Surface-Enhanced Infrared Absorption-Reflectance (SEIRA) Microspectroscopy – A Chemical/Biological Probe for Bacteria Localization in Geologic Materials

Hoi-Ying N. Holman, Dale L. Perry, Michael C. Martin, Wayne R. McKinney, and Jennie C. Hunter-Cevera
E.O. Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720 USA

INTRODUCTION

Pollution of subsurface geologic zones and the possibility of using the intrinsic endolithic (rock/mineral-inhabiting) bacteria to either detoxify or immobilize the pollutants have stimulated new interests in the exploration of endolithic bacteria and their long-term survival in the geologic environment. The location of bacteria within rocks and minerals affects the type and intensity of environmental stresses the bacteria are exposed to and thus affects their long-term survival potential. Localization of bacteria within rocks has been the subject of many ongoing research programs. Strategies must be devised to answer the question of relationships between the microbial localization and the microstructure of rocks. One approach is a microscopic system approach [1], using microscopy techniques to visually enumerate the distribution of microorganisms on an intact environmental sample. The distribution is related to the microscale physical and geochemical features measured on the same intact environmental sample. Other approaches include a global system [2], which uses different molecular probes to identify the presence of microorganisms in crushed rocks and conventional methods to identify the physical and geochemical properties of the rock materials.

The purpose of this study is to present the use of surface-enhanced infrared absorption-reflectance (SEIRA) microspectroscopy as a chemical/biological probe to analytically study — quickly and with minimum sample preparations — relationships between the microbial localization and the microstructure of geologic materials such as rocks. Specifically, it was to investigate if spatially resolved SEIRA microspectra, when recorded with a proper spatial scale and infrared beam area, could successfully illustrate the micro-scale spatial composition differences and to provide further insights into factors that control localization of bacteria in the specimen. The fundamental goal of our selecting spatially resolved SEIRA microspectroscopy was to identify qualitatively highly localized differences in the chemical composition and structure of clusters of living-bacteria within rocks that inherently have low infrared reflective surfaces.

PRINCIPLE OF SEIRA MICROSPECTROSCOPY

SEIRA microspectroscopy combines the traditional light-reflecting microscopy with the more recent but well-documented SEIRA spectroscopy technique. The fundamental working concept of SEIRA microspectroscopy is to use visible light and reflecting optics to view a magnified image of the sample and to select a microscopic surface area on the sample for infrared reflection-absorption spectroscopic analysis [3]. The selection of the area is relatively subjective and relies on a number of physical properties and other material-specific features of the sample surface. Once the sample area is selected, the molecular information of the selected surface area can be recorded spectroscopically in the infrared spectral region. For samples with surfaces of low infrared reflection, such as rock surfaces or surfaces with low analyte concentrations, such as those typically encountered in studies of environmental pollution, the measured spectra often do not have sufficient sensitivity for obtaining molecular information of the surface. Several researchers [e.g., 4-5] have found that SEIRA spectroscopy can increase the spectral sensitivity tremendously by

using different enhancement techniques that are commonly employed for chemical analyses for extremely small amounts of chemicals on surfaces. The increase is due to the enhancement of the incident infrared field at the surface. Recent literature has demonstrated that SEIRA spectroscopy has been successfully used in a wide variety of the analysis of water and thin organic films on semi-conductors, glasses, and polymers [6-10].

RESULTS AND DISCUSSION

The space-resolved SEIR microspectroscopy analysis was a line analysis conducted along axis BAB' inside a 400×500-μm study area, as shown in Fig. 1. Fig. 2 shows confocal micrograph images of the x-y section of bacterial microcolonies along axis BAB' under simultaneous excitation at 488 and 510 nm. Additionally, confocal micrograph images of the y-z section (not shown here) indicate that most microcolonies were less than 1 μm thick from the basalt surface, except for the colony "A". Fig. 3 shows the SEM (scanning electron microscopy) micrograph images of endolithic

