Instrument for the Identification of Live and Dead Bacteria

Arjun Krishnamoorthi

FUNCTIONAL SYSTEM REQUIREMENTS

REVISION – 1
4 December 2019

FUNCTIONAL SYSTEM REQUIREMENTS
FOR
Instrument for the Identification of Live and Dead Bacteria

PREPARED BY:

_____
Arjun Krishnamoorthi Date

APPROVED BY:

_____
Arjun Krishnamoorthi Date

_____
John Lusher, 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. 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.

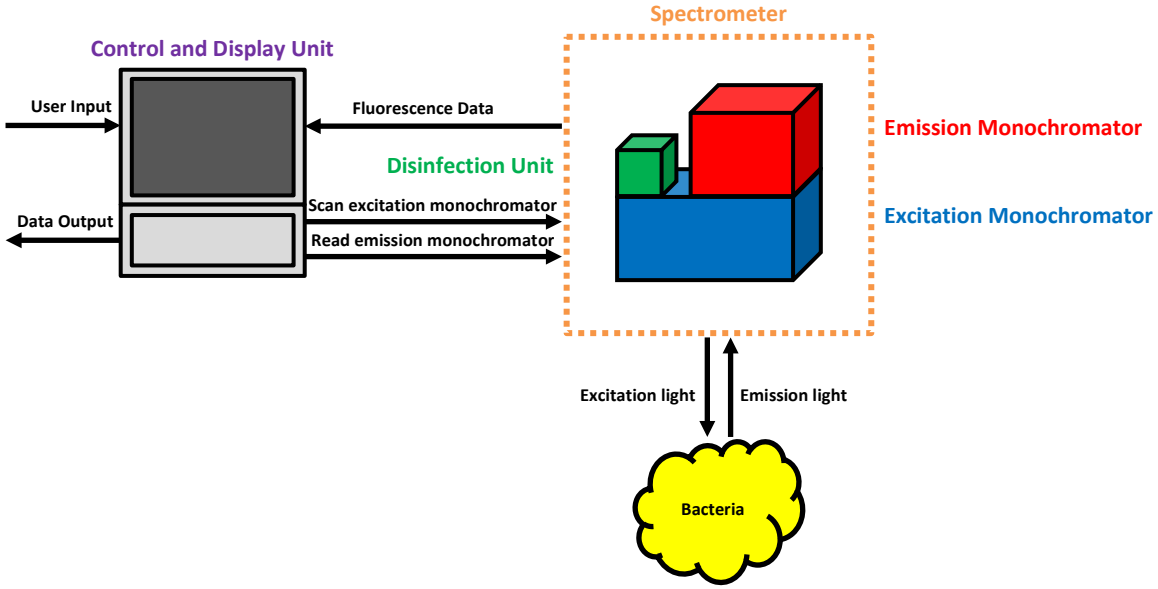

Figure 1: Conceptual Image of Proposed System.

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.

