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Off-Chip-Controlled Droplet-on-Demand Method for Precise Sample Handling

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ABSTRACT: We present a simple, stable, and highly reproducible off-chip-controlled method for generating droplets-on-demand. To induce the droplet generation, externally pre-programmed positive pressure pulses are applied to the dispersed phase input while the continuous phase channel remains at constant input pressure. By controlling solely one fluid phase, the method allows for connecting multiple independent dispersed-phase channels to a single continuous channel. Experimental results show that the method allows for a droplet generation frequency of 33 Hz and a high reproducibility of droplets with standard deviations less than 5% of the mean value. Moreover, utilization of the off-chip-controlled method results in the simplicity in chip design and allows rapid (∼5 min) and cost-efficient (0.5 USD) prototyping of the device.
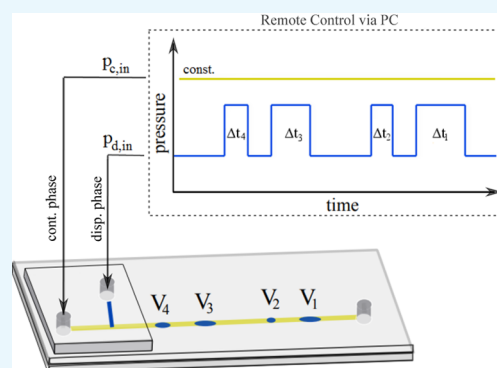
INTRODUCTION

Fundamental and applied research in chemistry and biology benefits from the unique opportunities provided by droplet-based microfluidic systems. Offering the possibility to perform laboratory operations on small scales, droplet microfluidic technologies are now a powerful analytical tool applied to many studies in chemistry,[1] microbiology,[2] detection and identification of pathogens,[3] and antibiotic susceptibility testing.[4] In such systems, droplets are used as reaction or incubation compartments that enable controllable confinement of biochemical samples and reagents to small volumes. This small-scale analysis, together with microfluidic technology, could deliver million-fold improvements in throughput when compared to conventional microplate-based sample screening.[5]

In order for droplets to serve as reliable reactors, precise and efficient control over the sample incubation, sample injection, and sample location is crucial. Since biochemical samples and reagents are encapsulated inside the droplets, fine control and manipulation over the samples and in-droplet reactions are actually realized through a highly controllable droplet generation process. Controllable methods for sample handling at the droplet generation site can therefore ensure that successful and fault-free biochemical processing inside the droplets as reactors takes place. After the droplet generation and sample/reagent encapsulation, droplets are submitted to a number of analytical screening and measurement tools, which have also been proven strongly sensitive to various droplet parameters. For example, for droplets with biochemical samples to be successfully detected and sorted using fluorescence-activated droplet sorting (FADS), both droplet

size and spacing between droplets were found to be an important factor affecting detection and sorting efficiency.[6] Therefore, precise control over the droplet parameters, especially droplet size and distance, is critical to ensure minimum errors or any unwanted noise both in biochemical in-droplet reactions and in succeeding measurements. Another recent example where it is too crucial to generate droplets at prescribed times with certain sizes is microfluidic networks.[7] Microfluidic networking aims to realize programmable and flexible LoC devices, through controlling the droplets' path purely based on hydrodynamic principles, i.e., channel geometry as well as droplet size and distance.[8,9]

Over the years, various means of performing the so-called droplet-on-demand (DoD) generation were introduced to address the need for achieving fine control over the droplet parameters.[1] To date, most of these methods focus on utilizing integrated microvalves for achieving on-chip control of the flow and the droplet parameters.[10,11] Although an elegant solution, the use of integrated valves is highly limited due to the (i) complex, multilevel fabrication, (ii) low biocompatibility of the device due to the limited choice of the fabrication materials, and (iii) the need for external equipment to control the valves, in addition to the pumps for the fluid supply.

