Abstract 01.08.2021

Droplet microfluidics disrupted analytical biology by introducing droplet digital polymerase chain reaction and droplet-assisted single-cell sequencing. The same highly parallel and high-throughput techniques for droplet generation, manipulation, and detection brought similar essential innovations in the analysis of bacteria and created new promising diagnostics approaches. The stochastic confinement of single cells into droplets allows for the faster accumulation and detection of metabolic products and secreted molecules compared to bulk cultures. Droplet microfluidics brings innovation, particularly in bacteria identification, precise quantification, antibiotic susceptibility testing (AST), and analysis of phenotypic and genotypic heterogeneity in the response of bacterial populations to antibiotics. These methods help to combat and more comprehensively understand antibiotic resistance and heteroresistance of bacterial populations. The conventional phenotypic AST methods (bacteria growth detection in the presence of antibiotic) like the broth microdilution method and E-test are based on a minimum inhibitory concentration (MIC) measurement. They are convenient and relatively easy approaches, but also time demanding and have a too low resolution to assess population heteroresistance.

The dissertation presents results of the improvement and development of microfluidic methods to analyze bacteria and their antibiotic susceptibility patterns, i.e., quantification, identification, growth detection, and susceptibility testing, including analysis of bacterial population towards heteroresistance and quantification of the subpopulation with reduced susceptibility to antibiotics.

The dissertation is composed of four Chapters. Chapter 1 describes the essential methods for the characterization of bacteria, introduces the concept of antibiotic resistance and heteroresistance of bacteria, reviews the standard and microfluidic methods for bacteria counting, identification, antimicrobial susceptibility testing, and detection of heteroresistance. Chapter 2 describes used materials and methods, while Chapter 3 presents results and discussion of the following research projects:

I. Droplet digital CFU (ddCFU) assay for precise quantification of bacteria over a broad dynamic range- standard droplet digital assay requires a large number of compartments for precise bacteria quantification over a broad dynamic range. We developed an optimized approach with a drastically reduced number of droplets. The technology is at par with gold standard plate count, has simplified experimental setup, and reduced manual workload.

II. Species-specific, accurate, and precise quantification and identification of bacteria in mixed samples- simultaneous differentiation and quantification of bacteria is commonly performed by real-time polymerase chain reaction, which requires laborious calibration, and the method is affected by errors associated with extraction and purification of DNA. We developed a direct droplet digital PCR assay (dddPCR) to identify and quantify bacteria in a bacterial mixture where purification of genetic material and calibration curves is eliminated.

III. High-throughput label-free readout of bacteria density in nanoliter droplets- the most general approach of bacteria growth detection in droplets is based on the measurement of fluorescence intensity. The lack of a high-throughput label-free method prohibits analysis of the most interesting strains and widespread use of droplet technologies in microbiology. To resolve that complication, we devised methods based on the measurement of scattered or native fluorescence light of unlabeled Gram-negative and Gram-positive species with a screening frequency of 1200 droplets/s.

IV. Droplet-based assay for high-resolution analysis of complex bacterial population- standard antibiotic susceptibility testing does not inform about the diversity of the bacterial population in response to antibiotics at the single-cell level. Besides, its low resolution constricts the quantification of low-abundant subpopulations with increased antibiotic susceptibility. We developed a method based on the determination of single-cell minimum inhibitory concentration (scMIC) to characterize and quantify complex bacterial populations, including heteroresistance subpopulations. The measurement of bacteria growth in droplets was conducted using our novel dual label-free detection of scattered and autofluorescence light.

Chapter 4 summarizes the results and provides limitations and an outlook for possible future modifications to improve demonstrated assays and systems.