Why do we need a Biofilm Imaging Library?
Current limitations when analyzing confocal 3D images

A. E. Parker*, H. Smith, B. Buttaro and J. Picone*
Biostatistician, Center for Biofilm Engineering
Montana State University, Bozeman, MT, USA
parker@math.montana.edu

Abstract:

Biofilms in the wild are composed of diverse microorganisms from multiple domains and are widespread in natural, human, and engineered environments. Biofilm-related problems exist across major economic and societal sectors, costing billions of dollars each year in energy losses, equipment damage, product contamination, agricultural losses, and medical infections. Understanding biofilms is crucial, particularly in how their heterogeneous structures and community organization affect their resilience, pathogenicity, nutrient access, and susceptibility to antimicrobial therapies.

Digital imaging has become an essential instrument in biofilm research because it provides 3D spatial information of living biofilms in real-time. Existing biofilm image analysis software is available: the venerable COMSTAT, Semiautomated microbial abundance and morphology (CMEIAS), Daime, Beers Analysis and the newfangled BiofilmQ. Fundamentally, these approaches seek to answer a very simple yet crucial question: Where is the biomass? The answer remains surprisingly elusive, especially when the biofilm is very sparse or very thick. The next question is: Where are the microbes in the biofilm? When the individual microbes are densely packed, these approaches, that rely on thresholding, fail spectacularly. Cell segmentation remains an active area of research, with existing approaches including BCM3D 2.0, StarDist OPP, DeepSeeded, and Cellpose–SAM.

Other more challenging research questions address higher order biofilm characteristics such as identification of microbial species, biofilm morphology and subcommunity arrangement. These require new robust image analysis methods that are easily accessible, centralized, computationally efficient, sensitive, and reproducible. The lack of such tools has hindered the meaningful and statistically robust integration of image analysis into biofilm studies.

The Biofilm Imaging Library will overcome these hurdles and lay the groundwork for an open-access, community-driven repository of standardized, high-quality biofilm images, metadata, and associated computational models, and accelerate the development of open-source easy-to-use computationally efficient tools aided by machine learning. More about this resource is available at *www.biofilmimagelibrary.org*.

Biography:

Dr. Al Parker has two decades of experience developing statistical learning methods, designing studies to efficiently collect microbial data, and statistically analyzing the resulting microbial and medical outcomes. As consulting statistician to US Environmental Protection Agency's Microbiology Laboratory Branch, the American Society for Testing and Materials International (ASTM) and the Association of Official Analytical Chemists International (AOAC), he has developed, assessed, and modified multiple standard methods that advance the regulatory science of microbial contamination and biofilms. Most recently he has been developing tools for the analysis of simultaneous field-of-view, multi-channel, multi-photon 3D confocal microscope movies of biofilms.