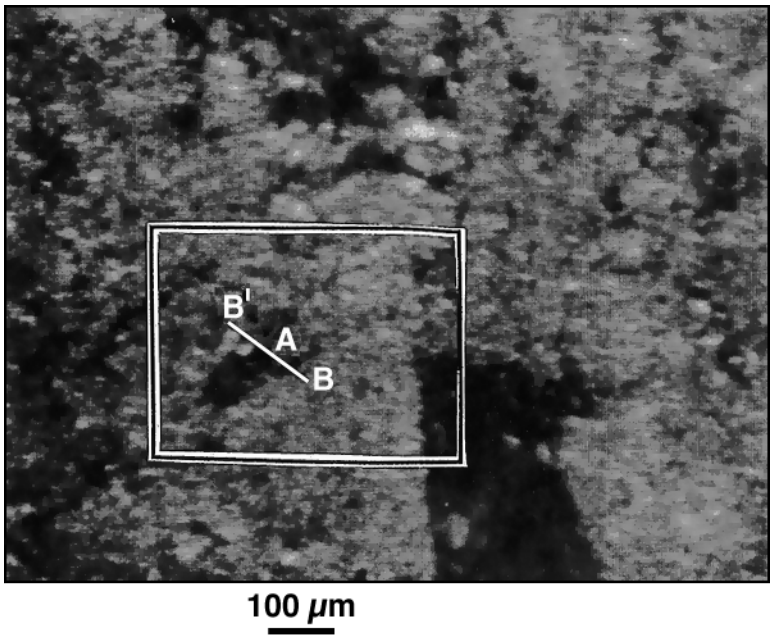

Figure 1. Microphotography of the study area. Bar = 100 μm.

