DUAL FORCE AGGREGATION OF MAGNETIC PARTICLES FOR LABEL-FREE DETECTION AND QUANTIFICATION OF DNA THROUGH IMAGE ANALYSIS

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ABSTRACT

Detection and quantification of analytes in biological specimens is integral to biomedicine, clinical diagnostics and forensic science. Previously, we described a label-free method for detecting the presence of DNA through magnetic particle aggregation. A stir plate-like device was used to generate a rotating magnetic field (RMF) for particle aggregation; however, the magnetic field was only effective for a single well at a time. Here, we present an improved method sufficient for multiplexing the assay, using a dual force approach to enhance the aggregation effect.

KEYWORDS: Label-free DNA detection, Magnetic particle aggregation

INTRODUCTION

We have developed two related label-free DNA detection modalities through bead aggregation, and these have been firmly established at previous uTAS conferences[1,2] and in a recent seminal paper [3]. Non-specific DNA sequences can be detected through chaotrope-driven aggregation (CDA), while specific DNA targets bind to oligonucleotides on the surface on the beads causing hybridization-induced aggregation (HIA). To fully implement CDA and HIA as an alternative to expensive DNA quantification assays, e.g., qPCR, they require increased throughput capabilities to allow for on-chip calibration sample testing. Ultimately, CDA and HIA provide an opportunity for faster pre-quantification and end-point detection, respectively, in a total analysis system.

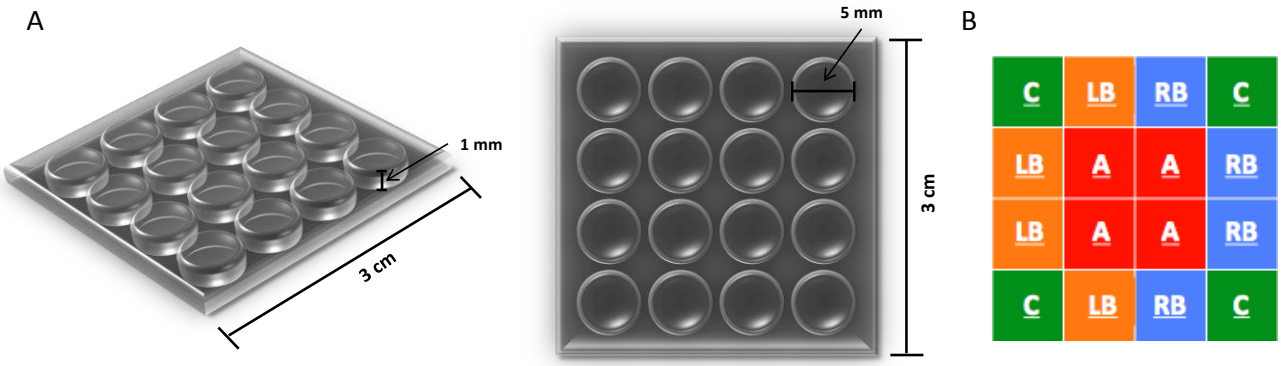

Figure 1: *Schematic of the microdevice used for bead aggregation. A) Each device is fabricated from poly(methyl methacrylate) (PMMA) and contains 16 wells that are 5 mm in diameter and 1 mm deep. Wells are vector cut in PMMA using a high powered CO_2 laser and thermally bonded to a second piece of PMMA. B) Zones of the microdevice. Defined regions on device under RMF. Each region grouped based on distance from center of device to center of each well.*

THEORY

Magnetic fields are parabolic, thus, lack homogeneity, compromising the ability to effectively quantitate DNA in multiple samples simultaneously. To compensate for the variation in the magnetic field across the microdevice, 'zones' of varying magnetic strength were established based on their relative distance from the center of the device (**Fig. 1B**).

However, RMF alone causes the particles to move to the side of the microwell, yielding irreproducible results (**Fig. 2A**). To distribute the particles homogeneously throughout the microwell (interacting with the sample), an additional force, provided by a vortexer, is required. The vortexer alone failed to produce visible aggregates, demonstrating the need for a magnetic field for effective binding of the DNA to the particle surface (**Fig. 2B**).

