Instrument for the Identification of Live and Dead Bacteria

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CONCEPT OF OPERATIONS

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CONCEPT OF OPERATIONS
FOR
Instrument for the Identification of Live and Dead Bacteria

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Table of Contents

List of Tables

No table of figures entries found.

List of Figures

1. Executive Summary

The identification of live and dead bacteria is a challenging task. Conventional methods of identification, requiring days and specialized equipment, are quite time-consuming and inappropriate in settings requiring prompt, *in-situ* diagnosis. With a rise in health care-associated infection (HAI) due to bacteria, as well as growing antibiotic resistance, a rather urgent need for the efficient detection and assessment of bacterial infections exists. To address this need, a portable fluorescence spectrometer will be constructed. The spectrometer will consist of excitation and emission monochromators, ultraviolet (UV) light-emitting diodes (LEDs), and a graphical user interface (GUI). An on-board UV LED will be utilized as a means of disinfection. The spectrometer will be capable of performing both normal fluorescence and synchronous fluorescence spectroscopy on bacterial samples. The normal fluorescence and synchronous fluorescence spectra of bacteria, before and after UV disinfection, may be recorded and processed through principal component analysis (PCA) on a computer. Moreover, a GUI will facilitate the acquisition, processing, and display of spectra. To that effect, live and dead bacteria will be rapidly distinguished, within minutes. These results will support a novel, efficient, and portable means of identifying live and dead bacteria *in-situ*.

2. Introduction

Bacteria pose a rather prevalent threat to life. Indeed, it is estimated that at least 2 million people, per year, incur infections due to antibiotic-resistant bacteria, and in addition, at least 23,000 people die each year following such infections [1]. Furthermore, almost 1.7 million hospital-acquired infections, along with approximately 98,000 resulting deaths, occur annually [2]. Such health care-associated infection (HAI) was noted to be among the top 10 causes of death in the United States with an estimated $4.5 billion to $6.5 billion cost of treatment [3]. Growing antibiotic resistance, coupled with a decline in antibiotic development, has further worsened the effects of HAIs [2]. The current, so-called "gold standard" for the quantification of bacterial species is Colonies Forming Units (CFU) counting, which requires a laboratory and, typically, 1 to 3 days to obtain results [4]. Such a procedure is rather time-consuming and unfeasible in settings requiring fast, *in-situ* diagnosis. To that effect, an efficient, portable means for detecting and assessing bacterial infections would prove invaluable. This project addresses such a need by means of a portable fluorescence spectrometer coupled with PCA. This system will be capable of recording the normal fluorescence and synchronous fluorescence spectra of bacteria, subjecting such spectra to PCA, and distinguishing live and dead bacteria. This system may ultimately be used, *in-situ*, in controlled environments, such as laboratories, operating rooms, and clinics, as well as in the field.

2.1. Background

Currently, bacterial infections account for a considerable and costly percentage of deaths within the United States [5]. The threat of bacterial infection is compounded by the fact that current detection and assessment methods are rather time-consuming and inefficient. The "gold standard," bacterial plating, necessitates the isolation, culturing (i.e., growth), and subsequent identification and quantification of the bacterial sample, a procedure which typically requires 1 to 3 days for completion [4]. Such a procedure requires extensive time and specialized equipment that are inappropriate for *in-situ* analyses and, therefore, may be detrimental to a patient's long-term health, especially in cases where such resources are not readily available. In addition, the ability to efficiently diagnose and assess bacterial infections has implications on the efficacy of the resulting antimicrobial therapy. Particularly, it has been noted that the inappropriate application of antibiotic drugs, in an attempt to treat HAI, has contributed to growing antibiotic resistance, toxic effects, and health care costs [2].

Previous studies have demonstrated the utility of optical spectroscopy, coupled with multivariate data analysis, in discriminating among various bacterial strains and concentrations [6-10]. Owing to the fact that bacterial cellular components, such as amino acids (tryptophan and tyrosine) and nucleic acids (DNA), possess rather intense absorption and fluorescence bands in the UV spectral region [11], normal fluorescence and synchronous fluorescence spectroscopy have been utilized, in conjunction with PCA, for the detection and identification of common commensal and pathogenic bacteria in foods [6], as well as clinically-important bacteria [12]. In addition, our previous studies [13-15] have demonstrated that the normal fluorescence and synchronous fluorescence spectra of bacteria, including its tryptophan and tyrosine components, undergo detectable changes following UV or antibiotic treatment. Subjecting these spectra to PCA allowed us to rapidly distinguish live and dead

bacteria following disinfection [13-15]. In many of these studies, however, the spectroscopic and disinfection instruments used were bulky and therefore unsuitable for usage in the field.

To that effect, the proposed system is a novel solution for the rapid identification of live and dead bacteria. This system offers several improvements compared to the conventional method of diagnosis: bacterial plating [4]. Namely, the system will be portable and therefore suitable for *in-situ* usage in controlled environments, such as laboratories, as well as in the field. This is rather critical for settings in which specialized equipment and resources are unavailable, such as in remote areas or clinics. Leveraging the techniques of normal fluorescence and synchronous fluorescence spectroscopy, coupled with PCA, the system will offer a rather rapid means of identification, requiring minutes rather than days. Such an improvement in diagnostic time is paramount in furthering timely, effective antibacterial therapy [2]. Furthermore, the system will require minimal sample preparation or enrichment compared to the conventional method of diagnosis and thus consume less resources.

It is worthy to mention that modular spectroscopy has been achieved for practically all forms of spectroscopy, including normal fluorescence spectroscopy. Therefore, this system will leverage several commercial, off-the-shelf components for its construction, including monochromators, a linear charge-coupled device (CCD) detector array, and LEDs. To our knowledge, however, no portable, spectroscopic system, capable of both normal fluorescence and synchronous fluorescence spectroscopy, has been created for identifying and distinguishing live and dead bacteria. Furthermore, this system will have the additional on-board capabilities of performing both PCA and bacterial disinfection *in-situ*. To that end, the proposed system is in itself a novel spectroscopic device.

2.2. Overview

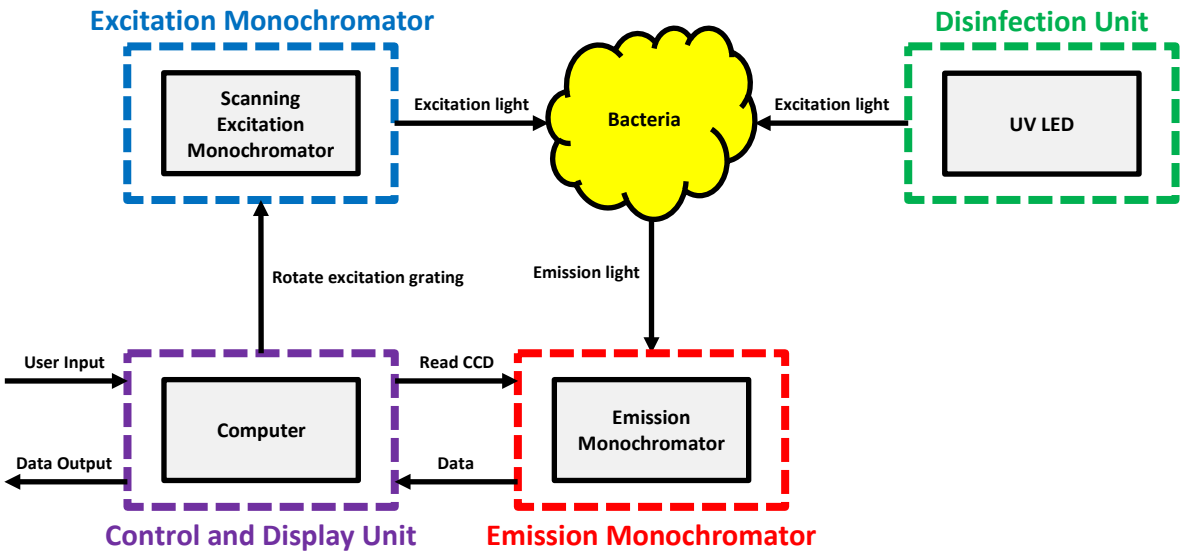

Figure 1: Block diagram of portable fluorescence spectrometer.

The block diagram of the portable fluorescence spectrometer is shown in Figure 1. The system consists of four subsystems: (1) a control and display unit, (2) a disinfection unit, (3)

an excitation monochromator, and (4) an emission monochromator. The proposed system is capable of performing both normal fluorescence and synchronous fluorescence spectroscopy, as well as UV disinfection of bacterial samples. To perform normal fluorescence spectroscopy, the excitation monochromator subsystem will be set to output a preselected excitation wavelength in the UV region. This wavelength of light will be transmitted to the bacterium, thereby inducing fluorescence. This fluorescence may then be collected, imaged, and recorded by the emission monochromator subsystem. Synchronous fluorescence spectroscopy may be similarly achieved by rotating the excitation monochromator subsystem through a range of excitation wavelengths, with the emission monochromator subsystem recording the sample's fluorescence at each given excitation wavelength. The resulting dataset will be a three-dimensional excitation-emission matrix (EEM) from which synchronous spectra may be easily derived. To perform UV disinfection, the disinfection unit may be utilized to irradiate bacterial samples with intense UV light. It is worthy to mention that the disinfection unit may also be utilized to excite bacterial samples and perform normal fluorescence spectroscopy. All commands issued to the excitation monochromator and emission monochromator subsystems, along with any recorded spectral data, will be transmitted via a serial communication link. Finally, the processing and display of recorded spectra, along with PCA, will be performed by the control and display unit.

2.3. Referenced Documents and Standards

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3. Operating Concept

3.1. Scope

The scope of this project is to design, construct, and validate a scaled-down prototype for a novel, portable fluorescence spectrometer which is capable of identifying and distinguishing live and dead bacteria within minutes. To that effect, the prototype is expected to be capable of recording the normal fluorescence and synchronous fluorescence spectra of bacterial samples before and after UV disinfection. These spectra may then be subjected to PCA for distinguishing live and dead bacteria. This spectroscopic, identification procedure is expected to be completed within minutes. To achieve these functions, the prototype is currently expected to consist of the following subsystems: (1) a control and display unit, (2) a disinfection unit, (3) an excitation monochromator, and (4) an emission monochromator. In addition to this hardware, software will be designed for (1) recording normal and synchronous fluorescence spectra, (2) scanning the excitation monochromator, and (3) performing PCA on recorded spectra. This prototype will be constructed, tested, and validated in a laboratory environment.

3.2. Operational Description and Constraints

This system is intended to be used by both scientists and medical personnel, depending on the particular application. Namely, scientists may utilize this system for further research and development, while medical personnel may use this system for the diagnosis and treatment of bacterial infections. This system is intended for use primarily in controlled environments, such as laboratories, hospitals, and clinics, although it may also be transported and used in the field and remote areas. Regardless of its particular use, the system is expected to be capable of recording the normal fluorescence and synchronous fluorescence spectra of bacterial samples, in addition to performing PCA of recorded spectra and thereby distinguishing live and dead bacteria. The on-board disinfection unit may additionally be used to inactivate any living bacteria. The resulting constraints from this operational description are as follows:

- The on-board computer must be capable of efficiently and reliably communicating with both the excitation and emission monochromator subsystems.
- The excitation monochromator must be capable of receiving and transmitting UV radiation of sufficient intensity to induce detectable fluorescence from both live and dead bacteria.
- The emission monochromator must be sensitive to the fluorescence of both live and dead bacteria, particularly in environments where background interference is high.
- The disinfection unit, namely its UV LED, must be effective in inactivating practically all types and strains of bacteria.
- Due to budget constraints, the quality of optical components used will be limited; this will affect the sensitivity and performance of the overall system.
- To promote safety to users as well as longevity of the system components, all subsystems should be housed in sturdy, yet easily transportable, enclosures.
- The overall system should be capable of being powered with either benchtop DC power supplies or batteries.
- The overall system must be portable.

3.3. System Description

The portable fluorescence spectrometer will consist of four subsystems: (1) a control and display unit, (2) a disinfection unit, (3) an excitation monochromator, and (4) an emission monochromator. Each subsystem is described in further detail as follows:

Control and Display Unit: The control and display unit will consist of a GUI on a computer which is responsible for receiving inputs from users, communicating with both the excitation monochromator and emission monochromator subsystems, and ultimately processing and displaying recorded data. This unit will interface with both the excitation monochromator and emission monochromator subsystems by means of a serial communication link, or cable, which may allow commands to be easily issued. Relevant inputs from users may include the following: (1) the type of spectrum (normal fluorescence or synchronous fluorescence) to record, (2) the initial and final excitation wavelengths of the spectrum, (3) the number of spectra to record, and (4) the range of fluorescence data to subject to PCA, among others. Following these user inputs, the unit will issue control signals for initializing the excitation and emission monochromators and initiating the spectrum acquisition. Furthermore, this unit will be responsible for receiving, processing, and displaying spectral data recorded by the emission monochromator. A detailed flowchart elucidating this process is provided in Figure 2 on the following page.

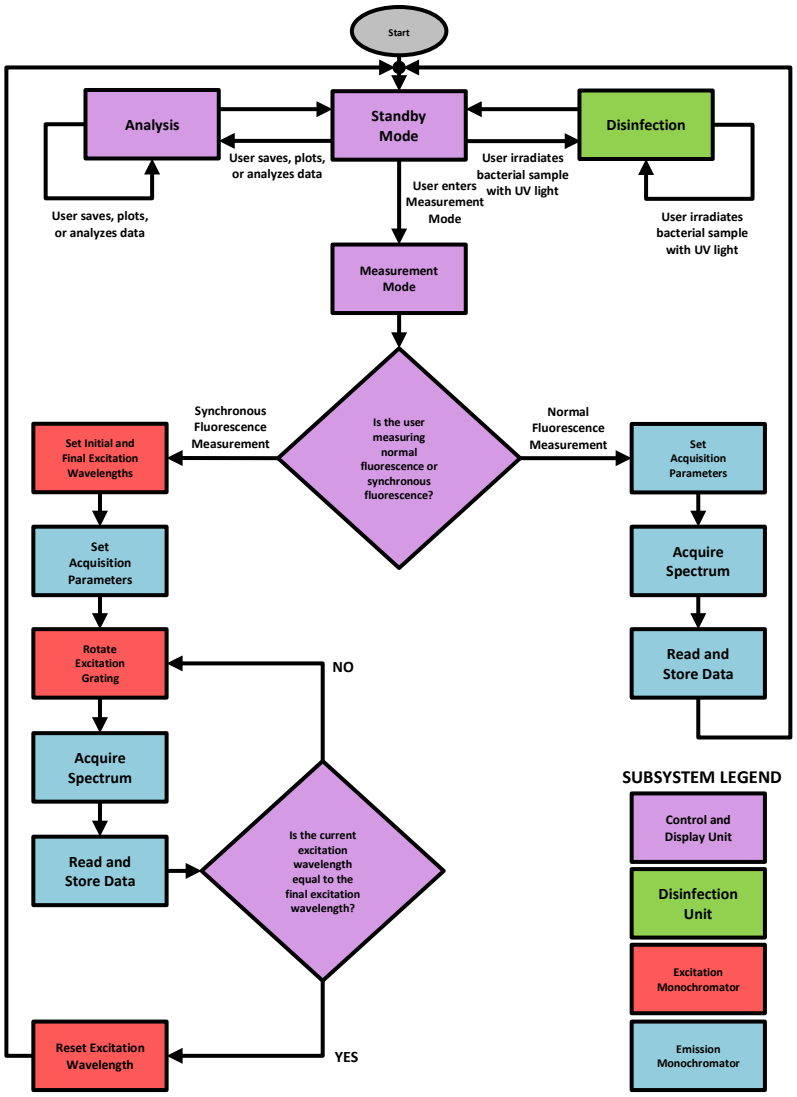

Figure 2: System flowchart detailing the various modes of operation.

Disinfection Unit: The disinfection unit will consist of a high-power, miniature UV LED which may be used to efficiently inactivate bacterial samples *in-situ*. The intensity of the UV LED may be increased rather easily by coupling it with a small ball lens, which would focus and impinge the light onto the sample. Besides this function, the UV LED may also serve as an excitation source and therefore be directly coupled with the emission monochromator to record the normal fluorescence spectrum of the bacterium at the UV LED's peak wavelength. Owing to the fact that the UV LED is not coupled to a monochromator, which would result in a reduction in the LED's output intensity, this could be a viable alternative in settings where the sample concentration or fluorescence signal is low. The output spectrum of a UVC LED is displayed in Figure 3 below, illustrating the rather narrow bandwidth of the source in the UV region, as desired. Finally, the UV LED may be easily powered by either a benchtop DC power supply or battery.

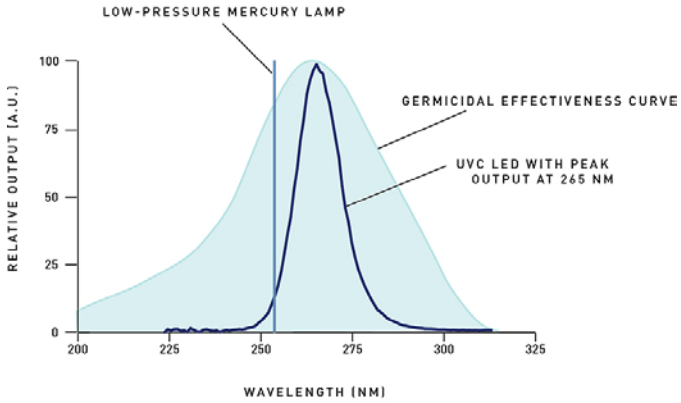

Figure 3: Output spectrum of UVC LED demonstrating its high-power, narrow profile, along with the associated germicidal effectiveness [1].

Excitation Monochromator: The excitation monochromator will consist of a high-power, miniature UV LED as an excitation source, an optical bench with mirrors and a reflective diffraction grating coupled with a stepper motor, and a stepper motor controller and driver for rotating the excitation grating. The schematic of the excitation monochromator, including the proposed optical bench and components, is shown in Figure 4 below. The optical bench houses a Fastie-Ebert configuration, consisting of two folding mirrors M1 and M3, a single curved mirror M2, and a reflective diffraction grating G1. The high-power, miniature UV LED excitation source will be coupled to the bench at the entrance slit, whereupon the input, broadband UV light will be reflected by M1 to M2. M2 will then collimate and direct the light to the reflective grating G1, which will diffract and disperse the light into its component wavelengths. The dispersed light will be reflected from G1 to M2 again, which will now focus the light to the exit slit by means of M3. The particular wavelength of the dispersed light focused on the exit slit will be dependent on the angular positioning of the excitation grating G1. A stepper motor, coupled with a stepper motor controller and driver, may rotate the grating to a desired output wavelength or, alternatively, scan through a range of excitation wavelengths, as desired in a synchronous fluorescence spectrum. An optical fiber may optionally be connected at the exit slit to transmit the excitation wavelength to the sample; alternatively, to maximize the excitation intensity, the sample may be directly situated at the exit slit itself. It is worthy to mention that all components in the optical bench must be optimized for performance in the UV spectral region.

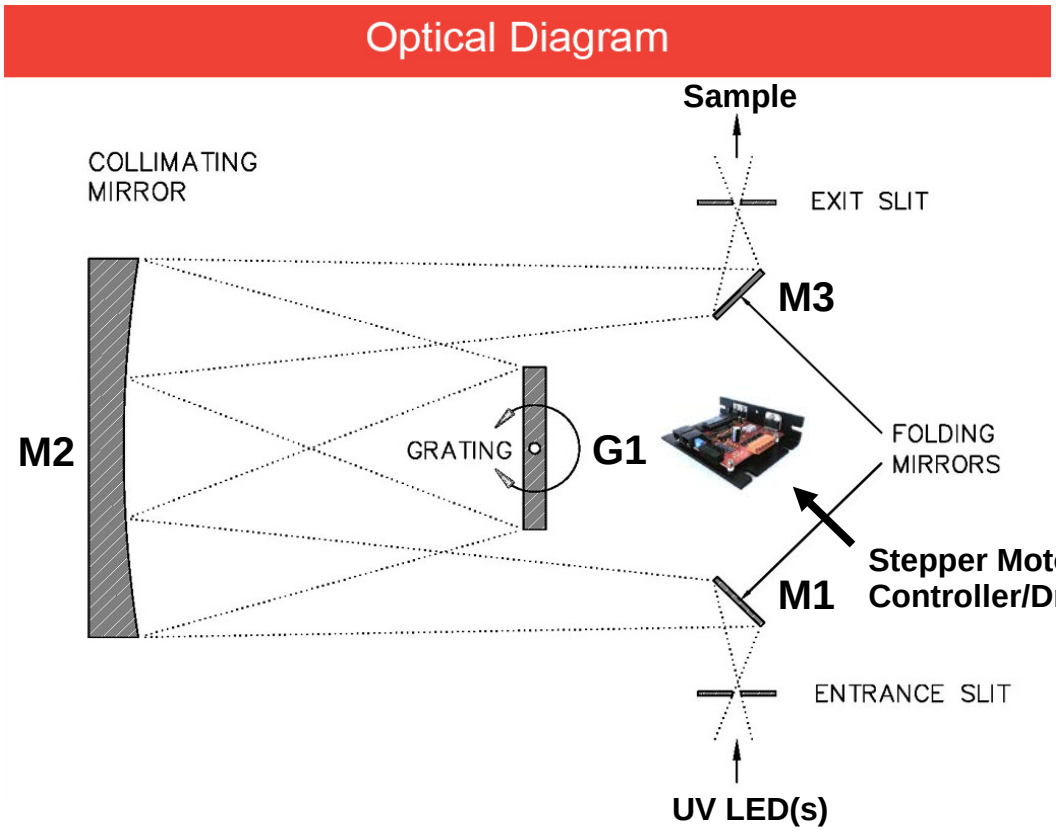

Figure 4: Schematic of the overall excitation monochromator subsystem, including the UV excitation source, Fastie-Ebert optical bench [2], and stepper motor controller/driver [3] for rotating the excitation grating G1.

Emission Monochromator: The emission monochromator will consist of an optical bench with mirrors and a reflective diffraction grating, a linear charge-coupled device (CCD) detector array, and control electronics for digitizing and transmitting data through a serial communication link. The schematic of the emission monochromator, including the proposed optical bench and detector, is shown in Figure 5. The optical bench houses a Czerny-Turner configuration, consisting of an entrance slit, collimating mirror, focusing mirror, and reflective diffraction grating. A linear CCD detector array records the full spectrum of radiation. Control electronics will be utilized for reading the detector and its recorded spectrum. Furthermore, an optical fiber may optionally be connected at the entrance slit to collect and transmit the sample fluorescence to the subsystem. Similar to the excitation monochromator subsystem, it is worthy to mention that all components in the optical bench must be optimized for performance in the UV spectral region, particularly the detector.

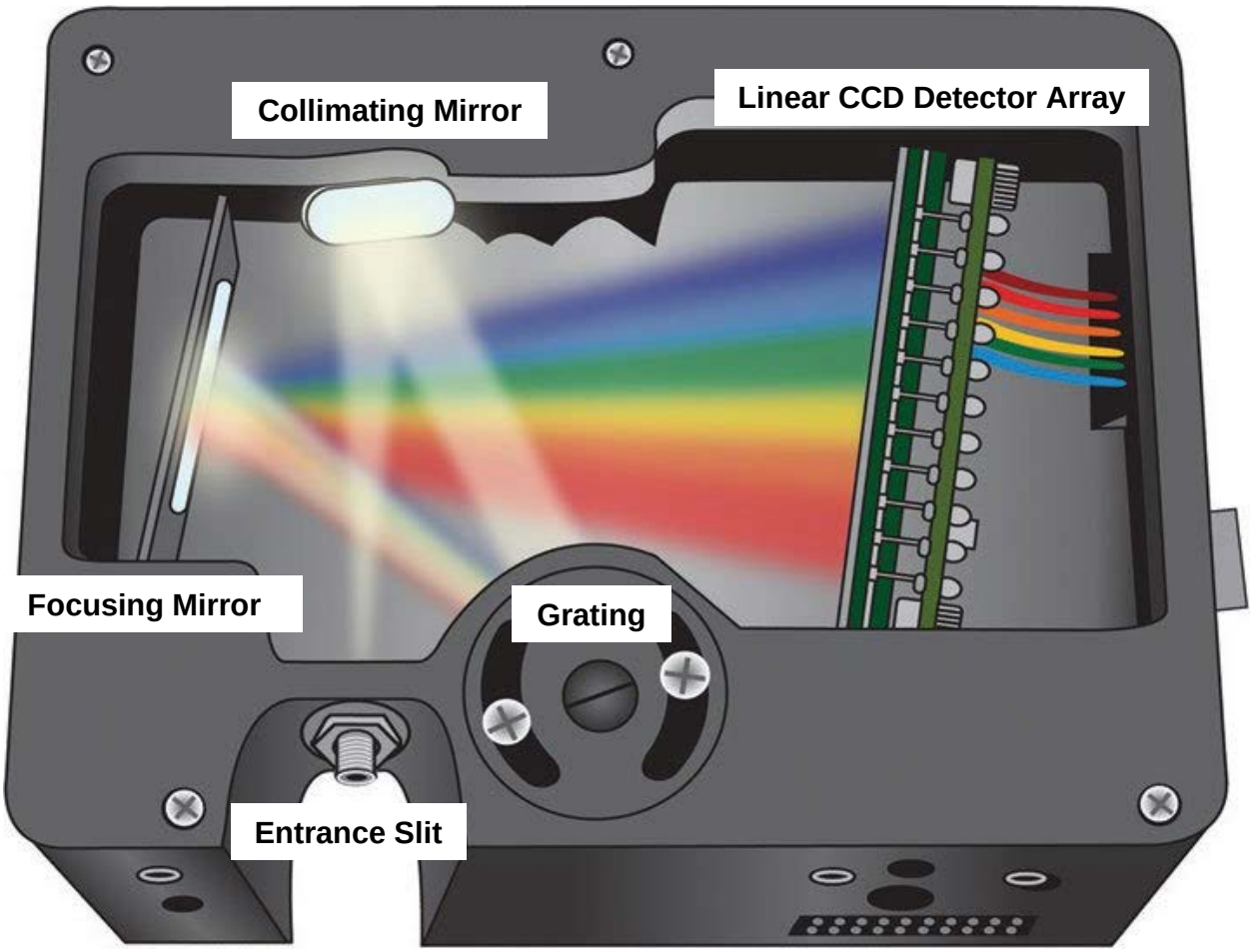

Figure 5: Schematic of the emission monochromator subsystem [4], including the Czerny-Turner optical bench and linear CCD detector array. Additional control electronics will be utilized for reading the detector.

3.4. Modes of Operations

This system shall possess two primary modes of operation as follows:

- In the “Standby” mode of operation, the user may choose to either (1) irradiate the bacterial sample with the on-board UV LED or (2) save, plot, and/or analyze previously recorded spectra. In this mode, no additional spectra can be recorded, although the full capabilities of displaying and processing previous spectra are available.
- In the “Measurement” mode of operation, the system is recording normal fluorescence or synchronous fluorescence spectra, with either the excitation monochromator subsystem or disinfection subsystem exciting the bacterial sample. All other processes are essentially halted until the measurement is either completed or cancelled by the user. In the case of completion, the system will output the desired data, reset the excitation and emission monochromator subsystems, and return to the “Standby” mode of operation. In the case of cancellation, the system will stop recording data,

reset the excitation and emission monochromator subsystems, and return to the “Standby” mode of operation.

3.5. Users

This system is intended to be used by primarily scientists and medical personnel. Scientists may utilize this system for research and development studies in scientific laboratories, while medical personnel may use this system for the rapid assessment and treatment of bacterial infections in such locations as hospitals, clinics, and even remote areas without specialized equipment. Owing to the fact that the system is a scientific instrument, training regarding its installation and usage will be a necessity. Since the system is portable and requires essentially no installation, however, greater emphasis may be placed on its proper usage. Indeed, many of the optical components are rather fragile and sensitive to human interference. Improper usage could therefore deteriorate the quality and performance of the instrument. In the case of medical personnel, who are less likely to be familiar with spectroscopy, additional training and knowledge regarding the spectroscopic techniques employed may also be necessary. Finally, proper training regarding the installation and usage of the software should be provided for all users as well.

3.6. Support

Support for this system will be provided in the form of a detailed user manual providing information on the system installation, usage, and maintenance. In addition, the user manual will provide an extensive discussion on the system’s theory of operation, its various subsystems, and any relevant recommendations for troubleshooting subsystem or system failures. Furthermore, typical use cases will be provided to facilitate understanding of the system. Finally, online support, in the form of a detailed user manual, relevant datasheets, and software, shall be provided as well.

4. Scenario(s)

4.1. Hospitals and Clinics

Due to the rather costly and life-threatening nature of health care-associated infection (HAI), this system would satisfy a critical need in hospitals and clinics. To that effect, this system could be utilized to efficiently detect bacteria present in operating rooms, medical equipment, and other such settings in which conventional methods of diagnosis, namely bacterial plating, would prove inefficient, unfeasible, and ultimately detrimental to those infected. Furthermore, this system offers an alternative means of disinfection, UV light, compared to antibiotics. The system would be able to additionally confirm the success of other forms of sterilization as well.

4.2. Remote Clinics (Field Environments)

In addition to hospital settings, this system would be appropriate for usage in remote clinics, or field environments, which often lack specialized equipment and resources. Instead of transporting bacterial samples from the field to laboratories for conventional diagnostics, which would incur substantial costs in terms of both time and equipment, this system could be utilized for detecting and sterilizing infections *in-situ*. Such measures could be lifesaving in situations requiring prompt, accurate diagnosis. Owing to the fact that the proposed system is portable, it may be taken to virtually any field environment and utilized for the rapid detection and identification of live and dead bacteria.

4.3. Scientific Laboratories

Furthermore, the system may be utilized in controlled environments such as scientific laboratories. Namely, laboratories may utilize the instrument for research and development studies. These studies may be biological in nature as well, ranging anywhere from pathogens to cancer cells, or may be extended to other fields of study, including instrumentation and product development. The system may be, easily, adjusted to operate in the particular spectral region appropriate for a given study. Indeed, as a prototype for a novel spectroscopic instrument, this system may be further refined, improved, and eventually miniaturized for use in, perhaps, daily life itself.

5. Analysis

5.1. Summary of Proposed Improvements

Several major improvements will be provided by the proposed system:

- The system is a novel spectroscopic device capable of performing both normal fluorescence and synchronous fluorescence spectroscopy.
- The system will have an on-board computer capable of performing PCA to identify and distinguish live and dead bacteria.
- The system will be portable and therefore suitable for *in-situ* usage in both controlled environments, such as laboratories and operating rooms, as well as in the field and remote areas.

- The system will offer a rapid means of detection and identification compared to conventional methods of diagnosis, requiring minutes rather than days.
- The system will require minimal additional equipment and thus consume less resources compared to conventional methods of diagnosis.
- The system will be capable of performing bacterial disinfection efficiently and *in-situ*.

5.2. Disadvantages and Limitations

Some disadvantages and limitations that the proposed system will have are as follows:

- The system, as an initial prototype, will not have wireless capabilities.
- The system, as an initial prototype, may not operate on solely batteries.
- The system will be optimized for operation in primarily the UV spectral region alone and may therefore be inappropriate for visible or infrared studies.
- Due to budget constraints, the sensitivity of optical components to UV light may be limited.
- The system may have difficulty in detecting bacterial samples of concentrations within some threshold.
- The system may have difficulty in distinguishing different types of bacteria with similar normal fluorescence and synchronous fluorescence spectra.

5.3. Alternatives

Some alternative solutions to the proposed system are as follows:

- One alternative solution is an automated counter of bacterial colonies on agar plates [5], which could tremendously reduce the time required for bacterial counting; however, owing to the fact that bacterial plating is still performed, this process requires a rather long time for completion and renders it unsuitable for *in-situ* applications.
- Another alternative solution is the staining of bacterial cells with fluorescent dyes such as CTC [6] to similarly accelerate the counting process; while yet again reducing the time required for bacterial counting itself, this process still requires hours, to as long as even days, for completion.
- Furthermore, an alternative solution could simply be to irradiate the suspected region of infection, be it a contaminated surface or wound, with high-intensity UV light for a considerable length of time; while this method will achieve disinfection of the region, it will certainly pose a harm to human beings and may even induce carcinogenic effects over prolonged periods.
- Alternative spectroscopic techniques, such as Raman spectroscopy, absorption spectroscopy, and reflectance spectroscopy, among others, should also be explored for their potential in rapidly identifying and distinguishing live and dead bacteria. The main factors, in comparing various spectroscopic techniques for this purpose, would include the required time for detection, portability, cost, accuracy, and safety in usage, among others.
- Finally, alternative methods of detection which do not rely on spectroscopy should also be explored. In particular, it may be possible to synthesize growth media, besides agar, in which bacteria rapidly colonize. This advancement could possibly accelerate the counting process to a degree which obviates the need for spectroscopy.

5.4. Citations

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FUNCTIONAL SYSTEM REQUIREMENTS

REVISION – 1
4 December 2019

FUNCTIONAL SYSTEM REQUIREMENTS
FOR
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PREPARED BY:

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John Lusher, P.E. Date

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Table of Contents

List of Tables

List of Figures

1. Introduction

1.1. Purpose and Scope

The identification of live and dead bacteria is a rather difficult task requiring extensive time and specialized equipment. Due to the large prevalence of bacterial infections in hospitals, along with growing antibiotic resistance, the inefficiency of current detection methods presents a life-threatening and costly problem. This is especially true in remote locations where such resources are unavailable or unfeasible, thereby necessitating prompt, *in-situ* diagnosis. To address such a pressing need, a portable fluorescence spectrometer will be designed and constructed. The spectrometer will consist of excitation and emission monochromators, a disinfection unit, and an onboard computer with a graphical user interface (GUI). Ultraviolet (UV) light-emitting diodes (LEDs) will be utilized for the excitation, as well as disinfection, of bacterial samples. The normal fluorescence and synchronous fluorescence spectra of bacteria, before and after UV disinfection, may be recorded and processed through principal component analysis (PCA) on a computer. In addition, a GUI will facilitate the acquisition, processing, and display of spectra. Through such means, live and dead bacteria will be rapidly distinguished, within minutes. This specification defines the technical requirements for the development items and support subsystems delivered to the client for the project. Figure 1 shows a representative integration of the project in the proposed Concept of Operations (CONOPS). The verification requirements for the project are contained in a separate Verification and Validation Plan.

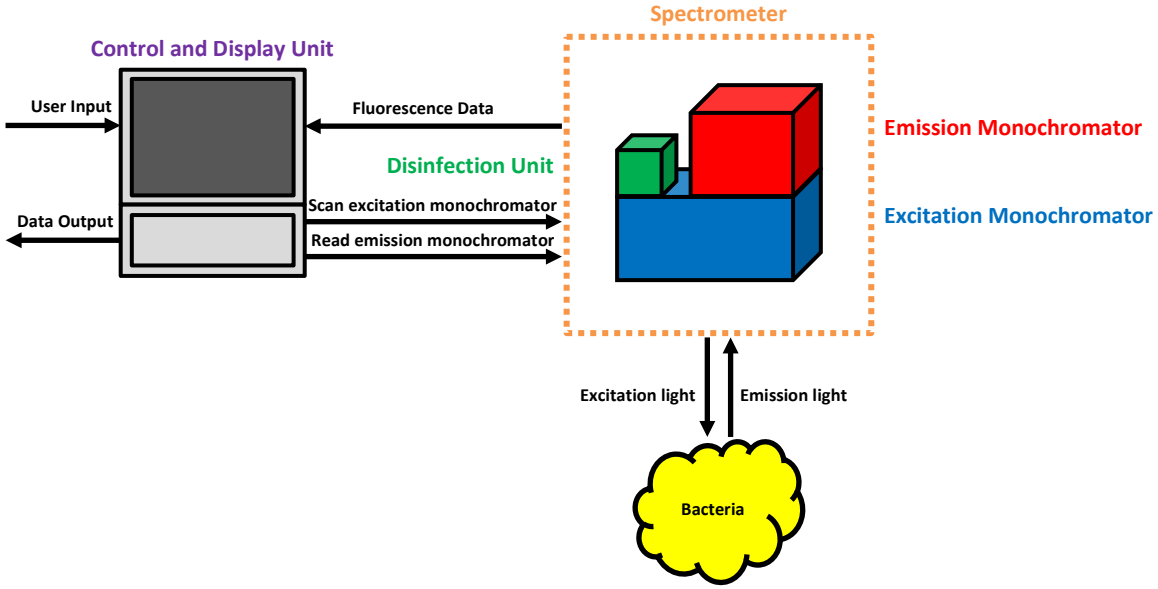

Figure 1: Conceptual Image of Proposed System.