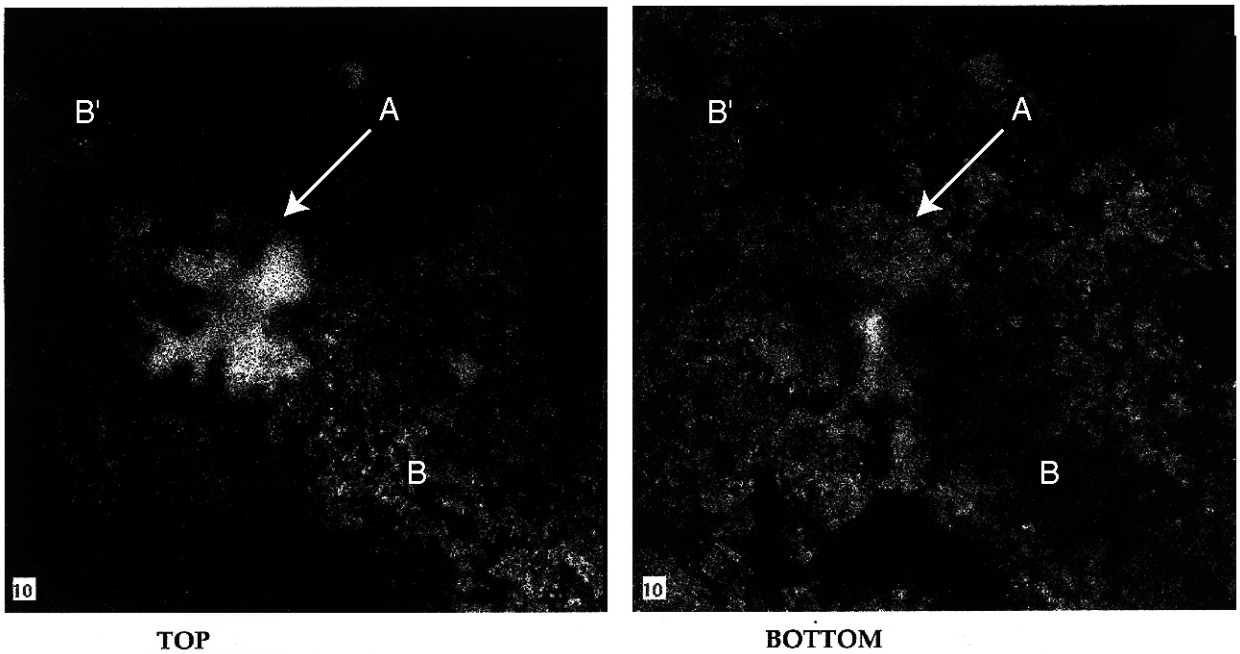

Figure 2. Confocal fluorescence micrograph of x-y sections showing images of microbial clusters "A" at two different optical depths on the vesicular basalt sample: 8.8 μm (top) and 1.1 μm (bottom) from the base of the brightest microbial cluster. The microbes were observed with simultaneous excitation at 488 and 514 nm. Microcolonies fluoresced naturally under simultaneous excitation at 488 and 510 nm. The fluorescence color was mostly pale yellow to yellow-green. Scale bar =10 μm.

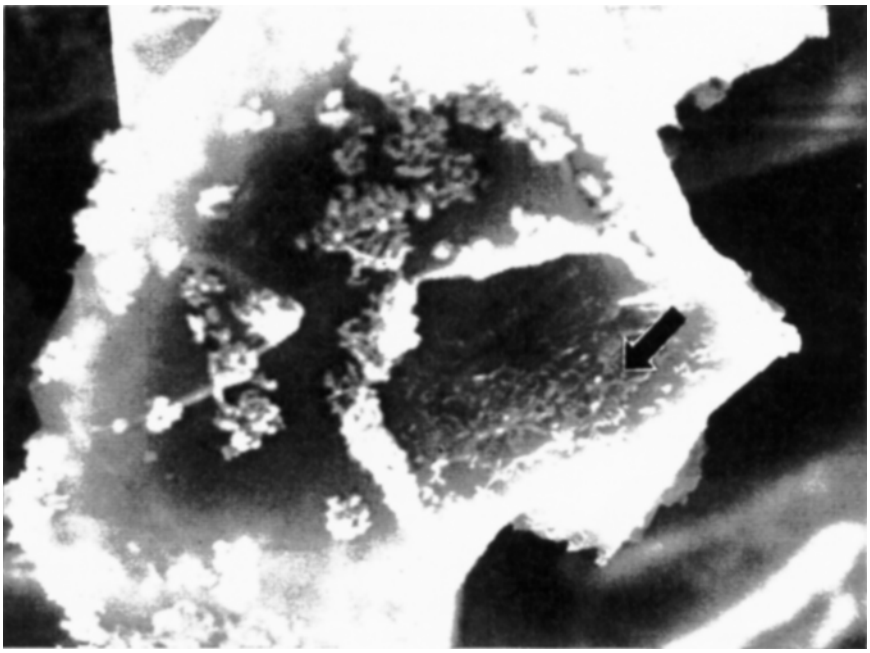

Figure 3. Scanning electron micrograph of rod shaped indigenous endolithic bacteria on the mineral surface inside a vesicle. (9,500x)

microbial populations on a mineral surface inside a basalt vesicle within the study area. Notice that the visible bacteria were rod shaped with approximate dimensions of 0.4-0.5 μm and were smaller than bacteria commonly found in the soil and aquatic environment.

All SEIRA microspectroscopic data were collected along the measurement axis BAB', starting at Point B and moving pass the edge of the fluorescent microcolony clusters. Fig. 4 shows the spectroscopic differences of FTIR scans as the sampling point was moved along BAB′. Notice the infrared spectral characteristics changed abruptly by scan 24 as the transition into the bacteria-free area was made. The continuous presence of biomolecule spectral peaks way into the dark area in the confocal micrograph (Fig.2) indicated that not all the indigenous bacteria naturally fluoresced under simultaneous excitation at 488 and 510 nm. The decrease of biomolecules marker peaks between scans 21 and 24 coincided with a change of mineral marker peaks. With this experimental effort, the practical aspects and the usefulness of SEIRA as a promisingly simple and fast analytical tool for studying the localization of living bacteria within rocks have been demonstrated.

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Principal investigator: Hoi-Ying N. Holman, Mail Stop 70A-3317, Ernest Orlando Lawrence Berkeley National Laboratory. E-mail: hyholman@lbl.gov. Telephone: 510-486-5943.

