

1 Supplementary Information to

2           A hydrogel beads based platform for single-cell  
3 phenotypic analysis and digital molecular detection

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12 **This PDF file includes:**

13 Supplementary Tables S1 to S2

14 Supplementary Figures S1 to S5

15 Supplementary Notes S1 to S3

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20 **Supplementary Tables**

		LAMP mix	PCR mix	TSB media
pH		8.8	8.3	7.3
7.5 w/v%	Sol-Gel	7.0	11.5	43.0
	transition time [min]	8.5	13.5	53.0
	Pore size	27nm		
10 w/v%	Sol-Gel	4.5	8.5	18.0
	transition time [min]	5.5	10.0	23.5
	Pore size	25nm		

21 **Table S1.** PEG hydrogel crosslinking characterization in bulk for LAMP mix, PCR mix, and TSB  
 22 media. Sol-Gel transition time was experimentally determined (See **Materials and Methods**). The  
 23 pH values were supplied by the manufactures. The pore sizes were estimated theoretically (20).

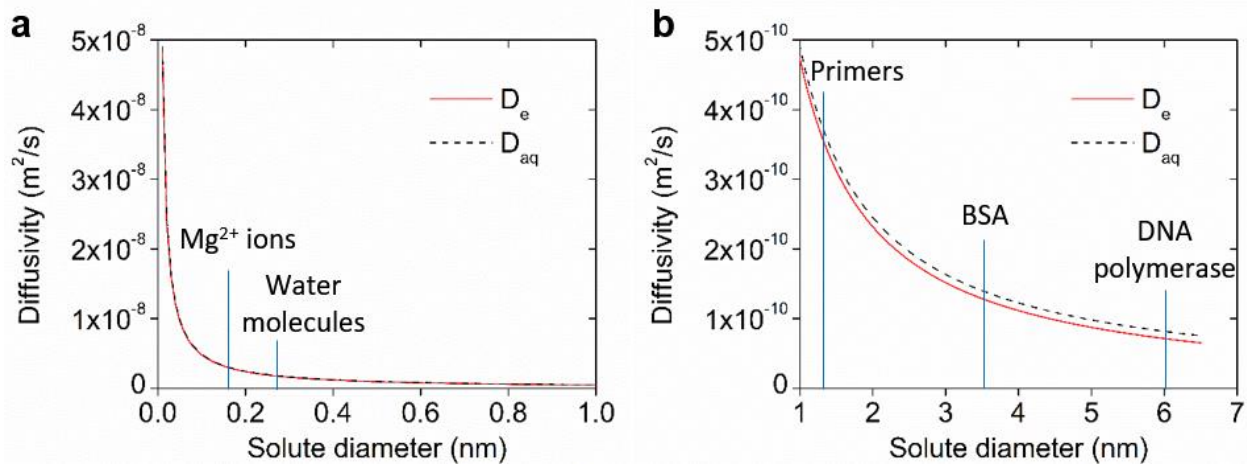
<b>PCR</b>	
Forward primer	5' CGCGAAGTCAGAGTCGACATAG 3'
Reverse primer	5' AAGACCTCAACGCCGATCAC 3'
Probe	5' FAM AAGACCTCAACGCCGATCAC 3'

<b>LAMP</b>	
FIP	5' AACTTGCTGCTGAAGAGTTGGACCGAATGACTCGACCATC 3'
BIP	5' CCTGGGGCCAAATGGCATTATGCACTAAGTAAGGCTGG 3'
F3	5' GACTTGCCTTTAAAAGATACCA 3'
B3	5' AGAGTGCGTTTGAACACTT 3'
LF	5' TCGGATGGCTTCGTTTCCT 3'
LB	5' CAAGGGTTTCAAGACTAAGTGGTTC 3'

24 **Table S2.** Sequences of primers and probe for PCR and LAMP assays. PCR primers and probes  
 25 target a region in gene STY0201 specific for *S. Typhi* for an amplicon size of 131 bp (32). LAMP  
 26 primers target a 196 bp region within the *S. Typhi* specific gene STY1607 (39). The target regions  
 27 for PCR and LAMP were both found to occur at one copy per cell, by searching the sequences  
 28 within the complete genome of *S. Typhi* strain CT18 (Accession no. NC\_003198) using Basic  
 29 Local Alignment Search Tool (BLAST).

