

# TOWARDS A HIGH THROUGHPUT DROPLET-BASED AGGLUTINATION ASSAY

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## ABSTRACT

This work demonstrates the detection method for a high throughput droplet based agglutination assay system. Using simple hydrodynamic forces to mix and aggregate functionalized microbeads we avoid the need to use magnetic assistance or mixing structures. The concentration of our target molecules was estimated by agglutination strength, obtained through optical image analysis. Agglutination in droplets was performed with flow rates of 150  $\mu\text{l}/\text{min}$  and occurred in under a minute, with potential to perform high-throughput measurements. The lowest target concentration detected in droplet microfluidics was 0.17 nM, which is three orders of magnitude more sensitive than a conventional card based agglutination assay.

**KEYWORDS:** Agglutination, Microbeads, Microdroplets, Biological Recognition, Point-of-Care testing

## INTRODUCTION

Agglutination is widely used in medical tests, immunology diagnostics, immunoassays techniques and Point-of-Care assays. In the presence of the target molecule, functionalized particles will aggregate (clump) together, enabling the macroscopic observation of a positive result. Microbeads in size of nm and  $\mu\text{m}$  and functionalized with recognition molecules are commonly used in such tests.

Conventional agglutination assays are performed on large cards and are semi-quantitative. Moving to a microfluidic format for the assay helps to decrease the sample volume required, increasing the sensitivity and speed of the assay. Both DNA [1] and antibody-assays [2] have been previously demonstrated in plug-based microfluidics [3]. At the low droplet speed (up to 5.5  $\mu\text{l}/\text{min}$ ), the mixing of agglutination components inside the beads was increased using windy, bumpy channels [2]. Also a magnetic field has been used to enhance the aggregation of agglutinates inside slow moving droplets (speeds up to 1  $\mu\text{l}/\text{min}$ ) [3].

Kurup et al. recently demonstrated that the circulation and aggregation of non-functionalized beads in a droplet can be controlled via simple droplet velocity control [4]. Our assay presented in this work uses simple velocity changes to control the hydrodynamics of the droplet's internal circulation to both increase mixing and aggregation. Addition of surfactant to the carrier oil allowed us to perform agglutination inside droplets with flow rates of 150  $\mu\text{l}/\text{min}$  without changing the channel surface and without any magnetic manipulation. This removes the need for magnetic interaction simplifying the apparatus and allowing a 150x increase in throughput over the previous magnetically assisted method and a 30x increase over the previous winding channel method.

We also verified the presence of the "prozone effect" in droplet-based agglutination by increasing the target concentration until active site saturation occurs and the agglutination signal decreases (Fig. 3b).

## EXPERIMENTAL

### Droplet microfluidics

Final concentration of 50k/ $\mu\text{l}$  Dynabeads M-270 streptavidin beads in the 1x PBS/ 1% BSA buffer were mixed with varying final concentrations (0, 1.7 pM, 17 pM, 170 pM, 1.7 nM, 17 nM and 170 nM) of biotinylated BSA (Sigma) in the tube (Axygen). Then 1  $\mu\text{l}$  of agglutination mix was transferred to the silicone tubing submerged into cured PDMS. External pump was used to control the droplet fluidics speed. The carrier fluid was silicone oil AR20 (Sigma) with 1% (w/w) nonionic surfactant Span 80. The agglutination droplet was pushed forward at the speed of 150  $\mu\text{l}/\text{min}$  and after reaching the end of tubing microfluidics, the droplet was pushed backwards at a speed of 75  $\mu\text{l}/\text{min}$  for observation.

### Optical image analysis

Canon EOS 5D MarkII camera fitted with 1-5x macro lens was used to record the droplet video from beginning to the end of each of experiment. Images from varying target concentration videos were extracted from the same location. Optical image processing was done using a script written in the Matlab programming environment. We used a greyscale method to determine the degree of agglutination. This method is based on the distribution of the image's greyscale values (ranging from 0 for the black to 1 for white). A negative sample has a narrow distribution and positive sample – a wide distribution (*i.e.*, a higher standard deviation).

