

Article

Application of a Low-Cost Fluorescence Detector for 3D-Printed Lab-on-a-Chip Microdevices

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Abstract

Lab-on-a-chip devices offer high efficiency, low volume and fast analytical measurement, but their use is still niche. A key component for these devices is the detector, and one common type of detection is fluorescence spectroscopy. However, in some cases the detector can be bulky and lose the appeal of small-footprint devices. To make lab-on-a-chip devices truly compact, detectors must also be compact. In this paper we discuss the use of simple and low-cost commercial multispectral sensors for use in lab-on-a-chip devices, more specifically for fluorescence detection, which we demonstrate to allow detection on nanomolar scale with a simple experimental setup.

Keywords: fluorescence; low-cost sensors; 3D printing; multispectral sensors; lab-on-a-chip

1. Introduction

Lab-on-a-chip (LoC) devices condense multiple laboratorial processes into a single compact platform [1], which can include pre-concentration, mixing, separation, detection, and others [2]. By condensing the analysis steps into a single chip, some advantages appear, such as low sample and reagent consumption, high efficiency and high throughput [3].

Despite these advantages, LoC devices still face challenges that hinder their widespread adoption [4–6]. A major limitation is the lack of standardization and the frequent dependence on bulky external laboratory equipment, such as detectors, pumps, and high-voltage power supplies [7]. These requirements reduce portability and increase system complexity and cost.

In recent years, various 3D printing techniques have been employed for fabricating LoC devices, enabling higher design flexibility, rapid prototyping and lower costs [8]. These techniques are based on layer-by-layer fabrication in an additive form, which can proportionate complex geometry fabrication, which would be impossible to achieve with conventional photolithographic techniques [9]. Due to the recent explosion in popularity of masked stereolithography (MSLA) and fused deposition modeling (FDM), the printers and materials have become very cheap and easily available, consequently making 3D printing LoCs increasingly popular [10].

A range of techniques are available for identifying and quantifying analytes, with fluorescence spectroscopy being a widely utilized approach. This method operates by exciting samples with a shorter wavelength and subsequently measuring the emission at a longer wavelength [11]. Fluorescence spectroscopy is valued for its high sensitivity, attributable to low background noise [12].



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Typically, fluorescence assays require an experimental setup comprising a light source, lens, wavelength selection filters, and photodetectors [13]. While these components may contribute to system bulkiness, miniaturized detectors [7,14] and compact light sources are highly desirable for lab-on-a-chip devices. Consequently, there is ongoing development of accurate and reliable detection systems that facilitate seamless integration with such platforms.

In this work, we explore the use of low-cost, compact commercial multispectral sensors for fluorescence detection in microfluidic LoC and capillary electrophoresis (CE) applications. These sensors are coupled with microcontrollers for simple data acquisition, offering a versatile and inexpensive solution for integrated fluorescence detection in miniaturized analytical systems.

2. Design

A multispectral photodetector was chosen for the sensor, due to its compact size, low cost (around 10 USD) and I²C serial communication bus. The used sensor was an AS7341 from ams-OSRAM, but many other similar sensors are available, such as the AS7343 and AS7261. The AS7341 has 11 different selectable channels with 8 different visible color filters. The spectral response of the AS7341 sensor was compared with a commercial USB ultraviolet–visible (UV-vis) spectrometer (USB 4000, Ocean Optics, Orlando, FL, USA) by measuring different light-emitting diode (LED) emission spectra (Figure 1).

