The two-photon fluorescence emission spectra of *P. aeruginosa* fluorophores were characterized with a hyperspectral imaging microscope (excitation = 740 nm, emission window = 400-690 nm) (Fig. S1

The fluorescence lifetime of reduced pyocyanin shifted depending on local conditions. Specifically, pyocyanin’s phasor position shifted left with increasing concentrations of TCEP (Fig. S2A).

The FLIM phasor position of pyocyanin was nearly on the universal circle when pyocyanin was mixed with a 1:1 ratio of TCEP (Fig. 2, S2B).

The spectra suggest the chemically and electrochemically reduced pyocyanin solutions consist of multiple fluorescent subspecies (Fig. S3).

Specifically, the florescent spectra shifted towards longer wavelengths with higher concentrations of reducing agent (Fig. S2).

The long lifetime signal has only been identified in *P. aeruginosa* cultures and not in other microbial cultures (Fig. S4)

The measured total fluorescence intensity was similar throughout the biofilm depths, suggesting effective excitation delivery (**Fig. S5**).

The phasor position of cultures was distinct from uninoculated media (Fig. S6),

When imaged with an air objective, the lifetime signal of the biofilm with a coverslip was closer to reduced pyocyanin than that of the sample without a cover slip (**Fig. S7**),