Received February 2, 2019, accepted February 8, 2019, date of publication February 13, 2019, date of current version March 5, 2019.

Digital Object Identifier 10.1109/ACCESS.2019.2899006

INVITED PAPER

Identification of Live and Dead Bacteria: A Raman Spectroscopic Study

RUNZE LI[1], DINESH DHANKHAR[1], JIE CHEN[2], ARJUN KRISHNAMOORTHI[1], THOMAS C. CESARIO[3], AND PETER M. RENTZEPIS[1]

[1]Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX 77843, USA
[2]Center for Ultrafast Science and Technology, Key Laboratory for Laser Plasmas (Ministry of Education), School of Physics and Astronomy, Collaborative Innovation Center of IFSA (CICIFSA), Shanghai Jiao Tong University, Shanghai 200240, China
[3]School of Medicine, University of California at Irvine, Irvine, CA 92697, USA

Corresponding author: Peter M. Rentzepis (prentzepis@tamu.edu)

This work was supported in part by the Welch Foundation under Grant 1501928, in part by the Air Force Office of Scientific Research under Grant FA9550-18-1-0100, and in part by the Texas A&M University TEES funds.

ABSTRACT Raman spectroscopy has been used to identify bacterial strains, bacterial components, such as protein and DNA bases, and the ratio of live to dead bacteria before and after exposure to ultraviolet (UV) radiation. New vibrational bands and changes in their intensity as a function of UV irradiation time have been recorded by high resolution Raman spectroscopy which made it possible to determine the mechanism of the UV inactivation of *Escherichia coli (E. coli)*, *Serratia marcescens (S. marcescens)*, and *Micrococcus luteus (M. luteus)* bacteria in saline solutions. We have also employed a novel, new, handheld spectrometer capable of recording, *in situ*, within minutes, the absorption, fluorescence, synchronous fluorescence, and Raman spectra of bacteria and other biological species and large molecules.

INDEX TERMS Bacteria inactivation, optical spectroscopy, principal component analysis, Raman scattering.

I. INTRODUCTION

Raman spectroscopy is one of the most often used spectroscopic methods for the identification of large biological molecules and other species such as bacteria, owing to the large information that is provided by their vibrational and rotational spectra. The application of spectroscopy for the studies of biological molecules has been extensive [1]–[6] and many Raman studies have been employed for the identification of various bacterial components [7]–[11]. Specifically relevant to the studies presented in this report, are the Raman spectra of aromatic amino acids [12], [13] and pyrimidine bases [14], [15]. In addition, DNA and several other components of bacteria such as peptidoglycans [16], [17] and isoprenoid quinines [18], [19], have been successfully identified even though the very large number of overlapping vibrational modes makes the identification of a specific vibration of a biomolecule rather difficult. However, owing to their active mode degeneracy, biopolymers display relatively simple vibrational spectra that can be accurately assigned. This suggests that Infrared (IR) and Raman spectroscopy can be considered as possible means for bacterial strain identification. In addition, the use of resonance Raman spectroscopy [20]–[25] provides an improved method for the analysis of complex spectra. It is possible to make accurate assignments using UV resonance Raman spectra of even a small concentration of active molecules, such as bacteria and viruses imbedded within a large number of spectroscopically similar species. This is aided by the fact that most of these molecules, dispersed in water, do not fluoresce in the UV region below 260 nm and allows for the use of UV excitation sources suitable for generating resonance Raman of several important bacterial components that absorb in the UV.

In this report we are also concerned with identifying the difference between the Raman spectra of live and dead bacteria and thus determine the ratio of live to dead bacteria before and after exposure to UV light. In previous studies we have utilized bacterial fluorescence combined with principal component analysis (PCA), and synchronous fluorescence to identify the bacteria strains and measure the ratio of live to dead bacteria as a function of UV irradiation dose and reaction time against antibiotics [26].

The ability to differentiate between dead and live bacteria may be critical in saving lives, preventing spread of infection,

The associate editor coordinating the review of this manuscript and approving it for publication was Derek Abbott.

and an important means for determining antibiotic efficacy. Traditional methods that measure antibiotic sensitivity rely on determining bacterial growth after reaction with an antibiotic [27], [28]. These processes commonly require days for growth, identification and subculture of the bacteria in the presence of the antibiotic panel before a selection of the correct antibiotic can be made with some degree of certainty. These are time-consuming processes where time can be the difference between lives saved or lives lost. Newer methods to accelerate this process require costly equipment and special expertise [29], [30]. Similarly, there is need to differentiate between dead and live bacteria after processes intended to sterilize lifesaving fluids or surgical equipment [31] including products intended for ingestion [32], [33]. In addition, the same determination is critical in supplying certain blood components for intravenous use [34], [35] and differentiate between live and dead bacteria in environmental samples as a means of tracking or even preventing the spread of an infectious agent [36], [37]. These examples have been done by a variety of processes and techniques that are aimed, primarily, at detecting bacterial growth. To simplify, accelerate and quantify the presence and species of live bacteria and differentiate them from bacteria killed, we have employed a hand held spectrometer capable of recording absorption, fluorescence and Raman spectra, *in situ*, within minutes, thus providing practically instantaneous identification of the strain and the ratio of live to dead bacteria.

The ''golden'' means for the measurement of bacterial (live) concentration, in unit of cells/ml, involves the plating of the bacterial strain on tryptone soya Agar (TSA) culture plates, for ∼24 hours, at 37°C, then counting the colony forming units. The number of dead bacteria, after irradiation or antibiotic treatment, is determined by subtracting the live bacteria, after treatment, from the original number of cells/ml. This widely used technique is very time-consuming and therefore may not be suitable for utilization in the field, operating rooms, or other time constrained situations. Considering that the bacterial replication occurs on the order of several minutes, the 'long time' required for incubation and culturing is obviously not acceptable for time sensitive situations. To eliminate those disadvantages and provide a method for recording spectra, *in situ*, at any remote place and practically instantaneous identification of the bacteria strain and ratio of live to dead bacteria, we have employed a newly designed and constructed handheld spectrometer capable of recording absorption, fluorescence and Raman spectra. Using this device, one may record in detail the fluorescence and Raman spectra of individual components such as DNA, amino acids and RNA bases, proteins and membrane components including tryptophan and tyrosine. These components provide a very powerful means for the identification of many bacteria strains and in addition record the response of bacteria to heat, antibiotics treatment or alterations in membrane integrity [38]–[41].

There is a critical need therefore to use this handheld Raman spectrometer to detect and assign the bands of several critical bacteria components, such as DNA, which either do not fluoresce, or their fluorescence is weak and occurs at a wavelength region where other bacterial components emit intensely and mask their fluorescence. To that effect our handheld Raman spectrometer can record *in situ*, within minutes, detailed Raman spectra that make it possible to monitor the changes in intensity and structure of these individual components as a function of UV dose or antibiotics treatment.

As more disease causing bacteria are becoming resistant to antibiotics, it is very important to utilize alternative techniques to inactivate them in order to prevent large scale epidemics caused by multi-drug resistant bacteria. An alternative, most effective inactivating method, is UV radiation. It has been shown that most antibiotic resistant bacteria and their wild type counterparts are equally sensitive to UV light [42], [43]. In many situations, such as operating rooms, it is important to differentiate between live and dead bacteria before and after inactivation with UV radiation or antibiotics treatment. The Raman spectroscopy utilized in this study may also achieve this goal: determining the ratio of live to dead bacteria, *in situ*, within a short period of time before and after each UV dose induced inactivation.

UV light penetrates through the membrane and mainly inactivates or kills, bacterial cells by damaging their DNA. This is affected by the UV light initiating a reaction between two thymine molecules to form a thymine dimer which results in cell inactivation by inhibiting bacterial DNA replication [44], [45]. In addition to these changes, proteins are also expected to undergo denaturation which is depicted by their absorption and fluorescence spectra changes as a function of UV light (∼280 nm) absorption by the bacteria cells [46], [47].

UV Damage to proteins is well known and we have verified it by irradiating pure protein (ovalbumin) with the same UV light dose as bacteria. The damage caused to pure proteins was compared with that of bacteria, under identical conditions, by comparing the Raman spectra of irradiated bacteria and pure proteins. The data presented in the results and discussion section show that the effect is practically identical. Damage to bacterial DNA was also verified by forming Thymine dimers from pure Thymine in solution [48] and recording the Raman spectra band maxima of irradiated and non-irradiated thymine and comparing it with the Raman spectra of *Escherichia coli (E. coli) and Micrococcus luteus (M. luteus)* bacteria. These data are used in this report as a means for determining the strain and the ratio of live and dead bacteria before and after irradiation with UV light. Moreover, changes in methylation due to UV damage of DNA were also observed previously [49].

