Development of a compact optical measurement system to quantify the optical properties of fluorescently labeled cervical cancer cells

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Outline

1. Introduction
2. Methods
3. Results and Discussions
4. Conclusions

1. Introduction

Motivation

- Flow cytometry is used for research on various diseases by simultaneously measuring various characteristics of a cell such as the number of cells, the degree of internal composition of the cells, the size of the cells, and the cell cycle.
- Flow cytometry is an expensive equipment and requires an operator with expertise for use and maintenance, so 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 requires irrational expense.

Goal

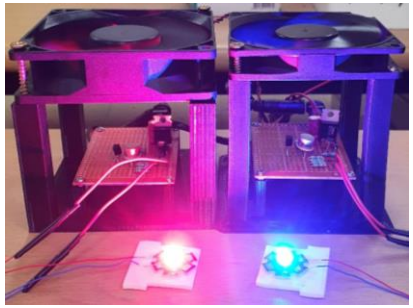
- Develop a compact optical signal measurement system that can acquire optical information of cells by using inexpensive LED, photodiode, and 3D printer.
- After fluorescence treatment of cervical cancer cells with Calcein-AM and DiD, fluorescence side scatter was measured using various optical filter and confirmed linearity in proportion to the number of cells.

2. Methods

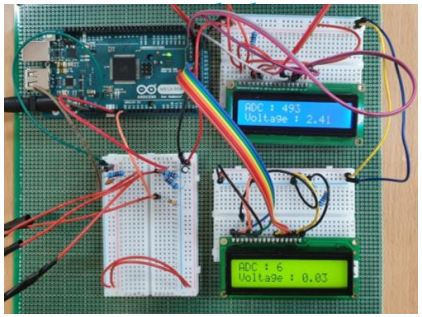
Experimental Process

Module Development

Constant Current Module

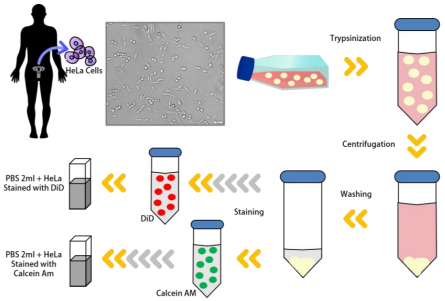


Optical Detection

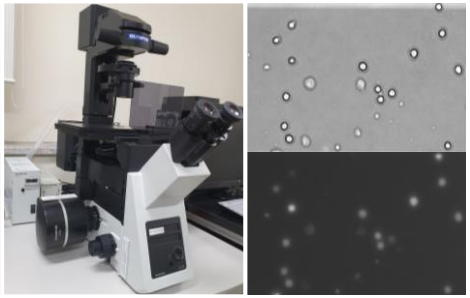


in-vitro Experiment

Fluorescence Treatment

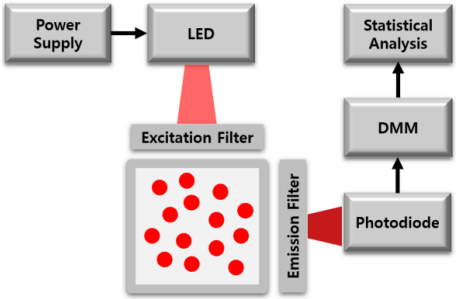


Cell Count

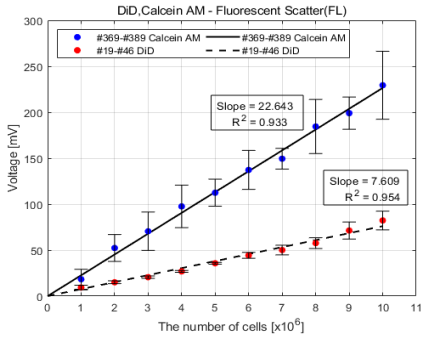


Characterization

SSC Measurement

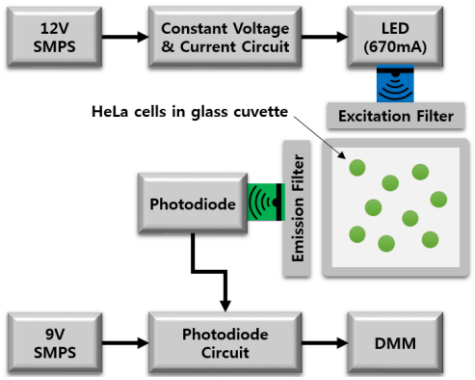


Statistical Analysis

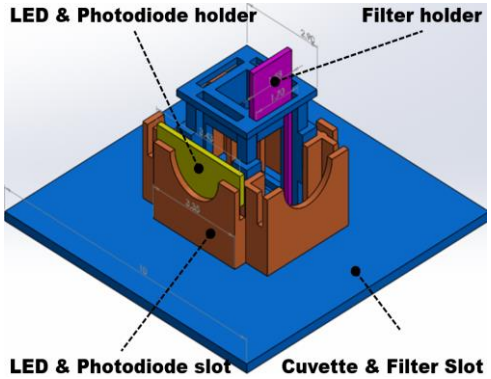


2. Methods

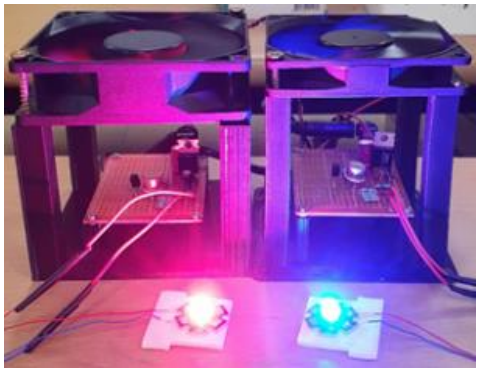
Module Development



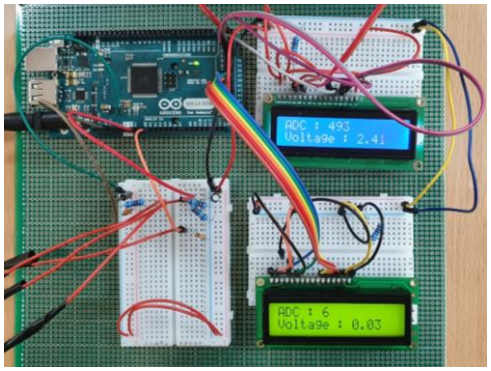
[Schematic Diagram of the System]



[3D design]



[Constant Current Module]

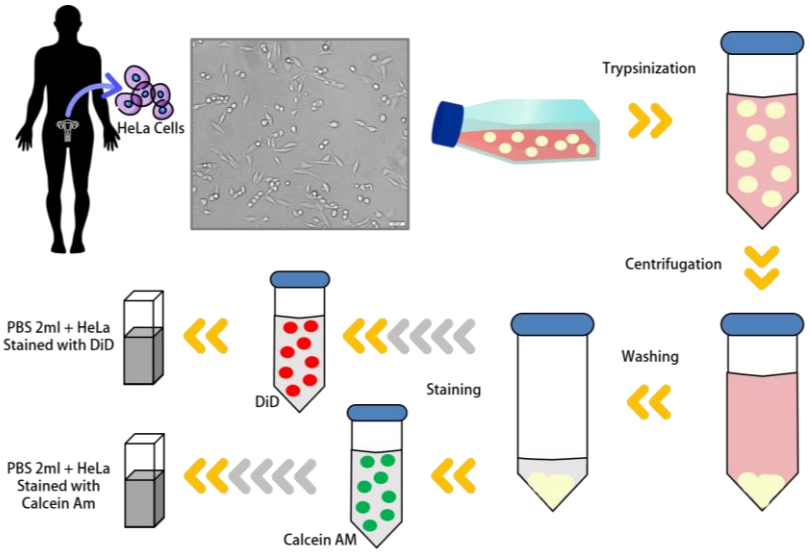


[Optical Detection Module]