The proposed system shall be capable of performing both normal fluorescence and synchronous fluorescence spectroscopy, as well as UV disinfection, on bacterial samples. The system shall interface with the user through a GUI executing on the onboard computer. All commands issued to the excitation monochromator and emission monochromator, along with any recorded spectral data, shall be transmitted via a serial communication link with the onboard computer. To perform normal fluorescence spectroscopy, the excitation monochromator shall be set to output a preselected excitation wavelength in the UV region.

This wavelength of light shall be transmitted to the bacterial sample, thereby inducing fluorescence. This fluorescence shall then be collected, imaged, and recorded by the emission monochromator. Synchronous fluorescence spectroscopy shall be similarly achieved by scanning, or rotating, the excitation monochromator through a range of excitation wavelengths, with the emission monochromator recording the sample's fluorescence at each given excitation wavelength. The resulting dataset will be a three-dimensional excitation-emission matrix (EEM) from which synchronous spectra shall be easily derived. To perform UV disinfection, the disinfection unit shall be utilized to irradiate bacterial samples with intense UV light. It is worthy to mention that the disinfection unit shall also be utilized to excite bacterial samples and perform normal fluorescence spectroscopy. Finally, the processing and display of recorded spectra, along with PCA, shall be performed and outputted by the control and display unit.

Additionally, the system may be entirely battery-operated. The system may also utilize miniature displays such as the Raspberry Pi Touchscreen Display for interfacing with the user.

1.2. Responsibility and Change Authority

The sole member of the team, Arjun Krishnamoorthi, shall be the team leader. The team leader, Arjun Krishnamoorthi, will be responsible for ensuring all requirements of the project are met. These requirements can only be changed with the approval of the team leader, Arjun Krishnamoorthi, and the project's sponsor, Dr. Peter Rentzepis.

Subsystem	Responsibility
Control and Display Unit	Arjun Krishnamoorthi
Disinfection Unit	Arjun Krishnamoorthi
Emission Monochromator	Arjun Krishnamoorthi
Excitation Monochromator	Arjun Krishnamoorthi

Table 1: Subsystem responsibilities.

2. Applicable and Reference Documents

2.1. Applicable Documents

The following documents, of the exact issue and revision shown, form a part of this specification to the extent specified herein:

Document Number	Revision/Release Date	Document Title
NMCPHC-TM6290.91-2	Revision C – 7/30/2019	Industrial Hygiene Field Operations Manual (Chapter 11 – Ultraviolet Radiation)
MIL-HDBK-263	Revision B – 7/31/1994	Electrostatic Discharge Control Handbook for Protection of Electrical and Electronic Parts, Assemblies and Equipment
IPC A-610E	Revision E – 4/1/2010	Acceptability of Electronic Assemblies
MIL-STD-464	Revision C – 2/1/2010	Electromagnetic Environmental Effects Requirements for Systems

Table 2: Applicable Documents.

2.2. Reference Documents

In addition to the documents noted in Table 3 below, refer to Section 2.3 in the CONOPS document. The following documents are reference documents utilized in the development of this specification. These documents do not form a part of this specification and are not controlled by their reference herein.

Document Number	Revision/Release Date	Document Title
C2-2017	2017	National Electrical Safety Code ®

Table 3: Reference Documents.

2.3. Order of Precedence

In the event of a conflict between the text of this specification and an applicable document cited herein, the text of this specification takes precedence without any exceptions.

All specifications, standards, exhibits, drawings or other documents that are invoked as “applicable” in this specification are incorporated as cited. All documents that are referred to within an applicable report are considered to be for guidance and information only, except ICDs that have their relevant documents considered to be incorporated as cited.

3. Requirements

This section defines the minimum requirements that the development item(s) must meet. The requirements and constraints that apply to performance, design, interoperability, reliability, etc., of the system, are covered.

3.1. System Definition

Bacterial infections currently account for a considerable percentage of deaths in the United States. The threat of bacterial infection is compounded by the fact that current detection and identification procedures are rather costly in terms of both time and equipment. Optical spectroscopy, coupled with multivariate analysis techniques such as PCA, has offered a fast, noninvasive, and accurate means of identifying various bacterial strains and concentrations. Our own studies have utilized normal fluorescence and synchronous fluorescence spectroscopy, along with PCA, to identify and distinguish live and dead bacteria following both UV and antibiotic treatment. To that effect, the proposed system offers a novel, efficient, and portable means of identifying and distinguishing live and dead bacteria in controlled environments, such as laboratories, and in the field. The portable fluorescence spectrometer shall consist of four subsystems: (1) a control and display unit, (2) a disinfection unit, (3) an excitation monochromator, and (4) an emission monochromator. The block diagram of the overall system is shown in Figure 2 below.

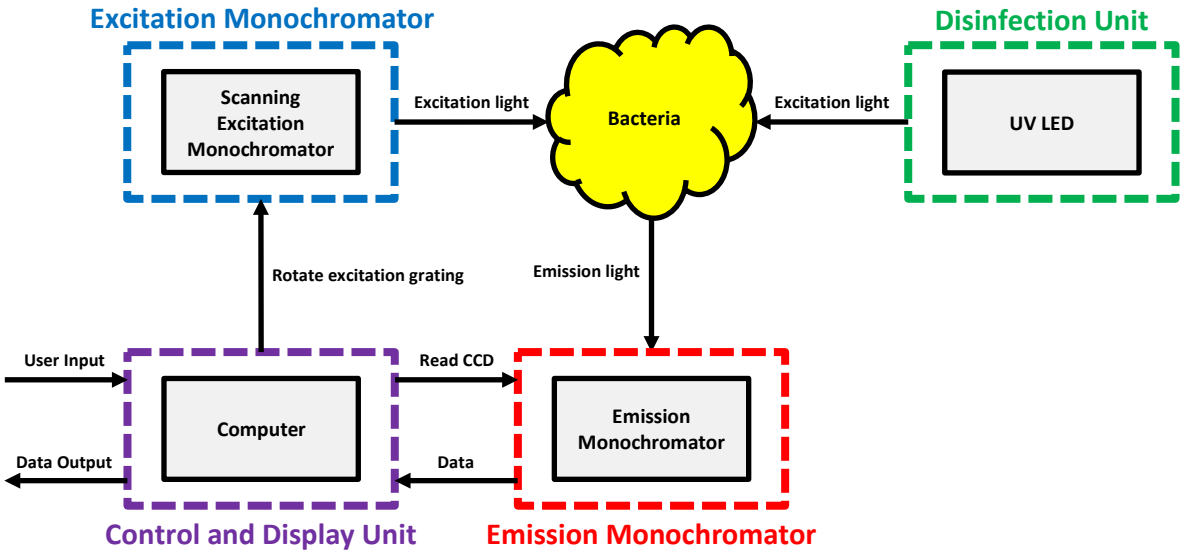

Figure 2: Block Diagram of System.

The control and display unit subsystem shall consist of a GUI on an onboard computer which is responsible for interfacing with the user, communicating with both the excitation monochromator and emission monochromator subsystems, and processing and displaying recorded spectral data. This subsystem shall interface with both the excitation monochromator and emission monochromator subsystems through a common serial communication link, or cable, which may allow control signals to be easily issued to each respective subsystem. Depending on the user input, the system will enter either a “Standby” or “Measurement” mode of operation. In the “Standby” mode of operation, the user may

choose to either (1) irradiate the bacterial sample with the on-board UV LED or (2) save, plot, and/or analyze previously recorded fluorescence spectra. In this mode, no additional spectra can be recorded, although the full capabilities of displaying and processing previously recorded spectra, in addition to performing PCA for distinguishing live and dead bacteria, are available. Conversely, in the “Measurement” mode of operation, the user may perform either normal fluorescence or synchronous fluorescence measurements. In this mode, relevant inputs from the user shall include the following: (1) the type of spectrum (normal fluorescence or synchronous fluorescence) to record, (2) the initial and final excitation wavelengths of the spectrum (in the case of synchronous fluorescence measurements), and (3) the acquisition parameters (integration time and number of spectra to average) for the emission monochromator. Following these user inputs, the unit will issue control signals for initializing the excitation and emission monochromators and initiating the spectrum acquisition. Furthermore, this unit will be responsible for receiving, processing, and displaying spectral data recorded by the emission monochromator. A detailed flowchart elucidating this process is provided in Figure 3 below.

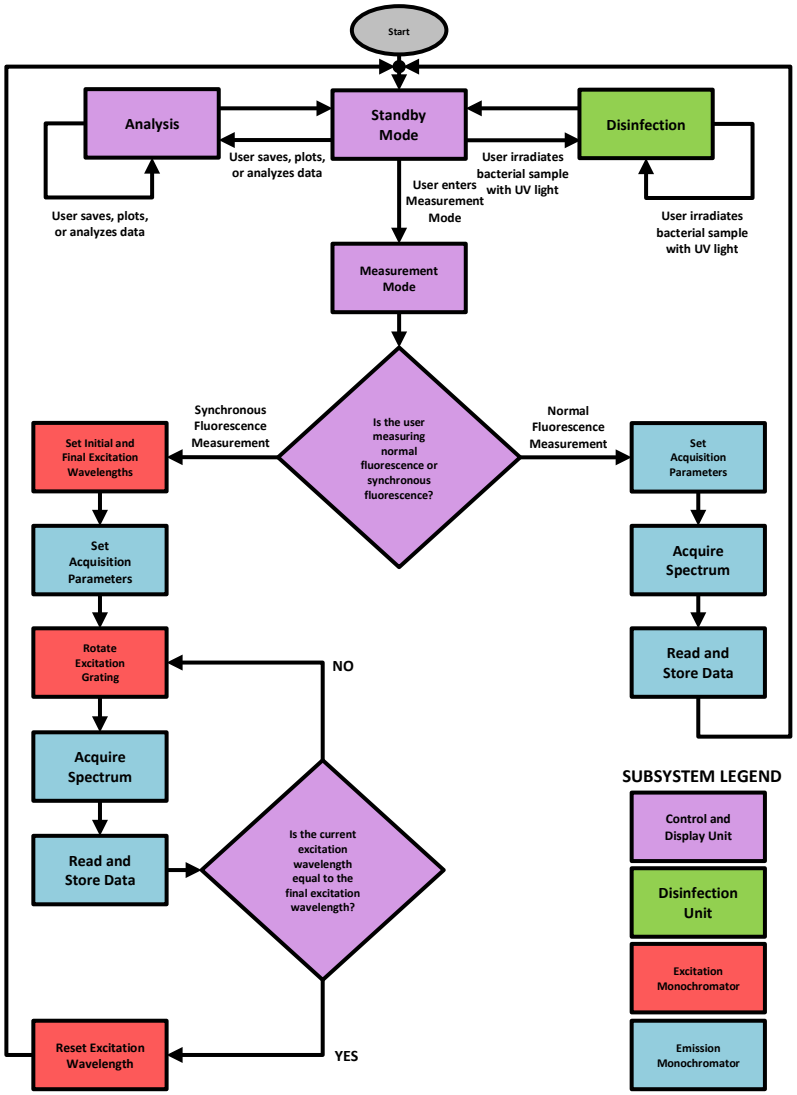

Figure 3: System flowchart detailing the various modes of operation.

The disinfection unit shall consist of a high-power, miniature UV LED which may be used to efficiently inactivate bacterial samples *in-situ*. The intensity of the UV LED may be increased by coupling it with a small ball lens, thereby focusing and impinging the light onto the sample. Besides this function, the UV LED shall also serve as an excitation source and therefore be directly coupled, through an optical fiber, with the emission monochromator to record the normal fluorescence spectrum of the bacterium at the UV LED's peak wavelength. Owing to the fact that the UV LED is not coupled to a monochromator, which would result in a reduction in the LED's output intensity, this could be a viable alternative in settings where the sample concentration or fluorescence signal is low. In fact, it is expected that the normal fluorescence spectra recorded through this unit, before and after UV disinfection, will be sufficient in distinguishing live and dead bacteria. Finally, the UV LED shall be powered by a benchtop DC power supply, although it may eventually be battery-operated.

Furthermore, the excitation monochromator shall consist of a high-power, miniature UV LED as an excitation source, an optical bench with mirrors and a reflective diffraction grating coupled with a stepper motor, and a stepper motor controller and driver for rotating the excitation grating. Refer to Section 3.3 in the CONOPS document for the schematic of the excitation monochromator, including the proposed optical bench and components. In brief, the optical bench shall house a Fastie-Ebert configuration consisting of two folding mirrors, a single curved mirror, and a reflective diffraction grating. The high-power, miniature UV LED excitation source shall be coupled to the bench at the entrance slit, whereupon the input, broadband UV light will be collimated and directed towards the reflective grating, which will diffract and disperse the light into its component wavelengths. The dispersed light will then be reflected and focused on the exit slit. The particular wavelength of the dispersed light focused on the exit slit will be dependent on the angular positioning of the excitation grating. The excitation monochromator shall interface with the control and display unit through a serial communication link attached to the serial port of the onboard controller. Commands shall be issued to the controller and driver when rotation of the excitation grating is necessary. Depending on the particular user input provided, a stepper motor shall then rotate the grating to a desired output wavelength or, alternatively, scan through a range of excitation wavelengths (as desired in a synchronous fluorescence spectrum, for example). An optical fiber may optionally be connected at the exit slit to transmit the excitation light to the sample; alternatively, to maximize the excitation intensity, the sample may be directly situated at the exit slit itself. All components in the optical bench shall be optimized for performance in the UV spectral region. Finally, the stepper motor controller and driver shall be powered by a benchtop DC power supply, although they may eventually be battery-operated.

Lastly, the emission monochromator shall consist of an optical bench with mirrors and a reflective diffraction grating, a linear charge-coupled device (CCD) detector array, and control electronics for digitizing and transmitting data through a serial communication link with the onboard computer. Refer to Section 3.3 in the CONOPS document for the schematic of the emission monochromator, including the proposed optical bench and detector. The optical bench shall house a Czerny-Turner configuration, consisting of an entrance slit, collimating mirror, focusing mirror, and reflective diffraction grating. A linear CCD detector array shall record the full spectrum of emitted radiation for both normal fluorescence and synchronous fluorescence measurements. The emission monochromator shall interface with the control and display unit through a serial communication link attached to the serial port of the onboard control electronics. Commands shall be issued to the emission monochromator for (1) setting acquisition parameters (e.g., integration or exposure time, number of spectra to average,

calibration of the detector, etc.) and (2) reading the detector and its recorded spectrum. Furthermore, an optical fiber shall be connected at the entrance slit to collect and transmit the sample fluorescence, induced by either the excitation monochromator or disinfection subsystem, to the optical bench. Similar to the excitation monochromator subsystem, all components in the optical bench shall be optimized for performance in the UV spectral region, including the detector. Finally, the emission monochromator shall be powered by a benchtop DC power supply, although it may eventually be battery-operated.

3.2. Characteristics

3.2.1. Functional / Performance Requirements

3.2.1.1. General Spectroscopic Requirements

The overall system shall be capable of performing both normal fluorescence and synchronous fluorescence spectroscopy in the UV region over an excitation range of at least 200-400 nm and an emission range of at least 280-500 nm. All optical components, including mirrors, lenses, gratings, and optical fibers, shall be optimized for operation in the UV region.

> *Rationale: These are the basic spectroscopic capabilities which the system is expected to perform. Normal fluorescence spectroscopy will allow the system to detect bacteria and, when coupled with PCA, distinguish live and dead bacteria. Synchronous fluorescence spectroscopy will allow the system to resolve the fluorescence bands of cellular components such as tryptophan and tyrosine. In addition to rendering the system as the first of its kind, this particular capability may allow the system to identify and distinguish specific bacterial species. The excitation and emission ranges noted overlap with the most intense absorption and fluorescence bands of bacteria. Finally, the optimization of optical components for operation in the UV region is a necessity to maximize the SNR of the normal fluorescence and synchronous fluorescence measurements.*

3.2.1.2. Control and Display Unit Requirements

The onboard control and display unit shall be a compact laptop capable of communicating with the excitation and emission monochromator subsystems via its USB port. In addition, the laptop shall be executing a MATLAB GUI to accept user inputs and display data outputs.

> *Rationale: The system shall utilize a serial communication link between the onboard laptop and the respective subsystems to execute normal fluorescence and synchronous fluorescence scans. Namely, commands will be issued through the GUI for scanning the excitation monochromator, in addition to acquiring spectra from the emission monochromator.*

3.2.1.3. Disinfection Unit Light Source

The disinfection unit shall utilize a single UVB or UVC LED with the following nominal characteristics: (1) a peak wavelength in the range of 270-290 nm, (2) an output power of at least 1 mW, and (3) a full width at half maximum (FWHM) of no more than 20 nm.

> *Rationale: The absorption maximum of bacteria is at approximately 280 nm, which ensures that the UVC LED, owing to its high output power at that wavelength, will induce the most*

intense, detectable fluorescence during normal fluorescence measurements. Furthermore, the usage of a UVB or UVC LED, as opposed to a UVA LED, maximizes the germicidal effectiveness of the unit. Lastly, the rather narrow bandwidth minimizes any possible harm to users due to the UVB or UVC radiation.

3.2.1.4. Scanning Capability of Excitation Monochromator

The excitation monochromator shall be capable of scanning its output wavelength by means of a stepper motor coupled with a controller and driver.

Rationale: The excitation monochromator must be capable of scanning its output wavelength in order to achieve synchronous fluorescence measurements. Without the scanning capability, the system will be unable to perform synchronous fluorescence measurements.

3.2.1.5. Operating Range of Excitation Monochromator

The excitation monochromator shall have an operating range of at least 200-400 nm with its maximum diffraction efficiency in the UV region.

Rationale: This minimum operating range is more than sufficient for resolving the fluorescence spectra of bacteria, which are excited primarily in the range of approximately 260-300 nm due to nucleic acids and proteins. Maximizing the diffraction efficiency in the UV region ensures minimal loss is present when coupling the UV excitation source to the monochromator.

3.2.1.6. Excitation Monochromator Light Source

The excitation monochromator shall utilize at least one UVB or UVC LED with the following nominal characteristics: (1) a peak wavelength in the range of 270-290 nm, (2) an output power of at least 10 mW, and (3) a full width at half maximum (FWHM) of at least 10 nm. Other UVB or UVC LEDs, with varying peak wavelengths, output powers, and FWHMs, may be utilized to induce more intense bacterial fluorescence for synchronous fluorescence measurements.

Rationale: The nominal output wavelength is again chosen in the region 270-290 nm, as this matches the region of maximum absorption for bacteria. In addition, the nominal power is chosen to be at least 10 times greater than that for the UV LED in the disinfection unit to account for optical losses (due to coupling and diffraction) associated with the excitation monochromator itself. Finally, it is now desirable to maximize the FWHM of the light source, as this provides a wider range of excitation wavelengths over which to scan. In the scenario that the output power is insufficient for inducing detectable bacteria fluorescence, more UVB or UVC LEDs may be utilized.

3.2.1.7. Operating Range of Emission Monochromator

The emission monochromator shall have an operating range of at least 280-600 nm with its maximum diffraction efficiency in the UV region.

Rationale: This minimum operating range is more than sufficient for resolving the fluorescence spectra of bacteria, which fluoresce primarily in the range of approximately 300-500 nm due to nucleic acids and proteins. Maximizing the diffraction efficiency in the UV region ensures the signal-to-noise ratio (SNR) in that region is maximized as well.

3.2.1.8. Emission Monochromator Detector Type and Sensitivity

The emission monochromator shall utilize a linear CCD detector array for recording normal fluorescence and synchronous fluorescence spectra. Furthermore, the linear CCD detector array shall possess sufficient sensitivity and SNR specifications in the UV region to record bacterial fluorescence with low integration or exposure times (< 60 seconds) and minimal averaging (< 10 spectra averaged per scan).

> *Rationale: Owing to the fact that a linear CCD detector array is capable of detecting and recording a full spectrum of emitted radiation, as opposed to a single wavelength, this type of detector would support more efficient measurement. Furthermore, this type of detector operates on low DC voltages, typically +5 VDC, and is quite compact and portable. Moreover, the linear CCD detector array should be sufficiently sensitive in detecting bacterial fluorescence so that low integration times and minimal averaging are required (the numbers provided may be subject to change depending on the sensitivity of components). This ensures that the system is quite efficient in detecting and distinguishing live and dead bacteria and, consequently, does not require the user to wait for long periods of time.*

3.2.1.9. Detection and Classification Time

The overall system shall be capable of recording the normal fluorescence of bacteria in no more than 30 seconds. Synchronous fluorescence measurements shall take no longer than 10-15 minutes, depending on the particular range of excitation wavelengths being scanned. Subsequent PCA processing shall take no more than a few minutes.

> *Rationale: The system is expected to perform normal fluorescence measurements quite rapidly through the onboard disinfection unit, as the high-power UVC LED is directly coupled to the sample without any monochromator. Synchronous fluorescence measurements will naturally take longer to complete due to the range of excitation wavelengths being scanned, the weaker excitation intensity and fluorescence signal, and, consequently, the longer integration or exposure time required for measurement. PCA processing of the recorded spectral data should also be quite rapid due to the onboard computer.*

3.2.2. Physical Characteristics

3.2.2.1. Mass

The mass of the portable fluorescence spectrometer may be less than or equal to 20 kilograms (~ 45 lbs.).

> *Rationale: This represents an approximate upper bound on the mass (and weight) of typical transportable spectrometers. It is expected that the system will weigh considerably less than this figure. As this is the first system of its kind, this requirement may change.*

3.2.2.2. Volume Envelope

The volume envelope of the portable fluorescence spectrometer may be less than or equal to 24 inches in height, 12 inches in width, and 8 inches in length.

> *Rationale: This represents an approximate upper bound on the height, width, and length of typical transportable spectrometers. It is expected that the system will be smaller along all dimensions. As this is the first system of its kind, this requirement may change.*
> *.*

3.2.2.3. Mounting

The mounting information for the portable fluorescence spectrometer shall be captured in the corresponding system ICD.

Rationale: As the portable fluorescence spectrometer consists of multiple subsystems, the interface between the subsystems includes mechanical, electrical, and thermal details.

3.2.2.4. Water Resistance

The subsystems of the portable fluorescence spectrometer, including its various optical components, shall be enclosed in a sealed environment to prevent excess moisture accumulation.

Rationale: Water and moisture deteriorate the quality of optical components and, in addition, may destroy any exposed electrical components.

3.2.3. Electrical Characteristics

3.2.3.1. Inputs

a. The presence or absence of any combination of the input signals in accordance with ICD specifications applied in any sequence shall not damage the portable fluorescence spectrometer, reduce its life expectancy, or cause any malfunction, either when the unit is powered or when it is not.

b. No sequence of command shall damage the portable fluorescence spectrometer, reduce its life expectancy, or cause any malfunction.

Rationale: The design of the system should limit the chance of damage or malfunction by user/technician error.

3.2.3.1.1 Power Consumption

a. The maximum peak power of the system may not exceed 10 watts.

Rationale: This figure is based on the nominal operating voltages and currents of the various subsystems and represents an upper bound on the expected power consumption of the device. Depending on the performance of components, though, the upper bound of the power consumption may be extended to as high as 20 W. This may be required in the case that the controller and driver performance is poor or the UV LED output power is too low to induce detectable fluorescence. It is worthy to mention that there is no requirement which must necessarily be met for this characteristic.

3.2.3.1.2 Input Voltage Level

The input voltage level for each subsystem of the portable fluorescence spectrometer will be in the nominal range of +5 VDC to +12 VDC. The upper bound of this range may be extended to as high as +32 VDC.

Rationale: Depending on the particular subsystem, the input DC voltage is expected to be in the nominal range of +5 to +12 VDC. The emission monochromator control electronics shall require +5 VDC, and the excitation monochromator controller and driver shall require +8 VDC to +12 VDC. The onboard UV LEDs, for both the excitation monochromator and disinfection unit subsystems, are expected to require no higher than +12 VDC as well. Depending on the performance of components, though, the upper bound of the input voltage level may be extended to as high as +32 VDC. This may be required in the case that the controller and driver performance is poor or the UV LED output power is too low to induce detectable fluorescence. It is worthy to mention that there is no requirement which must necessarily be met for this characteristic.

3.2.3.1.3 External Commands

The portable fluorescence spectrometer shall document all external commands in the appropriate ICD.

Rationale: The ICD will capture all interface details from the low level electrical to the high-level packet format.

3.2.3.2. Outputs

3.2.3.2.1 Data Output

The portable fluorescence spectrometer shall include a GUI for users to initiate normal fluorescence and synchronous fluorescence measurements, save and view recorded spectra, and perform PCA to identify and distinguish live and dead bacteria following UV disinfection. The GUI may also consist of additional data processing capabilities, such as curve fitting and peak extraction.

> *Rationale: The primary function of the GUI, in addition to interfacing with the excitation and emission monochromator subsystems, is to allow the user to view, analyze, and save the recorded spectral data. PCA will be the primary means of analysis for distinguishing live and dead bacteria, but additional processing capabilities may be added as well. Executing such an interface on a compact, onboard laptop provides a rather easy means for users to collect, view, and process data.*

3.2.3.2.2 Diagnostic Output

The portable fluorescence spectrometer may include a diagnostic interface for error logging and handling.

> *Rationale: This additional capability could prove useful in circumstances where certain components or routines fail during system operation.*

3.2.3.3. Connectors

The portable fluorescence spectrometer shall follow the relevant guidelines set forth in the National Electric Code regarding electrical connections.

> *Rationale: Following such guidelines will ensure that the system conforms to connection standards.*

3.2.3.4. Wiring

The portable fluorescence spectrometer shall follow the relevant guidelines set forth in the National Electric Code regarding electrical wiring.

> *Rationale: Following such guidelines will ensure that the system conforms to wiring standards.*

3.2.4. Environmental Requirements

The portable fluorescence spectrometer shall be designed to withstand and operate in the environments and laboratory tests specified in the following section.

> *Rationale: Satisfying this requirement ensures that the system may be used in both controlled environments, such as laboratories, operating rooms, and clinics, as well as in the field.*

3.2.4.1. Pressure (Altitude)

The portable fluorescence spectrometer may be designed to withstand and operate in air pressures of altitudes from sea level (0 feet) to an altitude of 4000 feet.

> *Rationale: Satisfying this requirement ensures that the system may be used in both controlled environments, such as laboratories, operating rooms, and clinics, as well as in the field.*

3.2.4.2. Thermal

The portable fluorescence spectrometer may be able to function properly in an environment with temperatures ranging from 40° F to 120° F.

> *Rationale: Satisfying this requirement ensures that the system may be used in both controlled environments, such as laboratories, operating rooms, and clinics, as well as in the field.*

3.2.4.3. External Contamination

All subsystems and optical components of the portable fluorescence spectrometer shall be enclosed to avoid external contamination from dust, moisture, and other environmental contaminants. In particular, each subsystem, and its respective optical and electrical components, shall be enclosed in a sturdy electronics project box.

> *Rationale: Many, if not all, of the optical components are rather fragile and sensitive to external interference and contamination. Contamination, in particular, would deteriorate the quality of optical components and may interfere with electrical components as well. In turn, the operation of the system could be compromised.*

3.2.4.4. Rain

The portable fluorescence spectrometer may be designed to withstand and operate in light rain environments.

> *Rationale: Satisfying this requirement would allow the system to be deployed in a wider variety of environments.*

3.2.4.5. Humidity

The portable fluorescence spectrometer may be designed to function properly in an environment with relative humidity ranging from 0% to 100%.

> *Rationale: Satisfying this requirement would allow the system to be deployed in a wider variety of environments.*

3.2.5. Failure Propagation

The portable fluorescence spectrometer shall not allow propagation of faults beyond the portable fluorescence spectrometer interface.

3.2.5.1. Failure Detection, Isolation, and Recovery (FDIR)

The portable fluorescence spectrometer shall have a user manual detailing relevant procedures for replacing optical components, such as the UV LEDs, linear CCD detector array, and optical fibers, among others, in the event that a particular component has either (a) reached the end of its lifetime of operation, (b) been mishandled and possibly broken, or (c) stopped satisfying the functional requirements of the system. In addition, tips and procedures for monitoring and maintaining the quality and operation of the optical components would be provided in the user manual. Furthermore, the system may have additional built-in tests for monitoring successful operation. In particular, the system may be capable of monitoring transmission failures in the serial communication link during operation. This may be accomplished through a watchdog timer which detects and signals when the system malfunctions. This could be particularly useful in the scenario when a spectrum measurement fails; the system could simply stop the measurement and reset the excitation and emission monochromator subsystems. Other features, such as subsystem initialization and validation upon turning the system on, along with internally tracking the number of hours each subsystem has been on, could further substantiate the system's capabilities.

3.2.5.1.1 Built In Test (BIT)

The portable fluorescence spectrometer may have an internal subsystem that will generate test signals and evaluate the subsystem responses and determine if there is a failure.

3.2.5.1.1.1 BIT Critical Fault Detection

The BIT should be able to detect a critical fault in the portable fluorescence spectrometer 95 percent of the time.

> *Rationale: This requirement would permit the system to detect critical faults and inform the user.*

3.2.5.1.1.2 BIT False Alarms

The BIT may have a false alarm rate of less than 5 percent.

> *Rationale: This requirement would limit the false alarm rate and save the user time.*

3.2.5.1.1.3 BIT Log

The BIT may save the results of each test to a log that may be stored in the onboard computer for retrieval and clearing by maintenance personnel.

> *Rationale: This requirement would allow the user to monitor failures over time and note any pertinent trends.*

3.2.5.1.2 Isolation and Recovery

The portable fluorescence spectrometer should provide for fault isolation and recovery by enabling subsystems to be reset or disabled based upon the result of the BIT.

> *Rationale: In the case of a fatal system error, the system may need to be fully reset.*

4. Support Requirements

The portable fluorescence spectrometer, in its current scaled-down, prototype form, shall consist of (1) excitation monochromator (all optical components, namely the mirrors, diffraction grating, and stepper motor, included), (1) emission monochromator (all optical components, namely the mirrors, diffraction grating, and linear CCD detector array, included), (1) disinfection unit (high-power, miniature UVC LED included), (1) GUI executable file, (1) stepper motor controller and driver for scanning the excitation monochromator, and at least (1) additional UVC LED to be used as an excitation source with the excitation monochromator. All subsystems and optical components shall be enclosed in an electronics project box. No onboard computer, however, will be directly provided to users. Therefore, users must provide both the onboard control and display unit and, in addition, DC power to the overall device. It is worthy to mention that the chosen control and display unit must be capable of executing the provided software. Technical support service may be provided in initially turning on the device and executing the software, along with reviewing the optical layout and spectroscopic capabilities of the system. Warranty will be of relevance in the scenario that any subsystems are uncalibrated, fail to meet the functional requirements of the system, or are simply broken and unusable. Issues may be resolved in the field by sending a trained technician or assisting through a service call.

Appendix A: Acronyms and Abbreviations

Below is a list of common acronyms and abbreviations used in this project.

BIT	Built-In Test
CCA	Circuit Card Assembly
EMC	Electromagnetic Compatibility
EMI	Electromagnetic Interference
EO/IR	Electro-optical Infrared
FOR	Field of Regard
FOV	Field of View
GPS	Global Positioning System
GUI	Graphical User Interface
Hz	Hertz
ICD	Interface Control Document
kHz	Kilohertz (1,000 Hz)
LCD	Liquid Crystal Display
LED	Light-emitting Diode
mA	Milliamp
MHz	Megahertz (1,000,000 Hz)
MTBF	Mean Time Between Failure
MTTR	Mean Time to Repair
mW	Milliwatt
PCA	Principal Component Analysis
PCB	Printed Circuit Board
RMS	Root Mean Square
SNR	Signal-to-Noise Ratio
TBD	To Be Determined
TTL	Transistor-Transistor Logic
USB	Universal Serial Bus
UV	Ultraviolet
VME	VERSA-Module Europe

Appendix B: Definition of Terms

Normal Fluorescence Spectroscopy:
A spectroscopic technique that analyzes the fluorescence from a sample.

Synchronous Fluorescence Spectroscopy:
A spectroscopic technique that resolves the fluorescence of individual components in a mixture.

Principal Component Analysis (PCA):
A multivariate analysis technique that finds the regions of greatest variation within a dataset.

Instrument for the Identification of Live and Dead Bacteria

Arjun Krishnamoorthi

INTERFACE CONTROL DOCUMENT

REVISION – 1
4 December 2019

INTERFACE CONTROL DOCUMENT
FOR
Instrument for the Identification of Live and Dead Bacteria

PREPARED BY:

_____
Arjun Krishnamoorthi Date

APPROVED BY:

_____
Arjun Krishnamoorthi Date

_____
John Lusher II, P.E. Date

_____
T/A Date

Change Record

Rev.	Date	Originator	Approvals	Description
-	9/30/2019	Arjun Krishnamoorthi		Draft Release
1	12/4/2019	Arjun Krishnamoorthi		Revision 1

Table of Contents

List of Tables

List of Figures

1. Overview

The Interface Control Document (ICD) for the portable fluorescence spectrometer will provide additional detail on how the subsystems noted in the Concept of Operations (CONOPS) and the Functional System Requirements (FSR) documents will be produced. Namely, the ICD will include physical descriptions and specifications for each of the subsystems, in addition to providing details regarding the physical, thermal, electrical, and communication interfaces between the subsystems. To that effect, the document will thoroughly explain how the subsystems will successfully interface together to achieve the goals and functional requirements noted in the CONOPS and FSR documents for the overall system.

2. References and Definitions

2.1. References

Refer to Section 2.3 of the CONOPS document.

2.2. Definitions

CCA	Circuit Card Assembly
LED	Light-emitting Diode
mA	Milliamp
mW	Milliwatt
MHz	Megahertz (1,000,000 Hz)
PCA	Principal Component Analysis
TBD	To Be Determined
TTL	Transistor-Transistor Logic
UV	Ultraviolet
VME	VERSA-Module Europe

3. Physical Interface

3.1. Weight

3.1.1. Weight of control and display unit

The current control and display unit will be a Dell Precision 5510 Mobile Workstation Laptop. This laptop is rather compact and weighs less than five pounds. The weight specification for the unit is noted in Table 1 below.

Component	Weight
Dell Precision 5510 Mobile Workstation Laptop	4.4 lbs.
Total	4.4 lbs.

Table 1: Weight Specifications for the Control and Display Unit.

3.1.2. Weight of disinfection unit

The weight specifications for the disinfection subsystem are noted in Table 2 below. These specifications include the weight of the enclosure for the unit, as well as the weight of the UV LED and heatsink.

Component	Weight
Enclosure	Estimate: 1 lbs.
UV LED and Heatsink	0.1875 lbs.
Total	1.1875 lbs.

Table 2: Weight Specifications for the Disinfection Unit.

3.1.3. Weight of excitation monochromator

The weight specifications for the excitation monochromator subsystem are noted in Table 3 below. These specifications include the weights of the proposed monochromator itself (including its enclosure, mirrors, diffraction grating, and stepper motor), the stepper motor controller and driver, the UV LED excitation source and heatsink, the electronics project box for enclosing all components, and the aluminum plate for mounting all components in the project box.

Component	Weight
Monochromator (includes enclosure, mirrors, diffraction grating, and stepper motor)	1.9 lbs.
Controller and Driver	0.5 lbs.
UV LED and Heatsink	0.1875 lbs.
Electronics Project Box	Estimate: 1.5 lbs.
Aluminum Mounting Plate	Estimate: 0.5 lbs.
Total	4.5875 lbs.

Table 3: Weight Specifications for the Excitation Monochromator.