II. MATERIAL AND METHODS

A. BACTERIAL CULTURE PREPARATION

E. coli bacteria (strain K-12, GM1655) were obtained from the Bacteriological Epidemiology and Antimicrobial Resistance unit, USDA-ARS. The bacteria were cultured on

tryptone soya agar (TSA) plate, sub-cultured in 10 ml of Luria Bertani (LB) growth medium and incubated overnight at 37°C. The bacteria were harvested in their stationary phase of growth by centrifugation at 3300 rpm for 5 minutes. Subsequently, the bacterial pellets were washed three times with 0.9%, w/v, saline solution to remove growth media. The pellets were diluted in saline to a concentration of $\sim 10^8$ cells/ml. The number of bacteria was determined by two methods: 1) counting CFU (Colony Forming Units) after culturing and 2) by their OD at 600 nm, recorded by a Shimadzu UV160 spectrophotometer.

The same procedure as the *E. Coli* bacteria was followed for the culturing of *M. luteus, Serratia marcescens (S. marcescens), and Bacillus thuringiensis (B. thuringiensis)* bacteria. The incubation temperature for *S. marcescens* was varied from room temperature, for prodigiosin rich growth, to 37°C for prodigiosin lower growth, in order to fit the experimental requirements.

B. UV IRRADIATION OF BACTERIA

A mercury lamp (Oriel model no. 66002) was used for UV irradiation. Its output passed through a 250 nm to 280 nm bandpass filter and impinged onto the sample. A Molectron detector (PM3Q with EPM1000) recorded the UV irradiation intensity, which was adjusted to 2 mW/cm^2. 9 ml of the bacteria solution were irradiated for various periods of time, ranging from 0 minutes to 20 minutes. After irradiation, the bacterial culture was concentrated, by centrifugation at 3300 rpm (Fisher Scientific Model 228) for 5 minutes. The supernatant was discarded and the bacterial pellets were re-suspended in 1000 ml of saline solution. A 2.5 ml aliquot portion, of this bacterial solution, was placed on an aluminum mirror, or a cuvette for Raman studies.

UV light emitting diodes (LEDs) are replacing mercury lamps, because the mercury lamps require a larger power supply and are environmentally hazardous and expensive. UV LEDs are very compact, energy efficient and may be used for many applications including bacterial inactivation, *in situ*, in the field, in conjunction with our novel handheld Raman spectrometer. The UV LEDs (Vishay Semiconductor VLMU60CL00-280-125) used for our bacteria inactivation experiments have an output wavelength of 280 nmand Full Width at Half Maximum intensity, FWHM, bandwidth of 10 nm.

C. RAMAN SPECTRA

A Horiba Xplora plus Raman microscope and the handheld spectrometer were used to record the Raman spectra of the bacterial and other samples. The bacteria Raman spectra measured by Horiba Xplora were, firstly, recorded immediately after the bacteria were placed on the aluminum mirror, or single crystal silicon cuvette, using the 10X microscope objective and 25 mW, 638 nm illumination laser. The acquisition time is 100 seconds per spectrum and usually 9 spectra were averaged for each measurement. At initial bacteria concentrations of 10^8 -10^9 cells/ml, an intense water band appeared at 1650 cm^{-1}, corresponding to the OH bending vibration, while other recorded bacteria Raman bands were much weaker in comparison.

At 10^{10}-10^{11} cells/ml concentrations, the 100X microscope objective was used to increase the bacteria Raman spectra band intensity and the signal to noise ratio.

The bacterial cells were examined under the microscope for visual signs of damage due to the Raman laser intensity, however no damage was observed. The temperature at the laser focal point was also measured, by focusing the Raman excitation beam directly onto a temperature sensor. Using two, different, thermocouples, the maximum temperature increase observed was 12 °C, from 23 °C (ambient room temperature) to 35 °C. These temperatures do not induce bacterial inactivation, or other deleterious effects. Moreover, Raman spectra observed in *E. coli* samples with different temperature were found to be different from those due to UV irradiation, which confirmed that the photo-thermal effects [50]–[52] were insignificant in our UV experiments.

D. BACTERIA COUNTING AND SEPARATION

Bacteria colony forming units (CFU) counting was performed using a 100 ml aliquot portion of the bacteria solution which was serially diluted to 1/10 concentrations and then plating it on tryptone soya agar (TSA) plates. Thereafter, these TSA plates were kept in an incubator for 24 hours for bacteria growth and then the colonies formed were counted. The Raman spectra of *E. coli* bacteria before and after UV irradiation with various doses were subjected to Principal Component Analysis (PCA). The spectra of a number of experiments, usually nine, irradiated with the same UV dose were normalized with respect to the 1450 cm^{-1} lipid band and subsequently subjected to PCA. Frozen Thymine solution was irradiated with UV LED and the Thymine dimers formation [48] was monitored during the irradiation period by observing the disappearance of the 260 nm absorption band of Thymine, while the Raman spectra of the formation of Thymine and Thymine dimers were recorded using the same Raman spectrometer. Similarly the effect of UV irradiation dose on bacterial proteins, was ascertained by preparing ovalbumin protein at a concentration of 0.4 mg/ml in distilled water and irradiating it with the same UV dose as the bacteria and comparing the changes recorded in the Raman spectra of each, pure protein and bacterial protein.

The Raman spectra of *E.coli* with assignments is shown in Figure 1, while the Raman spectra of thymine and thymine dimers formed after UV irradiation of thymine are shown in Figure 2. It is clearly that the Raman spectrum of Thymine has intense maxima near 1360 cm^{-1} and 1670 cm^{-1} whereas the Thymine dimer's intense band is located near 1700 cm^{-1}.

III. RESULTS AND DISSCUSSION

A. EFFECTS OF UV RADIATION ON PROTEINS

Two bacterial components that strongly absorb UV light are proteins and Nucleic acids (DNA & RNA). Proteins account

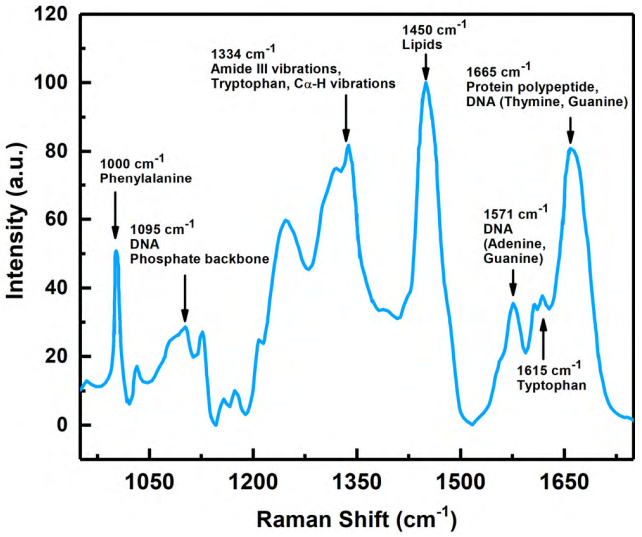

FIGURE 1. Raman spectrum of *E.coli* bacteria. Assignment based on refs. [53]–[58].

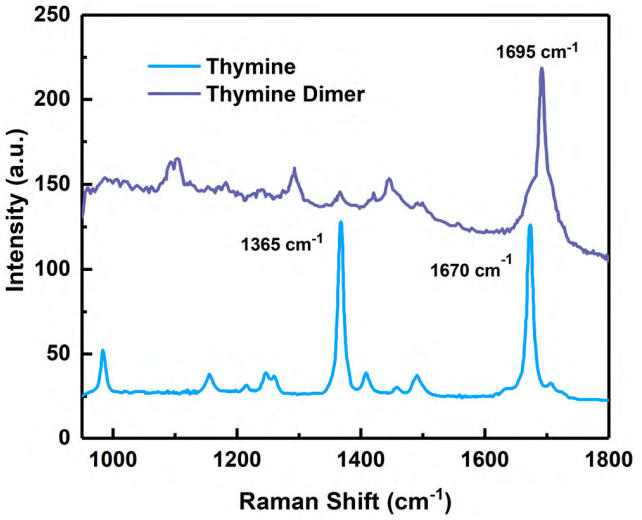

FIGURE 2. Raman spectra of thymine and thymine dimers.