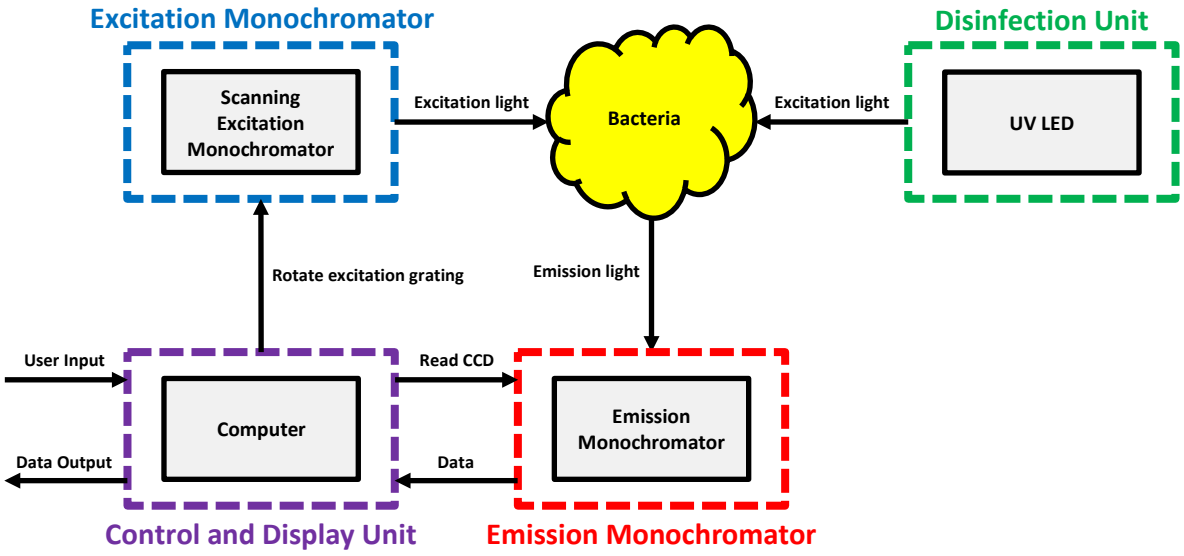

Figure 2: Block Diagram of System.

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.

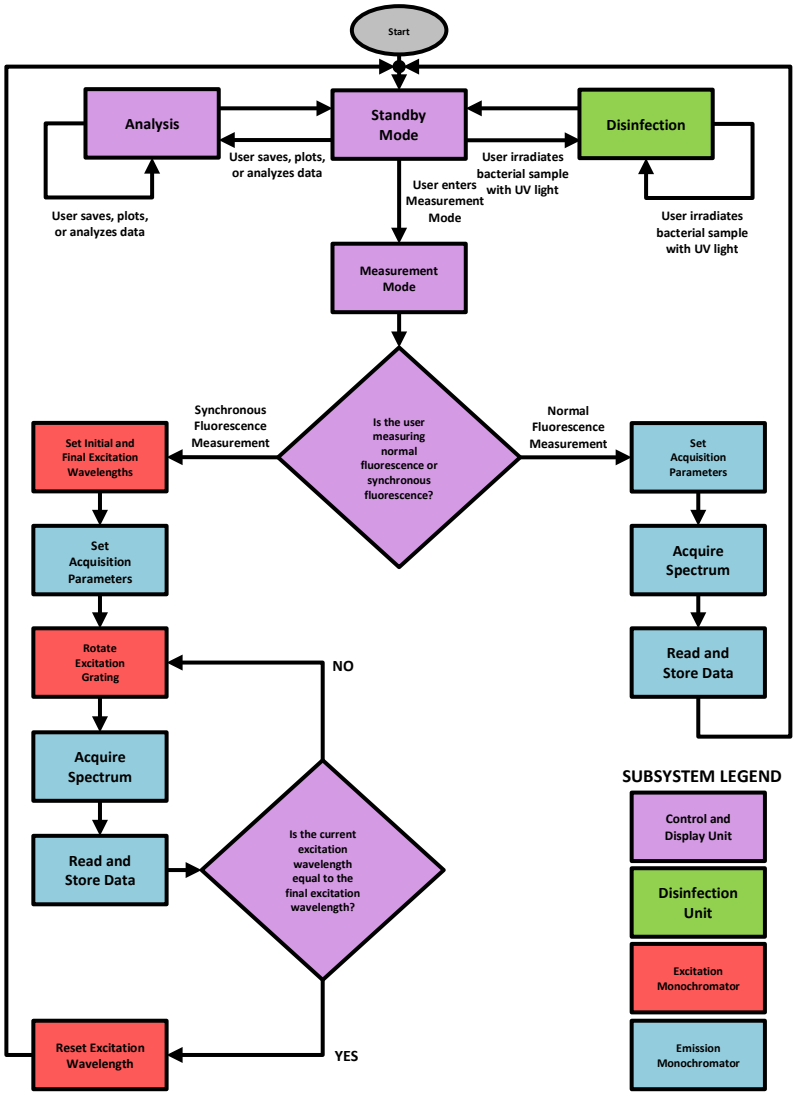

Figure 3: System flowchart detailing the various modes of operation.

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.