Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Microfluidic static droplet array for analyzing microbial communication on a population gradient

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Supplementary Information

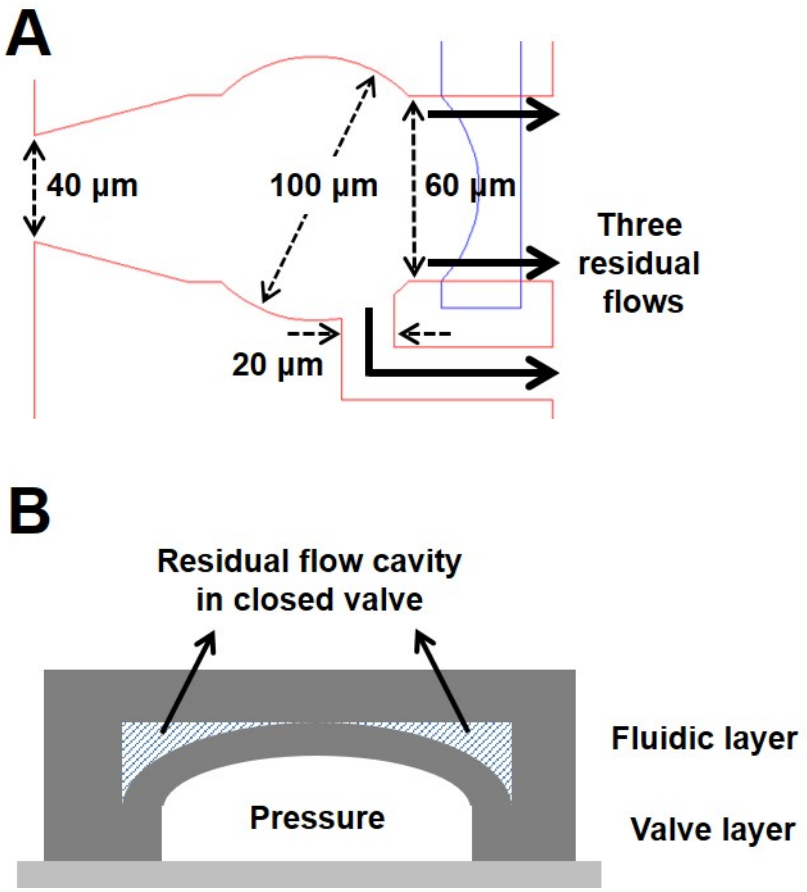

Fig. S1. Detailed design of a hydrodynamic trap for aqueous droplet trapping. (A) Dimensional view of one hydrodynamic trap. (B) Schematic diagram of residual flow cavity formed by closing the microvalve.

