LED-Based Optical Sensing Platforms for Multi-Analyte Detection

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LED-BASED OPTICAL SENSING PLATFORMS FOR MULTI-ANALYTE DETECTION

A Dissertation

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Louisiana State University and
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by
Young-Ho Shin
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Abstract

Real-time monitoring of phytoplankton groups provides important information about aquatic ecological states, nutrient abundance, and water pollution. A rapid and accurate method for monitoring phytoplankton in water is commonly performed by detecting fluorescence emission from the plankton; however, commercially available portable fluorescence sensors are still expensive, bulky, and limited in functions, such as lacking the capability of selectively detecting multiple phytoplankton groups. In this regard, a low-cost and portable fluorometer platform for phytoplankton detection was developed in order to address the issues that current portable fluorometers have.

This dissertation has four main goals: (1) perform a study on fluorescence measurement principles and a comprehensive review on portable fluorometers for phytoplankton detection; (2) characterize different phytoplankton groups and their photopigments; (3) develop and integrate necessary mechanical and electronical parts for the portable fluorometer system; and (4) demonstrate/validate the developed device.

The target analytes, including photopigments (chlorophyll a and phycocyanin) and different phytoplankton groups (green and blue algae), were fully characterized with a benchtop fluorometer (Horiba, Japan) using excitation-emission matrix (EEM) fluorescence spectroscopy. The fluorescence sensor utilized three different wavelengths of light emitting diodes (LEDs) for selective stimulation in order to concurrently measure and distinguish green and cyanobacteria samples. As a demonstration, the system was also tested on-site in order to validate the field deplorability of the system using an environmental water sample collected directly from a lake. The results suggest that our developed fluorometer could be used as a portable phytoplankton monitoring system for concurrent detection of different phytoplankton groups.
1. Introduction

1.1. Motivation

Accurate detection and quantification of biological and chemical substances are becoming important in many applications, such as environmental monitoring, clinical diagnostics, DNA sequencing, and even biological warfare agent detection [1–6]. A desirable sensor should not only be highly sensitive and selective but also capable of concurrently detecting and distinguishing multiple target analytes in a simple and rapid way. Optical sensing technology is one of the most promising methods in this aspect due to many advantages over other sensing methods. The main advantages include immunity to electromagnetic interference, durability under severe pressures and temperatures, lightweight and compact design configuration, and most importantly, high sensitivity and selectivity because the measurement is performed utilizing unique excitation and emission wavelengths specific to the target analytes [7–9]. In addition, a portable optical sensing platform can offer even more benefits to the applications where on-site detection and point-of-care (POC) are required. Therefore, the main focus of this dissertation is the development of a portable optical sensor system for selective detection of multiple target analytes.

Because of the benefits that optical sensing technologies provide, different optical detection techniques, such as absorbance [10], diffraction [11], reflection [12], scattering [13], chemiluminescence [14], and refractive index [15] have been utilized and reported as portable biochemical sensors. Amongst various optical sensing technologies, fluorescence technology has been considered highly useful in various optical sensing applications for its high sensitivity, specificity, and accuracy compared to other sensing technologies [16–18]. Although technologies in benchtop fluorescence instruments are well-established and advanced, challenges still remain in applying them to a fully portable fluorescence sensing system because most of the conventional
fluorescence sensing instruments (especially spectrometers) require complicated optical components and systems, which makes them expensive, large, and high-power consuming [19]. Hence, this work attempts to address these issues in order to develop a fully portable fluorescence sensing platform for selective detection of multiple biochemical analytes.

1.2. Objectives

The main objective of this dissertation is to develop a fully portable fluorescence sensor platform for selective detection of multiple biochemical target analytes. However, there are three main challenges in achieving this goal: (1) designing compact and suitable optomechanical compartments for the desired application; (2) development and integration of necessary mechanical and electronic parts for the portable system; and (3) selectively sensing multiple analytes. In the following subsections, detailed objectives of this research are described.

1.2.1 Development of a Portable Fluorescent Biochemical Sensor Platform

In order to develop a portable fluorescence sensor system while maintaining all of the benefits that benchtop fluorescence instruments offer, the bulky and high-power consuming parts of the conventional instrument need to be replaced with more compact alternative components. In general, fluorescence sensor systems consist of three essential functional parts: (1) excitation light with excitation monochromator; (2) detection monochromator/filter; and (3) photodetector.

For an excitation light source, conventional benchtop spectrofluorometers typically utilize a full range of wavelengths (ultraviolet [UV] to far-infrared [IR]) for analyzing the interaction between the light and target sample. Xenon arc or tungsten lamps combined with an excitation monochromator are used for specific excitation light wavelengths. These conventional light sources can be replaced with more compact and less power-consuming light sources, such as light
emitting diodes (LEDs), laser, and laser diodes (LDs). Since LEDs are compact, wavelength-specific, cheap, and easy to operate, they were chosen as alternative excitation light sources.

For wavelength selection of the excitation and emission, conventional monochromators can be replaced with much simpler and compact optical components, such as dichroic filters and color filters that offer more compact and economical wavelength selection configurations. For the photodetector, photomultipliers (PMTs) are one of the most widely used benchtop fluorescence sensing instruments due to their high gain (~up to 100 million-fold), fast responses (few nanoseconds or less), and low noise capabilities [20]. However, it is relatively large, requires high voltage for operation (1–2 kV), and expensive (a few thousand dollars); therefore, it is not suitable for a portable sensing system. Owing to recent advancements in the solid-state photodetector technology, highly sensitive silicon photomultipliers (SiPMs) were brought to the market to address the challenges and limitations that conventional silicon photodiodes have [21]. These SiPMs offer low-light detection capabilities comparable to that of a PMT while maintaining all of the advantages of solid-state photodetectors, such as low voltage requirements, immunity to magnetic field interference, mechanically durable, and uniformed response. An electronic circuit that controls the gain of an SiPM is required for a low-light detection, and an analog-to-digital converter is needed to read the photocurrent generated from the system’s photodetector. In addition, a microcontroller can be added to the system to offer more flexible and user-friendly controlling systems by using digital integrated circuits (ICs) with either the I2C or serial peripheral interface (SPI) communication protocol.

Lastly, almost every optical sensing instrument requires optomechanical components that help to mount optics and integrate mechanical compartments [22]. These components are typically expensive, require expertise when assembling, and are only available from a limited number of
vendors, thus creating barriers for inventors and researchers. The recent advancement in the three-dimensional (3D) printing technology allows for faster and easier prototyping of complex mechanical design with affordable costs [23]. Hence, custom designed optomechanical parts need to be fabricated using 3D printers, and the designs need to be verified using light simulation tools.

1.2.2 Characterization of Multiple Phytoplankton Species as Target Analytes and Implementation of Different Excitation Lights for Multi-Analyte Detection Capability

One common limitation of fluorescence sensors is that it can only detect particular molecules that interact with excitation light and subsequently emit fluorescence. While many substances, such as dyes and fluorophores, emit autofluorescence, the majority of naturally existing substances are unlikely to fluoresce (spectroscopically inactive). One way to detect non-fluorescence target analytes is via synthesis of fluorophores, which are conjugated to binding molecules, such as antibodies, in order to allow them to bind to the spectroscopically inactive target analytes and make them detectable [24]. In this study, different phytoplankton groups were selected as target analytes because they carry pigments that undergo autofluorescence and detecting them is important in environmental monitoring applications [25]. Characteristics of each phytoplankton species and their pigments were analyzed to define the absorption and emission spectra by analyzing the excitation and emission matrix (EEM). Once spectral information from the EEM is analyzed, the most efficient excitation LED lights and optical filters can be chosen to effectively stimulate the target analytes and measure the corresponding fluorescence signals. Utilization of multiple excitation lights generate different fluorescent responses from each sample, and these unique patterns can be used as a fluorescence fingerprint for classification purposes. However, fluorescence emission spectra of multiple analytes often overlap the excitation light, and statistical algorithms that help to classify and quantify the mixed target analytes are required. As
a demonstration, principal component analysis (PCA) and partial least squares (PLA) multivariate algorithms can be applied in order to classify and quantify multiple target analytes.

1.3. Dissertation Outline

The dissertation is divided into six chapters. Chapter 2 provides background information on both fluorescence principles and detection methods. The literature survey was conducted with state-of-the-art portable fluorometers for different applications and their challenges. Chapter 3 focuses on the fabrication of the fluorometer prototype for green algae detection and it was tested with green algal samples mixed with different turbidities of water to validate its selectivity. Chapter 4 focuses on the fabrication of the hand-held fluorometer that can selectively detect two different algae species (green algae and cyanobacteria). Chapter 5 describes development of a portable fluorometer with temperature controlling system.
2. Background

2.1. Introduction

Prior to the development and demonstration of a portable fluorescence sensor platform for multi-analyte detection, the fundamental principles of fluorescence and its measurement methods are introduced along with a literature survey on the use of portable fluorometers for phytoplankton detection. In addition, current limitations, potentials, and future challenges are addressed.

2.2. Fluorescence Sensing Fundamentals

2.2.1 Basic Principle of Fluorescence

Fluorescence is a unique phenomenon in which certain molecules absorb electromagnetic radiation, mostly from UV to visible range wavelengths, and subsequently emit the longer wavelength light as a result of energy stabilization. This section covers the physical fundamentals of light absorption and emission processes of the fluorescence phenomenon.

Light or electromagnetic radiation propagates through the medium as a wave and can be defined as,

\[ \lambda \nu = c \] (2.1)

where \( \lambda \) is the wavelength (m), \( \nu \) is the frequency (Hz), and \( c \) is speed of light (299,792,458 m/s in a vacuum). When light passes through a medium, it undergoes different phenomena based on the interactions between light and the type of medium as shown in Figure 2.1. Some of these interactions (reflection, diffraction, and Rayleigh scattering) neither obtain nor lose any energy during the interaction. However, when the medium interacts differently with the light by absorbing photon energy, the electron in the molecule promotes to the higher energy levels (excitation states).

Based on quantum theory, a photon is a fundamental unit of electromagnetic radiation, and this quantized energy of a photon \( E_{\text{Photon}} \) can be described as

\[
E_{\text{Photon}} = h \nu = h \left( \frac{c}{\lambda} \right)
\]  

(2.2)

where \( h \) is Plank’s constant \( (6.626 \times 10^{-34} \text{ J} \cdot \text{s}) \).

In bioscience and photochemistry, the amount of energy delivered by one mole of photons is often more useful for describing the energy absorption and emission associated with photosynthetic reactions. The energy of one mole of photons is expressed as an Einstein [E]:

\[
E = N h \left( \frac{c}{\lambda} \right)
\]  

(2.3)

where \( N \) is Avogadro’s number \( (6.023 \times 10^{23}) \).

The energy levels of electrons in molecules are known to only occupy discrete energy levels, thus transitions of their levels only occur by absorbing (excitation) or emitting (relaxation) a certain amount energy. The electron excitation and relaxation in a molecule can be explained with a Jablonski diagram as shown in Figure 2.2 (simplified). In this diagram, the excited triplet
state and intersystem crossing transition are not included since they are not related to fluorescence, which is not covered in this chapter. The electronic energy states illustrated by the bold horizontal lines, $S_0$, $S_1$, and $S_2$ are ground and first and second excited energy states, respectively. In each electronic energy state, thin horizontal lines represent different vibrational quantum energy levels.

Figure 2.2 Simplified Jablonski diagram includes the processes of absorbance (excitation), non-radiative decay, and fluorescence.

The first transition in the molecular excitation starts from absorption of particular energy from the incident photon as represented in Figure 2.2 as straight blue up-arrows. Since each electronic energy state is quantized, only certain photons having specific energy quantities can be absorbed by the molecule followed by the electron transition to a higher electronic state. This transition resulting from photon excitation occurs predominantly from the ground electronic state at room temperature (300K) since electron distribution is governed by the Boltzmann distribution.
Photon energy that excites an electron from the ground state ($S_0$) to the higher electronic energy level ($S_1$) can be described:

$$S_0 + h\nu = S_1 \tag{2.4}$$

As mentioned previously, $h\nu$ refers to the energy of photon as determined by the wavelength of the absorbed photon. This energy is transferred to the molecule in order to excite the electron from ground to any higher state. If the energy transferred from the photon is sufficiently high, the excited electron will be promoted to an even higher vibrational state. This is a relatively fast process that only takes about $10^{-15}$ seconds.

After the absorption process, the excited electron will release the energy and stabilize in two ways: (1) non-radiative and (2) radiative. First, the non-radiative process is vibrational relaxation, which is indicated in Figure 2.2 as bright orange downward arrow. This process occurs when the electron is excited to a higher vibrational energy level ($>S_1$), and the partial energy of the excited electron is transferred as kinetic energy to either the excited molecule itself or other neighboring molecules. This energy is normally dissipated as heat and occurs only between different vibrational levels; therefore, the electron will not experience electronic state change. This is also a rapid process that takes $10^{-12}$ seconds and can be described:

$$S_0 + h\nu = S_1 + E_V \tag{2.5}$$

where $E_V$ is the energy associated with vibrational relaxation. The transferred photon energy ($h\nu$) is high enough to excite the molecule so that it exceeds the $S_1$ electronic level but lower than the energy required to reach the $S_2$ electronic state.

Another type of non-radiative relaxation in which electron transition occurs between two different vibrational levels in the separate electronic states is called internal conversion, which is indicated in Figure 2.2 as a red zigzag arrow. As illustrated, when the energy transition in the
molecule is higher than that in the S₁ electronic state, the energy levels between vibrational and electronic state become closely distributed. Internal conversion occurs when the molecule is excited to the level in which electronic and vibrational energy states almost overlap, and the electron is prone to move to the lower electronic states. The processing time for the internal conversion is about $10^{-12}$ seconds. The internal conversion is also a rapid process; therefore, it is highly probable for the molecule to dissipate energy in the most excited states along with the vibrational relaxation state. However, this relaxation process typically occurs when electron is in a higher energy state and drops down to S₁ states due to the larger energy gap between the upper vibrational levels and the S₁ electronic level; therefore, there is a lack of overlap for those energy states. The absence of overlap causes a slower relaxation process of electron return to the ground state, and other relaxation process is likely to be engaged other than internal conversion. Two non-radiative relaxation processes can be described:

$$S_0 + h\nu = S_1 + E_V + E_{IC}$$  \hspace{1cm} (2.6)

where $E_{IC}$ is the internal conversion energy. The transferred photon energy ($h\nu$) is high enough to excite the molecule to exceed the S₂ electronic level or even higher levels.

Another way for a molecule to release the energy after excitation is via fluorescence, which is a radiative process as indicated in Figure 2.2 as a green straight downward arrow, dropping down to any of the vibrational states within the ground electronic state from the S₁ state. This process is relatively slower ($10^{-11}$–$10^{-7}$ seconds) than other non-radiative relaxations; therefore, this process is less likely to occur when an electron undergoing relaxation from an electronic state higher than S₁ and dropping to S₁ because non-radiative relaxation is more probable (faster) at this phase. Fluorescence occurs only after the excited molecule settles to the S₁ electronic state, and
subsequently, the fluorescence relaxation becomes competitive with respect to energy dissipation between $S_1$ and the ground state. This process can be described:

$$S_0 + h\nu_{ab} = S_0 + E_V + E_{IC} + h\nu_f$$

where $h\nu_{ab}$ is the energy of incident photons with the absorption wavelength ($\lambda_{ab}$), and $h\nu_f$ is the energy of fluorescence photons with the emission wavelength ($\lambda_f$). Since the energy of an absorbed photon is higher than that of emitted photon ($h\nu_{ab} > h\nu_f$) due to energy dissipation through non-radiative relaxation, the fluorescence-related wavelength is longer than the absorbed light in this case.

**2.2.2 Fluorescence Emission Characteristics**

Due to the energy differences between the absorbed excitation and emitted fluorescent light, the peak wavelengths of those two lights also present differences, which is called Stoke’s shift as illustrated in Figure 2.3. The general rule of spectral shapes for absorption and fluorescence is the mirror image rule, which is due to similar energy gaps in vibrational levels in the ground energy and excited energy states. As a result, absorption and fluorescence spectral shapes are often mirror images of each other. For example, fluorescein is one of the fluorophores that follows the mirror image rule by possessing a fluorescent spectral profile that is almost a mirrored pattern of its absorption spectral shape as illustrated in Figure 2.3. In fluorescence sensing, it is important to select the proper excitation wavelength in order to achieve the highest fluorescence intensity; therefore, it is desirable to select an excitation wavelength where maximum absorption occurs.
Figure 2.3 Normalized absorption and fluorescence fluorescein spectra. The difference in band maxima between absorption and fluorescence spectra is called Stoke’s shift. The plot was generated from the data of [26, 27].

However, since absorption and emission spectra of fluorophores often overlap as illustrated in Figure 2.3 (around 475–525 nm), careful selection of an excitation wavelength that is closest to the maximum absorption while not interfering with the emission spectrum is preferred. This study provides important guidelines for selecting appropriate excitation lights for multi-analyte detection applications and will be further discussed in a later section. Since fluorescence normally originates from the energy drop of the first electron state to the ground state, the emission spectrum is independent of the excitation wavelength as illustrated in Figure 2.4 (Kasha’s rule). Therefore, regardless of excitation wavelength, the fluorescence emission spectrum remains unchanged, but only its emission intensity will be affected. Based on this theory, the increasing excitation light bandwidth will promote much higher fluorescence emission intensity.
Based on Kasha’s rule, the emission fluorescence spectral shape remains unchanged independent of the excitation wavelength. However, intensities will be changed based on how many photons are being absorbed (different excitation intensities).

However, in cases in which obtaining the full emission spectral shape of the fluorophore are more critical, the excitation wavelength should not interfere with that of fluorescence emission even though selected band of excitation does not promote the maximum fluorescence intensity. For the multi-analyte detection case, each wavelength of excitation light should be carefully selected to stimulate only one type of fluorophore at a time in order to assure that the corresponding fluorescence emission is from the target of interest.

### 2.2.3 Quantitative Analysis of Fluorescence

In order to determine the concentration of single or multiple fluorophores of our interest, quantitative analysis of emitted fluorescence is required. Total fluorescence emission from a fluorophore is normally determined by the quantum yield and total absorbed rate of the light:

\[
I_F = \Phi \times I_A
\]
where $I_F$ is fluorescence, $\Phi$ is quantum yield of fluorophore, and $I_A$ is total absorbance of excitation light.

Fluorescence quantum yield is the ratio of the number of absorbed excitation photons to the number of the emitted fluorescence photons. The quantum yield for each fluorophore differs due to different molecular structures and determining the quantum yield is typically done in two ways: (1) absolute and (2) comparative methods. The absolute method is based on integrating spatial fluorescence emission distribution under a specific excitation wavelength (typically max absorption, $\lambda_{\text{max}}$). The other method is by comparison with a standard fluorophore sample with known quantum yields. Nowadays, these standard fluorophores are commonly available (Cy3, Cy5, fluorescein, rhodamine, etc).

