RESEARCH ABSTRACTS

Lab-on-a-Chip

Hydrodynamic-Flow-Enhanced Rapid Mixer for Isothermal DNA Hybridization Kinetics Analysis on Digital Microfluidics Platform

Mingzhong Li, Cheng Dong, Man-Kay Law, Yanwei Jia, Pui-In Mak, Rui P. Martins

FEATURES

Automated isothermal DNA hybridization kinetics on complete DMF platform

Stable droplet temperature (within ±0.1oC)

Rapid mixer with slow frequency AC actuation

Faster reaction rate than pure diffusion (>13×)

DESCRIPTION

This paper presents a DMF platform that can perform isothermal hydrodynamic-flow-enhanced droplet mixing to enhance the hybridization efficiency while ensuring a stable droplet temperature (within ±0.1℃). Specifically, with a single electrode, droplet-boundary oscillation under a slow AC actuation is studied for improving the reaction rate. The dependencies between the mixing efficiency and the actuation voltage, actuation frequency and the spacer thickness are also systematically studied. Reliable mixing efficiency improvement is further validated over a wide range of solute concentrations. The results from real-time on-chip DNA hybridization kinetics with stationary droplets using the complete sandwiched DMF system shows that the proposed rapid mixer can achieve the same hybridization equilibrium with >13 times faster reaction rate when compared to the reference one through pure diffusion, while preventing biased hybridization kinetics as demonstrated in the electrothermal technique.

Benefitting from the mechanical mixing process, the proposed method can achieve efficient mixing independently of the solute concentrations for flexible hybridization experiments. The negligible temperature change is also favourable for the discrimination of matched and mismatched binding for the DNA hybridization kinetics investigation.

Based on the hybridization experiments of Kras gene and its corresponding molecular beacon probe in a complete DMF platform, the hybridization equilibrium is more readily and accurately achieved with the proposed micro-mixing technique over the diffusion and the electrothermal counterparts on DMF platform.

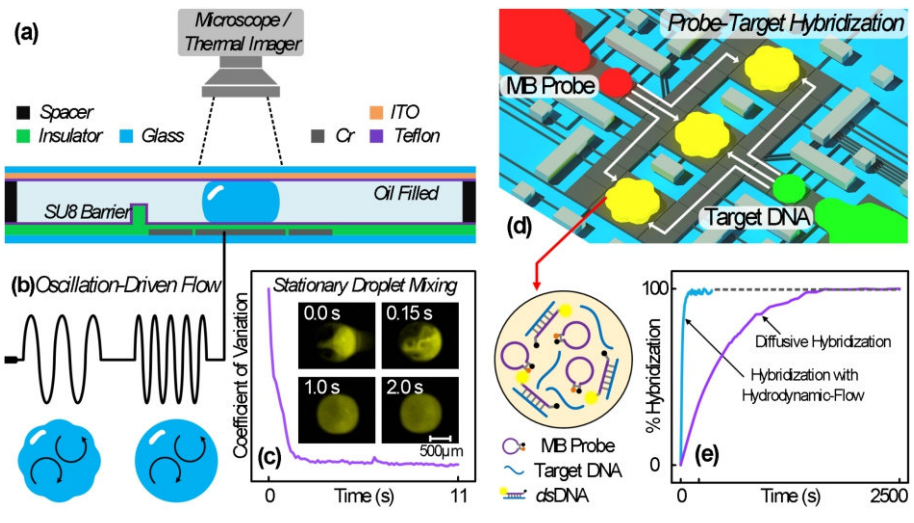

Fig. 1. (a) Sandwiched DMF device monitored by fluorescence microscope and thermal imager. (b) Droplet oscillation patterns and internal hydrodynamic flow at sub-kHz actuation frequency. (c) Stationary droplet mixing with 1-μM fluorophore DNA probe and 10-mM Tris-HCl buffer. (d) SE isometric view with the molecular beacon probe and target DNA dispensed and driven to the reaction chambers. (e) Real-time DNA hybridization kinetics through fluorescence with passive diffusion and the proposed hydrodynamic-flow enhancement method.