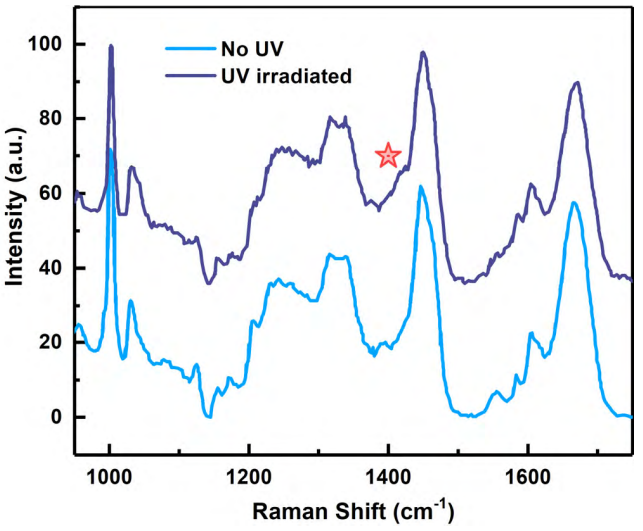

FIGURE 3. Protein (egg ovalbumin) raman spectra before and after UV irradiation. The new shoulder appeared near 1410 cm^{-1} after UV irradiation is marked with a star.

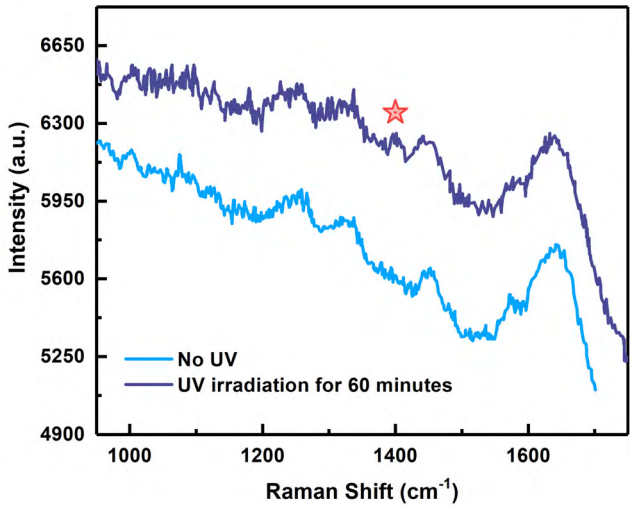

FIGURE 4. Raman spectra of *E. coli* in Saline solution (10^8 -10^9 cells/ml) before and after 60 minutes UV radiation. The band at 1400 cm^{-1}, formed after 60 minute UV radiation, is designated with a star.

for ∼55% of the bacteria dry weight and are located, majorly in the cytosol, and some in bacterial membrane. Proteins present in bacterial cell membrane are exposed directly to UV radiation and in some respects provide a protective shield for the inner located DNA. In order to be confident of our bacterial protein assignments we studied the changes in the Raman spectra of pure proteins as a function of UV radiation. To that effect, egg ovalbumin (pure protein) solution was irradiated with the same UV radiation dose as bacteria and the recorded Raman spectra are displayed in Figure 3.

B. EFFECTS OF ULTRAVIOLET RADIATION ON BACTERIA

The Raman spectra, of freshly prepared (10^8 - 10^9 cells/ml) *E. coli* bacteria in Saline solution, were recorded before and after UV radiation using the 10X microscope objective. As shown in Figure 4, the strong water band in the 1650 cm^{-1} region and the comparatively weak bacteria bands are observable; a new bacteria band near 1400 cm^{-1} is observed after 60 minutes UV irradiation. The Raman spectra of the *E. coli* bacteria, at a concentration of 10^{10} – 10^{11} cells/ml were also recorded using the 100X microscope objective. These spectra were normalized with respect to the lipid band at 1450 cm^{-1}, and after the removal of the water base, a new band at 1410 cm^{-1} was formed after UV irradiation.

The vibrational Raman spectra of *E.coli* bacteria after UV radiation from 0 minute to 20 minutes is depicted in Figure 5. The intensity of this band, 1410 cm^{-1},as a function of UV irradiation time is depicted in Figure 6, which show that the band intensity (I_{1410} vs UV dose) increases linearly as a function of UV dose.

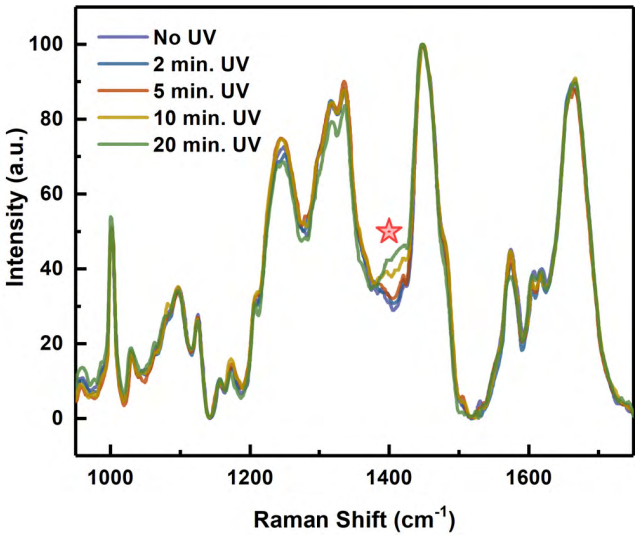

FIGURE 5. Raman spectra of *E. coli* in Saline solution before and after various UV radiation time. The 1410 cm^{-1} band formed after UV radiation is designated with a star.

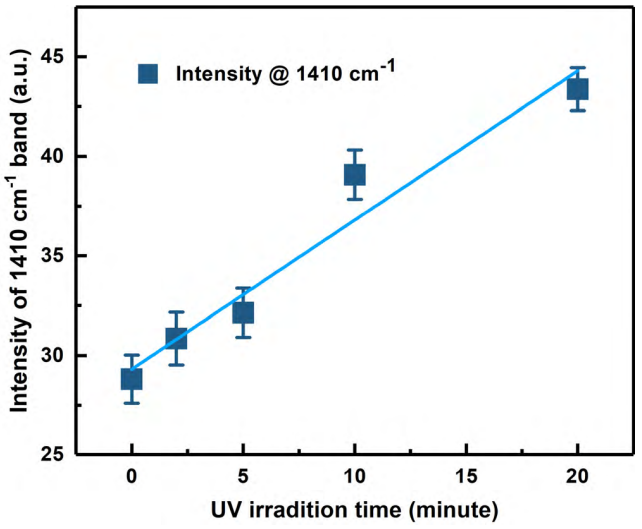

FIGURE 6. Intensity change of the 1410 cm^{-1} band as a function of irradiation time with ~2 mW/cm^2 UV light. Each point corresponds to the average of fifteen Raman spectra from five different points (three spectra from each point) at different locations on the respective samples.

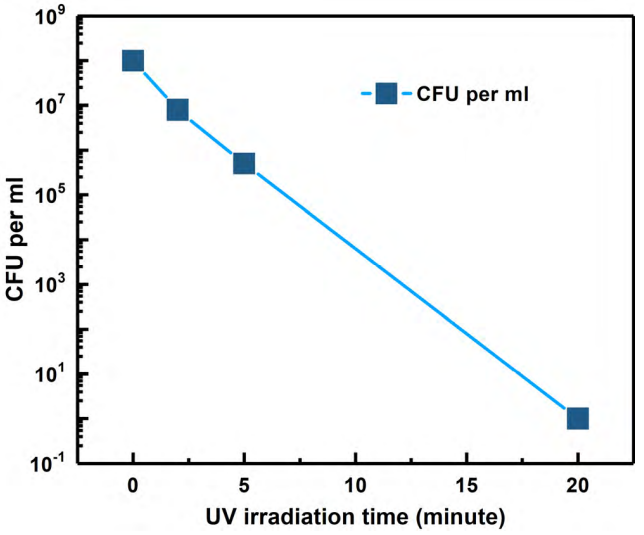

FIGURE 7. The CFU counts versus UV irradiation time.