The total absorption rate of light with a specific wavelength can be defined by the Beer–Lambert law:

$$I_A = \log \left( \frac{I_0}{I_T} \right) = \varepsilon \times c \times l = \text{OD (Optical Density)} \quad (2.9)$$

Figure 2.5 Beer–Lambert law. $I_0$ is initial intensity of light, $I_T$ is transmitted or attenuated light, $\varepsilon$ is molar extinction coefficient, $c$ is concentration of sample, and $l$ is light path length.
where $I_0$ is initial intensity of light, $I_T$ is transmitted or attenuated light, $\varepsilon$ is molar extinction coefficient, $C$ is concentration of sample, $l$ is light path length, and OD is the optical density. It is assumed that no scattering occurs within the medium through which the light travels. Normally, the target molecule’s extinction coefficient ($\varepsilon$) and light path length ($l$) are known; therefore, concentration of the sample can be easily estimated. In addition, quantitative estimation of absorbance as a function of wavelength (typically from UV to IR) often provides valuable information about the molecule, such as chemical bonding and conjugation [28–30]. Spectrophotometry, which utilizes this technique, is one of the most widely used optical measurement methods in analytical chemistry along with fluorometry. With the help of a spectrophotometer, the wavelength of maximum absorbance for any fluorophore can be determined prior to obtaining the fluorescence measurements. Therefore, the maximum total fluorescence is described using the equation:

$$I_{F(\text{max})} = \Phi(\lambda_{\text{max}}) \times I_A(\lambda_{\text{max}})$$ (2.10)

where $I_{F(\text{max})}$ is maximum total fluorescence, $\Phi(\lambda_{\text{max}})$ is quantum yield at $\lambda_{\text{max}}$, and $I_A(\lambda_{\text{max}})$ is the absorption at $\lambda_{\text{max}}$.

### 2.3. Review of Design Configurations for Portable Fluorescent Sensors

This section will introduce the basic functions of different fluorometers and review portable fluorometers that are especially designed for phytoplankton detection. Moreover, the subsections introduce the basic configurations of portable fluorometers for phytoplankton detection followed by a review of various types of fluorometers and their limitations.
2.3.1 Fluorescence Instrument Configuration

In the last few decades, significant advances in fluorescence-based instruments brought great impact in various areas of research, especially in bioscience. Now, the sensitivity of a fluorometer is as low as single photon level and the fluorescence microscope can distinguish two different particles spaced less than 10 nm distance thanks to state-of-the-art super resolution technology [31-33]. Fluorescence-based instruments can be categorized into several types based on which fluorescence parameters are being measured as illustrated in Figure 2.6. The basic function of the fluorometer will be to simply measure fluorescence intensity at fixed wavelength values of excitation and emission, which is often called a steady-state measurement (wavelength-based) [34]. The fluorescence lifetime is a measurement of the decay in the rate of emission intensity, which is a unique property of different fluorophores [35]. Anisotropy is the utilization of polarized excitation light for characterizing the fluorophores’ rotational motions via detection of fluorescence emission that has the same polarity as the excitation wavelength [36].

![Diagram of Fluorescence Instrument Configuration](image)

Figure 2.6 Different types of fluorometers are categorized based on which fluorescence parameters are being detected. Each parameter can be measured either separately or together based on the sensing mode and application. Generally, the instrument requires more sophisticated systems for measurement of more fluorescence parameters.
The focus of this review will address mostly the steady-state fluorescence sensors since steady-state fluorescence measurements are relatively simple and do not require sophisticated systems for the operation; thus, these types of measurement are more appropriate for portable applications. In the following section, two fluorescence sensor configurations will be introduced.

2.3.1.1 Spectrometer

A spectrometer contains five essential components as illustrated in Figure 2.7: (1) a light source for broad excitation spectrum; (2) an excitation monochromator for wavelength selection; (3) a sample container (usually cuvette and holder jig); (4) an emission monochromator for fluorescence wavelength selection; and (5) a photodetector. Light absorbance can be measured as an option (spectrophotometry function). Amongst all five main components, the quality of the measured fluorescence (resolution and intensity) is usually determined by the monochromator components. A monochromator consists of a collimator, focusing mirror, grating, slits, optomechanical components, and peripheral systems for controlling optics as illustrated in Figure 2.8. Monochromators and detectors are mainly responsible for making it difficult to miniaturize a spectrometer system unless its performance is being significantly compromised in terms of resolution and sensitivity [37, 38]. Simple handheld spectrometers have been reported to inspect the ripeness of fruits and measure the hemoglobin in blood [39, 40]. Smartphones are being used to capture the light rays via embedded cameras or to process and display captured spectra data through its processor (Figures 2.9 (a) and 2(b)). Wilkes et al. developed a spectrometer having 1 nm resolution with conventional optics and incorporating the Czerny-Turner monochromator configuration [41]. However, it requires relatively pricey optics and makes it difficult to achieve a compact form factor (Figure 2.9 (c)).
Regardless of the application of the device, all five fundamental components are critical in the spectrometer and none of them can be removed to be fully functional. However, some of the components that serve the same functions can be replaced for different applications and purposes. Different components have unique advantages and disadvantages, which requires careful selection of alternative components for the proper applications. A portable system may require alternative components to achieve compact size and less power-consuming features.

![Block diagram of the basic spectrofluorometer components. Although this configuration is old, it is still widely used in laboratory-based spectrofluorometer equipment.](image)

Figure 2.7 Block diagram of the basic spectrofluorometer components. Although this configuration is old, it is still widely used in laboratory-based spectrofluorometer equipment.
2.3.1.2 Filter-based Fluorometer

A filter-based fluorometer is the oldest configuration of all fluorescence-based instruments. Components of this type of instrument are similar to that of a spectrometer, but some components including excitation and emission wavelength selectors are replaced with optical filters as
illustrated in Figure 2.10. These excitation and emission optical filters are either single or multiple layers of color filters or dichroic mirrors; therefore, it is only possible to select fixed excitation and emission wavelengths. Nevertheless, multiple wavelengths can be selected with a filter-wheel configuration that contains a limited number of optical filters to obtain various wavelengths of interest [42].

Figure 2.10 Illustration of the filter-based fluorometer. Compared to the conventional spectrometer, filter-based fluorometers are simpler, cheaper, compact, and more application-specific.

Generally, the configuration for a filter-based fluorometer is simpler, cheaper, and smaller compared to a conventional spectrometer due to the absence of conventional monochromators used for excitation and emission wavelength selection. Therefore, for portable fluorescence sensing applications, the filter-based fluorometer configuration is frequently utilized to achieve a simple and compact system. However, the optical filters transmit only a single wavelength at a time; therefore, selectivity is limited to a set number of filters for both excitation and emission wavelengths. Filter-based fluorometers are usually appropriate for applications in which periodic quantitative analysis for a single analyte is needed. In addition, optical filters are often utilized to
minimize the autofluorescence noise. Autofluorescence is fluorescence emission from the materials other than the target analytes. There are several transparent materials that are widely used to load the samples, such as glass, polydimethylsiloxane (PDMS), Polymethyl methacrylate (PMMA), cyclic olefin copolymer (COC), and polycarbonate (PC). The comparison of autofluorescence of different materials was reported by Piruska et al. [43]. In this study, the highest autofluorescence emission was observed under blue excitation (403 nm). Relative autofluorescence of PDMS under 403 nm was about 4 times higher than that of borosilicate glass. PMMA exhibited 6 times higher, COC exhibited 20 times higher, and PC showed 41 times higher. Although thermoplastic materials exhibit relatively higher autofluorescence, they are durable against temperature and pressure, thus have been used in biological sensing applications with optical filters to minimize the autofluorescence noise.

2.3.1.3 Various Light Sources and Photodetectors for Portable Fluorescent Sensing Systems

A proper selection of excitation light is essential to improve the sensitivity, selectivity, and many other parameters of the fluorescent sensing system. There are various excitation light sources applied for different applications and each has its advantages and disadvantages. The spectral linewidth of excitation light should be as narrow as possible to minimize the interference with emission fluorescence. Characteristics of different light sources are described below: lamp, light-emitting diode (LED), and light amplification by stimulated emission of radiation (laser).

A lamp (low-pressure mercury and xenon arc lamp) is normally used for a laboratory-based benchtop spectrometer with monochromators and provides a broad spectral range of light (from deep ultraviolet to far infrared). This type of light source is large in size and usually requires high power. For this reason, a small flashing xenon lamp combined with an optical filter is an option for portable fluorometer applications [44]. A xenon lamp with excitation and emission filter wheels
was reported for a simpler design compared to the monochromator-based spectrometer [45]. This study utilized a charge-coupled device (CCD) as a fluorescence detector in order to obtain spatial information similar to information obtained from fluorescence microscopy. Replacing conventional monochromators and a photomultiplier tube (PMT) with filter wheels and CCD, respectively, provides rapid scanning of fluorescence capabilities with spatial information. Even though the power requirement is relatively smaller (5 ~60 W) than the conventional xenon lamps (300 ~ 450 W), the power consumption is still high for portable systems being powered by a battery. In addition, it requires an electronic circuitry for a high voltage discharging system, therefore, it becomes more challenging to incorporate into a compact system.

A laser is very useful when wavelength-specific light is required (≤ 1 nm). The light emitted from the laser is monochromatic, coherent, directional, and highly powerful. Cowles et al. reported a laser-based fluorometer for in situ measurements of different phytoplankton groups by stimulating their photopigments (phycoerythrin and chlorophyll) [46]. An argon laser (514 nm) coupled with a long optical fiber (200 m) was selected for stimulation and detection. Chen et al. developed a portable in situ fluorometer to measure the fluorescence signals of various dissolved organic matters in the sea [47]. By utilizing a narrow band laser with a center wavelength of 405 nm, fluorescence emission from the organic matter was successfully discriminated from the phytoplankton fluorescence. Although the laser is highly advantageous for fluorescent sensing systems, it requires a complicated, expensive, and large system, thus is not suitable for portable applications.

An LED is a semiconductor light that has many advantages over conventional light sources, especially for portable applications, due to its compact size, high-power efficiency, low-cost, narrow emission wavelength, and long lifetime. In addition, commercially available LEDs are
diverse in wavelength selection (ranging from ultraviolet to near-infrared); therefore, they are more appropriate as excitation light sources for a portable system than other sources. A laser diode, which is also widely used in portable systems, is a class of laser fabricated with semiconductor materials. The working principle of laser diode is very similar to that of LED, but it incorporates optical components that a laser has, thus it offers a more coherent and directional emission ray than LEDs. Velpula et al. reported the first axial AlInN ultraviolet core-shell nanowire LEDs in the ultraviolet wavelength range [48]. It exhibited high internal quantum efficiency of ~52% for emission at 295 nm. This can help to replace the bulky and high-power-consuming conventional ultraviolet lamps for portable applications. In addition, micro-LEDs (μLEDs) and mini-LEDs have been actively studied for next-generation displays for their low power consumption and extremely small size [49]. They can be highly useful for portable applications such as wearable sensors and internet-of-things (IoT). LED or laser diode is a practical excitation light source for portable fluorescence sensing applications.

Photodetectors used in portable fluorescence sensing application should be compact, inexpensive, and low-power consuming, yet sensitive. Depending on the requirements of a specific application, an appropriate photodetector should be selected. Wavelength range, light power, electrical bandwidth, gain, and response time (rise time) are among those requirements to consider in choosing the type of photodetectors. For example, photodiode (PD) or CCD is appropriate for applications expecting plenty of light. In order to detect weak light signals, PMT would be the best option unless applied to portable applications that require small size, low-cost, and low-power consumption. The following table compares four different photodetectors commonly used in portable fluorescence sensing applications: PD, CCD, avalanche photodiode (APD), and silicon photomultiplier (SiPM).
Table 2.1 Characteristics of different types of photodetectors.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PMT</th>
<th>PD</th>
<th>CCD</th>
<th>APD</th>
<th>SiPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>QE (%) at peak</td>
<td>&lt; 40</td>
<td>&lt; 90</td>
<td>&lt; 90</td>
<td>&lt; 90</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>Spectral range (nm)</td>
<td>115 – 1,700</td>
<td>190 – 13,000</td>
<td>200 – 1,200</td>
<td>190 – 1,700</td>
<td>320 – 900</td>
</tr>
<tr>
<td>Gain</td>
<td>$10^5$ – $10^7$</td>
<td>1</td>
<td>1</td>
<td>&lt; 300</td>
<td>$10^5$ – $10^6$</td>
</tr>
<tr>
<td>Rise time (ns)</td>
<td>0.15 – 13</td>
<td>0.23 – 10</td>
<td>$10^6$ – $10^8$</td>
<td>0.01 – 0.35</td>
<td>0.3 – 3</td>
</tr>
<tr>
<td>Gain</td>
<td>1 – 2 k</td>
<td>20 – 30</td>
<td>-</td>
<td>100 – 500</td>
<td>20 – 30</td>
</tr>
<tr>
<td>Noise</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
</tbody>
</table>

2.4. A Recent Progress in Portable Fluorescence Sensors

2.4.1 Fluorescent Sensing in Biochemical Detection

Portable biochemical sensors are widely used in various areas such as environmental monitoring [50], disease diagnosis [51], drug discovery [52], and food quality monitoring [53]. Fluorescence-based portable biochemical sensors are recognized as being highly advantageous over other methods for its high sensitivity, high selectivity, rapid response, and simple operation [54]. Unlike colorimetric or absorbance-based sensing techniques, the fluorescence signal is directly measured without comparing with a reference beam. Therefore, fluorescence-based detection is often a more attractive option for field applications where only small amount of biochemical samples are available. The development of new fluorophores increased the possibilities for the sensitive detection of numerous biochemical molecules. For example, near-infrared to far-red fluorescent probes have widely been used in bioimaging because of minimum photobleaching effect, deep penetration depth, and minimum auto-fluorescence by biomolecules [55, 56]. In addition, Wang et al. developed rhodamine-based fluorogenic probes in various colors and having increased cell permeability [57]. Halabi et al. reported a new type of fluorophore
possessing photoswitching capability and fluxionality, which enables to conduct long time-lapse and super-resolution microscopy experiments [58].

Kozma et al. developed a handheld fluorescent microarray reader for point-of-care diagnostics [59]. A laser diode with 635 nm was used as an excitation source. The ray of light was guided and focused by optical components including a pinhole array, waveguide, microlens array, and interference filter. Optical components were placed on top of CCD image sensor to selectively measure the fluorescence signal. A thin waveguide, which can be easily loaded into the device, was used to deliver the sample solution. For demonstration, a series of different concentrations of fluorescence-labeled antibody solutions were used. The limit of detection was 10 times lower than the laboratory-based fluorescence meter, 1 fluorophore/μm² (Figure 2.11(a)).

Ghosh et al. reported a miniaturized fluorescence-based microscope for cellular imaging purposes [60]. The device includes blue LEDs (470 nm peak) as an excitation light source soldered on a 6 mm x 6 mm printed circuit board (PCB) and assembled with optical components including a drum lens and excitation filter. A dichroic mirror was used for directing the excitation light towards the sample while selectively allowing the fluorescence signal. An additional bandpass filter with 535 nm peak was placed before a complementary metal-oxide-semiconductor (CMOS) image sensor to increase the signal-to-noise ratio. The size of the CMOS sensor was 5.8 mm × 5.8 mm having a pixel size of 5.6 μm × 5.6 μm. Electronic components were controlled with an interface PCB board containing field programmable gate arrays (FPGA) and an external PC. For the demonstration, cellular imaging of the active brain of mice and its activity was traced by a cell-permeant fluorescent Ca²⁺ indicator (Figure 2.11(b)).

Katzmeier et al. developed a pocket-sized fluorescence detector for point-of-care testing using a paper-based cartridge for a sample delivery [61]. The device has two parts: a detection unit
and an assay cartridge. The detection unit includes an excitation light source and a photodetector. The assay cartridge includes optical filters and a paper strip, and a 3D-printed frame that incorporates all the components. An LED with 466 nm peak wavelength was used as an excitation light source (70 mW/m²) and a thin blue filter (440 nm peak) was placed on top of the LED to have narrower excitation wavelength. A paper test strip was sandwiched between two slide glass covers to hold the samples and placed over the excitation filter for direct stimulation of the fluorophore samples. An orange filter (longpass 700 nm) was placed on top of the top cover slide glass to block the stray light while allowing the fluorescence emission from the sample to pass. A cadmium sulfide (CdS) light dependent resistor (LDR) was selected to measure the fluorescence emission from the paper strip. For a demonstration, Cas13a-based fluorescence assay was used to detect target RNAs. The reported limit of detection was 3.7 nM (Figure 2.11(c)).

Fang et al. developed a handheld laser-induced fluorescence (LIF) detector for various applications such as capillary electrophoresis, flow cytometry, and scanning detection [62]. For an excitation source, a laser diode with 450 nm peak was selected. The sample solution was loaded in the center of the capillary for testing. For an emission part, a 525 nm peak bandpass filter was coupled with a 1.0 mm diameter aperture. A miniaturized PMT was chosen for the sensitive detection of fluorescence emission. For demonstration, sodium fluorescein was used, and the detection limit was 0.42 nM (Figure 2.11(d)).

Chang et al. developed a handheld electronic tongue for determining the taste level of astringency and umami in different tea infusions [63]. Fluorescence quenching reaction of 3-aminophthalate (reaction with tannin) and o-phthalaldehyde (OPA) (reaction with amino acids) were utilized to detect astringency and umami, respectively. A single excitation ultraviolet LED with 340 nm peak was selected to stimulate both target analytes. The emission wavelength of
fluorescence was 425 nm peak and it was measured with an RGB photosensor. Electronic circuits and optical components are closely packed in a plastic housing with a dimension of 120 × 60 × 65 mm³. A standard disposable cuvette was used to hold the sample. The detection limits of theanine and tannic acid were 0.2 µg/ml and 1 µg/ml, respectively (Figure 2.11(e)).