Publication(s)

[1] M. Li, C. Dong, M. K. Law, Y. Jia, P. I. Mak and R. P. Martins, “Hydrodynamic-flow-enhanced rapid mixer for isothermal DNA hybridization kinetics analysis on digital microfluidics platform,” Sensors and Actuators B: Chemical, vol. 287, pp. 390-397, May 2019.

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau.

A 3D microblade structure for precise and parallel droplet splitting on digital microfluidic chips

Cheng Dong, Yanwei Jia, Jie Gao, Tianlan Chen, Pui-In Mak and Rui P. Martins

FEATURES

3D microstructures on DMF chip

Multiple droplet splitting in one step

Parallel DNA analysis on-chip

DESCRIPTION

Existing digital microfluidic (DMF) chips exploit the electrowetting on dielectric (EWOD) force to perform droplet splitting. However, the current splitting methods are not flexible and the volume of the droplets suffers from a large variation. Herein, we propose a DMF chip featuring a 3D microblade structure to enhance the droplet-splitting performance. By exploiting the EWOD force for shaping and manipulating the mother droplet, we obtain an average dividing error of <2% in the volume of the daughter droplets for a number of fluids such as deionized water, DNA solutions and DNA–protein mixtures. Customized droplet splitting ratios of up to 20 : 80 are achieved by positioning the blade at the appropriate position. Additionally, by fabricating multiple 3D microblades on one electrode, two to five uniform daughter droplets can be generated simultaneously. Finally, by taking synthetic DNA targets and their corresponding molecular beacon probes as a model system, multiple potential pathogens that cause sepsis are detected rapidly on the 3D-blade-equipped DMF chip, rendering it as a promising tool for parallel diagnosis of diseases.

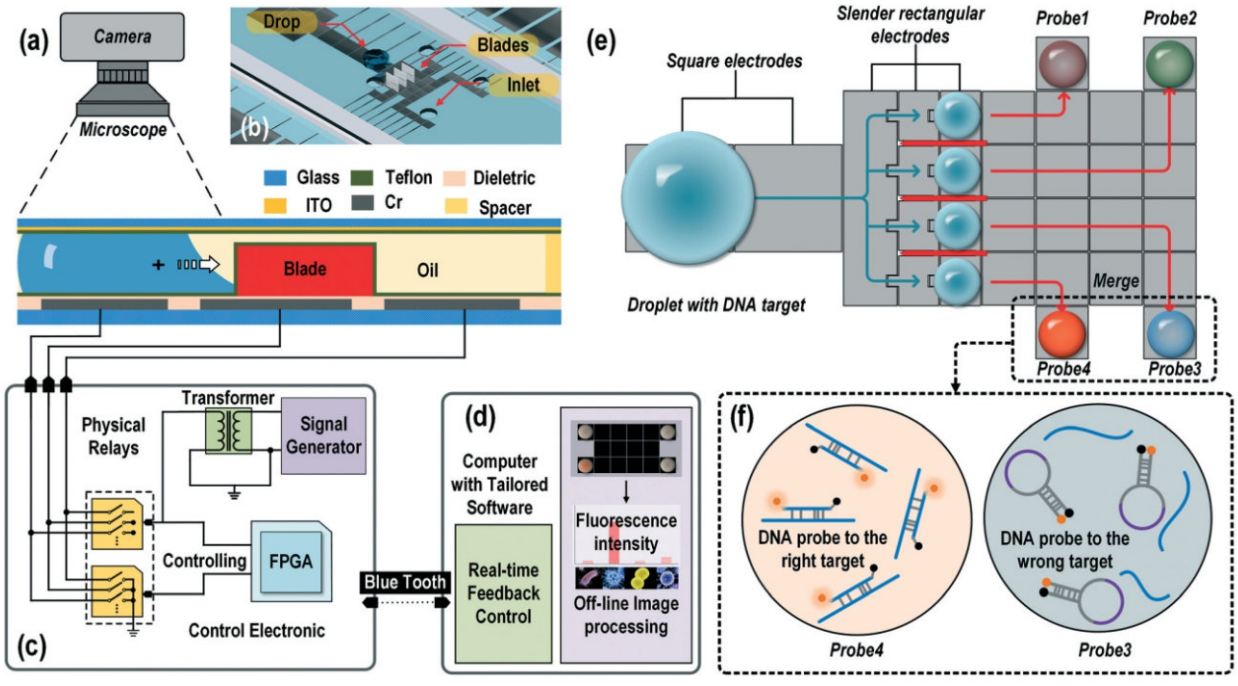

Fig. 1. Digital microfluidic device embedded with on-chip 3D blades for precise quantitative droplet splitting

Publication(s)

[1] C. Dong, Y. W. Jia, J. Gao, T. L. Chen, P. I. Mak, M. I. Vai and R. P. Martins, A 3D microblade structure for precise and parallel droplet splitting on digital microfluidic chips, Lab on a Chip, 17, 896-904, 2017.

