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Experimental Setup for Detecting Fluorescence Signals of Samples in Microfluidic Devices Reactors

P.M. Lanin[1], A.V. Tarasova[1], N.A. Esikova[2], N.V. Panteleev[2], I.E. Antifeev[2], K.V. Pantsialeyeu[3], D.A. Belov[2]

[1]Saint-Petersburg State University of Aerospace Instrumentation,
Bolshaya Morskaya str., 67, lit. A, St. Petersburg 190000, Russia

[2]Institute for Analytical Instrumentation of the Russian Academy of Sciences,
Ivan Chernykh str., 31-33, St. Petersburg 198095, Russia

[3]Belarusian National Technical University,
Nezavisimosti Ave., 65, Minsk 220013, Belarus

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Abstract

Genetic analysis in microfluidic devices commonly relies on detection of fluorescence intensity from dyes bound to DNA fragments. Aim of this work was to develop an experimental setup and a method for obtaining fluorescence intensity of liquids placed in microfluidic chips by analyzing digital fluorescence images under light excitation. This article describes an experimental setup for detection of fluorescence signal from sample placed into reaction chamber of microfluidic chips with a 25×40 mm field of view. The system directs excitation radiation from an LED (λ_{avg} = 480 nm, P = 3 W, $U_{\max}$ = 6 V, $I_{\max}$ = 0.6 A) through a bandpass filter (467–498 nm) and a lens onto the surface of a microfluidic chip. The selected parameters of the system enable efficient excitation of fluorescent dyes without overlapping their emission spectrum. Experiments were performed using fluorescein isothiocyanate solutions of different concentrations. Comparison of the signal-to-noise ratio values of the presented experimental setup with that of a commercially available real-time polymerase chain reaction device (ANK-48) has been carried out. The key advantages of the proposed system are its ability to perform full-area fluorescence detection on the microfluidic chip and its flexibility in terms of chip topology, i. e., the ability to work using chips of various designs and geometries.

Keywords: fluorescence, pixel array detector, microfluidic chip, real-time polymerase chain reaction, signal-to-noise ratio

Адрес для переписки:
Белов Д.А.
Институт аналитического приборостроения
Российской академии наук,
ул. Ивана Черных, д. 31-33, г. Санкт-Петербург 198095, Россия
e-mail belov.da@list.ru

Address for correspondence:
Belov D.A.
Institute for Analytical Instrumentation of the Russian Academy of Sciences,
Ivan Chernykh str., 31-33, St. Petersburg 198095, Russia
e-mail belov.da@list.ru

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П.М. Ланин, А.В. Тарасова, Н.А. Есикова, Н.В. Пантелеев, И.Е. Антифеев, К.В. Пантелеев, Д.А. Белов.
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Экспериментальная установка для детектирования сигналов флуоресценции образцов в реакторах микрофлюидных устройств

П.М. Ланин[1], А.В. Тарасова[1], Н.А. Есикова[2], Н.В. Пантелеев[2], И.Е. Антифеев[2], К.В. Пантелеев[3], Д.А. Белов[2]

[1]Санкт-Петербургский государственный университет аэрокосмического приборостроения, ул. Большая Морская, д. 67, лит. А, г. Санкт-Петербург 190000, Россия

[2]Институт аналитического приборостроения Российской академии наук, ул. Ивана Черных, д. 31-33, г. Санкт-Петербург 198095, Россия

[3]Белорусский национальный технический университет, пр-т Независимости, 65, г. Минск 220013, Беларусь

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Генетический анализ в микрофлюидных устройствах, как правило, основан на регистрации интенсивности флуоресценции красителей, связанных с фрагментами ДНК. Целью работы была разработка экспериментальной установки и методики определения интенсивности флуоресценции жидкостей в микрофлюидных чипах путём обработки цифровых флуоресцентных изображений, полученных при возбуждающем освещении. Представлена экспериментальная установка для одновременной детекции флуоресцентных сигналов из реакционных камер микрофлюидных чипов по полю зрения 25×40 мм. Установка обеспечивает направление возбуждающего излучения от светодиода ($\lambda_{ср}$ = 480 нм, P = 3 Вт, U_{max} = 6 В, I_{max} = 0,6 А) через полосовой светофильтр (467–498 нм) и линзу на поверхность микрофлюидного чипа. Выбранные параметры установки позволяют эффективно возбуждать флуоресцентные красители, не затрагивая спектр их эмиссии. Эксперименты выполнены с использованием растворов флуоресцеина изотиоцианата различных концентраций. Проведено сравнение значений отношения сигнал/шум разработанной установки и коммерчески доступного прибора (АНК-48). Ключевыми преимуществами предложенной системы являются возможность полноплощадной детекции флуоресценции на микрофлюидном чипе, а также гибкость по отношению к топологии чипа, т. е. способность работать с чипами различных конфигураций и геометрий.

