Supporting Information

Microparticle Parking and Isolation for Highly Sensitive MicroRNA Detection

Jae Jung Kim\textsuperscript{a†}, Lynna Chen\textsuperscript{b†}, and Patrick S. Doyle\textsuperscript{a*}

\textsuperscript{a} Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

\textsuperscript{b} Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

† These authors contributed equally to this work.

* E-mail: pdoyle@mit.edu

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**Table S1. Probe and target sequences.** Probe stocks were prepared by dissolving probe molecules in 1X TE (tris-EDTA buffer). In this study, we used previously reported probe concentrations, which were adjusted based on relative binding rates\(^1\). The concentrations of let7a, miR145, miR221 probes in the final prepolymer concentration were 50, 50, and 62 µM, respectively.

<table>
<thead>
<tr>
<th>Oligo Name</th>
<th>Type</th>
<th>Sequence</th>
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<tr>
<td>let7a probe</td>
<td>DNA</td>
<td>5Acryd/GAT ATA TTT TAA ACT ATA CAA CCT ACT ACC TCA/3InvdT</td>
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<tr>
<td>let7a target</td>
<td>miRNA</td>
<td>5’-UGA GGU AGU AGG UUG UAU AGU U-3’</td>
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<tr>
<td>miR145 probe</td>
<td>DNA</td>
<td>5Acryd/GAT ATA TTT TAA GGG ATT CCT GGG AAA ACT GGA C/3InvdT</td>
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<td>miR145 target</td>
<td>miRNA</td>
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<td>miR221 probe</td>
<td>DNA</td>
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<tr>
<td>miR221 target</td>
<td>miRNA</td>
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<tr>
<td>Universal linker</td>
<td>DNA</td>
<td>/5Phos/TAA AAT ATA TAA AAA AAA A/3Bio/</td>
</tr>
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**Fig. S1 Schematic of particle synthesis via stop-flow lithography (SFL).** We flowed prepolymer solution through the microfluidic channel. After stopping the flow, the prepolymer was exposed to mask-patterned ultraviolet light to photopolymerize microparticles in desired 2D-extruded shapes. Synthesized particles were flushed to the reservoir for collection. Flow, stop, and exposure steps were repeated to synthesize particles in a high throughput, semi-continuous manner.
Fig. S2 Parking/isolation channel design and dimensions. The parking/isolation channel used in this study has a total of 95 parking spots, 5 per row. Close-up view shows a microscope image of a representative PDMS channel. Average dimensions of channel features are indicated in microns, including widths of the trap entrance ($D_E$) and trap pore ($D_P$).
Fig. S3 Parking yield at different applied pressure. Parking yield is constant regardless of applied pressure. Parking yield is governed by channel geometry: number of traps per row, and the relative ratio of hydrodynamic resistance between the traps and the main channel. Parking yields are 96.2 ± 1.8 % at 1.0 psi, 96.7 ± 4.6 % psi, and 94.5 ± 5.3 % at 2.0 psi. We chose an applied pressure of 2 psi for our experiments to ensure the highest parking speed without significant particle deformation that can induce particle escape through the trap pore.

Fig. S4 Time-lapse images of parking of particles at different initial positions. Microparticles were successfully guided to traps regardless of their initial position: top (a), mid (b), or bottom (c) streamline. For each row, the particle of interest is marked by false color.
Fig. S5 Time-lapse of isolation using forward-flow. As FC-40 oil flows from the inlet, the oil/water interface penetrates into the traps, resulting in heterogeneous droplet sizes with severe deformation of some particles. Scale bar is 300 µm.
Fig. S6 Time-lapse of isolation using back-flow. As FC-40 oil flows from the outlet, the oil/water interface does not penetrate into the traps, resulting in homogeneous droplet sizes without any particle deformation. Scale bar is 300 µm.
Fig. S7 Empty droplet generation. Back-flow of oil ensures no trespassing of the interface on the trap. Therefore, our platform can isolate soft objects of any modulus. Even if there is no object in a trap, it is possible to generate empty, uniform-sized droplets. Scale bar is 300 µm.
Fig. S8 Signal amplification of biotinylated particles in isolated droplets. Particles synthesized with various biotinylated probe concentrations (in the prepolymer solution) are labeled with enzyme (SAB), parked, soaked with substrate (FDG), and isolated by oil. Florescent product (fluorescein) is generated and monitored over time within the isolated droplets.

Fig. S9 Calibration curves for the calculation of minimum detectable biotin probe concentration ($B_{\text{Bio}}$min) at each time point. Plots are made based on the data in figure S6. $B_{\text{Bio}}$min is defined by a net signal equal to three times the signal-to-noise ratio.
**Fig. S10** Plot of $Bio_{\text{min}}$ over time. $Bio_{\text{min}}$ reaches a constant value of 0.2 nM after the first 10 minutes of the enzyme reaction (calculated from data in Fig. S6 and S7). Although the signal is continuously amplified over time and can enable the detection of initially non-detectable signal, overall improvement in detection sensitivity is limited to a certain factor. For future optimization, it would be beneficial to find the upper limit of improvement, and minimum required process time.

**Fig. S11** Multiplex miRNA assays for three distinctive dysregulation patterns. This is an enlarged image of figure 5b. Particles for each miRNA (let7a, miR145, and miR221) have distinct 8-bit codes. – and + represent negative control and 1 amol dose, respectively. Scale bar is 50 µm.
Movie S1. Microparticle parking process.

Movie S2. Microparticle isolation process.

References