PhD Thesis Defense
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Roberts Hall 301
"A parallelized, ultrahigh-throughput droplet microfluidic platform for singe-cell antimicrobial susceptibility testing and heteroresistance detection"

ABSTRACT

Antimicrobial resistance (AMR) is an immediate threat to global health, as the spread of resistant strains of pathogens has reduced the efficacy of many classical antimicrobial therapy routes. Antimicrobial susceptibility testing (AST) is a critical phase in clinical infection therapy, where the susceptibility profile of causative organisms is deduced empirically, and used to guide antimicrobial therapy. Standard AST techniques such as broth microdilution are population-level analyses, in which an entire culture of bacteria is analyzed. This results in long assay times on the order of 18-72 hours, as well as poor ability to detect resistant subpopulations within an infection, both of which phenomena can have negative effects on patient outcomes. In response, AST techniques based on single cell analysis have demonstrated promise in advancing AST capabilities. Single cell ASTs allow for sensitive detection of early cell divisions, possibly expediting AST results, as well as description of cell-cell heterogeneity.

Recently, drop-based microfluidics (DBMF) has emerged as a powerful tool for characterizing bacterial response to antibiotics with single-cell resolution. Despite this, DBMF has not seen wide-scale implementation toward AST and AMR-related problems. The presented research details two separate but related projects which aimed to increase DBMF capabilities for AST. In the First project, we detail the development of a plate-interfacing parallel encapsulation (PIPE) chip, which rapidly emulsifies the contents of a microtiter plate into a multiplexed, barcoded droplet library. The PIPE chip provides advancements in conducting multiplexed experiments in droplets in parallel, an outstanding issue within the field. In the second project, we leverage the PIPE chip platform to conduct rapid droplet AST on single cells against a panel of antimicrobials, in parallel. The technique outputs quantitative susceptibility results by assaying ~150,000 single-cell antimicrobial responses, within a single working day. These results demonstrate advancements in developing single-cell screening technologies, and further the understanding of antimicrobial growth responses by bacteria.