Deconstructing the factors that modulate the mobility of molecules in microcolonies of *Pseudomonas aeruginosa* biofilms using SPIM-FCS

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Pseudomonas aeruginosa can shift between two different life styles, the unicellular planktonic form, and the multicellular, matrix encased form called the biofilm. These matrix substances surrounding the microcolony alter the mobility of molecules in and around the microcolony. *P. aeruginosa* biofilm development has been previously categorized into three different developmental stages, the attachment of the bacteria to the substrate, maturation leading to formation of microcolonies and finally dispersal leading to the release of freely swimming planktonic bacteria.

The axial sectioning provided by Single Plane Illumination Microscopy (SPIM) facilitates imaging at different depths of a single microcolony. Imaging in SPIM is carried out using water immersion objectives and hence *P. aeruginosa* is grown using flow-chamber in tubes made of a specialized polymer called fluorinated ethylene polymer (FEP) whose refractive index matches that of water. Fluorescence Correlation Spectroscopy (FCS) is a technique that quantifies the diffusion coefficient of molecules. Combining SPIM with FCS allows one to quantitate the mobility of molecules at µm resolution enabling visualization of the diffusion profile of an entire microcolony in 3D. Here the SPIM-FCS measurements were performed by coupling an EMCCD camera to a commercially available light sheet microscope (Zeiss Lightsheet Z.1).

Using light sheet and confocal FCS, we have probed the roles of size of individual microcolonies and charge of the molecule in determining the diffusion properties in live *P. aeruginosa* colonies in comparison to bacteria-free microcolony prepared from polymerizing one of the matrix ingredient - alginate. Our results suggest that the diffusion coefficient of fluorescent probes decreased with increasing size of individual microcolonies. Positively charged molecules localized into the biofilm and negatively charged molecules formed a ring around the microcolony. We next probed the changes in diffusion properties at two different developmental stages. Phosphodiesterases that cleave cyclic di GMP have been shown to promote dispersal of biofilms. Hence using a bacterial strain with a phosphodiesterase gene under the control of arabinose induction, we have monitored the real-time changes in diffusion properties at contiguous locations of a single microcolony before and after dispersal. Taken together, our results can help to delineate the roles of various physicochemical factors in altering the mobility of molecules in and around a biofilm microcolony.