Sasagawa *et al.* demonstrated a portable lensless fluorescence imaging device using multiple layers of interference filters [64]. A CMOS image sensor with a dimension of 67 mm² was selected for a large imaging area. A series of optical components were layered right on top of the image sensor to shift the focal plane and block the excitation light. First, a fiber optic plate (FOP) with a thickness of 2.54 mm was placed right on top of the image sensor. Two optical filters, an absorption filter (longpass above 500 nm) and a shortpass interference filter (< 560 nm), were placed to block the scattered stray (excitation with 448 nm) light. Another FOP with a longpass interference filter (> 490 nm) was placed right on top of the first FOP to block the excitation ray and autofluorescence. A sample can be directly loaded on top of the FOP surface. For demonstration, fluorescence images of the sliced brain of a mouse (stained with a green fluorescent protein (GFP), emission 515 nm) were obtained (Figure 2.11(f)).
Figure 2.11 Portable fluorescence-based biochemical detectors: (a) a handheld fluorescent microarray reader for point-of-care diagnostics [59]; (b) a miniaturized fluorescence-based microscope for cellular imaging purposes (after [60]); (c) a pocket-sized fluorescence detector for point-of-care testing using a paper-based cartridge for a sample delivery [61]; (d) a handheld LIF detector for various applications such as capillary electrophoresis, flow cytometry, and scanning detection [62]; (e) a handheld electronic tongue for determining the taste level of astringency and umami in different tea infusions [63], and (f) a lensless fluorescence imaging device using multiple layers of interference filters [64]. Copyright 2013 with permission from Elsevier for (a); licensed under a CC BY license for (c); copyright 2016 with permission from Elsevier for (d); copyright 2010 with permission from Elsevier for (e), and licensed under a CC BY license for (f).
2.4.2 Water Quality Monitoring

In this chapter, the basic principles of fluorescence and its measurement are introduced along with the literature survey of portable fluorescence sensor systems for phytoplankton detection. Fluorescence sensing technique is highly selective and sensitive; therefore, it is a powerful tool for multiple phytoplankton sensing application but requires relatively sophisticated components compared to other optical detection methods. Various types of fluorometers for phytoplankton detection were reviewed, and an LED-based excitation lighting system showed great potential for a portable fluorometer development. However, some major challenges were found, such as developing a completely standalone system and characterization of different phytoplankton species using the developed device.

2.4.2.1 Phytoplankton Detection

Phytoplankton monitoring is one of the important tasks for supporting human health and environmental issues, especially for water quality control. Detecting and analyzing different groups of phytoplankton in water provides important information on aquatic ecological states and nutrient compositions [65]. In addition, early detection of certain species of algae that cause harmful algal blooms (HABs) is essential to protect the water ecosystem and human health [66, 67]. There are various ways to detect and analyze phytoplankton species, namely microscopy [68], flow cytometry [69], high-performance liquid chromatography (HPLC) [70], spectrophotometry [71–73], spectrometry [74–76], and dry weight [77, 78]. However, these methods are difficult to be applied in the portable sensing system.

Different phytoplankton groups exhibit unique fluorescence properties due to different photopigment constituents [79-82]. A review on the photopigment constituent of different phytoplankton groups revealed that chlorophyll a can be found in every algal species due to its
essential role in oxygenic photosynthetic reactions [83]. Therefore, even though chlorophyll \( a \) fluorescence provides important information about the spatial mapping of overall phytoplankton abundance, it only offers information about microalgal species in the water and does not distinguish one group from another. Different photopigments within various phytoplankton species are responsible for absorbing photon energy with broader wavelengths for effective energy collection. Chlorophyll \( a \) is called a primary photosynthetic pigment, and all of the others are accessory photosynthetic pigments [46]. Although all the pigments in green algae are responsible for absorbing light and contributing to the photosynthetic reaction, chlorophyll \( a \) is responsible for majority of fluorescence due to the relatively longer lifetime of electrons. For this reason, chlorophyll \( a \) is referred to as a primary photosynthetic pigment, while all the others are referred to as accessory photosynthetic pigments [81, 84]. Accessory pigments, such as carotene and chlorophyll \( b \), show very weak or no fluorescence emissions since electrons are transferred to chlorophyll \( a \) immediately upon generation after absorbing photon energy. In this regard, careful selection of excitation light sources, optics, and detectors is necessary for multiple algal species detection capabilities. This section focuses on portable sensors that measure different phytoplankton species or toxins produced by some species of cyanobacteria.

Bickman et al. developed a portable toxin meter that can detect marine biotoxins (microcystin and cylindrospermopsin) released from cyanobacteria using multiplexed fluorescence immunoassay technology [85]. This approach is more accurate and direct when detecting the toxins released from HAB compared to the conventional indirect detection methods. The main reader device includes a laser diode (639 nm peak) that is coupled with a plastic light waveguide to evenly distribute and project the excitation lights on the sample holding substrate. A simple disposable cartridge that is pre-coated with antibodies was used to detect the toxins in the
collected sample. The presence of the toxins inhibited the binding of biotoxin-conjugate spots on the surface of the cartridge where fluorescence signal is reduced. The entire surface of a microarray was analyzed with a camera to detect the fluorescence emission. The limit of detection for microcystin and cylindrospermopsin were 0.4 μg/l and 0.7 μg/l, respectively (Figure 2.12(c)).

Gosset et al. developed a portable xurography-based microfluidic biosensor for green algae detection via total chlorophyll $a$ fluorescence measurement [86]. The sensor utilizes an LED with 470 nm peak wavelength as an excitation source. Optical components such as collimating lens and diffuser were placed underneath the LED to evenly distribute the light. A disposable microfluidic chip was fabricated with two slide glasses and a double-sided pressure adhesive film with 100 μm thickness to deliver the microalgal sample to the device. The chip has two chambers to read a control and a target of interest. Different concentrations of herbicide solutions were mixed with the algal samples to analyze the relationship between the fluorescence intensity changes to the photosynthesis disturbance rate of the samples. The fabricated portable fluorescence sensor was successfully demonstrated using three different microalgae species with the herbicide solution. The detection limit of the microalgae measurement was 1 μg/l. (Figure 2.12(d)). Although the use of those fluorometers was successfully demonstrated for phytoplankton detection, limitations for those sensors still exist. It is challenging to distinguish multiple species of phytoplankton, simultaneously, because the fluorescence signals from those species often overlap and interfere with each other. In addition, dissolved organic matter in the water becomes another source of the noise.
Figure 2.12 Portable fluorescence algae sensors: (a) a portable toxin meter for detecting microcystin and cylindrospermopsin [85], and (b) a portable xurography-based microfluidic biosensor for green algae detection via total chlorophyll \( \alpha \) fluorescence measurement [86]. Copyright 2018 with permission from American Chemical Society for (a), and copyright 2018 with permission from Elsevier for (b).
2.4.2.2 Dissolved Organic Matter Detection

The increase of human pollution and the effects of climate change significantly affected the quality of the water [87-89]. The pollutants in this natural water are closely related to the dissolved organic matter (DOM) concentration and its composition, therefore it is important to understand the characteristics of DOM in the water [90, 91]. DOM is defined as any organic matter dissolved in the water that can pass through the water filter with a pore size of 0.2 μm. For DOM detection, ultraviolet-visible fluorescence spectroscopy method was widely used to fully scan the absorption and the emission spectra [92, 93]. Since not all DOMs are light interactive, the light-absorbing portion, of which is defined as colored dissolved organic matter (CDOM), is measured by the fluorescence-based detection. Due to the advantages that fluorescence-based detection method offers, various studies have been conducted to concentrate and develop an on-site detection of CDOM fluorescence to aid the water quality monitoring. The main advantage of a portable fluorometer system is that in situ measurement of CDOM can report ecological conditions of the water in a timely manner, thus more accurate environmental monitoring is possible. Natural water typically has CDOM that is responsible for strongly absorbing light in the light range from 250 nm to 450 nm and fluorescing at 400–450 nm [94, 95]. However, the utilization of blue light excitation for CDOM detection is challenging due to the presence of chlorophyll pigments in natural bodies of the water, thus accurate assessment of the emission signal is essential [96]. Lewis et al. studied an algorithm to accurately estimate the CDOM in the arctic ocean to compensate for the overly estimated CDOM when chlorophyll a present in the water [97]. For remote sensing, satellite-based measurements were widely used to quickly assess the large water areas such as the ocean [98, 99]. In addition, hyperspectral remote sensing was reported to achieve higher resolution than the satellite-based sensing while covering a relatively larger area in the last decade and
successfully differentiated multiple many important water quality parameters such as CDOM, chlorophyll \( a \), diatoms, and turbidity [100]. However, those remote sensing techniques face challenges to report ecological state of deeper water body or continuous measurement of one single spot of an interest. This section focuses on reviewing the properties and results of water quality measurements by utilizing the portable fluorescence sensors for detecting CDOM excited by the light wavelength in the range of 250 –500 nm.

Brandl \textit{et al.} developed a portable fluorometer for detecting DOM and three different green algae by using different excitation LEDs [101]. The fluorometer has three different wavelengths of excitation LEDs (254, 310, and 370 nm) with two bandpass filters (370 and 310 nm). An adjustable filter wheel with four bandpass filters with 380, 430, 450, and 500 nm wavelengths was utilized to selectively measure the target fluorescence emission from different analytes. A PMT was used to detect the fluorescence signals. The DOM concentration was estimated by calculating the ratio of two fluorescence emissions from 380nm and 430 nm bandpass filters under 310 nm excitation. Consequently, the ratio of two fluorescence emissions from 450nm and 500 nm bandpass filter under 370 nm excitation, was calculated to determine if the measured DOM is from microbial nature or terrestrially derived. A standard cuvette was selected to hold the sample solution. (Figure 2.13(a)).

Bridgeman \textit{et al.} reported an LED-based portable fluorescence sensor to detect total organic carbon (TOC) and microbial activity of water [102]. Two UV excitation LEDs with 280 nm and 335 nm wavelengths were used to measure microbial activity and organic carbon, respectively. An additional UV LED with 310 nm was used to measure the water Raman signal, which is caused by the inelastic scattering of the excitation light. This allows to minimize the measurement error when the analyte concentration is low and the fluorescence is weak. A standard
quartz cuvette was used to hold the sample solution. Two PMTs were used to measure the fluorescence signal and water Raman signal, simultaneously. The dimension of the device was 425 (l) × 300 (w) × 225 (d) mm, with a weight of 3.5 kg. The results showed high correlations ($r^2$) between the new portable system and a conventional benchtop instrument (ranging from 0.83 – 1.00). (Figure 2.13 (b)).

Laser-based excitation sources have several benefits over LED-based excitation sources. Laser can generate highly monochromatic (equal or less than 1 nm), coherent, and collimated excitation light, therefore, they show minimal interference with the fluorescent emission spectra and require less optical components for beam shaping. However, it is difficult to apply conventional laser systems, such as gas lasers and solid-state lasers to portable systems because of their large size, high cost, and power consumption. On the contrary, LEDs with optical filters are desired for portable applications. A LIF technology was utilized to a portable fluorometer system by Chen et al. to detect chlorophyll $a$, CDOM, and total suspended matter (mainly slit and microorganisms) [103]. A laser with 405 nm wavelength was coupled with a fiber-optic probe to guide the light excitation and emission lights. As an excitation source, laser showed several benefits over other light sources such as high pulse frequency modulation capacity and narrower spectral profile. Those benefits can improve the signal-to-noise ratio of measured fluorescence signals. A quartz cuvette was selected to load the sample solution. A dichroic beam splitter and longpass filter were deployed to selectively scan the emission fluorescence spectra from the sample. Fluorescence emission from the sample was measured with a hyperspectral micro spectrometer. The fluorescence emissions in the peak near 685, 508, and 470 nm correspond to chlorophyll $a$, CDOM, and water Raman scattering, respectively. The measurement of water Raman signal was required to compensate the errors due to the spectral overlapping. The limit of detection for CDOM,
total suspended matter, and chlorophyll $a$ were estimated to be 0.75 µg/l, 1mg/l, and 0.2 µg/l, respectively (Figure 2.13 (c)).

Figure 2.13 Portable DOM fluorescence sensors: (a) a portable fluorescence sensor for DOM measurement using fluorescence index (FIX) and biological index (BIX) [101]; (b) a portable LED fluorescence instrumentation for the rapid measurement of TOC [102]; (c) a light-weight laser-based fluorometer for monitoring chlorophyll $a$ and CDOM [103], and (d) a miniature fluorometer is designed to measure chlorophyll and CDOM concentration in the aquatic environment (after [104]). Licensed under a CC BY license for (a) and (b), and copyright 2015 with permission from Elsevier for (c).

Blockstein et al. reported a portable fluorometer to measure chlorophyll pigments and CDOM concentration [104]. Light emitting diodes with 405 nm and 465 nm were utilized to selectively stimulate the CDOM and chlorophyll, respectively. Two custom fabricated thin optical filters were directly attached on a single sensor array to selectively measure two different fluorescence signals. The thin glass substrates were applied to absorb and attenuate the excitation
lights. For demonstration, standard fluorescein dye was selected to simulate chlorophyll a. The sensor was tested while it is completely submerged under the water. The limit of detection for the fluorescein was 0.7 nM. (Figure 2.13(d)).

Dissolved organic matter measurement in natural water is challenging since it is a collection of different constituents in water. The ratio of these elements in the water may highly vary depending on the region, time, and the weather, thus accurate assessment is required. CDOM showed a broad range of excitation spectrum, usually between 250 nm to 500 nm. The emission spectral range was normally between 350 nm to 500 nm.

2.4.2.3 Heavy Metal Ion Detection

Heavy metal pollutions in water have become a strong threat to marine animals and humans since they can bioaccumulate in living organisms either directly or through consumption [105]. Among many metal ions, copper, lead, mercury, chromium, and cadmium are known to be highly toxic for humans. For example, copper can cause liver damage, lead is known to damage the brain, and low doses of mercury exposure can cause severe damage to the nervous system for all animals including humans. Therefore, it is critical to rapidly detect those metal ions from the aqueous system.

Various methods have been developed for heavy metal detection such as electrochemical [106], spectroscopic [107], and optical detection [108-111]. Although both spectroscopic and electrochemical detection methods have been widely used, optical detection methods showed great potential for portable sensing platforms [123]. One of the widely used optical detection methods is colorimetric sensing that utilizes selective reagents and indicator dyes. They only react with desired target metal ions and absorb a specific wavelength of the color. For reactive agent material, colloidal gold nanoparticles (AuNPs) were widely selected to detect the target metal ions by
examining the color shifting of the solution, in which the particles aggregate with the target ions [113, 114]. Morais et al. developed a portable lead detector to demonstrate a low-cost colorimetric-based sensor using economical components such as LEDs and microcontroller module [115]. Chen et al. reported a mercury detector by utilizing the paper-based sensor combined with AuNPs to demonstrate a cheap/disposable sensing platform for resource-limited settings [116]. Wei et al. [117], Nguyen et al [118], and Xiao et al [119] demonstrated portable smartphone-based heavy metal detectors to show the benefits of incorporating the mobile phones with custom-designed sensing platforms. However, colorimetric-based sensing techniques face challenges when used at the natural water bodies where numerous elements exist as interference factors, therefore fluorescent-based detection method is desirable for a field-deployable application. The major interfering elements in natural water bodies include CDOMs and suspended particles. CDOMs strongly absorb the spectral range in 250 nm to 500 nm, which may interfere with AuNP-based colorimetric heavy metal sensors. Suspended particles are any particles that are bigger than 2 µm, such as clay, silt, sand, gravel, bacteria, and algae. It has been reported that suspended particles can negatively affect the detection performance of colorimetric-based sensors by increasing the optical density and scattering of light, however, the detection performance of fluorescence sensors was not affected by suspended particles [150]. This section focuses on portable fluorescence sensors for detecting heavy metal ions in water.

Xiao et al. developed a paper-based microarray using carbon nanodots (CDs) to detect the heavy metal ions (Hg^{2+}, Pb^{2+}, and Cu^{2+}) [120]. Three different CDs were prepared and drop casted on the pretreated filter paper for selective detection of different heavy metal ions. A 3D-printed apparatus was assembled with a smartphone to read out the fluorescence signals from the microarray using an image sensor on the phone. The apparatus includes an excitation LED (365
nm), a plastic diffuser, and an optical lens to evenly illuminate the light on the surface of the microarray. The emission spectra for Hg$^{2+}$, Pb$^{2+}$, and Cu$^{2+}$ were estimated to be 445, 450, and 475 nm, respectively. The image of the microarray was processed with the smartphone and the results were displayed on the screen using the custom-built application. The limit of detection for Hg$^{2+}$, Pb$^{2+}$, and Cu$^{2+}$ were 5.8 nM, 0.12 µM, and 0.076 µM, respectively. The sensor offers a great resolution to measure the World Health Organization (WHO) guideline values for the heavy metal ions in drinking water (Hg$^{2+}$ (0.006 mg/l), Pb$^{2+}$ (0.01 mg/l), and Cu$^{2+}$ (2 mg/l)) (Figure 2.14(a)).

Guo et al. reported a carbon dot doped hydrogel waveguide for detecting Hg$^{2+}$ in the water [121]. Although the reported work is not a stand-alone system, it showed great potential to be a key element of the device and easily incorporated into a portable device. PEG diacrylate (PEGDA) was selected as the material for the waveguide and the fluorescent carbon dots with 7.8 nm diameters were selected for the material. The waveguide exhibited a peak absorption at 352 nm and fluorescence emission at 475 nm. The detection range was between 0 to 5 µM and the limit of detection was 4 nM (Figure 2.14(b)).

Liu et al. reported smartphone-based fluorescence sensor for Hg$^{2+}$ detection [122]. CdSe/ZnS quantum dot modified optical fiber probe was coupled with a laser excitation light (405 nm) and an optical filter (bandpass with 620 ± 15 nm). The camera on the smartphone was placed behind the bandpass filter to measure the intensity of the fluorescence to quantify the Hg$^{2+}$ concentration in the solution. The detection range was between 1 nM to 1000 nM and the limit of detection was 1 nM (Figure 2.14(c)).
Lee et al. also introduced a smartphone imaging-based fluorescence microscope for monitoring Hg$^{2+}$ ions utilizing a biosensor cartridge [123]. The cartridge was pretreated with an Hg$^{2+}$ DNA probe to capture the Hg$^{2+}$ ions. The intercalated fluorescence dye that emits green (520 nm) fluorescence was quenched after binding with Hg$^{2+}$ ions while the quantum dot that emits red (655 nm) light became brighter after the reaction with the Hg$^{2+}$ ions. The captured image using a camera image sensor was analyzed in red and green channels to quantify the intensities and the results. The limit of detection was 1 pM (Figure 2.14(d)).
Heavy metal detection in an aqueous environment is challenging since it includes many elements and particles that can potentially interfere with the target metal ions and it may lower the detection accuracy [124]. There are several strategies to improve the detection performance of heavy metals in water. Cellulose filter papers can be used to pretreat the water before the test to get rid of the debris from the solution. In order to minimize the interference from the dissolved matter in water, accurate characterization of fluorescence emission spectra of the sample solution (noise mainly from CDOM and chlorophylls).