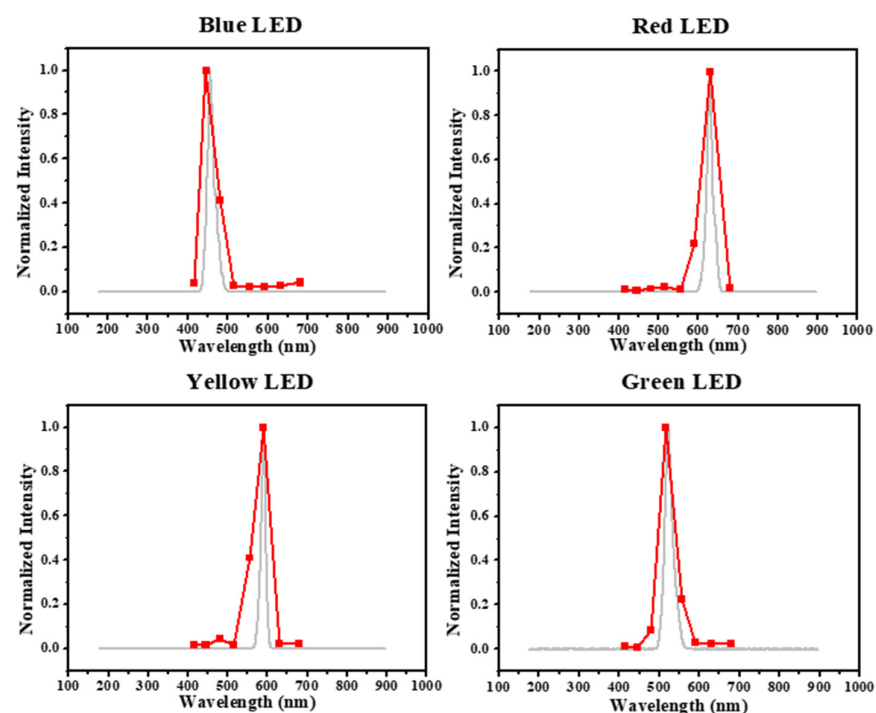


Figure 1. Comparison of spectra obtained by a commercial UV-vis (light-gray solid line) spectrometer and by the AS7341 multispectral sensor (red squares and lines) for different LEDs. The red squares are the signal output value obtained from each channel. The wavelength for each channel represents the maximum response of a given channel. The intensity was normalized.

As expected, the commercial spectrometer has a much higher spectral resolution, as observed in Figure 1; however, the graphs still maintain a similar overall shape and can be used to determine the LED color used. Another drawback for this sensor is the filter band pass, since each channel has a relatively wide response window, which is represented by its datasheet (Figure S1A), resulting in a wider band compared with the spectrometer.

Despite its drawbacks, this sensor offers great opportunities for miniaturized and low-cost systems, offering many outputs in a chip where traditional systems would need many photodiodes. Moreover, in most chemical analyses, only one channel is strictly necessary for keeping track of the chemical compound. So, having different channels to choose from brings more flexibility to use the same sensor in different situations.

Another advantage is the internal analog-to-digital converter (ADC) with communication with I²C. This lowers the possibility of noise between the sensor and microcontroller, since the signals from the sensors are in digital form. But since the photodiode outputs are directly connected to the channel selector and thus its ADCs, there is no way to directly access the photodiode signal (Figure S1B). Therefore, the maximum gain of 1024x can be a limitation for lower-light-intensity applications, such as very low concentrations of fluorescent material, especially considering the sensor's small photodiode area (Figure S1C). This sensor was nonetheless applied in fluorescence detection and initial results were promising.

3. Build Instructions

Two simple devices were utilized in this sensor prototype, one for validation of the sensor in a static system and the other for application in capillary electrophoresis detection. In both cases the AS7341 sensor was controlled through its I²C communication ports with a Raspberry Pi Pico microcontroller connected to a computer. A simple program was made in micropython (presented in Figure S2), with a pre-made library for this sensor, for monitoring and recording the data acquired in a .csv file, which was saved on the microcontroller and could be retrieved with Thonny IDE or other IDEs by directly accessing the data saved on the Pico onboard storage.

3.1. 3D Printing of the LoC Microfluidic Device

All 3D-printed parts were printed in an Anycubic M5s MSLA (Anycubic, Shenzhen, China) printer with Anycubic Standard Clear or Standard Grey resin; the layer height utilized was 20 µm with 1.2 s normal exposure and 30 s base exposure. After printing, the pieces were washed with excess ethanol by hand and then placed in an ultrasonic bath with ethanol for 15 min to remove uncured resin in the channels. Finally, the prints were placed in a post-curing chamber for 3 min. In addition to the channels and capillary support, a backplate for securing the AS sensor was printed in PLA in a FDM printer and fixed in place with nuts and screws.