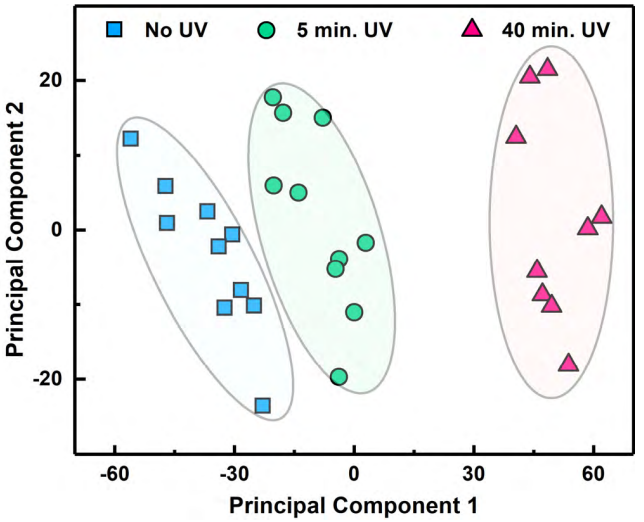

FIGURE 8. PCA plot of *E.coli* Raman spectra before and after UV irradiation. Each point corresponds to an average of three Raman spectra. Raman spectra were taken at different points of the corresponding samples and the data from two different experiments was combined for the analysis.

Figure 7 shows a plot of the corresponding CFU of live bacteria versus UV irradiation time. The Raman spectral features such as increase in the intensity of the 1410 cm^{-1} band was used, in PCA, to estimate the ratio of live to dead bacteria after UV irradiation. PCA was also performed on the *E.coli* Raman spectra in order to determine the separation of the live bacteria from the dead as a function of UV irradiation dose. PCA score plots and principle components are shown in Figures 8 and 9, respectively.

These figures provide additional information concerning the changes that bacterial components encounter as a result of UV radiation. The PCA plot in Figure 8 shows that UV irradiation causes the bacterial Raman spectra to be shifted to the right along the principal component 1 axis. Further examination of the principal component 1, Figure 9, reveals a sharp decrease in the 1350 cm^{-1} band intensity and an increase in the intensity of the 1700 cm^{-1} band. These spectral changes are commensurate with the degradation, decrease in thymine and formation, increase, in thymine dimers, (see Figure 2). In addition, changes in the Raman bands corresponding to DNA and tryptophan can be, clearly, seen in Figure 9 where the decrease in the DNA (Adenine and Guanine) 1571 cm^{-1} band and the ~1100 cm^{-1}DNA phosphate backbone intensity are displayed. In addition, decreases in the Raman intensity band(s) of Tryptophan, 1615 cm^{-1} and protein amide, 1650 cm^{-1} are depicted, also, in Figure 9. The expected formation of UV photoproduct(s) is evidenced

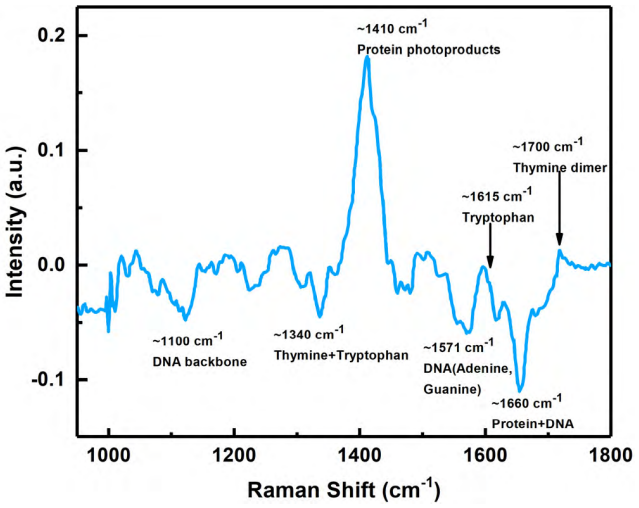

FIGURE 9. Principal component 1, derived from PCA.

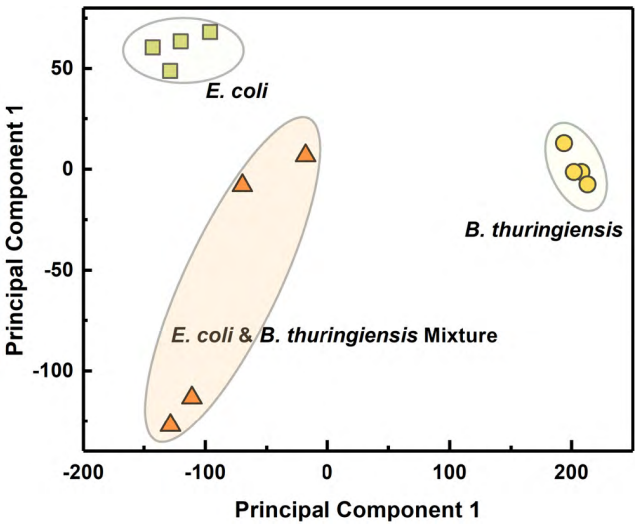

FIGURE 10. Raman spectra PCA plot used for the identification of *B. thuringiensis, E.coli bacteria* and their mixture. Each point corresponds to an average of three different Raman spectra of each bacterium. Spectra were taken at different points within the sample cuvette.

by the vibrational band at 1410 cm^{-1}. This assignment is verified by a shoulder at 1410 cm^{-1} that was also recorded in the Raman spectra of UV radiated pure proteins (Figure 3). Figure 10 shows the separation of *B. thuringiensis*, *E. coli* bacteria and their mixture before UV irradiation (together) and after UV irradiation (separated).

C. HANDHELD SPECTROMETER

We performed similar Raman experiments on bacteria and other molecules using the handheld Raman spectrometer. We illuminated the bacteria and the other species with the same 280 nm LED for periods of time varying from 0 minute to 150 minute. The spectra were processed in a similar manner to the ones recorded by the Horiba Raman microscope. The Raman spectra recorded by the handheld Raman spectrometer and Horiba spectrometer were identical, even though the handheld spectra were recorded remotely. Also the intensity changes as a function of UV irradiation time were practically identical. Therefore we are certain that the remotely recorded spectra are as suitable for the identification of bacterial strains and ratio of live to dead bacteria as the spectra recorded by normal bench-top spectrometers.

The data obtained from using LED irradiation is shown in Figure 11 which depicts the new Raman bands formed as a function of irradiation dose and changes in the intensity of *E.coli* bacteria Raman spectra after irradiation with the 280 nm LED for periods of 0 minutes to 150 minutes. Figure 12 depicts the 1410 cm^{-1} Raman band intensity vs UV

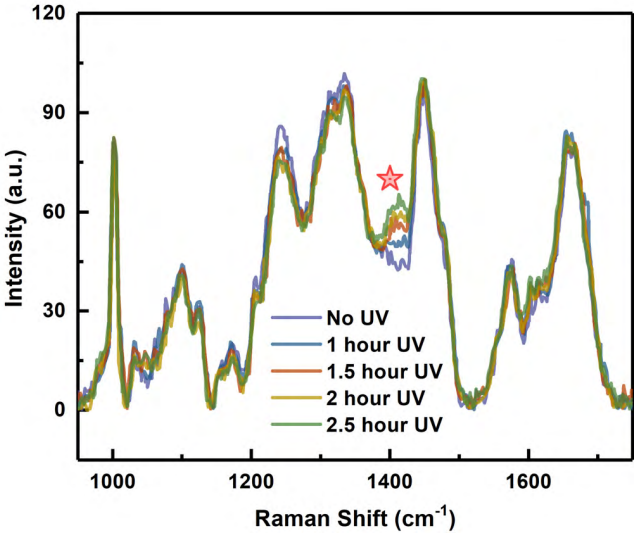

FIGURE 11. Changes in Raman spectra of *E.coli* bacteria as a function of irradiation time with a 280 nm LED. The spectra fluorescence baseline was removed and the spectra was normalized to the lipid band at 1450 cm^{-1}. The band at 1410 cm^{-1} is marked with a star.

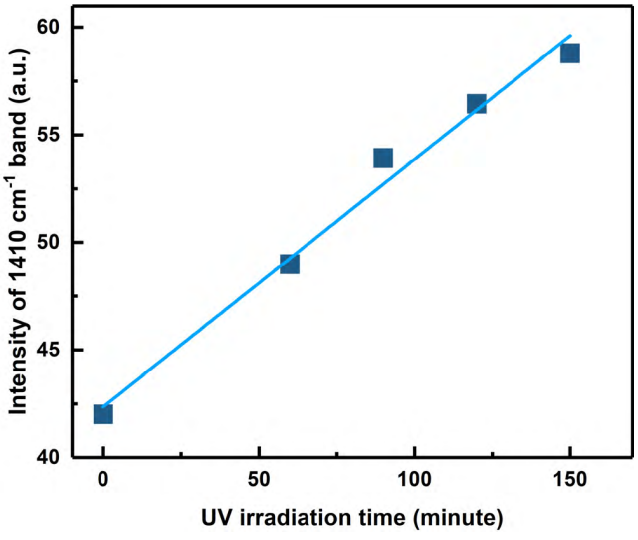

FIGURE 12. Increase of the 1410cm^{-1} Raman band intensity versus UV irradiation time.

