

Large-area electronics enabled high resolution digital microfluidics for single cell manipulations

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Abstract

Thin-film semiconductor devices as switching elements represent an ideal match for electrode-array-based digital microfluidics. With the support of large-area electronics technology, high-resolution digital droplets (diameter around 100 μm) containing single cells can be generated by pre-programmable addressing signals. Single-cell generation and manipulation is foundational to single-cell research, which demands tools that are easy to operate, multifunctional, and accurate. Herein, we report an active-matrix digital microfluidic platform for single-cell generation and manipulation enabled by large-area electronics technology. The active device contains 26,368 electrodes that can be independently addressed to perform parallel and simultaneous manipulation of droplets and even single cells. An on-chip-generated single-droplet volume limit of 500 pL has been reported, proving continuous and stable movement of droplets containing cells for over 1 hour. Furthermore, the success rate of single-droplet formation can exceed 98%, with the capability to generate approximately 10 single cells within 10 seconds. A pristine single-cell generation rate of 29% was achieved without any further sorting process.

Full Text

Preamble

Large-Area Electronics Enabled High-Resolution Digital Microfluidics for Single-Cell Manipulations

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Abstract

Thin-film semiconductor devices serve as ideal switching elements for electrode-array-based digital microfluidics. Leveraging large-area electronics technology, high-resolution digital droplets (approximately 100 μm in diameter) containing single cells can be generated through pre-programmable addressing signals. Single-cell generation and manipulation represent a foundation of single-cell research, demanding tools that are easy to operate, multifunctional, and accurate. Herein, we report an active-matrix digital microfluidic platform for single-cell generation and manipulation enabled by large-area electronics technology. The active device comprises 26,368 electrodes that can be independently addressed to perform parallel and simultaneous manipulation of droplets and even single cells. An on-chip generated single droplet volume limit of 500 pL has been demonstrated, with continuous and stable droplet movement containing cells for over 1 hour. Furthermore, the success rate of single droplet formation exceeds 98%, enabling generation of approximately 10 single cells within 10 seconds. A pristine single-cell generation rate of 29% is achieved without any further sorting process.

Keywords: Digital microfluidics; Active-matrix; Single cell manipulation; Large-area electronics

1. Introduction

Due to cellular heterogeneity in biological sciences, medicine, and biology, it is often necessary to isolate specific single cells from complex samples for subsequent scientific research or industrial production. For instance, in cancer research, isolating single cancer cells is a prerequisite for single-cell sequencing (1-3). In biomanufacturing cell lines, selecting a single cell that can derive sub-clones is essential for ensuring stable cell line acquisition (4, 5). However, single cells have extremely small volumes, typically in the nanoliter or picoliter scale, making precise control difficult to achieve with conventional laboratory methods. A complete single-cell analysis process generally requires three main steps: (1) cell manipulation, such as capture, selection, or sorting; (2) cell processing, such as transfection, injection, or lysis; and (3) detection of physical and chemical properties, homeostatic conditions, or functional responses. Overall, current single-cell research remains in its infancy, primarily limited by platform technology, which prevents many advantages of single-cell research from being fully realized (6, 7).

In response to the needs of single-cell research, numerous solutions for single-cell sorting and manipulation have emerged. Microfluidic technology, capable of handling microliter or even nanoliter-level liquid manipulation, has evolved into a platform-level technology for single-cell manipulation and analysis (8-10). Microfluidic technology offers many advantages unmatched by conventional bench-top methods. For cell sorting and manipulation, researchers have discovered and applied various microfluidic approaches, including fluidic (11), electrical (12-14), optical (15-17), magnetic (18), and acoustic (19, 20) methods. Electrowetting-on-dielectric (EWOD) digital microfluidic (DMF) technology, based on electrical principles, enables free manipulation of nanoliter or even picoliter droplets in a two-dimensional plane (21, 22), making it a highly promising platform for single-cell sorting and manipulation. Compared to other microfluidic technologies, EWOD DMF has demonstrated unique capabilities for integrating downstream analysis with single-cell sorting and manipulation (13, 23). The parallel-plate structure of EWOD-DMF systems allows easy integration of real-time feedback technology over relatively large areas based on optical or electrical properties (14, 24-27).