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau

A digital microfluidic system for loop-mediated isothermal Amplification and sequence specifc pathogen detection

Liang Wan, Tianlan Chen, Jie Gao, Cheng Dong, Ada Hang-Heng Wong, Yanwei Jia, Pui-In Mak, Chu-Xia Deng, Rui Martins

FEATURES

Isothermal DNA amplification on digital microfluidics

Specific DNA detection

No false positive DNA detection

DESCRIPTION

A digital microfluidic (DMF) system has been developed for loop-mediated isothermal amplification (LAMP)-based pathogen nucleic acid detection using specifc low melting temperature (Tm) Molecular Beacon DNA probes. A positive-temperature-coefcient heater with a temperature sensor for real-time thermal regulation was integrated into the control unit, which generated actuation signals for droplet manipulation. To enhance the specifcity of the LAMP reaction, low-Tm Molecular Beacon probes were designed within the single-stranded loop structures on the LAMP reaction products. In the experiments, only 1 µL of LAMP reaction samples containing purifed Trypanosoma brucei DNA were required, which represented over a 10x reduction of reagent consumption when comparing with the conventional off-chip LAMP. On-chip LAMP for unknown sample detection could be accomplished in 40 min with a detection limit of 10 copies/reaction. Also, we accomplished an on-chip melting curve analysis of the Molecular Beacon probe from 30 to 75 °C within 5 min, which was 3x faster than using a commercial qPCR machine. Discrimination of non-specifc amplifcation and lower risk of aerosol contamination for on-chip LAMP also highlight the potential utilization of this system in clinical applications. The entire platform is open for further integration with sample preparation and fluorescence detection towards a total-micro-analysis system

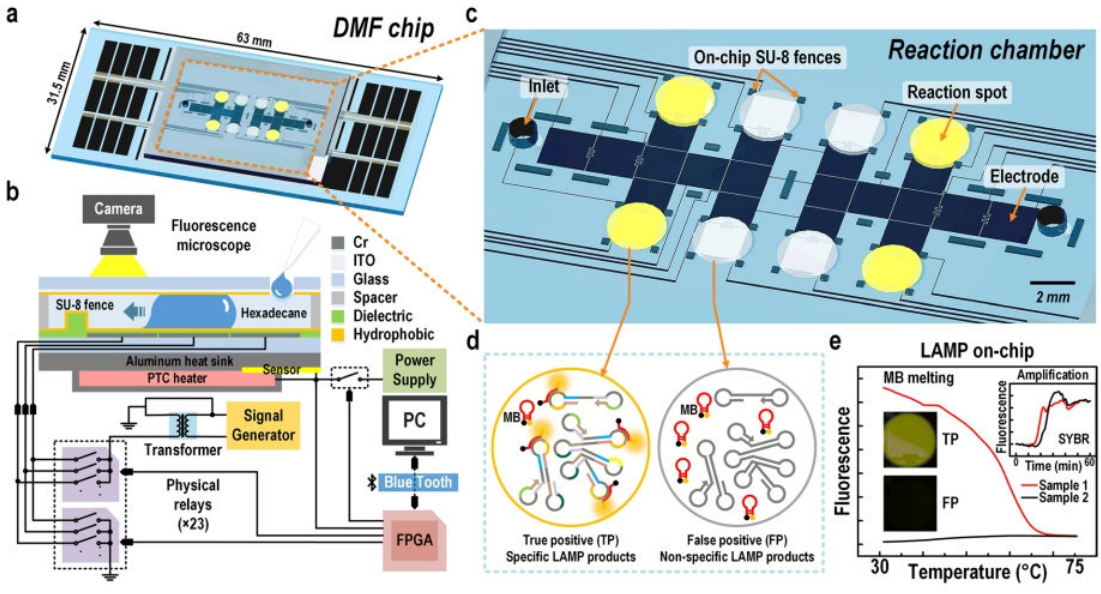

Fig. 1. Overview of the digital microfluidic system for loop-mediated isothermal amplification (LAMP) reaction and melting curve analysis using Molecular Beacon DNA probes.