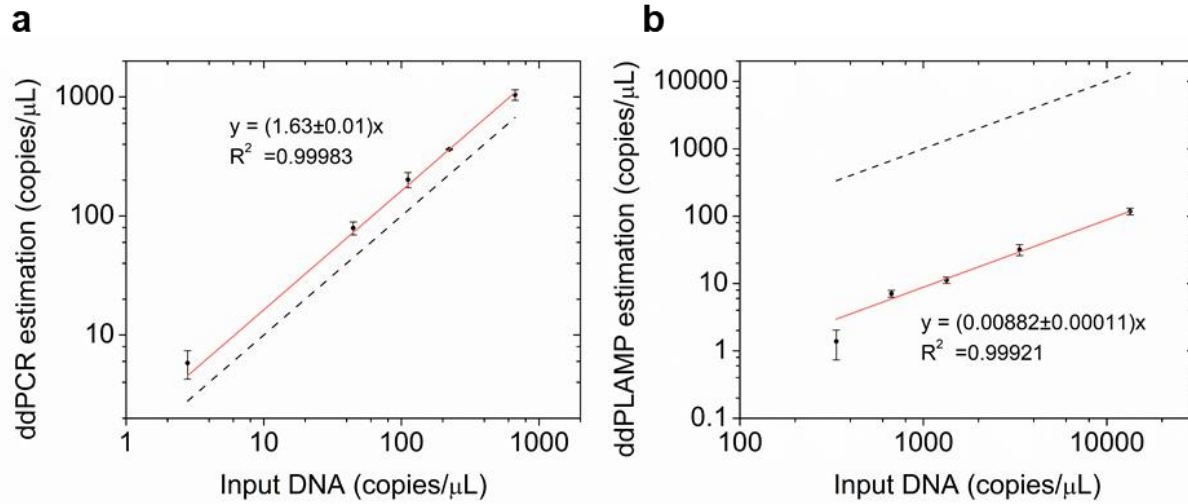
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31 **Supplementary Figures**



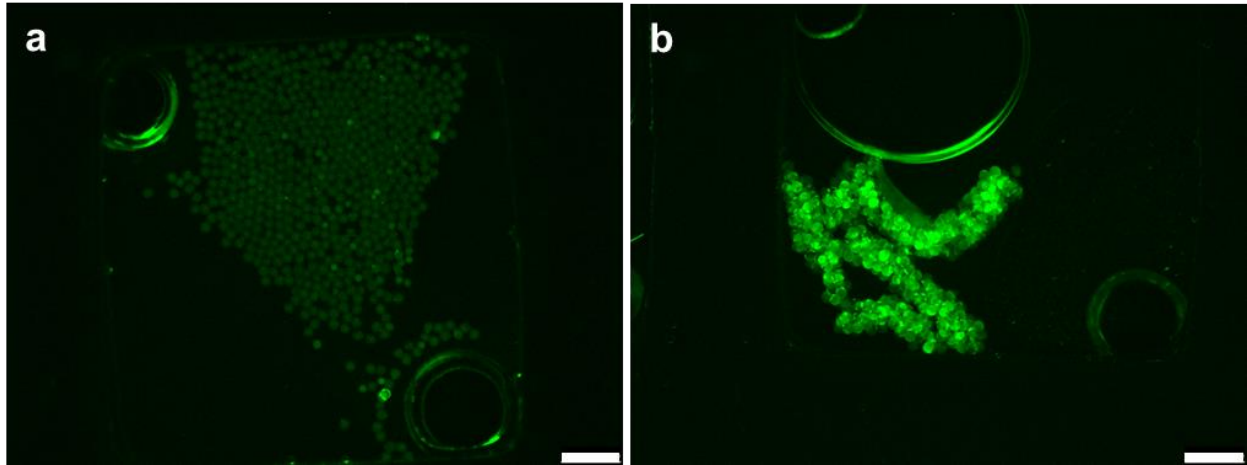
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33 **Figure S1.** Calculated effective diffusivity ( $D_e$ ) of solute in 7.5 w/v% PEG hydrogel matrix based  
34 on Weber et al. (30) and diffusivity in aqueous phase ( $D_{aq}$ ) at 37 °C based on Stokes-Einstein  
35 equation as a function of solute hydrodynamic radius (a) from 0-1 nm and (b) from 1-7 nm. For  
36 functional molecules in the molecular assays approximate sizes are indicated on the plots.