In single-cell research applications, high-throughput parallel manipulation and analysis of single cells can be achieved, enabling development of multiple time- and space-resolved analysis methods. Many studies have demonstrated that EWOD DMF can be applied to analyze suspended or adherent single cells (13, 23, 28). However, these studies are primarily based on DMF chips with passive electrode arrays (29), where each electrode is physically connected to a peripheral switch. The total number of electrodes in such DMF systems is typically less than 200, making scalability a true obstacle for advancing this technology in single-cell research (30, 31).

Active-matrix (AM) technology, which integrates thin-film transistors into each pixel to achieve control in large arrays, is widely used in flat-panel displays (32). Applying AM technology to EWOD presents an ideal solution to overcome the limitations of passive electrode arrays (33, 34). While a few groups have applied AM-DMF in molecular diagnostics (35, 36), few reports exist on its application in single-cell studies. To generate tiny droplets containing single cells, the resolution of EWOD electrodes and droplet diameter must reach approximately 100 nm scale (one order of magnitude larger than typical mammalian cells). This rigorous requirement can only be achieved through careful optimization of pixel circuits, drive signals, and material combinations in an AM-DMF device. In this work, we report an AM-DMF platform for high-efficiency single-cell generation and manipulation, providing a unique solution to the challenge of efficiently obtaining single-cell samples in current single-cell research. This method can be extended and universally applied in single-cell analysis research and integrated with many single-cell research tools to obtain single-cell information and applications across multiple dimensions.

2.1 AM-DMF Chip Design and Fabrication

The chip design is illustrated in Fig 1(b)-(d). The bottom glass plate with a thin-film transistor (TFT) electrode array was fabricated on a 0.5 mm glass substrate using LCD manufacturing processes, produced by Tianma Microelectronics (Shenzhen, China). The complete AM-DMF chip consists of an AM-DMF device array, a spacer, and a conductive top plate with a hole for liquid sample injection. The cross-sectional view of the chip is shown in Fig S1. The AM-DMF array obtained from Tianma already featured 1T-1C pixels and an electrowetting dielectric layer of 300 nm SiNx, after which we spin-coated the hydrophobic layer in our laboratory.

We primarily tested CYTOP (from Asahi Glass Company) dissolved in different solvents, including FC43 (from 3M), KBE-903 (from Shin-Etsu Chemical), Solv 180 (from Asahi Glass Company), and another hydrophobic material, Teflon (from Chemours Company), as shown in Fig 2(b). A plastic spacer was used to define a 30 μm gap between the top ITO-coated glass plate and the bottom plate. The ITO layer on the cover glass was also spin-coated with a hydrophobic layer, and the top and bottom hydrophobic layers were encapsulated facing each other. The conductive ITO was connected to system ground through silver glue. A custom-designed flexible printed circuit (FPC) was then attached to the chip for connection with the control board. The AM-DMF chip assembly and final cartridge construction are shown in Fig S1.

2.2 The AM-DMF System Setup

The AM-DMF system comprised four main components: the AM-DMF chip, the electronic control board, custom-written control software, and the optical detection module, as shown in Fig S2. The AM-DMF chip was fixed in a 3D-printed test fixture holder that contained the electronic control board. The software was used to acquire droplet generation and position information, with functions including drive signal modulation (voltage, frequency, time, etc.), droplet motion path generation and execution, and more. For the optical detection system, we established our own setup. The main frame, imaging tube, narrow-band filter, dichroic lens, and other components were purchased from Thorlabs Inc., while the microscopy objective lens was purchased from Nikon Inc. The imaging camera used was an sCMOS camera (C13440, Hamamatsu, Japan).

2.3 Reagents

Ethanol, acetone, and IPA were purchased from Sinopharm Chemical Reagent Ltd Co and primarily used for washing the chip. Silicone oil (5 cSt) and the surfactant Pluronic F68 were purchased from Sigma Aldrich (Oakville, ON, USA). HEK 293 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Modified Eagle's Medium (MEM), fetal bovine serum (FBS), trypsin-EDTA, and phosphate buffer solution (PBS) were purchased from Gibco. Cell viability was determined using a calcein/propidium iodide dual-staining as-

say (Invitrogen, Molecular Probes). All chemicals were used as received without further purification. Deionized (DI, $18.2 \text{ M}\Omega \cdot \text{cm}$) water used in all studies was purified using a Milli-Q water purification system.