In order to surpass the limitations of on-chip DoD systems, several other solutions that mostly rely on the geometry tuning and off-chip control have been proposed.[12,13] Teo et al.[12] introduced a DoD system that requires a negative pressure at the outlet of a flow-focusing device, and in Churski et al.,[13] both continuous and dispersed phases toggle between on and off states to achieve DoD. Although these systems eliminate the need for on-chip components and consequent complex fabrication, they suffer from high synchronization requirements and they lack in the possibility to connect multiple DoD generators on a shared continuous channel. Especially the cascade of multiple DoD systems on a single channel is very important in many applications. For example, in reaction engineering, it is important to investigate chemical interactions between two samples in a highly controllable manner.[14] This can be realized through controlled merging, the so-called merging-on-demand (MoD) where droplets encapsulating different samples are generated at appropriate times on the shared channel to allow immediate merging at the droplet generation site. Moreover, the concept of microfluidic networking requires precise and independent generation of two different droplet types on a shared channel. The works so far have only introduced the theoretical principles, which have only been experimentally validated to a very limited extent.[8,9] Thus, an off-chip controlled DoD system that can be cascaded will, for the first time, allow the experimental validation of the promising concept of microfluidic networking.

In this work, a novel off-chip controlled DoD method is proposed, which overcomes the limitations of the techniques discussed above. In this method, positive pressure pulses are applied to the dispersed phase, while the continuous phase is kept at a constant pressure level (see Figure 1). This allows the
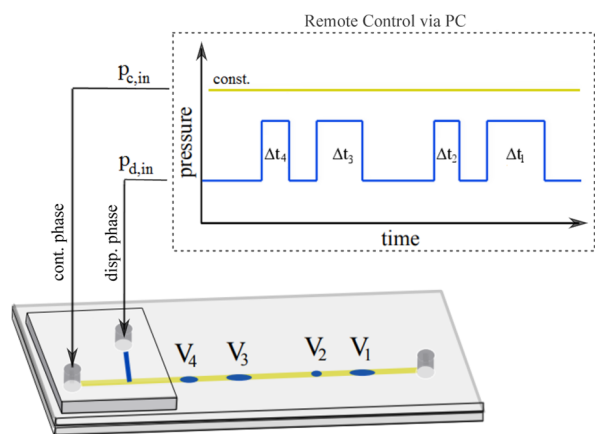
Figure 1. Droplet-on-demand realized by maintaining the continuous phase input at a constant input pressure and applying a series of positive rectangular pressure pulses (Δt) to the dispersed phase.

connection of multiple dispersed phase channels to shared continuous channels, enabling the concept of MoD and microfluidic networking. Due to the off-chip control, the simplicity in the chip design eliminates the need for complex and expensive fabrication techniques and allows the rapid (~5 min) prototyping of the device. Moreover, this technique enables a droplet generation frequency of 33 Hz and provides high droplet reproducibility with standard deviations less than 5% of the mean value.

RESULTS AND DISCUSSION

DoD Pressure Model. The droplet generation process starts at the equilibrium state, which is achieved through tuning the input pressures $p_{d,in}$ and $p_{c,in}$ until the dispersed $(p_{d,eq})$ and continuous $(p_{c,eq})$ equilibrium pressures are established inside the T-junction[2] under the condition

$$p_{d,eq} = p_{c,eq} + p_L \tag{1}$$

where p_L is the Laplace pressure and can be calculated as $p_L = \gamma(1/r_a + 1/r_r)$; γ is the interfacial tension between the two phases and r_a and r_r are the radii of curvatures due to the channel width and height, respectively.

To initiate the droplet generation, a positive pressure pulse is applied at the input of the dispersed phase, and consequently, the pressure of the dispersed phase inside the junction $p_{d,j}$ is also increased. At this stage,

$$p_{d,j} > p_{d,eq} \tag{2}$$

and thus,

$$p_{d,j} > p_{c,eq} + p_L \tag{3}$$

The increased pressure of the dispersed phase causes the tip of the phase to penetrate into the continuous channel and start forming a droplet. The droplet grows in the downstream direction and further blocks the continuous channel, leading to an increase in the junction pressure of the continuous phase $(p_{c,j})$. Consequently, the new pressure state is achieved where

$$p_{d,j} < p_{c,j} + p_L \tag{4}$$

Due to the increased pressure of the continuous phase, the droplet enters the break-up regime until it finally separates from the dispersed phase at the moment when Laplace pressure obtains its maximum value.[15] At this point, the tip of the dispersed phase recoils to the inlet channel and the pressures drop to their equilibrium values $p_{d,eq} = p_{c,eq} + p_L$, and a new droplet generation process can be initiated by applying another positive pressure pulse to $p_{d,in}$. The complete description of the proposed droplet generation process can be found in the Supporting Information (section 4, Figure S3).