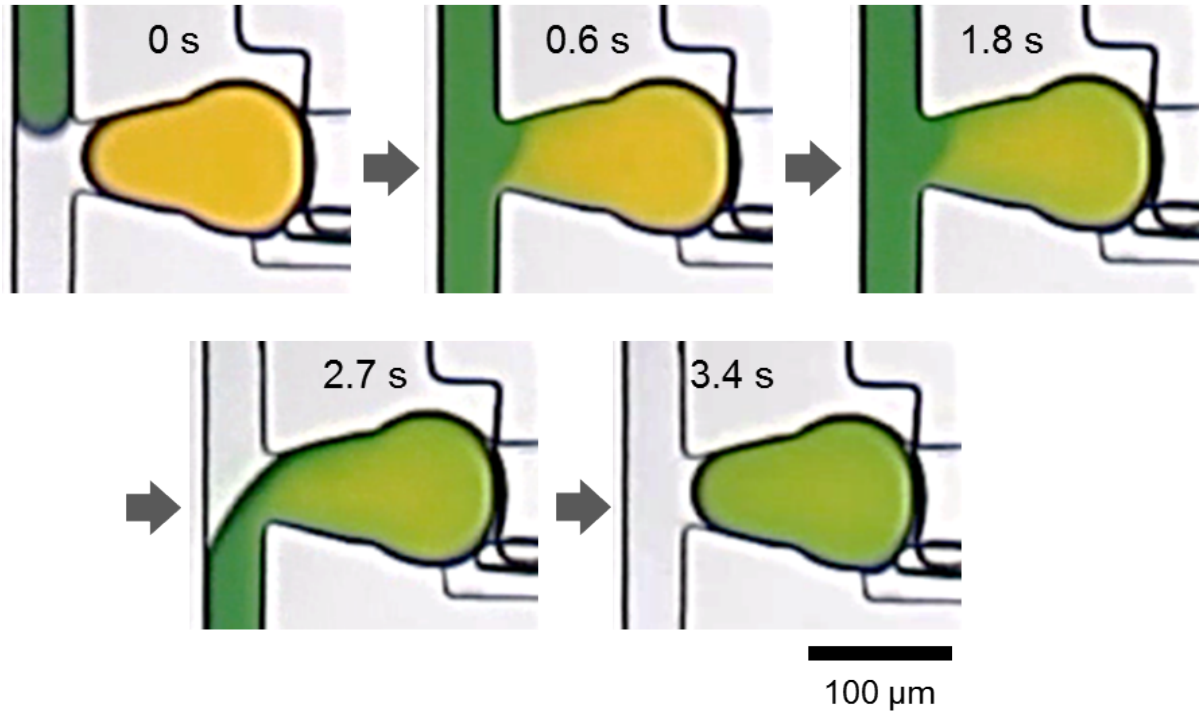

Fig. S2. Detailed sequential images of the exchange of materials between the moving plug and the trapped droplet.

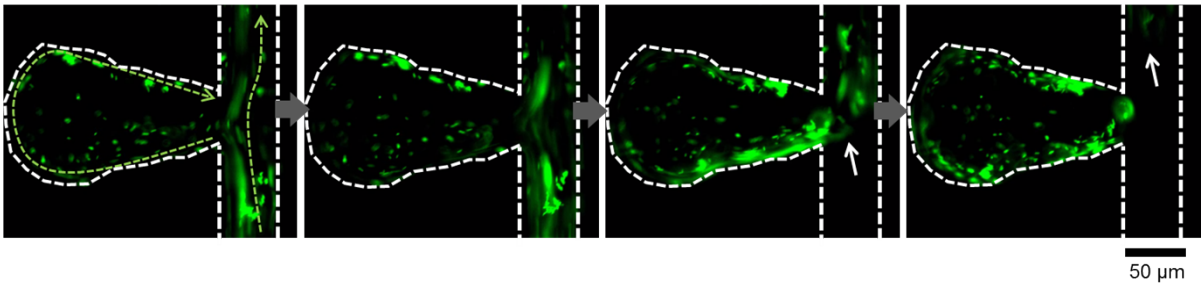

Fig. S3. Detailed sequential images of the exchange of bacteria between the moving plug and the trapped droplet.