LED radiation time of *E.coli* bacteria and Figure 13 shows the corresponding change in the colony forming units (CFU) versus irradiation time.

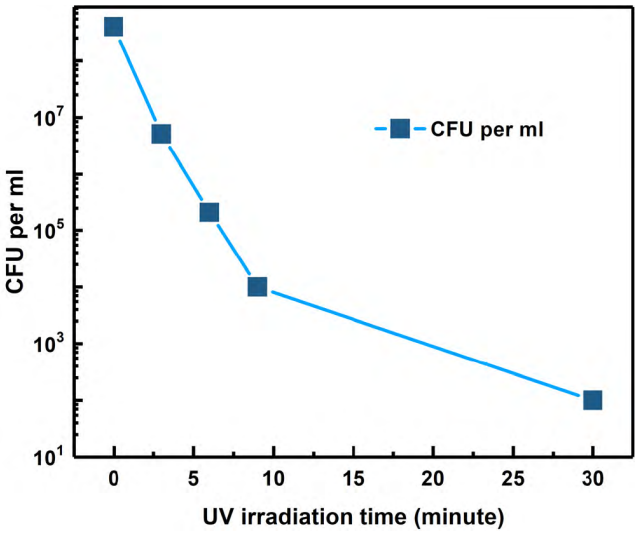

FIGURE 13. *E. coli* **CFU count versus 280 nm LED irradiation time.**

In addition to *E.coli* (Gram negative bacteria strain), several other bacteria including *M. luteus* (Gram positive bacteria) and *S. marcescens* (Gram negative bacteria) were studied and found to behave similarly under UV radiation. Namely, they experienced similar inactivation and spectroscopic changes as *E.coli*, including increase in the 1410 cm^{-1} Raman band intensity. Raman spectra and the changes in the intensity of the protein Raman bands, as a function of UV irradiation dose are depicted in the recorded Raman spectra of *M. luteus* and *S. marcescens* in Figures 14 and 15, respectively.

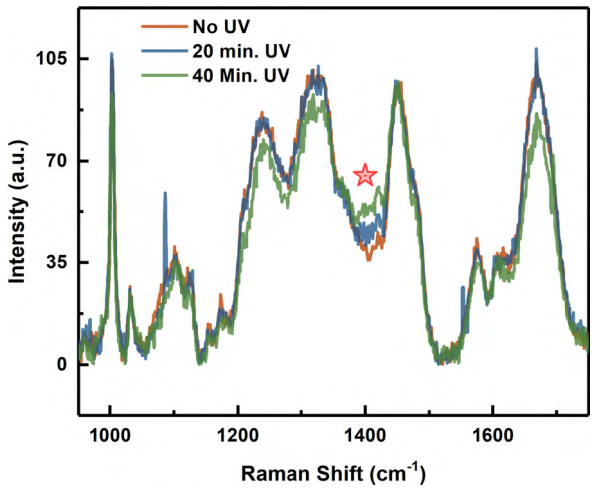

FIGURE 14. **Raman spectra of *M. luteus* bacteria before and after UV irradiation. The spectra were normalized with respect to the lipid 1450 cm^{-1} band. A star is designated to the 1410 cm^{-1} band whose intensity increases after UV irradiation.**

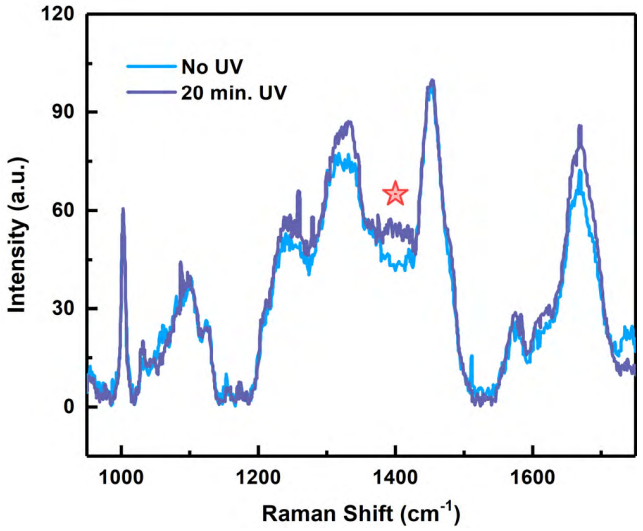

FIGURE 15. **Raman spectra of *S. marcescens* before and after UV radiation, which were normalized with respect to the lipid 1450 cm^{-1} band. The 1410 cm^{-1} band is designated by a star.**

IV. DISCUSSION

The Raman spectra recorded, after UV irradiation of the bacteria, show changes in both, the DNA bases and proteins. However, protein changes are much more pronounced than the changes observed for DNA bases, which are found to vary linearly with UV dose. The data suggest that proteins play a very important role in the radiation induced inactivation and death of bacterial cells. Interestingly, it has been shown that the extreme radiation resistance of bacteria such as *Deinococcus radiodurans*, depend on efficient proteome protection (but not DNA protection) against UV radiation induced protein carbonylation [59]. We have also shown previously that the bacterial fluorescence intensity of the protein amino acids, Tryptophan and Tyrosine, components can be used to determine the ratio of live and dead bacteria after UV irradiation or antibiotic treatment [5]. To that effect we have determined that when bacteria are exposed to UV light, Tryptophan and Tyrosine undergo a denaturation process which results in the decrease in the bacterial fluorescence intensity [60].

UV light inactivates and kills bacteria/cells by damaging their DNA. Recently, oxidative protein damage induced by UV radiation has been used as a means to assess the bacteria and other prokaryotic cells death [61], [62]. It was shown in those studies that protein damage causes the loss of necessary maintenance functions of bacterial cells, including DNA repair and that protein damage, not genomic (DNA) damage, correlates with eukaryotic and prokaryotic cell death after irradiation with UV. Data presented in this paper also point in a similar direction, namely that protein damage correlates with cell death and may be used as a means for determining live and dead bacteria.

V. HANDHELD INSTRUMENT

A novel handheld Raman instrument has been designed and constructed. This spectrometer is capable of

recording absorption, fluorescence and Raman spectra, *in situ*, which makes possible the fast and remote identification of bacteria strains in addition to providing information regarding the ratio of live and dead bacteria before and after UV irradiation. This system consists of two monochromaters, one provides the excitation wavelength while the other disperses the fluorescence or Raman spectra which is imaged onto the CCD detector. A schematic diagram of the Raman modules of this system is shown in Figure 16, while the components are shown in Figure 17.

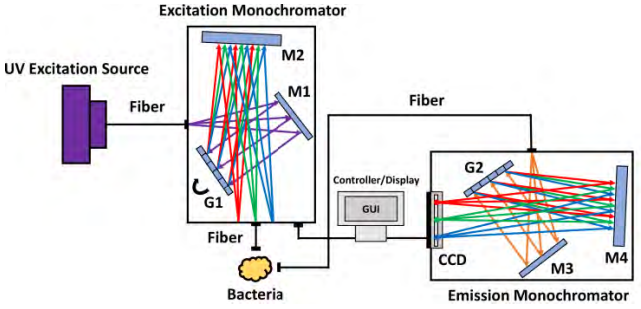

FIGURE 16. Schematic diagram of raman module for the handheld instrument.

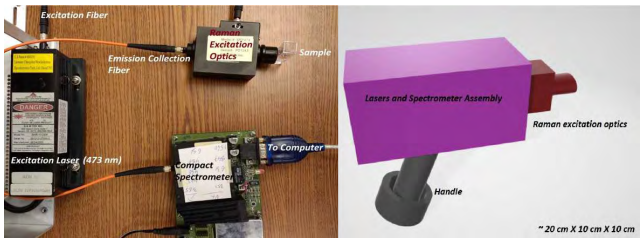

FIGURE 17. Components of the Raman module of the handheld device (left); 3D conceptual appearance (right).

