AGGREGATION-BASED DNA DETECTION IN MICROSEPARATOR
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ABSTRACT
We developed a novel aggregation-based method to execute oligonucleotide detection in a PFF (pinched flow fractionation) microseparator. Employing functionalized polystyrene microspheres, this method is capable of directly measuring the concentration of a specific DNA sequence. The label-free target DNA hybridizes with the probe DNA on the surface of microspheres and causes the formation of polymeric aggregate, thus enlarging the average size of the aggregated particles. Depending on the aggregated particle sizes, the microparticles were separated through a PFF microseparator, enabling subsequent concentration analysis of the target DNA with an optical microscope or even naked eyes.

KEYWORDS: aggregation-based, DNA, detection, microseparator

INTRODUCTION
Nucleic-acid analysis (e.g., DNA sequencing, detection and genetic variation, etc.) is a critical issue in the field of diagnostic medicine; methodologies based on electrochemical, magnetic [1], surface plasmon resonance (SPR), fluorescence resonance energy transfer (FRET) [2] and so forth were extensively developed. Although these methodologies possess advantages of high sensitivity and efficiency, they might require bulky or expensive apparatus [1]. In addition, sometimes a complicated sample pretreatment (e.g., labeling of target DNA) is required [2]. For practical point-of-care diagnosis, a direct, convenient and low cost method is essential and necessary.

EXPERIMENTAL
The notion and detection process of the method are illustrated with the schematic sketch shown in Fig. 1. Streptavdin-coated polystyrene microspheres (500 nm, yellow-green fluorescence) conjugate respectively with two kinds of probe DNA modified with biotin. By mixing the probe-labeled beads with the target DNA whose two ends is respectively complementary with the two DNA probes, hybridization occurs and brings the microspheres to aggregate. The binding of polystyrene microsphere with probe DNA (i.e., the conjugation of streptavdin with biotin), and the concentrations of the target DNA after hybridization was measured by UV absorption at 260 nm using a UV-Vis spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific Inc.). The aggregated particle sizes were determined by dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern Instruments Ltd.) and confocal microscope (A1R, Nikon). A PFF microseparator (20 and 1000 μm for the pinched and broadening segment widths, 25 μm for the depth) is used to analyze the size of the aggregated particles. The microstructure was fabricated in poly(dimethylsiloxane) replicas using soft lithography. The experimental images were captured and recorded by microscope and cooled CCD camera (Evolution VF Monochrome Cooled, Media Cybernetics).

RESULTS AND DISCUSSION
The optical images of the microparticles after hybridization are exhibited in Fig. 2. It is seen that the size of the aggregated microparticles varied with the concentration of the target DNA (C_{target}). The average size of the aggregated particle was enlarged from 1.43 to 8.80 μm as C_{target} increased from 0 to 10.0 nM (Table 1), meaning that the size of the aggregated

Probe DNA 1: Biotin-5’-GCGCT AGAGT CGTTT-3’
Probe DNA 2: 5’-CCTAT CGACC ATGCT-3’-Biotin
Target: 5’-AGCAT GGTCG ATAGG ...

Figure 1: Schematic diagram of the concept of aggregation-based DNA detection in a PFF microseparator.

Figure 2: Images of microparticles after hybridization with the target DNA at different concentrations.

Table 1: Average size of the aggregated particles at different target DNA concentrations.
Asymmetrical outlet design

(a) $C_{\text{target}}=0$
(b) $C_{\text{target}}=0.33$ nM
(c) $C_{\text{target}}=3.30$ nM
(d) $C_{\text{target}}=10.00$ nM

Figure 2: Microscopic image of the labeled beads (polystyrene microspheres + probe DNA) in different target-DNA concentrations ($C_{\text{target}}$). (a) 0 (without target DNA) (b) 0.33 nM (c) 3.30 nM and (d) 10.00 nM.

Table 1. The aggregated size of the microparticles in different target-DNA concentrations ($C_{\text{target}}$).

<table>
<thead>
<tr>
<th>$C_{\text{target}}$ (nM)</th>
<th>Min. Diameter (μm)</th>
<th>Max. Diameter (μm)</th>
<th>Mean Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.64</td>
<td>3.18</td>
<td>1.43</td>
</tr>
<tr>
<td>0.33</td>
<td>0.64</td>
<td>6.58</td>
<td>2.78</td>
</tr>
<tr>
<td>3.30</td>
<td>0.64</td>
<td>19.20</td>
<td>7.66</td>
</tr>
<tr>
<td>10.00</td>
<td>0.64</td>
<td>27.11</td>
<td>8.80</td>
</tr>
</tbody>
</table>

Figure 3: (a) SEM image of the PFF microseparator. And the optical microscope image of the aggregated particles with $C_{\text{target}}$ equal to (b) 0 (c) 0.33 nM (d) 3.3 nM (e) 0.33 nM and (f) 3.3 nM in microchannel. (g) The normalized fluorescence intensity distribution of different target DNA concentrations along the lateral direction of the broadening segment.

Microparticles can be indicative of the target-DNA concentration. The PFF microseparator was used to determine $C_{\text{target}}$, that is, the size of the aggregated microparticles. The size of the tested sample can be differentiated according to the lateral position of the streamline at the broadening segment (Fig. 3) [3]. The streamline pertaining to larger particles is closer to the center of the channel. The fluorescence intensity through the broadening segment of different target DNA concentrations is plotted as Fig. 3g. The size of aggregated particles can be evaluated by analyzing the average lateral position of the streamline, thus obtaining the concentration of target DNA (Fig. 4).

By using an asymmetrical outlet design [4], the particles of different sizes were eventually collected by different outlets. There is no need to label target DNA in this presented method, and the target-DNA concentration can be readily and directly
analyzed with an optical microscope or naked eyes (from the collected solution at outlet). Furthermore, this method can also be used to conduct multi-target detection on a chip.

**Figure 4:** The relation between the average lateral position of the streamline in the broadening segment and the concentration of target DNA.

**Figure 5:** The distribution of the aggregated particles in the 6-outlet asymmetrical design with $C_{\text{target}}$ equal to (a) 0 (b) 0.33 nM (c) 1.0 nM (d) 3.3 nM and (e) 10 nM.

**CONCLUSION**

This method features its simplicity, low cost and potential to detect other biochemical samples with distinct aggregation behaviors (e.g., Hg$^{2+}$, E. coli, SP-A and single nucleotide polymorphisms (SNPs) [4]). We suppose that the present method has the ability to apply in the field of disease diagnosis, home care, environmental monitoring and so on.

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**REFERENCES**


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