3.1.4. Weight of emission monochromator

The weight specifications for the emission monochromator subsystem are noted in Table 4 below. These specifications include the weights of the proposed monochromator itself (including its enclosure, mirrors, diffraction grating, linear CCD detector array, and control electronics), the electronics project box for enclosing all components, and the aluminum plate for mounting all components in the project box.

Component	Weight
Monochromator (includes enclosure, mirrors, diffraction grating, linear CCD detector array, and control electronics)	Estimate: 1 lbs.
Electronics Project Box	Estimate: 0.75 lbs.
Aluminum Mounting Plate	Estimate: 0.5 lbs.
Total	2.25 lbs.

Table 4: Weight Specifications for the Emission Monochromator.

3.1.5. Weight of overall system

The weight specifications for the overall system are summarized in Table 5 below. These specifications include the respective weights of each of the subsystems.

Subsystem	Weight
Control and Display Unit	4.4 lbs.
Disinfection Unit	1.1875 lbs.
Excitation Monochromator	4.5875 lbs.
Emission Monochromator	2.25 lbs.
Total	12.425 lbs.

Table 5: Weight Specifications for the Overall System.

Clearly, the overall system is quite portable and may easily be transported to the field.

3.2. Dimensions

3.2.1. Dimensions of control and display unit

As noted previously, the current control and display unit will be a Dell Precision 5510 Mobile Workstation Laptop. The dimensions of the unit are noted in Table 6 below.

Subsystem	Length (inches)	Width (inches)	Height (inches)
Control and Display Unit	14.06	9.27	0.66

Table 6: Dimensions of the Control and Display Unit.

3.2.2. Dimensions of disinfection unit

The dimensions of the disinfection unit will be determined by the dimensions of the enclosure for the unit. The dimensions of the unit are noted in Table 7 below.

Subsystem	Length (inches)	Width (inches)	Height (inches)
Disinfection Unit	Estimate: 3	Estimate: 3	Estimate: 3

Table 7: Dimensions of the Disinfection Unit.

3.2.3. Dimensions of excitation monochromator

The dimensions of the excitation monochromator will be determined by the dimensions of the electronics project box enclosing the entire subsystem. The dimensions of the subsystem are noted in Table 8 below.

Subsystem	Length (inches)	Width (inches)	Height (inches)
Excitation Monochromator	Estimate: 10	Estimate: 6	Estimate: 4

Table 8: Dimensions of the Excitation Monochromator.

3.2.4. Dimensions of emission monochromator

The dimensions of the emission monochromator will be determined by the dimensions of the electronics project box enclosing the entire subsystem. The dimensions of the subsystem are noted in Table 9 below.

Subsystem	Length (inches)	Width (inches)	Height (inches)
Emission Monochromator	Estimate: 8	Estimate: 5	Estimate: 3

Table 9: Dimensions of the Emission Monochromator.

3.2.5. Dimensions of overall system

The dimensions for the overall system are summarized in Table 10 below. These specifications include the respective dimensions of each of the subsystems.

Subsystem	Length (inches)	Width (inches)	Height (inches)
Control and Display Unit	14.06	9.27	0.66
Disinfection Unit	Estimate: 3	Estimate: 3	Estimate: 3
Excitation Monochromator	Estimate: 10	Estimate: 6	Estimate: 4
Emission Monochromator	Estimate: 8	Estimate: 5	Estimate: 3

Table 10: Dimensions of the Overall System.

3.3. Mounting Locations

3.3.1. Mounting of UV LED in Disinfection Unit

The UV LED in the disinfection unit will be mounted on either the side or bottom surface of the disinfection unit. Owing to the rather compact size of the UV LED, it is not necessary to

affix it with nails or screws. Mounting will instead be accomplished through sticky thermal adhesive tape. This will serve the dual purpose of mounting the UV LED in the disinfection unit and promoting efficient heat dissipation.

3.3.2. Mounting of Excitation Monochromator Components

The excitation monochromator subsystem components will all be mounted in a common electronics project box. This includes the excitation monochromator itself (including its enclosure, mirrors, diffraction grating, and stepper motor), the stepper motor controller and driver, and the UV LED excitation source and heatsink. This will be accomplished by drilling threaded holes in an aluminum mounting plate and utilizing set screws to mount and secure components in the project box.

3.3.3. Mounting of Emission Monochromator Components

The emission monochromator subsystem components will all be mounted in a common electronics project box. This includes the emission monochromator itself, along with its enclosure, mirrors, diffraction grating, linear CCD detector array, and control electronics. This will be accomplished by drilling threaded holes in an aluminum mounting plate and utilizing set screws to mount and secure components in the project box.

3.3.4. Mounting of Subsystems

The disinfection unit, excitation monochromator, and emission monochromator subsystems may also be mounted together. This may be accomplished through either double-sided tape or set screws. It is expected that the disinfection unit and emission monochromator subsystems will be mounted on top of the excitation monochromator subsystem, which has the largest dimensions. The subsystems may also simply be detached from each other, depending on the use case.

4. Thermal Interface

4.1.1. Cooling and Air Circulation

Cooling and air circulation shall be provided for the linear CCD detector array of the emission monochromator subsystem. This will be accomplished by means of a miniature fan, which will be powered through a +5 VDC input, situated on the optical bench by the detector. This is necessary in order to reduce the so-called “dark noise” associated with the detector, which increases as the detector overheats. Providing cooling and air circulation for the detector will therefore increase the signal-to-noise ratio (SNR) of the emission monochromator.

4.1.2. Heatsinks

Heatsinks shall be provided for most, if not all, onboard UV LEDs. This includes the UV LED in the disinfection unit, as well as the UV LED(s) present in the excitation monochromator subsystem. A heatsink may not be required if a relatively small voltage is being applied to the UV LED, which may be the case if the induced bacterial fluorescence is sufficiently intense for detection. The UV LEDs will be coupled with the heatsinks by means of either thermal adhesive tape or thermal paste to promote efficient heat conduction and dissipation. Therefore, no extra cold wall is required for the heatsinks. Finally, a heatsink shall be utilized for the stepper motor controller and driver as well to prevent overheating. This heatsink may be pasted directly on the IC itself.

5. Electrical Interface

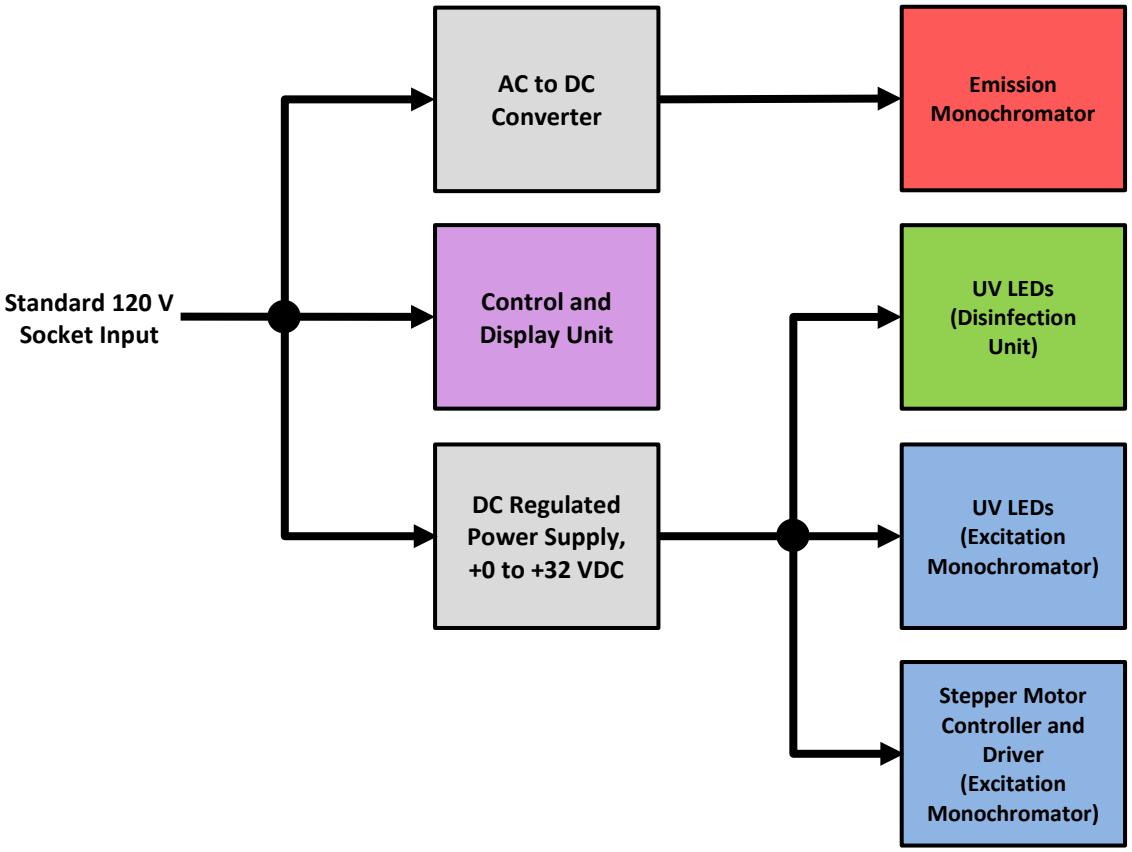

Figure 1: Electrical Interface Diagram.

5.1. Primary Input Power

5.1.1. Primary Input Power for Control and Display Unit

The control and display unit, a compact laptop, may be directly powered by a standard 120 V socket input. Alternatively, the unit may be charged before usage and therefore utilized without any charging cable.

5.1.2. Primary Input Power for Emission Monochromator

Owing to the fact that the emission monochromator will require the lowest input DC voltage, +5 VDC, an AC to DC converter will be utilized to power the subsystem directly from a standard 120 V socket input. The DC voltage will be regulated to ensure stability in the subsystem operation.

5.1.3. Primary Input Power for Disinfection Unit and Excitation Monochromator

A DC regulated power supply, capable of delivering DC voltages as high as +32 VDC, shall be utilized with the disinfection unit and excitation monochromator subsystems due to the higher nominal DC voltages of the stepper motor controller and driver and UV LEDs. Alternative components, such as batteries and boost converters, may be explored as more compact means of powering the system.

5.2. Voltage and Current Levels

Component	Voltage (V)	Current (mA)
UV LED (Disinfection Unit)	Estimate: 32	Estimate: 300
UV LED (Excitation Monochromator)	Estimate: 32	Estimate: 300
Stepper Motor Controller and Driver (Excitation Monochromator)	Estimate: 24	Estimate: 200
Emission Monochromator	Estimate: 5	Estimate: 600

Table 11: Absolute maximum voltage and current values for system components.

The excitation monochromator and disinfection unit subsystems are expected to consume more power than the emission monochromator and will therefore utilize a DC regulated power supply in the range of +0 to +32 VDC. Alternative means of powering the subsystems, perhaps with even only batteries, may be explored during the course of the project.

5.3. Signal Interfaces

5.3.1. Emission Monochromator Signal Interface

The emission monochromator control electronics will include a serial port which will be coupled with a USB to Serial adapter for interfacing with the onboard computer. Through this serial communication link, spectral data may be read and stored from the emission monochromator subsystem.

5.3.2. Stepper Motor Controller and Driver Signal Interface

The stepper motor controller and driver will include a serial port which will be coupled with a USB to Serial adapter for interfacing with the onboard computer. Through this serial communication link, commands may be issued for rotating the excitation grating and performing synchronous fluorescence measurements.

5.3.3. Excitation Monochromator Signal Interface

The excitation monochromator will house a DB9 connector which will be coupled with the stepper motor controller and driver for rotating the excitation grating of the monochromator. Control signals will be sent from the controller and driver to the connector based on user commands issued through the serial communication link between the controller and driver and onboard computer.

5.4. User Control Interface

The user control interface will be a GUI, executing on the onboard computer, which is responsible for receiving inputs from users, communicating with both the excitation monochromator and emission monochromator subsystems, and ultimately processing and displaying recorded data. This unit will interface with both the excitation monochromator and emission monochromator subsystems by means of a serial communication link, or cable, which may allow commands to be easily issued. Relevant inputs from users may include the following: (1) the type of spectrum (normal fluorescence or synchronous fluorescence) to record, (2) the initial and final excitation wavelengths of the spectrum, (3) the number of spectra to record, and (4) the range of fluorescence data to subject to PCA, among others. Following these user inputs, the unit will issue control signals for initializing the excitation and emission monochromators and initiating the spectrum acquisition. Furthermore, this unit will be responsible for receiving, processing, and displaying spectral data recorded by the emission monochromator.

6. Communications / Device Interface Protocols

6.1. Serial Communication

Serial communication will be achieved between the onboard computer and excitation and emission monochromator subsystems by means of a serial communication link. Through this serial communication link, spectral data may be read and stored from the emission monochromator subsystem. In addition, commands may be issued for rotating the excitation grating and performing synchronous fluorescence measurements.

6.2. RS-232 Communication Protocol

RS-232 is expected to be the standard protocol utilized for the serial communication process. All relevant practices regarding this protocol shall be enforced and followed. The serial port will be configured as needed to follow this particular communication protocol.

6.3. DB15 Connector

A DB15 connector will be utilized to interface the stepper motor controller and driver with the excitation monochromator. All relevant practices regarding this connector shall be enforced and followed. In particular, appropriate connectors will be utilized to interface these components together.

Instrument for the Identification of Live and Dead Bacteria

Arjun Krishnamoorthi

SCHEDULE

REVISION – 1
4 December 2019

Execution Plan for Instrument for the Identification of Live and Dead Bacteria

Schedule:

Status Legend:

- **Completed**
- **In progress**
- **Not started, on schedule**
- **Behind schedule**

TASK	END DATE	STATUS	DATE COMPLETED
Submit URS proposal to department	8/1/2019		7/22/2019
Obtain departmental approval for project	9/16/2019		7/26/2019
Apply for URS Program	9/16/2019		8/25/2019
Learn and understand the problem	9/16/2019		9/16/2019
Literature review and planning	9/16/2019		9/16/2019
Concept of Operations	9/16/2019		9/16/2019
Begin designing the spectroscopic instrument	9/24/2019		9/22/2019
Identify possible component vendors based on design, check laboratory for any spare parts	9/27/2019		9/27/2019
Finalize requirements and interfaces of instrument	9/29/2019		9/29/2019
Functional System Requirements	9/30/2019		9/30/2019
Interface Control Document	9/30/2019		9/30/2019
Execution Plan	9/30/2019		9/30/2019
Validation Plan	9/30/2019		9/30/2019
Order any remaining component parts which are not	10/2/2019		10/2/2019

available in the laboratory			
Begin constructing disinfection unit	10/3/2019		10/2/2019
Begin constructing emission monochromator	10/4/2019		10/2/2019
Begin constructing excitation monochromator	10/5/2019		10/5/2019
Begin writing and testing GUI software for excitation and emission monochromators through serial communication link	10/6/2019		10/6/2019
Continue optimizing existing subsystems as needed	10/8/2019		10/8/2019
Midterm Presentation	10/9/2019		10/9/2019
Test and validate disinfection unit for the inactivation of bacteria	10/10/2019		10/8/2019
Start scanning excitation monochromator with controller and driver	10/11/2019		10/10/2019
Test and validate emission monochromator for normal fluorescence measurements with disinfection unit, along with performing PCA	10/12/2019		10/10/2019
Visit Physics Machine Shop to finalize emission monochromator enclosure	10/13/2019		10/11/2019
Continue optimizing existing subsystems as needed	10/15/2019		10/15/2019
Receive any ordered component parts and order more as needed	10/16/2019		10/16/2019
Test and validate excitation monochromator for synchronous	10/18/2019		10/18/2019

fluorescence measurements			
Obtain emission monochromator enclosure	10/21/2019		10/20/2019
Visit Physics Machine Shop to finalize excitation monochromator enclosure	10/23/2019		10/23/2019
Continue optimizing existing subsystems as needed	10/25/2019		10/25/2019
Test and validate control and display unit, including ability to perform PCA	10/27/2019		10/25/2019
Continue optimizing existing subsystems as needed	11/6/2019		11/6/2019
Progress Update 1	11/6/2019		11/6/2019
Obtain excitation monochromator enclosure	11/9/2019		11/6/2019
Continue and finalize validation of subsystems	11/12/2019		11/12/2019
Prepare for subsystem demonstrations	11/18/2019		11/18/2019
Project Subsystem Demonstrations	11/18/2019		11/18/2019
Progress Update 2 (Final Presentations)	11/20/2019		11/20/2019
Work on Final Report	12/04/2019		12/04/2019
Final Report Due	12/04/2019		12/04/2019

Instrument for the Identification of Live and Dead Bacteria

Arjun Krishnamoorthi

VALIDATION

REVISION – 1
4 December 2019

Validation Plan for Instrument for the Identification of Live and Dead Bacteria

Validation Plan:

Status Legend:

- **Completed**
- **In progress**
- **Not started, on schedule**
- **Behind schedule**

TASK	END DATE	STATUS	DATE COMPLETED
Validate germicidal effectiveness of disinfection unit	10/10/2019		10/8/2019
Validate operating range and sufficient sensitivity of emission monochromator through measurement of bacterial fluorescence	10/12/2019		10/10/2019
Validate operating range and scanning capability of excitation monochromator by coupling subsystem with UV LED	10/18/2019		10/18/2019
Finalize enclosure of emission monochromator with Physics Machine Shop	10/21/2019		10/20/2019
Validate control and display unit requirements through serial communication link with subsystems and PCA	10/27/2019		10/25/2019
Finalize enclosure of excitation monochromator with Physics Machine Shop	11/9/2019		11/6/2019

Validate detection and classification time requirement of overall system in enclosures	11/12/2019		11/12/2019
Validate general spectroscopic requirements of system	11/12/2019		11/12/2019

Performance on Execution Plan

The execution plan was executed completely. Each task noted in the execution plan was completed on time and properly. All of the objectives outlined at the beginning of the project were ultimately completed and demonstrated.

Performance on Validation Plan

The validation plan was executed completely and thoroughly. Each individual subsystem was properly tested and validated. All major functionalities were achieved for each subsystem and will ultimately support the complete system integration for next semester.

Instrument for the Identification of Live and Dead Bacteria

Arjun Krishnamoorthi

SUBSYSTEM REPORTS

REVISION – Original
4 December 2019

SUBSYSTEMS REPORT
FOR
Instrument for the Identification of Live and Dead Bacteria

TEAM <52>

APPROVED BY:

_____
Arjun Krishnamoorthi Date

_____
John Lusher II, P.E. Date

_____
T/A Date

Change Record

Rev.	Date	Originator	Approvals	Description
1	12/4/2019	Arjun Krishnamoorthi		Original Release

Table of Contents

List of Tables

List of Figures

1. Introduction

The portable fluorescence spectrometer will be capable of recording the normal fluorescence and synchronous fluorescence spectra of bacteria, in addition to performing ultraviolet (UV) disinfection on bacterial samples *in-situ*. The fluorescence spectra of bacteria, before and after UV disinfection, will be recorded and processed through principal component analysis (PCA) as a means of rapidly distinguishing live and dead bacteria. A GUI will support the acquisition, processing, and display of spectral data. To that effect, the portable fluorescence spectrometer is composed of four subsystems: (1) a control and display unit, (2) a disinfection unit, (3) an excitation monochromator, and (4) an emission monochromator. All subsystems were successfully designed, constructed, and validated.

2. Control and Display Unit Subsystem Report

2.1. Subsystem Introduction

The main purpose of the control and display unit subsystem is to receive inputs from the user, communicate with both the excitation monochromator and emission monochromator subsystems, and process and display recorded spectral data. Relevant inputs from the user include (1) the type of spectrum (normal fluorescence or synchronous fluorescence) to record, (2) acquisition parameters (e.g., exposure time, number of spectra to record, etc.), and (3) PCA processing details, among others. Communication with the excitation monochromator subsystem consists of rotating the excitation grating, while communication with the emission monochromator subsystem consists of interfacing with the linear image sensor, setting acquisition parameters, and subsequently reading fluorescence spectra. Finally, once spectral data is successfully recorded, displayed, and stored, PCA and other data processing, such as median filtering, may be performed as well.

2.2. Subsystem Details

2.2.1. MATLAB GUIs and Laptop

The current embodiment of the control and display unit subsystem is a compact laptop (Dell Precision 5510 Mobile Workstation Laptop) executing custom MATLAB software. The software currently consists of two independent GUIs; one is an acquisition GUI for communicating with the excitation and emission monochromators, while the other is a processing GUI for performing PCA on recorded spectral data. These GUIs were designed through the App Designer feature of MATLAB to simplify the actual creation of the GUI layout and its various buttons, text fields, and checkboxes. To that effect, the majority of programming done for the GUI itself involved implementing various callbacks. The acquisition GUI and processing GUI are shown in Figure 1 and Figure 2, respectively, on the following page.

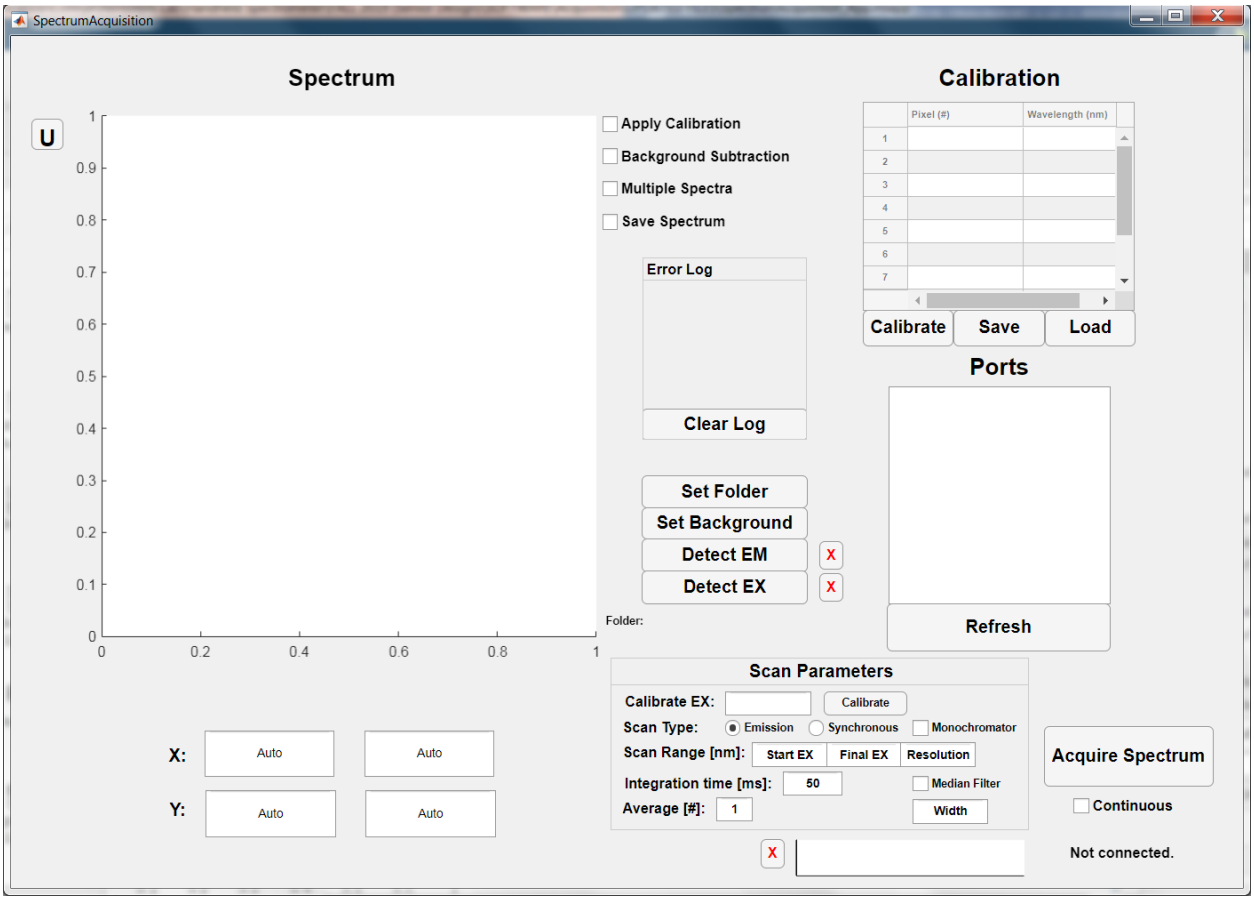

Figure 1: MATLAB acquisition GUI for communicating with excitation monochromator and emission monochromator subsystems.

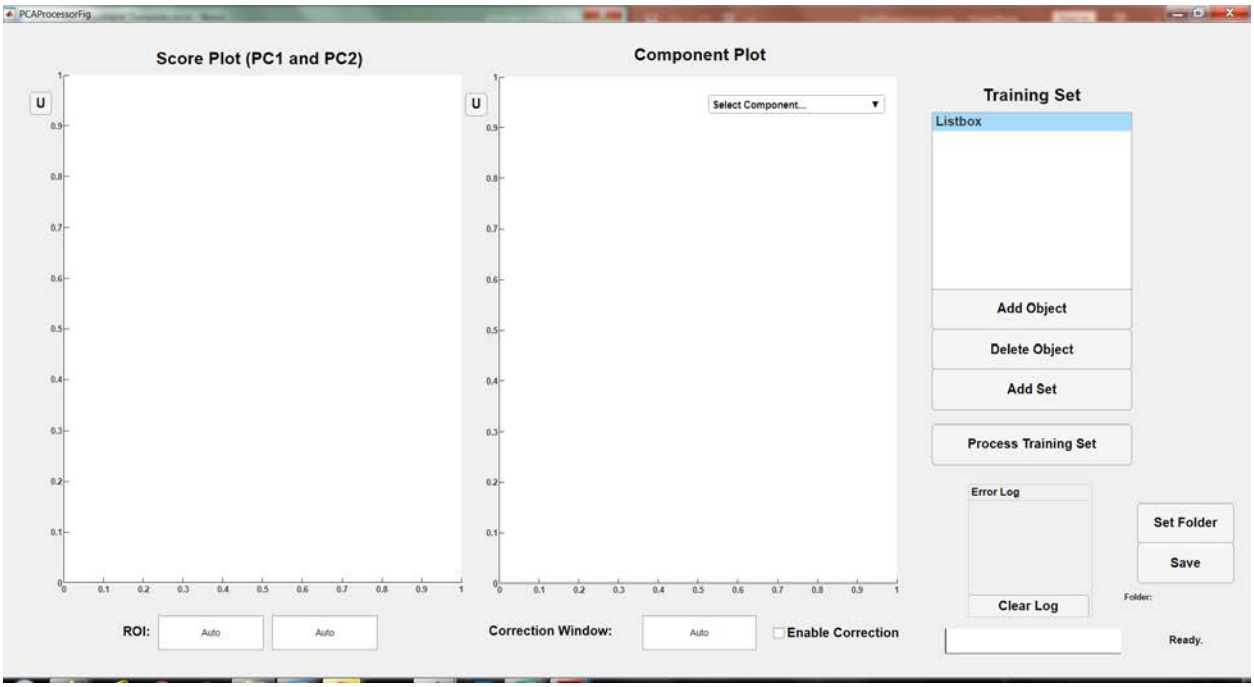

Figure 2: MATLAB processing GUI for performing and optimizing PCA on recorded data.

A variety of functionalities are currently available in the acquisition GUI, including the ability to record both normal fluorescence and synchronous fluorescence spectra, alter acquisition parameters, save spectra, calibrate the spectrometer and excitation monochromator, and perform background subtraction. In the processing GUI, the user may perform PCA on recorded spectral data and view the associated score and component plots. In addition, the user may also fine-tune the PCA processing by adjusting the region of interest (ROI), which essentially represents the range of fluorescence data subjected to PCA, or performing median filtering on the data to reduce noise and improve clustering.

Communication with the excitation and emission monochromator subsystems is achieved through a common serial communication link which allows commands to be issued to both subsystems simultaneously through separate serial ports. Namely, one serial cable connects to an RS-232 communication port integrated with the emission monochromator subsystem, while another serial cable feeds into the serial port of the microcontroller within the excitation monochromator subsystem. Figure 3 shows the basic functional block diagram (FBD) of this setup, while Figure 4 is a picture of its implementation.

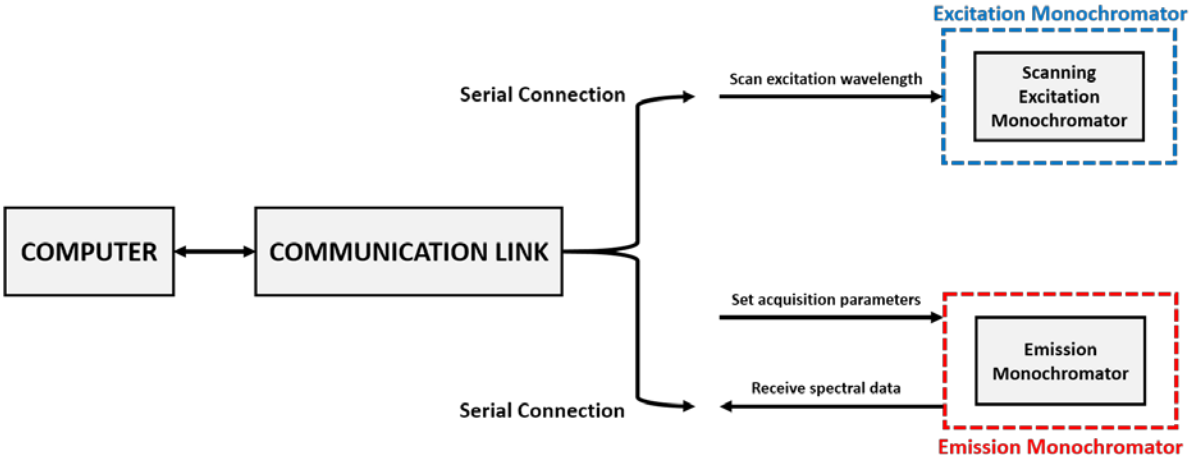

Figure 3: FBD for serial connection between computer and excitation monochromator and emission monochromator subsystems.

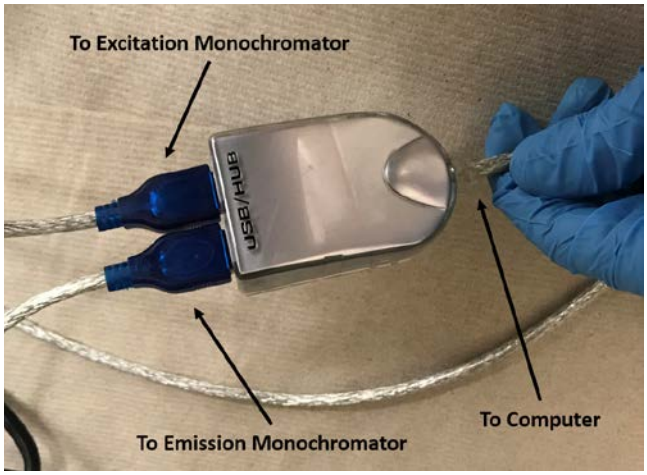

Figure 4: Implementation of serial connection between computer and excitation monochromator and emission monochromator subsystems.

To achieve communication with both subsystems simultaneously, individual serial port connections need to be established in MATLAB. The configuration of these serial port connections (e.g., baud rate, parity, data bit, etc.) was determined by the respective communication protocols associated with each subsystem.

2.2.2. Excitation Monochromator Subsystem Communication

Communication with the excitation monochromator subsystem is achieved through the serial port of the microcontroller within the subsystem. The microcontroller is a DCB-241, which is an integrated stepper motor driver-controller board that operates on an RS-422 communication protocol. To simplify the software development, a SIN-11 intelligent serial adapter, shown in Figure 5, was utilized as a means of converting the RS-232 bus from the computer into an RS-422 bus. The overall communication setup is shown in Figure 6. Furthermore, details concerning the serial port configuration for this communication are noted in Table 1 below.

Figure 5: SIN-11 intelligent serial adapter for converting RS-232 bus into an RS-422 bus for communication with the DCB-241 microcontroller.

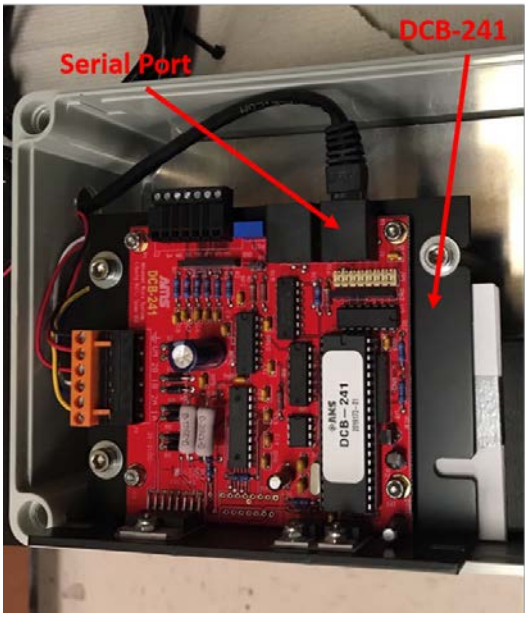

Figure 6: Overall communication setup for excitation monochromator subsystem, including the DCB-241 and its serial port.

Specification	Value
Baud Rate (bps)	9600
Data Bits	8
Stop Bits	1
Parity	None
Flow Control	Hardware
Handshaking	None

Table 1: Serial port configuration for excitation monochromator subsystem [1].

The primary purpose of the DCB-241 microcontroller is to rotate the excitation grating within the excitation monochromator subsystem by means of a stepper motor. To that effect, the serial port connection with the subsystem is utilized as a means of issuing high-level, ASCII commands for configuring and initiating the rotation of the stepper motor. A table of the most relevant commands is provided in Table 2 on the following page. The full set of commands is available in the associated DCB-241 documentation [1].

Command	Function	Value	Notes
I	Initial Velocity	18-23,000 SPS	The initial velocity specifies the start and stop speed, in steps per second (SPS), for the motor.
K	Ramp Slope	0-255	The ramp slope specifies the ramp acceleration and deceleration time.
V	Slew Velocity	18-23,000 SPS	The slew velocity is the final velocity following acceleration from the initial velocity.
+	Index in Plus Direction	0-16,777,215 Steps	This command steps the stepper motor in a positive direction for the specified number of steps.
-	Index in Minus Direction	0-16,777,215 Steps	This command steps the

			stepper motor in a negative direction for the specified number of steps.
S	Store Parameters	N/A	This command saves all operational parameters for recall during power-on reset.

Table 2: Relevant DCB-241 commands for rotating the stepper motor [1].

2.2.3. Emission Monochromator Subsystem Communication

Communication with the emission monochromator subsystem is achieved through an RS-232 port integrated with the onboard control electronics. The emission monochromator is an off-the-shelf BTC110-S spectrometer from B&W TEK. Owing to the onboard RS-232 port, a serial cable, shown in Figure 6, may be directly connected from the computer to the subsystem for communication. Details concerning the serial port configuration for this communication are noted in Table 3 below.