2.4 Cell Culture

HEK 293 cells were cultured in a cell culture incubator (5% CO_2 , saturated humidity, 37°C). The growth medium for HEK 293 cells was MEM containing 10% FBS, 100 g/mL penicillin, and 100 g/mL streptomycin. Cell lines were passaged every 2-3 days at 10^5 cells/mL per cm^2 . Prior to experiments, cells were dissociated and resuspended in fresh complete medium. Cell number and viability were measured using a cell counting plate (177-112C, Waston, Japan) and calcein/propidium iodide dual-staining assay kit.

2.5 Single-Cell Manipulation on the AM-DMF Chip

Before beginning the experiment, it is necessary to start the software to establish communication with the AM-DMF chip hardware system and edit the single-cell droplet generation path based on the One-to-Two method. Silicone oil is then injected into the chip through the injection hole. Next, we prepare the cell suspension for single-cell sorting. First, cells are digested from the culture flask with trypsin. After centrifugation at 1000 rpm for 5 min, the supernatant is removed and the cells are resuspended in 1 mL of PBS. We then count the cells and configure four concentrations of cell suspensions according to experimental requirements: 10^5 , 5×10^4 , 10^4 , and 5×10^3 cells/mL. Next, we add 0.01% (v/v) F68 surfactant to the cell suspension to enable more stable cell movement in the AM-DMF chip. We then run the sample injection program on the software, which applies a driving voltage to the electrodes around the sample injection port, rendering them hydrophilic to facilitate smooth loading of the cell suspension. Additionally, live-cell tracer dye analysis is used to assess cell viability on- and off-chip. We then start running the automated path for cell sorting, enabling single-cell droplet generation. In this study, we repeated each single-cell droplet generation experiment more than three times.

3.1 Cartridge and Control System Development of AM-DMF System

To build a platform for efficient and high-throughput single-cell sample preparation that integrates single-cell sorting, manipulation, parallel automated handling of picoliter-volume droplets, and optical detection, we developed an AM-DMF system to address the challenges of single-cell sorting and manipulation, as shown in Fig 1. The AM-DMF chip consists of two parallel glass plates: the bottom plate contains a thin-film transistor (TFT) electrode array, while the upper plate is ITO conductive glass with sample injection holes. The upper and lower layers are separated by a gap spacer (Fig S1). For the bottom active electrode array, we designed eight groups of parallel regions, which increased

the overall array scale while reducing the number of signal lines, as shown in Fig 1(b). With 64 row signals and 64 column signals, a total of 26,368 electrodes can be controlled independently. Based on droplet generation method and volume requirements, we primarily used three electrode designs, divided into square and hexagonal shapes, mainly 125 μm and 250 μm in size (Parts A, B, C in Figure 1(b) and Table S1). Each pixel contains a TFT and a capacitor, forming a 1T1C circuit. EWOD electrodes can be charged and discharged through row and column scanning. The minimum electrode size is 125 μm $\times$ 125 μm . Using a 30 μm spacer for the gap, droplet manipulation with a minimum volume of approximately 500 pL can be achieved, meeting the volume requirements for single-cell droplet manipulation. Based on the need to generate single-cell droplets, we designed the electrode arrangement of pixel electrodes with different shapes and sizes according to three functional areas: cell loading, cell droplet splitting, and single-cell droplet generation, ultimately forming an AM-DMF system for single-cell applications. The core assembly is a substrate with an active electrode array, while the upper ITO glass substrate serves as a ground electrode.

To form the complete system, we also designed and manufactured the main control board, including a micro-controller (MCU), power supply, and row and column address controller (multiplexer). The control board connects to the AM-DMF chip through a flexible printed circuit (FPC). Simultaneously, we integrated a large field-of-view microscopic optical imaging system above the AM-DMF chip for real-time monitoring and imaging of the experimental process. We designed the software control system ourselves, which can communicate continuously with the main control board and perform automatic process editing and real-time control of the experimental process (Fig S1).

3.2 AM-DMF System Characterization for Single-Cell Manipulation

To achieve stable manipulation of cell-containing liquid samples on the AM-DMF chip—including liquid distribution, droplet generation, and droplet movement—we must optimize and select relevant parameters affecting droplet motion. Previous studies (32) have shown that the array drive sequence of the AM chips used in our study is similar to that of standard flat-panel displays. The SCAN signal series primarily performs row selection, while the DATA signal parallel sets the column signal. Our previous research determined that when the SCAN period is set to 100 μs and DATA to 110 μs with a 10 μs offset, the array refresh rate is 160 ps. Combined with simulation data, when V_{data} is 40V and V_{scan} is 50V, pixel charging and discharging are simulated, and the pixel-on window is obtained sufficient to match driving requirements (Fig 2a).