DoD Validation. We fixed the input pressures $p_{d,in}$ and $p_{c,in}$ at 129 and 200 mbar, respectively, to achieve an equilibrium state. We initiate the droplet generation by applying a positive pressure pulse of 142 mbar and Δt = 3 s in duration. Using the microscope, we have closely followed the droplet generation process inside the junction and were able to identify and verify different pressure stages of the droplet generation process as shown in the Supporting Information (section 5, Figure S4, and Video S2).

In the second part of our investigations, we analyze the changes in the droplet volume, V, induced by the change in duration of the applied pressure Δt. A total of 5 pulses of different durations (Δt = {20,55,100,150,200} ms) were used, and the magnitude of each pressure pulse was kept at 142 mbar. A set of 1000 droplets was generated for each pulse duration resulting in a total of 5000 generated and processed droplets. The results were processed using the DMV software[16] and post-processed in Matlab. The experimental results depicted in Figure 2 show an almost linear increase in the droplet volume, V as the duration of the pulse, Δt, increases. Since longer pulse durations allow more fluid to enter the continuous channel and form a droplet, the increase in droplet volume was an expected result.
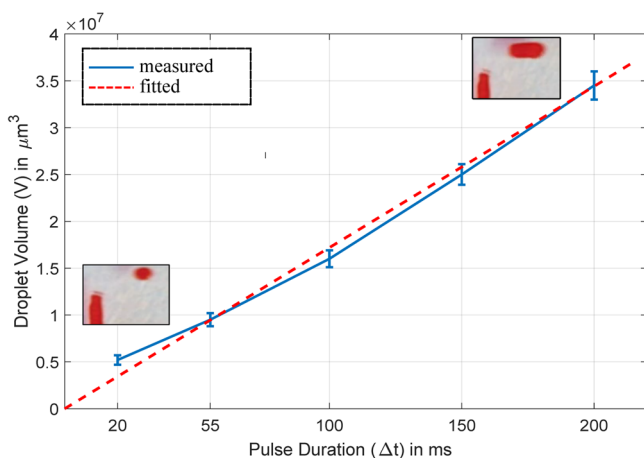
Figure 2. Change of the droplet volume, V, for various pulse durations Δt. The small error bars indicate high reproducibility and stability of the method for every pulse duration (Δt = {20,55,100,150,200} ms).

DoD Maximum Generation Frequency. For the purpose of identifying the maximum operational frequency, we reduce the duration of the positive pressure pulse (Δt) to its minimum feasible value, which is dominantly limited by the responsiveness of the pressure controller. The controller used in this work reports a response time of 9 ms, and for the reason of equipment safety and performance guarantee, it is recommended to use values higher than the minimum value of 9 ms. Following the technical recommendation and conducting a number of trial-and-error experiments, the minimum feasible pulse duration was selected to be Δt = 20 ms and the time period between two successive pulses was set to 10 ms as shown in the inset of Figure 3. This means that in each period of 30 ms, one droplet can be generated, which gives the maximum operational frequency of the proposed DoD method

$$f_{max} = 1/(30 \text{ ms}) = 33.3 \text{ droplets/s} \tag{5}$$

To verify that the frequency of 33 Hz is feasible, a set of roughly 1000 droplets was generated and the results were processed using the DMV and Matlab software. Figure 3
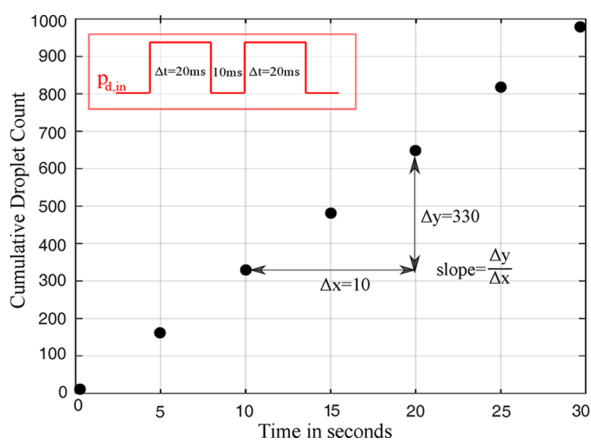
Figure 3. Cumulative droplet count plotted every 5 s, resulting in a total of 990 droplets in 30 s. The slope of the curve gives f_{max} = 33 Hz. The inset shows the dispersed phase pressure profile at the input where one pulse of duration Δt = 20 ms is applied for each 30 ms.