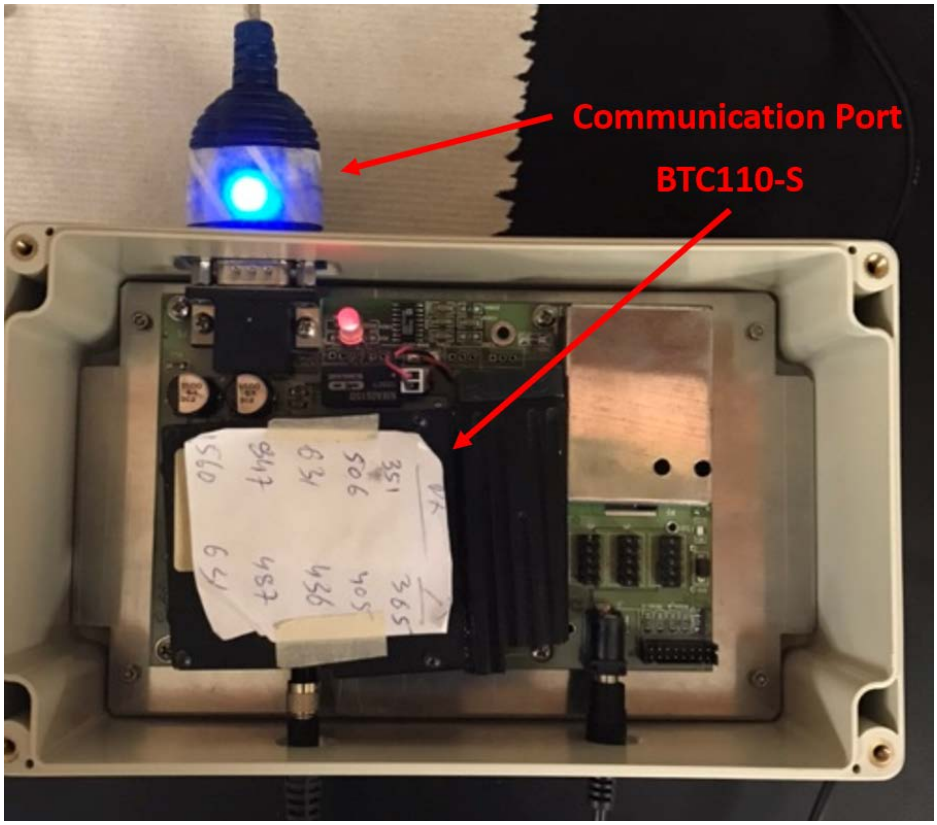

Figure 7: Overall communication setup for emission monochromator subsystem, including the BTC110-S and its serial port.

Specification	Value
Baud Rate (bps)	9600
Data Bits	8
Stop Bits	1
Parity	None
Flow Control	Hardware
Handshaking	None

Table 3: Serial port configuration for emission monochromator subsystem [2].

The primary purpose of the BTC110-S spectrometer is to detect, digitize, and record the fluorescence spectra of bacterial samples. To that effect, the serial port connection with the subsystem is utilized as a means of issuing high-level, ASCII commands for configuring acquisition parameters (e.g., integration time, averaging, etc.) and receiving spectral data from the linear image sensor within the optical bench. A table of the most relevant commands is provided in Table 4 below. The full set of commands is available in the associated BTC110-S documentation [2].

Command	Function	Value	Notes
K	Set Baud Rate	0	This command can be used to increase the baud rate (bps) to as high as 115,200 bps.
I	Set Integration Time	50-65535	This command can be used to set the integration time (milliseconds).
A	Set Averaging	1-1000000000	This command can be used to set the number of spectra to average in a given spectrum acquisition.
a	Set ASCII Mode	N/A	This command switches the communication into ASCII mode.
b	Set Binary Mode	N/A	This command switches the communication into binary mode.

?K	Query Baud Rate	N/A	This command queries the current baud rate (bps).
?I	Query Integration Time	N/A	This command queries the current integration time (milliseconds).
?A	Query Averaging	N/A	This command queries the current number of spectra to be averaged.
?a	Query Communication Mode	N/A	This command queries the current communication mode (ASCII or binary).

Table 4: Relevant BTC-110S commands for configuring and reading the spectrometer [2].

2.2.4. Acquiring Normal Fluorescence Spectra

Normal fluorescence spectra describe the fluorescence of bacteria at a fixed, constant excitation wavelength λ_{EX}. This data is recorded by utilizing either the disinfection unit or excitation monochromator as a fixed excitation source and subsequently measuring the fluorescence of the bacterial sample through the emission monochromator subsystem. Briefly, this process requires identifying the excitation source (i.e., disinfection unit or excitation monochromator), configuring acquisition parameters, acquiring spectral data, processing the data, and outputting the data through a MATLAB figure and CSV file. The control logic for this process is detailed in Figure 8 on the subsequent page. It is worthy to mention that the excitation intensity of the disinfection unit at the sample location is much higher compared to that of the excitation monochromator. Therefore, for normal fluorescence measurements, it is preferable to utilize solely the disinfection unit.

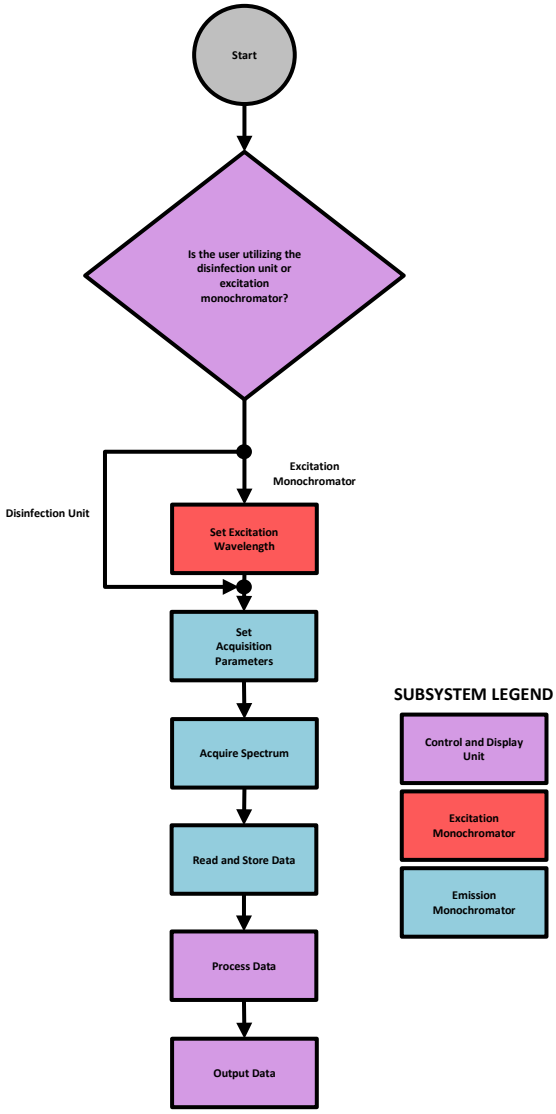

Figure 8: System flowchart detailing the normal fluorescence measurement.

2.2.5. Acquiring the Excitation-Emission Matrix (EEM)

An Excitation-Emission Matrix (EEM) is a three-dimensional dataset which consists of a sample's fluorescence spectra across a set of excitation wavelengths. The EEM can offer a complete, detailed characterization of the fluorescence from an unknown sample. This data is recorded by utilizing the excitation monochromator as a scanning excitation source and subsequently measuring the fluorescence of the bacterial sample over a range of excitation wavelengths. Briefly, this process requires calibrating and configuring the excitation monochromator, configuring acquisition parameters, acquiring spectral data over a range of scanned excitation wavelengths, processing the data, and outputting the data through a MATLAB figure and CSV file. The EEM may be provided as either a three-dimensional (3D)

surface plot or two-dimensional (2D) contour plot. The control logic for this process is detailed in Figure 9 below.

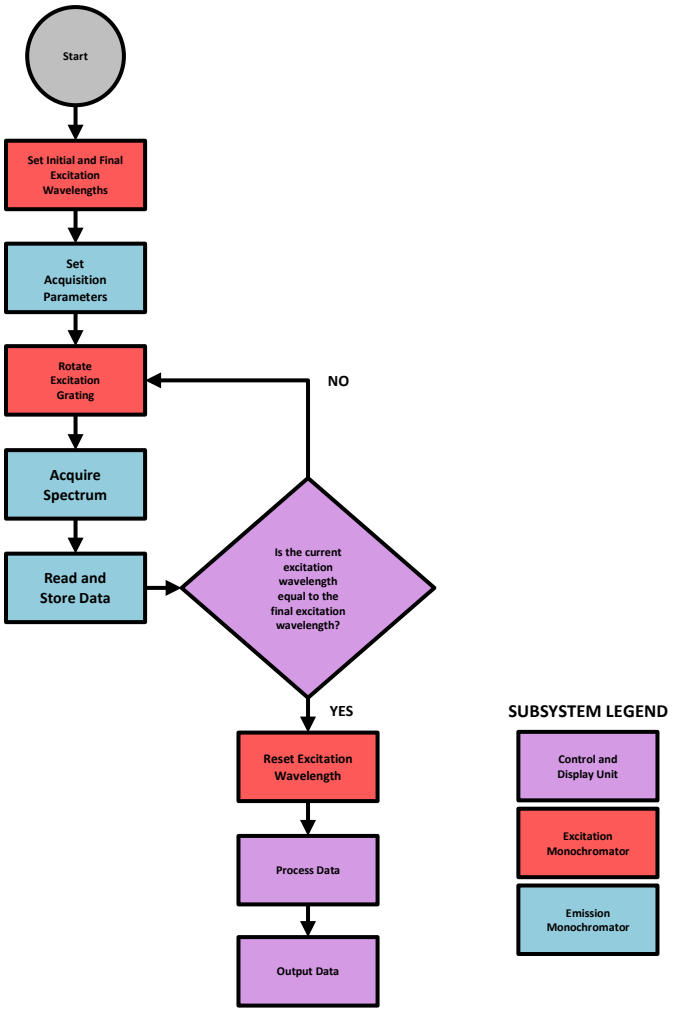

Figure 9: System flowchart detailing the EEM measurement.

2.2.6. Acquiring Synchronous Fluorescence Spectra

Synchronous fluorescence spectra are a particular type of fluorescence spectra which can resolve the fluorescence of individual components in a mixture or multicomponent sample [3]. With bacteria, synchronous fluorescence is an especially useful technique for resolving the fluorescence of its cellular components, namely tryptophan and tyrosine. To that effect, this technique can offer more detailed information on a molecular level than normal fluorescence. In commercial, benchtop spectrometers, this technique is generally achieved by synchronously scanning both the excitation monochromator and emission monochromator such that the excitation wavelength, λ_{EX}, and emission wavelength, λ_{EM}, satisfy the following relation:

$$\Delta\lambda = \lambda_{EM} - \lambda_{EX}.$$

$\Delta\lambda$ is a quantity which denotes the constant offset in wavelength maintained between the monochromators. This quantity is a rather critical parameter in determining which fluorescence bands are resolved in a mixture. Alternatively, synchronous fluorescence spectra may simply be extracted from a sample's EEM by considering the fact that the relation $\lambda_{EM} = \lambda_{EX} + \Delta\lambda$ is simply a linear equation with a slope equal to unity and intercept equal to $\Delta\lambda$. Synchronous fluorescence spectra may therefore be simply interpreted as a set of parallel lines with varying intercepts that exist within a sample's EEM. To that end, once an EEM is acquired, synchronous fluorescence spectra of varying $\Delta\lambda$ may be efficiently acquired within the software itself. Currently, once a bacterial EEM is acquired, synchronous spectra across a range of $\Delta\lambda$ are automatically extracted and written to a separate CSV file.

2.3. Subsystem Validation

2.3.1. Acquisition GUI

The validation of the acquisition GUI was performed through validation of the excitation monochromator and emission monochromator subsystem communication itself. Alongside, functionalities such as the ability to save spectra, calibrate the spectrometer, and perform background subtraction, among others, were thoroughly tested by performing experiments on bacterial samples. Many of these experimental results will be provided in the subsystem reports for the excitation monochromator and emission monochromator subsystems. Debugging and further development of this GUI will continue into next semester.

The normal fluorescence, excitation-emission matrix (EEM), and synchronous fluorescence of *E. coli* bacteria are provided on the subsequent pages to illustrate typical outputs of the acquisition GUI. Outputs are generated as both MATLAB figures and CSV files to simplify plotting of the data.

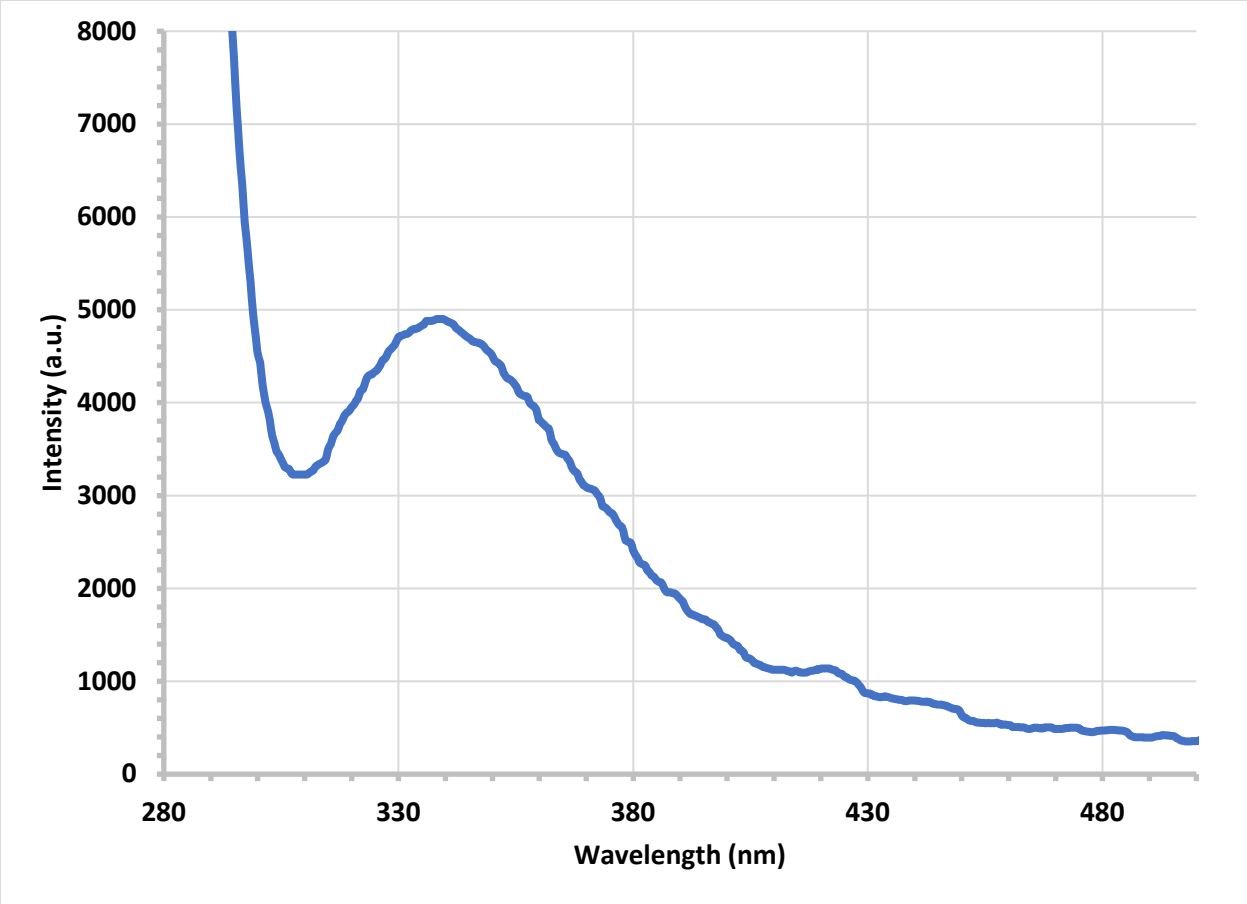

Figure 10: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectrum of *E. coli* bacteria ($\approx 10^8$ cells/mL).

Figure 10 is a plot of the normal fluorescence spectrum of *E. coli*. The vertical axis is the fluorescence intensity, while the horizontal axis is the emission wavelength. This spectrum was acquired with the disinfection unit as the fixed excitation source. The initially high intensity is due to Rayleigh (elastic) scattering of the excitation beam. The fluorescence band of *E. coli* is clearly observed with a maximum at approximately 340 nm. This fluorescence is due, primarily, to tryptophan and tyrosine residues within the bacterial cells. Because the excitation wavelength is fixed, we are unable to resolve the fluorescence of tryptophan and tyrosine individually. The validation of this measurement is provided in the emission monochromator and excitation monochromator subsystem reports.

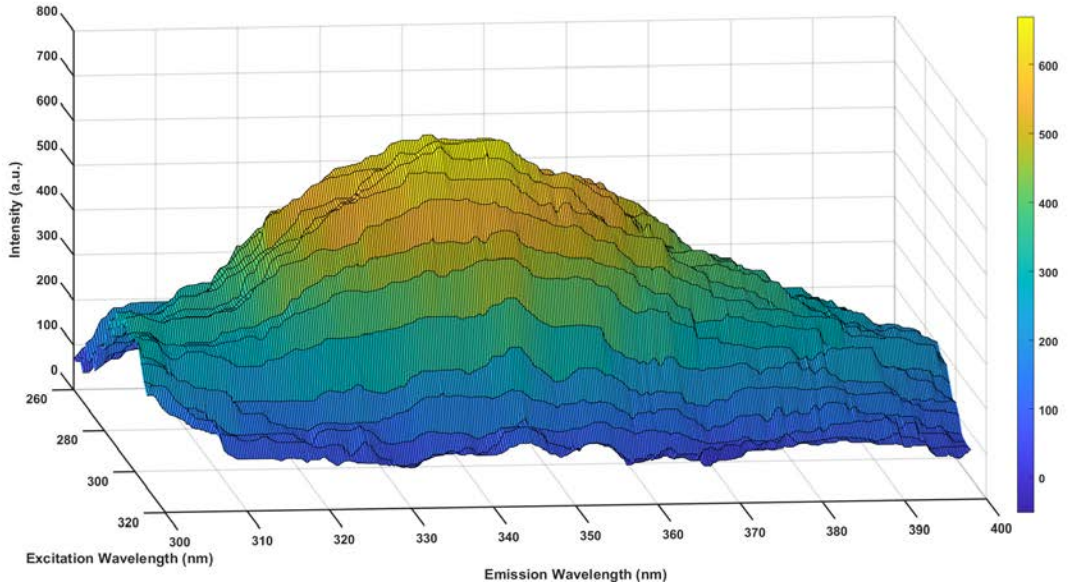

Figure 11: 3D surface plot of EEM for *E. coli* bacteria (≈ 10^8 cells/mL) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

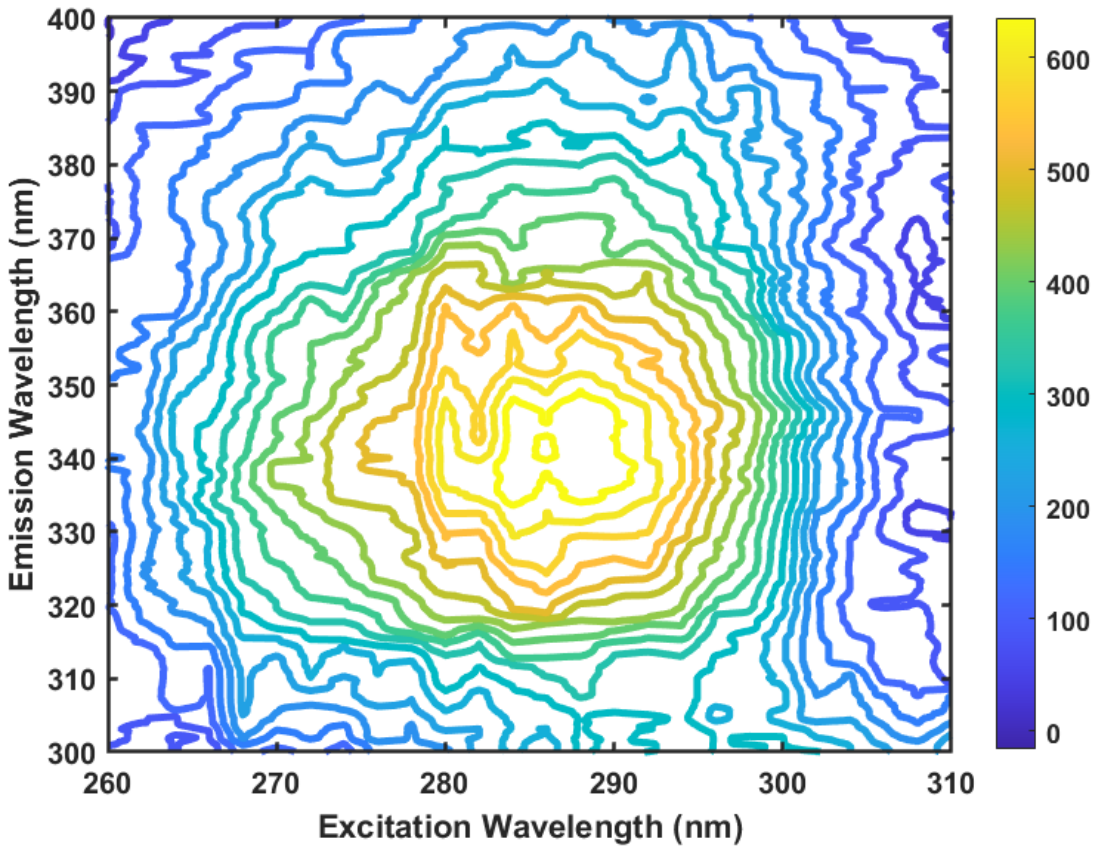

Figure 12: 2D contour plot of EEM for *E. coli* bacteria (≈ 10^8 cells/mL) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

The EEM shown in Figure 11 and Figure 12 was acquired across an excitation range of 260 to 310 nm in 2 nm steps with the excitation monochromator as a scanning excitation source. Clearly, the EEM offers a complete, detailed characterization of the fluorescence bands of *E. coli*. From this EEM, we may extract the synchronous spectra of the tryptophan and tyrosine components in *E. coli*.

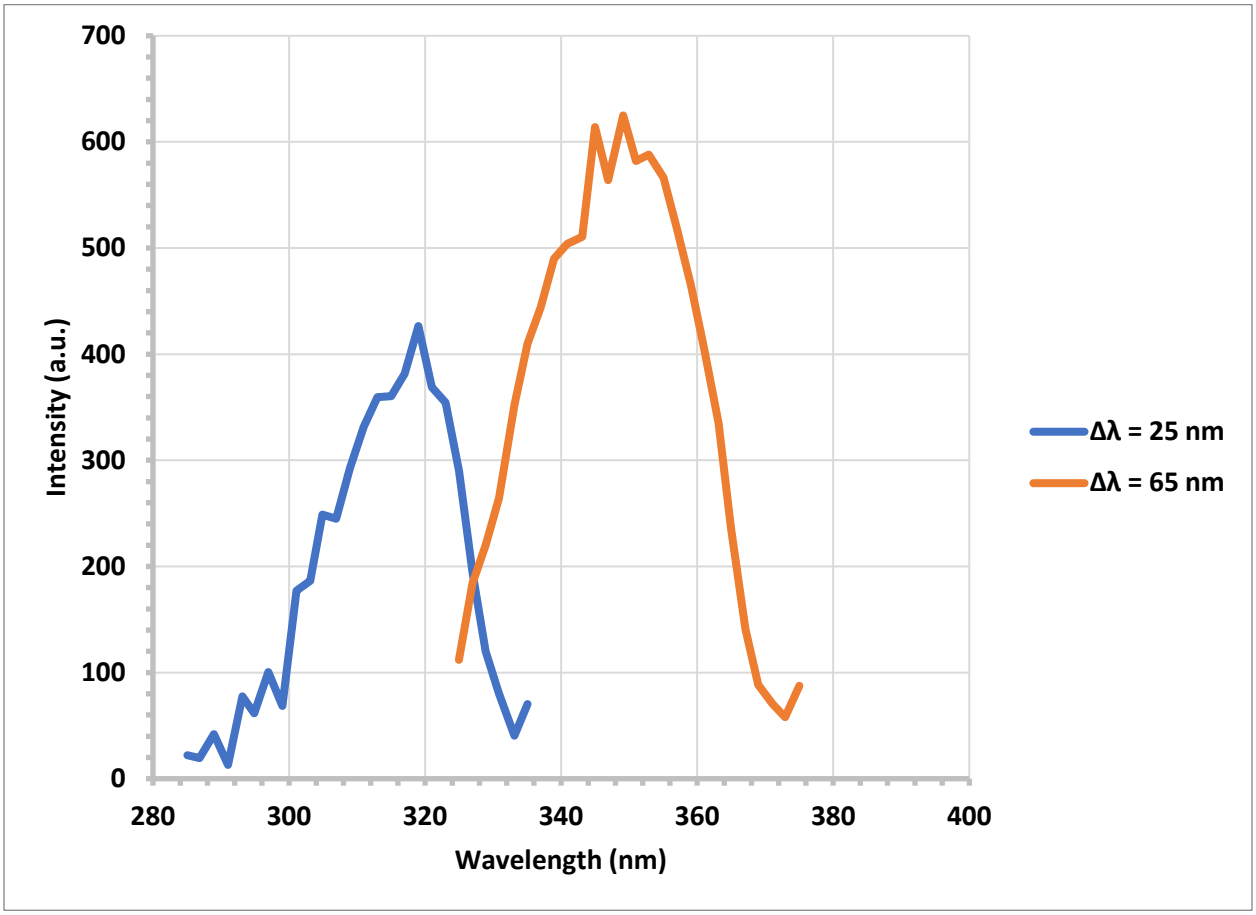

Figure 13: Synchronous fluorescence spectra ($\Delta\lambda = 25$ nm and $\Delta\lambda = 65$ nm) of *E. coli* bacteria ($\approx 10^8$ cells/mL).

Figure 13 is a plot of the synchronous fluorescence spectra of *E. coli* at $\Delta\lambda = 25$ nm and $\Delta\lambda = 65$ nm, which correspond, respectively, to the tyrosine and tryptophan components within the bacterial cells. These synchronous fluorescence spectra were extracted from the EEM shown in Figure 11 and Figure 12. The EEM and synchronous fluorescence spectra thus provide a means of resolving the fluorescence of individual components within bacterial cells. The validation of the EEM and synchronous fluorescence measurements is provided in the emission monochromator and excitation monochromator subsystem reports.

2.3.2. Processing GUI

The validation of the processing GUI was performed through the generation of PCA score and component plots for experimental data recorded through the acquisition GUI. Alongside, functionalities such as the ability to tune the ROI, perform median filtering, and save PCA results were thoroughly tested. Many of these PCA results will be provided in the subsystem reports for the excitation monochromator and emission monochromator subsystems. Debugging and further development of this GUI will continue into next semester.

PCA score and component plots for experimental data are provided below and on the subsequent page to illustrate typical outputs of the processing GUI. Outputs are generated as both MATLAB figures and CSV files to simplify plotting of the data.

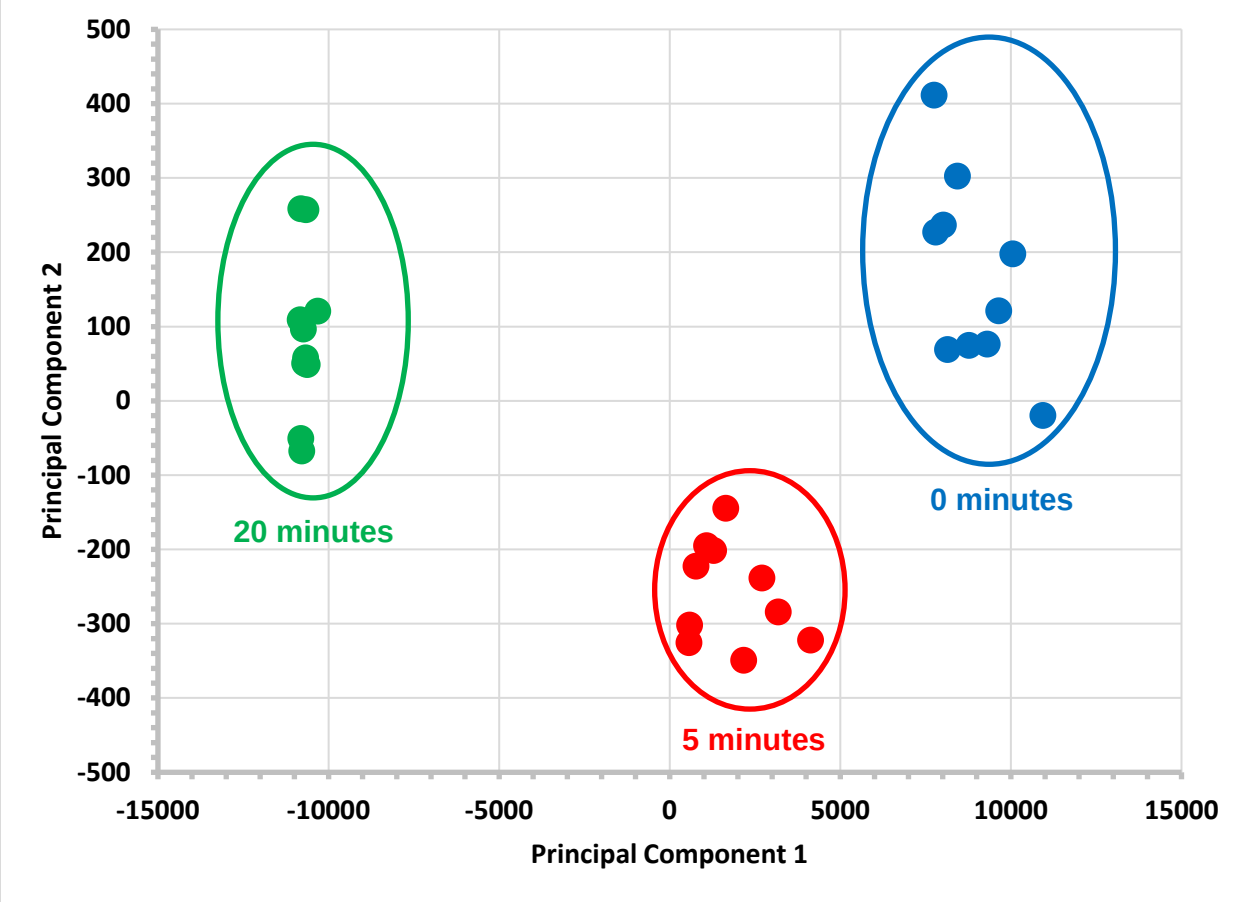

Figure 14: PCA score plot for UV irradiation experiment on *E. coli* bacteria ($\approx 10^8$ cells/mL).

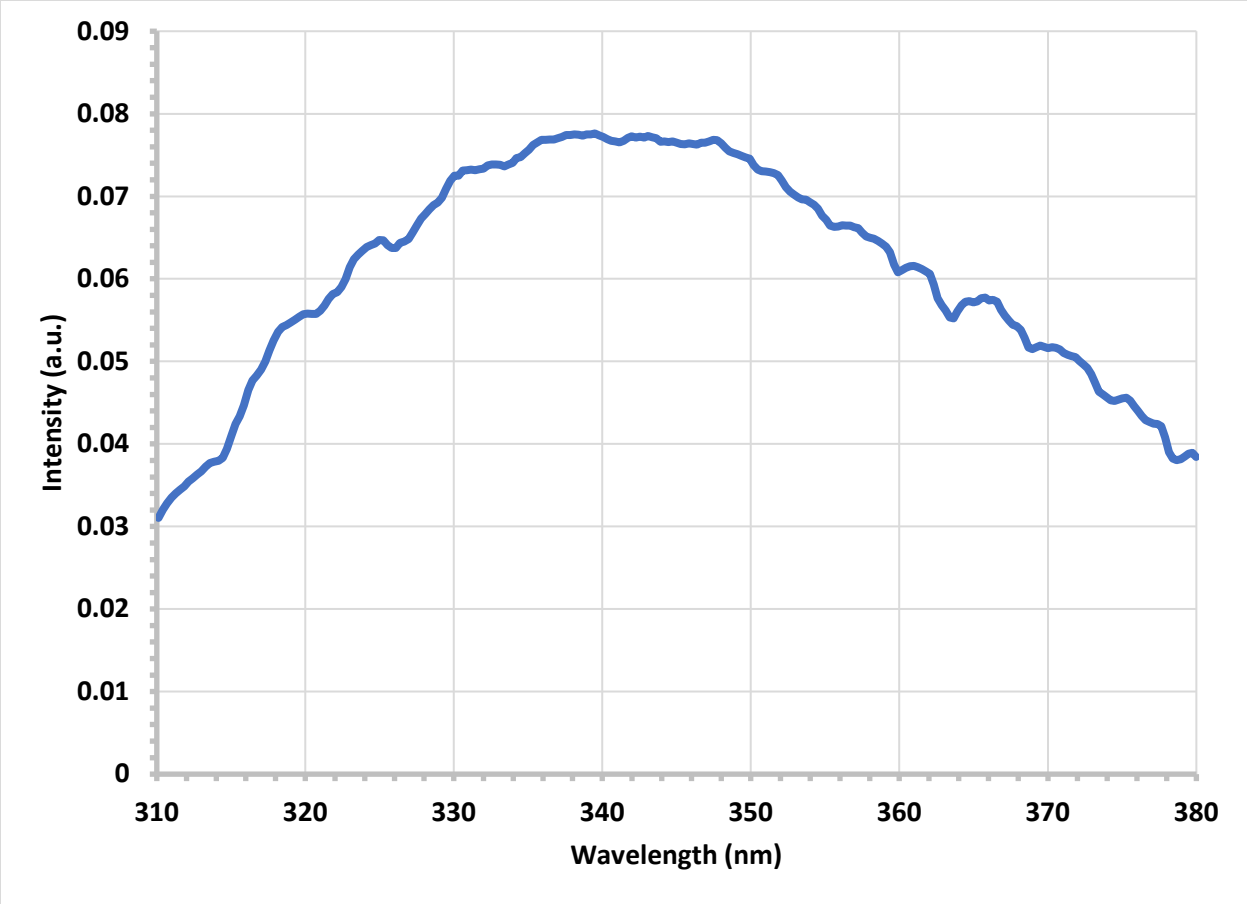

Figure 15: PCA Principal Component 1 plot for UV irradiation experiment on *E. coli* bacteria ($\approx 10^8$ cells/mL).

For this experiment, *E. coli* bacteria ($\approx 10^8$ cells/mL) were irradiated for 0 minutes, 5 minutes, and 20 minutes by means of the onboard disinfection unit. At each time step, 10 normal fluorescence spectra ($\lambda_{EX} \approx 285$ nm) were acquired with the disinfection unit as the excitation source. The fluorescence data in the range of 310-380 nm, which was found to provide the best clustering, was then subjected to PCA by means of the processing GUI. Essentially, the PCA score plot shown in Figure 14 treats each fluorescence spectrum as a single object, thereby reducing the dimensionality of the original dataset; the objects are separated along the principal component 1 (PC1) and principal component 2 (PC2) axes, with the majority of variation occurring along the PC1 axis, as expected. This plot serves as a visual means of separating and identifying bacteria as a function of the UV dosage, which we expect to be correlated with the fraction of bacteria inactivated. Therefore, this procedure allows for the identification of live and dead bacteria samples. It is worthy to mention that the clustering in this score plot may be improved by either (a) acquiring more spectra for processing or (b) performing median-filtering on the data to further reduce noise.

The PC1 component plot shown in Figure 15 demonstrates that while the score plot reduces the dimensionality of the dataset, the principal components, which account for the variation between PCA objects, maintain the same dimensionality as the dataset. Such component plots provide critical information as to the exact regions of difference, or variation, between

each PCA object. For our experiments, it was found that practically all variation was captured in PC1 alone. Therefore, the PC2 component plot is excluded from this subsystem report.

2.3.3. Excitation Monochromator Subsystem Communication

The validation of the excitation monochromator subsystem communication is already provided through the previous EEM and synchronous fluorescence data shown in Figure 11, Figure 12, and Figure 13. The acquisition of this data involved communication with the excitation monochromator, namely the calibration and rotation of the excitation grating across a range of excitation wavelengths, and therefore validates this subsystem requirement. Further validation on the accuracy of the experimental data is provided in the excitation monochromator and emission monochromator subsystem reports.

Additionally, it is worthy to mention that at any point when a communication failure with the excitation monochromator subsystem was detected, the program would write an error message and cease execution.

2.3.4. Emission Monochromator Subsystem Communication

The validation of the emission monochromator subsystem communication is already provided through the previous normal fluorescence, EEM, and synchronous fluorescence data shown in Figure 10, Figure 11, Figure 12, and Figure 13. The acquisition of this data involved communication with the emission monochromator, namely the configuration of acquisition parameters and subsequent receival of spectral data, and therefore validates this subsystem requirement.

Additionally, it is worthy to mention that the query commands noted in Table 4 were utilized in the MATLAB code as a means of ensuring acquisition parameters were properly communicated to the emission monochromator subsystem. At any point when a communication failure was detected, the program would write an error message and cease execution.

