# 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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# 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*

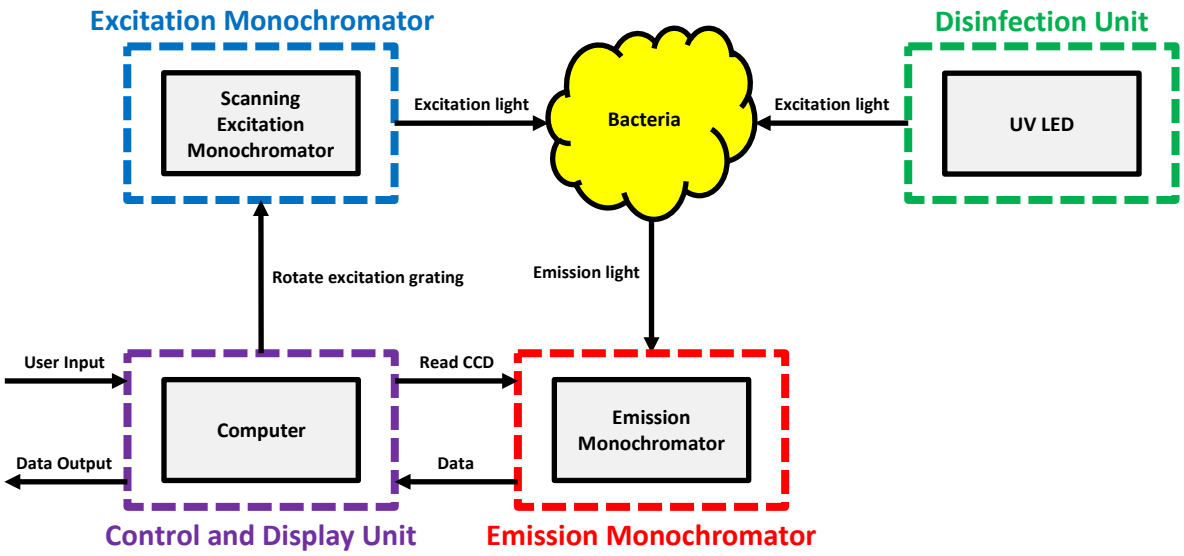


**Figure 1:** Block diagram of portable fluorescence spectrometer.

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.