2.4.2.4 Smartphone-based Fluorescence Microscope

Fluorescence microscopy is a very effective method in bioscience, however, its use in portable applications is difficult due to the complexity and the size of its instrument. Recently, smartphones have been widely used for remote sensing applications owing to their multiple built-in sensors and communication modules. In addition, smartphones were able to achieve great improvements in image sensor and computing power. This allowed to demonstrate a compact fluorescence microscope for analytical imaging applications in field settings. Wei et al. demonstrated a smartphone-based fluorescence microscope to detect single bacteria [125]. In addition, advanced image processing algorithms such as machine learning and deep learning techniques were applied to improve sensing accuracy [126, 127]. In this section, smartphone-based fluorescence microscopes used in various applications were reviewed.

Wei et al. reported a smartphone-based portable fluorescence microscope for imaging of nanoparticles and viruses [125]. For an excitation source, a laser diode with 450 nm peak was used with an optical lens (focal distance = 4 mm). Two longpass optical filters (> 500 nm) were placed above the image sensor to block the stray lights from the excitation source. An aliquote volume of sample solution was loaded with a standard cover glass slide, which was held by a 3D-printed tray.
The light ray was projected with a high incident angle (75°) towards the sample to minimize the excitation rays from reaching to the image sensor. For demonstration, green fluorescent polystyrene (PS) particles (ex/em: 505/515 nm) and human cytomegaloviruses were tested. The microscope had 0.6 mm x 0.6 mm field of view. (Figure 2.15(a)).

Yu et al. demonstrated smartphone-based fluorescence spectroscopy for demonstrating microRNA sequencing [128]. Two different lasers with a peak wavelength of 532 nm and 653 nm were selected as excitation sources. A series of optical components including pinhole, optical fiber, diffraction grading (1200 lines/mm), and three different optical lenses were used to scan broadband fluorescence emission spectra. The sample solution was loaded via standard cuvette. For testing, molecular beacon Förster resonance energy transfer (FRET) assay was selected to observe the changes in the quenching efficiency of a miRNA sequence. The limit of detection was 10 pM. Although the laser excitation sources and the sample holder were not completely incorporated into the cradle, the authors claimed that they can be easily integrated for a standalone application. (Figure 2.15(b)).

Snow et al. developed a smartphone-based fluorescence microscope for imaging and detecting pathogenic spores in honeybees [129]. For excitation light source, four ultraviolet (specific wavelength is unclear) LEDs were selected. A longpass filter (> 460 nm) and a simple optical lens were placed before the smartphone camera lens to minimize the noise from the excitation light. For sample delivery, a standard microscopic slide glass was selected and it was easily inserted with a 3D printed slide-holder. For sample preparation, midgut tissues of honeybees were stained with fluorescent brightener to detect the spores. A smartphone application was developed to transmit the captured image to the main server for image processing. The detection limit was $0.5 \times 10^6$ spores per bee (Figure 2.15(c)).
Figure 2.15 Smartphone-based fluorescence microscopes: (a) smartphone-based imaging device for nanoparticles and viruses [125]; (b) smartphone spectrophotometry for detecting fluorescence biological assays [128]; (c) a smartphone-based fluorescence microscope for imaging and detecting pathogenic spores in honeybees [129]; (d) a smartphone-based fluoresce microscope
combined with surface-enhanced fluorescence created with thin metal-film [130], and (e) a smartphone-based fluorescence microscope for pathogenic bacteria identification [131]. Copyright 2013 with permission from American Chemical Society for (a); copyright 2014 with permission from American Chemical Society for (b); copyright 2019 with permission from Royal Society of Chemistry for (c), and Licensed under a CC BY license for (d) and (e).

Wei et al. reported a smartphone-based fluorescence microscope combined with surface-enhanced fluorescence created with thin metal-film [130]. A compact laser diode with 465 nm peak wavelength was utilized as an excitation source and the light was guided through a glass hemisphere at an incident angle of 58°. The beam from the laser was then filtered by a polarizer to deliver p-polarized rays onto the silver-coated glass coverslip (22 × 22 mm). A bandpass filter (525 ± 25 nm) and a collimating lens were placed before the phone camera to block the excitation background noise. For demonstration, DNA origami nanobeads (average diameter of 23 nm) and quantum dots (ex: 468nm/ em: 508 nm) were selected. The limit of detection for the DNA origami nanobeads was reported as 80-fluorophore DNA origami nanobeads (Figure 2.15(d)).

Müller et al. developed a smartphone-based fluorescence microscope for pathogenic bacteria identification [131]. The laser diode with 488 nm wavelength was selected for an excitation source and a half ball lens was chosen to focus the light on the sample. Both the laser diode and ball lens were moved vertically by a miniature dovetail stage. An aliquot volume of sample droplet (20 µl) was placed on a glass coverslip (22 mm × 50 mm) for sample delivery. A longpass emission filter (> 514 nm) was placed before the smartphone camera with an external lens to block the excitation light. A smartphone LED (white) was utilized to locate the reference dot and preliminary focusing of the sample. For testing, bacteria with various concentrations were prepared with fluorescence labels. The sensor was able to selectively detect the target strand even
Figure 2.16 Smartphone-based fluorescence microscopes (cont.): (a) reported a smartphone-based fluorescence microscope for monitoring of OOC [132] and (b) a portable fluorescence-based mercury detector using smartphone microscope [133]. Copyright 2016, with permission from Elsevier for (a) and copyright 2019, with permission from Elsevier for (b).
when mixed with different bacterial strain and infant formula. The fluorescence emission wavelength was 525 nm. The limit of detection was $10^4$ CFUs/ml (Figure 2.15(e)).

Cho et al. reported a smartphone-based fluorescence microscope for monitoring of organ-on-a-chip [132]. White LED with a bandpass filter (480 ± 10nm) was selected as an excitation source. A longpass filter (> 500 nm) with an objective lens was placed in front of the image sensor to block the noise from the stray light. Organ-on-a-chip was fabricated using PDMS, which is a transparent silicon-based organic polymer. The microfluidic channel was etched on the glass substrate and bonded with PDMS chip. Sample solutions were loaded through the inlet and outlet holes. For demonstration, cancerous cells were selected as target analytes. These cells were functionalized on the glass substrate and fluorescently labeled (ex: 480 nm/ em: 510 nm). The solution including the fluorescent particles was introduced into the organ-on-a-chip (OOC) with 500 µl/h flow rate during the testing. The limit of detection was 10 pg/ml (Figure 2.16(a)).

Shan et al. developed a portable fluorescence-based mercury detector using a smartphone microscope [133]. A compact laser diode with a wavelength of 405 nm peak was selected for an excitation source. Optical components including micro-objective and eyepiece were arranged for focusing and collimation. A bandpass filter (469 ± 35 nm) was placed before the phone camera to obtain noiseless fluorescence images. A standard slide glass with a 3D printed tray was used to deliver the sample droplet. The size of a sensor device was 170mm (length) ×113mm (width) ×168mm (height). For detection, fluorescent microspheres (ex: 405 nm, em: 450 nm) were utilized for selected detection of mercury. The limit of detection was 1 nM (Figure 2.16(b)).

A variety of types of smartphone-based fluorescence microscopes were reviewed. Smartphones and optical components were easily incorporated into custom-designed jigs to demonstrate compact stand-alone systems for remote sensing applications. Smartphones have
shown great potential to be used in a resource-limited setting and point-of-care testing. It has been reported that even in-built ambient light sensors on the smartphones were utilized for biochemical sensing applications [134-136]. Table 2 summarizes the characteristics of different types of fluorescence-based portable sensing devices that are reviewed. Typical detection speeds for fluorescence-based sensors were almost instantaneous, however incubation and pretreatment times increased the overall test duration.

<table>
<thead>
<tr>
<th>Excitation λ_{\text{ex}} (nm)</th>
<th>Emission λ_{\text{em}} (nm)</th>
<th>Target analytes</th>
<th>Detection limit</th>
<th>Stand-alone</th>
<th>Specificity</th>
<th>Sample volume</th>
<th>Detection speed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>635</td>
<td>647</td>
<td>Antibodies (Alexa Fluor 647-conjugated)</td>
<td>1 fluorophore/µm^2</td>
<td>Yes</td>
<td>Single measure</td>
<td>2 ml</td>
<td>Incubation: 30 min</td>
<td>[59]</td>
</tr>
<tr>
<td>470</td>
<td>535</td>
<td>Cellular imaging</td>
<td>~ 1.5 µm (optical resolution)</td>
<td>Partially</td>
<td>Single measure</td>
<td>1 nl</td>
<td>Incubation: 2 hr Operation: 15 s</td>
<td>[60]</td>
</tr>
<tr>
<td>466</td>
<td>700</td>
<td>RNAs</td>
<td>3.7 nM</td>
<td>Yes</td>
<td>Single measure</td>
<td>30 µl</td>
<td>Incubation: 10 min</td>
<td>[61]</td>
</tr>
<tr>
<td>450</td>
<td>525</td>
<td>Sodium fluorescein</td>
<td>0.42 nM</td>
<td>Yes</td>
<td>Single measure</td>
<td>2 ml</td>
<td>Incubation: 30 min</td>
<td>[62]</td>
</tr>
<tr>
<td>340</td>
<td>425</td>
<td>(1) 3-aminophthalate and (2) o-phthalaldehyde (OPA)</td>
<td>(1) 0.2 µg/ml and (2) 1 µg/ml,</td>
<td>Yes</td>
<td>High</td>
<td>1 ml</td>
<td>Incubation: 10 sec</td>
<td>[63]</td>
</tr>
<tr>
<td>448</td>
<td>515</td>
<td>Mice brain (stained with green fluorescent protein)</td>
<td>36 line pairs/mm (USAF 1951 test target)</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
<td>Operation: 15 ms</td>
<td>[64]</td>
</tr>
<tr>
<td>639</td>
<td>N/A</td>
<td>Myrocystin, Cylindrospermopsis</td>
<td>0.4 µg/l (MC) and 0.7 µg/l (CYN)</td>
<td>Yes</td>
<td>High</td>
<td>5 ml</td>
<td>Total: 10 min</td>
<td>[85]</td>
</tr>
<tr>
<td>470</td>
<td>680</td>
<td>Green algae and herbicide (Diuron)</td>
<td>1 µg/l (green algae)</td>
<td>Partially</td>
<td>N/A</td>
<td>15 µl</td>
<td>Operation: 400 ms</td>
<td>[86]</td>
</tr>
<tr>
<td>254, 310, 370</td>
<td>450, 500</td>
<td>DOM and three different algae species</td>
<td>10 - 50 pM (DPH fluorophore)</td>
<td>Yes</td>
<td>Moderate</td>
<td>3.5 ml</td>
<td>N/A</td>
<td>[101]</td>
</tr>
<tr>
<td>280, 310, 335</td>
<td>310, 380</td>
<td>DOM and microbial matter</td>
<td>N/A</td>
<td>Partially</td>
<td>High</td>
<td>3.5 ml</td>
<td>Operation: 2 sec</td>
<td>[102]</td>
</tr>
<tr>
<td>405</td>
<td>470, 508, 685</td>
<td>Chlorophyll a, CDOM, TSM</td>
<td>0.2 µg/l (Chl-a), 0.75 µg/l (CDOM), and 1 mg/l (TSM)</td>
<td>Partially</td>
<td>High</td>
<td>N/A</td>
<td>N/A</td>
<td>[103]</td>
</tr>
<tr>
<td>405, 465</td>
<td>650, 400-500</td>
<td>Chlorophyll a, CDOM</td>
<td>0.7 nM (fluorescein), and 2.55 nM (QSD) 5.8 nM (Hg^{2+}), 0.12 µM (Pb^{2+}), and 0.076 µM (Cu^{2+})</td>
<td>Partially</td>
<td>Moderate</td>
<td>Immersible</td>
<td>Operation: &lt; 1 sec</td>
<td>[104]</td>
</tr>
<tr>
<td>365</td>
<td>445, 450, 475</td>
<td>Hg^{2+}, Pb^{2+}, and Cu^{2+}</td>
<td>Yes</td>
<td>High</td>
<td></td>
<td>30 ml</td>
<td>Total: 20 min</td>
<td>[120]</td>
</tr>
<tr>
<td>352</td>
<td>475</td>
<td>Hg^{2+}</td>
<td>4 nM</td>
<td>No</td>
<td>High</td>
<td>N/A</td>
<td>N/A</td>
<td>[121]</td>
</tr>
</tbody>
</table>

(table cont’d)
2.5. Discussion and Challenges

Fluorescence-based portable sensing systems have been considerably studied and developed in the past decade. The main benefits of fluorescence-based portable sensors are compact size, low power consumption, low-cost, and fast speed. Although fluorescence-based detection method is highly useful for portable applications, there is still room to improve.

First, a fluorescence labeling process is required to detect the analytes that are not inherently fluorescent. This may add laborious steps for sample preparation to bind the fluorescent tags to the biomolecules such as proteins. Furthermore, the sample solution can contain unwanted matters, which interfere with the target analyte signals. Several studies have demonstrated an integration of microfluidic channels with sample separation/filtration, mixing, target labeling, and washing to minimize the preparation steps [137, 138]. In addition, biochemical reagents can be prefabricated in cheap & disposable materials such as paper. Chu et al. reported a nanoprobe-based
fluorescent paper strip to monitor the glutathione level in human serum [139]. A mixture of quantum dot and carbon dot solution was deposited on a piece of filter paper to fabricate a paper strip sensor. With those approaches, laborious steps can be significantly reduced and increase the practicality of the system.

Secondly, many demonstrated systems still require external instruments to aid the device operation. For example, a laptop or PC was frequently required to control the device or process the data. Single-board computers can be selected as a viable option to handle complicated tasks such as image processing, data analysis, and controlling peripheral devices, simultaneously. They are compact, low-cost, and low-power, hence a great option for portable applications. Jian et al. demonstrated a portable fluorescence-based imaging system for intraoperative display of biliary structure and prevention of iatrogenic injuries during cholecystectomy [140]. A single-board computer (Raspberry Pi, Raspberry Pi Foundation, UK) was able to handle multiple tasks, such as displaying the LCD screen, controlling the image sensor, and processing the data. As reviewed in the previous section, smartphones can be an alternative option as well, since they are equipped with advanced imaging systems and powerful processors to handle complicated tasks [125-133]. Another issue is finding an alternative option to replace the bulky micropumping system. Laksanasopin et al. developed a dongle that can conduct an immunoassay test without the help of complex instruments such as a syringe pump [141]. The custom-designed dongle demonstrated a power-free fluidic pump system by using a chamber with a diaphragm that created a negative pressure when pushed.

For the future perspective, miniaturization and performance improvement of those sensors will lead to broad sensing networks coupled with IoT, thus providing real-time identification of analytes for many other applications such as healthcare and environmental monitoring. Liu et al.
reported an IoT-enabled paper sensor platform for pathogen detection [142]. This opens up the possibility of rapid and early diagnosis of the disease in the future. In addition, new image processing algorithms such as machine learning and deep learning techniques can be applied to improve image quality and increase detection performance [143, 144]. Göröcs et al. developed an in-field portable imaging flow cytometer to detect ocean planktons [145]. Deep learning algorithm has been proven to improve image classification performance.

2.6. Conclusions

We have reviewed the recent development in portable fluorescence sensors and different applications using them. The fluorescent sensing technologies offered many benefits such as high sensitivity, specificity, and accuracy compared to other sensing technologies. Portable fluorescence-based sensors showed great potential for fulfilling critical demands for simple, rapid, and accurate testing in remote and resource-limited settings. These sensors can be utilized in a wide variety of biochemical detection platforms to monitor healthcare, environmental pollution, and biological phenomenon. However, more research is required to address some of the challenges regarding laborious preparation steps and bulky external peripherals. Significant work is currently ongoing to address those limitations, such as utilization of silicon-based photomultiplier to replace conventional photodetectors for better sensitivity and miniaturization. In addition, a compact single-board computer, smartphone, and power free self-operated microfluidic system are great solutions to address current limitations in portable fluorescence sensors. With those improvements, it is expected to increase the use of portable fluorescence sensors in a wide range of biochemical sensing applications.
3. Preliminary Portable Fluorescent Sensor Design for Green Algae Detection

3.1. Introduction

As a first step of accomplishing the final goal of developing a fully portable fluorescent sensor platform, a preliminary design that includes essential parts of the system such as excitation light sources, photodetector, 3-D printed optomechanical parts, and PDMS microfluidic chip were developed. The main purpose of this chapter is to describe the optomechanical design of the fluorescence detection platform, to illustrate the experimental condition and method, and to present the measurement results and the analysis of the fluorescent signals obtained from the target analytes. The fluorescent sensor was characterized and tested with different concentrations of green algae (*Chlorella vulgaris*). For the sample delivery, a microfluidic chip was used. The microfluidic chip consists of a glass slide and a PDMS microfluidic channel with a passive vacuum pump, which delivers a small volume of the sample solution (<10 ul). The fluorescence sensor was calibrated with different concentration of green algal samples and demonstrated its capability of measuring green algal concentrations. The sensor was also tested with green algal samples mixed with different turbidity water to validate its selectivity.

