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Supplementary Information

Counting of Enzymatically Amplified Affinity Reactions in Hydrogel Particle-Templated Drops

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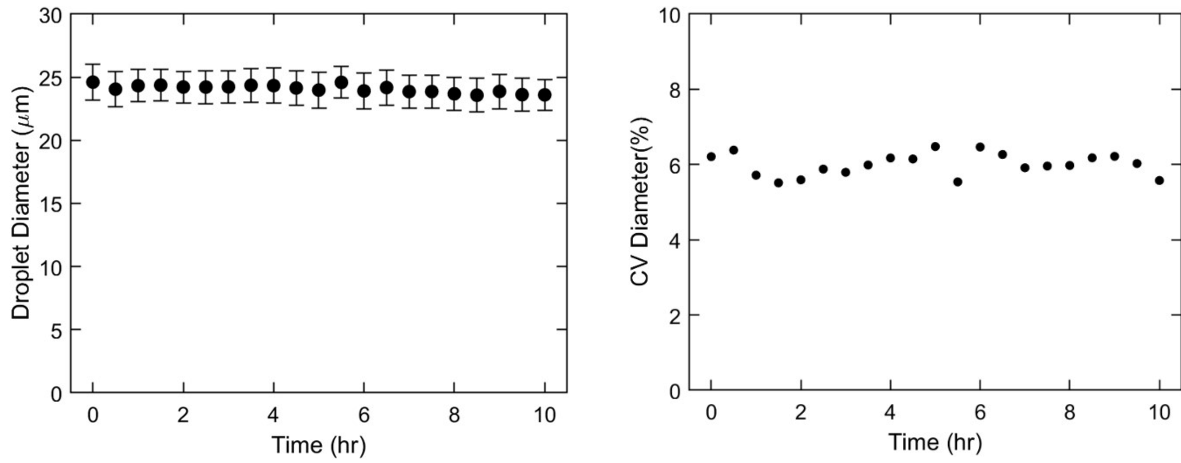

Figure. S1 The mean diameter of droplets produced over a 10-hour period using the step emulsifier microfluidic device remains consistent, with CV values around 6%. (n = 150-300 for each 30-minute time point).

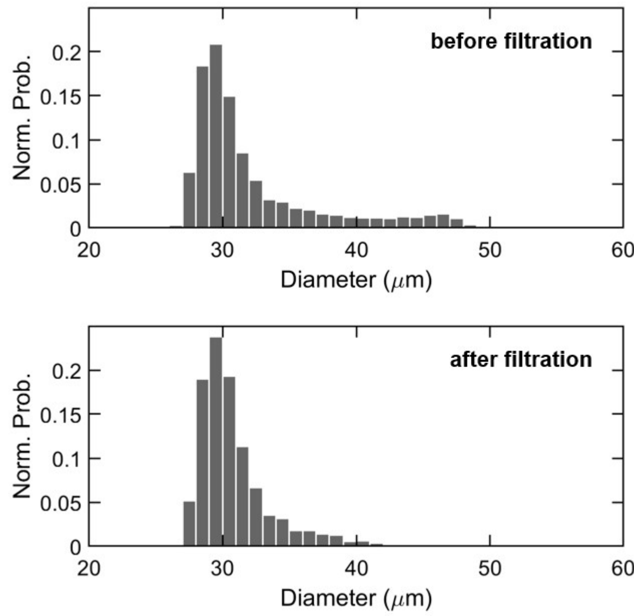

Figure. S2 Size distribution of the fabricated particles before and after being filtered by a 40 μm cell strainer to remove those with malformed shapes. About 10% of all particles were filtered out.