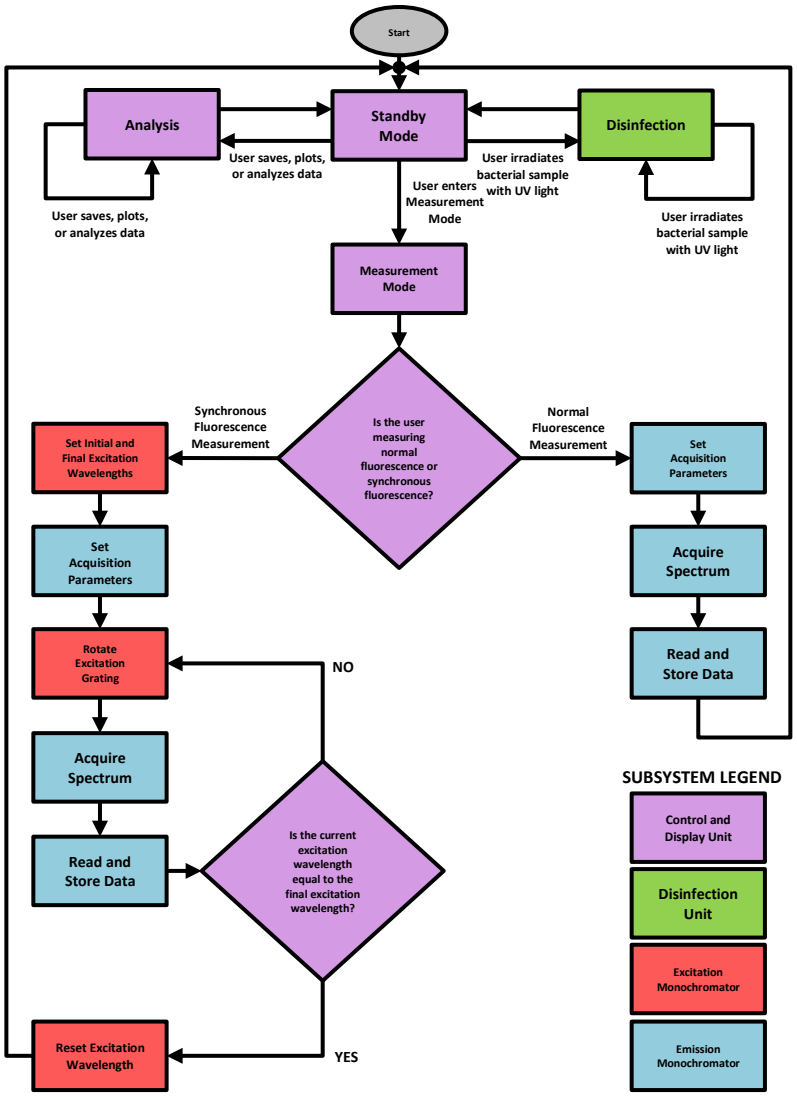


**Figure 2:** System flowchart detailing the various modes of operation.

**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.

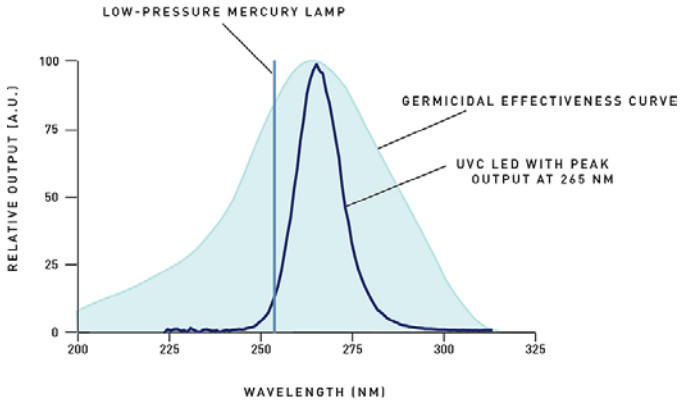


**Figure 3:** Output spectrum of UVC LED demonstrating its high-power, narrow profile, along with the associated germicidal effectiveness [1].

**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.

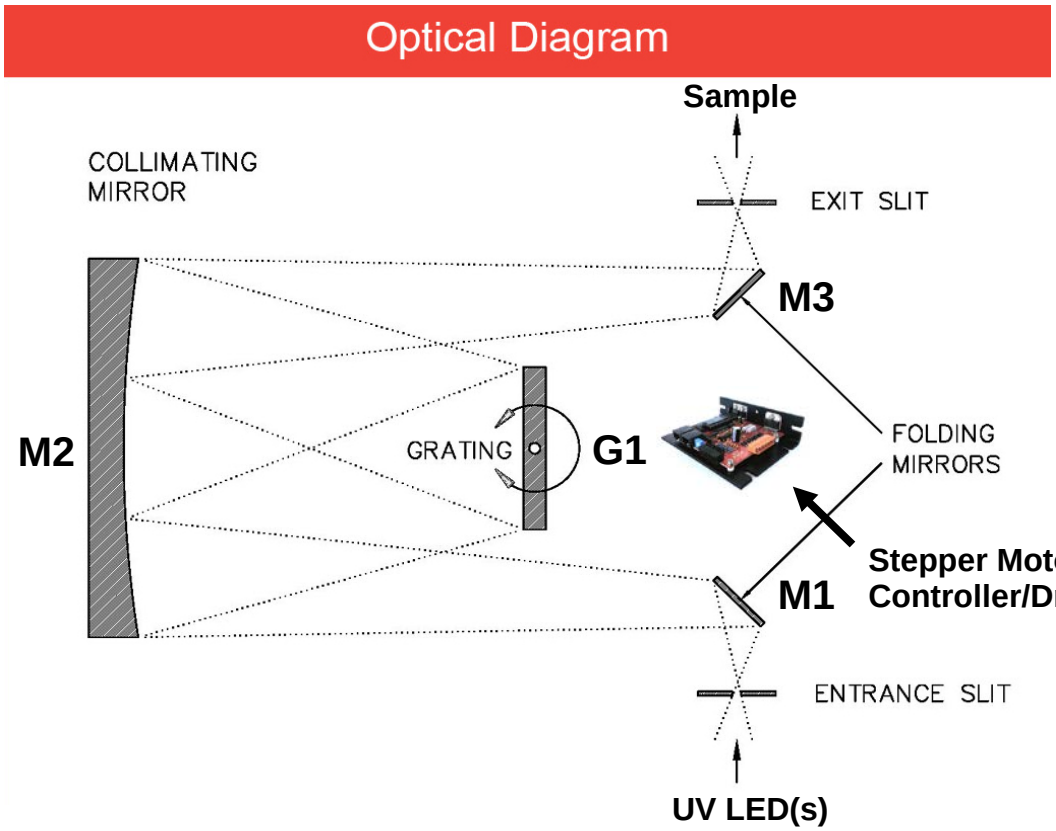


**Figure 4:** Schematic of the overall excitation monochromator subsystem, including the UV excitation source, Fastie-Ebert optical bench [2], and stepper motor controller/driver [3] for rotating the excitation grating G1.