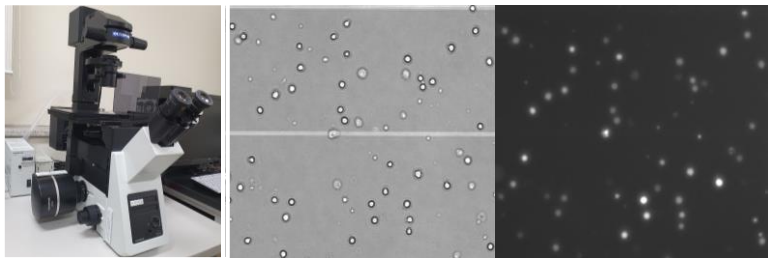
- **Constant current module design (LED)**
  - Switch Mode Power Supply (12V, 1A)
  - System Cooler (12V, 0.2A)
  - LED (Photron, 3W, 470nm-Blue, 620nm-Red)
- **Optical detection module (Photodiode)**
  - Switch Mode Power Supply (9V, 1A)
  - Photodiode (FDS-1010, Thorlabs, 350-1100nm)
  - Arduino Mega ADK (Atmega 2560)
- **Main module(3D printing)**
  - SolidWorks (Dassault Systems, USA)
  - 3D Printer (3DP-310F, Cubicon Inc., Korea)

2. Methods

in-vitro Experiment



[Cell Incubation and Fluorescence Treatment]

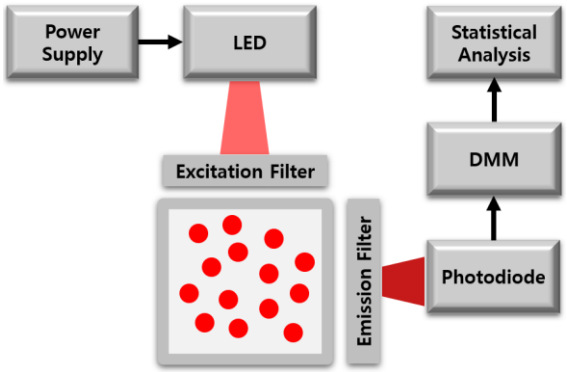


[Observing Fluorescence and Cell Count]

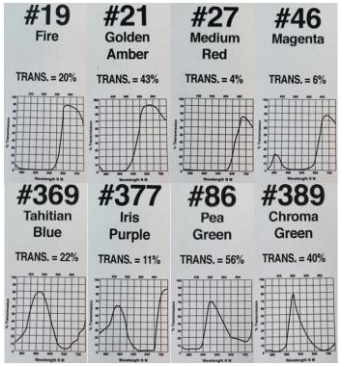
- **Cell information**
  - Human cervical cancer cells (HeLa, Korean Cell Line Bank, Korea)
  - CO_2 Incubator 72 hours incubation
- **Excitation / emission wavelength of fluorescent dyes**
  - DiD (644nm / 665nm)
  - Calcein-AM (495nm / 516nm)
- **Observing fluorescence and cell count**
  - Inverted fluorescent microscope (IX73, Olympus, Japan)
  - Using Hemocytometer to count

2. Methods

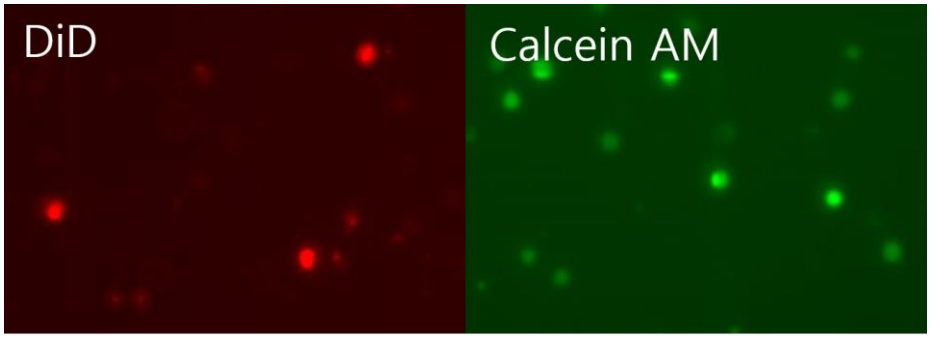
Characterization



[Method of measuring SSC]



[Characteristics of optical filter]