After determining the driving parameters, chip hardware factors such as the hydrophobic layer, the gap between top and bottom plates, and droplet properties become the main factors affecting droplet movement. When droplet characteristic size is reduced to nanoliter or even picoliter levels, microdroplet properties

change and size effects emerge. As microdroplet size decreases, the surface-area-to-volume ratio increases, meaning larger specific surface area and manifestation of surface effects. The size-effect relationships for various forces and feature sizes are not equivalent. When feature size decreases, body force decreases faster than adhesion force, causing adhesion force to replace body force as the dominant force for droplet movement. For microliter-scale droplets, surface tension dominates among the three adhesion forces. Compared to Van der Waals and electrostatic forces, changing surface tension to drive microdroplets is the easiest to implement.

Based on the classical Young's equation (shown in Supplementary Materials) describing wetting characteristics of the solid-liquid-gas three-phase interface, droplet wetting level is closely related to surface tension at the liquid, gas, and solid three-phase interface. Simultaneously, according to the Young-Laplace formula (shown in Supplementary Materials), the relationship between gas-liquid interface pressure difference and surface tension can be observed. These two equations constitute the fundamental equations for surface tension-driven actuation. Therefore, methods for changing droplet surface tension are based on generation methods and working principles. In applying DMF technology, we primarily use two approaches: adding special reagents to microdroplets and surface modification. Surface modification mainly refers to surface treatment of chip substrate materials to change surface tension and form gradients by modifying solid surface wettability.

We also tested contact angle changes in different functional regions under driving signals (Fig 2(c)-(e)). We selected three commonly used reagents—deionized water (DI water), phosphate buffered saline (PBS), and serum-free media (MEM)—for testing. The test volume was approximately 0.5 μ L. Reagents were placed in silicone oil medium, and a tungsten alloy probe was inserted into the droplet as a ground electrode. Initial contact angles were all around 170°, decreasing with increasing DATA voltage. We further compared regions A, B, and C. When the same DATA voltage was applied, the contact angle corresponding to the larger electrode in region C was smallest, with stronger control force on the droplet. This phenomenon may occur because edge charge effects in large electrodes are more pronounced, promoting greater surface tension on large electrodes.

Based on these principles, we tested contact angles of liquid reagents on different hydrophobic coatings involved in single-cell sorting and manipulation tests. Liquid reagents included DI water, PBS, serum-free media (MEM), serum-containing media (MEM+FBS), and cell-containing solutions (Cell). The hydrophobic coating selection was based on previous research and literature (27, 31, 33), focusing on Teflon and CYTOP, two commonly used hydrophobic coatings for EWOD. For CYTOP, we tested dissolution in four different solvents. Using 0.5 μ L droplets for testing, results showed that Teflon's contact angle for various liquid reagents was larger than other hydrophobic coatings, particularly for cell-containing liquids where the contact angle on Teflon was much larger than on other coatings. Therefore, we used Teflon layers for hydrophobic

coatings on both upper and bottom plates, applied via spin coating.

Droplet movement and generation stability are key performance indicators for evaluating AM-DMF chip performance. We selected commonly used biological solvents as test samples, including DI water, PBS, MEM, and MEM with serum (with 0.01% F68 surfactant, MEM+FBS(F68)). Previous studies have shown that adding surfactant to protein-containing biological reagents can improve mobility stability and reduce protein adsorption on electrode surfaces. For droplet movement stability, we selected a row of 5 electrodes in area A, allowing a single droplet to make continuous reciprocating motion while video-monitoring movement state. Test results are shown in Fig. 3(a). Except for MEM solvent, the other three solvents achieved continuous movement for over 1 hour while maintaining good movement form when the mentioned exercise forms were repeated. PBS and MEM+FBS(F68) are the main solvents for single-cell experiments. Additionally, droplet generation stability is a parameter requiring verification. We selected the “One-to-Two” method to generate single droplets in region A. The final generated droplet is controlled by two electrodes, with each droplet volume approximately 1 nL. This “One-to-Two” method can efficiently and quickly divide a large droplet into multiple small droplets to form a regularly arranged droplet matrix, making it more suitable for high-throughput large-scale single-cell droplet generation and sorting. We tested 32 droplet generation events for each solvent through pre-edited automatic droplet generation, repeating each reagent test more than 3 times. Results are shown in Fig 3(b), demonstrating that the probability of generating 32 droplets for the above solvents exceeds 82%, meaning more than 26 effective single droplets can be obtained at once. Among them, the probability of single droplet generation for PBS is close to 100%, reflecting high stability in single droplet generation. This proves that our selected “One-to-Two” droplet generation path is effective.

