Ultrastructure and Imaging

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Abstract

Most characterization techniques use bulk approaches to study microbes in either their planktonic or their biofilm state. Such bulk analysis methods however ignore the large heterogeneities that exist with respect to protein expression and metabolism. Visualizing the cell-to-cell differences in protein and metabolite abundance that exist in planktonic cultures as well as regional differences that can be found in biofilms require imaging approaches with adequate resolving power and spatial coverage. Various optical light and electron microscopy techniques are most frequently employed, often in a correlative manner. Samples must be faithfully preserved, and imaging often requires the use of affinity-based or genetically encoded tag-based specific labeling approaches, however label-free imaging is a promising developing field. Light and electron microscopy, particularly when well integrated, have excellent potential to allow mechanistic insight into biological processes in hydrocarbon and lipid research.

Keywords Biofilms, Electron microscopy, Heterogeneity, Imaging, Light microscopy

Most characterization techniques studying microbes in planktonic culture or in biofilms are bulk approaches resulting in measurements of an averaged characteristics or response. It is now widely accepted that the assumption of homogeneity in a cell culture is incorrect, instead that even monocultures display heterogeneities possibly stemming from the fact that different cells in solution are likely in a different stage in their life cycle and show vastly different protein expression profiles. This heterogeneity is augmented when considering microbial communities, where different cells face different micro-niche environments. Likewise, the proximity to a hydrocarbon liquid or solid surfaces is likely to affect microbial physiology and further complicates the idealized view of a unified homogenous response and/or metabolic and protein expression profile.

Imaging is one of the most promising routes to deal with such heterogeneities, as imaging in principle allows the study of individual microbes and the interaction with their respective micro-niche environment. Given that the size of the microbes being typically in its shortest dimension is less than 1 μ, only a small window of the

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electromagnetic and/or discrete subatomic particle spectrum can be utilized for imaging, and thus it is no surprise that the vast majority of contributions in this volume/chapter is based on fluorescence light microscopy and electron microscopy. Often these two approaches are employed in a correlative manner or at least consecutively, since they probe different aspects of the system, and if planned carefully with adequate controls can yield comprehensive insight into microbial function.

To be clear, in most cases, the task goes beyond the simple taking of image snapshots that may serve as eye candy for an otherwise solid scientific story. Instead, the imaging itself is the vehicle that allows to test hypotheses and to reveal mechanistic insight. Therefore, adequate experimental controls, extensive optimization of probes, labeling schemes, contrasting schemes, and sample preservation all are crucial in order to provide adequate spatiotemporal context to microbial responses and physiological properties.

Sample preparation, the often unsung hero, obviously is key for any meaningful analysis: avoiding the trash-in/trash-out trap cannot be overstated. This ranges from resolution-faithful preservation of 2D and 3D (biofilm or protein) organization, over the elimination or reduction of background noise, e.g., autofluorescence of the abiotic surface area or through minimizing out-of-focus contributions.

Furthermore, devising adequate and specific molecular targeting schemes that exploit distinctions in microbial phylogenetic identity, metabolic activity, or functional protein inventory is at the heart of label-dependent imaging, whereas label-free imaging approaches at the optical microscopy level may be affected by nuisances like high autofluorescence or spectral overlap.

Naturally, it is important to obtain statistically sufficient data, either by high-throughput imaging of individual cells or by large area/volume imaging, in order to reveal a comprehensive picture of microbial properties or response, ideally by clustering the heterogeneous observations into a small set of interpretable categories through computer-assisted image analysis. All optical microscopy imaging approaches are designed to yield information at the level of individual cells or clusters of cells in microbial communities localizing to different micro-niches or biofilm regions.

For information at the subcellular level, one typically needs to resort to the high-resolution imaging capability ensured by electron microscopy (EM). However, careful sample preparation is even more important for EM studies of hydrocarbon-rich samples in order to preserve both the hydrocarbon-rich sample portion as well as the microbial ultrastructure. Cryogenic and/or correlative sample preparation approaches offer a solution but can be more difficult to implement, with some approaches being limited to very few labs around the world, whereas other approaches can be

mastered by a wider community. For certain applications, it may be desirable to combine fluorescence and electron microscopy imaging, whereas with others, they appear somewhat mutually exclusive or somewhat difficult to combine (e.g., CARD-FISH and EM).

All in all, it seems clear that an integrated approach that combines several of these approaches is superior to conventional approaches and will yield a more complete picture of microbial physiology in the presence of hydrocarbons and lipids. Beside architectural imaging and localization imaging, one would like to have a strong footprint in label-free compositional imaging, such as FTIR, Raman, or mass spectrometry imaging, but hydrocarbons are not always very easy to detect and visualize or technical issues like autofluorescence can be problematic, and this there is clearly room for innovation and technology improvement.

In summary, some of the more frequently encountered techniques like fluorescence microscopy are widely accessible and can be readily adapted by a novice researcher, whereas others are so specialized that only few people will have access to it; however, we believe that they make a useful contribution as they demonstrate the potential that ultrastructure and imaging have in the context of hydrocarbon and lipid research.