EMBRACING CHAOS – A SIMPLIFIED PLATFORM FOR MULTIPLEXING DIGITAL ASSAYS IN POLYDISPERSE DROPLETS

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ABSTRACT

Combinatorial experiments in microfluidics typically require complex fluidic handling systems that are too expensive for many of their desired use-cases. To overcome these limitations and improve on previous efforts, we have developed a simplified platform for performing multi-step, sample-to-answer digital assays that enables control of reagent conditions at the droplet level. We have used this approach to demonstrate a sample-to-result assay that combines pathogen lysis and duplexed ddPCR within a droplet. We have also developed a simplified reader with comparable results to commercial systems such as BioRad's ddPCR machine.

KEYWORDS: digital assays, multiplexing, portable reader, sample-to-result

INTRODUCTION

Droplet-based digital assays partition bulk reaction volumes into pL-sized droplets containing zero or one target, resulting in absolute quantification without a standard curve [1,2]. In recent years, commercial digital assay systems have become available, but the equipment is often expensive, cumbersome, and challenging to operate [3]. Additionally, these systems suffer from the same constraints as traditional microfluidics, namely limited capabilities for multiplexing and performing multiple assay conditions simultaneously. This lack of combinatorial experiments arises because traditional digital assays mix all reagents prior to droplet formation, therefore the contents of every droplet is the same. Ideally, a more powerful solution would enable controlled delivery of reagents at the individual droplet level, enabling dozens of conditions to be tested in a single droplet reaction.

EXPERIMENTAL

We have developed a multiplexing approach utilizing polydisperse digital assays combined with novel controlled-release particles that dramatically simplifies the microfluidic requirements of these assays. We utilize specially designed controlled-release particles containing reaction-specific reagents and a simple shaken emulsion preparation that results in droplets with volumes ranging over multiple logs. Distributing our controlled-release particles within the polydisperse droplets results in multiple logs of dynamic range for reagent concentrations within a single assay – which would take dozens or hundreds of assays using traditional methods.

RESULTS AND DISCUSSION

We have demonstrated multiple assay types using this platform including a one-pot digital droplet immunoassay (ddIA) using proximity ligation to remove the need for wash steps, **Figure 1A/B** [4]. We have also developed an octaplexed ddPCR and a one-pot assay that combines pathogen lysis and duplexed ddPCR within a droplet using controlled-release particles containing lytic reagents.

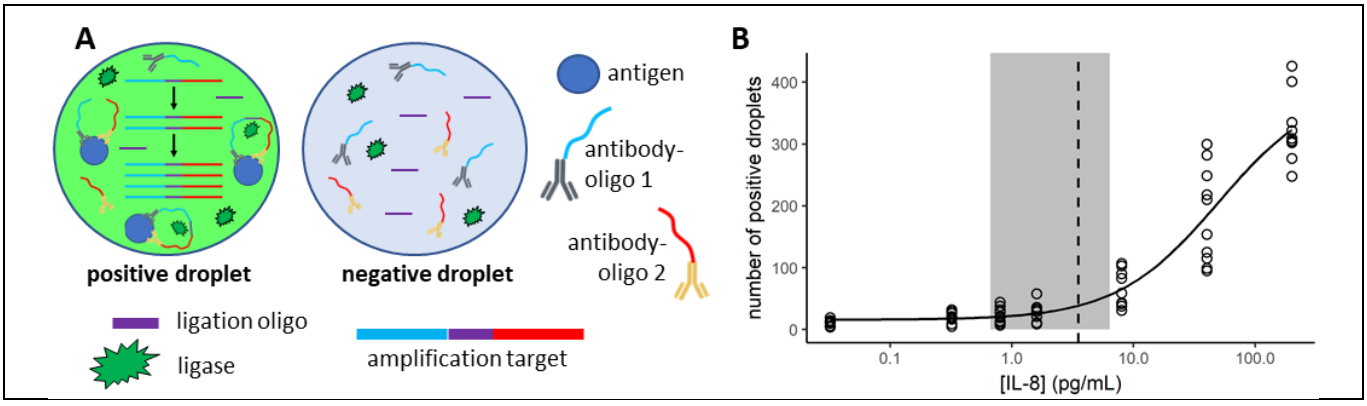
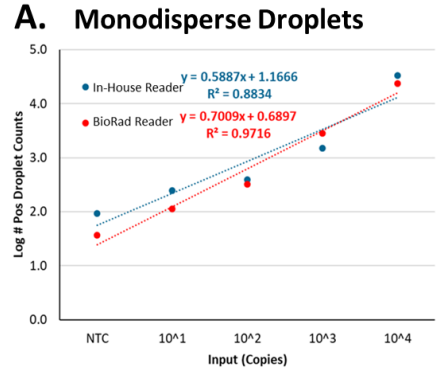
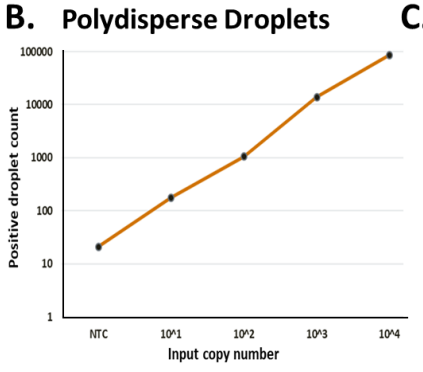


