

Proceedings

Development of a Compact Optical Measurement System to Quantify the Optical Properties of Fluorescently Labeled Cervical Cancer Cells [†]

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Abstract: The flow cytometer is an instrument that can measure the characteristics of cells such as the number of cells, the degree of internal composition of the cells, the size of the cells, and the cell cycle etc. This equipment has been used to study leukemia, DNA and RNA analysis, protein expression, cell death, and immune response. However, flow cytometer is an expensive equipment and requires an operator with expertise for use and maintenance. When only simple data are needed, such as measuring the number of cells or quantitative analysis of cell growth and inhibition, the use of a flow cytometer is not suitable in terms of cost and requires unnecessary measurement time consumption. In this study, a compact optical measurement system using commercially available LED, photodiode, and Arduino Mega ADK was developed, and the body structure was printed and utilized by a 3D printer. Cervical cancer cells, known as one of the major cancers of women, were fluorescently treated with fluorescent dyes such as Calcein-AM and DiD, and performance of the system was verified. The side scattering measured using various filters with different transmission wavelengths of light showed high linearity in proportion to the number of cells. By measuring the side scattering of the untreated cervical cancer cells, fluorescence scattering could be confirmed from the difference in the side scattering intensity according to the fluorescence treatment.

Keywords: cancer cells; fluorescence detection; light-emitting diode; photodiode; 3D printer

1. Introduction

The flow cytometer that has been used for cell analysis is an instrument that can measure the characteristics of cells such as the number of cells, the degree of internal composition of the cells, the size of the cells, and the cell cycle, etc. [1]. It has been used to study leukemia, DNA and RNA analysis, protein expression, cell death, and immune response [2]. This equipment causes the fluorescently treated cells to collide with photons emitted from the light source, leading to light scattering and the expression of fluorescent substances [3]. In order to collect such scattered light and fluorescence, the commercial flow cytometer mainly uses a laser as a light source to detect forward scatter (FSC), side scatter (SSC), and fluorescent scatter (FL) through a photomultiplier tube (PMT) [3]. These optical signals are converted to digital signals through analog-digital converter (ADC) and processed by computers, allowing precise analysis of various optical properties of cells [4]. However, the flow cytometer not only includes expensive equipment such as laser and PMT, but also requires an operator with expertise for use and maintenance. When only simple data are needed, such as measuring the number of cells or quantitative analysis of cell growth and inhibition, the use of a flow

cytometer is not suitable in terms of cost and requires unnecessary measurement time consumption. In addition, when these data are measured using hemocytometer, the disadvantage is that the number of samples analyzed is limited and depends on the subjective judgment and proficiency of the user [5]. In this study, a system that can quantify optical properties was designed using cost-effective light-emitting diode (LED), photodiode, and Arduino Mega ADK, and some modules were produced to implement it. To evaluate the feasibility of the system, Cervical cancer cells (HeLa), known as one of the major cancers of women, were fluorescently treated with fluorescent dyes such as Calcein-AM and DiD [6]. After that, SSC of the treated HeLa was measured using various filters with different emission wavelengths of light. Consequently, FL was able to calculate through the difference in SSC intensities according to whether or not to be subjected to fluorescence treatment.

2. Methods

The proposed optical measurement system for quantifying properties of fluorescently labeled cells is composed of constant current supply module, main module, optical detection module as shown in Figure 1. The constant current supply module is for using LED (Photron, 3W, USA) instead of expensive laser. It consists of a circuit and a system cooler to supply stable voltage and rated current to the LED. The main module is a structure to fix quartz cuvette, LED, photodiode (FDS-1010, 350–1100nm, Thorlabs, USA) and optical filter, which was printed and utilized by a 3D printer (3DP-310F, Cubicon Inc., Korea). The optical detection module is used for acquiring SSC of HeLa which is irradiated from the LED light and consists of a photodiode, circuit for applying reverse bias and Arduino Mega ADK with text LCD. Since the reverse current generated in the photodiode is proportional to the intensity of light incident on the photodiode, the photodiode and the load resistor were connected in series and then, the voltage applied to both ends of the load resistor was applied through the analog pin of the Arduino board and displayed on the text LCD. Therefore, the proposed system can measure the scattered light via emission filter generated when the light emitted from the LED collides with the cell in the quartz cuvette through the excitation filter.

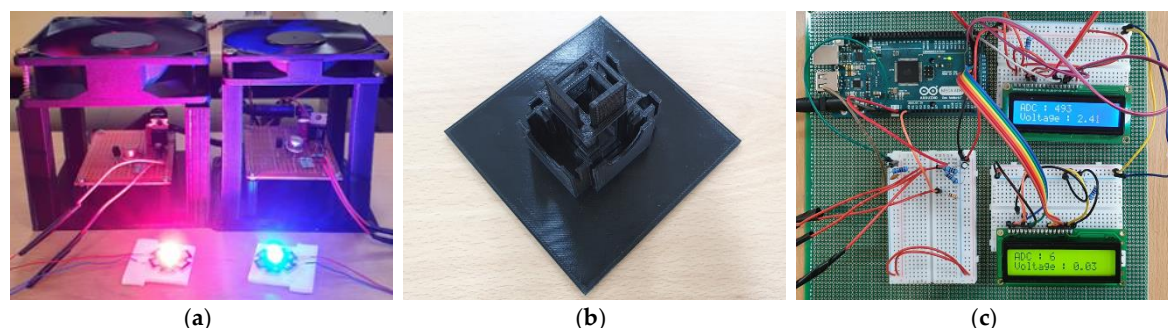


Figure 1. Each module of the proposed system: (a) constant current supply module; (b) main module; (c) optical detection module