Ключевые слова: флуоресценция, двумерный пиксельный детектор, микрофлюидный чип, полимеразная цепная реакция в реальном времени, отношение сигнал/шум

Адрес для переписки:
Белов Д.А.
Институт аналитического приборостроения Российской академии наук,
ул. Ивана Черных, д. 31-33, г. Санкт-Петербург 198095, Россия
e-mail belov.da@list.ru

Address for correspondence:
Belov D.A.
Institute for Analytical Instrumentation of the Russian Academy of Sciences,
Ivan Chernykh str., 31-33, St. Petersburg 198095, Russia
e-mail belov.da@list.ru

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Introduction

Application of microfluidic technologies enables precise control of microscale fluid flows and their controlled manipulation [1]. Use of these technologies in applied research offers several advantages over conventional approaches including reduced chemical reaction times, lower reagent consumption, enhanced portability and improved analytical efficiency [2]. Over the past decade substantial advances in microfluidics and microfabrication have been achieved allowing implementation of these technologies across various fields including diagnostics, food safety assurance and materials manufacturing [3].

Planar microfluidic devices are widely used in genetic analysis particularly for real-time polymerase chain reaction (qPCR) applications [4]. qPCR is a genetic analysis method for the quantitative and qualitative assessment of target DNA fragments based on the increase in fluorescence intensity of dyes within the analyzed sample [5]. In conventional commercial qPCR systems, reactions are typically performed in 96-well plates or 0.2-mL tubes. The integrated detection system excites the fluorescent dyes and measures the total fluorescence signal emitted from each individual tube/well.

qPCR is widely implemented on microfluidic platforms. This conventional approach enables a significant reduction in sample volume and reaction time while improving accuracy [6]. Portable PCR analyzers based on microfluidic technology and optical detection (such as the Cepheid GeneXpert or FilmArray systems) are commercially available.

Commercially available fluorescence detection systems are often not suitable for full-area or large-scale parallel imaging of multiple reaction chambers for microfluidic chips (MFCs). Various detection approaches are described in the literature most of which utilize CMOS or CCD matrix detectors that enable image acquisition from multiple reaction chambers within one or several fields of view [7, 8]. However moving the detector relative to the chip and subsequently merging the obtained images increases the overall scanning time. Moreover for certain applications such as high-resolution DNA melting analysis it is necessary to monitor fluorescence signals across the entire area of the microfluidic chip.

Aim of this work was to develop an experimental setup and a method for determining the fluorescence intensity of liquids placed in microfluidic chips by analyzing digital fluorescence images acquired under excitation illumination. This article describes an experimental setup providing a 25×40 mm field of view for detection of fluorescence signals from samples in the reaction chambers of microfluidic chips.

Materials and Methods

Schematic diagram of the experimental setup is shown in Figure 1. The setup enables projection of the excitation radiation from an LED source (λ_{avg} = 480 nm, P = 3 W, $U_{\max}$ = 6 V, $I_{\max}$ = 0.6 A) through a single-band excitation filter E×F (OTF 467–498 nm) and lens OL2 onto the surface of MFC. The selected spectral and power characteristics of the LED ensure efficient excitation of fluorescent dyes while avoiding overlap with their emission spectra.

LED operation generates significant heat. To ensure effective cooling, the experimental setup equipped with a finned heat sink that maintains thermal contact with the LED. The design also allows for movement of the LED relative to the housing and the lens, providing the ability to adjust the area of the excitation radiation projected onto the detection plane.

We implemented three-axis movement of the detection system relative to the chip.

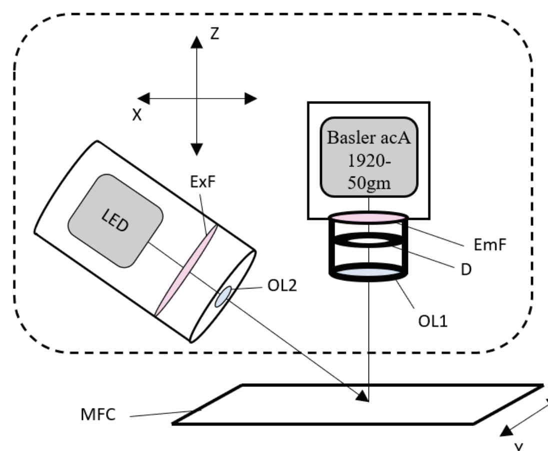


Figure 1 – Schematic diagram of the detection system: MFC – microfluidic chip; LED – light-emitting diode; ExF – single-band filter (467–498 nm); OL1 and OL2 – lenses; D – diaphragm; EmF – single-band filter (510–530 nm), Basler acA 1920-50gm – digital camera for detection

To obtain images in fluorescent rays, a monochrome Basler acA 1920-50gm CMOS camera with a Sony IMX174LLJ-sensor with a resolution of 1920×1200 pixels with a single-band OTF 510-530 nm light filter and a Computar 2/3" 16mm F2.8 lens were used. The images were processed in 16-bit grayscale.