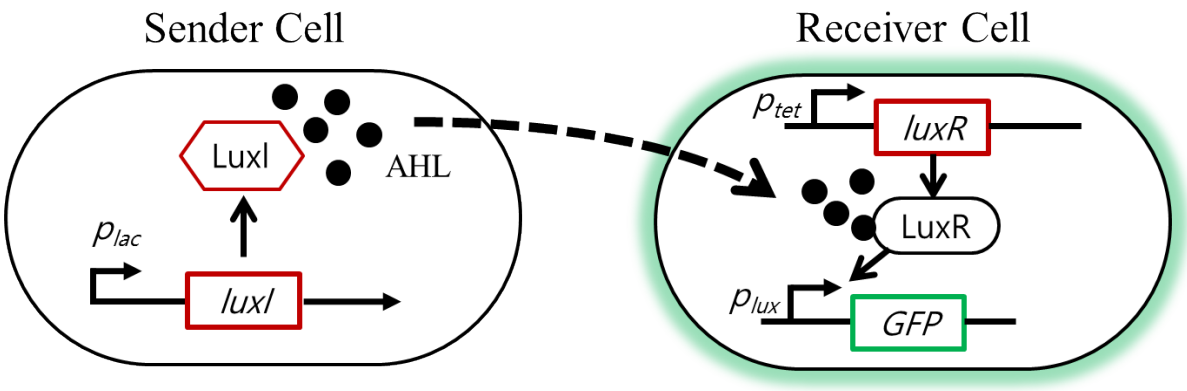

Fig. S4. Schematic diagram of cell-cell interaction between sender cells (SCs) and receiver cells (RCs). In the gene circuit of SCs, they continuously produce acyl-homoserine lactone (AHL) by LuxI protein. Produced AHL is released from SCs and transfer to RCs. And then, the internalized AHL signal molecule into RCs forms a complex with the LuxR protein that activate the transcription of the GFP gene, resulting in the expression of fluorescence in RCs.