3.2. Design and Characterization of a Portable Fluorescent Sensor

3.2.1 Characteristics of Green Algae and Chlorophyll Fluorescence

Different types of fluorophores have distinctive responsiveness of excitation spectra, therefore characterization of target fluorescent molecule prior to the development of a device is required. In this study, green algae (*Chlorella vulgaris*) were chosen as our target analytes which contain chlorophyll as light harvesting photopigment (or fluorophore). Green algae primarily

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The contents of this chapter are taken from the previously published journal article titled “A portable fluorescent sensor for on-site detection of microalgae,” published in 2014 in *Microelectronic Engineering* [146]. Reprinted by permission of Elsevier.
absorb blue and red wavelength of the light while reflecting 500 nm – 600 nm wavelength of the light, making them appear green color. Although all the pigments in the green algae are responsible for absorbing the light and contributing to the photosynthetic reaction, Chlorophyll $a$ is the only pigment that emits significant fluorescence due to the relatively longer lifetime of electrons [147]. Other accessory pigments such as carotene and chlorophyll $b$, show very weak or no fluorescence emissions since electrons are transferred to chlorophyll $a$ immediately upon generation due to the photon energy [46]. During this process, chlorophyll $a$ fluorescence emitted from the green algae provides valuable information such as ecological state of water and physiological state and population of the species. In Figure 3.1, absorption spectrum for chlorophyll $a$ show that the maximum absorbance of the light occurs between 420 nm – 440 nm wavelength range and the fluorescence emission peak was measured in the 665 nm – 675 nm wavelength range. In order to properly excite the chlorophyll $a$ in the green algae, light source with peak emission spectrum in 430 nm while having narrow bandwidth would be desirable. For the fluorescence detection, photodetector only need to detect and measure the light wavelength of 665 nm or longer should be measured only.
Figure 3.1 Optical property measurement of chlorophyll $a$ in diethyl ether: (a) light absorption and (b) fluorescence emission measurements. Plot generated from the data from [148, 149].
3.2.2 Fluorescence Sensing Configuration

Figure 3.2 illustrates the sensing configuration of the fluorescence sensor device. The system consists of three major functional parts: excitation lights, photodetector, and sample delivery. For the excitation light source, LEDs were used for lower power consumption and miniaturization. Since chlorophyll $a$ mostly absorbs the blue and red spectral ranges [149], Philips Lumileds Rebel color LED (Philips, Netherlands) with 448 nm wavelength was chosen as an excitation light source to stimulate the green algae which emits 680 nm fluorescent light from chlorophyll $a$. For a photodetector, a silicon photodiode (FDS100, Thorlabs, USA) with optical filters was used to detect the fluorescent signal emitted from green algae while blocking the noise. Two optical filters with similar cut-off wavelength were used to improve the signal to noise ratio (SNR). The first optical filter on the top side of the printed circuit board (PCB) where excitation LEDs are mounted is a dichroic filter (PIXELTEQ, USA) that only allows the fluorescent light to pass (674 nm lowpass). The second optical filter below the PCB is a color filter (Edmund Optics, USA) working as low pass filter for 650 nm wavelength fluorescent light. For the sample delivery, green algal sample solution is introduced into a disposable PDMS microfluidic chip placed on top of the PCB board with excitation LEDs.
3.2.3 Optomechanical Design, Optical Filters, and Electronic Circuitry Configuration

The schematic configuration of the sensor system is illustrated in Figure 3.3 (a). The sensor jig was fabricated with a three-dimensional (3-D) printer. Polylactic acid (PLA), a biodegradable thermoplastic material derived from renewable resources, was used as the structural material for 3-D printing. The photodetector and the color filter are mounted in the bottom cover (90 × 50 × 20 mm$^3$) and covered with the PCB. The PCB’s aperture was aligned with photodetector’s window to receive the fluorescent signal from green algal sample. Tray guide and the tray with a PDMS microfluidic chip containing the sample were aligned precisely to have maximum overlapping excitation light projected on the microfluidic chamber and to obtain maximum fluorescent light signal. The top cover is to achieve the fluorescence measurements in the dark environment. The top and the bottom cover block ambient light when fully assembled as shown in Figure 3.2 (a). The actual device is shown in Figure 3.3 (b). The PCB comprises six excitation LEDs with 448 (±10) nm peak wavelength. The aperture in the middle of the PCB allows for the fluorescent light from the microalgae to pass through to the photodetector. A dichroic mirror filter was installed on
the top side of the PCB and a color filter was installed on the bottom side of the PCB to reduce the noise. Thelen and Chu [150] have demonstrated a portable low current sensing circuit design for a fluorescence optical detection. For a fully integrated system, a nanoampere range current meter for the photocurrent detection can be easily implemented and integrated with our proposed device for the portable detection of the green algae.

Figure 3.3 The designed sensor system: (a) an explode and assembled view of the hand-held fluorescent detection system and (b) the 3-D printed sensor housing.

3.2.4 Microfluidic Chip Design for a Passive Pumping Sample Delivery

The microfluidic PDMS chip design is shown in Figure. 3.4 (a). The dimension of the sensing chamber is 5 mm in diameter and 200 µm in thickness. The PDMS chip consists of a vacuum pumping square chamber and the sensing chamber of 10 µl in volume. Since our suggested microfluidic PDMS chip was fabricated with a single SU-8 main mold, the sensing chamber thicknesses of the PDMS chips were supposed to be identical. The thicknesses of ten random PDMS chips have been measured, and the thickness variation was negligible. The vacuum treated PDMS chip pumps up the green algal sample solution into the sensing chamber as shown in Figure. 3.4 (b). The microalgal sample solutions were well shaken to ensure uniform dispersion of
microalgae right before loading into the vacuum treated PDMS chip. Simple and cheap food vacuum sealing system was utilized to demonstrate that our PDMS chip can be vacuum sealed for collecting algal samples on-site.

Figure 3.4 Sample loading microfluidic chip: (a) schematic of the PDMS microfluidic chip and (b) a photograph of the microalgal sample loaded chip.

Figure 3.5 Microfluidic chip was vacuum sealed and ready to be used for on-site detection applications.
3.3. Detection of *Chlorella vulgaris* Using a Fluorescence Sensor

For measuring the fluorescent signal, the PDMS chips containing different microalgal populations were made separately. The microfluidic PDMS chips were produced with SU-8 standard lithography techniques. After carefully attaching PDMS with a glass slide, fabricated microfluidic PDMS chips were vacuum treated to remove gas molecules trapped in the chips. Prepared microalgal sample (*C. vulgaris*) with different populations were injected into the PDMS microfluidic chips immediately after the vacuum treatment. The 100% concentration of microalgal sample contained 19,000 cells/µl measured by flow cytometer (BD Accuri C6). Lower concentration microalgal samples were obtained by diluting the 100% concentration of microalgal sample with culture media. Also, predetermined microalgal sample was mixed with turbid water studying the effects of suspended particle in water. The slide glass with a PDMS microfluidic chip was deployed on the 3D printed tray to insert easily into the sensor system. Six excitation LEDs were connected in series and driven with 50 mA forward current when microfluidic PDMS chip was inserted into the device. The photodiode was reverse biased with −20 V to acquire linear response to the fluorescent input light, and it was connected to a picoammeter (Keithley 6485, USA) to measure and record the photocurrent. The picoammeter collected a total of 300 sampling points with second interval for each microfluidic PDMS chips.

3.3.1 Characterization of the LED and Optical Components

The blue LED from Philips Lumileds was selected due to the relatively cheap price and narrow wavelength range (447 nm ± 10 nm). Moreover, since it is a surface-mountable LED, heat is easily dissipated through the PCB thermal pad, thus experiencing no light intensity drop while in operation. The emission spectrum of the blue LEDs was measured using a portable spectrometer (Ocean Optics, USA). The result shows that the blue LED has its peak at 443 nm wavelength with
13.2 µW/cm² light intensity. Transmittance of a dichroic mirror filter and a color filter was also measured using a spectrophotometer (Hach Company, USA). The dichroic mirror has a cut-off wavelength at 674 nm and the transmittance at 680 nm was 95%. The color filter has a cut-off wavelength at 645 nm and the transmittance at 680 nm was 65%. The color filter is 3 mm in thickness and the dichroic mirror is only 1 mm in thickness showing better transmittance in the bandpass range. The dichroic filter selectively transmits or reflects the light based on the wavelength of the light that is angle-dependent. The incident angle of the reflected excitation light is not always normal and as a result, a small amount of blue light passes through the dichroic filter without being reflected. On the other hand, the color filter is less sensitive to the incident light angle, but due to the dye material on the glass, it shows autofluorescence contributing to background noise. Therefore, a two-filter system can minimize the background noise condition during the operation (see Figure. 3.6).

Figure 3.6 Measured spectra of the excitation LED and the optical filters used in the fluorescent detection system.
3.3.2 Characterization of a Portable Fluorescent Sensor Using *Chlorella vulgaris*

We analyzed the performance of our fluorescent sensor by measuring the microalgal cell concentration. To test the microalgae fluorescence, six different concentrations were made with different ratios of the stock microalgal sample (*C. vulgaris*) with a cell concentration of 38,000 cells/ll read from a BD Accuri C6 Flow Cytometer. The test for microalgae fluorescence with turbid water will add an extra dilution; therefore the microalgae only solutions were prepared to keep the same cell concentration as the samples mixed with turbid water. The following is the ratio of microalgae and water (algae volume:water volume) along with the approximate cell concentration (cells/ll): 1:9 (3800 cells/ll), 1:4 (7600 cells/ll), 3:7 (11,400 cells/ll), 2:3 (15,200 cells/ll), 1:1 (19,000 cells/ll). The microfluidic chips with microalgal sample solutions were kept in the dark for more than 20 min before the test as a means to maximally oxidize the primary quinone electron acceptor of Photosystem II and open Photosystem II reaction center [150]. Then, the microfluidic chip was loaded into the sensor and the excitation light was turned on. Figure 3.7 shows a microscope pictures of the green algae cells tested. Figure 3.8 (a) shows that the excitation light creates background noise signal due to the light leakage. Some reflected LED light rays penetrate the optical filters even though most of them were filtered out. However, we also confirmed that the excitation light intensity was stable and not degraded over the duration of measurement. Since the LED light does not require a time for stabilization, it can reduce the power consumption and preparation time before the measurement. The photocurrent signal data was recorded and saved with a 6485 picoammeter (Keithley, USA). Figure 3.8 (b) shows that fluorescent light intensity from a microalgal sample (19,000 cells/ll) decreases over time due to the photochemical quenching effect [151]. When the excitation light was on, the photocurrent quenching occurred for 20 s and the signal was stabilized. Microfluidic chips for testing microalgal
samples at varying concentrations were prepared and corresponding stabilized photocurrent values were correlated with the cell count values obtained from the BD Accuri C6 Flow Cytometer.

Figure 3.7 Microscopic image of C. vulgaris (microalgae or green algae) sample. Therefore, the LED was allowed to run for 120 s and photocurrent was measured every 1 s interval.
Figure 3.8 Measured photocurrent values: (a) background noise with a water only sample due to the leakage of excitation light and (b) fluorescent light signal over time with a microalgal sample (19,000 cell/µl). The fluorescent light intensity decreases over time due to the quenching effect.

### 3.3.3 Chlorophyll *a* Fluorescence vs. *Chlorella vulgaris* Concentration

Figure 3.9 is the test result of the fluorescent sensor with different microalgae concentration measurements. The result shows that fluorescent sensor has a linear response compared to the flow cytometer cell counting measurement. The critical issue is the excessive noise from the light
source. The highest concentration measurement data of the developed sensor was found to be 19,000 cells/ll according to our experimental results, which is higher than the harvesting concentration of the microalgal products from the HISTAR system (15,000 cells/ll) [152, 153].

Figure 3.9 Fluorescent photocurrent vs. microalgae concentration. The error-bars indicate one standard deviation with a sample number of n =3.

### 3.3.4 Fluorescence Signal Integrity of Turbid Microalgal Sample

The culture media of outdoor raceway cultivation system for microalgae may include various suspended particles such as dirt. Moreover, lake and pond water will contain suspended particles with microalgae. The suspended particles in turbid water are one of the possible error sources for the microalgal sensors [154]. The fluorescent signal from microalgal cell can be attenuated by the scattering effect.

To investigate the influence of turbid water mixed with a microalgal sample solution, different mixtures of turbid water and microalgal sample were prepared. Six stock cultures of turbid water at different turbidity levels were prepared and measured using a 2100P ISO HACH Turbidimeter and BD Accuri C6 Flow Cytometer. The soil used to make turbid water is similar in
size and properties as that found in samples from unlined algal ponds in Louisiana (Table 3.1). Figure 3.10 shows water sample at the different turbidity levels, from 0 to 157 NTU or nephelometric turbidity units.

Table 3.1 Soil composition of the turbid water

<table>
<thead>
<tr>
<th>Property</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand content</td>
<td>%</td>
<td>73.2</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
<td>16.3</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>10.5</td>
</tr>
<tr>
<td>Median grain size</td>
<td>mm</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Figure 3.10 Image of different turbidity level of mixed water.

Table 3.2 shows the test result of the fluorescent sensor with different microalgae concentration mixed with turbid water. The result confirms that the fluorescent sensor has a linear response compared to the flow cytometer cell counting measurement regardless of the turbidity level of the mixed water.
Table 3.2 also validates that the turbidity level of the water mixed with microalgae sample was not affected by the photocurrent signal obtained from the photodetector. This experimental result demonstrates that the fluorescent sensor has an advantage over turbidity sensor in terms of selectivity, especially in detecting microalgal sample with contaminants.

Table 3.2 Photocurrent measurement results of the microalgal samples mixed with different turbidity water. Photocurrent values of the microalgal sample with same cell count are constant regardless of the turbidity level of mixed water.

<table>
<thead>
<tr>
<th>Measured photocurrent [nA]</th>
<th>Turbidity of the mixed water NTU</th>
<th>0</th>
<th>23.5</th>
<th>48.2</th>
<th>73.1</th>
<th>109</th>
<th>121</th>
<th>157</th>
<th>Avg.</th>
<th>SD (σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cells/µl</td>
<td>15.7</td>
<td>15.7</td>
<td>15.9</td>
<td>15.9</td>
<td>16.3</td>
<td>16.0</td>
<td>15.7</td>
<td>15.9</td>
<td>0.206</td>
<td></td>
</tr>
<tr>
<td>3,800 cells/µl</td>
<td>16.6</td>
<td>17.4</td>
<td>16.9</td>
<td>17.2</td>
<td>17.2</td>
<td>17.7</td>
<td>16.1</td>
<td>17.0</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td>11,400 cells/µl</td>
<td>18.8</td>
<td>19.2</td>
<td>19.1</td>
<td>18.8</td>
<td>18.6</td>
<td>18.8</td>
<td>19.0</td>
<td>18.9</td>
<td>0.215</td>
<td></td>
</tr>
<tr>
<td>19,000 cells/µl</td>
<td>22.8</td>
<td>21.5</td>
<td>23.6</td>
<td>22.0</td>
<td>21.1</td>
<td>22.5</td>
<td>22.4</td>
<td>22.3</td>
<td>0.826</td>
<td></td>
</tr>
</tbody>
</table>

3.4. Conclusions

In conclusion, a portable and low-cost fluorescent sensor was developed for on-site detection of microalgae with a disposable PDMS microfluidic chip. A 448 nm wavelength LED was selected to excite the microalgae that emit 680 nm wavelength fluorescent light. A photodiode with a 645 nm long-pass optical color filter and a dichroic mirror was mounted below the PDMS microfluidic chamber to detect the fluorescent light signal from the samples. The experimental results have confirmed that the fluorescent sensor has a linear response comparable to the flow cytometry cell counting measurement. Selective microalgae detection to a turbid sample was also demonstrated. The fluorescent signal was independent of the turbidity level of the sample and the result from microalgal concentration measurements in different levels of turbid samples showed a linear response comparable to the cell counting measurement as well. In the following chapter,
improved second prototype that incorporates the electronic circuit system to enable the on-site detection.


4. Multi-Phytoplankton Detection with a Hand-Held LED-Based Fluorescent Sensor

4.1. Introduction

From the previous chapter, it has been demonstrated that the preliminary design of a portable fluorescence sensor was capable of measuring green algal samples with various concentrations and even in a turbid water. In this chapter, there are two main goals for a newly suggested design of a fluorescence sensor platform. Firstly, an improved fluorescence sensor platform should be able to simultaneously detect multiple analytes using different excitation lights when more than one kind of analytes coexist. Lastly, a fluorescence sensor platform should be field-deployable. A portable system offers many advantages especially for the environmental monitoring applications where early warning is critical such as microbiological hazards or harmful algal bloom (HAB) [155, 156]. To demonstrate the ability of the sensor system to selectively detect multiple analytes, a mixture of two phytoplankton species is utilized in this study: green algae (Chlorella vulgaris) and cyanobacteria (Spirulina). The contents of this chapter are taken from the previously published journal articles titled “A hand-held fluorescent sensor platform for selectively estimating green algae and cyanobacteria biomass” published in 2018 in Sensors and Actuators B: Chemical [157] and “A field-deployable and hand-held fluorometer for environmental water quality monitoring” published in 2018 in Micro and Nano Systems Letters [158].

4.2. Design of a Hand-Held LED-Based Fluorescent Sensor

4.2.1 Characterization of Different Algal Species: Green Algae and Cyanobacteria

In order to detect and distinguish two different algal species simultaneously, determination of absorption and emission spectra of green algae and cyanobacteria is required. Figure 4.1 shows the absorption and emission spectra of different photopigment components and the spectral power distribution of the three excitation LEDs used in our system. Green algae contain chlorophyll $a$ and $b$ photopigments, which are mainly stimulated by both blue (448 nm) and UV (385 nm) excitations, but minimally stimulated by amber (590 nm) excitation. Cyanobacteria contain phycocyanin as the main light harvesting photopigment, which is mainly stimulated by amber excitation. Simultaneously detecting and differentiating green algae and cyanobacteria is a challenging task because the photopigments in both species are stimulated at the same excitation wavelength. For example, green algae and cyanobacteria both have chlorophyll $a$ that fluoresces under the blue light excitation, therefore, contains the signals emitted from both species, as illustrated in Figure 4.2.
Figure 4.1 Normalized spectral distributions of three LEDs used for excitation and absorption/emission spectra of pigments in green algae and cyanobacteria.

Figure 4.2 Different fluorescence intensity levels under the blue light illumination for chlorophyll $a$, $b$, and phycocyanin pigments.
4.2.2 Excitation-Emission Matrix (EEM) Analysis

The photopigment components for green algae and cyanobacteria and their absorption/emission spectra are studied in 4.2. Prior to selecting excitation LEDs for selective stimulation and optical filters to measure the corresponding fluorescence emission from the samples, full range of excitation and emission spectral scanning is required for an accurate characterization. Figure 4.3 shows the normalized EEMs of chlorophyll a and C-phycocyanin pigments measured with a spectrofluorometer (Fluorolog3, Horiba, Japan). The pseudo-colored (red being highest and blue being lowest) fluorescence intensity map is produced by scanning fluorescence signal over a range of excitation (350 nm - 600 nm) and emission wavelength (400 nm - 700 nm). The plot shows that chlorophyll a absorbs the light most strongly in the blue region (360 nm - 440 nm) and fluoresces in the red region (660 nm - 680 nm) while phycocyanin absorbs the light most strongly in the amber region (550 nm - 660 nm) and fluoresces in the red region (630 nm - 650 nm). Note that chlorophyll a and phycocyanin also slightly absorb the light in the red and ultraviolet region, respectively. Figure 4.4 shows that the EEM patterns of green algae and cyanobacteria agree well with that of chlorophyll a and phycocyanin, respectively. Green algae show relatively stronger fluorescence compared to that of chlorophyll a under the UV LED stimulation because the green algae contain extra pigments such as chlorophyll b, beta-carotene, and xanthophylls.
4.2.3 Detection Principle of Muti-Excitation LED-Based Fluorescent System

A schematic illustration of the proposed LED-based fluorescent sensor platform is shown in Figure 4.5. Color LEDs (Lumileds, CA, USA) with 448 nm (blue) and 590 nm (amber) wavelengths are used to excite the photosynthetic pigments. The blue LED stimulates chlorophyll a and b photopigments in green algae and emits peak fluorescent light at 680 nm, while the amber LED stimulates phycocyanin photopigment in cyanobacteria and emits peak fluorescent light at 645 nm. An ultraviolet (UV) LED with wavelength 385 nm (Vishay Semiconductor, PA, USA) is
employed to stimulate both chlorophyll a and phycocyanin for the total phytoplankton measurement. A dichroic mirror (PIXELTEQ, FL, USA) and a color filter (Edmund Optics, NJ, USA) are placed to block the excitation light while selectively allowing the fluorescent signals to pass through it. The corresponding fluorescent emission from each species is collected with a highly sensitive silicon photomultiplier (SiPM) (SensL, Cork, Ireland). An aliquot volume of the sample solution can be easily delivered to the system with a disposable glass micro-vial (0.9 ml, Specialty Bottle, USA).