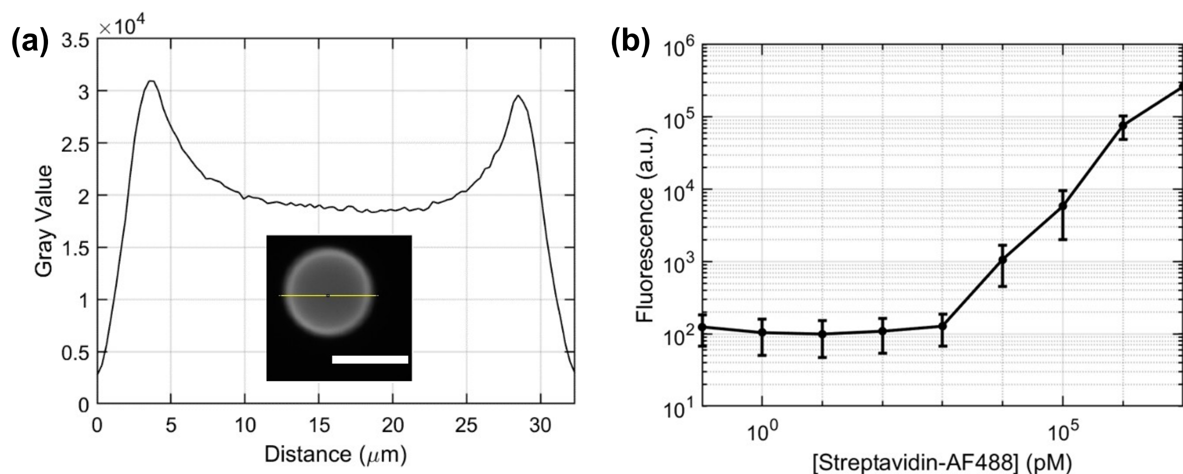

Figure. S3 Streptavidin binding to biotinylated particles. **(a)** Fluorescence signal is localized to the outer surface of the hydrogel particle, forming a bright edge on the boundary of the particle in a 1D fluorescence intensity slice. **(b)** The integrated fluorescence signal from particles with bound streptavidin-Alexa Flour 488 after incubation are linearly correlated to the concentration of the streptavidin solution across 5 orders of magnitudes. The lowest resolvable signal was around 1 nM, representing the limit of detection for an unamplified affinity assay on the particles using our microscopy setup. (n = 10,000 for each streptavidin concentration.)

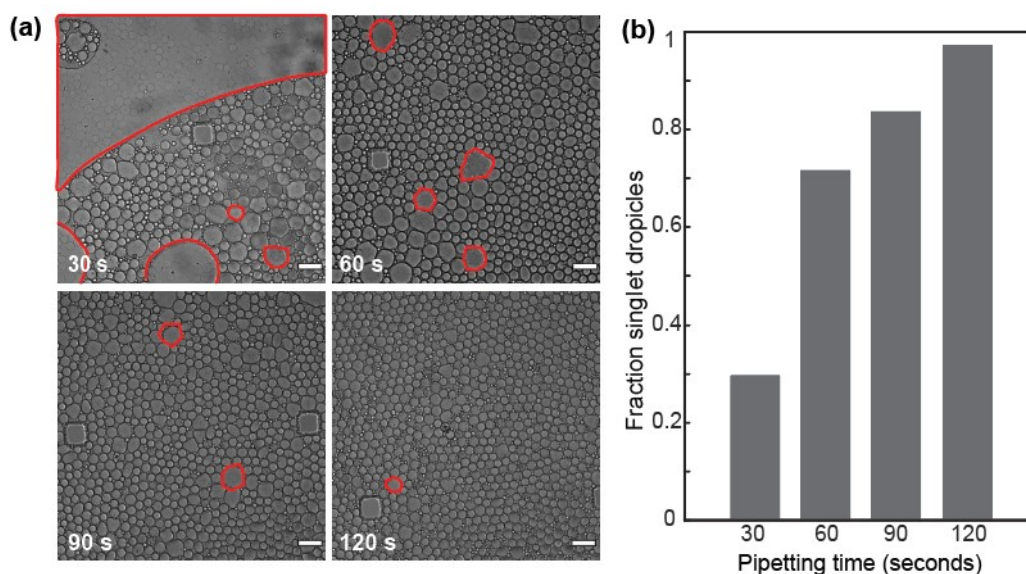

Figure. S4 Optimizing the quality of dropicle formation with increased pipetting time. **(a)** Bright field images showing the formation of dropicles at 30 second intervals of vigorous pipetting. The number of droplets containing multiple hydrogel particles (highlight by red contour) decreased with increased pipetting time. Scale bar = 100 µm. **(b)** The fraction of singlet dropicles (droplets templated by only one particle) increased with time, approaching 100% after 120 seconds of pipetting.