Figure 1. Summary of the ddIA. **A.** Reaction schematic of the wash-free, single-step ddIA. **B.** Assay LoD was determined to be 3.53 pg/mL [95% CI: 0.67 – 6.40 pg/mL] (0.424 pM [95% CI: 0.080 – 0.767 pM]; $1.27x10^6$ antigens [95% CI: $2.39x10^5$ – $2.30x10^6$ antigens]).

We pair these assays with the development of a simplified reader with comparable results to commercial systems such as BioRad's ddPCR machine, **Figure 2A**. Our reader has many advantages over currently available commercial systems including the ability to accurately quantify positive counts from a population of polydisperse droplets, **Figure 2B**, to process a larger range of sample volumes therefore improving the dynamic range, **Figure 2C**, to quantify targets from hexaplexed reactions, **Figure 2D**, and requiring significantly less equipment and time to execute. We have also developed a statistical framework to apply to reactions performed in polydisperse droplets to overcome any bias as a result of the polydisperse droplet population.





C. Comparing BioRad and In-House

System	In-House	BioRad
Channels	Six	Two
Volume Limitation per Sample	µL – mL per sample	25 µL per sample
Max Read Speed (Droplets/s)	~7000	~1500
Droplet Diameter Limitation (µm)	>1 to 1000s	100 ± 20

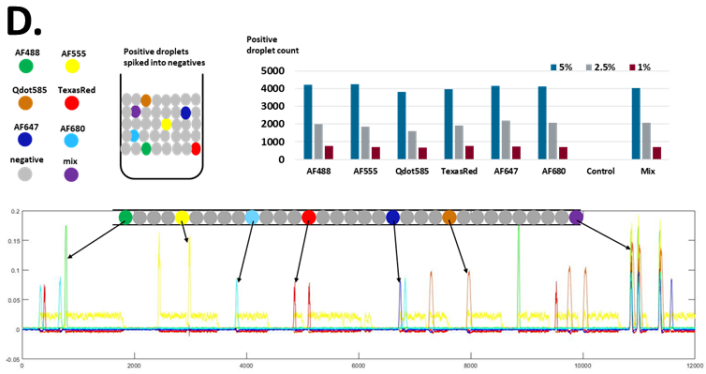


Figure 2. Development of an improved droplet reader. **A**. Our in-house digital droplet reader performs as well as BioRad's commercial ddPCR reader. **B.** Our reader is also able to accurately quantify positive counts from a population of polydisperse droplets whereas BioRad's reader only works with monodisperse droplet populations. **C.** Additionally, our reader can process larger volumes than commercial systems, therefore improving the dynamic range. **D.** Example data from our reader quantifying a hexaplexed reaction.

CONCLUSION

The combination of rapid droplet preparation, the statistical framework, the increased ability to multiplex, and our simplified reader results in significantly reduced complexity for an end user which can lead to faster adoption of digital assay technology in a variety of settings.

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