

ACS Infectious Diseases

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Dear Editor,

I am writing to submit our manuscript entitled “**Fluorescence lifetime imaging detects reduced pyocyanin at the surface of *Pseudomonas aeruginosa* biofilms and in cross-feeding conditions.”** Using a combination of fluorescence lifetime imaging and hyperspectral microscopy, we investigated the interplay between bacterial metabolites and environmental factors within *Pseudomonas aeruginosa* biofilms. We found that the fluorescent lifetime signal of biofilms is correlated with reduced pyocyanin, an alternative electron acceptor that helps *P. aeruginosa* persist in low-oxygen environments and is also indicative of infection-state.

The state-of-the-art optical approach used to visualize shifts in bacterial metabolism within biofilms contributes to our understanding of bacterial physiology in naturally-forming oxygen gradients, with implications for the development of therapeutic strategies aimed at addressing biofilm-associated infections. We believe that the interdisciplinary nature of our study, its focus on infection-relevant conditions, and the correlation of imaging data with clinically relevant biomarkers align well with the objectives and standards of ACS Infectious Diseases.

***Short Summary (150 words):***

In order to improve treatment of infections, bacterial physiology in real-world conditions needs to be better understood. Infections are often characterized by chemical gradients, which bacteria must be able to survive to colonize an infection-site. By integrating two advanced, non-invasive microscopy techniques (fluorescence lifetime imaging and hyperspectral imaging), the interplay between metabolites and environmental factors was investigated. Imaging approaches were used to detect and spatially resolve pyocyanin, a metabolite produced by the opportunistic pathogen, *Pseudomonas aeruginosa*. Reduced pyocyanin was prominent at the surface ofbiofilms, where bacterial growth was dense and oxygen levels limited. Pycoyanin was also detected in the presence of fermentation metabolites produced by a co-colonizing microbe. As pyocyanin is linked to *P. aeruginosa* infection progression and survival in low-oxygen, this study highlights the utilization of non-invasive fluorescence imaging approaches in driving forward our understanding of biofilms in relevant conditions.

Sincerely,

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