Publication(s)

[1] L. Wan, T. L. Chen, J. Gao, C. Dong, A. H. H. Wong, Y. W. Jia*, P. I. Mak, C. X. Deng and R. Martins, A digital microfluidic system for loop-mediated isothermal amplification and sequence specific pathogen detection, Scientific Reports, 7, 14586, 2017.

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau

A digital microfluidic system with 3D microstructures for single-cell culture

Jiao Zhai, Haoran Li, Ada Hang-Heng Wong, Cheng Dong, Shuhong Yi, Yanwei Jia, Pui-In Mak, Chu-Xia Deng, Rui P. Martins

FEATURES

3D microstructures on digital microfluidic chip
Low voltage for drop actuation
Single cell drug screening
No evaporation of drop on-chip

DESCRIPTION

Despite the precise controllability of droplet samples in digital microfluidic (DMF) systems, their capability in isolating single cells for long-time culture is still limited: typically, only a few cells can be captured on an electrode. Although fabricating small-sized hydrophilic micropatches on an electrode aids single-cell capture, the actuation voltage for droplet transportation has to be significantly raised, resulting in a shorter lifetime for the DMF chip and a larger risk of damaging the cells. In this work, a DMF system with 3D microstructures engineered on-chip is proposed to form semiclosed micro-wells for efficient single-cell isolation and long-time culture. Our optimum results showed that approximately 20% of the micro-wells over a 30 × 30 array were occupied by isolated single cells. In addition, lowevaporation-temperature oil and surfactant aided the system in chieving a low droplet actuation voltage of 36V, which was 4 times lower than the typical 150 V, minimizing the potential damage to the cells in the droplets and to the DMF chip. To exemplify the technological advances, drug sensitivity tests were run in our DMF system to investigate the cell response of breast cancer cells (MDA-MB-231) and breast normal cells (MCF-10A) to a widely used chemotherapeutic drug, Cisplatin (Cis). The results on-chip were consistent with those screened in conventional 96-well plates. This novel, simple and robust single-cell trapping method has great potential in biological research at the single cell level.

