level is

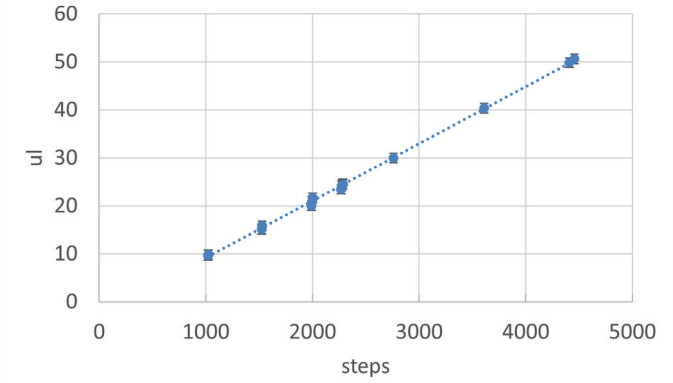

Fig. 2. Dosing calibration curve

based on OOP principles, each separate electrical board is a class object with specific methods in according to its functions. The lower level is based on ARM-architecture chip with HAL.

Results and Discussion

As a result of testing the position module, a dosing calibration curve with deionized water was obtained. Dose volume was taken as the average of 50 measurements (Fig. 2). Table presents the results of testing the temperature control module's thermostats. Where t is stabilization time of thermostats and T_i is temperature of thermostat, i is the thermostat number.

Table

Results of testing the thermostats

Measurement №	t, s	T_1, °C	T_2, °C	T_3, °C	T_4, °C
1	714	8.0 ± 0.3	8.1 ± 0.2	8.1 ± 0.2	8.0 ± 0.2
2	508	7.9 ± 0.2	8.0 ± 0.1	8.0 ± 0.2	7.9 ± 0.3
3	809	8.0 ± 0.1	8.0 ± 0.2	7.9 ± 0.2	8.0 ± 0.1

Conclusion

An analysis of the obtained experimental data allows us to establish the following. The developed sample preparation unit allows automatic dosing of liquid samples with an accuracy of 1 μl and maintains the set thermostat temperature with an accuracy of 0.5 K. The achieved sample dosing accuracy is sufficient for genetic sequence sequencing studies. The accuracy of maintaining the temperature of the thermostat at this stage of research meets the experiment requirements. In the future, it will need to be improved, which will be the subject of our further work.

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