For testing the setup, a fluorescein isothiocyanate solution in 0.1 N NaOH was used. Solutions with concentration ranging from 3.0×10^{-7} to 6.0×10^{-8} M were prepared by serial dilution followed by further dilution.

Microfluidic chips shown in Figure 2 were developed at the Institute of Analytical Instrumentation of the Russian Academy of Sciences (IAI RAS). The chips measuring have dimension 28×38 mm and contain four reaction chambers, 14 μL each. The fabrication method of the chips is described in [9]. The chips are made of Novattro polycarbonate, which has low autofluorescence, high chemical and thermal resistance, is easy to mold, and does not inhibit PCR. The mold was fabricated from LS-59 brass using a CNC milling machine.

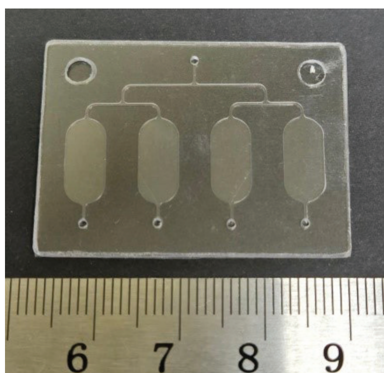


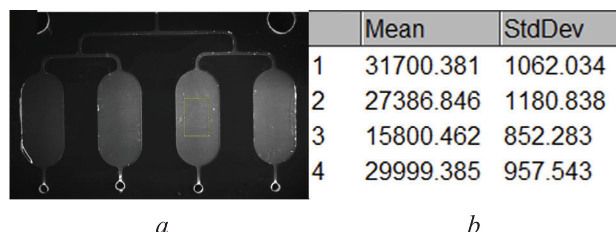
Figure 2 – Image of a microfluidic chip

The signal-to-noise ratio (SNR) of the proposed system was assessed by comparing its SNR_{mf} to SNR_t of the commercial device ANK-48 (IAI RAS, Russia) using identical samples.

The SNRt value was calculated based on the mean fluorescence intensities of the fluorescein sample F_{t1} and the buffer F_{t2}, as well as the standard deviation of the fluorescence intensity in the buffer tube SD_t:

$$SNR_t = (F_{t1} - F_{t2}) / SD_t. \quad (1)$$

The SNR_{mf} values for the proposed system were evaluated using ImageJ software. For each of the four reaction chambers of the MFC, a region of interest was manually defined using the Rectangle tool. Regions (Figure 3*a*) were selected to fully capture the fluorescent signal inside the chamber while excluding: boundary artifacts (chamber edges, out-of-band illumination) and imaging defects (individual dead pixels or overexposed areas resulting in signal saturation). For each defined rectangular region, analysis was performed using the command Analyze → Measure. The results are presented in Figure 3*b*, which shows the following key parameters for each measured region: Mean (average signal intensity within the region) and StdDev (standard deviation of pixel intensity). Additionally, the fluorescent signal from the MFC filled with buffer was detected separately to assess the background signal F_{mf2}.



	Mean	StdDev
1	31700.381	1062.034
2	27386.846	1180.838
3	15800.462	852.283
4	29999.385	957.543

a *b*

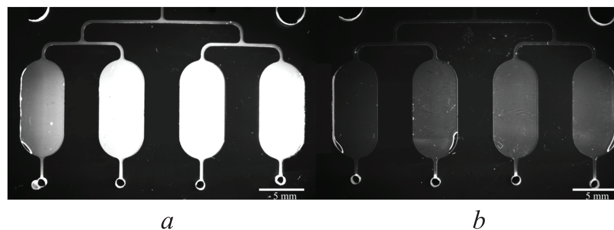
Figure 3 – Example of selecting an area for detecting a fluorescent signal inside the microfluidic chip chamber (*a*) and an example of the results obtained using ImageJ (*b*)