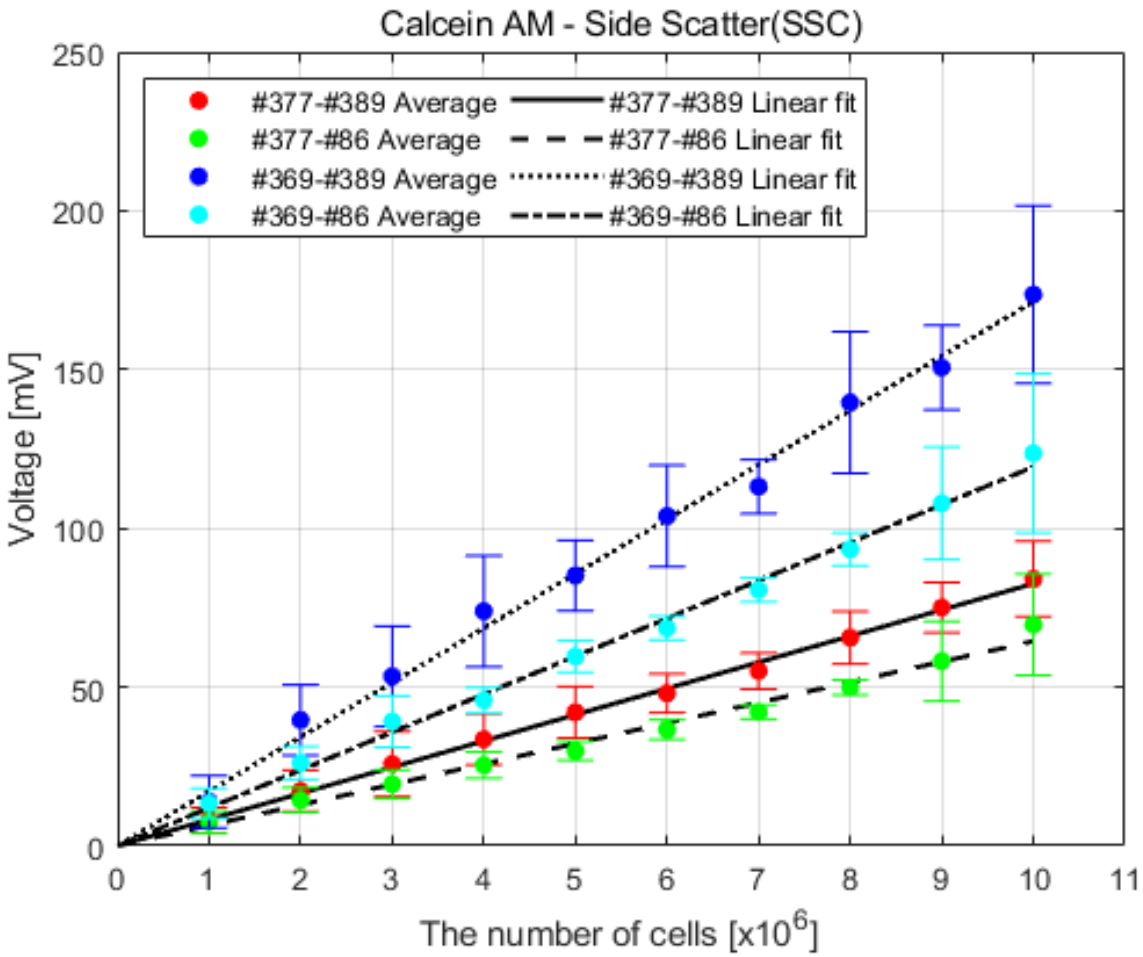


[HeLa treated with DiD & Calcein AM]

- **SSC measurement and analysis**
  - Fluorescently treated HeLa cell suspension with DiD and Calcein AM 10×10^6 cells/2ml loaded on the quartz cuvette of the main module
  - Change the cell concentration and measure SSC (10×10^6 cells/2ml $\rightarrow 1 \times 10^6$ cells/2ml)
  - Red / Blue LED and optical filter optimization by reflecting the excitation / emission [nm] optical properties of fluorescent dyes.
  - Confirmation of fluorescence scattering (FL) from the difference in SSC intensity and verification through Hemocytometer