graphically illustrates the measured data of the change of the droplet count over the time. The maximum operational frequency can be found from the slope of the curve as $\Delta y/\Delta x$ = 33. Since the maximum droplet generation frequency is mainly limited by the responsiveness of the pressure controller, the proposed DoD method can achieve droplet generation frequencies beyond 33 Hz if a pressure controller with a lower response time is used.

DoD Reproducibility. We define reproducibility as the possibility to achieve consistent droplet parameters (size/volume) when the same pulse (same magnitude and duration) is applied to the dispersed phase input. Reproducibility of the droplet generation is crucial for measurements where the results of many single droplet experiments are averaged.[17] For the sake of consistency of our characterization, we use the pulse durations from Figure 2 to investigate the reproducibility. A set of 1000 droplets was generated for each pulse (Δt = {20,55,100,150,200} ms) and the mean value (μ) of the droplet area, as well as the standard deviation (σ), was measured for each set of droplets. The obtained results are shown in Figure 4. For all pulse durations, the standard deviation is less than 5% of the mean value, which confirms the high reproducibility of the proposed DoD method.
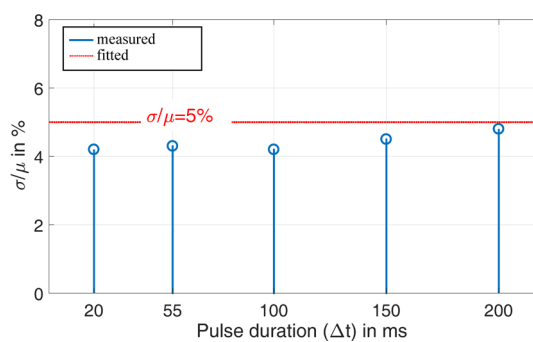

Figure 4. Reproducibility of the proposed DoD method. Standard deviation (σ) with respect to the mean value (μ) was measured for each pulse Δt = {20,55,100,150,200} ms. For all pulse durations, the standard deviation is less than 5% of the mean value.

Cascading Multiple DoDs. The uniqueness of the proposed DoD method lies in the possibility to connect two (or more) dispersed channels to a single continuous channel. This way, it is possible to (i) feed multiple reagents/samples on demand to the microfluidic chip, (ii) produce a large number of different droplets in very short times, and (iii) perform basic droplet operations, such as droplet mixing and reacting in a simple, rapid, and straightforward manner.

A microfluidic chip with two dispersed phase channels (two DoD generators) connected to a single continuous channel is shown in Figure 5. Enabling a high level of controllability over each dispersed channel, the DoD method allows synchronization of the connected channels in time. This way, it is possible to achieve time-controlled droplet merging and reacting immediately at the droplet generation site, yielding MoD.

The principle of MoD is illustrated in Figure 6 and can be described as follows. The precise time of droplets generated by DoD_1 to reach DoD_2 can be calculated as $\delta t = d/v$ (s) where d denotes the distance between DoD_1 and DoD_2 and v is the velocity of the droplet inside the continuous channel. In order to achieve MoD, the DoD_2 generator uses this traveling time (δt) for the precise timing of its activation. This means that if
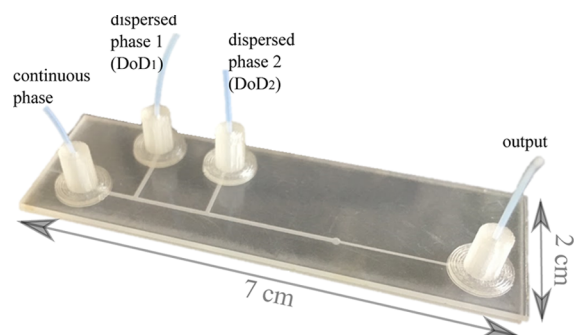
Figure 5. Microfluidic device with two DoD generators, i.e., two dispersed phase channels are connected to a single continuous channel.
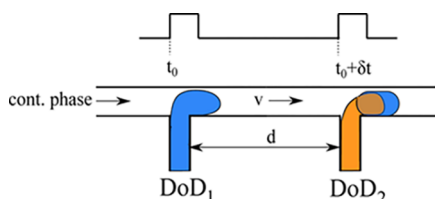
Figure 6. Principle of MoD as a promising application for cascading multiple DoDs.