3.2. Construction of Static Detection System

The static measurement system is composed of a 3D-printed piece, one LED diode, the AS7341 sensor, a backplate, and an LED driving circuit. The 3D-printed piece has a simple 0.6 mm channel with a 3 mm optical path (Figure 2B). The light source was placed perpendicular to the sensor, minimizing the amount of light from the LED that directly hit the sensor. To drive the LED a simple LM317 current source was utilized; this source was powered by the 5 V from the USB cable that powered the microcontroller, and a 470 Ω trimpot was used to adjust the current to 15 mA for the blue LED and 2.5 mA for the green LED (electric diagram presented in Figure S2A). The microfluidic chip inlet and outlet were connected to silicone tubes for loading solutions and cleaning the microfluidic channel (Figure 2C).

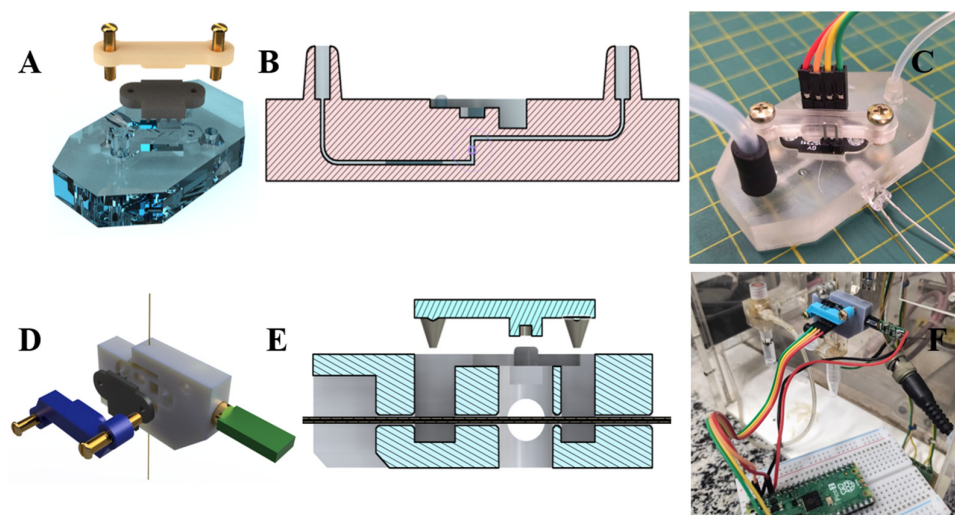


Figure 2. Render (A), cross-section (B), and photo (C) of the system utilized for the static analytical curves for fluorescein and rhodamine B. Render (D), cross-section (E) and photo (F) of the detection system coupled with the homemade CE system.

3.3. Construction of the CE Detector System

The CE detector system is composed of a 3D-printed piece, one laser diode with a driving circuit, the AS7341 sensor, a backplate, and a silica capillary. The printed piece has an opening for passing a fused silica capillary of 100 μm internal diameter (Figure 2D,E); the AS7341 sensor was aligned with the capillary and a 450 nm laser diode (450MD-5-0610-QS33) was placed. This laser was powered by the 5 V line from the microcontroller and formed a 90-degree angle between the light source and detector, as seen in Figure 2D,E. This detection system was used in a homemade CE system previously reported by our group [15,16].

Detailed photos can be found for each system in Figure 2, and all CAD models are also added in the Supplementary Materials (Files S3 and S4). In both systems, the sensor was held in place by a backplate screwed onto the main device.

3.4. Materials

Fluorescein and rhodamine B were purchased from Synth (Diadema, SP, Brazil). Fluorescein 5 isothiocyanate (FITC), L-arginine and L-histidine were purchased from Sigma-Aldrich. The resins used for 3D printing were Anycubic Standard Clear and Anycubic Standard Grey, which were acquired from Slim3D (Curitiba, PR, Brazil). The blue laser diode (450MD-5-0610-QS33) and AS7341 on a breakout board were purchased online from AliExpress (Hangzhou, China). Raspberry Pi Pico, green LEDs (HLMP-CM3G-Y10DD), blue LEDs (HLMP-CB1G-XZ0DD) and other electronic supplies were obtained from MakerHero (Palhoça, SC, Brazil).

4. Operating Instructions

4.1. Construction of Analytical Curves for Probes

For the construction of analytical curves for each data point, the channel was first loaded with deionized water and a blank was recorded. Afterwards, the channel was loaded with the probe, and the full spectrum was recorded 10 times for each replicate; then, the average was calculated and used as the data point. Within measurements of the same replicate, the channel was washed with deionized water, and between replicates the channel was washed with 5 mL of ethanol followed by 10 mL deionized water.