Figure 4.5 Working principle of the proposed LED-based fluorescent detection method.

4.2.4 Optomechanical Design, Optical Filters, and Electronic Circuitry Configuration

The portable fluorescent sensor platform, shown in Figure 4.6. (a), consists of an electronic circuitry, an optomechanical guide, excitation LEDs, an LCD module, a replaceable 9-V battery, and a 3D-printed housing made of a durable plastic material, acrylonitrile butadiene styrene (ABS). Three different wavelengths of surface-mountable LEDs were covered with a 3D-printed optomechanical guide to decrease the reflection noise. The aperture on the PCB, 3mm in diameter,
was aligned to the central hole of the optomechanical guide and the photodetector window to maximize the fluorescent signal reading. The color filter and the dichroic filter were positioned between the PCB aperture and photodetector window. The excitation LEDs and the sensing operation were controlled by a custom designed circuitry. The LCD module with selection buttons displays the measurement results and allows the user to navigate through different menu functions, such as sensor calibration, display of measured results, or loading from the saved data. To easily inject and deliver the sample to the system, a glass micro-vial with a 3D-printed cap blocking ambient light was used (Figure 4.6. (b)). The top cover was designed to readily accommodate a glass micro-vial and block ambient light when closed.
The sensor system has three major functional circuitries as shown in Figure 4.7 (a): an LED-driving circuit, a temperature compensation circuit for the photodetector, and a signal amplification circuit. The LED-driving circuit generates a configurable switching current to control the intensity of each LED while sequentially operating three different wavelengths of excitation LEDs. Each LED set having two identical LEDs coupled in series was turned on for 5 ms and subsequently turned off for 5 ms, and the corresponding fluorescent signals were measured using a photodetector, as illustrated in Figure 4.7 (b).

Figure 4.6 Photograph and schematics of the fabricated portable fluorescent sensor platform: (a) images of the 3D-printed algae fluorescent sensor with an exploded view of the fluorescent detection module and (b) a glass micro-vial for sample loading.

The photodetector (SiPM) was biased at −29.5 V for maximum photon sensitivity at room temperature. As the amplification gain of the photodetector varied with both reverse bias and temperature, a high-precision temperature monitoring module was implemented for active gain feedback to maintain a stable gain. The photocurrent collected from the fluorescent signals was amplified with a transimpedance circuit and digitized by the microprocessor at a sampling rate of 2 kHz. The fluorescent readout data were simultaneously displayed on the LCD screen and saved to the on-board storage module. A block diagram of the system functions is illustrated in Figure 4.7 (c).
Figure 4.7 Electronic circuit design: (a) image of the designed electronic circuit; (b) data acquisition process synchronized with an LED control signal, and (c) block diagram of the circuitry functions.

4.3. Detection of Green Algae and Cyanobacteria Fluorescence using Hand-Held LED-Based Fluorescent Sensor

For measuring fluorescent signals, glass micro-vials were loaded with different concentrations of green algae, cyanobacteria, and mixed samples. The biomass of Chlorella vulgaris (500 mg/l) and Spirulina (500 mg/l) samples was initially measured with the dry weight method. Low biomass concentrations of green algae and cyanobacteria samples were obtained by serially diluting the highest biomass sample stocks.
Experiments were carried out with three replicates of micro-vials for each test. The prepared sample solutions were well agitated using a shaker (Vortex Genie 2, Fisher, USA) before loading them into the micro-vials. After loading the sample solution, the micro-vial was inserted into the sensor system and the cap was affixed to block ambient light. The LEDs were set to emit light intensity of 0.6 µE m\(^{-2}\) s\(^{-1}\) (or 122 mWm\(^{-2}\)), 0.5 µE m\(^{-2}\) s\(^{-1}\) (or 109 mWm\(^{-2}\)), and 0.35 µE m\(^{-2}\) s\(^{-1}\) (or 134 mWm\(^{-2}\)) for the amber, UV, and blue excitation LEDs, respectively.

### 4.3.1 Characterization of Green Algae and Cyanobacteria Fluorescence using a Hand-Held LED-Based Fluorescent Sensor

Figure 4.8 depicts the fluorescent signal patterns measured with the single species of green algae and cyanobacteria samples (both at a 500 mg/l biomass concentration). The blue line represents the base noise level measured from each excitation LED when tested with a control sample (distilled water). The green line denotes the fluorescent signal pattern when tested with green algae. The green algae signal shows that the blue light excitation induced the highest magnitude of the relative fluorescence value, whereas the amber light excitation induced the lowest fluorescence. This reveals that the blue and UV LEDs successfully stimulated the chlorophyll a and b components in green algae.

For the cyanobacteria sample, the highest fluorescence response was observed from the amber light excitation in the same graph. The lowest response was from the blue light excitation, which is denoted by orange lines. Thus, the phycocyanin component in the cyanobacteria was successfully stimulated by the amber LED, while chlorophyll a stimulation was minimal as discussed in [159]. Moreover, measured fluorescence patterns were used to identify photopigment components of each sample. The obtained unique pattern was also used as a fingerprint for classifying the different phytoplankton groups (green algae and cyanobacteria). Fluorescent light intensity of algae decreases under the excitation light over time due to the photochemical
quenching effect, which we also observed in our previous work [75]. In order to prevent the cell damage due to the photo bleaching effect on the photopigments, the excitation light intensity of each LED was carefully controlled and maintained at a relatively low level not to damage the cells during experiments. Figure 4.8 also shows that the measured fluorescent signals are stable.

Figure 4.8 Different patterns of fluorescence response from green algae and cyanobacteria.

### 4.3.2 Calibration of Green Algae and Cyanobacteria Biomass

A single analyte sample was tested to obtain its calibration curve by plotting the fluorescence emission as a function of the biomass concentration for all three excitation lights. The biomass samples of green algae and cyanobacteria were diluted with distilled water to prepare a series of varying concentration samples. For each analyte, seven different concentrations were prepared and tested: 25, 50, 100, 200, 250, 300, 400, and 500 mg/l. Three replicate measurements were performed for each test, and 100 µl volume of the sample solution was loaded into a micro-vial.
Figure 4.9 presents the fluorescence measurement results with different green algae and cyanobacteria concentrations. For the green algae samples, the photocurrent increase was higher with the increasing concentration of the sample under the blue and UV LED lights. Conversely, its change was relatively low under the amber LED light. For the cyanobacteria samples, the photocurrent increase was higher with the increasing concentration of the sample under the amber LED light, while its change was relatively low under the blue and UV LED lights. These measurement results agree well with the fluorescent characteristics of both samples shown in Figure 4.8. The lower limit of detection of the developed sensor for the green algae and cyanobacteria were found to be 1 mg/l and 4 mg/l, respectively, according to our experimental results.

![Graphs showing fluorescence measurements](image)

Figure 4.9 Measured fluorescence emission from different concentrations of (a) green algae and (b) cyanobacteria using three different excitation LEDs. The error-bars represent the standard deviation of a data set (n = 3).
4.4. Identification and classification of Green Algae and Cyanobacteria using Multivariate Analysis

As mentioned in 4.2, simultaneously detecting and differentiating green algae and cyanobacteria is challenging due to the interference of different fluorescence emission from multiple photopigments. The result of a recent study suggested that the phytoplankton fluorescence conjunction with multivariate analysis is highly effective in predicting chlorophyll a concentration from mixed species samples [160]. Therefore, applying a multivariate algorithm should provide an accurate prediction of the sample biomass regardless of any unknown effects.

The collected corresponding fluorescent signals are analyzed by the multivariate calibration algorithm for differentiation and quantification of each phytoplankton species. Multivariate calibration is a widely used spectroscopic analysis method for estimating the constituent concentrations of analytes when the signals from multiple analytes overlap [26]. Amongst multivariate analytic methods, including classical least squares (CLS) and principal component regression (PCR), the partial least squares regression (PLSR) method reportedly provides the most accurate biomass prediction estimate for mixed phytoplankton species [27–29]. In this study, we leverage the PLSR algorithm to selectively estimate the biomass of each species using a training sample dataset of fluorescence responses obtained from single and mixed phytoplankton species.

To apply the PLSR algorithm, a training dataset was collected by measuring the fluorescence from each species and mixed samples of known concentrations. PLSR is a multivariate regression method that correlates one data matrix (fluorescent signal variables, X) to another matrix (biomass variables, Y). The PLSR algorithm builds a predictive model using correlations between the sample biomass values and the fluorescence signal data. To this end, it decomposes both biomass and fluorescence signal data into eigenvector and score components to
identify the most relevant eigenvectors for predicting the biomass of the samples. Four different concentrations of green algae and cyanobacteria sample mixtures were tested in order to validate our predictive model.

The fluorescence signals obtained from the mixture of the two species were also obtained using the sample mixtures listed in Table 4.1. The acquired data were combined with the results shown in Figure 4.9 from single analyte samples to plot all three corresponding fluorescent patterns in 3D space, as illustrated in Figure 4.10. Increasing concentrations of green algae show ascending patterns of blue and UV fluorescence while increasing concentrations of cyanobacteria show a corresponding escalation in amber fluorescence for both the single and the mixed analyte samples.

Table 4.1 Dataset tested for the mixture of two species

<table>
<thead>
<tr>
<th>Co-culture sample no.</th>
<th>Biomass (mg/l)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Green algae</td>
<td>Cyanobacteria</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>250 (fixed)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>250 (fixed)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>250 (fixed)</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>250 (fixed)</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>250 (fixed)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>250 (fixed)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>250 (fixed)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>250</td>
<td>250 (fixed)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.10 3D plot of the measured fluorescent emission from the green algae and cyanobacteria samples for the three different excitation LEDs.

4.4.1 Principle Component Analysis (PCA)

To simplify the graph, the principal component analysis (PCA) algorithm was employed for easy visualization of the raw data mapped onto the principal component (PC) vector space. The PCs were defined as vectors that best describe a dataset while being represented in lower dimensional space. The PCA algorithm is often used to classify multiple analytes to minimize the redundancy of the raw dataset by linearly reducing the dimensionality \[161\]. Two new coordinates that explain the variation in the dataset were constructed for the first PC (PC1) representing the direction of the largest variation in fluorescent signal and the second PC (PC2) correspondingly representing the second largest variation. A dataset matrix of the fluorescence measurement was mapped onto a new two-dimensional score plot space via two base vectors, PC1 (accounts variance \(= 77.63\%\)) and PC2 (accounts variance \(= 21.93\%\)), as shown in Figure 4.11 (total accounts variance
The plot shows that the two PCs are appropriate for separating the sample groups with similar species while describing most of the information from the dataset. Figure 4.9 further explains the correlation between the variables. As can be seen, PC1 is more relevant for explaining the blue and UV fluorescence variables, while PC2 is more relevant for explaining the amber fluorescence variable. Furthermore, a positive correlation was obtained between the blue and UV fluorescence variables. It is apparent that PCA is an effective qualitative representation method for visualizing different groups of clustered data.

Figure 4.11 2D mapping of fluorescence data measured from the single and the mixed analytes with loadings (PCA bi-plot). The data label beside each point represents the sample species and concentration (mg/l). Al, Cy, and Al/Cy indicate green algae, cyanobacteria, and algae/cyanobacteria mixed sample, respectively.

4.4.2 Partial Least Squares Regression (PLSR)

To quantitatively estimate the biomass of the two analytes, the PLSR method was employed for the 23 datasets (fluorescence measurements of 15 single species and eight mixtures
of species) measured from three dependent variables (LEDs). The PLSR algorithm extracted the set of components (or latent variables) that accounted for the greatest variation possible in the data while maximizing the covariance of data matrices, $X$ (fluorescence measurements) and $Y$ (biomass of samples). The component vectors obtained from $X$ were then used in the regression step to predict $Y$.

To maximize the accuracy of the predictive model, it is essential to select the optimum numbers of components. This was achieved by evaluating the root mean square error of the prediction (RMSEP) value with different numbers of components. The result is the standard deviation of differences between the predicted and referenced data. The RMSEP is defined as,

$$\text{RMSEP} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (y_i - y'_i)^2},$$

where $N$ is the number of samples, $y$ is the true biomass, and $y'$ is the predicted biomass.

The RMSEP unit is same as that of our prediction values (mg/l). The RMSEP values for both green algae and cyanobacteria changed in accordance with the number of components included for each constructed predictive model. The optimal numbers of components were determined by selecting the minimum RMSEP value for each species. Table 4.2 shows that the RMSEP value was the lowest with the model of three components for both the green algae and cyanobacteria biomass, indicating that choosing three components gives the lowest prediction error although choosing two components still provides reasonable results.

The dataset matrix was plotted on a new two-dimensional space based on first two components, as shown in Figure 4.12. Component 1 accounted for 61% variation in $X$ and 60% variation in $Y$, while component 2 accounted for 38% variation in $X$ and 39% variation in $Y$. 

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Unlike PCA, the components were selected to maximize the correlation between $X$ and $Y$ in such a manner that $Y$ can be accurately predicted. The PLSR bi-plot shows that the direction of the main variance is different from that of PCA, although the distributions of the dataset are virtually identical in both plots. The blue and UV variables were strongly correlated with the green algae biomass variable, while the amber variable is highly correlated with the cyanobacteria biomass variable. In other words, the fluorescence signals from the blue and UV LEDs were effective in predicting the green algae biomass while fluorescence signals from the amber LED were effective in predicting the cyanobacteria biomass. Thus, when equal amounts of green algae and cyanobacteria are mixed (Al250/Cy250), the direction of the sample vector is centered between the amber and the blue variables.

Table 4.2 Variance of $X$, $Y$ and RMSEP described by the components.

<table>
<thead>
<tr>
<th>Number of components</th>
<th>Percentage of described variance for $X$</th>
<th>Percentage of described variance for $Y_1$</th>
<th>RMSEP of $Y_1$ (mg/l)</th>
<th>Percentage of described variance for $Y_2$</th>
<th>RMSEP of $Y_2$ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>153</td>
<td>0</td>
<td>146</td>
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<td>32</td>
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<td>100</td>
<td>99</td>
<td>18</td>
<td>99</td>
<td>17</td>
</tr>
</tbody>
</table>
Figure 4.12 2D mapping of fluorescence data measured from the single and the mixed analytes with loadings of $X$ and $Y$ (PLSR bi-plot). Red dotted lines indicate $X$ loadings and green dotted lines indicate $Y$ loadings. The data label beside each point represents the sample species and concentration (mg/l). Al, Cy, and Al/Cy indicate green algae, cyanobacteria, and algae/cyanobacteria mixed sample, respectively.

4.4.3 Classification of Green Algae and Cyanobacteria

To evaluate the developed green algae and cyanobacteria biomass predictive model with three components, plots comparing the predicted and the reference biomass were obtained, as shown in Figure 4.13. The squares of the correlations for the green algae and cyanobacteria cases were 0.9862 and 0.9794, respectively, indicating that most of the variations in our data were captured by the components in our model. To further validate our model, four different mixtures of green algae and cyanobacteria samples were prepared and blindly tested. The corresponding fluorescent signal data were used to predict the biomass of the mixed species. The mixture concentration for the test and the predicted results are listed in Table 4.3.

The predicted values show good matching results with an error rate in the range 2–16% of the biomass. The prediction accuracy of PLSR modeling can be improved even more by decreasing...
the noise. The sources of noise can be device artifacts, measurement errors, and including predictor variables (X) that do not explain the prediction values (Y). Moreover, including more independent variables that are highly correlated with prediction values will improve the prediction accuracy. In our model, the UV LED is strongly correlated with the blue LED in explaining the green algae biomass, for which prediction performance was marginally better than that for the two variables (amber and blue LEDs) PLSR model. However, UV LED would be highly useful for differentiating other analytes, especially when it is used in an outdoor environment, such as a lake or a pond, where a CDOM is present [162].

Figure 4.13 Predicted vs. reference biomass (mg/l) for (a) green algae and (b) cyanobacteria.

Table 4.3 Various mixtures of green algae (Y_1) and cyanobacteria (Y_2) samples and estimated biomass.

| Test sample no. | Tested biomass [mg/l] | Predicted biomass [mg/l] | Error [%] | Actual deviation [mg/l] (|Test – Predicted|) |
|-----------------|-----------------------|--------------------------|-----------|--------------------------|
|                 | Y_1 | Y_2 | Y_1 | Y_2 | Y_1 | Y_2 | Y_1 | Y_2 |
| 1               | 250 | 125 | 238 | 128 | 15 | 2 | 38 | 3 |
| 2               | 250 | 200 | 246 | 227 | 2 | 14 | 4 | 27 |
| 3               | 125 | 250 | 118 | 289 | 6 | 16 | 7 | 39 |
| 4               | 200 | 250 | 186 | 269 | 7 | 8 | 14 | 19 |
4.5. Conclusions

In this chapter, a hand-held fluorescence sensing platform was developed and demonstrated for selective and quantitative detection of multiple analytes. To demonstrate the efficacy of the developed system, it was used to quantify and differentiate two types of phytoplankton species: green algae and cyanobacteria. The green algae were predominantly stimulated by blue and UV LED lights owing to the presence of chlorophyll \(a\) and \(b\), while the phycocyanin in the cyanobacteria was mainly stimulated by amber LED light. Each LED was sequentially turned on and off using a microcontroller and the corresponding unique patterns of fluorescent signals measured using a highly sensitive photodetector (SiPM) with a long-pass filter. PCA algorithm was used to visualize the clusters of the different sample concentrations. Furthermore, the partial least squares regression (PLSR) algorithm was used to build a predictive model for estimating the biomass of each sample. The results indicated that the use of different excitation lights was effective in selectively stimulating the target photopigments. Moreover, the application of the PLSR algorithm effectively differentiated and quantified the two algal species. Thus, it is clear that the developed fluorescent sensor system could simultaneously detect multiple analytes.

