PhD Thesis Defense
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Via Zoom
"Development of droplet microfluidic technologies for negative-strand RNA viruses"

ABSTRACT

Viruses infect all organisms and play a key role in host population dynamics and evolution. RNA viruses evolve extremely rapidly, infect a wide range of host cell types, and cause severe diseases. The incredible diversity of RNA virus populations, combined with the heterogeneity of host cells they infect, makes studying RNA virus infection quite challenging. Averaging outcomes across RNA virus infection in bulk often misses minor variants and phenotypes that can drive overall population dynamics. Therefore, it is increasingly important to study RNA virus infections at the single-cell level. Droplet microfluidics is a powerful tool for single-cell analysis. By physically isolating individual cells within microfluidic droplets, and manipulating those droplets to conduct various biological assays, we can study RNA virus infection one cell at a time. In this work I describe the establishment of three new droplet microfluidic methods for studying clinically relevant negative-strand RNA viruses, influenza A virus (IAV) and measles virus (MV), at the single-cell level. The first method involves culturing IAV from single infected cells encapsulated in microfluidic droplets. We show that cell viability, genome replication, and viral infectivity in droplets is comparable to bulk infection conditions. The second method builds on this model system by developing a way to directly quantify the number of viral particles produced by each cell. We find that IAV production varies widely between individual cells and across different IAV strains. Together, these platforms provide a high-throughput solution for studying IAV infection at the single-cell level that can be used to explore the effects of antiviral interventions on IAV replication. The third method focuses on viral genome diversity at the single-cell level. We developed a single-cell sequencing approach to study variation in the MV P gene, which contains attenuation markers important for MV function as a cancer immunotherapy treatment. Preliminary data shows the development of custom single-cell sequencing beads to capture the MV P gene attenuation site and generation of Illumina sequencing libraries. Further optimization is required to improve sequencing yield for diversity analysis. Overall, this work introduces novel methodologies for high-throughput single-cell analysis of negative-strand RNA virus infection dynamics and population diversity.