the DoD_1 generator was activated at the moment $t = t_0$, then the DoD_2 generator will be activated at $t = t_0 + \delta t$. The complete system can be automated and once started does not require any manual control. More details on the MoD principle and the practical realization is provided in the Supporting Information (section 6, Figure S5, and Video S4).

The cascading of multiple DoDs not only allows the realization of MoD but also increases the generation frequency of a complete microfluidic device. For example, since two dispersed phase channels are used for the device in Figure 5, two DoD methods can be simultaneously applied to each dispersed phase channel where as shown before, each channel can support 33 Hz generation frequency. Supporting 33 droplets/s on each channel, the device with two cascaded channels can in principle provide a droplet generation frequency of 66 Hz. This means that by cascading more dispersed phase channels, the droplet generation frequency of the microfluidic device can be increased according to $f_{device} = n \times f_{max}$ where n denotes the number of cascaded DoD systems.[3] Such a device, with multiple cascaded DoD systems, is highly suitable for studies where precise and detailed investigation of chemical interactions between many samples needs to be carried out on a high number (thousands to millions) of droplets.

Possible Influence of Surfactants. Surfactants are used in many applications in order to stabilize the droplet generation process. Although we added no surfactants to either phase for the results reported in this work, we observed high stability in the generated droplet as shown in Figure 4. Nevertheless, if surfactants would be added, then we expect that the stability would be further increased. Moreover, it can be expected that due to surfactants, the presented MoD method only works under limited conditions. However, a detailed investigation on the behavior of the proposed DoD and MoD methods with surfactants would be very interesting. Since such an analysis is beyond the scope of this work, it would be an interesting future research direction.

CONCLUSIONS

We have proposed a simple and efficient method for off-chip-induced control over the droplet generation process. We have shown that positive pressure pulses applied to the dispersed phase can be employed on a T-junction geometry to generate droplets-on-demand. Moreover, for the first time, a method where sole control over the dispersed phase is required while the continuous phase remains at a constant input pressure was proposed. In particular, we have proposed a pressure model and presented practical on-chip realization of the novel DoD method. We have observed linear correlation between the duration of the applied positive pulse and the volume of the generated droplets. We have shown that the proposed method slightly exceeds the performance of other off-chip-controlled methods by enabling an operational frequency of 33 Hz and providing high reproducibility of droplets with standard deviations less than 5% of the mean value. For the first time, a DoD method where connecting of multiple dispersed phase inputs to a single continuous channel is possible was presented. Utilizing two DoD generators, the first realization of the merging-on-demand concept was presented. Moreover, the potential for increasing the droplet generation frequency by cascading multiple dispersed phase channels was highlighted.

EXPERIMENTAL SECTION

Chip Fabrication. The microfluidic device was constructed from PMMA (polymethylmethacrylate) sheets (Modulor, Germany) of 0.8 and 1.5 mm thicknesses. The process starts with a laser engraving the desired microfluidic design in the PMMA sheets using a CO_2 laser cutter (Trotec, Speedy 300). After laser engraving, the inlet holes for the fluid supply were drilled using a drilling machine (Proxxon, D-54343). In the next step, the PMMA sheets were cleaned with a clean-room tissue and later with isopropanol (Apatina, Austria) and pressurized air to remove dust. For sealing the channels and assembling the final device, a thin layer of ethanol was spread between the engraved PMMA layer and a PMMA cover layer until the interface area was completely wetted. The two layers were aligned, covered with a thermoconducting, protective membrane, and placed in a hot press (Unibind, Austria) that heats the chip only from one side at a temperature of 145 °C for 40 s. Using this rapid prototyping method, the droplet generation chip with continuous and dispersed channel widths of 400 and 200 μm, respectively, was fabricated. The height of the channels was measured to be 100 μm. The complete fabrication process can be seen in the Supporting Information (section 2, Video S1).[4]