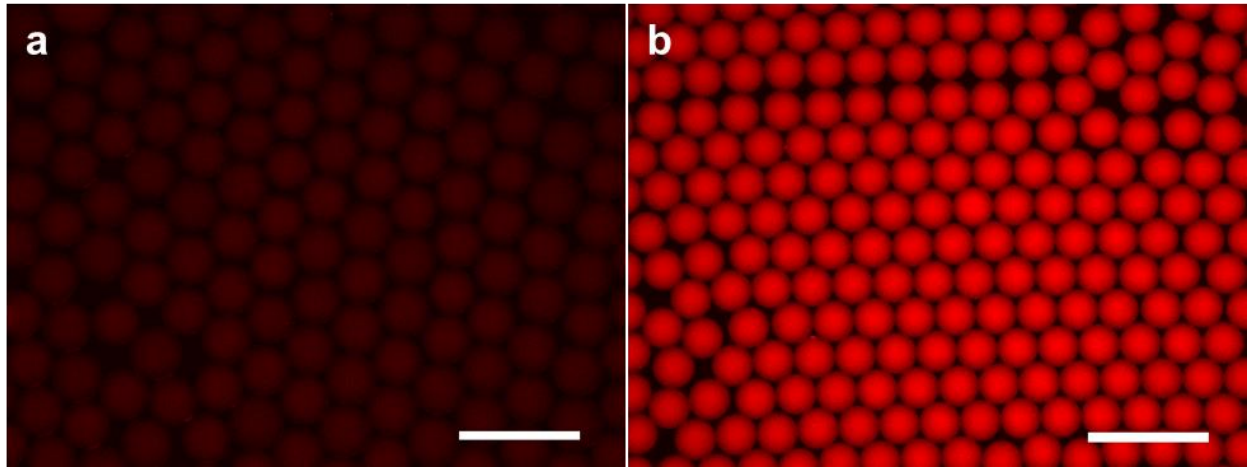
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38 **Figure S2.** Estimated DNA concentration by (a) ddPCR for 24000, 1500, 600, 300, 100 times  
 39 dilution of harvested *S. Typhi* DNA. and (b) ddLAMP for 200, 100, 50, 20, 5 times dilution of  
 40 harvested *S. Typhi* DNA. compared with input DNA concentration. Input DNA concentration was  
 41 calculated from dilution factor and OD600 measurement of cultured cells before DNA extraction  
 42 using commercial kit. The dashed lines reference an exact match with input DNA concentration.