2.4. Subsystem Conclusion

In conclusion, this subsystem satisfies all required functionalities. Namely, it is capable of receiving inputs from the user, communicating with both the excitation monochromator and emission monochromator subsystems, processing data, and outputting data, including both acquired spectra and PCA results. Communication with both the excitation monochromator and emission monochromator subsystems was validated, along with the functionalities of the acquisition and processing GUIs. For next semester, the acquisition and processing GUIs will be combined into a single GUI, and perhaps deployable application, which integrates the acquisition and processing capabilities.

3. Disinfection Unit Subsystem Report

3.1. Subsystem Introduction

The disinfection unit subsystem may serve as both a (1) disinfection source for inactivating bacteria *in-situ* and as an (2) excitation source for normal fluorescence measurements. This provides a very efficient means of inactivating bacteria, observing the resulting changes in the normal fluorescence spectra, and confirming the disinfection was successful. To accomplish these functionalities, a high-power, miniature UV LED is used, along with excitation and collection optics for maximizing the fluorescence intensity. These components are housed in a lightweight, portable enclosure.

3.2. Subsystem Details

3.2.1. Components

A picture of the current embodiment for the disinfection unit subsystem, along with its major components, is shown in Figure 16 below.

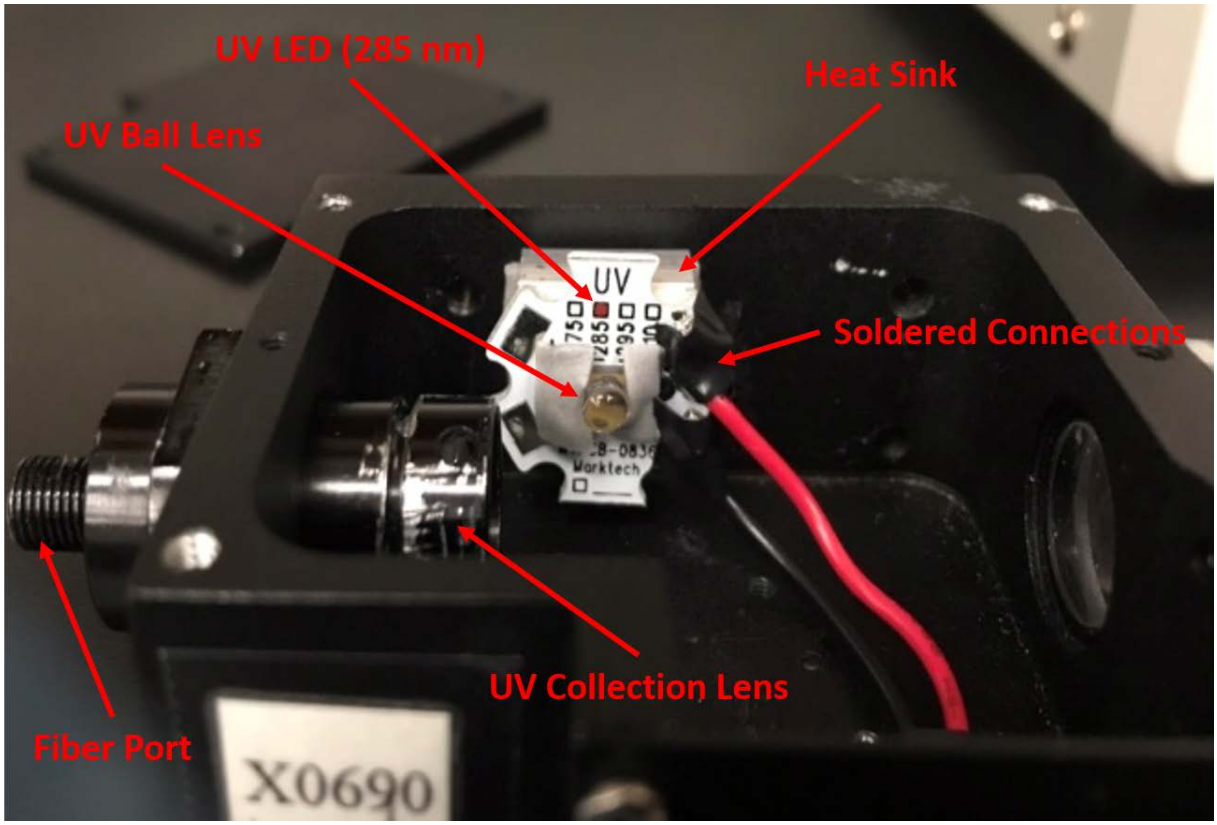

Figure 16: Disinfection unit subsystem and its major components.

The disinfection unit currently consists of a high-power, miniature UV LED (MTSM285UV-F1120S) mounted on a heat sink and coupled with a UV ball lens. The UV LED is attached to the heat sink by means of a thermal adhesive to promote efficient heat dissipation, which is critical in ensuring stable LED operation. A UV ball lens is utilized as a means of collecting

and focusing the output of the LED, which is otherwise diverging. This increases the excitation intensity and subsequent fluorescence from the bacterial sample. Since the fluorescence of the sample is diverging as well, a UV collection lens is utilized for collecting and focusing the fluorescence onto an optical fiber positioned at the fiber port. This optical fiber is coupled to the input port of the emission monochromator. The enclosure, fiber port, and collection lens holder are all off-the-shelf components from B&W Tek.

3.2.2. UV LED

Selection of the UV LED is a particularly critical design choice in ensuring that the functionalities of disinfection and excitation are both satisfied by a single LED. To that effect, the germicidal effectiveness curve, indicating the efficacy of various UVB and UVC wavelengths on inactivating bacteria, is provided in Figure 17 below.

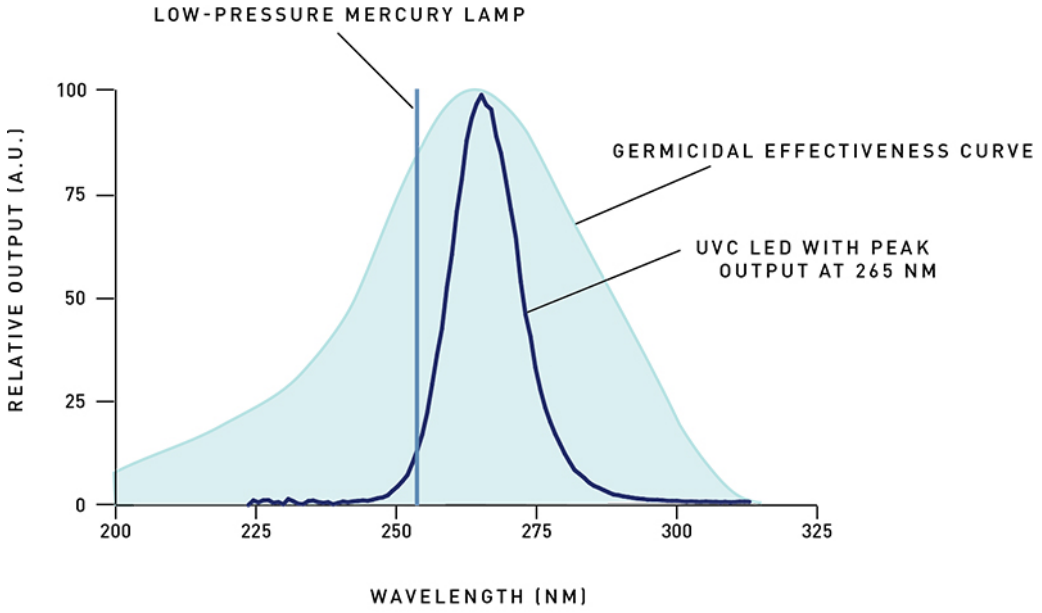

Figure 17: Germicidal effectiveness curve [4].

The plot in Figure 17 suggests that to achieve maximum germicidal effectiveness, a UVC wavelength of approximately 265 nm should be used. The primary reason for this is that this wavelength corresponds to the absorption band of DNA. The absorption of UV light by DNA results in damage to its structure [4], which consequently inhibits bacterial replication and is the mechanism by which bacteria are inactivated. The fluorescence of bacteria, however, is primarily due to tryptophan and tyrosine residues, rather than DNA, which possess absorption bands at higher wavelengths in the UVB and UVC region (≈ 270-290 nm) [5]. Tryptophan, in particular, is known to be the most intensely fluorescing component of bacteria with an absorption band maximum at (≈ 280 nm) [5]. Therefore, a central wavelength of 285 nm was selected for the UV LED to provide both the excitation and disinfection functionalities. As shown in Figure 17, a UV wavelength of 285 nm still provides a rather high germicidal effectiveness.

To that effect, the nominal output spectrum of the selected UV LED is shown in Figure 18 below, along with its nominal characteristics in Table 5.

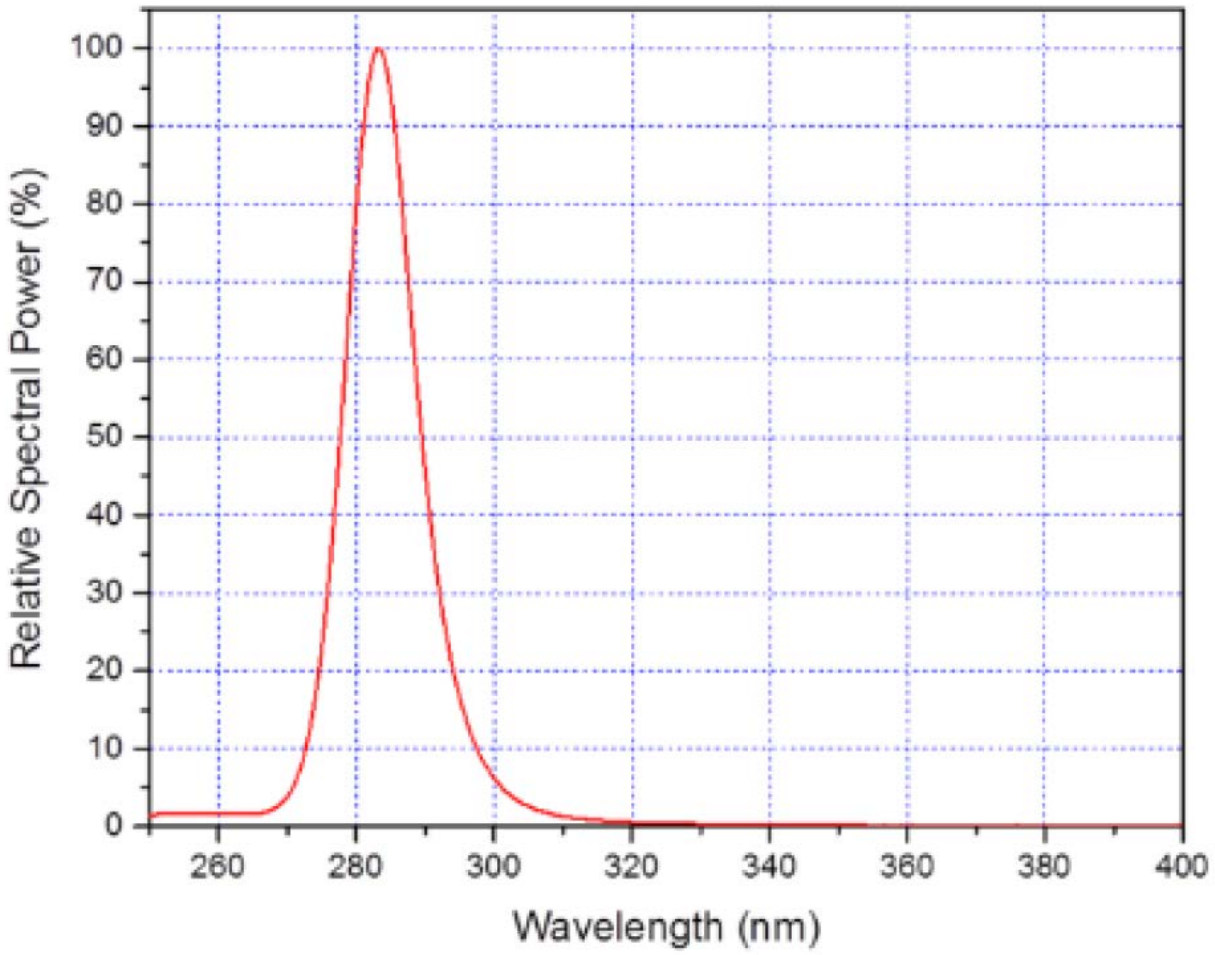

Figure 18: Output spectrum of selected UV LED for disinfection unit (MTSM285UV-F1120S) [6].

Specification	Condition	Minimum	Maximum
Peak Wavelength (nm)	$I_F = 20$ mA	280	290
Power Output (mW)	$I_F = 20$ mA	1.0	2.0
Forward Voltage (V)	$I_F = 20$ mA	5.0	7.0
FWHM (nm)	$I_F = 20$ mA	10.0	15.0

Table 5: Nominal electrical and optical characteristics of selected UV LED [6].

The specifications for the peak wavelength and FWHM of the UV LED indicate that it is a suitable component for functioning as both a disinfection and excitation source, as its most intense emission wavelengths correspond to the absorption bands of tryptophan and tyrosine, along with possessing high germicidal effectiveness. Slight deviations in the exact peak emission wavelength are not expected to drastically affect the excitation and disinfection functionalities.

3.2.3. Operation

The operation of the disinfection unit subsystem is shown in Figure 19 below.

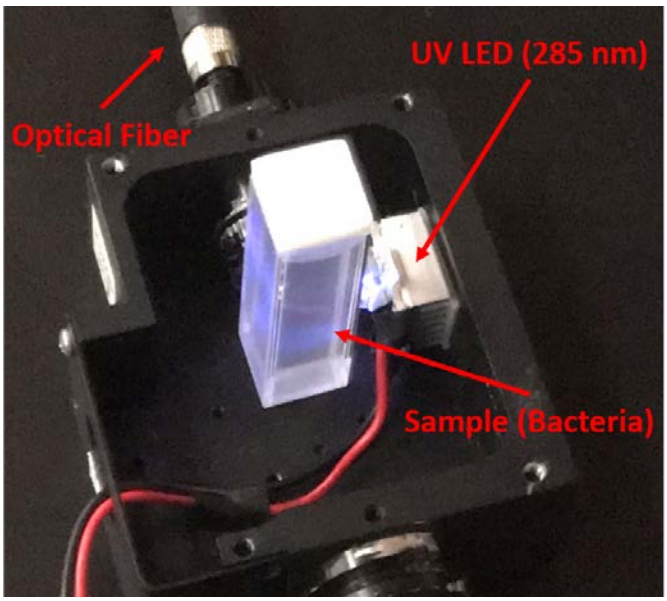

Figure 19: Operation of disinfection unit.

The UV LED was biased to operate at a forward current of approximately 25 mA, which is within its absolute maximum ratings. Power was supplied by a benchtop, regulated DC power supply. The bacterial sample is located at approximately the center of both the UV collection lens and UV LED. This maximizes the fluorescence coupled to the optical fiber and transmitted to the emission monochromator subsystem. A cover for the enclosure will be designed and fabricated next semester to minimize background (stray) light interference. Figure 20 below shows the effect of attaching the UV ball lens to the UV LED when it is turned on.

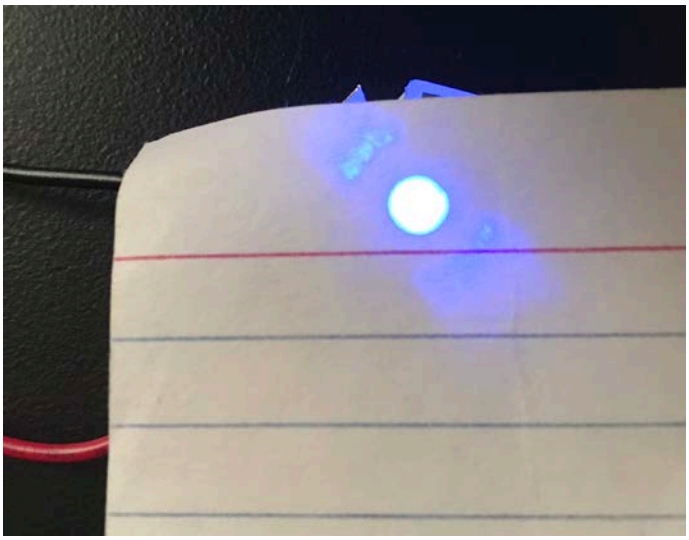

Figure 20: Effect of attaching a UV ball lens to the UV LED.

With the UV ball lens, the output light is more focused and results in a higher excitation intensity, which increases both the sample fluorescence and disinfection rate. When coupled to the emission monochromator subsystem, the disinfection unit may simultaneously inactivate bacteria and serve as an excitation source for normal fluorescence measurements. This serves as an efficient means of monitoring changes in the fluorescence of a bacterial sample, indicative of the fraction of bacteria inactivated, as a function of the UV irradiation time.

3.3. Subsystem Validation

3.3.1. Excitation Source Functionality

The functionality of the disinfection unit as an excitation source was validated by recording the normal fluorescence of tyrosine (≈ 1 mg/mL), tryptophan (≈ 1 mg/mL), and *E. coli* bacteria (≈ 10^8 cells/mL) with the emission monochromator subsystem.

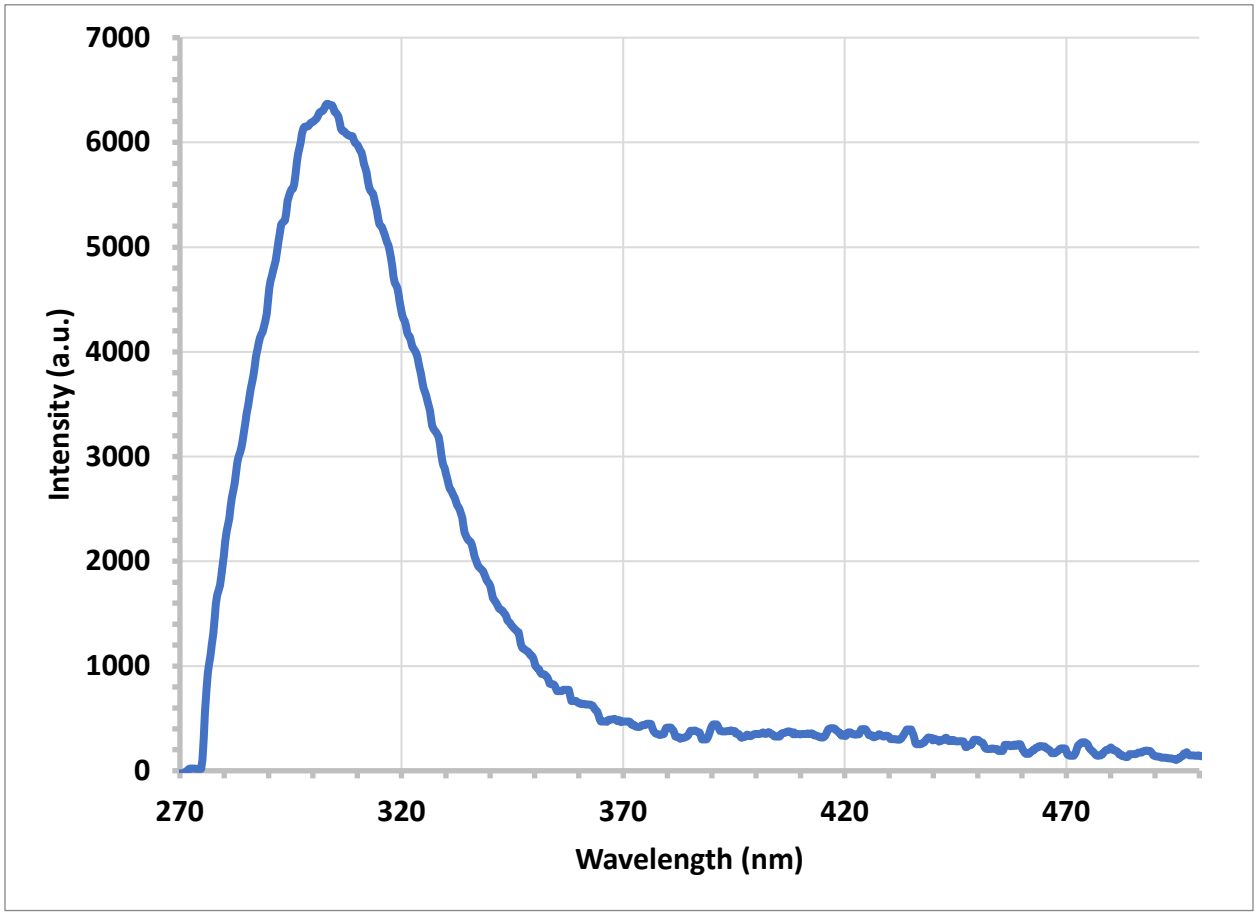

Figure 21: Normal fluorescence (λ_{EX} ≈ 285 nm) spectrum of tyrosine (≈ 1 mg/mL) acquired with the disinfection unit as the excitation source.

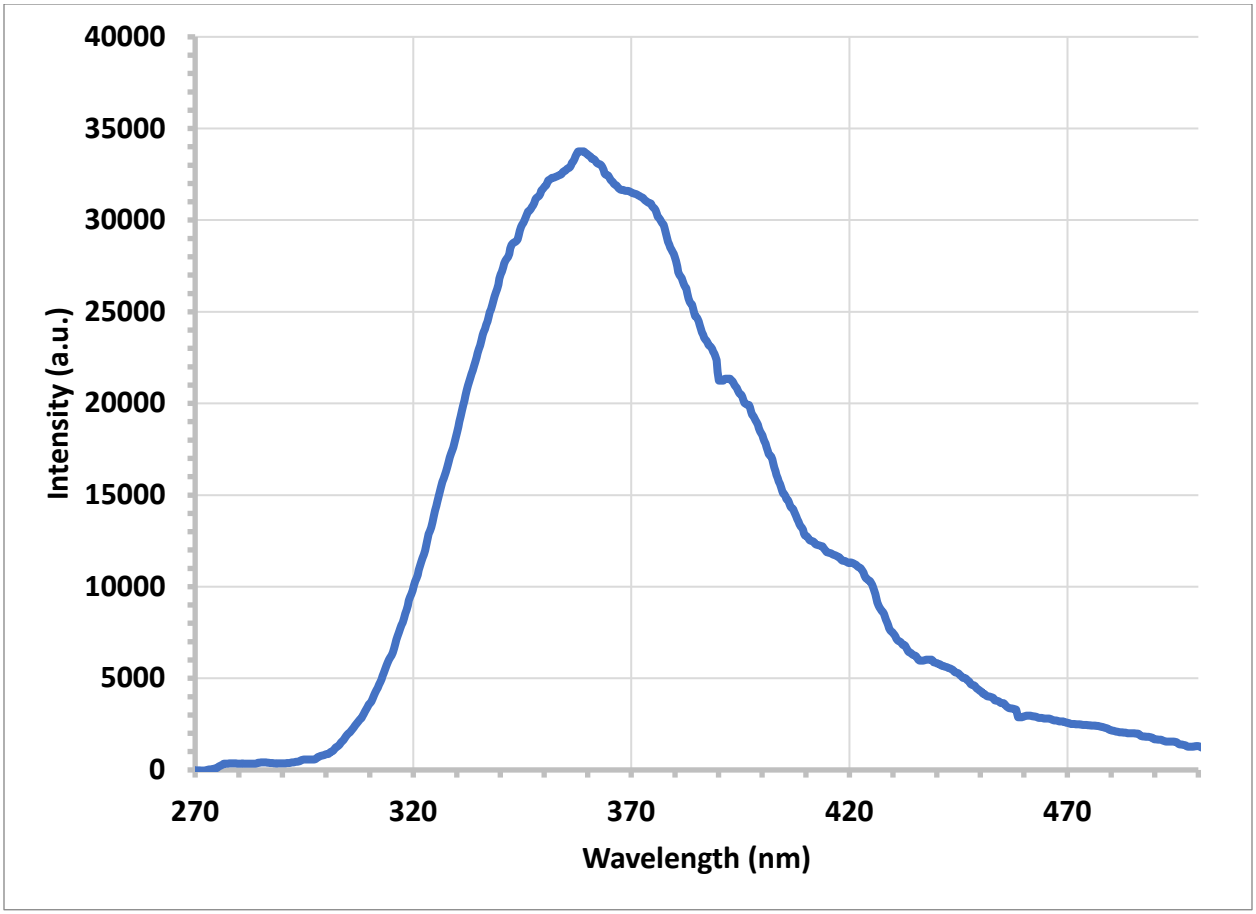

Figure 22: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectrum of tryptophan (≈ 1 mg/mL) acquired with the disinfection unit as the excitation source.

In comparing Figure 21 and Figure 22, it is of relevance to observe the differences in the normal fluorescence spectra of tyrosine and tryptophan at $\lambda_{EX} \approx 285$ nm, namely the region of maximum fluorescence and the peak fluorescence intensity itself. A more comprehensive validation of these normal fluorescence measurements is provided in the excitation monochromator and emission monochromator subsystem reports.

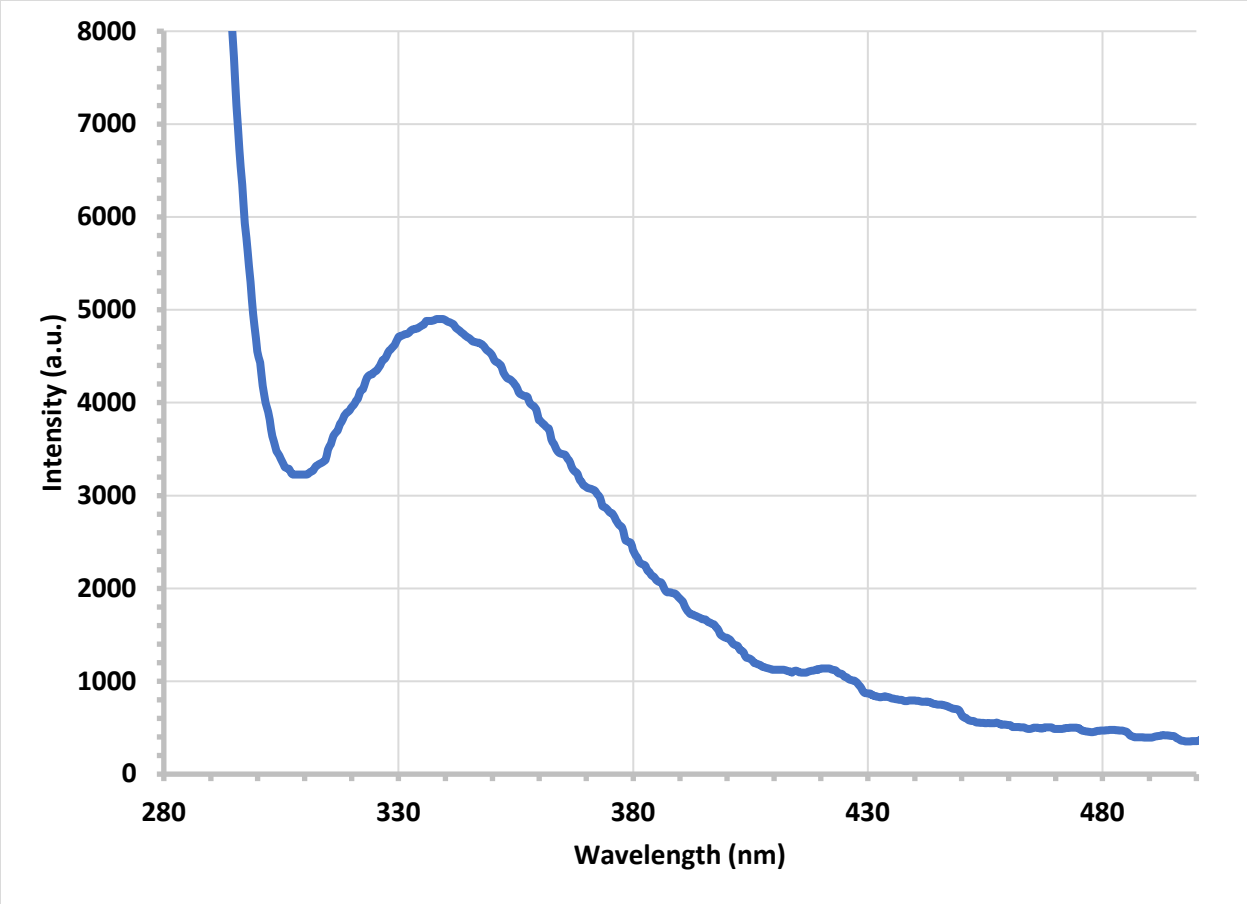

Figure 23: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectrum of *E. coli* bacteria ($\approx 10^8$ cells/mL) acquired with the disinfection unit as the excitation source.

The initially high intensity is due to Rayleigh (elastic) scattering of the excitation beam and may be used as a means of estimating the peak emission wavelength of the LED. The fluorescence band of *E. coli* is clearly observed with a maximum at approximately 340 nm. This fluorescence is due, primarily, to tryptophan and tyrosine residues within the bacterial cells. The detection of this band is therefore a validation of the excitation functionality of the disinfection unit. A more comprehensive validation of this normal fluorescence measurement is provided in the excitation monochromator and emission monochromator subsystem reports.

3.3.2. Disinfection Source Functionality

The functionality of the disinfection unit as an excitation source was validated by recording the normal fluorescence of *E. coli* bacteria ($\approx 10^8$ cells/mL) as a function of varying irradiation, or disinfection, times. The disinfection unit was therefore used as both an excitation and disinfection source for this experiment.

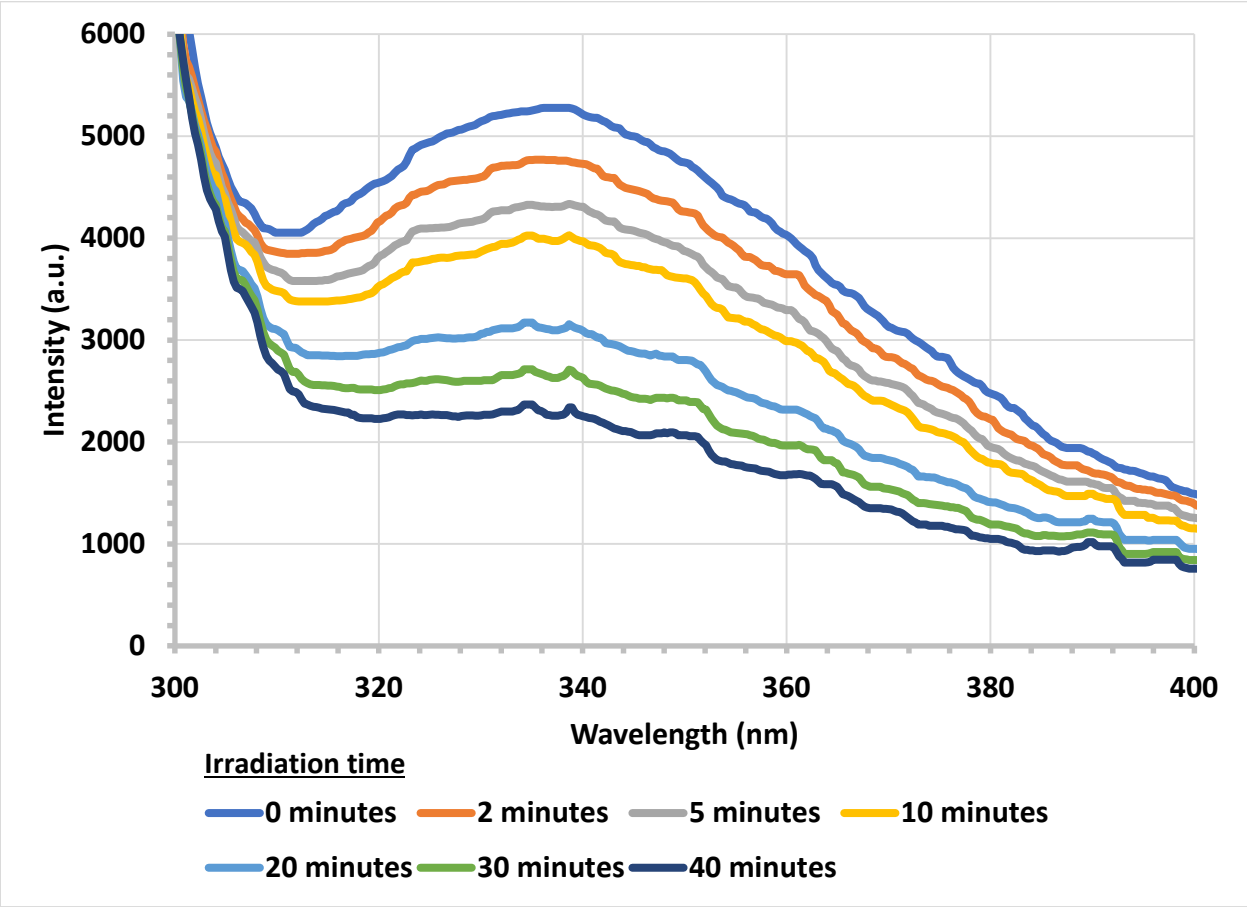

Figure 24: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectra of *E. coli* bacteria ($\approx 10^8$ cells/mL) as a function of disinfection time.

In this experiment, the irradiation time varied from 0-40 minutes in varying time steps. Clearly, the fluorescence spectra of *E. coli* decrease continuously as a function of the irradiation time. To further investigate this decrease, the fluorescence band maximum, or peak intensity, was plotted as a function of irradiation time.

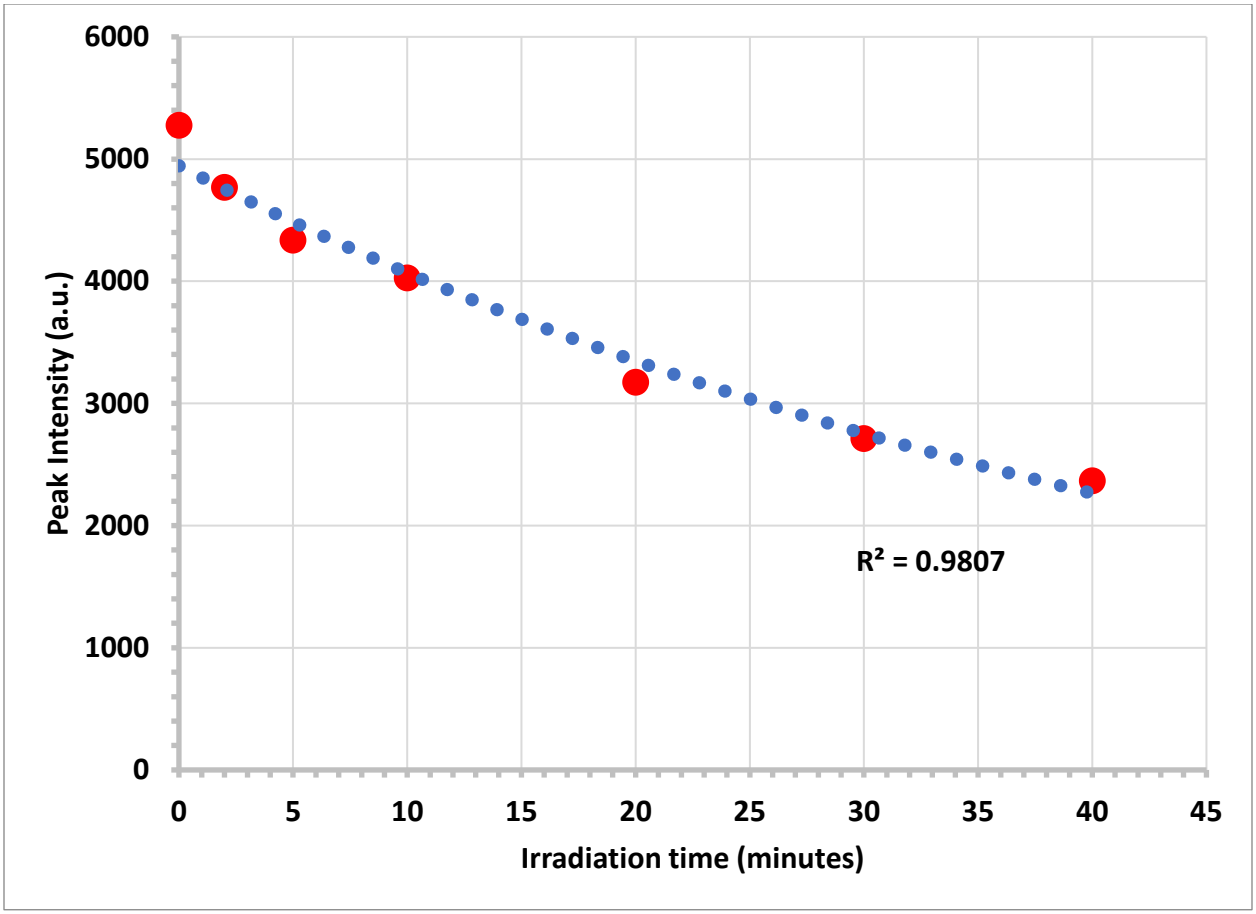

Figure 25: Peak fluorescence intensity as a function of irradiation time.