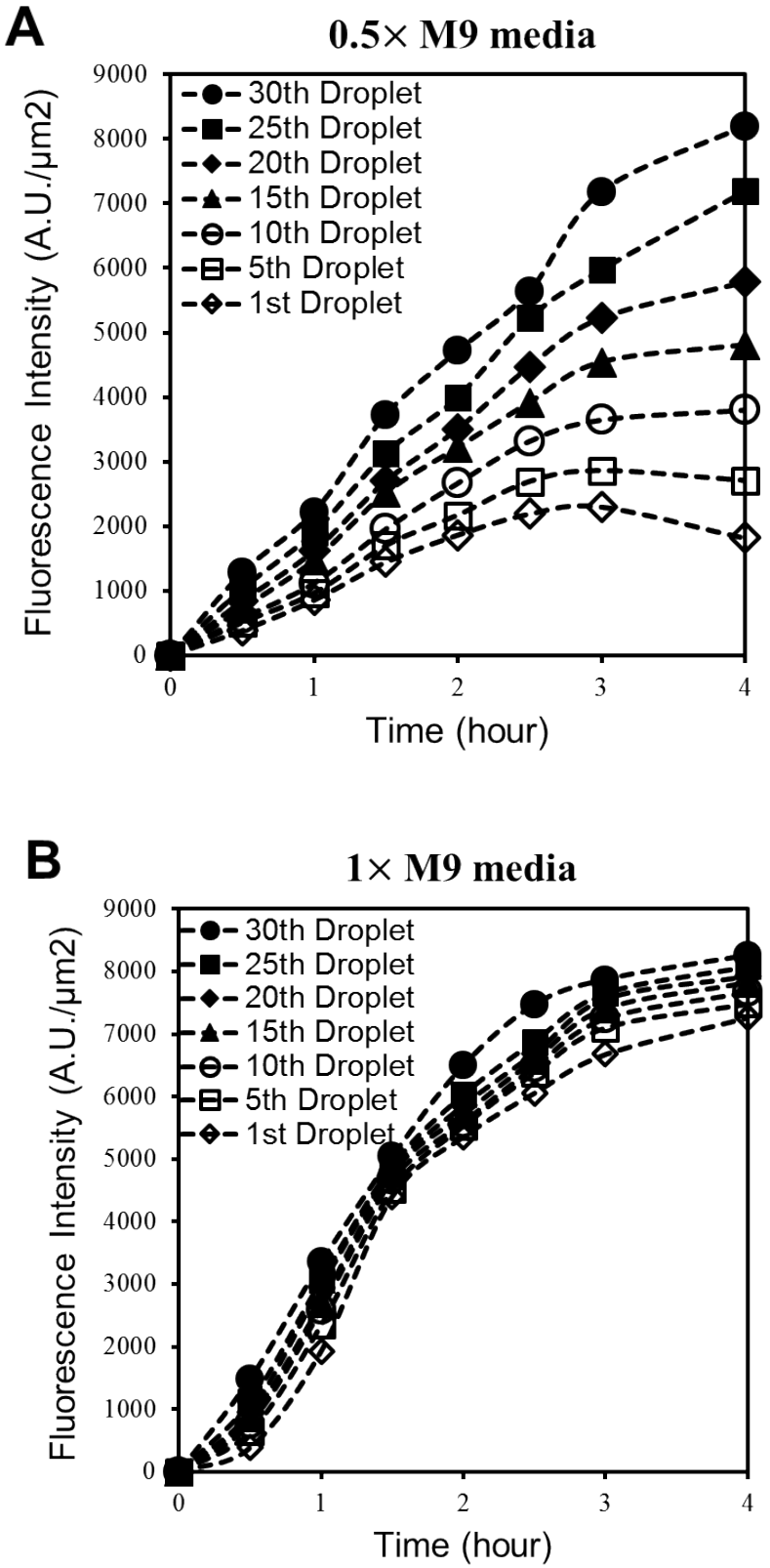

Fig. S5. Population-dependent cell-cell interactions in an SDA. Time course of GFP expression according to various population ratios in (A) 0.5X M9 media and (B) 1X M9 media.