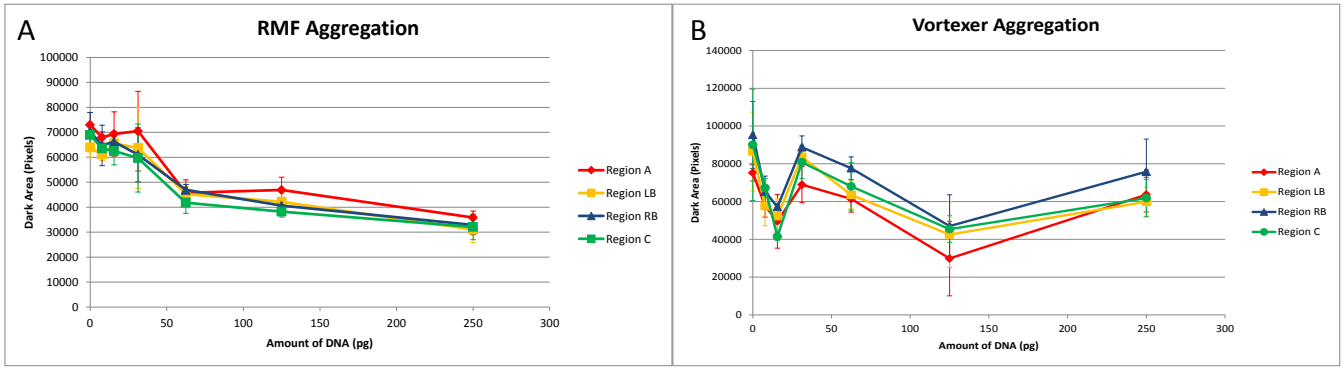

Figure 2: *Serial dilution curves generated using a single force. A) RMF alone - Slight trend observed, however reproducibility at lower concentrations limits its use. [Red – Region A, Orange/Blue – Region B, Green – Region C] B) Vortexer alone - No statistical relevance between the each concentration point, and exponential decay not seen compared to DFA system. (Same color legend)*

With a combination of a RMF and agitation, for the first time we show that dual force aggregation (DFA) allowed for CDA and HIA to be multiplexed (**Fig. 3**); here we show results with a 16-plex assay.

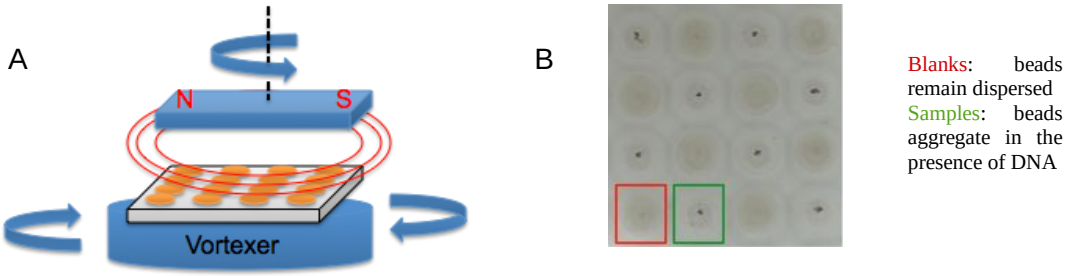

Figure 3: *The dual force aggregation system for DNA detection. A) Schematic showing the dual force set-up, with the RMF above the microchip and a vortexer below. B) Image of 16-well microchip after an assay on the DFA system.*

EXPERIMENTAL

Each assay is performed in 140 seconds in a 5 mm poly(methyl methacrylate) PMMA microwell device (**Fig. 1A**), fabricated through laser ablation and thermal bonding. The device is contained within the vortexer, with the RMF operating from above at 2500 rpm. The aggregates are photographed from above for image processing.

RESULTS AND DISCUSSION

Initially, DFA was applied to the quantification of pre-purified λ-phage DNA using CDA for generic DNA quantification. In addition to allowing for multiplexing, DFA allowed for a 2-fold reduction in time-to-aggregate and an order of magnitude increase in sensitivity over the magnetic field-only method (**Fig. 4A**).