The experimental data in Figure 25 is plotted with an exponential fit. This suggests that the peak fluorescence intensity decays exponentially as a function of the irradiation time. The nature of this fluorescence decay will be investigated in more detail next semester. Nonetheless, it is well known that both tryptophan and tyrosine degrade in response to UV exposure, which results in a decrease in fluorescence [7]. This degradation, in turn, is correlated with damage to DNA and the subsequent inactivation of bacteria. We may therefore interpret the decrease in fluorescence as a validation of the disinfection capability of this subsystem. Furthermore, the inherent germicidal effectiveness of the UV LED wavelength is a rather strong confirmation of this functionality.

3.4. Subsystem Conclusion

In conclusion, this subsystem satisfies all required functionalities. Namely, it is capable of serving as both an excitation source for normal fluorescence measurements, as well as a disinfection source for irradiating and inactivating bacterial samples. The design and fabrication of a cover for the disinfection unit will be completed next semester. In addition, the exact nature of the inactivation process will be further investigated and quantified next semester.

4. Excitation Monochromator Subsystem Report

4.1. Subsystem Introduction

The excitation monochromator subsystem serves as a scanning excitation source for performing EEM acquisition and acquiring synchronous fluorescence spectra. It is composed of a (1) high-power, miniature UV LED as an excitation source, (2) a scanning monochromator coupled with a stepper motor for selecting a particular excitation wavelength from the LED spectrum, and (3) a stepper motor controller and driver for rotating the excitation grating. Commands are issued to this subsystem through a serial communication link between the computer and controller. These components are housed in a lightweight, portable enclosure.

4.2. Subsystem Details

4.2.1. Components

A picture of the current embodiment for the excitation monochromator subsystem, along with its major components, is shown in Figure 26 below.

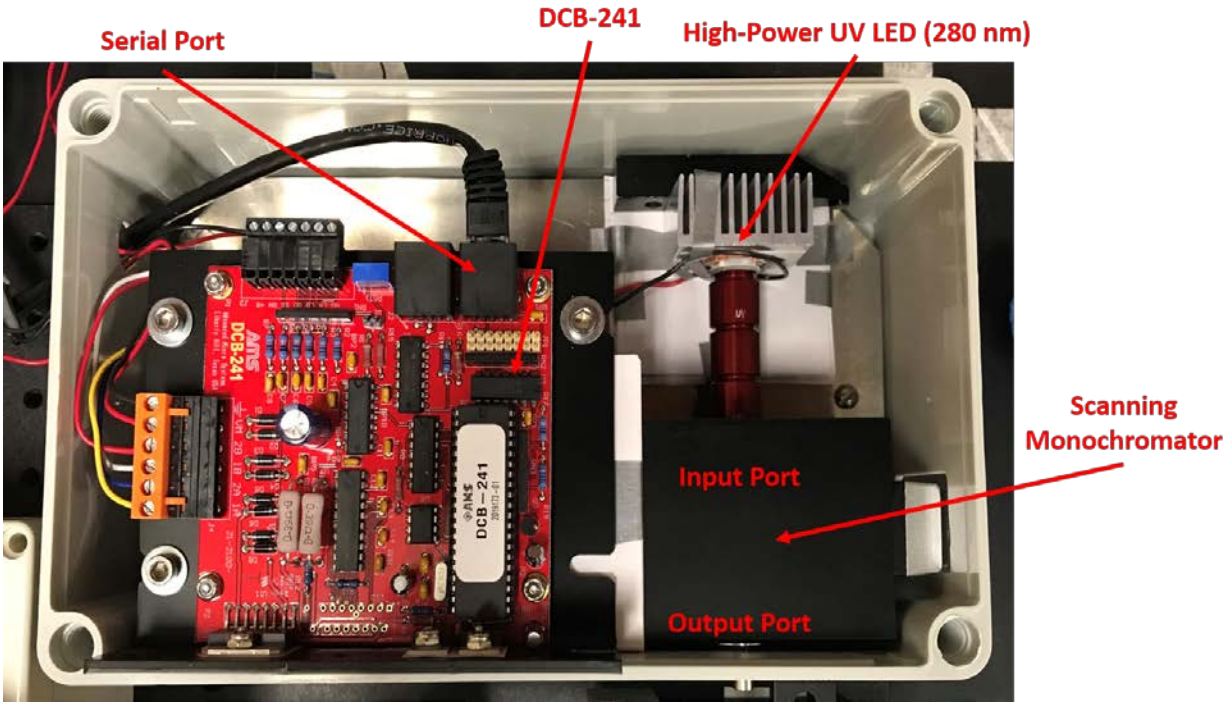

Figure 26: Excitation monochromator subsystem and its major components.

The excitation monochromator currently consists of a high-power miniature UV LED (RVXP4-280-SM-077132) mounted on a heat sink and coupled with a set of three UV lenses. Similar to the disinfection unit, the UV LED is attached to the heat sink by means of a thermal adhesive to promote efficient heat dissipation, which is critical in ensuring stable LED operation. The three UV lenses are necessary for collecting and reducing the divergence of the LED output and ensuring good coupling is achieved with the scanning monochromator,

which principally determines the excitation intensity at the sample location and consequent fluorescence intensity.

The scanning monochromator is an off-the-shelf component from Dynasil. The monochromator consists of an optical bench housing two folding mirrors, a single curved mirror, and a reflective diffraction grating. The function of this component is to disperse the broadband UV light from the LED and focus a particular wavelength of the dispersed light on the exit slit of the monochromator. The particular wavelength of the dispersed light focused on the exit slit will be dependent on the angular positioning of the excitation grating and controlled by an onboard stepper motor. To that effect, EEM acquisition can occur by rotating the excitation grating and therefore scanning over a range of excitation wavelengths.

The integrated stepper motor controller and driver is the DCB-241, which was previously described in detail in the control and display unit subsystem report. The controller is wired to the stepper motor within the monochromator through an external DB-15 connector. Communication with the controller is achieved through the onboard serial port.

A mounting plate for this subsystem was designed and fabricated through the Physics Machine Shop at Texas A&M University with the assistance of Mr. Garrick Garza. A cheap, compact electronics project box was utilized as the enclosure. A picture of the overall design is shown in Figure 27 below.

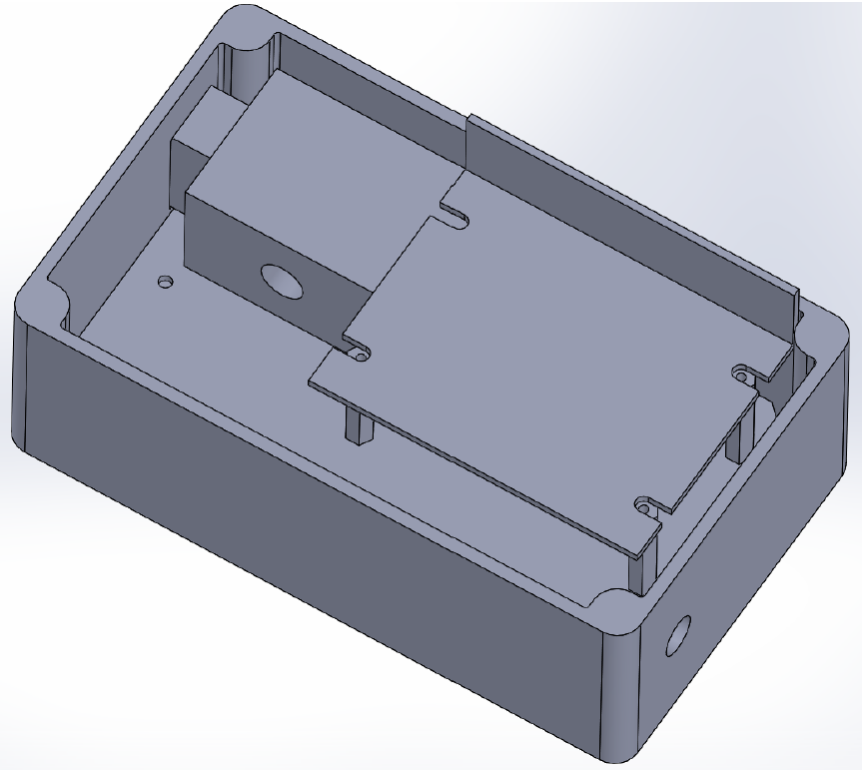

Figure 27: Design for the excitation monochromator subsystem, including the enclosure and mounting plate.

4.2.2. UV LED

Selection of the UV LED wavelength was guided, mainly, by the same considerations noted in the disinfection unit subsystem report. Owing to the fact that the LED output passes through a monochromator in this subsystem, however, an LED with a significantly higher output power was selected. This is necessary to account for both (a) coupling losses at the input of the monochromator and (b) diffraction losses within the monochromator itself, which will significantly reduce the excitation intensity at the sample location.

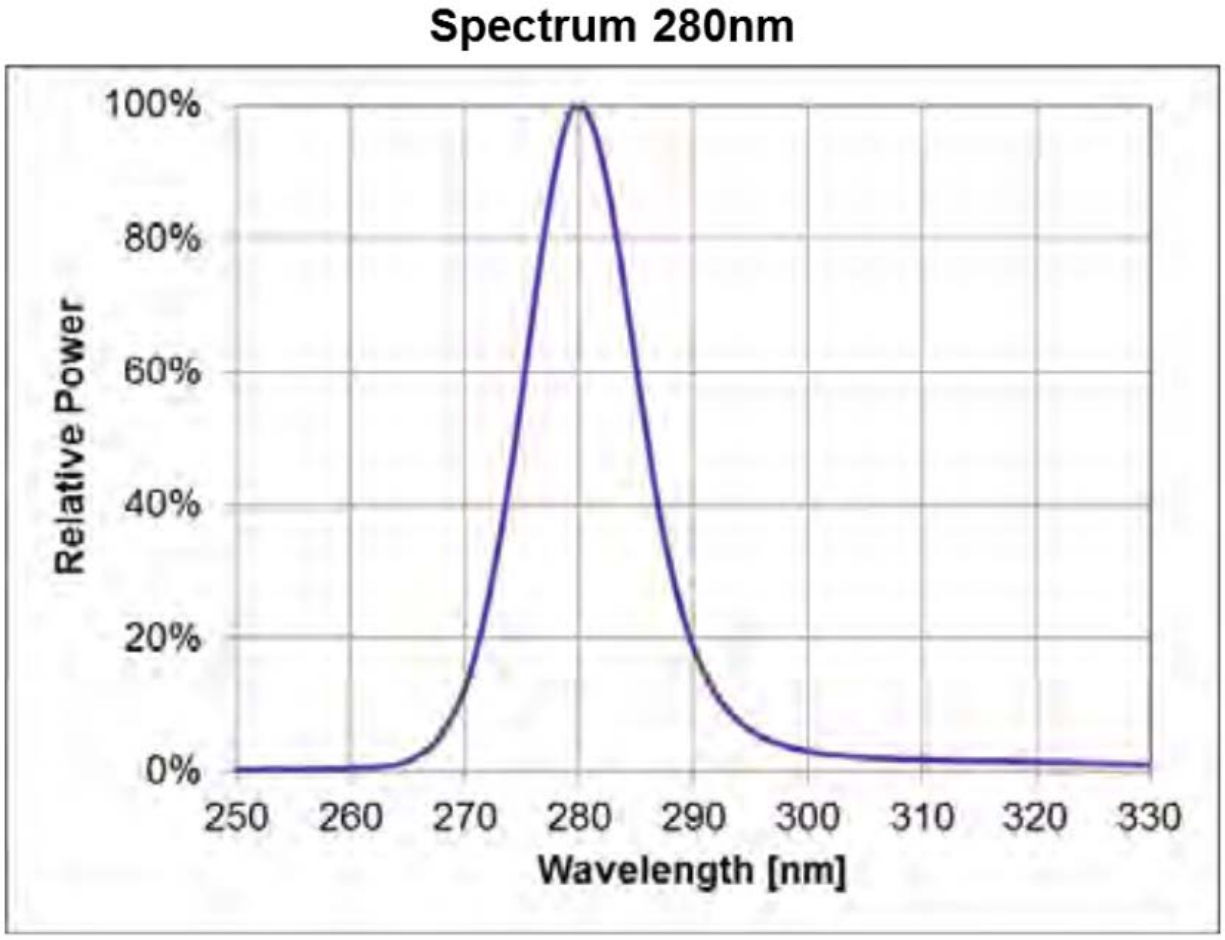

Figure 28: Output spectrum of selected UV LED for excitation monochromator (RVXP4-280-SM-077132) [8].

Specification	Condition	Minimum	Typical	Maximum
Peak Wavelength (nm)	$I_F = 200$ mA	275	280	285
Power Output (mW)	$I_F = 200$ mA	30	50	70
Forward Voltage (V)	$I_F = 200$ mA	20	26	32
FWHM (nm)	$I_F = 200$ mA	Not given.	15	Not given.

Table 6: Nominal electrical and optical characteristics of selected UV LED [8].

Clearly, the selected UV LED for the excitation monochromator has a significantly higher optical power than that used for the disinfection unit. This output power is among the highest, if not the highest, radiant output available for current UV LED technology.

4.2.3. Scanning Monochromator

The scanning monochromator selected for this subsystem is the Scanning Digital Mini-Chrom Monochromator (SDMC1-02) sold by Dynasil. The optical layout for this component is provided in Figure 29 below.

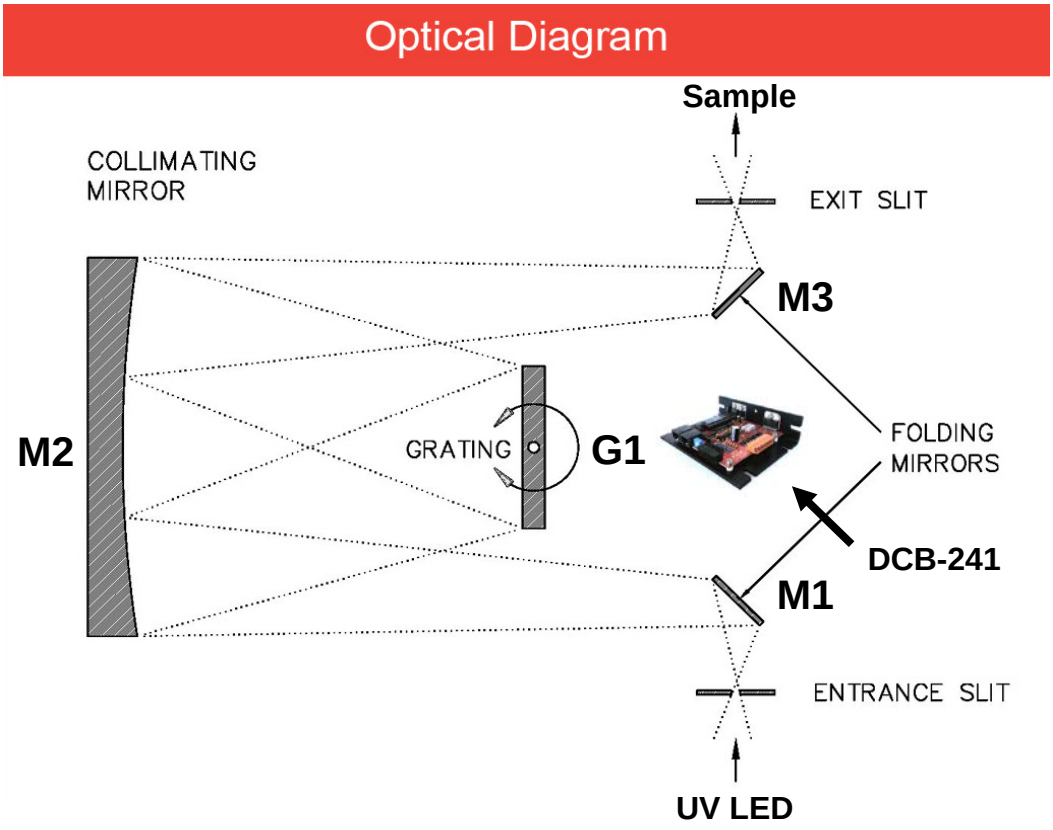

Figure 29: Schematic of the scanning monochromator in the excitation monochromator subsystem [9].

The monochromator optical bench houses a Fastie-Ebert configuration, consisting of two folding mirrors M1 and M3, a single curved mirror M2, and a reflective diffraction grating G1. The high-power, miniature UV LED is coupled to the bench at the entrance slit, whereupon the input, broadband UV light is reflected by M1 to M2. M2 then collimates and directs the light to the reflective grating G1, which diffracts and disperses the light into its component wavelengths. The dispersed light is reflected from G1 to M2 again, which focuses the light to the exit slit by means of M3. The particular wavelength of the dispersed light focused on the exit slit is dependent on the angular positioning of the excitation grating G1. To that effect,

the DCB-241 rotates a stepper motor within the monochromator connected to the excitation grating. This allows for rotation to a desired output wavelength or, alternatively, through a range of excitation wavelengths, as desired in an EEM acquisition. An optical fiber may optionally be connected at the exit slit to transmit the excitation wavelength to the sample; alternatively, to maximize the excitation intensity, the sample may be directly situated at the exit slit itself. It is worthy to mention that all components in the optical bench are optimized for performance in the UV spectral region, particularly the grating.

4.2.4. DCB-241

The DCB-241 was described in detail in the control and display unit subsystem report. The commands provided in Table 2 of that report were utilized to set the initial and final slew velocity of the stepper motor within the scanning monochromator, rotate the stepper motor and excitation grating, and thereby perform EEM acquisitions when coupled with the emission monochromator subsystem. To supplement that information, some additional electrical specifications of the component are provided below.

Specification	Value
Input Voltage (V)	+24 to 40 V DC
Motor Step Resolution	Half Step

Table 7: Electrical specifications of the DCB-241.

4.2.5. Operation

The UV LED was biased to operate at a forward current of approximately 100 mA, which is well within its absolute maximum ratings. This bias point was selected to ensure stable operation but can be changed for future experiments as needed to increase the excitation intensity. Figure 30 shows the high-power UV LED mounted on a heat sink with the thermal adhesive.

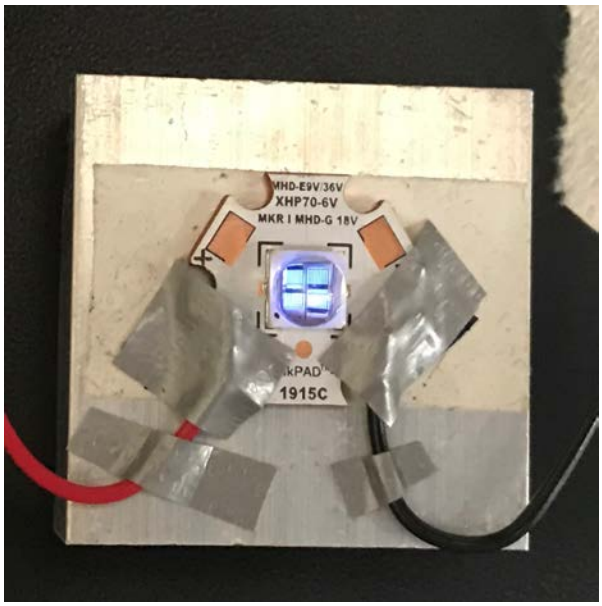

Figure 30: High-power UV LED mounted on heat sink with thermal adhesive.

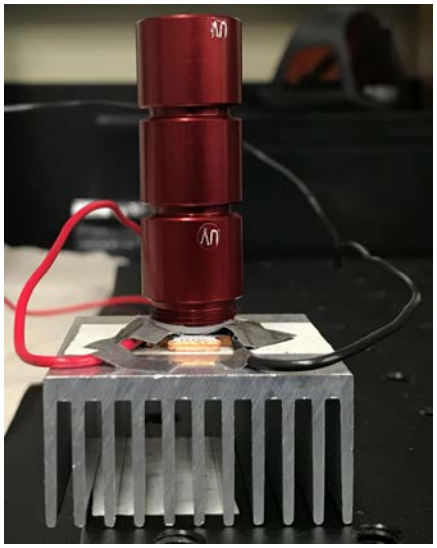

Figure 31: UV coupling lenses mounted on top of high-power UV LED for maximizing excitation intensity.

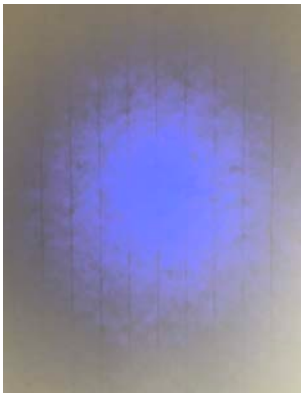

Figure 32: Image of UV LED output without coupling lenses.

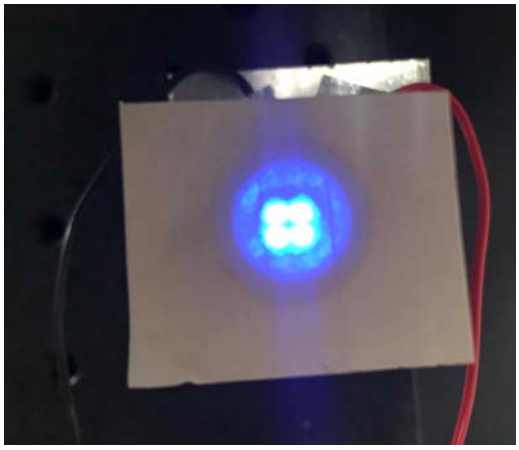

Figure 33: Image of UV LED output with coupling lenses.

As demonstrated in Figure 32 and Figure 33, the UV coupling lenses are critical in capturing a large fraction of the rapidly diverging UV light from the LED; the use of three lenses increases the optical power of the overall setup and results in a sharp image of the emitting surfaces within the LED. Alignment was then performed between the UV LED and input slit of the excitation monochromator to maximize the coupling between the two components.

The DCB-241 was then biased through its onboard potentiometer to output a run current which resulted in stable rotation of the stepper motor within the monochromator. This process involved rotating the onboard potentiometer in small increments and observing the resultant behavior and performance of the stepper motor in rotating the excitation grating. Once a minimum run current was established, it was increased by ≈ 10%. This was done to ensure reliable operation of the stepper motor and minimize heating due to large run currents.

The initial and final slew velocities of the stepper motor were then set to the lowest possible values and increased until the stepper motion was deemed sufficiently smooth. This was determined to occur at initial and final slew velocities of 20 steps/second.

To confirm the linearity of the stepper motor, the time required to traverse a range of steps as low as 160 to as high as 1600 was measured and compared with the theoretical time set by the slew velocities. The results of this test are shown in Figure 34 below.

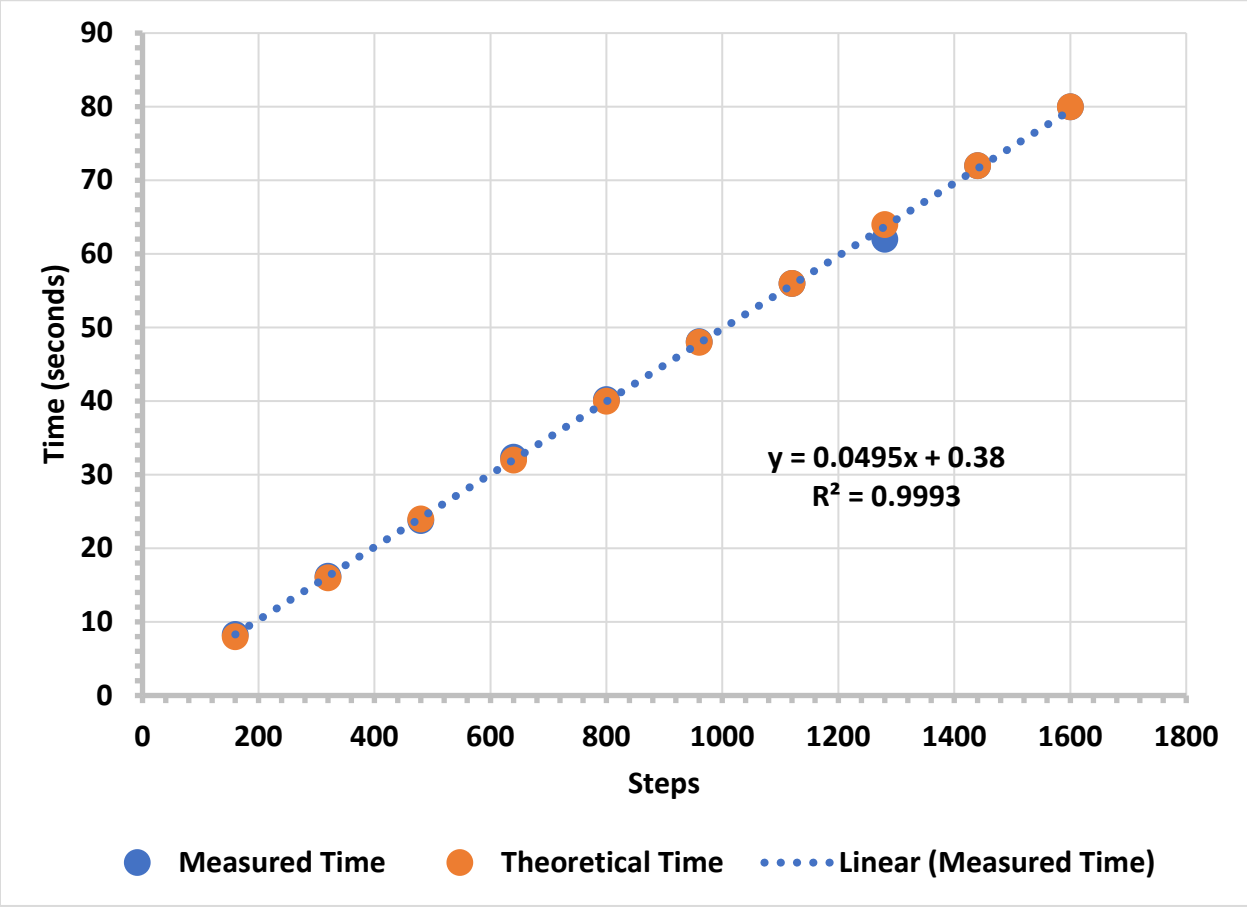

Figure 34: Comparison of measured time and theoretical time for stepper motor rotation.

Figure 34 clearly demonstrates the proper operation of the stepper motor at the biased, run current and initial and final slew velocities. These parameters can be easily adjusted and refined if the stepper motor operation is deemed insufficient at any time. For the experimental data collected this semester, though, such parameters were sufficient in achieving the desired performance of the subsystem.

Power was supplied to both the UV LED and DCB-241 by a benchtop, regulated DC power supply to simplify the validation of the subsystem.

4.3. Subsystem Validation

4.3.1. Experimental Setup for Acquiring EEM

The functionality of the excitation monochromator in recording the EEM of a sample was validated by recording the EEM of tyrosine (≈ 1 mg/mL), tryptophan (≈ 1 mg/mL), a mixture of tyrosine and tryptophan, and *E. coli* bacteria (≈ 10^8 cells/mL). This was accomplished by utilizing the excitation monochromator as a scanning excitation source and detecting the sample fluorescence with the emission monochromator subsystem. A picture of the overall experimental setup that was built for this validation is shown in Figure 35 below.

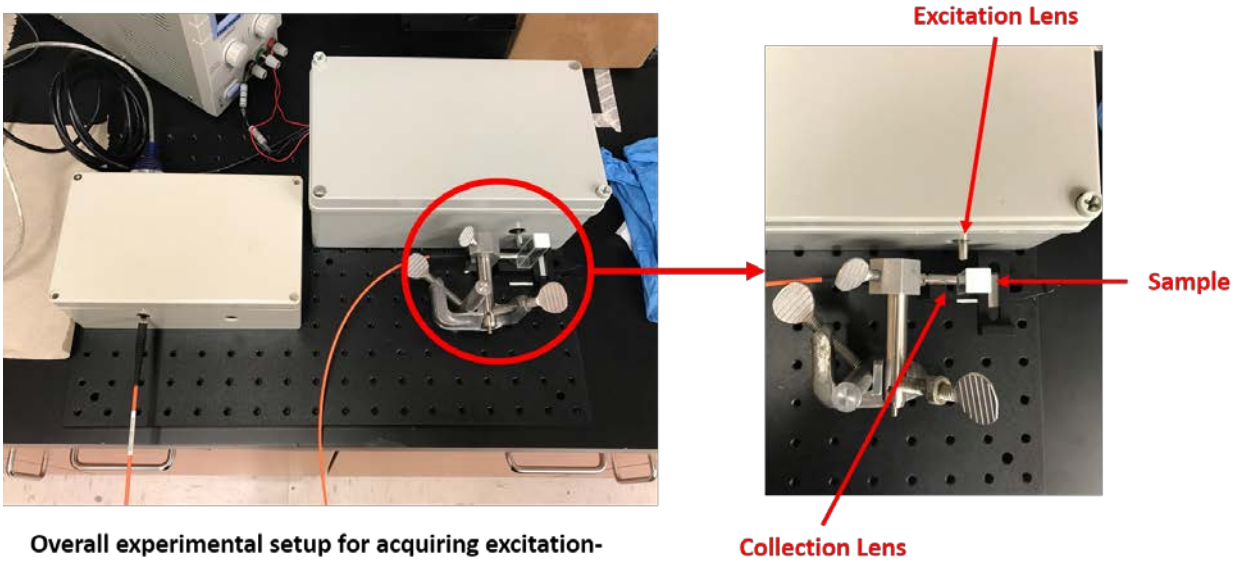

Figure 35: Overall experimental setup for acquiring the excitation-emission matrix (EEM) of a sample.

In brief, the output of the excitation monochromator was focused onto the sample by means of a UV excitation lens. The sample was located at, approximately, the focal point of the excitation lens to maximize the excitation and fluorescence intensity. The sample fluorescence was then captured by a UV collection lens coupled with an optical fiber. The optical fiber then transmitted the sample fluorescence to the input slit of the emission monochromator subsystem. By recording the sample fluorescence over a range of excitation wavelengths, the sample EEM may be acquired.

4.3.2. EEM Acquisition

The EEMs of tyrosine (≈ 1 mg/mL), tryptophan (≈ 1 mg/mL), a mixture of tyrosine and tryptophan, and *E. coli* bacteria (≈ 10^8 cells/mL) are presented below. Synchronous fluorescence spectra are compared with those acquired by a benchtop, commercial spectrometer as validation of the subsystem's operation.

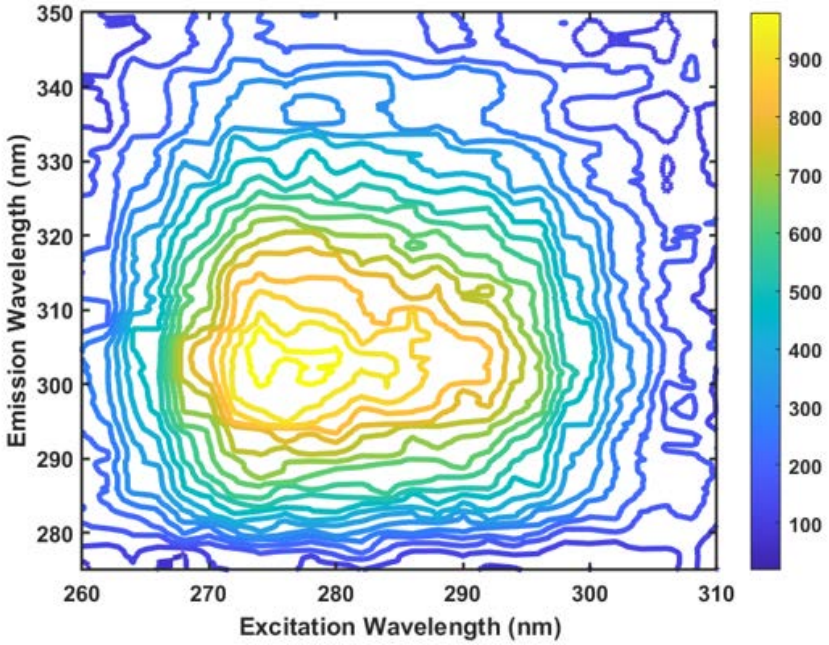

Figure 36: 2D contour plot of EEM for tyrosine (≈ 1 mg/mL) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

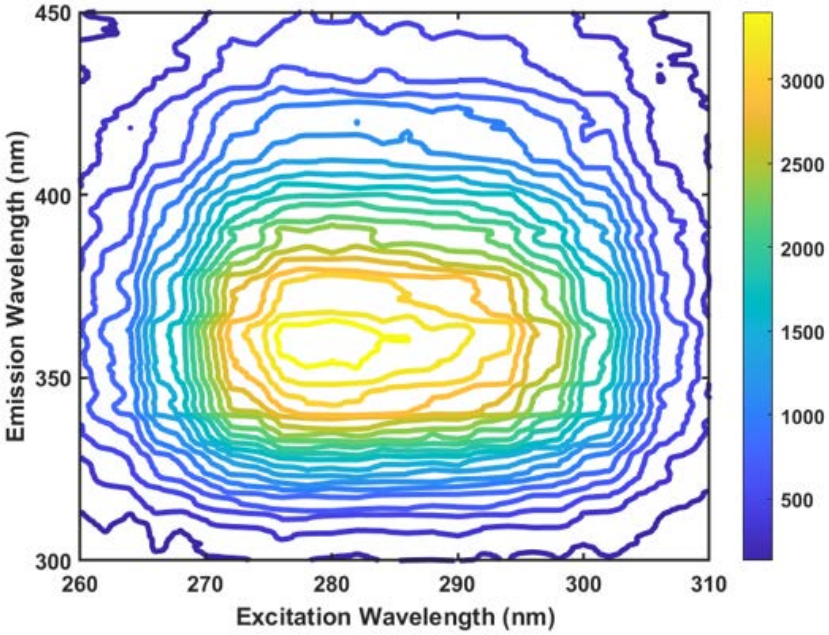

Figure 37: 2D contour plot of EEM for tryptophan (≈ 1 mg/mL) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

Figure 36 and Figure 37 offer a detailed characterization of the fluorescence bands associated with tyrosine and tryptophan. In particular, while both amino acids absorb light at an excitation wavelength of $\lambda_{EX} \approx 280$ nm, the fluorescence band maximum occurs at a distinctly higher wavelength and intensity for tryptophan.

After acquiring these EEMs, a mixture of tyrosine and tryptophan (≈ 1 mg/mL concentration for each component) was created. The resulting EEM and synchronous fluorescence spectra are shown in Figure 38 and Figure 39 below.

