Raman Spectroscopy and Digital Imaging for Identification and Enumeration of Bacteria in Water

Maria F. Escoriza*, Jeanne M Van Briesen*, Shona Stewart #, John Maier #
* Department of Civil and Environmental Engineering, Carnegie Mellon University; Pittsburgh PA
# ChemImage Corporation; Pittsburgh PA

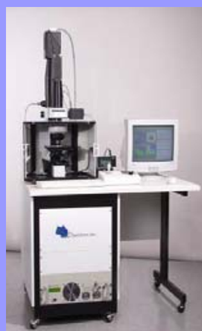
Abstract

Molecular spectroscopy is a reliable, universally accepted technique for confirmation of the identity of unknown chemicals. We report on evaluation of Raman Spectroscopy for rapid identification of waterborne pathogens in complex aquatic environments. Samples of pathogenic strains obtained under laboratory cultivation techniques were analyzed using Raman Spectroscopy and Digital Imaging (Chemimage Corporation, Pittsburgh PA). Different bacterial species exhibit characteristic signals. Further, quantification of bacterial concentration based on Raman signal strength was demonstrated. *Staphylococcus epidermidis* in different concentrations were analyzed using Raman Chemical Imaging. Raman Chemical Imaging data set of each region was collected in 10 cm-1 increment in the range of 2700 to 3100 cm^{-1} (wavenumber), 100x magnification, 90 seconds of exposure time, 1 average and a laser power of 0.2 Watt. The number of microorganisms present in each region was obtained (manually counted) and correlated with the integrated area of each image spectra in the range of interest (2700 to 3100 cm^{-1}). A linear correlation between the number of cells and the integrated area of the Raman intensity was observed. Concentrations of *Escherichia coli* in test samples were validated with plate counting, optical density and through cell dry weight measurements. Inter-method consistency was evaluated.

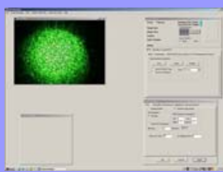
Objective

The objective of this project is to evaluate the suitability of Raman spectroscopy as a tool for rapid identification of naturally-occurring and maliciously-introduced waterborne pathogens in complex aquatic environments.

Equipment, Materials and Methods



Falcon Raman Chemical Imaging Microscope, ChemImage Corporation, Pittsburgh



ChemAcquire c, ChemImage Corporation

Falcon Equipment

The instrument used in the experiments is the Falcon Raman Chemical Imaging Microscope (ChemImage Corp., Pittsburgh). It combines the benefits of dispersive Raman spectroscopy and Chemical Imaging. The scientific research grade microscope is equipped with 5x,20x,50x and 100x objectives, transmission & reflectance illumination and polarization optics. It delivers real-time simultaneous Imaging and Spectroscopy. Imaging of chemical species below 1 µm is routine. ChemAcquire c is the software used for data acquisition in the Falcon microscope.

Cultures

Staphylococcus epidermidis (ATCC 35984) and *Escherichia coli* (ATTC 11775) were used as representative micro organisms. *Staphylococcus epidermidis* is a species of bacteria that is a normal inhabitant of the skin of healthy humans. It causes infection when is introduced into the body through foreign objects, such as sutures, indwelling catheters, and implanted artificial joints. It is an important cause of hospital-acquired infections. *Escherichia coli* is a bacterial species present in the normal intestinal flora of man and warm-blooded animals. Some strains of this species are responsible for gastrointestinal illness.

Standard Plate Count. *S.epidermidis colonies*

Bacterial solutions at different concentrations

S. epidermidis bright field image at 100x magnification on aluminum slide

13 mm diameter silver membrane

Filtration equipment

Cultivation

Staphylococcus epidermidis was cultivated from a loop of pure culture (ATCC 35984) that was aseptically transferred and incubated in a liquid media (Trypticase Soy Agar) for 24 hours at 37°C without shaking. The same procedure was followed for *Escherichia coli* (ATTC 11775) but with an incubation period of 18 hours.

Sample preparation

Raman Spectra and Imaging

Suspended cells were pelletized by centrifugation and re-suspended in distilled water. Then, several droplets were placed on an aluminum-coated slide and dried. The slide was examined under the microscope and the laser source was focused on the area of interest where Raman images and spectra were collected.

Raman protocol for bacteria quantification by filtration

After 18 hours of incubation for *E.coli*, dilutions of the growth media containing the cells were prepared using distilled water. Each of the dilutions were plated following the Standard Plate Count Method. Also, optical density (600 nm) and dry weight were measured for each of the samples (dilutions). One milliliter of each sample was filtered through a silver membrane of 0.2 µm pore size and 13 mm diameter (Sterlitech Corp.). Thirty spectra were acquired per filter and their average intensity was correlated with Standard Plate Count, optical density and dry weight results.