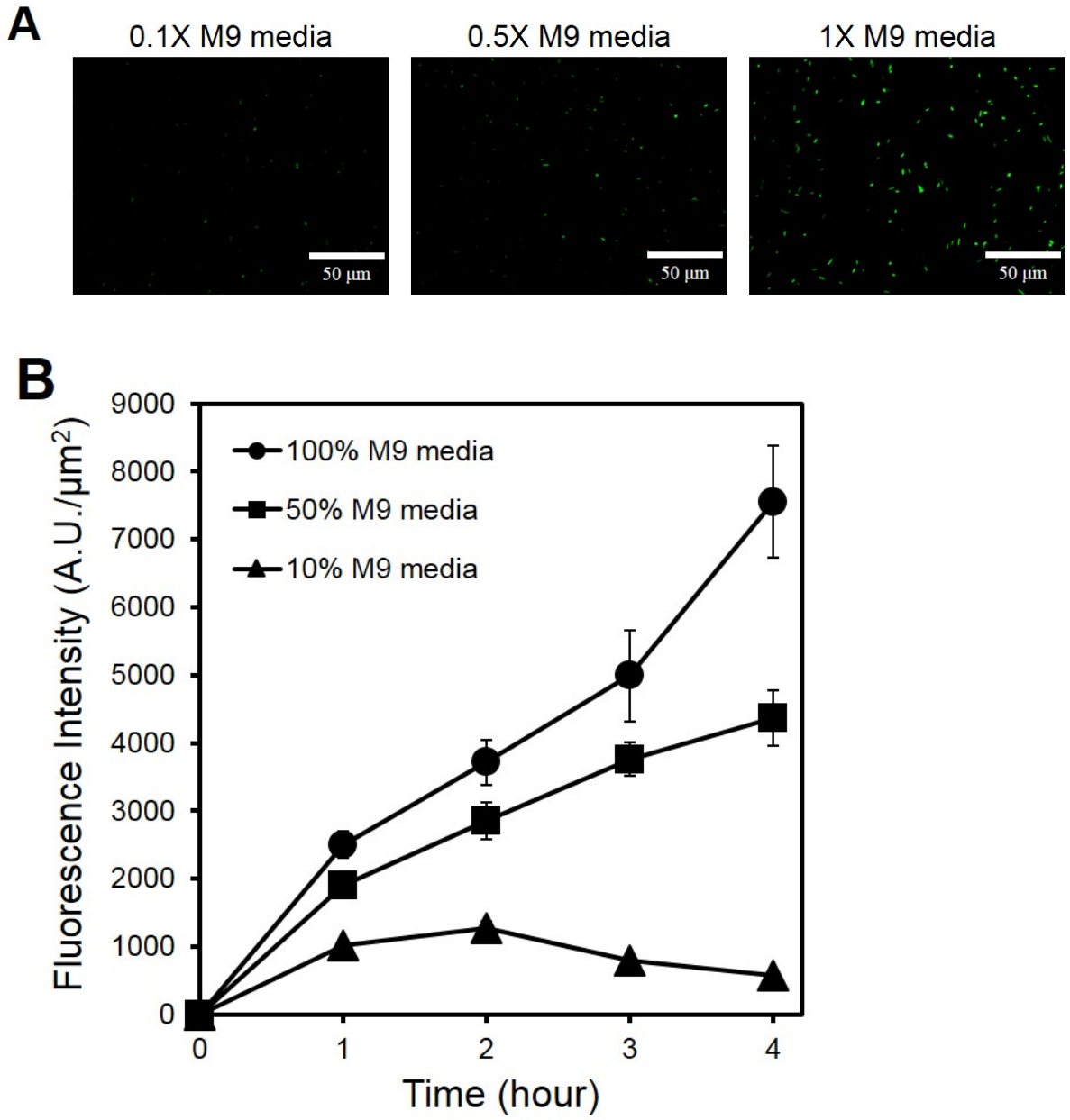

Fig. S6. (A) Fluorescence images of bacteria cultured in three different conditions. (B) GFP expression kinetics at different concentrations of nutrients in bulk flask culture.

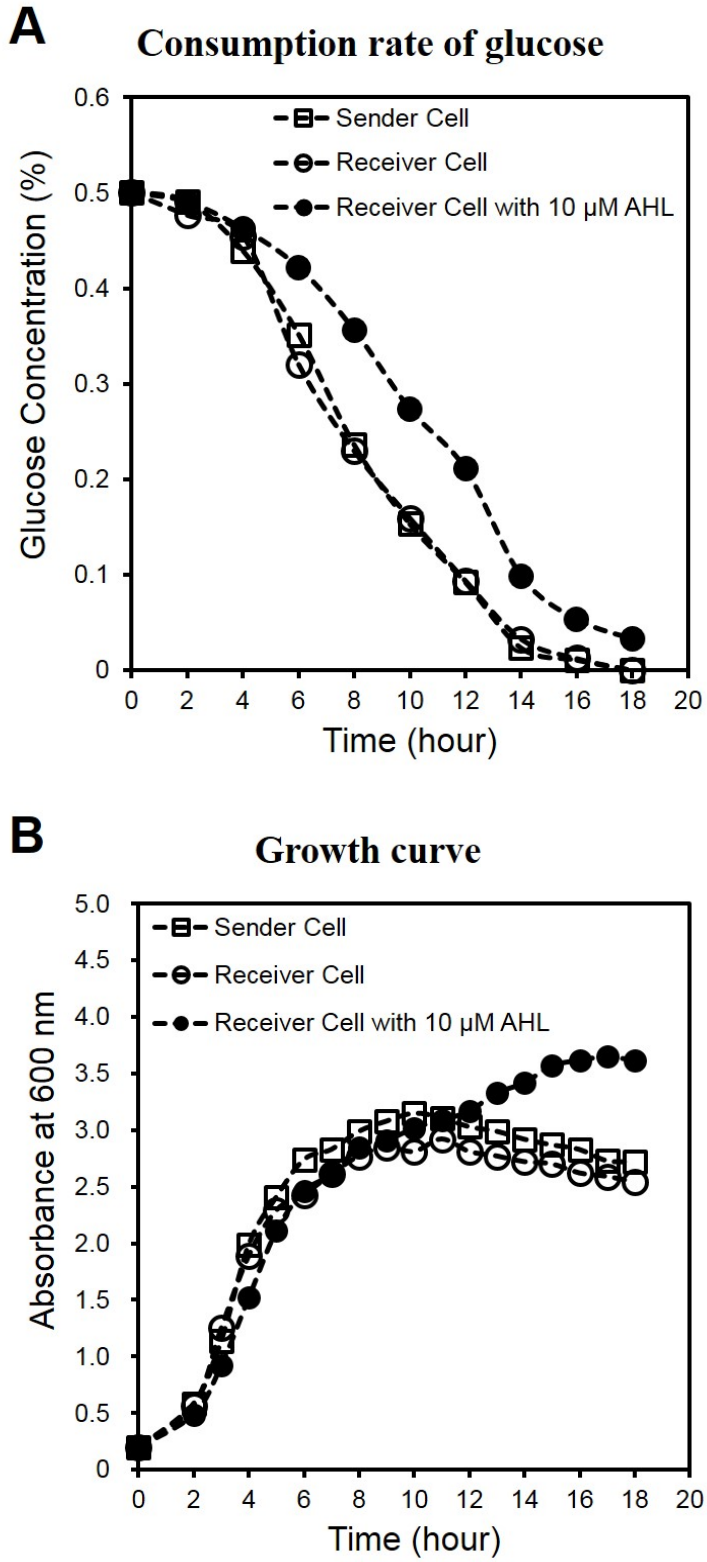

Fig. S7. Characterization of bacterial growth. (A) Changes in the consumption rate of glucose with sender cells, receiver cells, and receiver cells with AHL in medium supplemented with AHL. (B) Growth curves were observed for three cases (sender cells, receiver cells, and receiver cells with AHL).