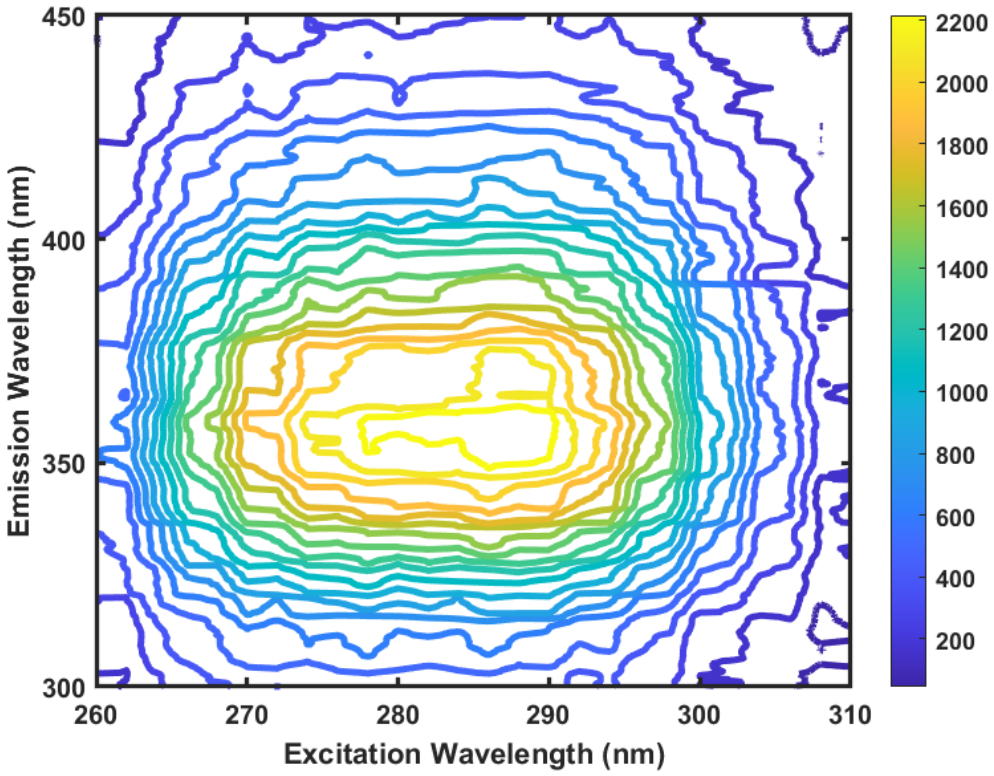

Figure 38: 2D contour plot of EEM for mixture of tyrosine and tryptophan (≈ 1 mg/mL concentration for each component) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

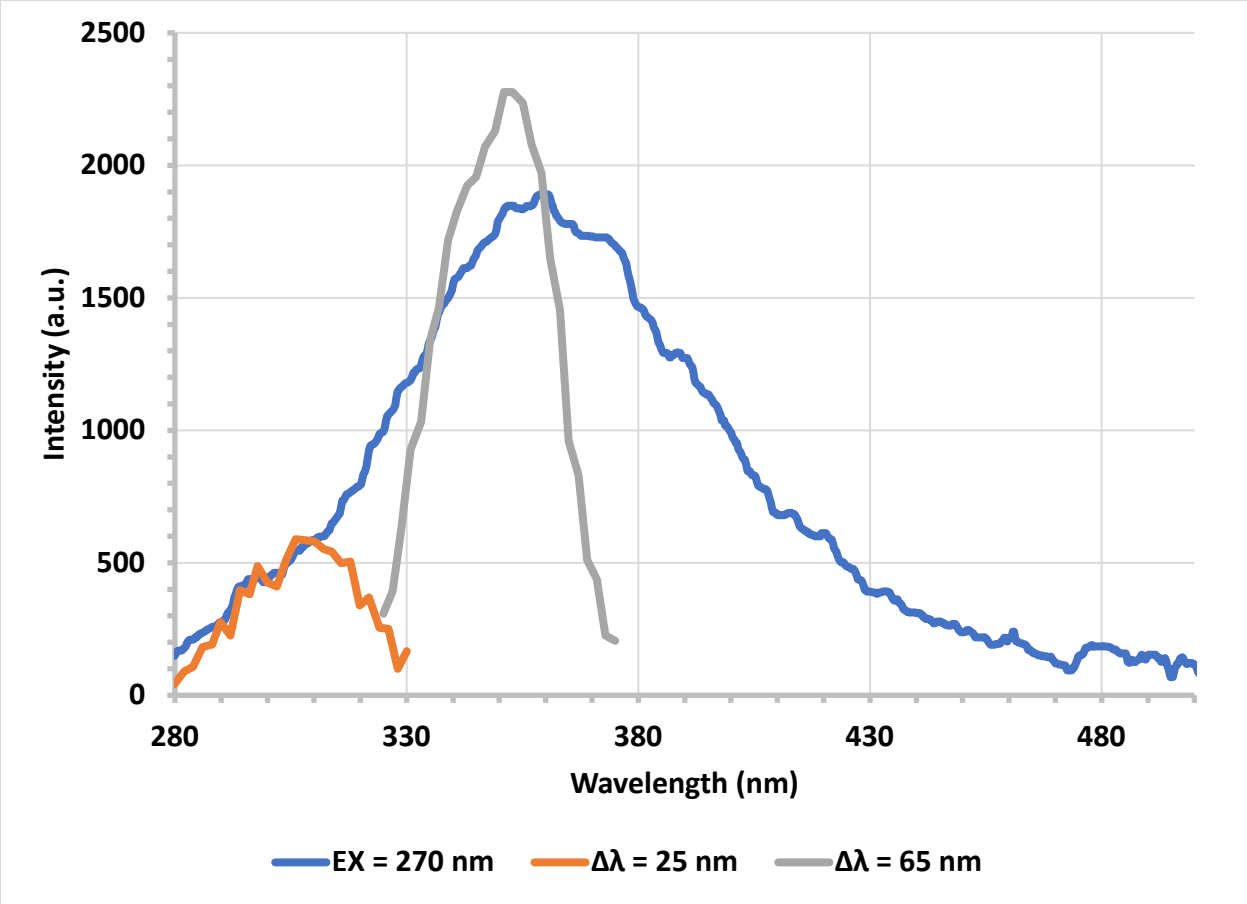

Figure 39: Normal fluorescence and synchronous fluorescence spectra of tyrosine and tryptophan mixture extracted from EEM.

Figure 39 demonstrates the utility of synchronous fluorescence in resolving the fluorescence bands of individual components within a mixture. Namely, the normal fluorescence spectrum is simply a broad, structureless band that cannot be uniquely assigned to either tryptophan or tyrosine individually. In contrast, the synchronous fluorescence spectra at $\Delta\lambda = 25$ nm and $\Delta\lambda = 65$ nm resolve the tyrosine and tryptophan components, respectively.

To further validate this procedure, the EEM and synchronous fluorescence spectra of *E. coli* bacteria were acquired. The synchronous fluorescence spectra were then compared with those acquired on a benchtop, commercial spectrometer (Shimadzu RF5301PC).

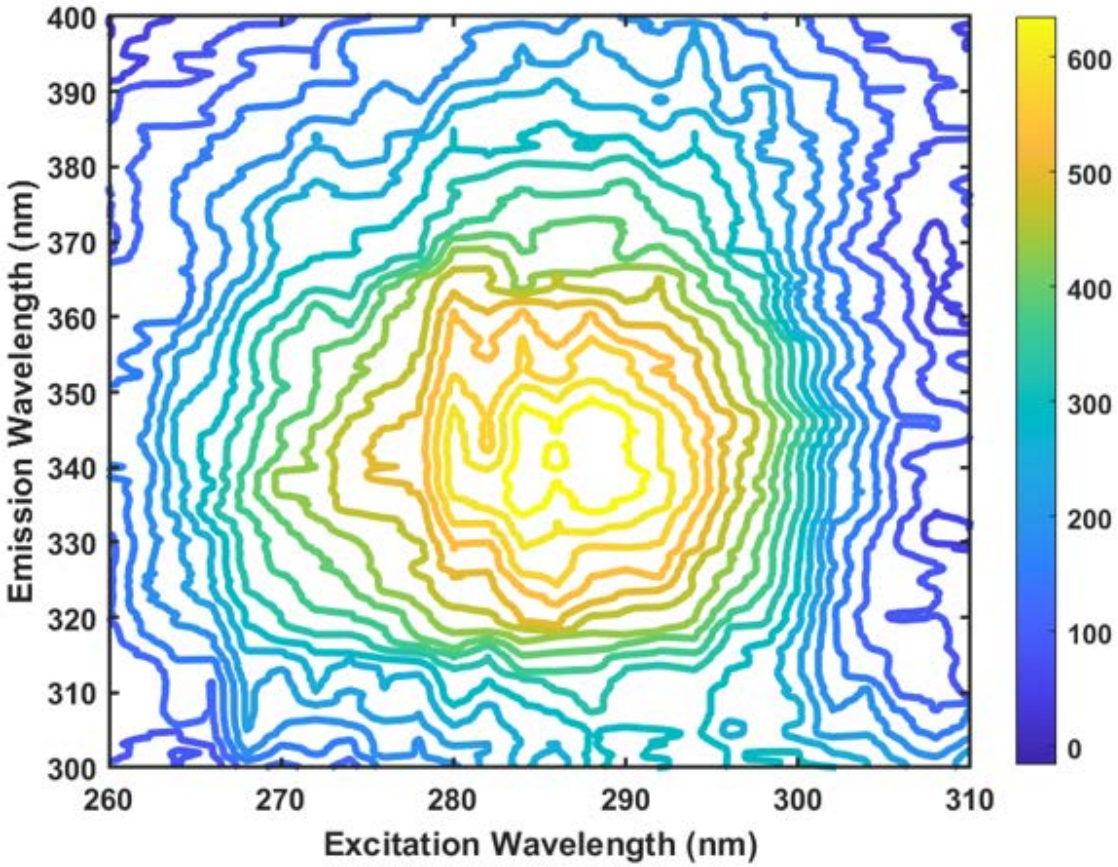

Figure 40: 2D contour plot of EEM for *E. coli* bacteria (≈ 10^8 cells/mL) acquired over an excitation range of 260 to 310 nm in 2 nm steps.

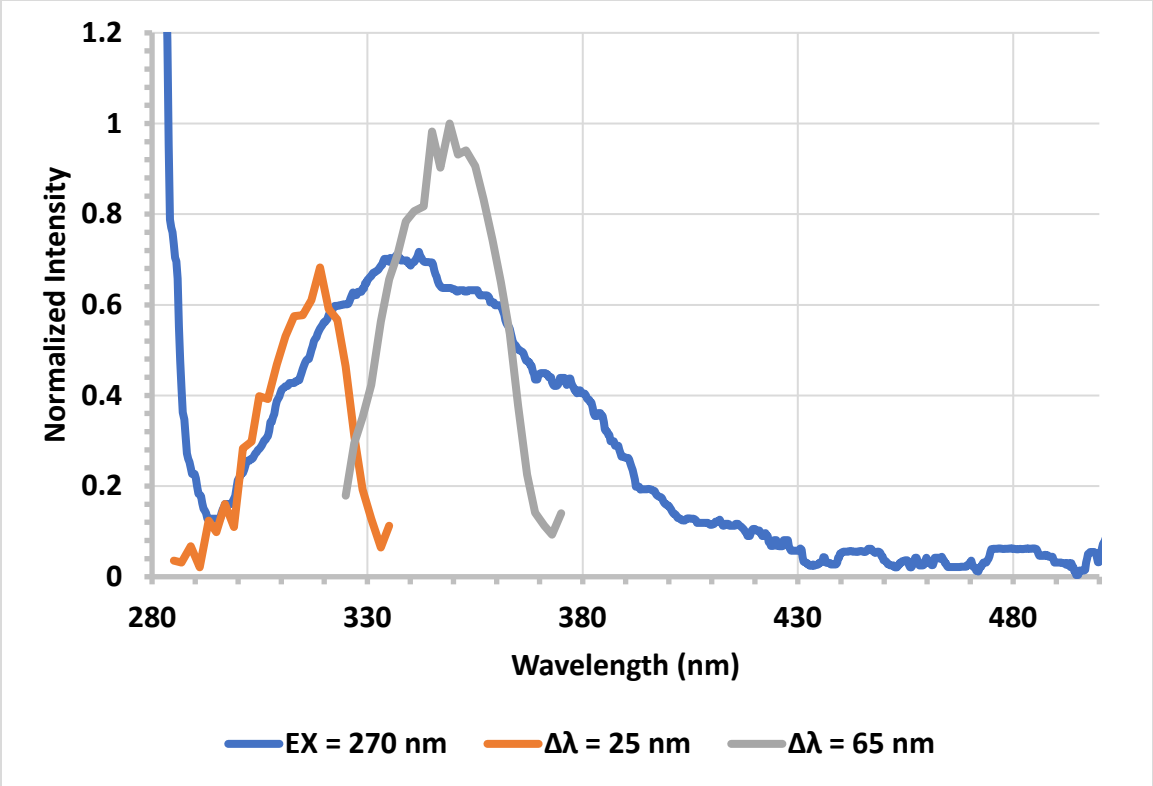

Figure 41: Normal fluorescence and synchronous fluorescence spectra of *E. coli* bacteria extracted from EEM. The spectra are normalized.

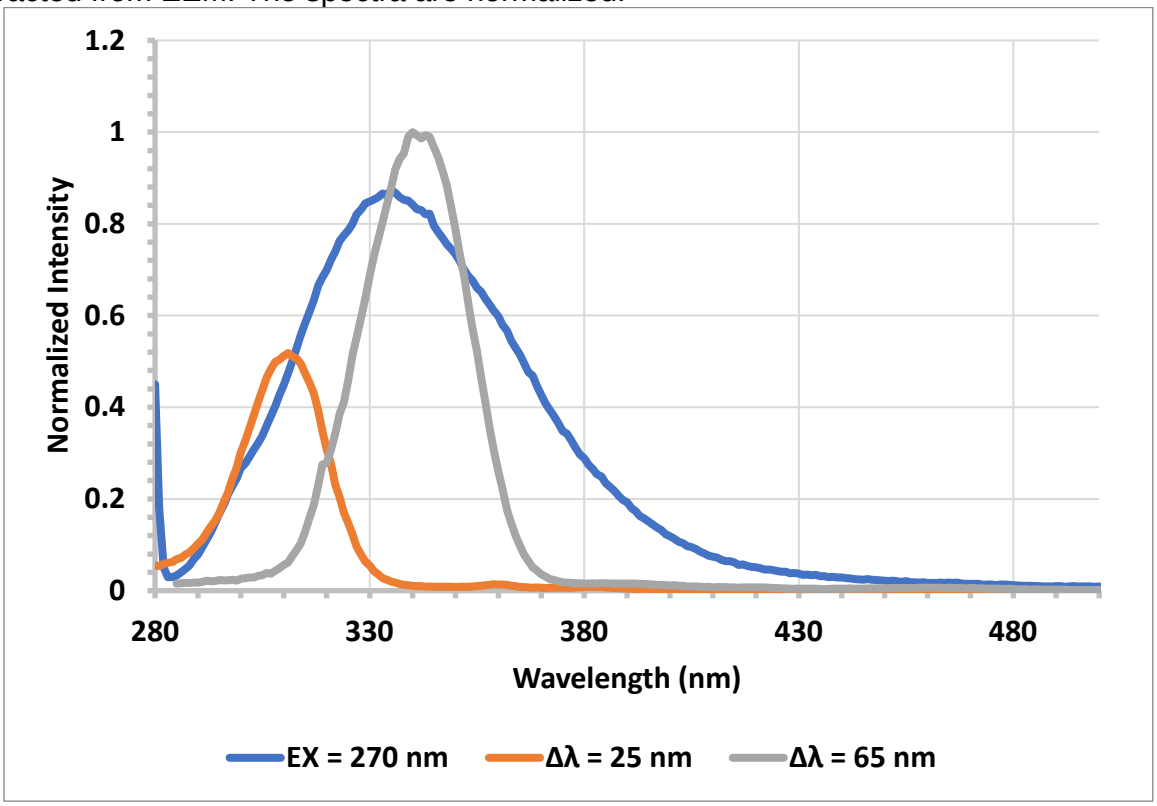

Figure 42: Normal fluorescence and synchronous fluorescence spectra of *E. coli* bacteria recorded with benchtop, commercial spectrometer. The spectra are normalized.

The portable and benchtop spectra are plotted in normalized units. In general, the agreement between the two set of spectra is very good, indicating that the components and techniques utilized in the portable instrument for acquiring synchronous spectra are validated. It is worthy to mention that the exact intensities and locations of certain bands may be slightly different due to variations in the detector sensitivity and excitation source intensity. For example, the benchtop instrument utilizes a Xenon lamp as its excitation source, which has a much broader output spectrum than the UV LED source in the portable instrument; this accounts for the difference in the range of the synchronous spectra and would also introduce variations in the source intensity. The detector used in the benchtop instrument, a photomultiplier tube (PMT), also possesses its own sensitivity curve which would differ from the linear image sensor used in the portable instrument. Nonetheless, the individual fluorescence bands of tyrosine and tryptophan, in both a mixture and bacteria, are successfully resolved through the synchronous fluorescence technique, thus validating the excitation monochromator subsystem.

4.4. Subsystem Conclusion

In conclusion, this subsystem satisfies all required functionalities. In particular, it is capable of serving as a scanning excitation source for acquiring the EEM of a sample and extracting synchronous fluorescence spectra. These capabilities were validated for *E. coli* bacteria and its cellular components, tryptophan and tyrosine. For next semester, additional UV excitation sources will be investigated to increase the output power of the excitation monochromator and sensitivity of the overall system.

5. Emission Monochromator Subsystem Report

5.1. Subsystem Introduction

The emission monochromator subsystem serves as the imaging spectrometer for detecting and recording the fluorescence spectra of bacteria under either fixed or scanning excitation. The subsystem is composed of a (1) optical bench with mirrors and a diffraction grating, (2) linear charge-coupled device (CCD) detector array, and (3) control electronics for digitizing the spectral data and communicating with the control and display unit subsystem. Commands are issued to this subsystem through a serial communication link between the computer and controller. These components are housed in a lightweight, portable enclosure.

5.2. Subsystem Details

5.2.1. Components

A picture of the current embodiment for the emission monochromator subsystem, along with its major components, is shown in Figure 43 below.

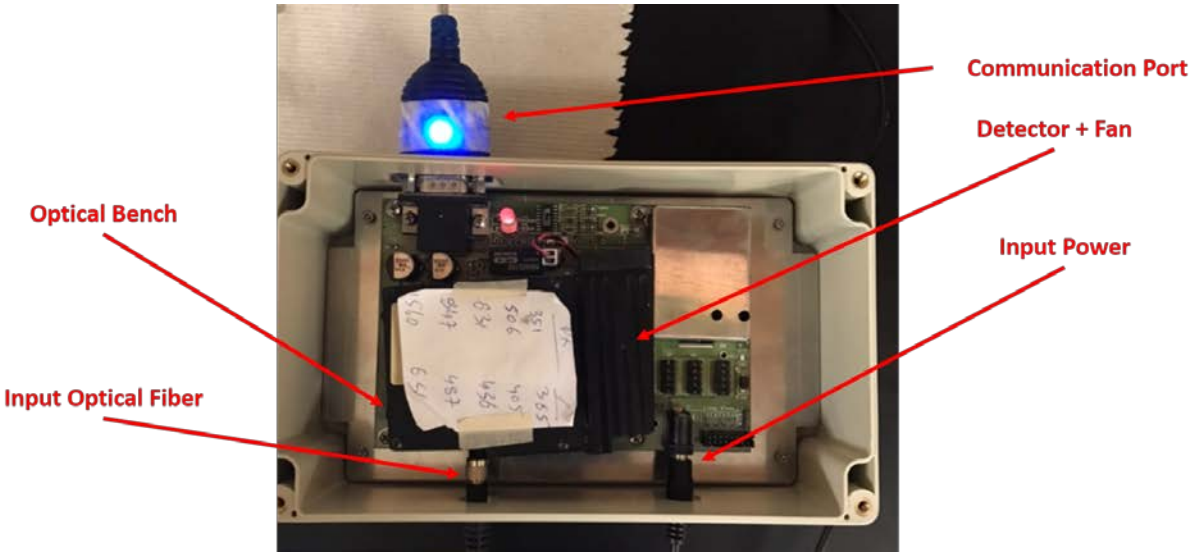

Figure 43: Emission monochromator subsystem and its major components.

The emission monochromator subsystem is an off-the-shelf spectrometer (BTC110-S) from B&W Tek. As shown in Figure 43, this system already includes an optical bench, input fiber port, input power port, detector and cooling fan, and communication port for setting acquisition parameters and acquiring spectral data. The optical bench itself is easily configurable and was modified with optical components, namely mirrors and a diffraction grating, with high efficiency in the UV region to increase the spectrometer's sensitivity to bacterial fluorescence. Details regarding the communication with the BTC110-S, and the associated procedures for setting acquisition parameters and receiving spectral data, were provided in the control and display unit subsystem report.

A mounting plate for this subsystem was designed and fabricated through the Physics Machine Shop at Texas A&M University with the assistance of Mr. Garrick Garza. A cheap, compact electronics project box was utilized as the enclosure. A picture of the overall design is shown in Figure 44 below.

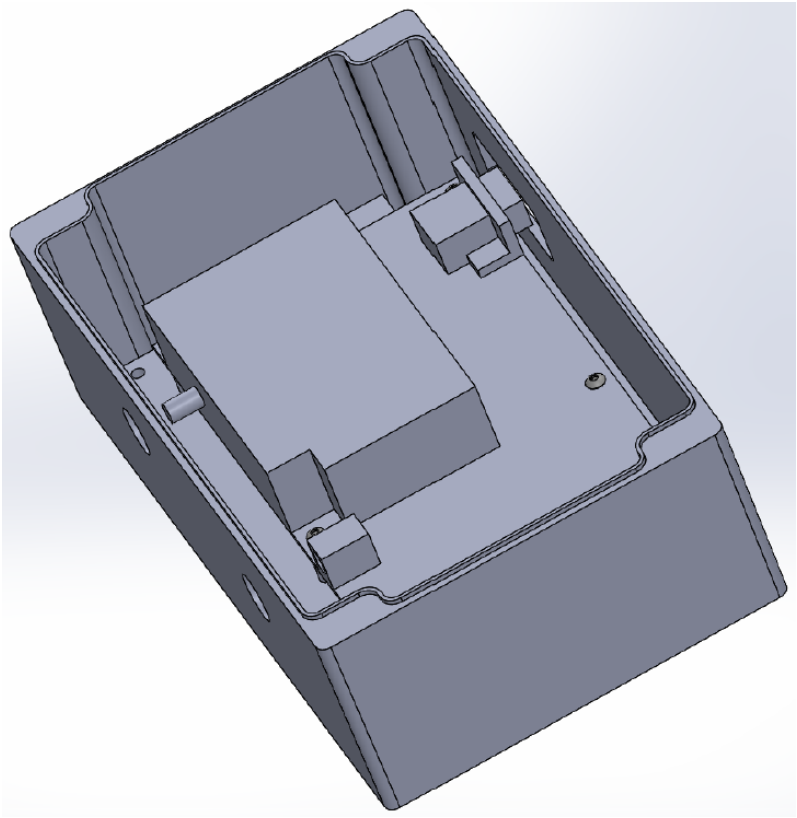

Figure 44: Design for the emission monochromator subsystem, including the enclosure and mounting plate.

5.2.2. Optical Bench

The design of the optical bench, namely its optical components, is critical in determining the sensitivity of the spectrometer in the UV region. The components of the optical bench are shown in Figure 45 below.

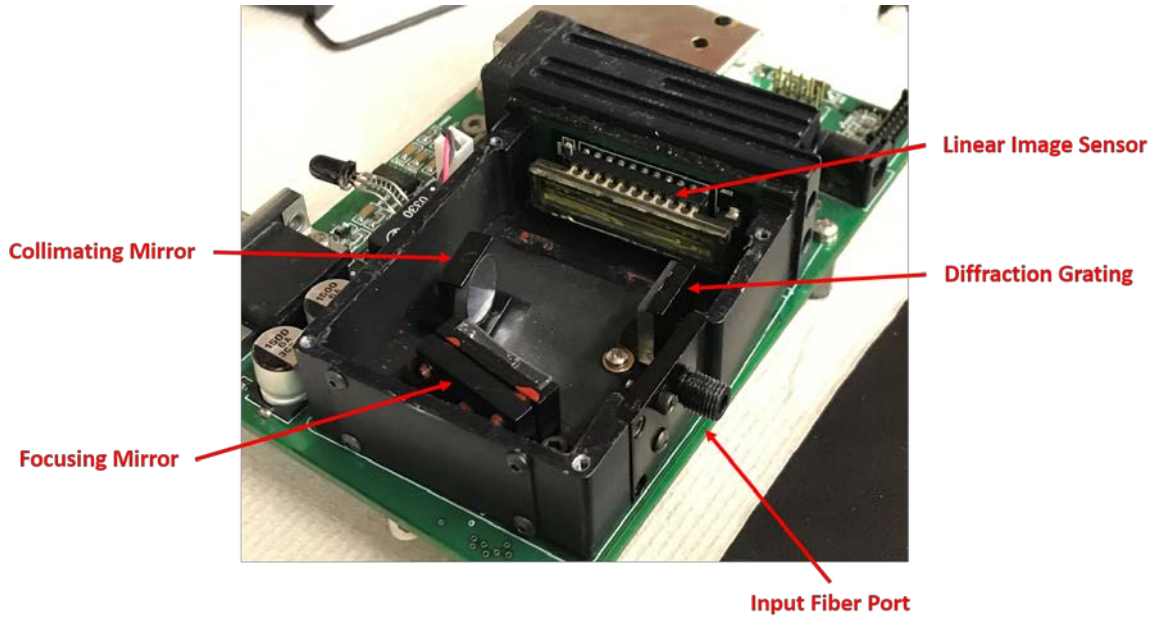

Figure 45: Optical bench and its major components.

Briefly, fluorescence entering the input fiber port will be collected and collimated by the collimating mirror, dispersed by the diffraction grating, and then focused onto the pixels of the linear image sensor, whereupon the spectrum will be digitized, stored, and transmitted to the computer. To achieve this, the optical bench had to be properly aligned to maximize the intensity of light falling onto the detector pixels. This was accomplished by performing horizontal and vertical adjustments on the mirrors and grating through the set screws in the optical bench. Such a process is depicted in Figure 46 below.

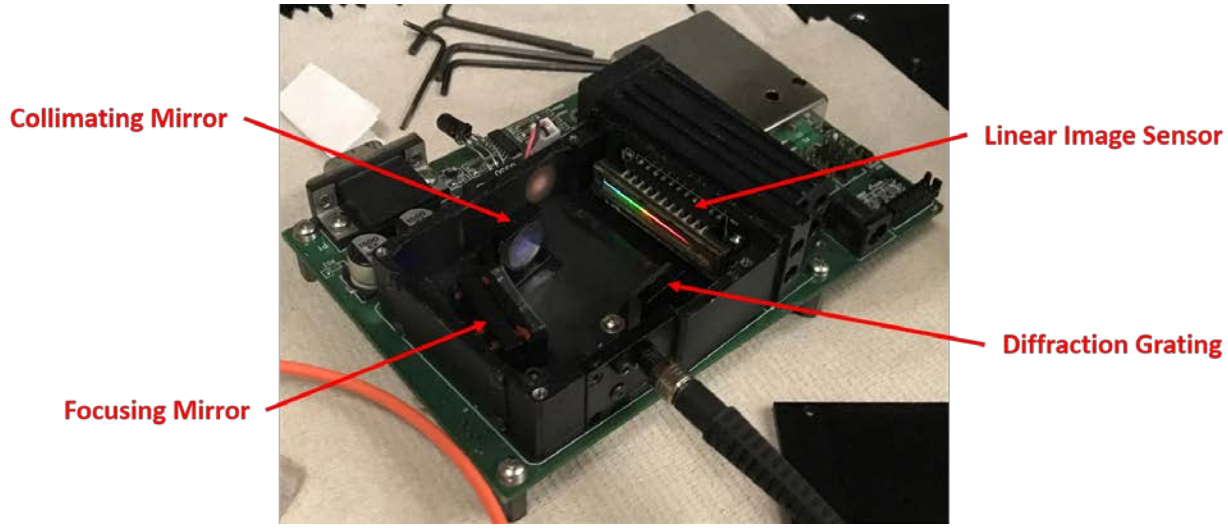

Figure 46: Optical bench during alignment process.

To increase the UV sensitivity of the spectrometer, the diffraction grating provided by B&W Tek was replaced with a UV diffraction grating possessing very high diffraction efficiency in the UV region (Newport Corporation 33025FL01-060R). The usage of this grating ensures that the fluorescence of bacteria and its components will not be significantly attenuated within the optical bench. The efficiency curve of this diffraction grating is provided in Figure 47 below and can be found to be as high as 75% in the UV region.

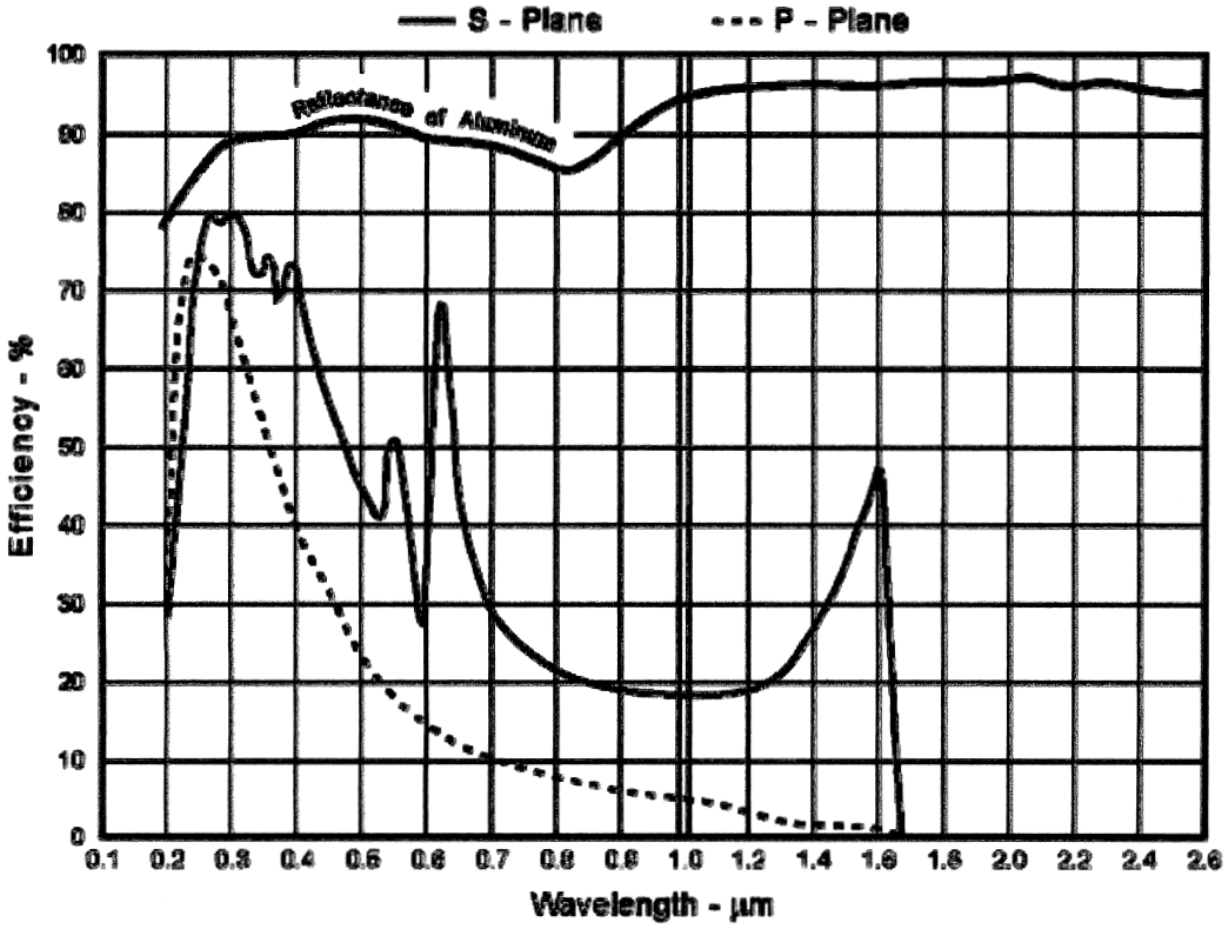

Figure 47: Diffraction grating efficiency curve [10].

In addition to the diffraction grating, the collimating mirror was replaced with a UV-enhanced mirror of the same size to further increase the efficiency of the spectrometer in the UV region.

5.2.3. Detector

The detector which is included in the BTC-110S is the Sony ILX511B linear image sensor, which is a CCD detector with 2048 pixels. This sensor natively has a very high sensitivity in the visible region, but poor sensitivity in the UV region. Therefore, this sensor was ordered with an additional dye coating known as Lumogen F Violet 570. This dye absorbs strongly in the UV region and fluoresces in the visible region, thereby increasing the UV sensitivity of the detector. This was another modification made to the optical bench in order to increase its UV sensitivity.

5.2.4. Operation

To utilize the spectrometer, it must first be calibrated. Calibration is performed by taking a source with known emission lines at distinct wavelengths, recording the spectrum, and deriving a fitting which provides the wavelength as a function of the detector pixel. This calibration is then utilized, in software, to convert the pixel-dependent intensity read out by the detector into a wavelength-dependent spectrum. A rather simple source to use in this process is the mercury-vapor lamp, also known as the compact fluorescent lamp (CFL), which is the same lamp typically used for overhead lighting in rooms within a building, for example. Figure 48 provides the well-known CFL spectrum, which possesses many distinct emission lines, while Figure 49 shows the CFL spectrum recorded by the emission monochromator.

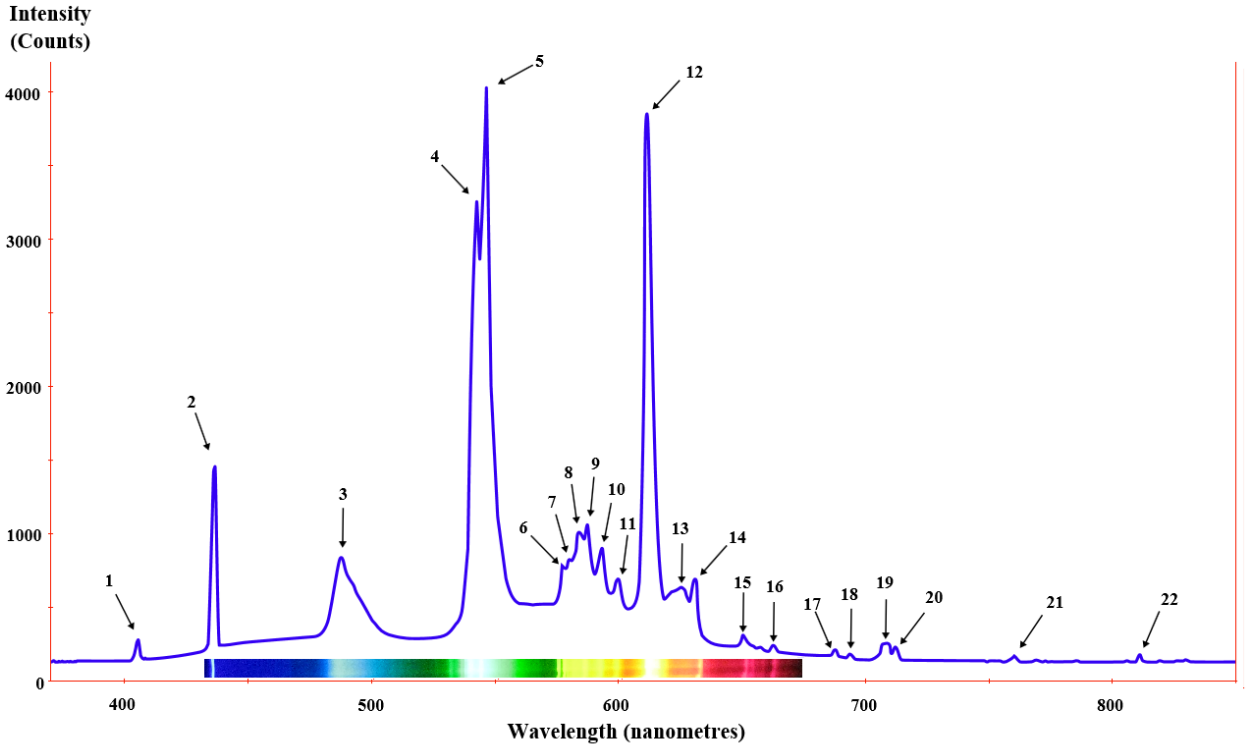

Figure 48: CFL (mercury-vapor lamp) spectrum [11].