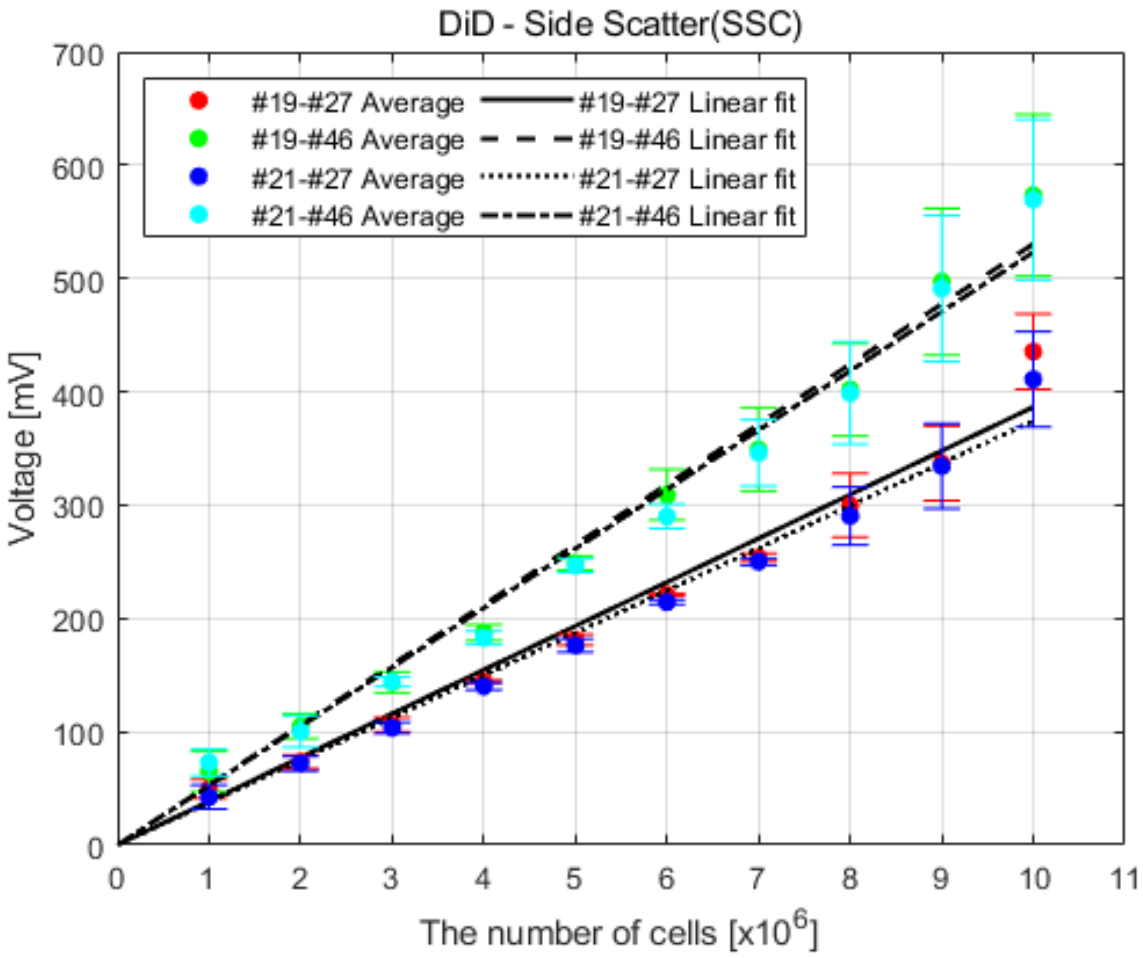
3. Results and Discussions

SSC of the HeLa treated with Calcein-AM



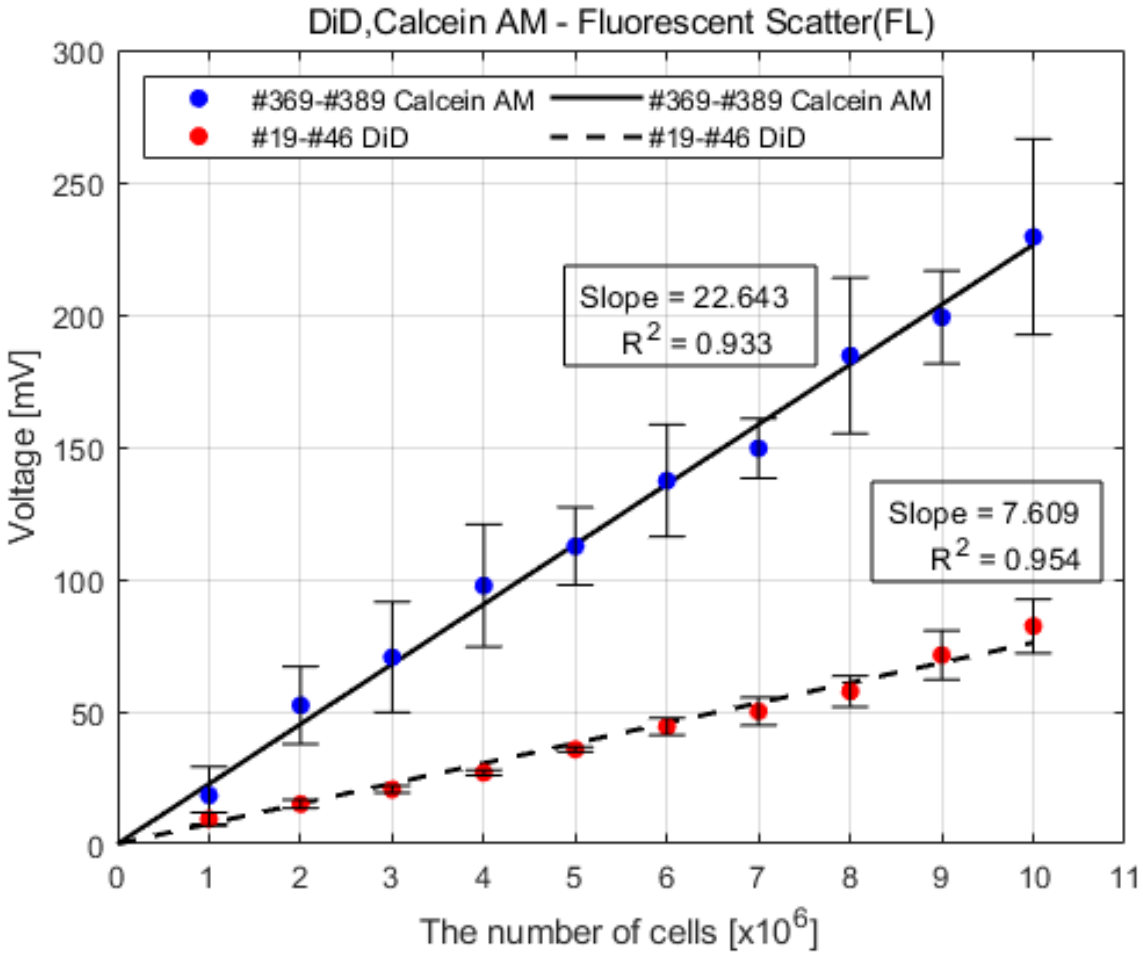
3. Results and Discussions

SSC of the HeLa treated with DiD



3. Results and Discussions

FL of HeLa treated with Calcein-AM vs. DiD



4. Conclusions

- In this study, instead of the commercially available expensive flow cytometry system, a small optical signal measurement system using relatively inexpensive LED and photodiode was developed.
- As a result of measuring SSC of HeLa treated with Calcein AM and DiD using various filters, high linearity in proportion to the number of cells was confirmed, and an optimized optical filter was able to select
- As a result of confirming FL from the difference in SSC intensity between experimental group and the control group, high linearity in proportion to the number of cells was confirmed.
- The proposed system enables quantitative comparison of cells and optical characteristics analysis within a relatively fast time with low cost of use.
- Since it is easy to replace the LED and optical filter, it is possible to use light sources and filters in various wavelength ranges, and the freedom to select target cells and fluorescent dyes is expanded.
- As a follow-up study, a system capable of simultaneous measurement for each cell will be constructed by improving the 3D structure of the main module and the performance of the optical detection module.

Thank You

Q & A