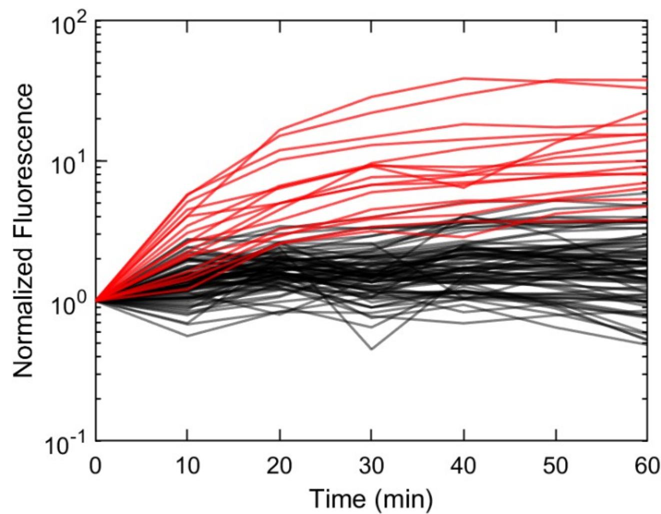

Figure. S5 Signals of dropicles loaded with the HRP/ADHP/resorufin system analyzed at 10-minute intervals indicating the enzymatic amplification completed at around 30 minutes. The fluorescence signals of each dropicle were normalized against their starting fluorescence at t=0. Red lines refer to signals from positive dropicles (containing particles bound with at least 1 HRP enzyme), black lines correspond to negative dropicles. (n = 101)

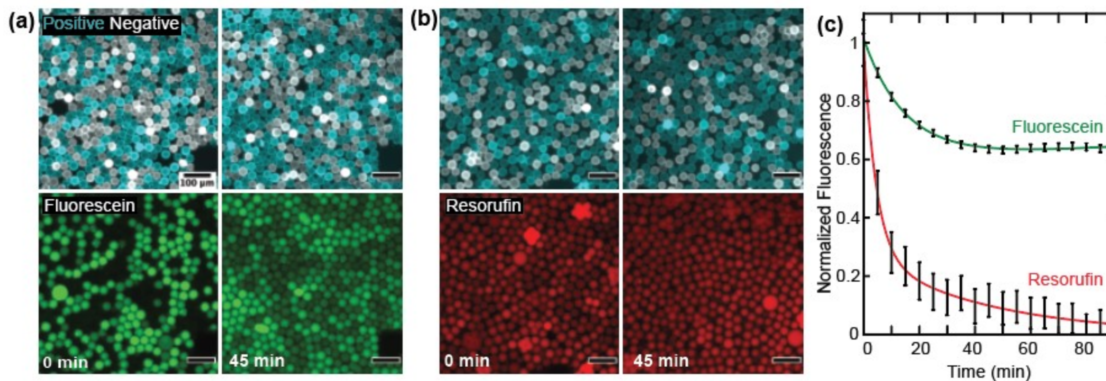

Figure. S6 Transport of resorufin and fluorescein in dropicles. **(a)** Fluorescence signals from fluorescein containing dropicles, the location of which indicated by the cyan color filter on the top images, transported towards negative dropicles during a 45-minute incubation. Scale bar = 100 µm. **(b)** Fluorescence signals from resorufin containing dropicles transported towards negative dropicles during a 45-minute incubation. **(c)** Normalized fluorescence intensities over 90 minutes observation showing the transport of fluorescein is slower than resorufin, and ~65% of fluorescein signal was retained at equilibrium. Error bars indicate the standard deviation of all dropicles observed.

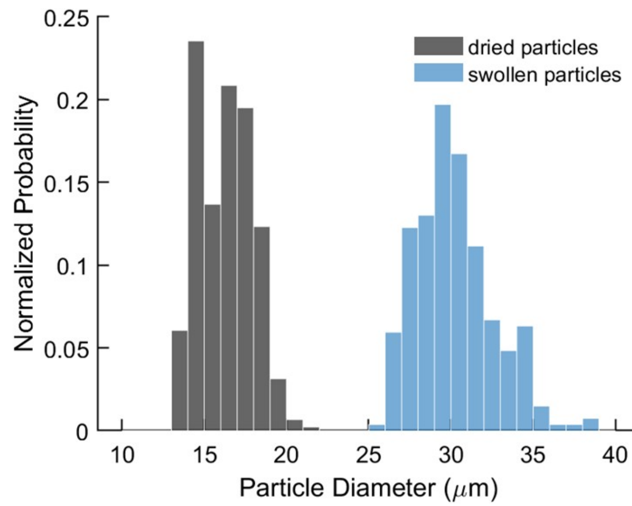

Figure. S7 Change of particle size during the rehydration process of active absorption. Dried particles (d_1 = 16.3 ± 1.6 μm, n_1 = 446) swelled into hydrated particles (d_2 = 30.3 ± 2.4 μm, n_2 = 269), yielding a 6.4-fold in the total volume of the spherical hydrogel particles.

