

Raman Chemical Imaging for Detection of Biological Agents in Water

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Abstract

Molecular Spectroscopy is a reliable, universally accepted technique for confirmation of the identity of unknown chemicals. However, detection of pathogens in water systems using this technique presents a new challenge. This poster introduces a project whose objective is the evaluation of molecular spectroscopy as a tool for rapid identification of naturally-occurring and maliciously-introduced waterborne pathogens in complex aquatic environments. In the first stage of the project; samples of pathogenic strains obtained under laboratory cultivation techniques were analyzed using Raman Spectroscopy and Digital Imaging. These preliminary results indicate that bacteria exhibit a characteristic signal when they are analyzed with Raman Spectroscopy and Digital Imaging. The next stages in the project will be the creation of a signal library for pathogenic organisms of interest and the evaluation of detection limits and interferences.

Background

Raman Imaging

Raman Imaging combines Raman spectroscopy and optical methods to provide molecular images that can reveal material morphology, composition, structure and concentration. The principle of Raman Imaging is explained in Figure 1. The conventional image or bright field image provides low contrast between the different materials A, B and C in Figure 1. As each compound has a characteristic spectrum, chemical imaging technique maps the spatial arrangement of each constituent (A, B and C) of the inhomogeneous sample by isolating from the Raman scattered photons those which originate from a characteristic Raman line of the specific compound. Thus, each component can be visualized in high-contrast images (Raman images) at intrinsic wave numbers where the Raman signal is strong.

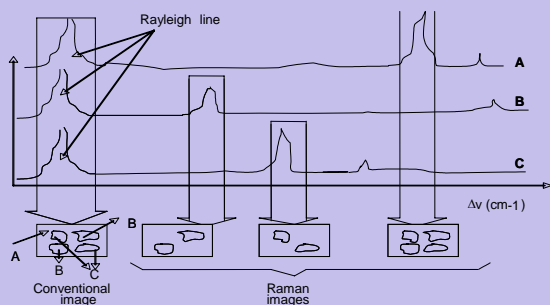


Figure 1. Principle of Raman image. Y-axis is relative scattering intensity. Adapted from Turrell, G., & Corset, J. 1996. *Raman Microscopy, Developments and applications*. Academic Press Inc, San Diego.



Carnegie Mellon

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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.

Approach and methodology

- 1-Verify that bacteria generate a characteristic Raman Spectrum-Digital Imaging.
- 2-Verify that pathogens concentration will not limit the suitability of molecular spectroscopy as a detection technique
- 3-Verify that the presence of non-pathogenic bacteria in natural waters will not interface with identification of pathogenic organisms

Equipment, Materials and Methods



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.

Culture

Staphylococcus epidermidis (ATCC 35984) was the pathogenic microorganism sample. *Staphylococcus epidermidis* is a species of bacteria that is a normal inhabitant of the skin of healthy humans. It causes infection when it 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.

Cultivation

A loop of pure microbes culture (ATCC 35984) was aseptically transferred and incubated in a liquid media (Trypticase Soy Agar) for 24 hours at 37°C.

Sample preparation

Suspended cells were pelleted by centrifugation and re-suspended in deionized water three times. Then, several droplets were placed on a aluminum coated slide and dried. The slide was examined under the microscope and several Raman spectra were collected. Figure 2 and 3 show *Staphylococcus epidermidis* at microscope field of view of 100x.



Figure 2. *S. epidermidis* brightfield image 100x magnification

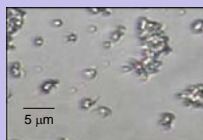


Figure 3. *S. epidermidis* brightfield image 100x magnification

S. Epidermidis Identification

Figure 4 shows a spectrum of *S. epidermidis* 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. The microscopic area of material utilized to acquire the spectrum is shown on top of the spectrum. In figure 5, different-microorganisms spectra are plotted together to point out that *S. epidermidis* (as well as each microorganism) has a distinctive Raman signal, specially in the 700 cm⁻¹ to 1800 cm⁻¹ region.

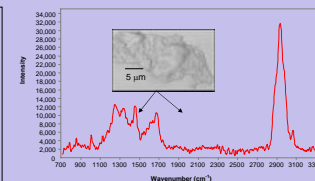


Figure 4. Spectrum of *S. epidermidis* bacteria collected on the spectrometer mode of the Falcon equipment. Brightfield image of the area where the spectrum was acquired is also shown.

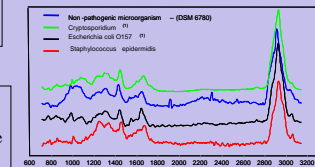


Figure 5. Comparison of different-microorganisms spectrum. (I) Spectrum provided by ChemImage Corp.

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).

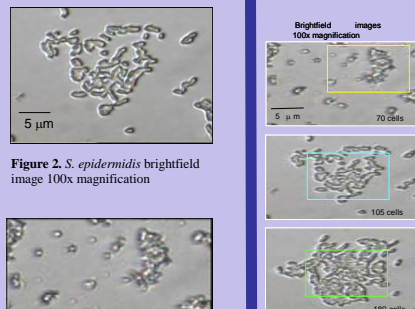


Figure 6. Raman intensity for each of the regions

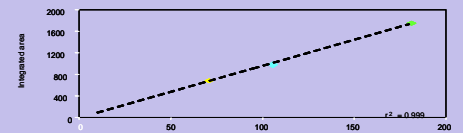


Figure 7. Correlation between integrated area and number of cells

Conclusions

1. *S. epidermidis* bacteria exhibit a characteristic signal when are analyzed using Raman Spectroscopy
2. The results obtained reveal a linear correlation between the number of microorganisms and the intensity of the Raman response
3. The Raman Imaging data were acquired in 60 minutes, using a very simple preparation procedure, suggesting this method may be suitable for bacterial pathogen detection in water samples.