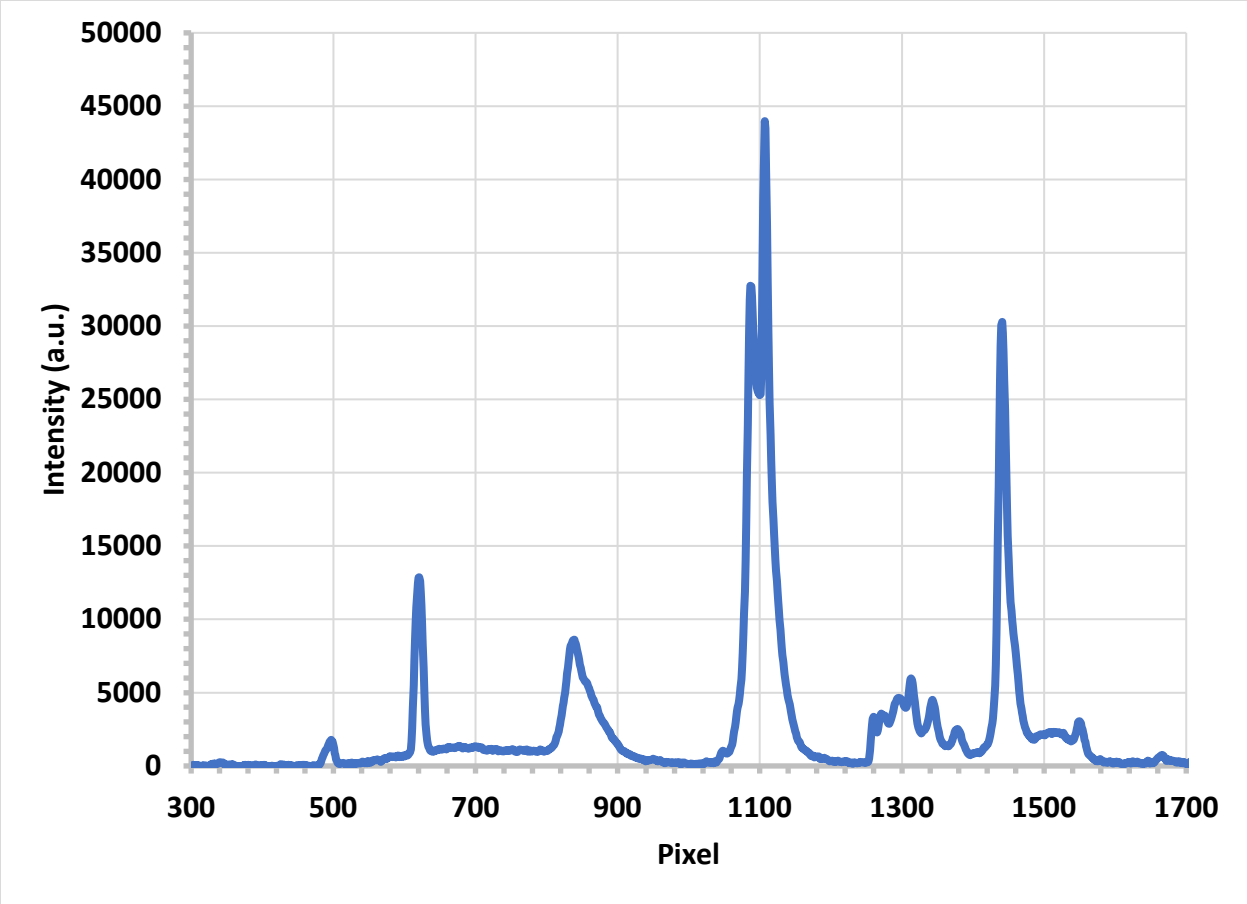

Figure 49: CFL (mercury-vapor lamp) spectrum recorded by the emission monochromator as a function of pixel.

From Figure 48 and Figure 49, distinct emission lines of the CFL spectrum could be identified at certain pixels. The resulting pixel-wavelength pairs were utilized to derive a second-order polynomial using the least squares method. This fitting accounts for slight nonlinearities along the focal plane within the spectrometer, although the relation is expected to be highly linear in general. Table 8 provides the pixel-wavelength pairs utilized in deriving this fitting.

Pixel	Wavelength (nm)
351	365
506	405
631	436
847	487
1560	651

Table 8: Pixel-wavelength pairs for calibrating spectrometer.

Figure 50 shows the resulting fitting which is derived from the measurements of the CFL spectrum.

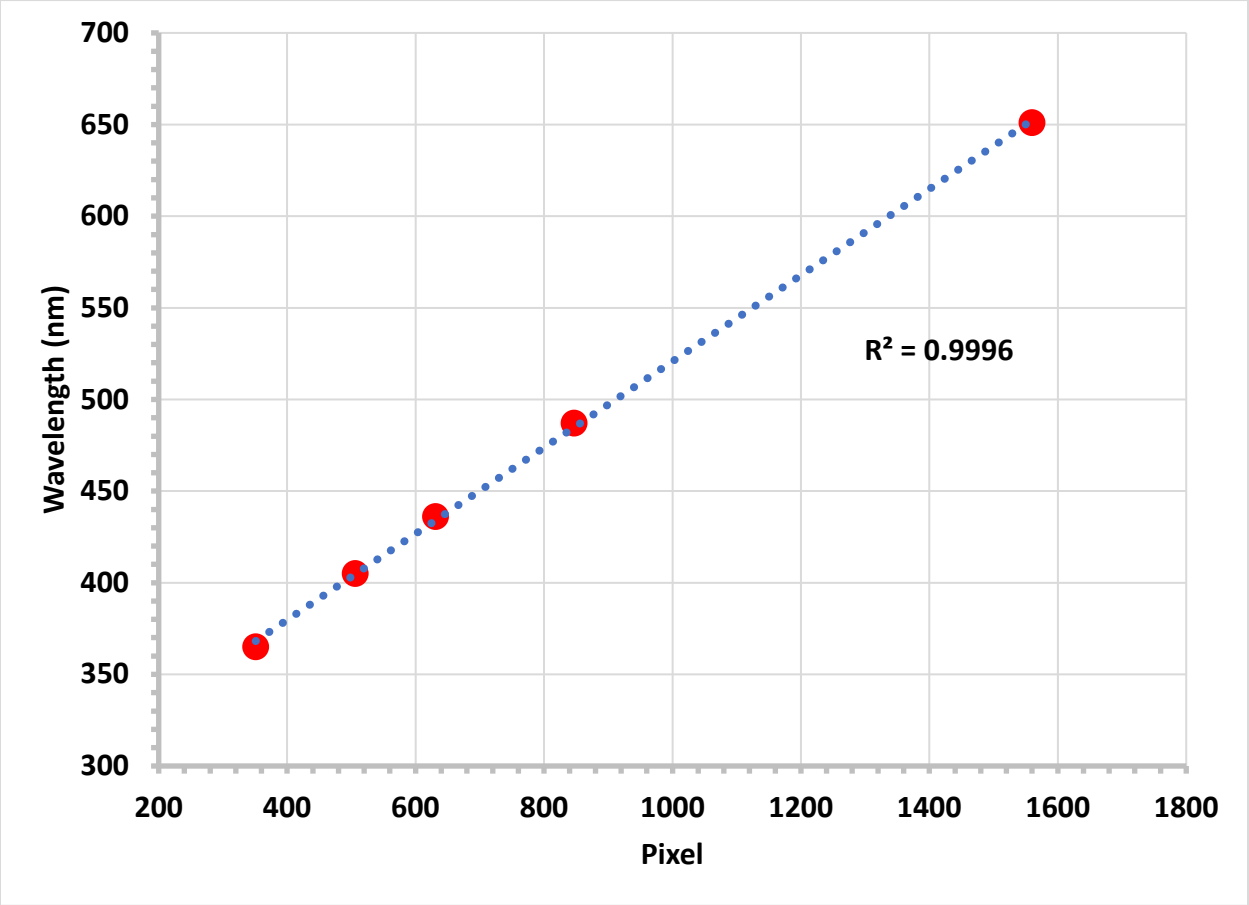

Figure 50: Calibration of emission monochromator subsystem by fitting the pixel-wavelength pairs of the CFL spectrum.

Power was supplied to the emission monochromator subsystem through its input power port, which simply requires a 5 V DC source.

5.3. Subsystem Validation

5.3.1. Normal Fluorescence Spectra of Bacteria Components

The UV sensitivity and calibration of the emission monochromator subsystem was first validated by recording the normal fluorescence spectra of tryptophan and tyrosine. The excitation source for these spectra was the disinfection unit. These spectra were compared against those recorded by a benchtop, commercial spectrometer (Shimadzu RF5301PC) for validation.

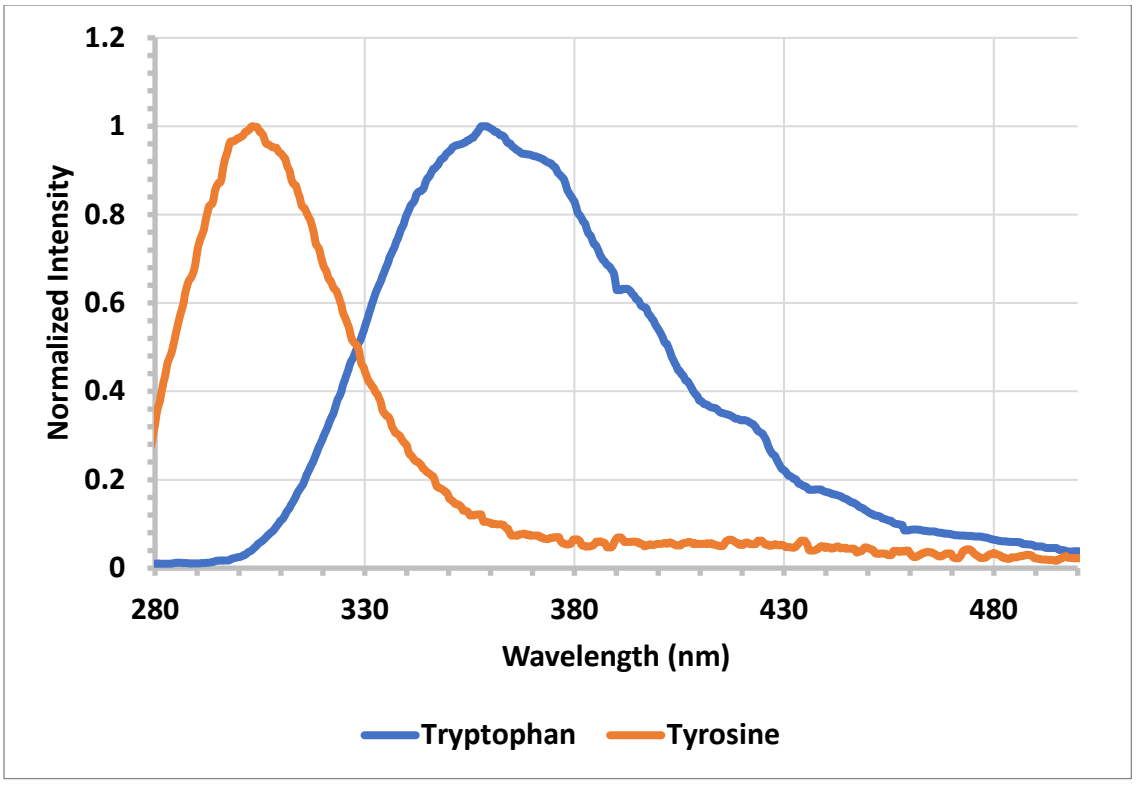

Figure 51: Normal fluorescence spectra ($\lambda_{EX} \approx 285$ nm) of tryptophan and tyrosine recorded by the portable spectrometer. The spectra are normalized.

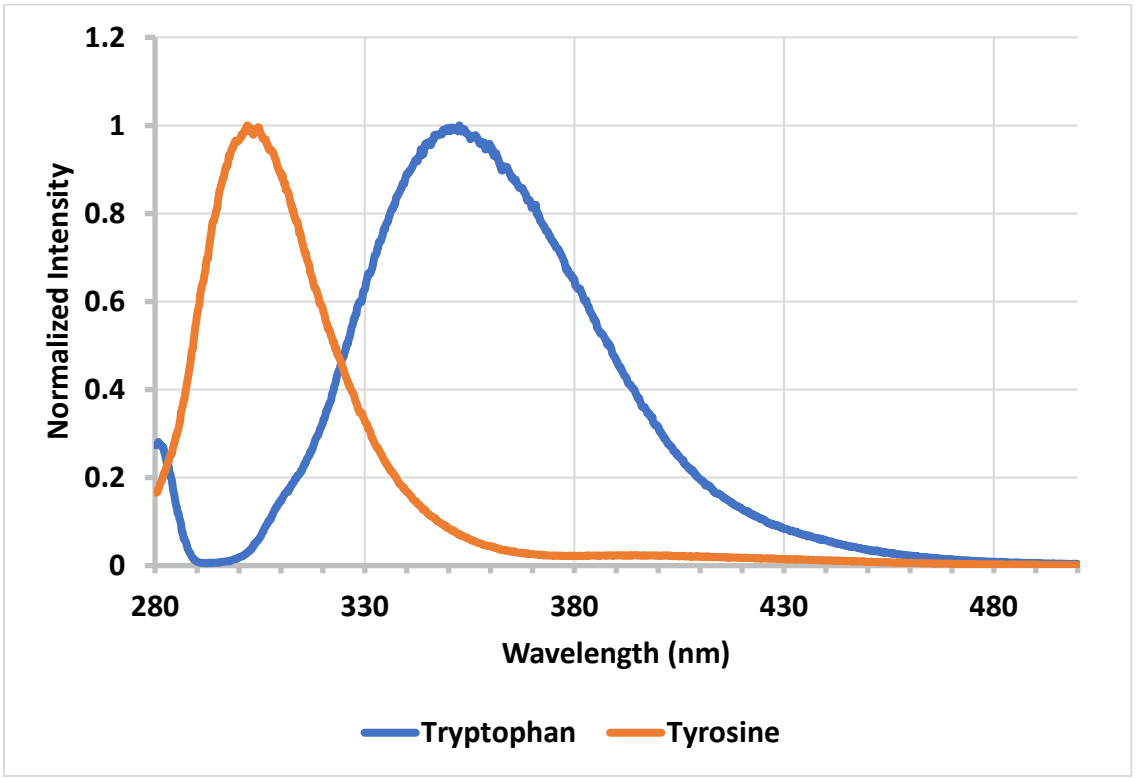

Figure 52: Normal fluorescence spectra ($\lambda_{EX} \approx 285$ nm) of tryptophan and tyrosine recorded by the benchtop spectrometer. The spectra are normalized.

The portable and benchtop spectra are plotted in normalized units. In general, the agreement between the two set of spectra is very good, indicating the spectrometer calibration was sufficiently performed. It can be noted that the resolution and signal-to-noise ratio (SNR) of the portable spectrometer are worse compared to those for the benchtop spectrometer, but that is expected.

5.3.2. Normal Fluorescence Spectra of *E. coli* Bacteria

The UV sensitivity and calibration of the emission monochromator subsystem was further validated by recording the normal fluorescence spectra of *E. coli* bacteria. The excitation source for these spectra was the disinfection unit. These spectra were again compared against those recorded by a benchtop, commercial spectrometer (Shimadzu RF5301PC) for validation.

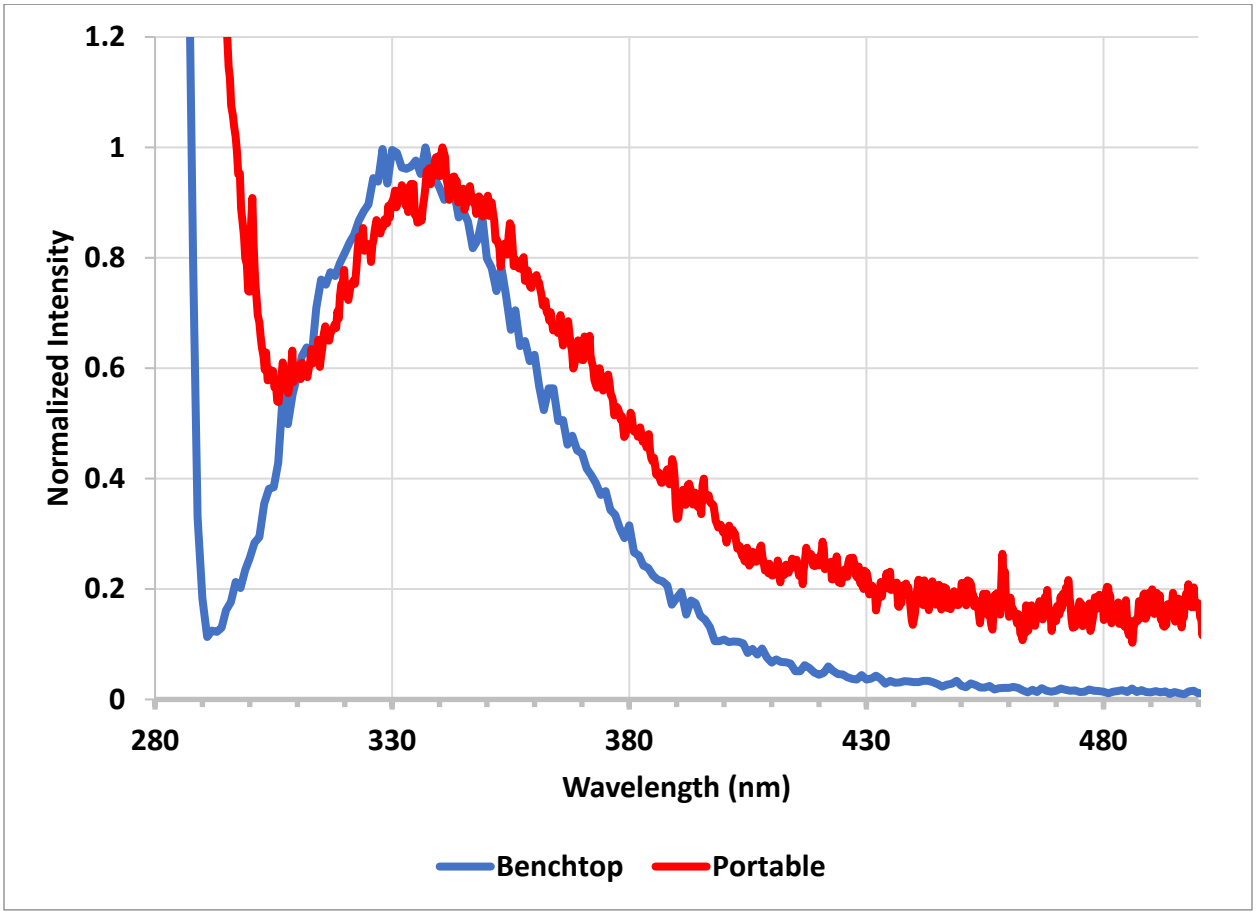

Figure 53: Normal fluorescence spectra ($\lambda_{EX} \approx 285$ nm) of *E. coli* bacteria recorded by the portable spectrometer. The spectra are normalized.

The portable and benchtop spectra are plotted in normalized units. In general, the agreement between the two set of spectra is very good, indicating the spectrometer calibration was sufficiently performed. It can be noted that the resolution and signal-to-noise ratio (SNR) of the portable spectrometer are worse compared to those for the benchtop spectrometer, but that is expected. This is also a validation of the spectrometer's enhanced UV sensitivity for bacterial detection.

During this subsystem testing, it was found that the CCD noise could become particularly prevalent in the acquired fluorescence spectra when the emission monochromator was on for long periods of time. To that effect, consider the fluorescence decay plot provided in Figure 54 below.

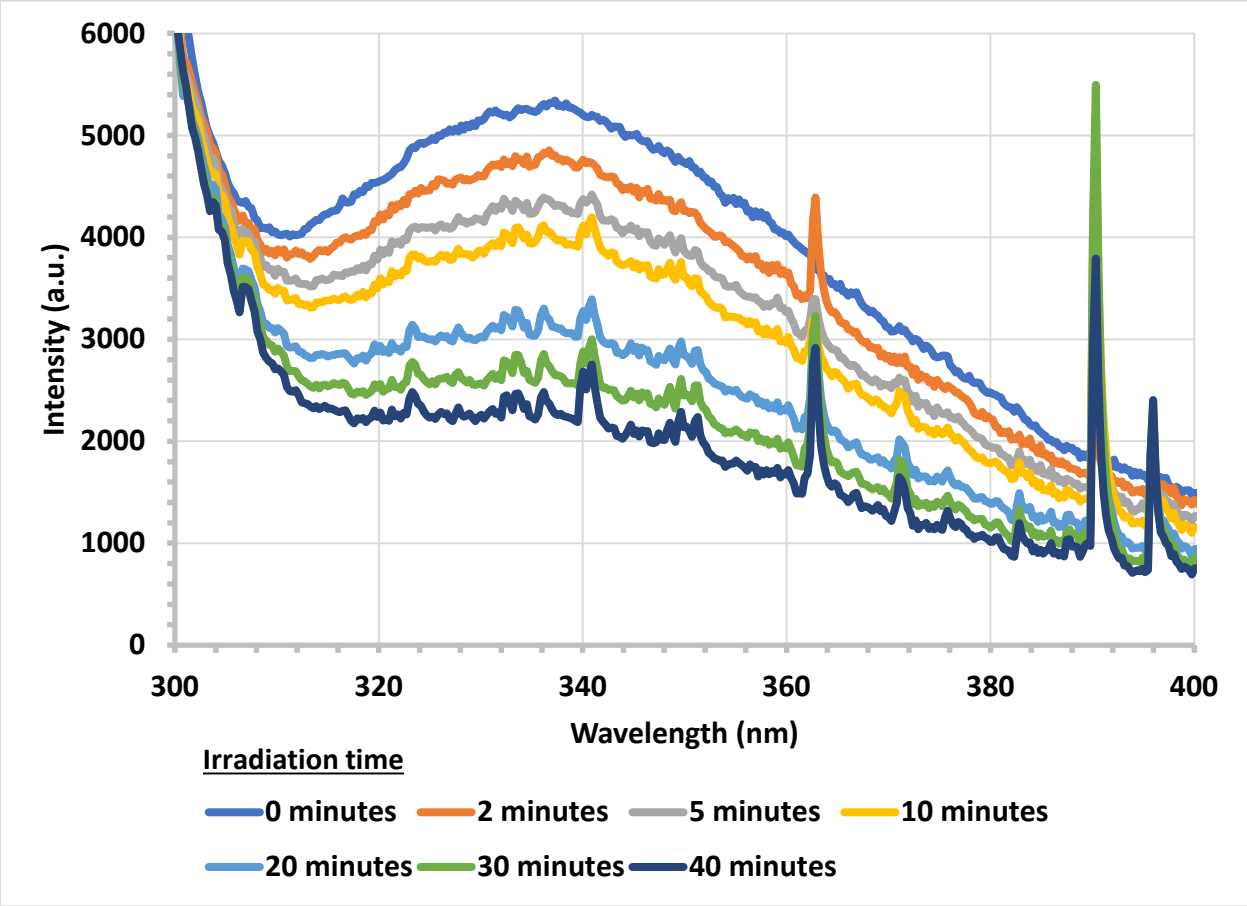

Figure 54: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectra of *E. coli* bacteria ($\approx 10^8$ cells/mL) as a function of disinfection time.

Clearly, the spikes in the spectra, which are essentially noise spikes in the CCD readout itself, increase as subsystem remains on for a longer period of time. These spikes may therefore be attributed to the heating of the detector. To correct these spikes, a median filter with a width of 20 points was applied to all spectra, resulting in the plot shown in Figure 55.

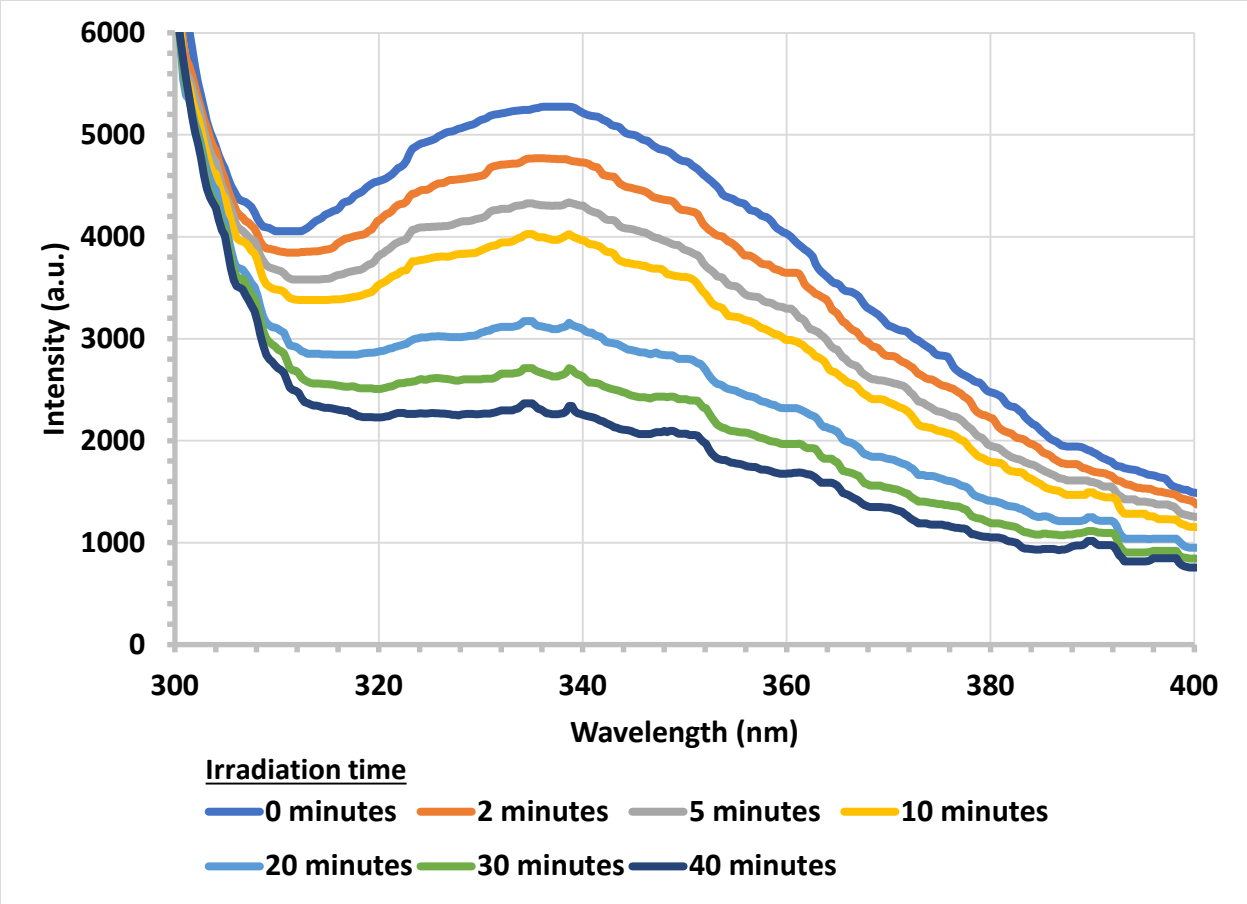

Figure 55: Normal fluorescence ($\lambda_{EX} \approx 285$ nm) spectra of *E. coli* bacteria ($\approx 10^8$ cells/mL) as a function of disinfection time with a median filter of width 20 points applied.

After applying a median filter, the spectra are significantly smoothened without any distortion occurring to the band shape or peak intensity. Median filtering is thus a rather useful technique in removing the CCD noise during spectral acquisitions. The effects of median filtering on the PCA clustering are quite dramatic as well, as shown in Figure 56 and Figure 57.

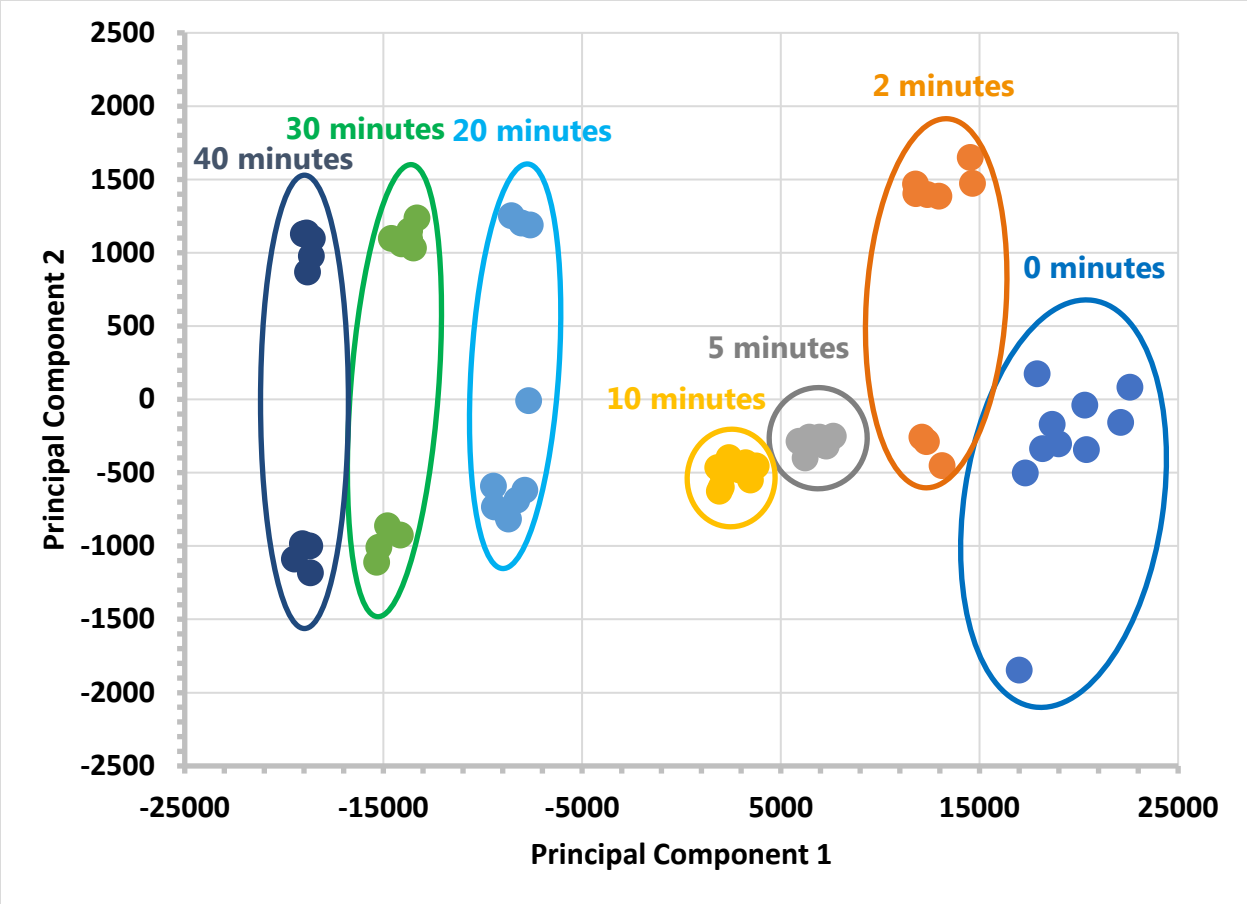

Figure 56: PCA score plot for UV irradiation experiment on *E. coli* bacteria ($\approx 10^8$ cells/mL) before performing median filtering.

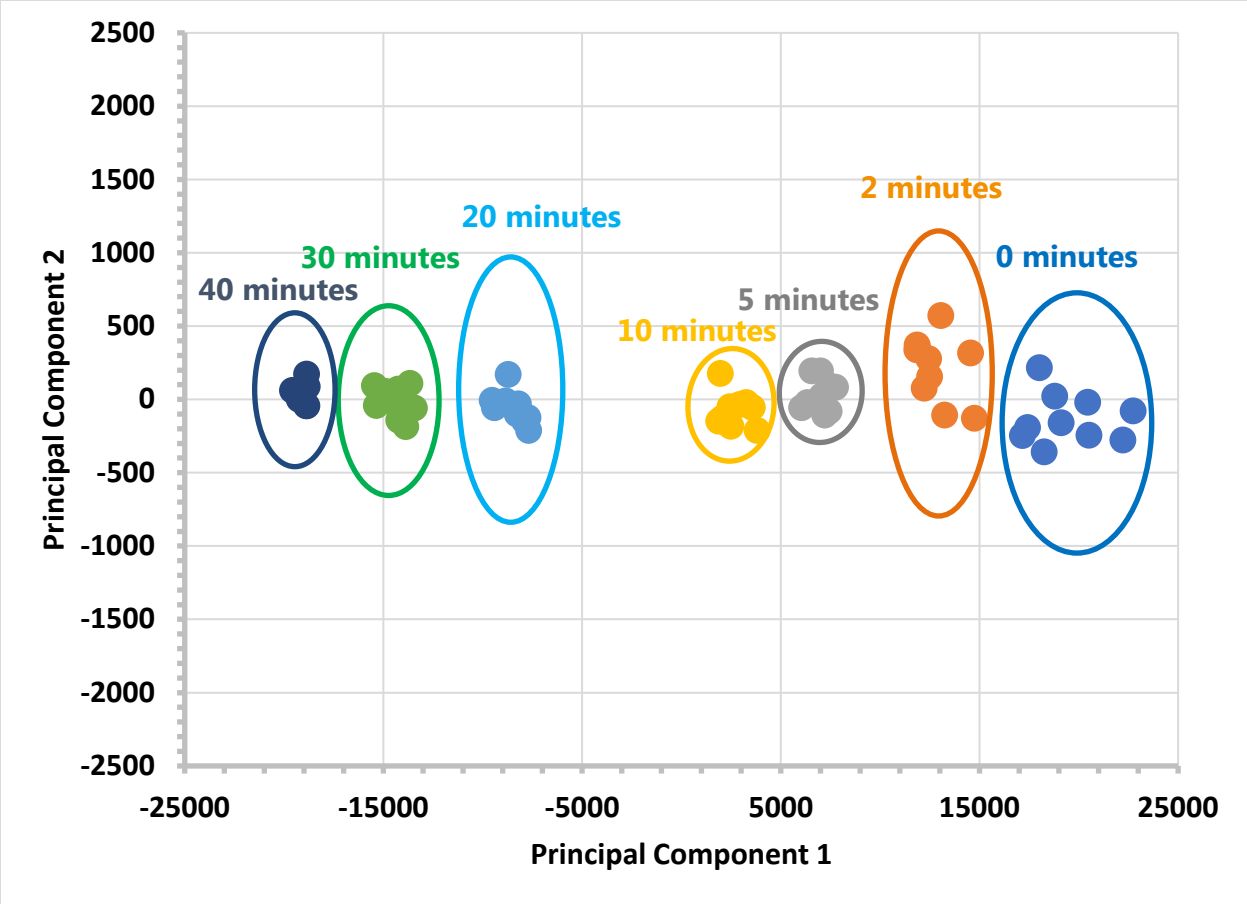

Figure 57: PCA score plot for UV irradiation experiment on *E. coli* bacteria (≈ 10^8 cells/mL) after performing median filtering.

Thus, median filtering provides a powerful means of improving the clustering in PCA score plots as well.

5.3.3. Operation with Disinfection Unit and Excitation Monochromator

The operation of the emission monochromator subsystem with the disinfection unit and excitation monochromator subsystems was already thoroughly discussed and validated. This validation includes the validation of the spectrometer's capability to record EEMs and synchronous fluorescence spectra.

5.4. Subsystem Conclusion

In conclusion, this subsystem satisfies all required functionalities. In particular, it possesses sufficient UV sensitivity for recording the fluorescence of bacteria components and bacteria itself. The calibration of the unit was validated as well. In regards to next semester, the sensitivity of the unit will continue to be optimized to achieve the smallest exposure times possible.

5.5. Citations

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