This novel handheld, fluorescence and Raman spectra recording system employs a 473 nm Diode Pumped Solid State (DPSS) excitation laser (variable output power: 3 mW to 30 mW) which is suitable for generating Raman spectra of species that absorb in the blue-green region of the spectrum. The output of this 473 nm excitation laser is attached to one end of an optical fiber, while the other end, of the fiber, is coupled to a lens which collimates the laser light output. The collimated beam is then reflected by a dichroic beam splitter and focused, by an objective lens, on to the sample. This focusing increases the light intensity on the sample and results in more intense and higher resolution Raman spectra. The backscattered light, from the sample, is collected with the same objective lens. The dichroic mirror blocks the Rayleigh light scattered and allows the Stokes shifted Raman component of the light to pass through. The Stokes shifted Raman light is then imaged onto an emission monochromator where it is filtered once more, by a sharp long pass filter with cut-off below at 485 nm, in order to remove the remaining excitation light (Rayleigh scattering).

In order to be able to record high resolution Raman spectra the spectrometer input slit was reduced to 50 microns in width and the numerical aperture of the input optical fiber matches the numerical aperture of the spectrometer collection optics. After the light enters the spectrometer it is collimated and directed to the diffraction grating. The diffraction grating, as expected, has high dispersion efficiency in the UV and visible spectral regions. The dispersed light is then imaged, by means of a concave mirror, onto a linear CCD sensor (Sony ILX 511 B). All the mirrors inside the handheld spectrometer are highly reflecting in the UV and visible regions of the spectrum. This ensures that the same spectrometer can be used to record fluorescence and Raman spectra in both the UV and visible regions. The pixels of the linear CCD sensor are coated with a dye which converts the incident UV light to visible light where the quantum efficiency of this CCD is high. The data from the CCD are processed by a compact tablet PC using a serial communication link and displayed on top of the device. A graphical user-interface (GUI) allows for the selection of acquisition parameters such as integration time, number of spectra to be averaged and to perform further data analysis, using PCA.

The Raman capabilities of this handheld spectrometer were tested by recording the Raman spectra of red *S. marcescens* bacteria (grown at room temperature), and excited by the 473 nm laser source because red *S. marcescens* bacteria contain Prodigiosin, a red color pigment that absorbs at 473 nm [63]. Figure 18 shows the Raman spectra of *S. marscescens* bacteria obtained using the handheld Raman instrument (473 nm excitation, 3 mW, 5 seconds integration time, 25 accumulations). Figure 19 shows the same spectra recorded by the Horiba Raman spectrometer (excitation at 532 nm, 20 mW, for 10 second integration time, 10 accumulations).Vibrational spectra of the red pigment prodigiosin are clearly visible in the Raman spectra displayed in Figures 18 and 19. The resolution of the Raman spectral bands obtained from the handheld instrument is $\sim$70 cm^{-1}

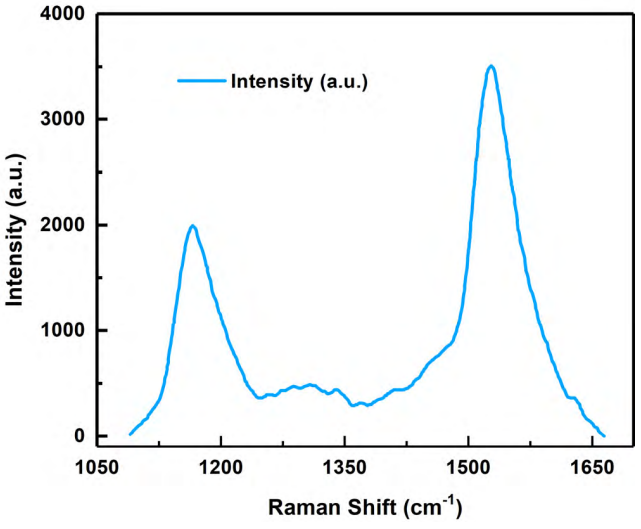

FIGURE 18. Spectra of Red *S. marcescens* recorded by the Handheld Raman spectrometer.

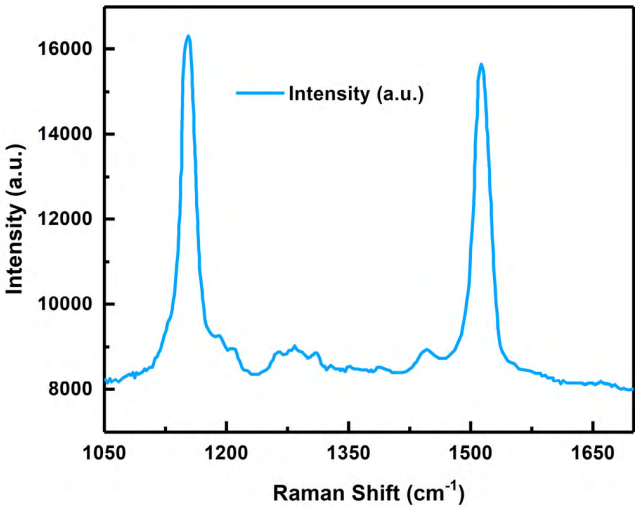

FIGURE 19. Raman spectra of red *S. marcescens* recorded by Horiba Raman spectrometer.

which is sufficient for most bacteria identification owing to their inherent broad Raman bands. However, this resolution can be improved by replacing the current diffraction grating by a higher resolution grating.

The scanning monochromator uses a stepper motor mounted on the diffraction grating, which is rotated by an Arduino microcontroller. This allows us to scan continuously the excitation wavelength, enabling the acquisition of 3-D excitation-emission matrices (EEMs) and the recording of synchronous fluorescence spectra for the identification of bacterial components.

In order to record high-resolution spectra at lower concentrations, UV Resonance Raman Spectra (UVRR) will be employed. Lasers emitting in the wavelength range of 220 nm and 250 nm will be used for resonance Raman excitation [64]. Choosing the appropriate UV excitation wavelength enables the recorded Raman spectra of DNA and proteins to be widely separated. For example, excitation at 222 nm makes it possible to record the Raman spectra of proteins whereas excitation at 251 nm allows for recording the Raman spectra of DNA [64].

Such procedures, we expect, will allow us to investigate the effects of UV radiation on bacteria with better resolution and at lower concentrations. The handheld device when it utilizes UV and red lasers, in addition to the current 473 nm laser, is expected to enable it to record resonance Raman spectra of most bacteria strains and other large molecules. In addition, the gratings will be replaced, as needed; with the most suitable ones in order to record highly resolved vibrational Raman bands of the species under investigation.

We are aware that the field applications of Raman spectroscopy for detection and identification of bacteria, especially in complex sample environments can be challenging due to high detection limits and interference from other molecules present, such as in wounds and food items. These issues and some of the approaches to overcome them have been detailed in [65]. Sample enrichment or preparation such as filtration or scraping and washing could be performed in the field and then record the sample's spectra using our handheld spectrometer, thereby eliminating the need to transport samples to a laboratory for testing. Meanwhile, it is relatively easy to couple a Raman spectrometer with a small microscope having high numerical aperture objectives. This approach may allow one to record the Raman spectra of a single bacterium. Visible and UV resonance Raman spectroscopy can be an efficient technique for high quality Raman measurements, with minimal sample preparations, or in the presence of interfering impurities. In addition, SERS substrates can also be used to enhance the sensitivity of the Raman measurements where the concentration of samples is low. This handheld spectrometer may be used when an object cannot or should not be touched or physically manipulated such as ancient texts and art objects.

VI. CONCLUSION

A novel, new, handheld spectrometer which is capable of recording fluorescence and Raman spectra has been designed, constructed and utilized to determine bacterial strains and differentiate between live and dead bacteria before and after irradiation with UV light. The Raman spectra recorded before and after UV irradiation display changes in the intensity of several vibrational bands as a function of irradiation time, which correspond to proteins, DNA bases and photodegradation products. We find that new Raman bands were formed after UV light irradiation. The new band located in the 1410 cm^{-1} region display a linear intensity increase with UV dose. We assigned this band to protein photoproducts. We have also established that its intensity increase correlates with the number of inactivated live bacteria. Additional uses of the handheld spectrometer are proposed.

ACKNOWLEDGMENT

The authors would like to thank Prof. Maria King for providing bacteria and valuable discussions.

REFERENCES

[1] T. R. Globus *et al.*, "THz-spectroscopy of biological molecules," *J. Biol. Phys.*, vol. 29, pp. 89–100, Jun. 2003. doi: 10.1023/A:1024420104400.

[2] F. Siebert, "Infrared spectroscopy applied to biochemical and biological problems," *Methods Enzymology*, vol. 246, pp. 501–526, Jan. 1995.

[3] P. Carmona, R. Navarro, and A. Hernanz, *Spectroscopy of Biological Molecules: Modern Trends*. Springer, 1997.