Materials and Device Operation. Silicone oil (Rhodorsil, 47 V 350) was used as a continuous phase, and as a dispersed phase, deionized (DI) water with dissolved water colors (Thalia, Austria) was used. No surfactant was added to either phase. Each fluid was stored in a custom plastic container and fed in the chip using a pressure controller (Elveflow, OB1MK3). The microfluidic device was then mounted on a microscope (Leica, Germany; model S6D) and imaged with an integrated high-speed camera (Leica S6D digital camera). The complete system was monitored and controlled using a PC equipped with a pump control software (Elveflow, ESI). We measured different droplet parameters such as droplet area and droplet diameter using the open source droplet morphometry and velocimetry (DMV) software. For post-processing, we used the Matlab software. Moreover, for conducting all

experiments, we used a temperature-controlled laboratory, with low temperature variations of 0.1 °C. Due to these small variations and a stable-temperature environment, we assume that any changes in the physical properties of the liquid samples (viscosity, surface tension, and density) that could potentially affect the droplet generation process are negligible. The schematic of the experimental setup is shown in Figure 7. More details on the experimental setup is provided in the Supporting Information (section 2, Figure S1).
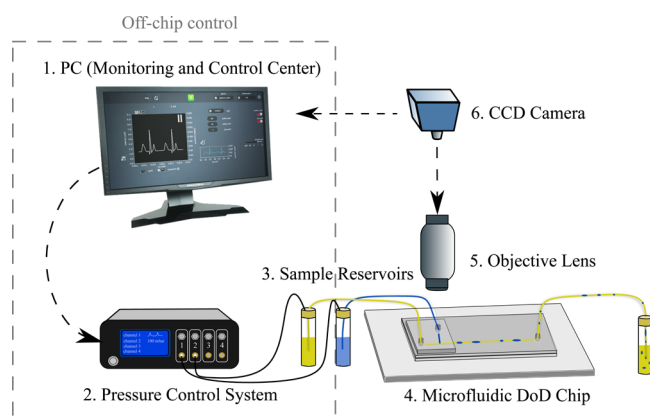
Figure 7. Schematic of the complete system required for DoD generation. Using the PC, a pressure controller is activated to supply fluids from the reservoirs to the DoD chip. The droplet generation process is followed using the CCD camera and objective lenses. The imaging data from the camera is sent back to the monitoring center for further processing.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03883.

> (Section 1, Table S1) State-of-the-art DoD methods; (Section 2, Figure S1, and Tables S2−S4) rapid prototyping and experimental setup; (Section 3, Figure S2, and Table S5) T-junction geometry; (Section 4, Figure S3, and Table S6) theoretical pressure model; (Section 5 and Figure S4) DoD validation; (Section 6 and Figure S5) merging-on-demand (MoD) (PDF)
>
> (Video S1) Fast prototyping of microfluidic devices (AVI)
>
> (Video S2) Droplet-on-demand generation by tuning of the input pressure of both phases (AVI)
>
> (Video S3) Droplet-on-demand generation by varying the applied positive pressure pulses (AVI)
>
> (Video S4) Merging-on-demand (AVI)

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https://pubs.acs.org/10.1021/acsomega.9b03883

Notes

The authors declare no competing financial interest.

ADDITIONAL NOTES

[1] More details on these methods can be found in the Supporting Information (section 1, Table S1).
[2] More details on these methods can be found in the Supporting Information (section 1, Table S1).
[3] Please note that the complete characterization of this approach is beyond the scope of this work.
[4] Please note that for a PDMS T-junction droplet generator, a simple and low-cost fabrication method based on 3D printing has been proposed.[18]

REFERENCES

(1) Mashaghi, S.; Abbaspourrad, A.; Weitz, D. A.; van Oijen, A. M. Droplet microfluidics: a tool for biology, chemistry and nano-technology. *TrAC, Trends Anal. Chem.* **2016**, *82*, 118−125.

(2) Vincent, M. E.; Liu, W.; Haney, E. B.; Ismagilov, R. F. Microfluidic stochastic confinement enhances analysis of rare cells by isolating cells and creating high density environments for control of diffusible signals. *Chem. Soc. Rev.* **2010**, *39*, 974−984.

