

Characterizing and Mapping Bacteria in Plant Tissue

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Researchers at Lawrence Berkeley National Laboratory (LBNL) have acquired CytoViva's Hyperspectral Microscopy System. The long-term goal of this acquisition is to increase the dimensionality of the real-time multi-mode imaging platform at the Berkeley Synchrotron Infrared Structural Biology (BSISB) user's program. This program is part of the Advanced Light Source of LBNL (<http://infrared.als.lbl.gov/content/structuralbiology>), funded by the Department of Energy (DOE). The LBNL approach is to continue building upon the unique nature (high brightness and the broadband) of a synchrotron light source, while extending the current use of mid-infrared to include near infrared, visible and soft-UV. This will allow LBNL to characterize, for example, microbial/plant biomass systems associated with bio-energy research, with the goal of increasing molecular information and improved spatial resolution from micrometers to nanometers.

Some of the images captured by LBNL show the ability of the CytoViva system below. **Figure 1** illustrates a hyperspectral scan of the clostridium bacteria at 100X. From this image, we select different pixels to build a spectral library (**Figure 2**) which is used to confirm the presence of the clostridium in other environments. **Figure 3** is a hyperspectral scan at 100X of plant tissue where the clostridium bacterium has been exposed. Pixels in red map the presence of clostridium spectra, illustrating the bacteria's presence and location in the tissue.



Figure 1. Clostridium

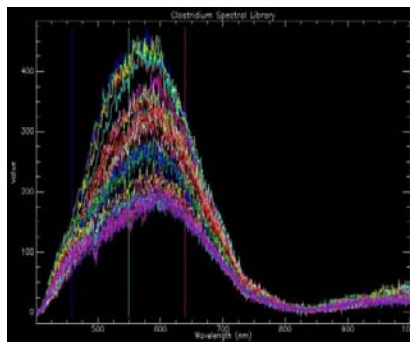


Figure 2. Clostridium Spectral Library



Figure 3. Plant Tissue with Mapping of Clostridium

One important advantage illustrated from this approach is the ability to detect intracellular spectral differences and thus chemical information at a sub-cellular spatial scales (e.g., sub-micrometers), as shown in the single cell image (**Figure 1**).

Another advantage of this broadband spectral analysis method occurring over the soft UV/VIS range is its flexibility for many applications. For example, it supports both non-fluorescent and fluorescently labeled components in live cells and other plant materials. It will also be perfectly suitable to obtain a much more comprehensive picture of the chemistry and microbiology of a microbe/lignocellulose system by combining detailed chemical-molecular information from the Synchrotron Infrared Fourier Transform (SIR-FT) spectromicroscopy. This can include information regarding the presence and fate of different components of lignocellulose as well as the microbial cellular states from the microbe/lignocellulose interface, along with structural and chemical information from this soft-UV/VIS imaging technology.

The soft-UV/VIS spectra measure electronic excitation/electronic state in the molecules, which can change with surrounding environment. This will enable the ability to probe for additional information which may not be detectable in the mid-infrared region. In the end, the combined instrumentation allows LBNL to investigate the interactions between live bacteria and the plant materials in a three dimensional manner with nanometer resolution. This provides (in real time) the "true-colored" images carrying distinguished spectral information from sample elements such as the cell membrane and nucleus of bacteria, as well as, plant cell wall and membrane.