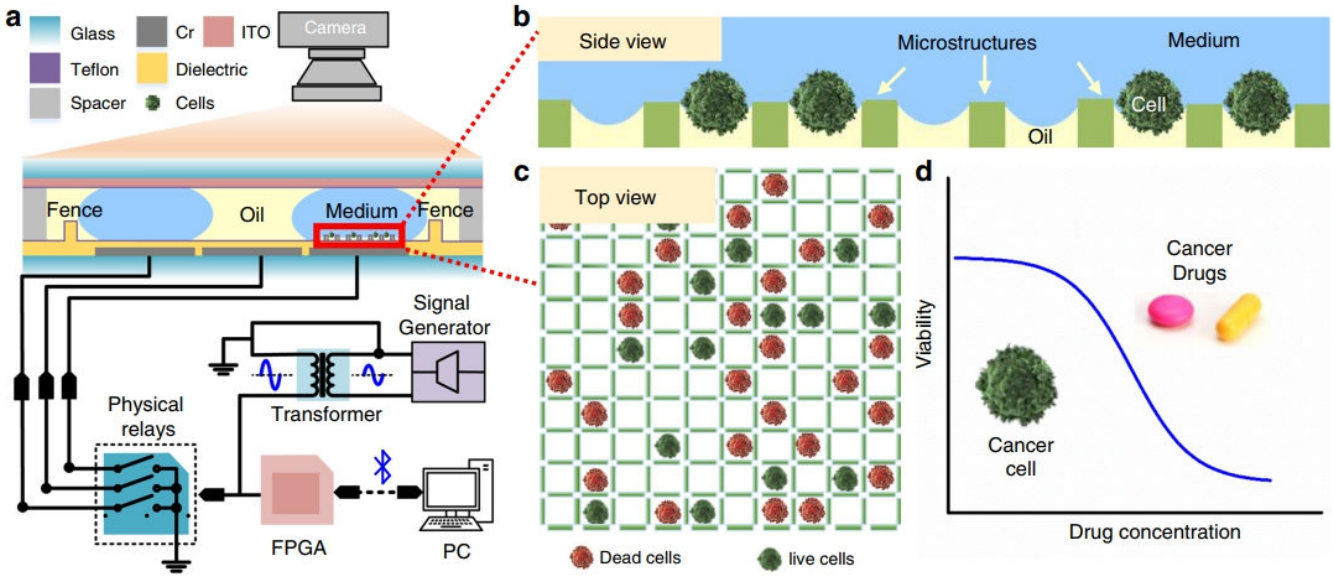

Fig. 1. Schematic of the digital microfluidic system for single cell culture and drug toxicity tests

Publication(s)

[1] J. Zhai, H. R. Li, A. H. H. Wong, C. Dong, S. H. Yi, Y. W. Jia*, P. I. Mak, C. X. Deng and R. P. Martins, A digital microfluidic system with 3D microstructures for single-cell culture, Microsystems and Nanoengineering, 6, 6, 2020.

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau

Clip-to-release on amplification (CRoA): a novel DNA amplification enhancer on and off microfluidics

Ren Shen, Yanwei Jia, Pui-In Mak and Rui P. Martins

FEATURES

Novel reagent for DNA amplification on/off microfluidics

10 x brighter amplification signal

No false negative results

DESCRIPTION

Despite its high sensitivity, low cost, and high efficiency as a DNA amplification indicator with a yes/no answer, dsDNA-binding dye encounters incompatibility when used in microfluidic systems, resulting in problems such as false negative amplification results. Besides, its inhibition of amplification at high concentrations hinders its application both on-chip and off-chip. In this study, we propose a novel DNA amplification enhancer to counteract the drawbacks of dsDNA-binding dyes. It acts as a temporary reservoir for the free-floating dyes in solution and releases them on demand during the amplification process. Through this clip-to-release on amplification mechanism, the enhancer lowered the background fluorescence of sample droplets before amplification, enhanced the signal-to-background ratio of positive samples, and eliminated the false negative signal of on-chip PCR. Moreover, the enhancer increased the off-chip polymerase chain reaction (PCR) efficiency, boosted the fluorescence signal up to 10-fold, and made less nonspecific amplification product. All the factors affecting the enhancer's performance are investigated in detail, including its structure and concentration, and the types of dsDNA-binding dye used in the reaction. Finally, we demonstrated the broad application of the proposed amplification enhancer in various DNA amplification systems, for various genes, and on various amplification platforms. It would reignite the utilization of dsDNA dyes for wider applications in DNA analysis both on-chip and off-chip.

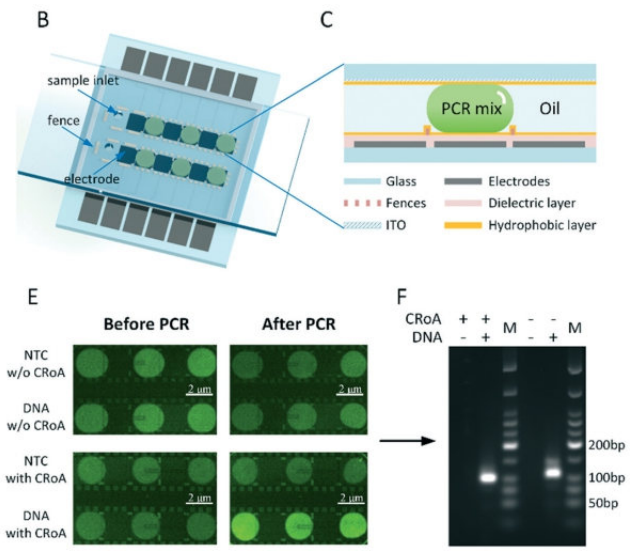

Fig. 1. proof of principle experiments of the CRoA PCR enhancing function for on-chip PCR.