3.3 Single-Cell Generation and Manipulation on the AM-DMF Chip

After identifying the hydrophobic coating and solvent, we determined and optimized conditions for single-cell droplet generation. To generate large numbers of droplets in short time, we selected the “One-to-Two” droplet generation method, as shown in Fig. 4(a). This method enables exponential growth in the number of individual droplets. Compared with traditional microchannel-based single-cell droplet generation methods, this AM-DMF chip approach is not limited by physical microchannels and structures, which further restrict droplet movement paths and volumes. Moreover, each single-cell droplet generated can be assigned an electronic code without traditional barcodes, and target single-cell droplets can be individually manipulated through EWOD electrodes. Simultaneously, precise droplet volume control can be achieved through EWOD electrodes. Therefore, this AM-DMF system-based “One-to-Two” droplet generation method is more efficient and flexible than traditional droplet generation methods.

Applying this automated “One-to-Two” droplet generation approach, we suc-

successfully achieved automatic generation of 32 droplets from cell-containing suspension in region A. Generation from a single droplet to 32 droplets required 8 seconds after 5 splitting operations, as shown in Fig 4(b) and Movie S1. We then identified droplets containing single cells from fluorescence microscopic images (Fig 4(b)), where it is clearly visible that the droplet contains one single cell. We can select target droplets containing single cells according to research requirements and move them directionally. They can be moved to idle electrode areas where other single-cell-based operations could be performed, as shown in Movie S2. If cell samples are relatively rare and precious, droplets containing multiple cells can also be collected and recovered in situ, then subjected to further droplet generation until all obtained droplets are single-cell droplets—something traditional microfluidic systems cannot achieve.

To efficiently obtain droplets containing single cells, initial cell concentration is an important parameter. We tested cell suspensions with four concentration gradients and compared their effective droplet generation probability and probability of generating single-cell-containing droplets, with results shown in Fig 4(c). The effective generation probability of droplets is close to 100%, proving that the droplet generation path and driving parameters are stable and accurate. When cell concentration decreased from 10^5 cells/mL to 10^4 cells/mL, the probability of obtaining a single droplet containing one cell increased from 3.1% to 43.3%. As cell density continued decreasing to 5×10^3 cells/mL, the probability of droplets containing single cells decreased. Therefore, based on experimental results, 10^4 cells/mL was selected as the optimized cell concentration for single-cell generation in subsequent experiments.

Using HEK 293 cell suspension at 10^4 cells/mL concentration, with driving signal V_{scan} at 40V, V_{data} at 30V, holding time of 100 μ s, and delay time of 1000 μ s, we applied the “One-to-Two” automatic path. Generation of 32 droplets was performed to obtain droplets containing single cells. Results shown in Fig 5(a) and Fig S3 were obtained by repeating the test experiment 5 times. The average probability of successfully generating 32 single droplets is 98.8%, demonstrating high success rate that meets single-cell droplet generation needs. The probability of obtaining single-cell droplets is 29.7%, which exceeds the probability achieved in some traditional microfluidic systems and meets current single-cell omics research requirements. After demonstrating single-cell droplet generation and manipulation using AM-DMF chips, we investigated AM-DMF effects on cell viability, with results shown in Fig 5(b). Here we applied electrodes to drive single droplets with volume of 2 nL. Using live-cell tracer dye, we proved that cells manipulated by the DMF chip maintain good viability. Results show that green fluorescence signal indicates living cells, while red signal indicates dead cells. These studies demonstrate that our AM-DMF chip can efficiently and rapidly generate droplets containing single cells with good biocompatibility, maintaining good activity for direct use in subsequent single-cell research applications. This addresses shortcomings of existing single-cell sorting methods that can damage cells.

