jbei
Joint BioEnergy Institute

# Electron Microscopic Imaging at JBEI

Bernhard Knierim, Lina Prak, Seema Singh, Danielle, Jorgens, Marcin Zemla, Kristen DeAngelis, Amitha Reddy, Jean Vandergheynst, Terry C. Hazen, Brad Holmes, Rajat Sapra, Blake Simmons, Paul Adams, Manfred Auer
Joint BioEnergy Institute, Emeryville, CA

Advanced imaging approaches at JBEI include sophisticated sample preparation such as high-pressure freezing/freeze substitution, resin-embedded transmission electron microscopy (TEM), cryo-EM and electron tomography, as well as scanning electron microscopy (SEM). In addition we have advanced optical imaging techniques including confocal fluorescence microscopy, Raman microscopy, as well as atomic force microscopy. These techniques are applied to characterize feedstock cell walls, to determine subcellular protein localization in feedstocks, to monitor at high resolution the consequences on cell wall properties of ionic liquid pretreatment of biomass, to visualize microbial communities, and to analyze in detail the macromolecular lignocellulose degradation strategies of selected candidate microbes. Specifically, we have examined the cell wall of Brachypodium, Miscanthus, Equisetum and found in 2D TEM projection views differences in density texture, most likely reflecting real differences in cell wall architecture. Second, we have characterized the effect of pretreatment on cell walls as a function of length of exposure of ionic liquid pretreatment on Switchgrass biomass using high-resolution wide-field electron microscopy. We are planning to subject specimen from selected time points to electron tomographic 3D analysis. Third, we have studied Puerto Rico rain forest and compost microbial communities and found an abundance of bacterial shapes and sizes, as well a variety of interesting extracellular features likely to be involved in lignocellulose degradation. Our images will complement phylogenetic profiling of such samples and may allow a spatial mapping of the respective position of community members and their interaction. Fourth, we have studied Sulfolobus samples incubated in the presence of a variety of different substrates. Only in the presence of cellulose did we find an organelle-like feature that appears to be a novel membrane-bound cellulose-degrading extracellular specialization. We are currently in the process of characterizing this novel feature in more detail.

U.S. DEPARTMENT OF ENERGY | Office of Science