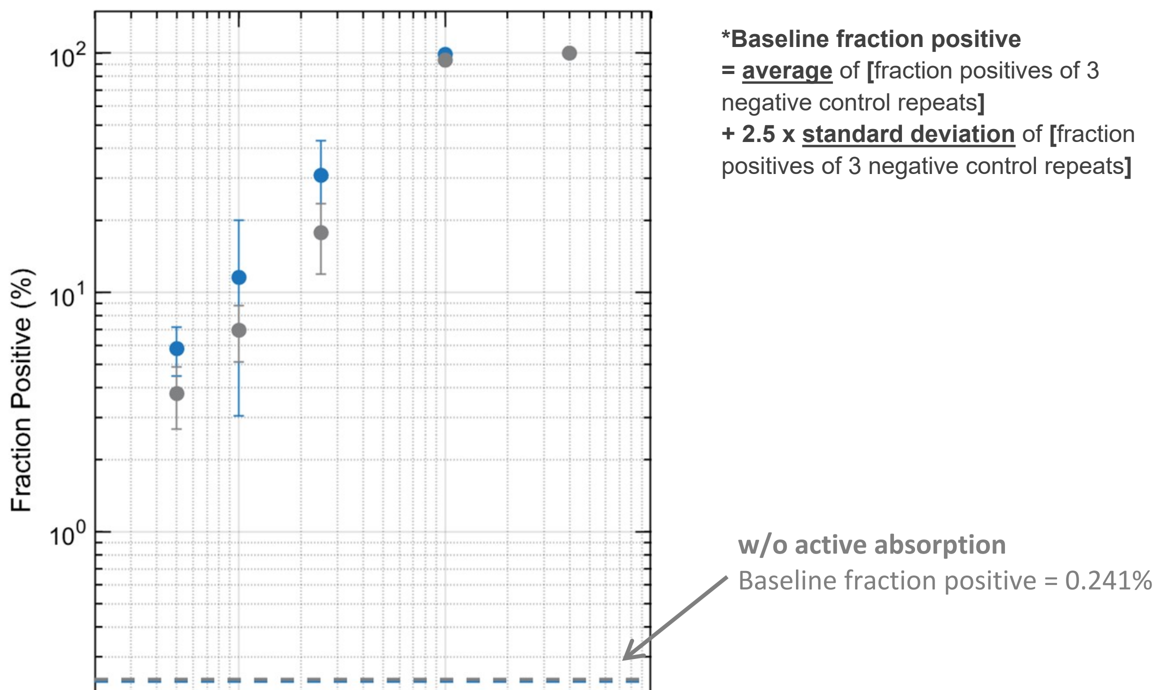

Table S1 Assay metrics comparison with other digital enzyme counting publications

Reference	Partition Method	# of partitions analyzed	Limit of Detection	Partition Volume	Signal Incubation Time
Enzyme Detection					
Rissin et al., 2010[1]	Particles in microwells	50,000	220 zM	40 fL	2 minutes
Kim et al., 2012[2]	Particles in microwells	700,000	10 zM	59 fL	5 hours
Obayashi et al., 2015[3]	Enzymes in microwells	900,000	7 fM	44 fL	10 minutes
Guan et al., 2014[4]	Enzymes in droplets	200,000	40 fM	4-300 pL	4-20 hours
Ono et al., 2018[5]	Enzymes in microwells	1,000,000	100 fM	200 aL	30 seconds
This work	**Enzymes in dropicles**	**50,000**	**4.5 fM**	**20 pL**	**20 hours**
Sandwiched ELISA					
Rissin et al., 2010[1]	Particles in microwells	50,000	200 aM (PSA)	40 fL	2 minutes
Kim et al., 2012[2]	Particles in microwells	700,000	2 aM (PSA)	59 fL	5 hours
Shim et al., 2013[6]	Particles in droplets	20,000 droplets 1,900 particles	46 fM (PSA)	32 fL	10 minutes
Leirs et al., 2016[7]	Particles in microwells	7,000	4 fM (Influenza A Nucleoprotein)	38 fL	20 minutes
Yelleswarapu et al., 2019[8]	Particles in droplets	10 M droplets 1M particles	300 aM (GM-CSF)	22.5 pL	2 minutes
Cohen et al., 2020[9]	Particles in droplets	60,000 particles	20 aM (IL-2)	1.4 pL	overnight
Kan et al., 2020[10]	Particles in microwells	5,000	0.31 aM (IL-12p70)	44 fL	10 minutes

Video S1 Microscopic recording of dried hydrogel particles being rehydrated, expanding 6.4x in volume in the process.

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