4.2. Application to Capillary Electrophoresis Detection

For separation of amino acids, a fluorescent derivatization was made with FITC following a procedure adapted from Takizawa and Nakamura [17]. A 1 mg/mL FITC solution was prepared in pure acetone; a fresh solution was prepared every day due to FITC low stability [18]. A wide range of concentrations of L-arginine and L-histidine were tested, varying from 0.25 to 25 $\mu\text{mol L}^{-1}$. The concentration of FITC was fixed at 100 $\mu\text{mol L}^{-1}$ for all solutions, which made the amino acids the limiting reagent. The solutions were prepared in borate buffer pH 9 at 20 mmol L^{-1} and were incubated at 50 $^{\circ}\text{C}$ for 5 h. After incubating, the solutions were injected directly into the CE equipment. The separation conditions were 20 kV in a 100 μm fused silica capillary with 50 cm length (42 cm to the detector), the run buffer was the same as the dilution buffer, and the injection was made with hydrodynamic pressure at 11 kPa for 1.5 s.

5. Validation

5.1. Use of AS7341 in Analytical Curves

An analytical curve was constructed varying concentrations of fluorescein and rhodamine B in a simple microfluidic channel, which is presented in Figure 3. These probes were chosen for the wide availability of derivatives with similar fluorescence characteristics and due to their excitation wavelength being higher than the resin absorption wavelength (as discussed in Figure S3).

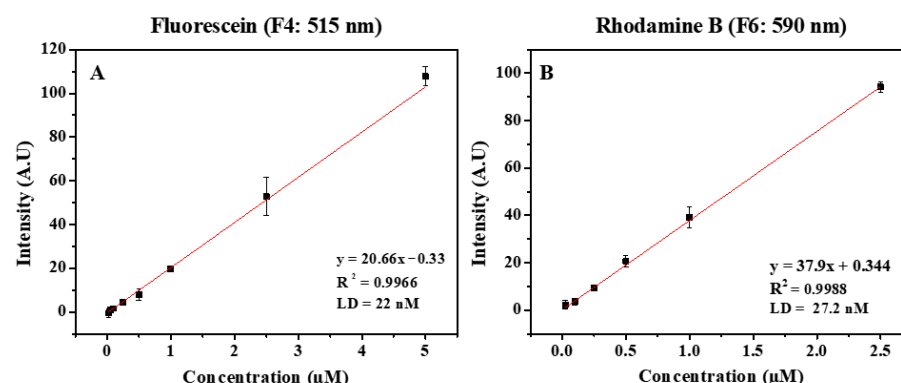


Figure 3. Calibration curves recorded with AS7341 sensor in the device present in Figure 2C. (A) shows the signal recorded on channel F4 when a fluorescein solution is present, while (B) presents the data recorded on channel F6 when rhodamine B solution is used. In both cases the integration time was 0.11 s. All measurements were recorded in triplicate.

Since fluorescein and rhodamine B have emissions at 510 nm and 627 nm [19,20], respectively, channels F4 and F6 were chosen for each sample, since those are the channels closest to the probes' emission maximums [21]. Each probe has a different optimal channel, and its choice is a combination of high sensitivity to the probe emission and low background noise from the LED emission.

Despite the sensor having 8 visible channels, only 6 are used in each measurement. This is because only 6 ADCs are present, which can be individually assigned to each channel. For a full 8-channel spectra, it would be necessary to reassign the ADC mid-measurement, which can be done by modifying the python code, but for simplicity and faster integration time, only 6 channels were recorded in each measurement.

Although conditions were not optimal for each probe (i.e., pH, ionic strength), this setup was still able to achieve nanomolar limits of detection, as seen in Figure 3, with 22 and 27 nM LoDs for fluorescein and rhodamine B, respectively. This method of measurement

relies heavily on background-level stability. But, due to variations in LED intensity and sensor drift, a stable background proved to be a challenge in this simple setup.

Recording a blank before each measurement made this curve construction possible, but a high variation is still visible when the residuals are analyzed (Figure S5).

5.2. Application in Capillary Electrophoresis

To demonstrate the applicability of this sensor in separation analyses, this sensor was used for detecting two amino acids derivatized with FITC and separated by using capillary electrophoresis. The separation was carried out in a standard 100 μm fused silica capillary and a cheap blue laser (450 nm) was utilized as the excitation source.