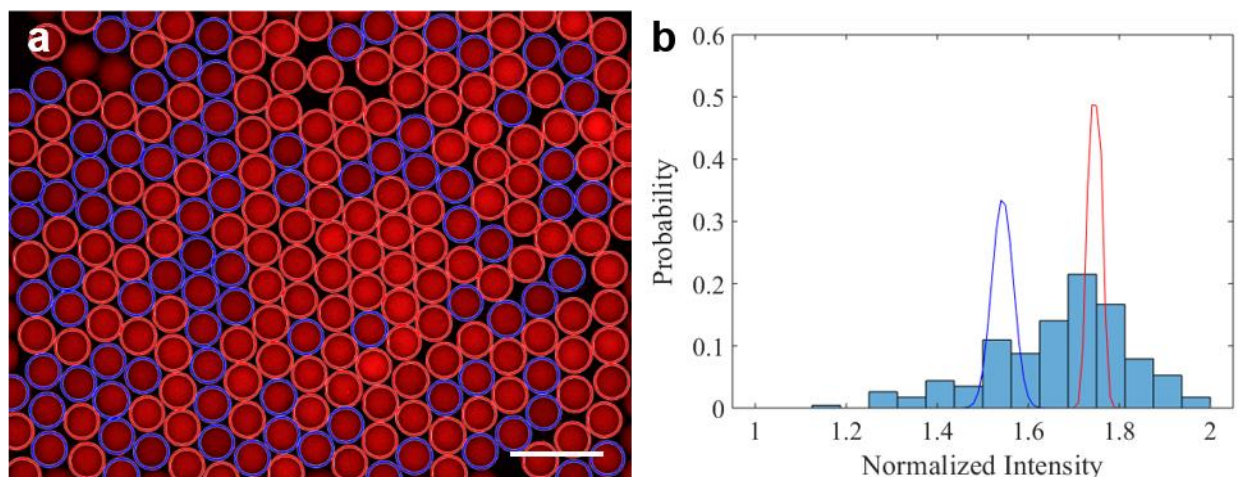
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44 **Figure S3.** Example fluorescent images from preliminary gdLAMP experiments with (a) no target  
45 template and (b) 100 times dilution of harvested *S. Typhi* DNA. Scale bars, 1 mm. For preliminary  
46 gdLAMP experiments, LAMP MasterMix was used (New England BioLabs) instead of the customizable  
47 LAMP recipe specified in **Online Methods**. Each 20  $\mu$ L of reaction mix for contained 1 $\times$  LAMP  
48 MasterMix, 1.6  $\mu$ M FIB and BIP, 0.2  $\mu$ M F3 and B3, 0.8  $\mu$ M LF and LB, 1 $\times$  LAMP dye. 7.5 w/v% PEG  
49 hydrogel was added as 10 $\times$  PEG monomers. The heating protocol involved 65  $^{\circ}$ C for 30 min and then 80  $^{\circ}$ C  
50 for 5 min. Aggregation of Gelbeads observed for positive samples but not for no-template controls. The  
51 extent of aggregation indicates occurrence of severe crosstalking.



52

53 **Figure S4.** Example images of preliminary cell phenotyping experiments. With alamarBlue and  
54 media only, Gelbeads appeared to be much brighter than droplets before incubation even when  
55 imaged under a lower fluorescence (300 ms exposure for droplets and 25 ms exposure for  
56 Gelbeads). Scale bars, 500  $\mu\text{m}$ .



57

58 **Figure S5.** MATLAB analysis and threshold setting for images from phenotyping experiments.

59 (a) An example image of phenotyping assay analyzed in MATLAB. Scale bar, 500  $\mu\text{m}$ . The blue  
60 circles represent identified dark Gelbeads, and the red circles represent identified bright Gelbeads.

61 (b) The histogram presents the occurrence probability of mean normalized intensity of Gelbeads  
62 analyzed based on the source image of (a). Gaussian fitting of the occurrence probability data  
63 generated two peaks, represented by the blue and red curves. The threshold suggested by this

64 MATLAB script was set as the average of the mean ( $\mu$ ) of two peaks. This threshold was used to  
65 categorize negative and positive Gelbeads and produced the identification results on the left. We

66 note that for 2 hours and 5 hours of incubation, the differences in normalized intensity of Gelbeads  
67 were too small for this thresholding method. In these cases, threshold enumeration and visual

68 inspection were used instead for an approximately appropriate threshold.



