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"Single cell encapsulation, detection, and sorting of pseudomonas syringae using drop-based microfluidics"

Abstract:
Bacteria can survive antibiotic or bactericidal treatment through genetic mutations. Even within bacterial populations that are fully susceptible to treatment, a small proportion of cells can have enhanced survival capacity in a phenomenon called persistence. Traditional microbiology methods can fail to identify or isolate these persister cells present within the population. A novel method for high-throughput single cell analyses of microbial populations is that of drop-based microfluidics, in which individual cells can be isolated within picoliter-sized drops. In this work, fluorescent detection and dielectrophoresis-based sorting of drops was developed for isolating Pseudomonas syringae persister cells following antimicrobial treatment. We demonstrate: (1) the dielectrophoresis-based sorting of dye-filled 25 µm drops based upon two colors, (2) differences between laser-induced fluorescent detection of dyes compared to single bacterial cells, (3) single-cell isolation of P. syringae into 25 µm droplets with ~10% of droplets containing single-cells, and (4) the treatment, staining, and fluorescent characterization of P. syringae at 0.5´, 5´, and 50´ the minimum inhibitory concentration of carbonyl cyanide m-chlorophenyl hydrazone (CCCP), an antibiotic which resulted in 6.2%, 10.2%, and 88.6% cell death of the population, respectively. These results provide the groundwork for studying antibiotic-treated P. syringae and the isolation of surviving cells that will lend insight into the molecular basis of persistence for preventing recurrent infections and decreasing the likelihood of antibiotic resistance.

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