## RESULTS

We demonstrated our droplet assay with flow rates of 150  $\mu\text{l}/\text{min}$ , with potential suitability for high-throughput assays. The agglutination occurred in less than a minute in the droplets and was observed in real-time video (Fig. 1). Image analysis demonstrated the lowest biotinylated BSA target concentration of 0.17 nM detected, with the maximum agglutination strength observed at 17 nM (Fig. 2). The detection concentration is well under the range of agglutination-based commercial tests, such as for inflammation marker C-reactive protein (*i.e.* “Dutch diagnostics” test kit lower detection limit is 6 mg/l or 240 nM). The “prozone effect” could be observed in agglutination mixture containing 50k/ $\mu\text{l}$  final concentration beads with high target concentration of 170 nM (Fig. 3a and 3b). The concentration at which the “prozone effect” occurs can be adjusted by optimizing the number of microbeads present.

The agglutinated beads are widely distributed through the 1  $\mu\text{l}$  droplet. The detection limit can be further lowered down by improving the optical setup that we are using to lower the signal to noise ratio. Further droplet processing can also be added to allow sample splitting for redundant testing. In summary we demonstrated a droplet based agglutination assay with potential for high-throughput analysis.

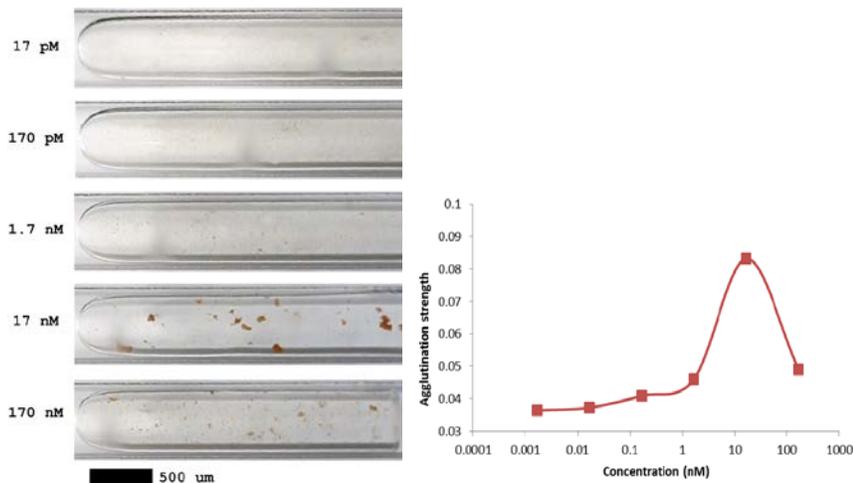


Figure 1: Image sequences of agglutination mix, using different target concentrations in droplet microfluidics.

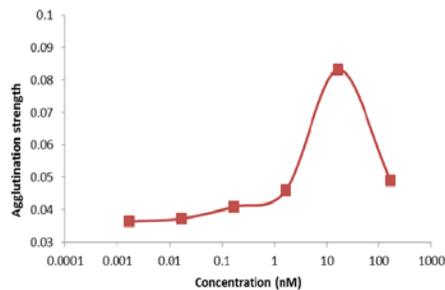


Figure 2: Evaluation of 50k bead agglutination in droplets experiment. The “prozone effect” visible with the higher than 17 nM target concentration.

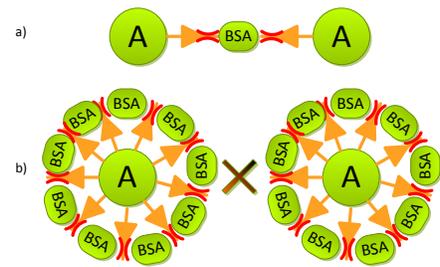


Figure 3: a) the ideal situation promoting agglutination, b) high analyte concentration causes active site saturation (“prozone effect”).

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