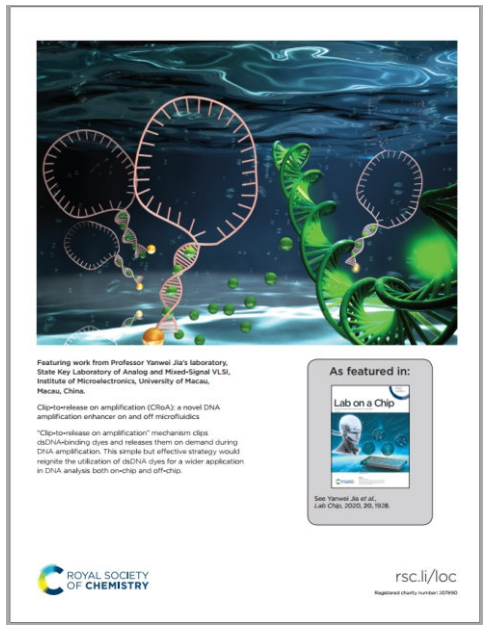

Fig. 2. Back Cover featured story in Lab on a Chip.

Publication(s)

[1] R. Shen, Y. W. Jia*, P. I. Mak, and R. P. Martins, Clip to release on amplification (CRoA): a novel enhancer for DNA amplification on and off microfluidics, Lab on a Chip, 20, 1928-1938, 2020 (Outside Back Cover).

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau

Drug screening of cancer cell lines and human primary tumors using droplet microfluidics

Ada Hang-Heng Wong, Haoran Li, Yanwei Jia, Pui-In Mak, Rui P. Martins, Yan Liu, Chi Man Vong, Hang Cheong Wong, Pak Kin Wong, Haitao Wang, Heng Sun, Chu-Xia Deng

FEATURES

Droplet microfluidics
High throughput multiple drug screening on-chip
Primary tumor drug screening
Precision medicine

DESCRIPTION

Precision Medicine in Oncology requires tailoring of therapeutic strategies to individual cancer patients. Due to the limited quantity of tumor samples, this proves to be difcult, especially for early stage cancer patients whose tumors are small. In this study, we exploited a 2.4 ×2.4 centimeters polydimethylsiloxane (PDMS) based microfluidic chip which employed droplet microfluidics to conduct drug screens against suspended and adherent cancer cell lines, as well as cells dissociated from primary tumor of human patients. Single cells were dispersed in aqueous droplets and imaged within 24 hours of drug treatment to assess cell viability by ethidium homodimer 1 staining. Our results showed that 5 conditions could be screened for every 80,000 cells in one channel on our chip under current circumstances. Additionally, screening conditions have been adapted to both suspended and adherent cancer cells, giving versatility to potentially all types of cancers. Hence, this study provides a powerful tool for rapid, low-input drug screening of primary cancers within 24 hours after tumor resection from cancer patients. This paves the way for further technological advancement to cutting down sample size and increasing drug screening throughput in advent to personalized cancer therapy.

