Electronic Supplementary Information

“Droplet CAR-Wash: Continuous Picoliter-Scale Immunocapture and Washing”

Steven R. Doonan, Melissa Lin, and Ryan C. Bailey

\(^1\)Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

Supplementary Video 01. CAR-Wash Operation. Input droplets (141 ± 1 pL each) electrocoalesce at 550 Hz with >98% 10 µm magnetic bead capture during droplet reformation at 560 Hz (189 ± 2 pL each).

Supplementary Video 02. CAR-Wash Frequency Modulation. Reinjection of loosely packed droplets at 100 Hz with output droplet production at 500 Hz prevents coencapsulation of beads from different input droplets during resegmentation. This frequency mismatch also increases the proportion of empty droplets formed.
Figure S1. Magnetophoresis Flow Rate Dependence. a) Micrograph of the CAR-Wash magnetophoresis region during droplet processing at ~500 Hz input and output droplet frequencies. Approximate flow rates for each species are 75 µL/min for the washing buffer, 8.5 µL/min for the oil co-flow, and 4.5 µL/min for input droplets. Magnetic particles are circled in red, and the washing buffer-oil co-flow interface is highlighted with a white line. B) Micrograph of the same CAR-Wash magnetophoresis region during droplet processing at ~200 Hz input and output droplet frequencies. Approximate flow rates for each species are 60 µL/min for the washing buffer, 4 µL/min for the oil co-flow, and 2.5 µL/min for input droplets. c) Plot of particle migration lengths under each frequency condition. We define “migration length” as the distance each particle travels down the channel length before magnetically-driven orthogonal forces pull it into first contact with the washing buffer-oil co-flow interface. Decreasing frequencies and flow rates significantly decreases resulting migration lengths. Magnetic migration velocity in the direction of the flow interface (48 ± 2 mm/s at 500 Hz and 45 ± 4 mm/s at 200 Hz) did not differ significantly between frequency conditions. Significance was assessed at 95% confidence for N = 75 particles under each condition (evaluated in N = 5 bins based on distance from the co-flow interface when evaluating magnetic migration velocity). Micrographs are aligned and in scale with the plot’s migration length axis.