Future improvements to the system presented in this chapter will include integration of additional excitation LEDs with various wavelengths for broader spectral stimulation, which will increase the number of detectable analytes of the system. In addition, compact spectrometer module will be embedded to the system instead of optical filters to embed emission wavelength scanning capability. Furthermore, several useful functions that no other portable fluorescence system offered will be included, such as temperature controlling and monitoring of solution, and solution mixing capabilities for improve the accuracy for the fluorescence sensing. This will offer
new portable fluorometer platform that can be utilized not only for the phytoplankton detection, but also many different applications.
5. Development of a Portable Fluorometer with Temperature Controlling System

5.1. Introduction

From the previous chapter, a hand-held fluorescence sensing platform using multiple LED excitation light was developed and demonstrated for selective and quantitative detection of different species of algal samples, green algae (C. vulgaris) and cyanobacteria (Spirulina). In this chapter, a new design of a portable fluorometer system was suggested to expand the multi-analyte detection capability and increase the practicality of the system for on-site detection applications. The newly suggested design is an extension of the work from the previous two chapters by presenting four major improvements. First, an increased number of excitation LEDs are implemented to offer broader excitation wavelength information. Previously, the number of excitation wavelengths was three, but the new device delivers thirteen different wavelengths of LEDs, spanning from 280 nm to 725 nm. Secondly, absorbances of the samples will be measured simultaneously along with the fluorescence measurements. Absorbance measurement of a sample provides a molar absorptivity which is an important parameter in classifying and distinguishing the chemical composition and material’s structure. Thirdly, a monochromator was developed to separate the color elements of the fluorescent emission and selectively detect the wavelength of interest. The previous system provided a simple and inexpensive solution by using an optical filter-based configuration. However, the use of this system has been limited because it can only be used in applications where the fluorescence emission is longer than 645 nm. The newly developed monochromator can sequentially scan a range of wavelength (400 nm – 780 nm) with 0.5 nm resolution to offer details of fluorescent emission profile. Lastly, a copper-based cuvette holder.

The contents of this chapter are partially taken from the previously published journal articles titled “A thermoelectric temperature control module for a portable fluorescent sensing platform” published in 2020 in Journal of The Electrochemical Society [163]. ©The Electrochemical Society. Reproduced with permission. All rights reserved.

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will offer temperature controllability of the sample solution for a stable reading of fluorescence emission. The temperature of the solution will be maintained at our desired value in the range from 20 °C to 40 °C (with +/- 1 °C tolerance). The fluorometer is calibrated and tested with different types of fluorescent dyes (rhodamine B and fluorescein) to verify its capacity of distinguishing different dyes of our interest while maintaining a stable reading of fluorescent signals. Design of a portable fluorometer with temperature controlling system

5.1.1 Characteristics of Fluorescent Dyes: Rhodamine B and Fluorescein

Fluorescent dyes are widely used in bioscience, especially for cell staining, water tracing, fluorescent microscopy, and diagnostics [164]. Different types of fluorescent dyes have unique absorption and emission spectra characteristics. In this study, rhodamine B and fluorescein (MilliporeSigma, USA) were selected as our target analytes to measure the fluorescence signals and validate the temperature controlling capability of the system. Normalized absorption and emission spectra of rhodamine B are shown in Figure 5.1. The maximum absorbance wavelength is 545 nm and the peak fluorescence emission is 565 nm when dissolved in ethanol. The peak of absorption and emission may shift when dissolved in a different solvent. Since excitation and emission peaks are only 20 nm apart in bandwidth, 535 nm excitation LED with 5 nm FWHM was selected to achieve maximum excitation efficiency while not interfering with the fluorescent emission peak of rhodamine B. Normalized absorption and emission spectra of fluorescein are shown in Figure 5.2. The maximum absorbance wavelength is 485 nm and the peak fluorescence emission was 514 nm when dissolved in ethanol. The peak of absorption and emission may shift when dissolved in a different solvent. For an excitation source, 465 nm LED with 5 nm FWHM was selected to achieve maximum excitation efficiency while minimally interfering with the fluorescent emission peak of fluorescein.
Figure 5.1 Normalized absorption and emission spectra of rhodamine B.

Figure 5.2 Normalized absorption and emission spectra of fluorescein.
5.1.2 Detection Principle of a Portable Fluorometer

A portable fluorometer is composed of four main functional parts: a light source module, an absorbance measurement module, a fluorescent detection module, and a peripheral module that controls the motors, Peltier’s, and fans. Figure 5.3 illustrates the overall device configuration of the portable fluorometer system. For the excitation light source module, 13 different LED/LD were used to offer a broad range of excitation wavelengths, ranging from 280 nm to 725 nm (280, 367, 410, 450, 470, 500, 530, 550, 570, 593, 629, 660, and 725 nm). Each LED was coupled with a color filter (Edmund Optics, USA) and a collimator lens to carry out a narrow excitation spectrum (FWHM < 10 nm). The excitation light sources were positioned in circular arrangements on the circuit board and the emission light ray of each LED was collimated toward the center of the circuit board. Subsequently, an off-axis parabolic mirror was placed in the center of the circuit board to redirect the light towards the sample solution through the aperture in the middle of the board (2 cm in diameter). The rotational movement of the mirror was controlled by a gear motor and a magnetic encoder. The magnetic encoder consisted of a hall effect sensor (TLE4946-2K, Infineon, Germany) and a magnetic disk with 4 different magnetic poles. Magnetic disk was connected to the back shaft of the motor and the hall effect sensor was placed close to the magnetic disk to monitor the magnetic pole changes of the disk. This allowed us to control the angular movement of the mirror with 0.2° step resolution. The maximum intensity of each light source was achieved at an angle perpendicular to the mirror and excitation light source.

For the absorbance measurement, an adjustable aperture was placed right behind the mirror to optimize the beam width and reduce the stray noise. Then, the beam was split into two beams (7:3 optical power ratio) by a beam splitter. One of the beams with higher optical power (70%) was guided towards the quartz cuvette where sample solution was contained and the attenuated
light due to the molecular absorption will be measured with a silicon photodiode. Another split beam with lower optical power (30%) was guided directly to the reference silicon photodiode which is identical to the one used for the absorbance measurement. A reference silicon photodiode was implemented to monitor any intensity changes of the excitation lights to cancel the DC offset, drift, and thermal noise errors of the measurement.

For the fluorescence measurement, the sample contained in the cuvette was first illuminated with the excitation light. During this process, the absorbance of the sample solution is being measured simultaneously. The fluorescent light emitted from the sample solution was collected with a monochromator module. A monochromator is an optical instrument that separates the multiwavelength light into individual components (or wavelengths) of the light. Monochromators can be categorized into different types depending on the type of optical components and their configurations used in the system. One of the classical types of the monochromator is the Czerny-Turner as illustrated in Figure 5.3 (b). First, the incident light is focused onto the input slit by a mirror. The input slit has 50 µm in width and 3 mm in length. This light is polychromatic, which is mixed with fluorescent and excitation lights. After passing through the input slit, the light is being collimated by another focusing mirror and the direction of the light was guided towards the reflective diffraction grating. Diffraction grating deflects different wavelength elements in different directions and the dispersion angle is directly proportional to the number of grooves per length of the grating. Therefore, it is a key component of determining the resolution of the spectrometer. A diffraction grating with a high groove density (3400 grooves/mm) was selected for high resolution for a portable spectrometer system with 400 – 780 nm scanning range. The diffracted light rays were guided to the second focusing mirror and focused onto an exit slit. The exit slit has the same dimension as the input slit to allow the 0.5 nm
region of the light can get through the slit. This monochromatic light is being measured by a highly sensitive silicon photomultiplier (SIPM) (On Semiconductor, USA) which is placed behind the exit slit. By controlling the rotational angle of the diffraction grating, the direction of the dispersed light can be controlled as well, therefore any monochromatic light of interest can be selected to pass through the exit slit. Since fluorescence emission efficiency can be readily affected by several parameters such as pH, polarity of the solvent, and temperature, it is essential to stabilize those parameters to achieve a stable reading of fluorescence [165]. In our system, the temperature of the sample solution was stabilized to offer a more steady and reliable reading of fluorescence throughout the test. To control the temperature of the solution contained in the cuvette, a Peltier device (71035-506, Laird Thermal Systems, USA) was attached to the copper-based cuvette holder and controlled by a microcontroller unit. When a Peltier device is being used to lower the temperature of the solution, the temperature of the opposite side of the device will increase since the heat (or charge) is being moved from the cold side to the hot side. In order to protect the device from being damaged from the excessive heat, a water-cooling system was selected. The pump pushes water through the water-cooling block (copper), which removes heat collected on the side of the Peltier device. The heated water is then pumped into the radiator with the fan to lower the water temperature. Later the cooled water is pumped into the 3D-printed water reservoir to get pumped back to the cooling block. In addition to the temperature controllability, a magnetic stirring module was placed underneath the cuvette holder to offer horizontal and vertical mixing of the solution for homogeneous temperature distribution and particles.
Figure 5.3 Working principle of the proposed portable fluorometer: (a) schematic illustration of the portable fluorometer platform; (b) Czerny-turner monochromator configuration.
5.1.3 Optomechanical Design, and Electronic Circuitry Configuration

The portable fluorometer, shown in Figure 5.4, consists of two main components: a light source unit and a fluorescence measurement unit. The dimension of the entire device is $240 \times 130 \times 150$ mm$^3$ and the housing of the entire device was constructed with biodegradable plastic, Polylactic Acid (PLA).

![Figure 5.4](image)

(a) An illustration of the assembled portable fluorometer and (b) a photograph of the fabricated device using 3D-printing technology.

Light Source Module:

As illustrated in figure 5.5, the light source module consists of an infrared (IR) temperature sensor (Maxim Integrated, USA), excitation LEDs coupled with color filters and collimators, a motor with a parabolic mirror, a printed circuit board (PCB) for excitation light control and data collection, an adjustable aperture, a beam splitter, a mirror, a copper-based cuvette holder attached with a Peltier device for heating/cooling, a custom-designed water cooling system for the al device, a magnetic cuvette stirrer, six rechargeable 3.7-volt standard lithium cell pack (Anker, USA), and a PCB module for a light transmittance measurement.

A top cover was designed to block the ambient light and hold the IR temperature sensor (MLX90614, Melexis, Belgium). A pair of magnets was utilized to tightly close the lid during the operation. An IR temperature sensor with a narrow view angle ($5^\circ$) was positioned in the center of
the lid to remotely monitor the temperature of the sample solution contained in the cuvette. 13 different wavelengths of LEDs were assembled with beam collimators and color filters to focus the light to the sample (Figure 5.5 (b)). The PCB board has a center hole where an off-axis parabolic mirror was placed to guide the excitation light rays towards the sample solution. The rotational angle of the mirror was controlled by the motor with a magnetic encoder that is attached at the end of the motor shaft (Figure 5.5 (d)). An adjustable aperture was placed on the straight path of the light to optimize the beamwidth of the excitation rays for noise reduction (Figure 5.5 (c)). Two stationary optics, a beam splitter (Φ12.5 mm) and a mirror (10 × 10 mm²) were used to redirect a portion of the excitation light beam to the reference photodetector to monitor the optical power output. The excitation light beam and the reference light beam were measured with an absorbance measuring module. A copper-based cuvette holder (16 mm × 16 mm × 42 mm) was fabricated with 3-D printing technology to efficiently deliver the temperature from the Peltier device to the sample solution contained in the quartz cuvette (Figure 5.5 (e)). Three digital temperature ICs, MCP9808 (Microchip Technology, USA), were attached to three different spots of the copper surface to monitoring the temperature distribution of the copper body. The sensor provides the temperature detection capacity in the range of -20 °C < T < +100 °C with ± 0.25 °C accuracy. For the communication protocol, the sensor has an industry-standard 2-wire, SMBus/I2C compatible serial interface with 400 kHz speed. A custom-designed PCB boards were used to solder the surface-mount device (SMD) temperature sensors for a compact design. The measured values through these digital temperature sensors were cross-validated with a type K thermocouple attached directly on the surface of the holder body and the temperature variations between two types of sensors were below 1 °C. A Peltier device (12.3 x 12.3 mm²) with 4.5 W maximum power output was attached to the copper-based cuvette holder using silver epoxy. The Peltier can control
the temperature of the solution from 20 °C to 40 °C. The Peltier device allows a maximum power of 4.4 W with 2.1 A of drive current and the maximum temperature difference between the hot and cold sides is 67 °C (@ 25 °C). When the Peltier device operates as a cooling element, the opposite side of its face gets heated due to the thermoelectric effect, therefore it requires a heat dissipation method to prevent the device from damaging due to the high temperature [166]. For the heat dissipation method, the liquid cooling method was selected. The liquid cooling system includes a water-cooling block (copper), a water reservoir, a water pump, and a radiator with a fan (Figure 5.5 (f)). The pump pushes water through the water-cooling block (copper), which removes heat collected on the side of the Peltier device. The heated water is then pumped into the radiator with the fan to lower the water temperature. Later the cooled water is pumped into the 3D-printed water reservoir to get pumped back again to the cooling block to dissipate the heat from the Peltier device. For the power supply, a rechargeable lithium-ion battery pack with 97 Wh energy capacity was placed at the bottom of the device with the water-cooling unit.

The light source unit has two electronic boards: an LED controller board and an absorbance measurement board. The images of the LED controller board are shown in Figure 5.4 (a). The LED controller board has a microcontroller, an LED driving circuitry, and a parabolic mirror controller. For a microcontroller, ATMEGA328P (Atmel, USA) was selected for basic operations of controlling digital/analog output signals, storing data, and communicating with ICs through I2C and SPI communication protocols. For an LED driving circuitry, an LED driver IC (AL8843SP-13, Diodes Incorporated, USA) was selected to supply constant current to LEDs with ±4% output accuracy. The output current of the chip can be digitally adjusted by applying feedback voltage to its control pin to offer a linear output current in the range of 0 to 300 mA.
Figure 5.5 Illustration and photograph of the fabricated light source module in an exploded view: (a) a cross-sectional view of a light source module; (b) images of LED and optical parts; (c) image of an adjustable aperture; (d) an image of motor and mirror for LED light guide; (e) images of a temperature control unit and magnetic stirrer; (f) images of battery pack and water cooling system.
The feedback voltage was applied by a digital-to-analog (DAC) IC (MCP4725, Microchip, USA) with 12-bit digital steps of resolution (1.22 mV/bit). In order to control the angle of the parabolic mirror, a 5V DC motor and a motor controller IC (TB6612FNG, Toshiba, Japan) were selected. The motor controller IC controls the rotational speed with a PWM signal and the direction of the lotion is altered by digital control signals.

Images of an absorbance measurement board are shown in Figure 5.6 (b). The absorbance measurement board has two photodiodes, transimpedance circuits, and analog-to-digital (ADC) IC. Two photodiodes (Edmund Optics, USA) with UV enhanced response were selected to measure the optical transmittance of the sample and monitor the excitation LED intensity variation throughout the test. A transimpedance circuit was used to amplify the photocurrent generated from the photodiodes and convert them into voltage outputs. Consequently, 2nd order Sellen-key low pass filter with a cut-off frequency of 1 kHz was implemented to increase the signal to noise ratio (SNR). The voltage signals from both photodiodes were measured with an ADC IC (Texas Instruments, USA) that offers 16-bit digitization resolution with 860 samples per second sampling rate. The ADC IC can measure either single or differential reading of both voltage inputs. The signal reading from the reference photodiode was the excitation light that was split by an optical beam splitter.
<Top layer>

Microcontroller module

LED coupler CNT x13

Switch (LED) x 15

LED current Monitoring (ADC)

DC motor with an encoder

Multiplexer for LED control

Light pass hole

Motor connector

<Bottom layer>

IR sensor CNT

FTDI connector

Motor controller

Power input

SPI CNT

I2C CNT

Voltage regulator

Temp sensor for a board

IR sensor CNT2

LED controller and current motor IC

Low-pass filter

(fig. cont’d)
Figure 5.6 Electronic circuit designs of light source module: (a) images of the electronic circuit of the LED control board and (b) images of the electronic circuit of the absorbance measurement module.

Fluorescence Detection Module:

The fluorescence detection module consists of various optics (including parabolic mirrors, aluminum air slits, and a diffraction grating), a DC motor, magnetic encoder, an electronic board for diffraction grating control, an electronic board for fluorescence signal reading, and a Peltier device for cooling the fluorescence readout board.
An illustration of an exploded view of a fluorescence detection module is shown in Figure 7 (a). A top cover and a mid cover were fabricated with a 3-D printer to incorporate optical components. The mid cover has an inlet hole that is perpendicular to the excitation light direction. This minimizes the stray light noise from the excitation source while maximizing the fluorescence signal. A first parabolic mirror (1) collects and collimates the polychromatic light that is mixed with fluorescence and excitation stray light. Consequently, the second parabolic mirror (2) focuses the light on an input aluminum slit whose width and length is 50 µm by 3 mm. A third parabolic mirror (3) collimates the light towards the diffraction grating to break down the polychromatic light to monochromatic light. The basic expression of diffraction grating can be defined as,

\[ m\lambda = d (\sin \theta_{\text{inc}} + \sin \theta_{\text{dif}}) \] (5.1)

where \( d \) is the groove spacing (lines per mm), \( \theta_{\text{inc}} \) is the angle of incidence of the light, \( \theta_{\text{dif}} \) the angle of the diffraction, \( \lambda \) is the wavelength of the light, and \( m \) is the diffraction order. This means that the diffraction angle of the reflected light depends on the incident light angle and the wavelength of the light when \( m \) and \( d \) values are fixed. Therefore, it is possible to control the direction of the wavelength of interest by adjusting the grating angle. The angle of the diffraction grating was adjusted by a DC motor and its angular step was controlled by a magnetic encoder attached at the end of the motor shaft. Diffracted light rays were focused by the fourth parabolic mirror (4) to the exit slit plane as illustrated in Figure 5.7 (b). The exit slit, which has the same size as the input slit, allows only light in the 0.5 nm waveband to pass through. A highly sensitive silicon photomultiplier (SiPM) was deployed under the exit slit to measure the monochromatic light that passed through the slit. A Peltier device was attached to the electronic board to maintain the temperature of the SiPM to 20 °C. The low temperature of SiPM. This benefits the SiPM to have stable signal readings and low dark noise characteristics. In order to minimize the stray noise
due to the internal reflection of the rays, 3D printed stray light reducers (thorn-like structures) were placed along with the optical components.