4. Discussion and Conclusion

Although existing single-cell sorting and analysis platforms can rapidly acquire single cells, and single-cell manipulation can be achieved with optical, magnetic, and acoustic technologies, the complexity of current methods limits high-throughput parallel acquisition. Typically, devices with high-throughput single-cell capabilities have relatively complex structures that are not easily integrated with related single-cell analysis technologies, thus limiting promotion and development of single-cell analysis systems. The AM-DMF we developed can simplify the single-cell acquisition process, realizing high-throughput parallel manipulation of nanoliter or even picoliter single-cell droplets. It can also replace traditional barcoding approaches by assigning addressable electronic codes, further simplifying experimental steps for single-cell sorting. The AM-DMF technology platform can achieve high-throughput single-cell sorting and free manipulation of single cells on a two-dimensional plane based on EWOD principles, because each EWOD electrode can be independently controlled. Our AM-DMF technology platform differs from passive DMF technology platforms. Existing passive DMF platforms are limited by hardware and manufacturing processes, with maximum control electrodes currently not exceeding 200, constrained by space for electrode and wire arrangement. These limitations make large-area matrix electrode arrangements difficult to achieve. Our AM-DMF technology platform currently supports 26,368 electrodes. More than 1000 single cells can be processed in parallel on a single chip when all electrodes are rationally utilized. The platform can be easily combined with optical detection, magnetic attraction, and temperature control modules. Functions such as editing, culture, and molecular detection based on single cells can be expanded and integrated in the future. Faced with the need for high-throughput single-cell sorting, manipulation, and enrichment processes in digital cell biology derived from single-cell omics, AM-DMF represents a very promising technology. We expect that with the technology introduced here and subsequent continuous improvement and enrichment of functional modules, AM-DMF can serve as a powerful single-cell sample processing platform for more applications based on single-cell omics, while extending this platform technology to many research areas in biological sciences and chemistry.

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Supporting Information

Young' s Equation

Among them, θ indicates the contact angle—the angle between the tangent line and contact surface between the liquid droplet and solid. Contact angle describes droplet wetting properties on solid interfaces. γ_{sg} , γ_{sl} , and γ_{lg} represent surface tension of solid-gas, solid-liquid, and liquid-gas interfaces, respectively. The values of γ_{sg} , γ_{sl} , and γ_{lg} determine θ .

Young-Laplace Formula

Among them, P is the pressure difference generated by the contact surface between the body and droplet, γ_{lg} is the surface tension at the gas-droplet contact interface, and R_1 and R_2 are the radii of curvature of the contact surface between droplet and gas phase. Therefore, as long as γ_{lg} is changed, a surface tension gradient forms, creating different pressure differences between the two ends of the droplet and resulting in different contact angles between the two ends. This change in contact angle at both ends drives droplet movement toward the direction with smaller contact angle.

Table S1. Summary of Pixel Parameters

Pixel	Shape	Pixel Number	Storage Capacitor @Gap=30 μ m	Droplet Volume
A	Hexagon	288 μ m	4.34 pF	2.2 nL
B	Hexagon	144 μ m	4.08 pF	0.55 nL
C	Square	125 μ m	3.78 pF	0.47 nL

Fig S1. The structure and cross-section view of the AM-DMF chip

Fig S2. The software and hardware system setup of the AM-DMF chip

Fig S3. Single-cell droplet generation experiment based on one-to-two method in the AM-DMF

Movie S1. Automated single-cell droplet generation in the AM-DMF chip

Movie S2. The target single-cell droplet movement in the AM-DMF chip

Figures

Fig. 1. Design of the AM-DMF chip for single-cell sorting and manipulation. (a) Schematic diagram of workflow and chip function allocation area; (b) Layout and microscopy images of the AM-DMF chip electrode; (c) Shape, pitch, and array scale of different function areas of the AM-DMF chip; (d) Pixel circuit design of the AM-DMF chip.

Fig. 2. (a) Pixel driving signals simulation with pixel charge and discharge; (b) Contact angles of different liquid reagents on different hydrophobic layers;

(c)-(e) Contact angle of DI water, PBS buffer, and MEM at various applied Data voltages.

Fig. 3. Droplet movement and generation stability. (a) Stability test of droplet movement, where the droplet continuously reciprocates on a row of 5 electrodes. (b) Stability test for generation of 32 single droplets using a one-to-two approach.

Fig. 4. Single-cell droplet generation on the AM-DMF chip. (a) Schematic diagram of droplet generation path using the One-to-Two method; (b) Screenshots of each step of the 32-droplet generation process and fluorescence microscopic images of droplets; (c) Probability of generating single-cell droplets with different concentrations of cell reagent.

Fig. 5. Single-cell generation and cell culture on the AM-DMF. (a) Probability of cell solution generating droplets and single cells. (b) Cell viability test on- and off-chip.

Note: Figure translations are in progress. See original paper for figures.

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