[4] G. McDermott *et al.*, "Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria," *Nature*, vol. 374, pp. 517–521, Apr. 1995.

[5] R. Li *et al.*, "Hand-held synchronous scan spectrometer for *in situ* and immediate detection of live/dead bacteria ratio," *Rev. Sci. Instrum.*, vol. 88, 2017, Art. no. 114301.

[6] M. Sohn, D. S. Himmelsbach, F. E. Barton, and P. J. Fedorka-Cray, "Fluorescence spectroscopy for rapid detection and classification of bacterial pathogens," *Appl. Spectrosc.*, vol. 63, no. 11, pp. 1251–1255, 2009.

[7] H. Trüper and C. S. Yentsch, "Use of glass fiber filters for the rapid preparation of in vivo absorption spectra of photosynthetic bacteria," *J. Bacteriology*, vol. 94, no. 4, p. 1255, 1967.

[8] I. Sondi and B. Salopek-Sondi, "Silver nanoparticles as antimicrobial agent: A case study on E. Coli as a model for Gram-negative bacteria," *J. Colloid Interface Sci.*, vol. 275, pp. 177–182, Jul. 2004.
[9] P. Carmona, "Vibrational spectra and structure of crystalline dipicolinic acid and calcium dipicolinate trihydrate," *Spectrochimica Acta A, Mol. Spectrosc.*, vol. 36, no. 7, pp. 705–712, 1980.
[10] J. M. Benevides, S. A. Overman, and G. J. Thomas, Jr., "Raman, polarized Raman and ultraviolet resonance Raman spectroscopy of nucleic acids and their complexes," *J. Raman Spectrosc.*, vol. 36, no. 4, pp. 279–299, 2005.
[11] B. Lorenz, C. Wichmann, S. Stöckel, P. Rösch, and J. Popp, "Cultivation-free Raman spectroscopic investigations of bacteria," *Trends Microbiol.*, vol. 25, pp. 413–424, May 2017.
[12] K. A. Britton, R. A. Dalterio, W. H. Nelson, D. Britt, and J. F. Sperry, "Ultraviolet resonance Raman spectra of *Escherichia* coli with 222.5–251.0 nm pulsed laser excitation," *Appl. Spectrosc.*, vol. 42, no. 5, pp. 782–788, 1988.
[13] C. R. Johnson, M. Ludwig, S. O'Donnell, and S. A. Asher, "UV resonance Raman spectroscopy of the aromatic amino acids and myoglobin," *J. Amer. Chem. Soc.*, vol. 106, no. 17, pp. 5008–5010, 1984.
[14] S. F. Al-Khaldi and M. M. Mossoba, "Gene and bacterial identification using high-throughput technologies:: Genomics, proteomics, and phonemics," *Nutrition*, vol. 20, no. 1, pp. 32–38, 2004.
[15] A. F. Bell, L. Hecht, and L. D. Barron, "Vibrational Raman optical activity of pyrimidine nucleosides," *J. Chem. Soc., Faraday Trans.*, vol. 93, no. 4, pp 553–562, 1997.
[16] S. Chadha, W. H. Nelson, and J. F. Sperry, "Ultraviolet micro-Raman spectrograph for the detection of small numbers of bacterial cells," *Rev. Sci. Instrum.*, vol. 64, p. 3088, Jul. 1993.
[17] D. Nauman, G. Barnickel, H. Bradaczek, H. Labischinski, and P. Giesbrecht, "Infrared spectroscopy, a tool for probing bacterial peptidoglycan," *Eur. J. Biochem.*, vol. 125, pp. 505–515, Jul. 1982.
[18] R. A. Dalterio, W. H. Nelson, D. Britt, and J. F. Sperry, "An ultraviolet (242 nm excitation) resonance Raman study of live bacteria and bacterial components," *Appl. Spectrosc.*, vol. 41, pp. 417–422, Mar. 1987.
[19] M. D. Collins and D. Jones, "Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication," *Microbiol. Rev.*, vol. 45, pp. 316–354, Jun. 1981.
[20] R. P. Rava and T. G. Spiro, "Resonance enhancement in the ultraviolet Raman spectra of aromatic amino acids," *J. Phys. Chem.*, vol. 89, no. 10, pp. 1856–1861, 1985.
[21] S. R. Samanta and F. E. Lytle, "Raman excitation profiles of purine and pyrimidine nucleic acid bases," *Appl. Spectrosc.*, vol. 36, no. 3, pp. 306–309, 1982.
[22] Y. C. Cao, R. Jin, and C. A. Mirkin, "Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection," *Science*, vol. 297, pp. 1536–1540, Aug. 2002.
[23] H. Stammreich and T. T. Sans, "Molecular vibrations of quinones. IV. Raman spectra of p-benzoquinone and its centrosymmetrically substituted isotopic derivatives and assignment of observed frequencies," *J. Chem. Phys.*, vol. 42, p. 920, Jul. 2004. doi: 10.1063/1.1696083.
[24] S. A. Asher, "UV resonance Raman spectroscopy for analytical, physical, and biophysical chemistry. Part 1," *Anal. Chem.*, vol. 65, no. 2, pp. 59A–66A, 1993.
[25] R. Manoharan, E. Ghiamati, R. A. Dalterio, K. A. Britton, W. H. Nelson, and J. F. Sperry, "UV resonance Raman spectra of bacteria, bacterial spores, protoplasts and calcium dipicolinate," *J. Microbiol. Methods*, vol. 11, pp. 1–15, Feb. 1990.
[26] R. Li, D. Dhankhar, J. Chen, T. C. Cesario, and P. M. Rentzepis, "Determination of live: Dead bacteria as a function of antibiotic treatment," *J. Microbiol. Methods*, vol. 154, pp. 73–78, Nov. 2018.
[27] A. W. Bauer, W. M. M. Kirby, J. C. Sherris, and M. Turck, "Antibiotic susceptibility testing by a standardized single disk method," *Amer. J. Clin. Pathol.*, vol. 45, no. 4, pp. 493–496, 1966.
[28] J. H. Marymont, Jr., and R. M. Wentz, "Serial dilution antibiotic sensitivity testing with the microtitrator system," *Amer. J. Clin. Pathol.*, vol. 45, no. 5, pp. 548–551, 1966.
[29] M. Li *et al.*, "Rapid antimicrobial susceptibility testing by matrix-assisted laser desorption ionization–time of flight mass spectrometry using a qualitative method in Acinetobacter baumannii complex," *J. Microbiol. Methods*, vol. 153, pp. 60–65, Oct. 2018.
[30] K. Rathore *et al.*, "Evaluation of multiplex polymerase chain reaction as an alternative to conventional antibiotic sensitivity test," *Veterinary World*, vol. 11, no. 4, pp. 474–479, 2018.
[31] W. A. Rutala and D. J. Weber, "The Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for disinfection and sterilization in healthcare facilities," Centers for Disease Control and Prevention, Atlanta, Georgia, Tech. Rep., 2008, p. 42. [Online]. Available: https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines.pdf
[32] G. Kultur, N. N. Misra, F. J. Barba, M. Koubaa, V. Gökmen, and H. Alpas, "Microbial inactivation and evaluation of furan formation in high hydrostatic pressure (HHP) treated vegetable-based infant food," *Food Res. Int.*, vol. 101, pp. 17–23, Nov. 2017.
[33] R. Sevenich *et al.*, "High-pressure thermal sterilization: Food safety and food quality of baby food puree," *J. Food Sci.*, vol. 79, pp. M230–M237, Feb. 2014.
[34] S. M. Erony *et al.*, "The epidemiology of bacterial culture–positive and septic transfusion reactions at a large tertiary academic center: 2009 to 2016," *Transfusion*, vol. 58, no. 8, pp. 1933–1938, 2018.
[35] E. M. Bloch *et al.*, "Implementation of secondary bacterial culture testing of platelets to mitigate residual risk of septic transfusion reactions," *Transfusion*, vol. 58, no. 7, pp. 1647–1653, 2018.
[36] N. Rastogi *et al.*, "Epidemiological investigation and successful management of a Burkholderia cepacia outbreak in a neurotrauma intensive care unit," *Int. J. Infectious Diseases*, vol. 79, pp. 4–11, Feb. 2019.
[37] V. Decraene *et al.*, "A large, refractory nosocomial outbreak of klebsiella pneumoniae carbapenemase-producing *Escherichia* coli demonstrates Carbapenemase gene outbreaks involving sink sites require novel approaches to infection control," *Antimicrobial Agents Chemotherapy*, vol. 62, no. 12, p. e01689, 2018.
[38] M. F. Escoriza, J. M. Van Briesen, S. Stewart, and J. Maier, "Raman spectroscopic discrimination of cell response to chemical and physical inactivation," *Appl. Spectrosc.*, vol. 61, pp. 812–823, May 2007.
[39] T. J. Moritz, C. R. Polage, D. S. Taylor, D. M. Krol, S. M. Lane, and J. W. Chan, "Evaluation of Escherichia coli cell response to antibiotic treatment by use of Raman spectroscopy with laser tweezers," *J. Clin. Microbiol.*, vol. 48, no. 11, pp. 4287–4290, 2010.
[40] A. I. M. Athamneh, R. A. Alajlouni, R. S. Wallace, M. N. Seleem, and R. S. Senger, "Phenotypic profiling of antibiotic response signatures in *Escherichia* coli using Raman spectroscopy," *Antimicrobial Agents Chemotherapy*, vol. 58, no. 3, pp. 1302–1314, 2014.
[41] I. Notingher, S. Verrier, H. Romanska, A. E. Bishop, J. M. Polak, and L. L. Hench, "*In situ* characterisation of living cells by Raman spectroscopy," *J. Spectrosc.*, vol. 16, no. 6, pp. 43–51, 2002.
[42] T. A. Conner-Kerr, P. K. Sullivan, J. Gaillard, M. E. Franklin, and R. M. Jones, "The effects of ultraviolet radiation on antibiotic-resistant bacteria *in vitro*," *Ostomy/Wound Manage.*, vol. 44, no. 10, pp. 50–56, 1998.
[43] T. Dai, M. S. Vrahas, C. K. Murray, and M. R. Hamblin, "Ultraviolet C irradiation: An alternative antimicrobial approach to localized infections?" *Expert Rev. Anti-Infective Therapy*, vol. 10, no. 2, pp. 185–195, 2012.
[44] J. C. Chang *et al.*, "UV inactivation of pathogenic and indicator microorganisms," *Appl. Environ. Microbiol.*, vol. 49, no. 6, pp. 1361–1365, 1985.
[45] R. P. Rastogi, A. Kumar, M. B. Tyagi, and R. P. Sinha, "Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair," *J. Nucleic Acids*, vol. 2010, Sep. 2010, Art. no. 592980.
[46] H. Durchschlag *et al.*, "Effects of X- and UV-irradiation on proteins," *Radiat. Phys. Chem.*, vol. 47, pp. 501–505, Mar. 1996.
[47] E. Kristo, A. Hazizaj, and M. Corredig, "Structural changes imposed on whey proteins by UV irradiation in a continuous UV light reactor," *J. Agricult. Food Chem.*, vol. 60, no. 24, pp. 6204–6209, 2012.
[48] K. C. Smith, "Photochemical reactions of thymine, uracil, uridine, cytosine and bromouracil in frozen solution and in dried films," *Photochemistry Photobiol.*, vol. 2, no. 4, pp. 503–517, 1963.
[49] E. Lipiec, R. Sekine, J. Bielecki, W. M. Kwiatek, and B. R. Wood, "Molecular characterization of DNA double strand breaks with tip-enhanced Raman scattering," *Angew. Chem. Int. Ed.*, vol. 53, no. 1, pp. 169–172, 2014. doi: 10.1002/anie.201307271.
[50] B. R. Wood, L. Hammer, L. Davis, and D. McNaughton, "Raman microspectroscopy and imaging provides insights into heme aggregation and denaturation within human erythrocytes," *J. Biomed. Opt.*, vol. 10, no. 1, 2005, Art. no. 014005.
[51] K. K. Ramser, E. J. Bjerneld, C. Fant, and M. Kaell, "Importance of substrate- and photo-induced effects in Raman spectroscopy of single functional erythrocytes," *J. Biomed. Opt.*, vol. 8, no. 2, p. 173, 2003.