Results

***S.epidermidis* and *E.coli* identification**

Figure 1 shows spectra of *S.epidermidis* and *E.coli* bacteria collected on the spectrometer mode of the Falcon equipment at 100x magnification, 100 seconds of exposure time, 3 averages and a laser power of 0.2 Watt for *S.epidermidis* (300 seconds total time). For *E.coli* the exposure time was 20 seconds with 10 averages (200 seconds total time) .

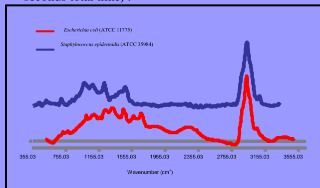


Figure 1. Spectrum of *S.epidermidis* and *E.coli* bacteria collected on the spectrometer mode of the Falcon equipment.

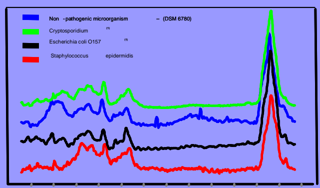


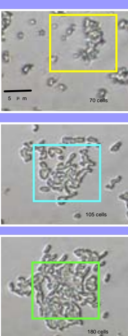
Figure 2. Comparison of different-microorganisms spectrum.

(1) Spectrum provided by ChemImage Corp.

***S.epidermidis* quantification**

An experiment was conducted to obtain quantitative data to correlate Raman response to microorganism numbers. Three regions of the slide containing *S.epidermidis* in different concentrations were analyzed using Raman Chemical Imaging. Raman Chemical Imaging data set of each region was collected in 10 cm^{-1} increments in the range of 2700 to 3100 cm^{-1} (wavenumber), 100x magnification, 90 seconds of exposure time, 1 average and a laser power of 0.2 Watt. Also, the number of microorganisms present in each region was obtained (manually counted) and correlated with the integrated area of each image spectra in the range of interest (2700 to 3100 cm^{-1}). See figures 3 and 4.

Brightfield images at 100x magnification



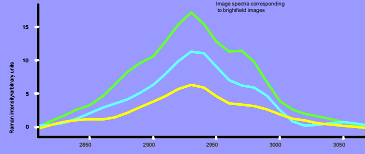


Figure 3. Raman intensity for each of the regions

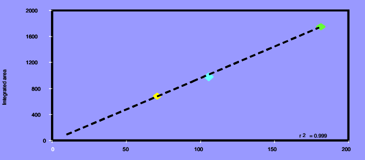


Figure 4. Correlation between integrated area and number of cells

***E.coli* quantification**

Another experiment was conducted to obtain quantitative data to correlate Raman response to microorganisms' counts using Standard Plate Count, optical density and dry weight methods. Four filters containing different bacteria concentrations were analyzed using Raman Spectroscopy. Thirty Raman spectra were collected per filter. Each spectrum was collected was collected in the range of 2700 to 3200 cm^{-1} (wavenumber), 50x magnification, 4 seconds of exposure time, 10 averages and a laser power of 0.2 Watt. Also, the concentration of microorganisms present in each solution used for filtration was estimated by the Standard Plate Count method, optical density and dry weight methods. The results obtained by each of the methods were correlated with the integrated area of each filter's average spectrum (Figures 5 to 7) .

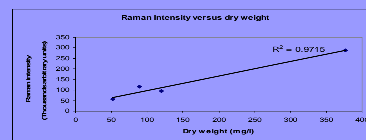


Figure5. Correlation between Raman intensity and dry weight

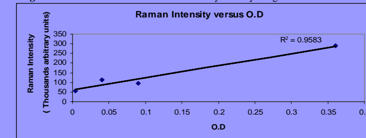


Figure6. Correlation between Raman intensity and optical density (600nm)

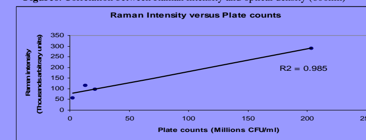


Figure7. Correlation between Raman intensity and Plate counts

Conclusions

1. *S. epidermidis* and *E.coli* bacteria exhibit a characteristic and distinct signals when analyzed using Raman Spectroscopy .
2. A relationship exists between the number of organisms in a sample and the intensity of the Raman response in the 2700-3200 cm^{-1} (wavenumbers).
3. Quantification with Raman spectroscopy compares favorably with standard quantification methods including plate counts , optical density and dry weight measurements.
4. Raman spectra and images were acquired in less than one hour with simple preparation methods, indicating this method may be suitable for bacterial identification and quantification.

Acknowledgments

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