(3) Avraham, R.; Haseley, N.; Brown, D.; Penaranda, C.; Jijon, H. B.; Trombetta, J. J.; Satija, R.; Shalek, A. K.; Xavier, R. J.; Regev, A.; Hung, D. T. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell* **2015**, *162*, 1309−1321.

(4) Akselband, Y.; Cabral, C.; Shapiro, D. S.; McGrath, P. Rapid mycobacteria drug susceptibility testing using Gel microdrop (GMD) growth assay and ow cytometry. *J. Microbiol. Methods* **2005**, *62*, 181−197.

(5) Price, A. K.; Paegel, B. M. Discovery in droplets. *Anal. Chem.* **2016**, *88*, 339−353.

(6) Baret, J.-C.; Miller, O. J.; Taly, V.; Ryckelynck, M.; El-Harrak, A.; Frenz, L.; Rick, C.; Samuels, M. L.; Hutchison, J. B.; Agresti, J. J.; Link, D. R.; Weitz, D. A.; Griffiths, A. D. Fluorescence-activated

droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. *Lab Chip* **2009**, *9*, 1850−1858.

(7) De Leo, E.; Galluccio, L.; Lombardo, A.; Morabito, G. Networked Labs-on-a-Chip (NLoC): Introducing networking technologies in microfluidic systems. *Nano Commun. Networks* **2012**, *3*, 217−228.

(8) De Leo, E.; Donvito, L.; Galluccio, L.; Lombardo, A.; Morabito, G.; Zanoli, L. M. Communications and switching in microfluidic systems: pure hydrodynamic control for networking labs-on-a-chip. *IEEE Trans. Commun.* **2013**, *61*, 4663−4677.

(9) Donvito, L.; Galluccio, L.; Lombardo, A.; Morabito, G. μ-NET: a network for molecular biology applications in microfluidic chips. *IEEE/ACM Trans. Networking* **2016**, *24*, 2525−2538.

(10) Zeng, S.; Li, B.; Su, X.; Qin, J.; Lin, B. Microvalve-actuated precise control of individual droplets in microfluidic devices. *Lab Chip* **2009**, *9*, 1340−1343.

(11) Babahosseini, H.; Misteli, T.; DeVoe, D. L. Microfluidic on-demand droplet generation, storage, retrieval, and merging for single-cell pairing. *Lab Chip* **2019**, *19*, 493−502.

(12) Teo, A. J. T.; Li, K.-H. H.; Nguyen, N.-T.; Guo, W.; Heere, N.; Xi, H.-D.; Tsao, C.-W.; Li, W.; Tan, S. H. Negative pressure induced droplet generation in a microfluidic flow-focusing device. *Anal. Chem.* **2017**, *89*, 4387−4391.

(13) Churski, K.; Nowacki, M.; Korczyk, P. M.; Garstecki, P. Simple modular systems for generation of droplets on demand. *Lab Chip* **2013**, *13*, 3689−3697.

(14) Song, H.; Chen, D. L.; Ismagilov, R. F. Reactions in droplets in microfluidic channels. *Angew. Chem., Int. Ed.* **2006**, *45*, 7336−7356.

(15) Garstecki, P.; Fuerstman, M. J.; Stone, H. A.; Whitesides, G. M. Formation of droplets and bubbles in a microfluidic T-junction scaling and mechanism of break-up. *Lab Chip* **2006**, *6*, 437−446.

(16) Basu, A. S. Droplet morphometry and velocimetry (DMV): a video processing software for time-resolved, label-free tracking of droplet parameters. *Lab Chip* **2013**, *13*, 1892−1901.

(17) Vaughn, B. S.; Tracey, P. J.; Trevitt, A. J. Drop-on-demand microdroplet generation: a very stable platform for single-droplet experimentation. *RSC Adv.* **2016**, *6*, 60215−60222.

(18) Donvito, L.; Galluccio, L.; Lombardo, A.; Morabito, G.; Nicolosi, A.; Reno, M. Experimental validation of a simple, lowcost, T-junction droplet generator fabricated through 3D printing. *J. Micromech. Microeng.* **2015**, *25*, No. 035013.