Based on the obtained results, the average pixel intensity (gray level) in each region of interest corresponding to the sample-filled MFC F_{mf1}, the average pixel intensity for the buffer-filled chamber F_{mf2}, and the standard deviation of pixel intensity in the buffer chamber – SDmf were determined. Grayscale pixel intensity is directly proportional to the intensity of the detected fluorescent signal under the given experimental conditions. SNR_{mf} values were calculated using formula:

$$SNR_{mf} = (F_{mf} - F_{mf}) / SD_{mf}. \quad (2)$$

Results and Discussion

Images obtained with the experimental setup from fluorescein isothiocyanate solutions at concentrations of $C = 0.3 \cdot 10^{-6}$ M (*a*) and $C = 0.6 \cdot 10^{-7}$ M (*b*) are shown in Figure 4.



a *b*

Figure 4 – Image of microfluidic chip chambers with a fluorescein isothiocyanate concentration $C = 0.3 \cdot 10^{-6}$ M (*a*) and $C = 0.6 \cdot 10^{-7}$ M (*b*)

The obtained SNR_t and SNR_{mf} values for seven different fluorescein concentrations are shown in Figures 5 and 6. In Figure 5, the vertical rectangles

represent the range of signal variation for each concentration (mean ± standard deviation) obtained with the ANK-48 device. Similarly, in Figure 6, the rectangles denote the spread of SNR_{mf} values calculated from multiple image regions for each concentration.

Comparison of the graphs shows that the SNR values of the commercial device is higher: the SNR_t values exceed the SNR_{mf} values by a factor of 1.5 to 2. Obtained SNR values can be increased by detector with higher SNR, optimized optical filtering that minimizes background noise, and the use of high-power LED excitation, which ensures a strong fluorescence signal even at low dye concentrations.

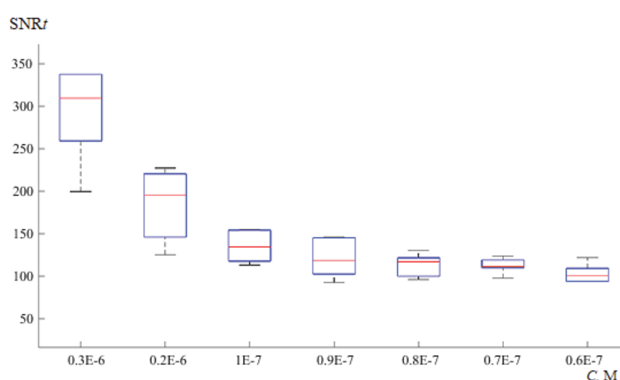


Figure 5 – The signal-to-noise ratio values of the commercial ANK-48 device

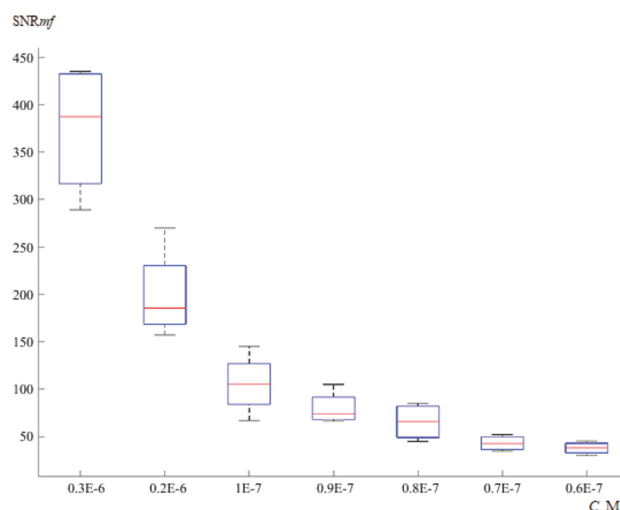


Figure 6 – The signal-to-noise ratio values of the proposed experimental setup

The field of view of the setup is adjustable. However, increasing the field of view may affect the illumination uniformity and requires optimization to maintain a high SNR.

Conclusions

The proposed optical scheme for detecting fluorescent signals from samples in microfluidic chip ensures the signal-to-noise ratio values comparable to those of commercially available devices designed for tube-based real-time polymerase chain reaction analysis. Advantages of the proposed design include its versatility enabling use of various microfluidic chip topologies and detection of fluorescence signals with an adjustable field of view of up to 40×25 mm.

The developed setup is required for conducting research with microfluidic chip of different purposes and geometries, as well as for testing experimental hypotheses. It can also be applied to practical tasks such as implementation of genetic analysis methods, real-time polymerase chain reaction and high-resolution DNA melting analysis in a microfluidic approach. Further development of the setup should focus on enabling detection in more than one spectral channel and improving the uniformity of LED illumination across the detection field of view.

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