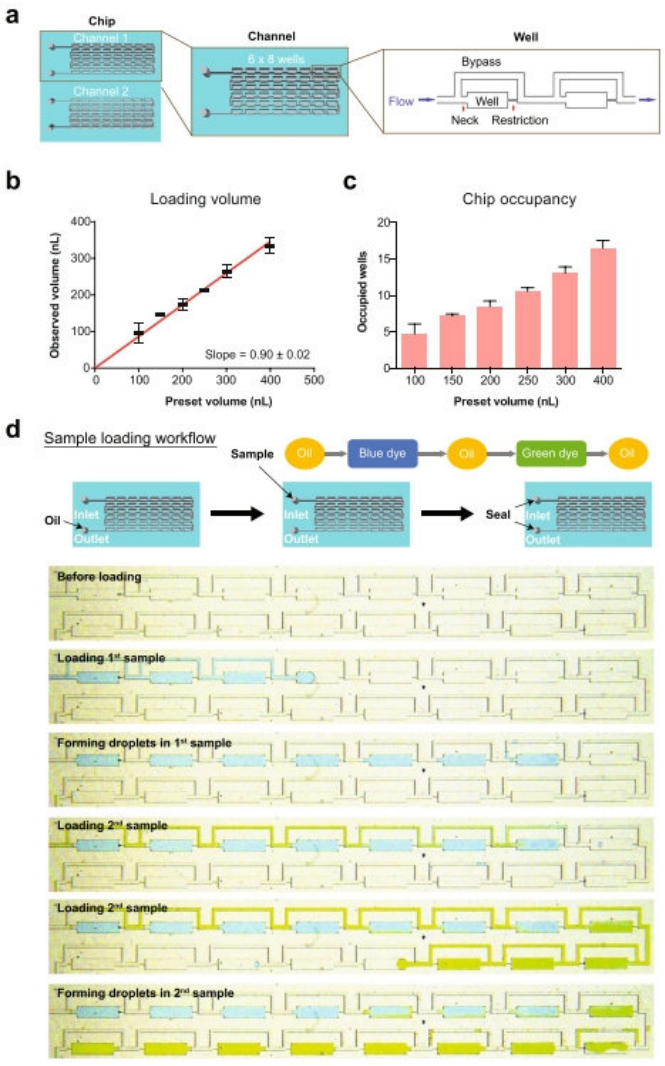

Fig. 1. Microfluidic chip design and validation

Publication(s)

[1] A. H. H. Wong, H. R. Li, Y. W. Jia, P. I. Mak, R. P. Martins, Y. Liu, C. M. Vong, H. C. Won, P. K. Wong, H. T. Wang, H. Sun, C. X. Deng, Drug screening of cancer cell lines and human primary tumors using droplet microfluidics, Scientific Reports, 7, 9109, 2017.

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau

Turning on/off satellite droplet ejection for flexible sample delivery on digital microfluidics

Haoran Li, Ren Shen, Cheng Dong, Tianlan Chen, Yanwei Jia, Pui-In Mak and Rui P. Martins

FEATURES

Sample delivery on digital microfluidic chip

Easy electric controlled sample delivery

Wide range from pL to nL

DESCRIPTION

Digital microfluidics has the potential to minimize and automate reactions in biochemical labs. However, the complexity of drop manipulation and sample preparation on-chip has limited its incorporation into daily workflow. In this paper, we report a novel method for flexible sample delivery on digital microfluidics in a wide volume range spanning four orders of magnitude from picoliters to nanoliters. The method is based on the phenomenon of satellite droplet ejection, triggered by a sudden change in the strength of the electric field across a drop on a hydrophobic dielectric surface. By precisely modulating the actuation signal with convenient external electric controls, satellite droplet ejection can be turned on to dispense samples or turned off to transport picking-up drops. A pico-dosing design is presented and validated in this work to demonstrate the direct and flexible on-chip sample delivery. This approach could pave the way for the acceptance of microfluidics as a common platform for daily reactions to realize lab-on-a-chip.

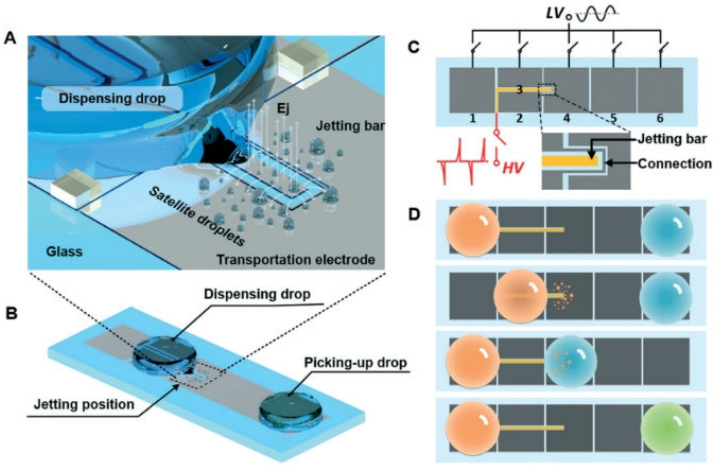

Fig. 1. Design of the pico-dosing technique on digital microfluidics

Fig. 2. Front Cover featured story in Lab on a Chip.

Publication(s)

[1] H. R. Li, R. Shen, Y. W. Jia*, P. I. Mak, R. P. Martins, Turning on/off satellite droplet ejection for flexible sample delivery on digital microfluidics, Lab on a Chip, 20,3709-3719, 2020 (Inside Front Cover).

Sponsorship

Macau Science and Technology Development Fund (FDCT), Research Committee of University of Macau