**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.

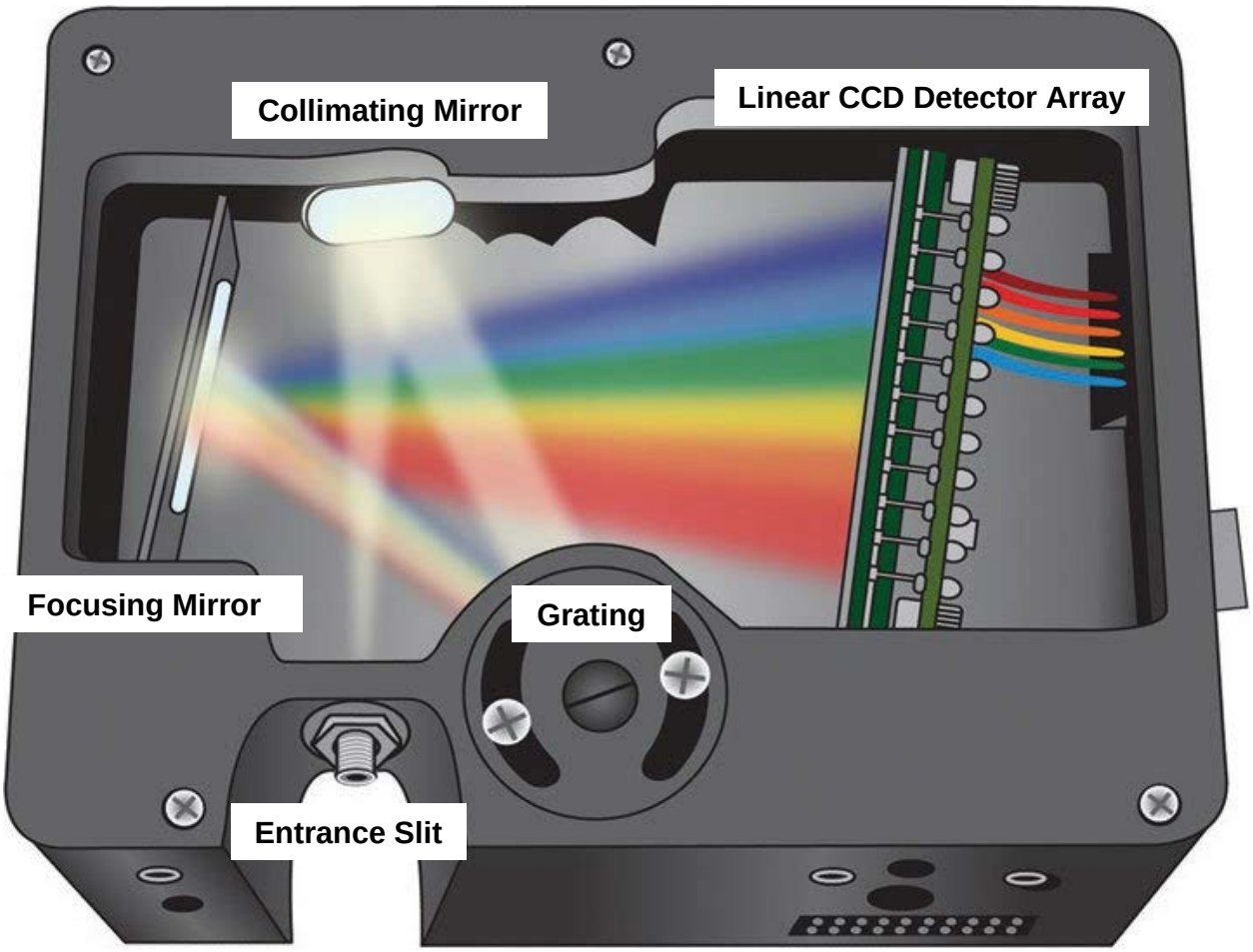


**Figure 5:** Schematic of the emission monochromator subsystem [4], including the Czerny-Turner optical bench and linear CCD detector array. Additional control electronics will be utilized for reading the detector.

## *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.

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