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DEVELOPMENT OF DROP-BASED MICROFLUIDIC METHODS FOR HIGH-THROUGHPUT BIOLOGICAL ASSAYS

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ABSTRACT

Drop-based microfluidics enables single cell resolution in biological assays by encapsulating individual cells in picoliter scale drops. Adapting biological assays to drop-based microfluidics requires novel approaches to meet the method requirements of each assay. For example, microtiter plates are a common tool for storing many unique samples in some assays. An equivalent strategy for drops involves labeling samples with a barcode prior to drop encapsulation and storing the barcoded drops in a single mixture, thereby creating a drop library. Other assay adaptions, such as drop-based reverse transcription quantitative polymerase chain reaction (RT-qPCR) require that drops be stabilized during the high temperatures used for thermal cycling. Drop-based RT-qPCR is useful for studying single-cell dynamics in drops, such as influenza A virus (IAV) infection. Conventional methods for measuring IAV output from individual cells are labor intensive and low-throughput. Thus, there is a need to adapt RT-qPCR to drop-based microfluidics for the purpose of high-throughput single cell analysis of infected cells.

The research presented here focuses on the characterization of the Pressure Cooker Chip (PCC) to rapidly encapsulate drop libraries and the development of a drop-based RT-qPCR method to measure IAV output from infected cells. The PCC was used to make drop libraries by rapidly generating drops of up to 96 different conditions in parallel by interfacing individual drop makers with a standard microtiter well plate. The drop library was optically barcoded using a two-color combination of fluorescent microbeads or quantum dots with 24 or 192 unique combinations, respectively. To adapt RT-qPCR in drops, known PCR additives were systematically tested to optimize drop stability and limit dye diffusion during thermocycling. A novel qPCR data analysis method was developed to convert drop fluorescence data collected at a single thermocycle to an initial RNA template concentration. Together, the additive screening and novel qPCR data analsyis method enabled the use of drop-based RT-qPCR to quantify the highly heterogeneous IAV burst size from single cells in thousands of drops. Our method is the first to measure single cell IAV burst size using a high-throughput, drop-based RT-qPCR assay.

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