[52] B. R. Wood and D. McNaughton, "Raman excitation wavelength investigation of single red blood cells *in vivo*," *J. Raman Spectrosc.*, vol. 33, no. 7, pp. 517–523, 2002.
[53] P. Rösch, M. Harz, K.-D. Peschke, O. Ronneberger, H. Burkhardt, and J. Popp, "Identification of single eukaryotic cells with micro-Raman spectroscopy," *Biopolymers*, vol. 82, no. 4, pp. 312–316, 2006.
[54] A. Rygula, K. Majzner, K. M. Marzec, A. Kaczor, M. Pilarczyk, and M. Baranska, "Raman spectroscopy of proteins: A review," *J. Raman Spectrosc.*, vol. 44, no. 8, pp. 1061–1076, 2013.
[55] H. H. Torkabadi, C. R. Bethel, K. M. Papp-Wallace, P. A. J. de Boer, R. A. Bonomo, and P. R. Carey, "Following drug uptake and reactions inside *Escherichia* coli cells by Raman microspectroscopy," *Biochemistry*, vol. 53, no. 25, pp. 4113–4121, 2014.
[56] K. C. Schuster, I. Reese, E. Urlaub, J. R. Gapes, and B. Lendl, "Multidimensional information on the chemical composition of single bacterial cells by confocal Raman microspectroscopy," *Anal. Chem.*, vol. 72, no. 22, pp. 5529–5534, 2000.
[57] W. E. Huang, R. I. Griffiths, I. P. Thompson, M. J. Bailey, and A. S. Whiteley, "Raman microscopic analysis of single microbial cells," *Anal. Chem.*, vol. 76, no. 15, pp. 4452–4458, 2004.
[58] K. A. Okotrub, N. V. Surovtsev, V. F. Semeshin, and L. V. Omelyanchuk, "Raman spectroscopy for DNA quantification in cell nucleus," *Cytometry*, vol. 87, no. 1, pp. 68–73, 2015.
[59] A. Krisko and M. Radman, "Protein damage and death by radiation in Escherichia coli and Deinococcus radiodurans," *Proc. Nat. Acad. Sci. USA*, vol. 107, no. 32, pp. 14373–14377, 2010.
[60] R. Li, U. Goswami, M. King, J. Chen, T. C. Cesario, and P. M. Rentzepis, "*In situ* detection of live-to-dead bacteria ratio after inactivation by means of synchronous fluorescence and PCA," *Proc. Nat. Acad. Sci. USA*, vol. 115, no. 4, pp. 668–673, 2018.
[61] M. J. Daly, "Death by protein damage in irradiated cells," *DNA Repair*, vol. 11, no. 1, pp. 12–21, 2012.
[62] M. J. Daly *et al.*, "Small-molecule antioxidant proteome-shields in Deinococcus radiodurans," *PLoS ONE*, vol. 5, 2010, Art. no. e12570.
[63] V. L. Yu, " Serratia marcescens—Historical perspective and clinical review," *New England J. Med.*, vol. 300, pp. 887–893, Apr. 1979.
[64] W. H. Nelson, R. Manoharan, and J. F. Sperry, "UV resonance Raman studies of bacteria," *Appl. Spectrosc. Rev.*, vol. 27, no. 1, pp. 67–124, 1992.
[65] S. Pahlow, S. Meisel, D. Cialla-May, K. Weber, P. Rösch, and J. Popp, "Isolation and identification of bacteria by means of Raman spectroscopy," *Adv. Drug Del. Rev.*, vol. 89, pp. 105–120, Jul. 2015.

RUNZE LI received the Ph.D. degree from Shanghai Jiao Tong University, Shanghai, China. He is currently an Assistant Research Scientist with the Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, USA.

DINESH DHANKHAR received the B.Tech. degree in avionics engineering from the Indian Institute of Space Science and Technology, Trivandrum, India, in 2011, and the M.S. degree from the Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, USA, in 2018, where he is currently pursuing the Ph.D. degree.

JIE CHEN received the Ph.D. degree from the University of California at Irvine, Irvine, CA, USA. She is currently a Distinguished Researcher with Shanghai Jiao Tong University, Shanghai, China. She has published over 40 papers on ultrafast X-ray and electron diffraction. She holds three patents.

ARJUN KRISHNAMOORTHI is currently pursuing the B.E. degree in electrical engineering specializing in device science and nanotechnology (optics) with the Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, USA.

THOMAS C. CESARIO received the M.D. degree in infectious diseases from the University of Wisconsin School of Medicine and Public Health. He is currently a Professor and the Dean of the School of Medicine, University of California at Irvine.

PETER M. RENTZEPIS received the Ph.D. degree from the University of Cambridge. He is a TEES Distinguished Professor with the Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX, USA.