Figure 5.7 Illustration and photograph of the fabricated fluorescence detection: (a) illustration of an exploded view of the fluorescent detection module and (b) photograph of the fabricated fluorescence detection module under operation.
The fluorescence detection module has two electronic boards: a monochromator controller board and a fluorescence measurement board. The images of a monochromator board are shown in Figure 5.8 (a). The monochromator board has a microcontroller, Peltier controlling circuitries, magnetic stirrer/water pump/radiator fan controllers, and a grating motor controller. A microcontroller controls the speed of motors including a magnetic stirrer, a water pump, a radiator fan, and a grating motor. The speeds of the motors are adjusted via PWM signals from the microcontroller. The current supplied to the Peltier device is in the range 0 - 1.8 A at room temperature (25 °C). The Peltier device's output current was digitally adjusted by applying linear feedback voltage to the driver IC (AL8843SP-13, Diodes Incorporated, USA).

The images of the electronic circuit of the fluorescence measurement board are shown in Figure 5.8 (b). The fluorescence measurement board has SiPM, temperature sensor, transimpedance circuit, Sallen-key lowpass filter, ADC chip, and a Peltier. For fluorescence detection, SiPM was selected to detect a weak fluorescence signal. Transimpedance circuit converts the photocurrent generated from the SiPM to the voltage output. Consequently, the Sallen-key lowpass filter removes the high-frequency noise from the converted voltage signal with a 2 kHz cut-off frequency. The voltage output signal was measured with an ADC IC that offers 16-bit digitization resolution with 860 samples per second sampling rate. In order to minimize the thermal noise from SiPM, a Peltier device was attached to the board to control and maintain the temperature of SiPM to 20 °C. A digital temperature IC sensor (MCP9808) was positioned right next to the SiPM to closely monitor the temperature and offer feedback to the Peltier controller.
Figure 5.8 Electronic circuit designs of fluorescence detection module: (a) an image of an electronic circuit of monochromator board and (b) images of the electronic circuit of fluorescence measurement board (SiPM board).
5.2. Detection of Rhodamine B and Fluorescein Fluorescent Dyes Using a Portable Fluorometer with Temperature Controlling System

For measuring fluorescent signals, standard UV Fused Quartz Cuvette (Thorlabs, USA) was chosen to load the dye solution sample (3.5 ml). Different concentrations of rhodamine B and fluorescein samples were obtained by serially diluting the highest dye solution samples.

5.2.1 Characterization of the Excitation LEDs and a Driving Circuitry

Figure 5.9 depicts the normalized spectra of excitation LEDs. The spectra of LEDs were measured with a benchtop spectrometer (Green-Wave, StellarNet Inc, USA). Each LED was assembled with an optical filter to offer a narrow bandwidth of the light. 280 nm (VLMU60CL00, Vishay Intertechnology, USA) and 367 nm (QBHP684E-UV365AS, QT-Brightek, USA) excitation LEDs were implemented to utilize in pathogen detection applications [167]. Other LEDs (400 – 730 nm) from Lumileds (USA) were selected to offer various wavelengths of stimulations without having any spectral overlapping. Each LED was assembled with an optical filter to offer a narrow bandwidth of the light and FHWM bandwidth was less than 10 nm.

A block diagram of LED driving control functions is illustrated in Figure 5.10. A microcontroller controls the DAC via I2C communication to adjust the feedback voltage of an LED driver IC in the range of 0.4 – 2.5 V. The output current of the IC provides a linear output current in the range of 0 to 300 mA. The output current is monitored by ACS724 current sensing IC (Allegro, Poland). A multiplexer IC (CD74HC4067SM96, Texas instrument, USA) was implemented to sequentially turn on one of the selected LEDs.
Figure 5.9 Normalized spectra of excitation LEDs ranging from 280 nm to 730 nm.

In order to demonstrate the stability of the driving current, various current values were supplied through the LED driver IC to show that a constant current is flowing through the LED.
In Figure 5.11 (a), different values of control voltage were applied to the driver IC to ensure the linearity of the corresponding current levels. It was demonstrated that the relationship between the control voltages and the driving currents are highly linear. Figure 5.11 (b) shows the results of output stability tests driven at different levels of current (ranging from 30 mA to 295 mA). The current was driven for 100 seconds and monitored with both current monitoring IC and a benchtop multimeter (Tektronix, USA) with a 1 Hz sampling interval. The current maintained <0.5% stability for all levels of driving current throughout the test. The operation time for each LED is expected to be less than 10 seconds, therefore the LED output intensity will be highly stable as well.

![Figure 5.11 LED driving current test: (a) relationship between control voltages and corresponding current levels; (b) output stability test driven at different levels of current (30 – 295 mA)](image)

5.2.2 Demonstration of Temperature Controllability of a Portable Fluorometer

Fluorescence emission efficiencies of fluorescent dyes are easily affected by many other parameters, such as temperature, pH, solvent, and excitation wavelength. In order to minimize the detection error due to those parameters, the solvent temperature of the device was controlled and
stabilized using a custom-designed temperature controlling module. Figure 5.12 shows the system configuration of the temperature controlling module. The temperature controlling capacity of the module was tested before implemented into a portable fluorometer system to validate its operation and performance. For the temperature monitoring of the copper body, digital temperature IC sensors were used. Three surface-mounted temperature modules were attached via silver epoxy to the surface of the copper holder to monitor the temperature of three different regions of its body. The measured values through these digital temperature sensors were cross-validated with a type K thermocouple attached directly on the surface of the holder body, and the temperature variations between the two types of sensors were below 1 °C. An IR temperature sensor was mounted above the quartz cuvette for contactless temperature monitoring of the sample solution. The sensor provides the temperature detection capacity in the range of −20 °C < T < +120 °C with ±0.14 °C accuracy and 5° of a field of view (FOV). Conventional temperature controlling systems may fail to measure an accurate temperature reading of the solution in the cuvette because the sensors are only reporting the temperatures of the heating element or the metal holder body. Therefore, the IR temperature sensor was selected to directly monitor the solution temperature for higher accuracy. The temperature reading of the solution was cross-validated with a waterproof digital temperature sensor (DS18B20, Adafruit Industries, USA), and the variations between the two sensors were below 1 °C.

Figure 5.13 shows the temperature values at different spots on the copper body and the reading at various target temperatures (25, 25, 45, and 45°C). It was demonstrated that the temperature distribution of the copper cuvette holder was almost uniform (within ±1 °C variation) as well as the solution temperature in the cuvette. 3 ml volume of D.I water was used for the test solution. Throughout the test, the cuvette was stirred with a magnetic stirrer to ensure that the
temperature of the solution was uniform.

Figure 5.12 Schematic illustration and photograph of the fabricated thermoelectric temperature control module: (a) images of the module with an exploded view of each component and (b) illustrations of the temperature monitoring IR sensor and a temperature sensor IC.

<table>
<thead>
<tr>
<th>Target Temperature (°C)</th>
<th>Solution (°C)</th>
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<tbody>
<tr>
<td>25 °C</td>
<td>25.08 °C</td>
</tr>
<tr>
<td>35 °C</td>
<td>35.57 °C</td>
</tr>
<tr>
<td>45 °C</td>
<td>44.73 °C</td>
</tr>
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<td>55 °C</td>
<td>54.41 °C</td>
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<table>
<thead>
<tr>
<th>Back Top (°C)</th>
<th>Front Top (°C)</th>
</tr>
</thead>
<tbody>
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<td>25.30 °C</td>
</tr>
<tr>
<td>35.77 °C</td>
<td>35.83 °C</td>
</tr>
<tr>
<td>45.35 °C</td>
<td>45.40 °C</td>
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<td>55.47 °C</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Back Bottom (°C)</th>
<th>Front Side of Peltier</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.46 °C</td>
<td></td>
</tr>
<tr>
<td>30.25 °C</td>
<td></td>
</tr>
<tr>
<td>40.08 °C</td>
<td></td>
</tr>
<tr>
<td>59.34 °C</td>
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</table>

Figure 5.13 Copper cuvette illustrated with the distribution of temperature in various target temperatures (25, 25, 45, and 45°C).

For analyzing the temperature stabilization module’s viability for fluorophore analysis, the temperature dependency of rhodamine B at five different temperatures is analyzed, as seen in Figure 5.14. The solution is dissolved in methanol with a 5 µg/ml concentration. Fluorescence emission was monitored with a benchtop spectrophotometer (Fluorolog 3 FL3-22, Horiba, Japan).
The absorption peak of rhodamine B dissolved in methanol was 553 nm and the emission peak of fluorescence was 553 nm. Fluorescence emission spectra show a linear degradation as the temperature increases (Figure 8). With a 30 °C change, the fluorescence emission drops to ~0.6 of its original value. This degradation on par with what literature has reported, where a 30 °C change leaves ~0.5 of original fluorescence emission yield [168]. The performance of the temperature stabilization module is illustrated, indicating its viability for on-site analysis of florescent solutions.

![Fluorescent emission spectra of rhodamine B showing normalized fluorescent emission spectra of rhodamine B at varying temperatures (Inset: peak fluorescence emission as a function of temperature). The excitation wavelength used was 553 nm, and the resulting emission peak was observed at 577 nm. This experiment was performed at room temperature stabilization for each of the four temperature cases were achieved in under 90 s.](image)

5.2.3 Measurement of Fluorescein and Rhodamine B Using a Portable Fluorometer

A portable fluorometer was tested using fluorescein and rhodamine B fluorescent dyes. For fluorescein detection, an excitation LED with 434 nm wavelength was selected. The fluorescein was dissolved in methanol with 220 mg/ml concentration for fluorescence
measurement test. The spectra of the excitation LED and fluorescein fluorescence signals were measured with our portable fluorometer as shown in Figure 5.15. The peak fluorescence of fluorescein was around 530 nm. The spectral profile of the excitation LED and the peak fluorescence of fluorescein was well-matched with previously reported references.

Figure 5.15 Normalized spectral emission of 434 nm excitation LED and fluorescein fluorescence measured with a portable fluorometer.

For rhodamine B detection, an excitation LED with a 535 nm wavelength was selected. The rhodamine B was dissolved in methanol with 100 mg/ml concentration for fluorescence measurement test. The spectra of the excitation LED and rhodamine B fluorescence was measured with our portable fluorometer as shown in Figure 5.16. The peak fluorescence of rhodamine B was around 602 nm, which is about ~ 20 nm off from the value measured from the benchtop fluorometer. This means our portable fluorometer requires calibration in the longer wavelength range to increase the accuracy. However, the spectral trend and contour were well matched with
that of benchtop fluorometer measurement results, thus, calibration will offer more accurate spectral scanning in the future.

Figure 5.16 Normalized spectral emission of 535 nm excitation LED and rhodamine B fluorescence measured with a portable fluorometer.

5.3. Conclusions

In this chapter, a portable fluorometer was developed and demonstrated for multiple excitation LEDs with 13 different wavelengths and spectral scanning capacity in the range of 400 – 780 nm wavelength. In addition, a temperature controller module was implemented for the stabilization of the solution temperature for accurate fluorescent signal measurement. To demonstrate the efficacy of the developed system, rhodamine B was tested under various solvent temperature conditions to monitor the fluorescent emission efficiency change and temperature
control capacity of the device. It was reported that the temperature increment of the solvent temperature decreased the fluorescence emission efficiency. Therefore, the temperature controllability of the solvent is essential not only for the stability of the fluorescence emission but also for the maximization of the output emission intensity for sensitive detection of the target fluorescent analytes. The spectral scanning of the monochromator module was demonstrated with rhodamine B and fluorescein under 525 nm excitation and 434 nm excitation, respectively. Spectral scanning results of our monochromator module was compared with a commercially available spectrometer and well-matched with both excitation LED and fluorescence readouts. However, the device needs more calibration to increase the accuracy of the spectral reading, especially in the longer wavelength range.

Future improvements to the system presented in this chapter will include applying advanced algorithms such as PCR for spectrum to detect multiple fluorescent dyes. In addition, the sensitivity of the monochromator can be improved by replacing the optical parts, such as light-collecting mirror and optical slits. Our new portable fluorometer platform can be utilized in many different applications, such as pathogen detection and POC device.
6. Conclusions and Future Work

6.1. Summary

The main objective of this dissertation is to develop a fully portable fluorescence sensor platform for selective detection of multiple biochemical target analytes. Amongst various optical detection techniques, fluorescent sensing is considered highly useful in practical applications for its high sensitivity, specificity, and accuracy compared to other optical sensing techniques. However, three main challenges in associated with fluorescence measurement technology are detection of an extremely weak fluorescent signal, maintaining the photostability of fluorescent analytes, and differentiation of multiple fluorescence signals. To overcome such challenges, this work was done to propose possible solutions for these constrictions. This work also demonstrates the possibility of a portable fluorescent sensor that is capable of multiple analyte detection using multiple excitation sources and advanced multivariate algorithms. The following is the summary of the three main contributions from this thesis:

1. Development of highly sensitive fluorescence detection of necessary optomechanical and electronic compartments for the portable system.
2. Development and integration of necessary systems to provide temperature stability of fluorescent analytes.
3. Implementation of multivariate algorithm for multianalyte capacity.

In the following subsections, detailed achievements and conclusions from each chapter are given in detail.
6.1.1 Preliminary Portable Fluorescent Sensor Design for Green Algae Detection

In chapter 3, a preliminary design that includes essential parts of the system such as excitation light sources, photodetector, 3-D printed optomechanical parts, and PDMS microfluidic chip was developed. The fluorescent sensor was characterized and tested with different concentrations of green algae (Chlorella vulgaris). For the sample delivery, a microfluidic chip was used. The microfluidic chip consists of a glass slide and a PDMS microfluidic channel with a passive vacuum pump, which delivers a small volume of the sample solution (<10 ul). A 448 nm wavelength LED was selected to excite the microalgae that emit 680 nm wavelength fluorescent light. A photodiode with a 645 nm long-pass optical color filter and a dichroic mirror was mounted below the PDMS microfluidic chamber to detect the fluorescent light signal from the samples. The fluorescence sensor was calibrated with different concentrations of green algal samples and demonstrated its capability of measuring green algal concentrations. The sensor was also tested with green algal samples mixed with different turbidity water to validate its selectivity. The fluorescent signal was independent of the turbidity level of the sample and the result from microalgal concentration measurements in different levels of turbid samples showed a linear response comparable to the cell counting measurement as well.

6.1.2 Multi-Phytoplankton Detection with a Hand-Held LED-Based Fluorescent Sensor

In chapter 4, a hand-held fluorescence sensing platform was developed and demonstrated for selective and quantitative detection of multiple analytes. To demonstrate the efficacy of the developed system, it was used to quantify and differentiate two types of phytoplankton species: green algae and cyanobacteria. The green algae were predominantly stimulated by blue and UV LED lights owing to the presence of chlorophyll $a$ and $b$, while the phycocyanin in the cyanobacteria was mainly stimulated by amber LED light. Each LED was sequentially turned on
and off using a microcontroller and the corresponding unique patterns of fluorescent signals measured using a highly sensitive photodetector (SiPM) with a long-pass filter. PCA algorithm was used to visualize the clusters of the different sample concentrations. Furthermore, the partial least squares regression (PLSR) algorithm was used to build a predictive model for estimating the biomass of each sample. The results indicated that the use of different excitation lights was effective in selectively stimulating the target photopigments. Moreover, the application of the PLSR algorithm effectively differentiated and quantified the two algal species. Thus, it is clear that the developed fluorescent sensor system could simultaneously detect multiple analytes.

6.1.3 Development a Portable Fluorometer with Temperature Controlling System

In chapter 5, a portable fluorometer with a temperature controlling system was developed. The system offers 13 different wavelengths of excitation LEDs to offer broader excitation (spanning from 280 to 725 nm). A monochromator was developed to separate the color elements of the fluorescent emission and selectively detect the wavelength of interest. The developed monochromator can sequentially scan a range of wavelength in 400 - 780 nm with 1 nm resolution. In addition, a temperature controller module was implemented for stabilization of the solution temperature to offer accurate fluorescent signal measurement. To demonstrate the efficacy of the developed system, rhodamine B was tested under various solvent temperature conditions to monitor the fluorescent emission efficiency change and temperature control capacity of the device. It was demonstrated that the temperature increment of the solvent temperature decreased the fluorescence emission efficiency. Therefore, the temperature controllability of the solvent is essential not only for the stability of the fluorescence emission but also for the maximization of the output emission intensity for sensitive detection of the target fluorescent analytes. The spectral scanning of the monochromator module was demonstrated with rhodamine B and fluorescein
under 525 nm excitation and 434 nm excitation, respectively. Spectral scanning results of our monochromator module was compared with a commercially available spectrometer and well-matched with both excitation LED and fluorescence readouts.

6.2. Suggested Future Work

For future improvements of this research, the following applications and techniques could be further investigated to advance this concept as a viable choice for future portable sensing technology.

6.2.1 Test with Increased Number of Samples to Validate the Multianalyte Detection Capacity of a Portable Fluorometer Platform

Although fluorescence-based sensing is highly selective and sensitive, it is very common to observe spectral overlap of fluorescence emission in different analytes. In addition, they are often stimulated by common excitation light sources, which makes it even more difficult to differentiate multiple fluorescent target analytes. Multivariate algorithms are useful especially when multiple signals are mixed. It is essential to train the algorithm with as many parameters as possible to increase the accuracy and detection capacity. A portable fluorometer with temperature controllability can offer viable options for increasing the parameters that can potentially improve the detection accuracy of the multivariate algorithm. You et al. reported that different fluorescent dyes have different fluorescent emission efficiency under different temperature conditions [169]. Different quenching rates of various fluorescent dyes can be utilized for the characterization of different dyes and can be easily demonstrated with our portable fluorometer platform.
6.2.2 Developing an Advanced Algorithm for Effective Multi-Analyte Sensing (Deep Learning and Neural Network)

Using PCR and PLS as pattern recognition algorithms for classification and quantification of multianalyte was the first step in developing a generic fluorescence-based sensing platform for biochemical detection applications. Deep learning and neural network algorithms are widely used in many applications even in the sensing area, especially in image sensing for diagnostics and object detection. With the help of the real-time sensing capacity of portable sensors with IoT functionality, metadata collected from those sensors can offer valuable data in environmental monitoring and healthcare monitoring applications.
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[99] C. Coelho, B. Heim, S. Foerster, A. Brosinsky, and J. C. De Araújo, "In situ and satellite observation of CDOM and chlorophyll-a dynamics in small water surface reservoirs in the Brazilian semi-arid region," *Water*, vol. 9, no. 12, 913 (22pp), 2017.


Vita

Young-Ho Shin is a graduate assistant in the Division of Electrical and Computer Engineering. He has received a B.S. degree in Electrical and Computer Engineering at Kyunghee university in South Korea in 2010. His research focuses on the biosensor systems, microfluidic devices, and 3D printing technology. He has been working on developing a portable fluorescent detection system for microalgal samples. This research is funded by the Louisiana Board of Regents Industrial Ties Research Subprogram, contract LEQSF(2013-2015)-RD-B-02. He is advised by Dr. Choi in the Division of Electrical and Computer Engineering.