To evaluate the performance of proposed fluorescence detection system, the SSC was measured on the human cervical cancer cells (HeLa, Korea Cell Line Bank, Seoul, Korea). The HeLa cells were cultured in high-glucose Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin. The cells were incubated at 37°C humidified incubator with 5% CO₂ for 72 h. They were washed two times with phosphate-buffered saline (PBS) and were isolated from cell culture flasks with trypsin-EDTA solution. To enable fluorescence emission by absorbing light in a specific wavelength range of the LED light source, the isolated cells were stained with each Calcein-AM and DiD solution. After observing the fluorescence emission of the stained cells using an optical microscope (IX73, Olympus, Tokyo, Japan), the number of cells was measured using a hemocytometer. The prepared cells were put in a quartz cuvette at a concentration of 10×10^6 cells/2ml with PBS and then, SSC were measured for each number of cells (from 10×10^6 cells/2ml to 1×10^6 cells/2ml) by reducing concentration of 1×10^6 cells/2ml. Considering different absorption wavelengths of each fluorescent dye, blue LED (470 nm) and red LED (630 nm)

were used to measure SSC of HeLa stained with Calcein-AM and DiD, respectively. In addition, two filters suitable for absorption and emission wavelengths of fluorescent dyes were selected from cost-effective filter booklet (Roscolux series swatchbook, Rosco, USA) to optimize excitation and emission filters. In this way, optical filter sets showing the most sensitive change in the number of cells was able to select. Using selected excitation and emission filters, fluorescence emission from stained HeLa was calculated through the difference in SSC intensities by dividing into an experimental group and a control group according to whether or not to be subjected to fluorescence treatment. All the experiments were conducted in a dark room at room temperature to measure under the same conditions.

3. Results and Discussion

The SSC of HeLa treated with Calcein-AM and DiD was measured using various optical filters, and the combination of filters showing the most sensitive change depending on the number of cells was examined. Linear regression analysis was performed for 10 different concentration of cells (from 10×10^6 cells/2ml to 1×10^6 cells/2ml by reducing concentration of 1×10^6 cells/2ml, $n=4$), and the excitation filter and emission filter with the largest slope of the estimated equation were selected. As a result, when using blue LED and measuring the SSC of HeLa fluorescently treated with Calcein-AM, the most sensitive changes were observed when the excitation filter #369 Tahitian Blue and the emission filter #389 Chroma Green were used. Next, when using red LED to measure the SSC of HeLa fluorescently treated with DiD, the most sensitive changes were observed when the excitation filter #19 Fire and the emission filter #46 Magenta were used. In addition, as a result of examining the intensity of side scattering of the experimental group and the control group to confirm fluorescence from HeLa fluorescently treated with Calcein-AM and DiD using an optimized filter, it was confirmed that there was a statistically significant difference. It can be seen that the difference occurred due to the expression of the fluorescent substance in the experimental group. The high linearity ($R^2 > 0.9$) of the FL proportional to the number of cells was confirmed as shown in Table 1.

Table 1. Summary of FL of HeLa treated with Calcein-AM and DiD using optimized filters.

Fluorescent dye	Slope [mV/#cell]	R^2
Calcein-AM	22.64	0.933
DiD	7.61	0.954

4. Conclusions

In this study, a compact optical signal measurement system was designed using relatively inexpensive LED, photodiode, and Arduino Mega ADK instead of expensive equipment and systems of commercially available flow cytometers. Unlike the system proposed in the previous study, the constant current supply circuit was improved to increase the amount of light emitted from the LED to induce a sensitive change in the measured light intensity according to the number of cells. HeLa were fluorescently treated with two fluorescent dyes such as Calcein-AM and DiD, and SSC was measured using various filters considering the expression wavelength of the fluorescent substance, and high linearity in proportion to the number of cells was confirmed. In addition, it was possible to confirm FL from the difference in SSC intensity between the experimental group and the control group, which means that the cells can be analyzed with an optical approach through the scattering of light emitted from the light source and the expression level of the fluorescent substance. Therefore, when simple data such as quantitative comparison and analysis of cells are required through the proposed system, relatively objective data can be obtained in a short time and this system can be implemented at a low price. It is also believed that it is possible to design according to the purpose of the user's research using LED and optical filters of various wavelength bands through replacement of the light source and optical filter. As a follow-up study, additional research will be conducted on not only cervical cancer, but also other diseased cells such as liver cancer cells and lung cancer cells

by using various optical filters and optical lenses, as well as structural improvement by modification of 3D design.

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