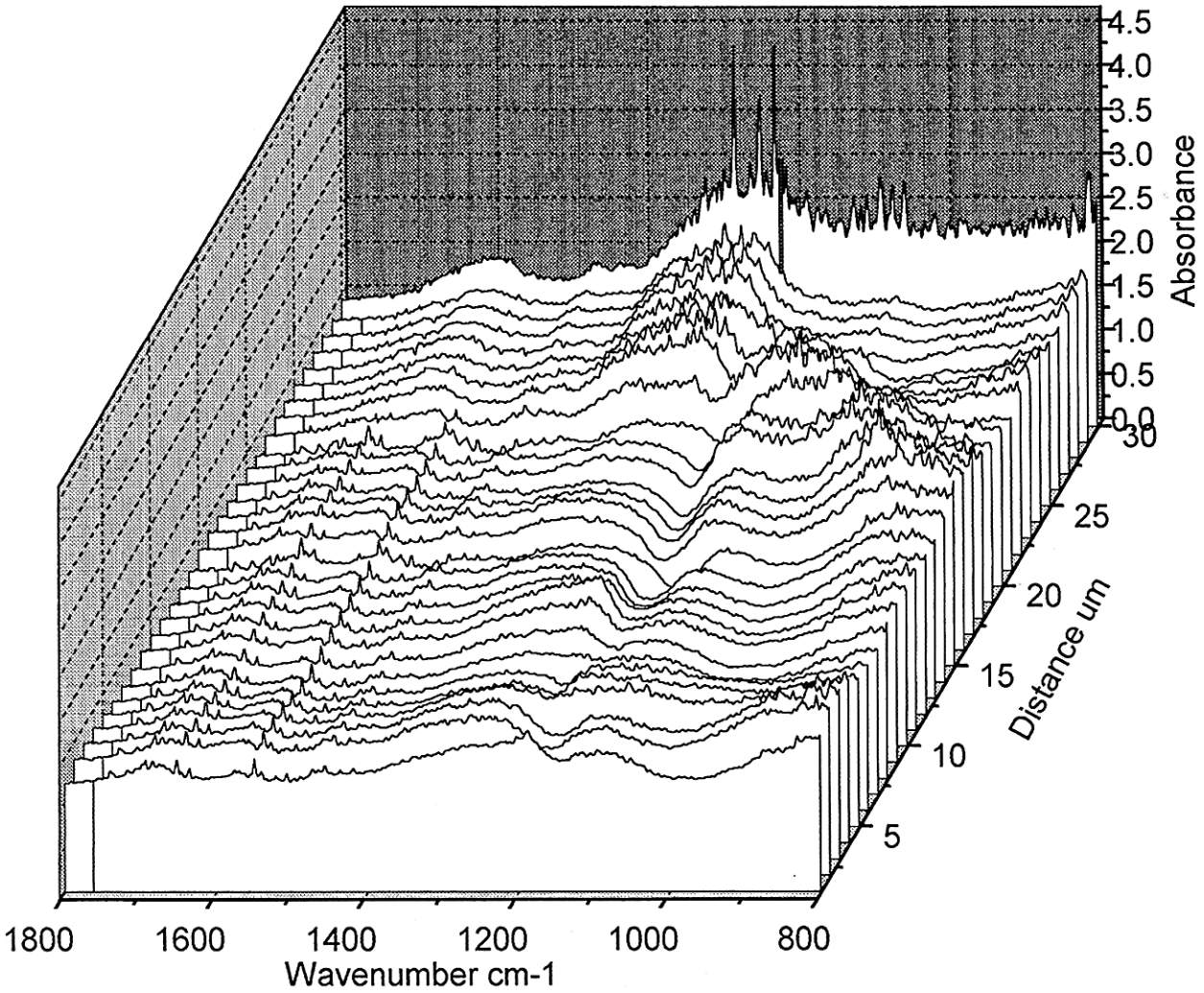

Figure 4. A series of SEIRA spectra showing the transition from bacteria-containing to bacteria-free basalt surface. Each step = 5 μm.