Table S1 *E. coli* strains and plasmids used in this study

Strain/plasmid	Description/genotype	Source
Strain		
E. coli MG1655	Wild-type	**
E. coli DH10B	F–*mcrA*Δ(*mrr-hsdRMS mcrBC*) ϕ80d*lacZ*ΔM15 Δ*lacX74 deoR recA1 ara*Δ*139* Δ(*ara leu*)*7697 galU galK* λ^-*rpsL endA1 nupG* Str^r	Life Technologies
Plasmids		
pTKU4-2	Cmr; pBR322 replicon, $P_L$tetO-1-gfp	This study
pTKU4-65	Cm^r; pBR322 replicon, $P_L$tetO-1-*rfp*	This study
pTKU1-11S	Cmr; pBR322 replicon, P_{lac} Promoter	This study
pTKU1-12R	Cm^r; pBR322 replicon, P_{Lux}-*gfp*	This study

** S. H. Lee, A. J. Heinz, S. Shin, Y. G. Jung, S. E. Choi, W. Park, J. H. Roe and S. Kwon, Anal. Chem., 2010, 82, 2900-2906.

Pressure drop and design criterion for microfluidic SDA

Basically, we apply previously well-established Darcy-Weisbach equation for determining the pressure drop in hydrodynamic trap (1-5).

$$\Delta P = \frac{C(\alpha)}{32} \cdot \frac{\mu L Q (2W + 2H)^2}{A^3} \quad \text{(Eq.1)}$$

Where, C(α) denotes a constant that is a function of α (aspect ratio of channel). L is length of the channel, Q is the volumetric flow rate, W and H is a channel width and height, respectively, and μ is the fluid viscosity.

Due to the both flow paths in hydrodynamic trap are connected to each other, the pressure drop in both paths is the same. Therefore, the ratio of volumetric flow rate can be expressed as follow. (1-5)

$$\frac{Q_1}{Q_2} = \left(\frac{C_2(\alpha_2)}{C_1(\alpha_1)}\right) \cdot \left(\frac{L_2}{L_1}\right) \cdot \left(\frac{W_2 + H}{W_1 + H}\right)^2 \cdot \left(\frac{W_1}{W_2}\right)^3 > 1 \quad \text{(Eq.2)}$$

Where subscript 1 and 2 represent trapping flow and bypassing flow in each path.

Table S2 lists the dimensions of microfluidic SDA, which meet the design criterion (Eq. 2).

Table S2 Geometric dimension of microfluidic SDA.

	Width of cavity flow channel (W_1)	Length of cavity flow channel (L_1)	Width of bypass channel (W_2)	Length of bypass channel (L_2)	Height (H)	Q_1/Q_2
Off-microvalve	60	70	40	830	20	20.5
On-microvalve	20	80	40	830	20	3.19

For a simplified calculation, we have assumed three residual flows in the hydrodynamic trap as a cavity flow in an artificial flow channel (Fig. S1).

Reference

1. W. H. Tan and S. Takeuchi, *Proc. Natl. Acad. Sci. U. S. A.,* 2007, 104, 1146-1151.

2. J.-P. Frimat, M. Becker, Y.-Y. Chiang, U. Marggraf, D. Janasek, J. G. Hengstler, J. Franzke and J. West, Lab on a Chip, 2011, 11, 231-237.

3. J. Kim, J. Erath, A. Rodriguez and C. Yang, Lab on a Chip, 2014, 14, 2480-2490.

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5. J. Chung, Y.-J. Kim and E. Yoon, Applied Physics Letters, 2011, 98, 123701.