**Title:** Correlative Fluorescence and Cryo-Electron Microscopy for Biofilm Matrix Ultrastructure Analysis

**Abstract:**
Staphylococcus aureus biofilms pose a significant challenge in clinical settings due to their resilience against antimicrobial treatments. Understanding the ultrastructure of the biofilm extracellular matrix (ECM) is crucial for developing targeted interventions. However, the ECM’s hydration-sensitive nature and structural complexity make high-resolution imaging particularly challenging.

This study explores a correlative fluorescence and cryo-electron microscopy (cryo-EM) approach to visualize biofilms in near-native conditions. Biofilms are grown directly on EM grids and vitrified using the ‘waffle’ method to preserve ultrastructure. Fluorescent markers targeting key ECM components—extracellular DNA, polysaccharides, and amyloid-like proteins—are evaluated for cryo-compatibility. We integrate two complementary cryo-EM workflows: volume cryo-focused ion beam scanning electron microscopy (cryo-FIB-SEM) in a slice-and-view mode to assess overall architecture and cryo-lamella preparation for high-resolution cryo-electron tomography.

Preliminary results demonstrate the successful retention of fluorescence signals in vitrified biofilms, enabling targeted high-resolution imaging. Future work will refine labeling strategies, optimize correlative workflows, and apply this approach to study biofilm adaptation under stress conditions. By bridging fluorescence-based biofilm characterization with high-resolution cryo-EM, this study advances structural insights into biofilm ECM organization and function.