The main parameter that is tunable in this sensor is the integration time. A higher integration time will result in higher sensitivity and lower noise but will also result in a lower data acquisition frequency, which will lead to a worse peak resolution in the electropherograms. So, a balance between sensitivity and resolution must be made. The measurements displayed in Figure 4 were recorded in 0.1 s, which made it possible to achieve a limit of detection of 210 nmol L^{-1} of arginine while maintaining a high-resolution signal. A higher integration time could be used in this case to increase sensitivity even more. But the data acquisition rate was fixed at 10 Hz because the ultimate goal is to apply this sensor as the detector in LoC devices, and LoC devices require more data due to their higher throughput.

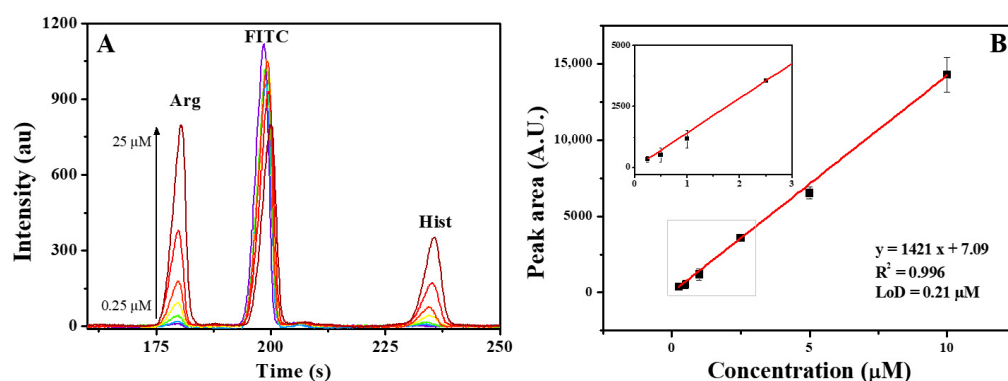


Figure 4. Electropherograms recorded using AS7341 with baseline correction (A) and analytical curve constructed using the peak area of arginine peaks (B) ($n = 3$).

This LoD is far from the limits offered by commercial laser-induced fluorescence (LIF) detection systems, which often have detection limits in pM [12]. However, considering the simplicity of the electronic design and that arginine and histidine concentrations in urine are, on average, 88 and 61 $\mu\text{mol L}^{-1}$ [22], respectively, this sensor is sensitive enough for many applications involving monitoring biomarkers.

In addition, since the signal is baseline-corrected, the background drift is not as significant as is the case in static measurements, resulting in lower error measurements as observed in Figure S6.

Another aspect that can improve the sensitivity is the light source. In this case a 450 nm 5 mW diode laser was used. This light is far from the ideal 495 nm for exciting fluorescein. But, considering that this laser diode cost only 10 USD, the whole detection system was built for around 25 USD, including the sensor, laser, 3D-printed parts and microcontroller, showing the potential of this compact sensor for bioanalyses in low-cost devices.

6. Conclusions

This new class of sensors shows great potential for lab-on-a-chip devices, allowing for small devices with multispectral capabilities, while costing only 10 USD, maintaining

analytical accuracy and providing more flexibility for different analyses. The fabricated device achieved a limit of detection of 22 nM for fluorescein in direct measurements in the microfluidic channel. When coupled with a capillary electrophoresis separation system, it was possible to detect concentrations of arginine as low as 210 nM, reflecting its potential for applications in bioanalysis. In future work, this sensor will be coupled with on-chip electrophoretic separation and compact systems powered by microcontrollers, further demonstrating the capability to design low-cost and compact systems that offer great analytical performance and automation in complex analyses.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/hardware4020008/s1>, File S1: Supplementary figures; File S2: Micropython code for data acquisition with AS7341; File S3: CAD model for AS7341 with silica capillary; File S4: CAD model for AS7341 simple channel device.

Name	Type	Description
S1	Word (.docx)	Supplementary figures
S2	Python Program (.py)	Micropython code for data acquisition with AS7341
S3	CAD (.step)	CAD model for AS7341 with silica capillary
S4	CAD (.step)	CAD model for AS7341 simple channel device

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