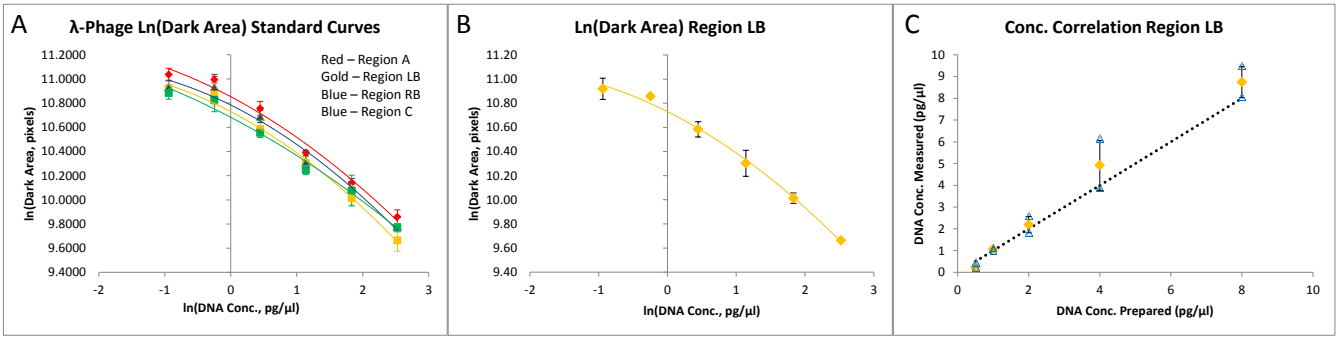

Figure 4: *Quantifying λ-phage DNA. A) Natural log standard curves were used to accurately predict concentrations of λ-phage DNA. B) Individual standard curve for region LB (from A). C) Correlation between prepared values and measured values of a second set of standards using the curve seen in B. Blue points represent individual assays, gold represent the average.*

Moreover, the limit of detection (LOD) was as low as 350 fg/µL, an order of magnitude improvement over fluor-based quantification. Using 'separate zone calibration', CDA functions over a dynamic range of 350 fg/µL – 12.5 pg/µL (**Fig. 4B**). Preliminary data on a second set of standard samples (500 fg/µL–8 pg/µL) shows concentrations successfully measured to within 20% of their actual values, with three of the five points within 10% (**Fig. 4C**), all in ~2 minutes, with only 0.05 USD in reagents. Further studies look to improve correlation to within 5%.

Application of DFA to the sequence-specific method, HIA allowed for the detection of as few as one, and as many as three, single base substitutions (SBS's) in target DNA (**Fig. 5**), demonstrating that HIA [3] has the potential to detect strands differing by a single base. The lack of completely complimentary binding is seen as a 20% decrease in % Maximum Aggregation for a SBS. This suggests that DFA-HIA has the potential to be exploited for multi-array assays for the detection of single-point mutations – critical in diagnostics.

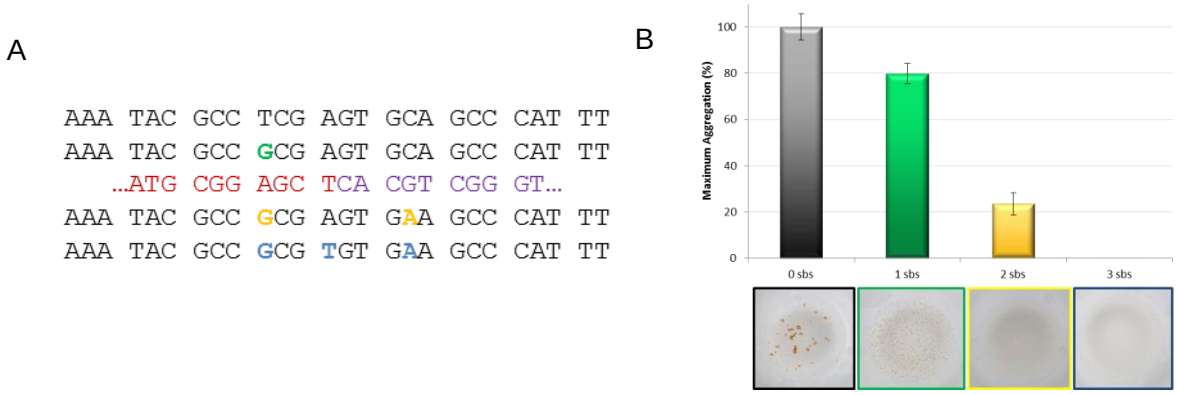

Figure 5: *Using the DFA system to detect single base changes in a given sequence. A) Sequences illustrating the insertion of a base change. Red and purple strands represent the bead-bound oligonucleotide probes, with blue nucleotides representing their complementary bases (target). B) Quantitative HIA response as an increasing number of single bases changes are introduced (n=3). Representative photographs of each aggregation are shown below.*

CONCLUSIONS

Overall, DFA presents a novel system, multiplexing CDA and HIA for the first time, while improving significantly the sensitivity and selectivity of the assays. CDA has been shown to be quantitative over a dynamic range of 350 fg/µL – 12.5 pg/µL with an LOD of 350 fg/µL using λ-phage DNA. Further, HIA has demonstrated sequence specificity with the ability to detect as few as one single base substitution in target DNA.

REFERENCES

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