69 **Supplementary Notes**

70 **Supplementary Note 1: Characterization of PEG hydrogel crosslinking**

71 To identify the reasonable time frame for Gelbead generation, PEG gelation time in our targeted  
72 reaction matrices by bulk phase sol-gel transition experiments (online methods) was first measured.  
73 Ideally the compartmentalization process should be completed before the sol-gel transition starting,  
74 after which further crosslinking would considerably alter fluid properties such as viscosity and  
75 surface tension. For the three types of reaction matrix examined, results showed that the sol-gel  
76 transition start time spanned from 4.5 min to 43.0 min, and the crosslinking was accelerated by  
77 higher pH and higher monomer concentration (**Table S1**). Accordingly, the gelation time might  
78 be further extended by decreasing the crosslinking temperature. We then estimated if the lower gel  
79 concentration considerably affects the hydrogel properties. The theoretical pore sizes for our  
80 crosslinked PEG were close for 7.5% and 10% gel (**Table S1**), and both would allow diffusion of  
81 functional molecules in our applications including water, ions, small DNA fragments, and proteins  
82 that size from below an angstrom to ~ 6 nm (31). This estimation neglects non-ideality such as  
83 dangling ends or monomer self-interlinking, which might result in larger actual pore size. Using  
84 the same PEG monomers, 10 w/v% hydrogel concentration has been utilized in cell encapsulation,  
85 multiple displacement amplification (MDA) and LAMP (20, 21). We reason that lowering the  
86 hydrogel concentration to 7.5 w/v% would benefit our applications by allowing more time for  
87 Gelbead generation and creating looser hydrogel network for reagent diffusion. Therefore, 7.5 w/v%  
88 PEG was used in further experiments.

89 **Supplementary Note 2: Droplet generation performance and sources of error**

90 To achieve high throughput analysis of droplets with limited available instruments, we chose to  
91 analyze droplets through fluorescence imaging. As in the reported protocol, droplets were  
92 extracted into a viewing chamber, made by bonding a commercial plastic chamber onto a glass  
93 slide, for fluorescence imaging at 1.25× objective. We acknowledge that this protocol might have  
94 introduced systematic error in size characterizations by 1) pipetting droplets from the  
95 microcentrifuge tube into the viewing chamber, 2) noise difference from focus point to the edges  
96 within an image, and 3) image processing bias by MATLAB when identifying the circular-shaped  
97 edges. These sources of error would lead to overestimation of size distribution. Therefore, it is  
98 anticipated that the actual CV of the generated droplets or Gelbeads should be lower than reported.  
99 Generally, the reported sizes and CVs of generated droplets, optimized at 175 μm diameter with  
100 CV of 5%, are comparable to those generated by centrifugal microfluidics reported in literature.  
101 For example, Haeberle et al. used polymer-tube micronozzles for alginate bead generation, and  
102 reported beads generated at diameters tunable from 180 to 800 μm with CV of 7–16% (24). As  
103 another example, using a lab-on-a-disk centrifugal droplet generation with, Schuler et al. reported  
104 droplet diameters of 120 to 170 μm with CV of 2–4%. It should be noted that these values represent  
105 only 20 measurements of droplet under high microscope objective (22).

106 **Supplementary Note 3: Microscope objective choice**

107 For ddPCR and gdPCR, 5× objective was used in fluorescent microscope imaging to more  
108 accurately capture the assay quantification performance. Taqman probe, commonly used in PCR  
109 for enhanced detection specificity by hydrolysis upon encounter of specific sequence target, was  
110 employed in our PCR assay recipe. In this case, the fluorescence exhibited by negative  
111 compartments was too low to be distinguishable from the oil phase under 1.25× objective. To be  
112 able to count negative compartments, a zoomed-in view using 5× objective had to be used instead  
113 of 1.25× objective, which was employed to image ddLAMP and gdLAMP results. A commercial  
114 DNA-intercalating dye was used in LAMP so that the higher background fluorescence was  
115 observed in negative compartments. 1.25× objective could image an area of 0.785 cm<sup>2</sup> to include  
116 the whole viewing chamber at one image shot. The smaller view at 5× objective was compensated  
117 by taking 5 images of different areas in the viewing chamber. A similar strategy was applied in  
118 Gelbeads imaging for phenotyping experiments to better distinguish the Gelbeads with varying  
119 fluorescence levels.