Lab on a Chip

ARTICLE

Electronic Supplementary Information (ESI) for

Capture and Enumeration of mRNA Transcripts from Single Cells Using a Microfluidic Device†

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I. Materials and Methods

1. Materials and equipment

All chemicals are of American Chemical Society reagent or higher grade. Synthetic oligonucleotides (oligos) were purchased from Integrated DNA Technologies. Deionized water refers to MilliQ-purified 18 MΩ·cm water. A monofunctional amine-PEG with MW of ~2000 g/mol (amine-PEG2000) and a homo-bifunctional carboxy-methyl polyethylene glycol with a molecular weight of 1000 g/mol (dicarboxyl-PEG1000) were purchased from Laysan Bio, Inc. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was purchased from Thermo Fisher. 3-aminopropyltriethoxysilane and 3-glycidoxypropyl triethoxysilane were from Gelest Inc. Dulbecco's Modified Eagle's Medium (DMEM), 10% fetal bovine serum and 0.25% trypsin/EDTA solution for cell culture and treatment were purchased from Mediatech, Inc. Cell lysis buffer solution (500 mM LiCl, 100 mM Tris-HCl, pH 7.5, 10 mM EDTA, 5 mM DTT, 1% lithium dodecyl sulfate (LiDS)) was purchased from Life Technologies Inc. For fluid control, the syringe pump and 9-port rotary valve (Cavro Rocket Pump and SmartValve) from Tecan Systems were used. Bioprecision 2 automated XY stage for inverted microscope (Ludl Electronics Products) and a custom-built adaptor were used for positioning of the microfluidic device onto the microscope for imaging.

2. PDMS device fabrication

The PDMS devices were designed and fabricated essentially according to the procedures of Unger *et al*.[1] with some modifications as described in our previous work.[2, 3] A two-step lithography process was utilized to fabricate the mold for the PDMS fluidic layer with larger cylindrical channels for valving and smaller channels with vertical walls for cell trapping and fluid flow. All fluidic channel features were fabricated to a height of 25 µm. The width and length of the processing chamber are 250 µm. The mold for the valve control layer was patterned with a channel height of 40 µm. Mold height and profile measurements were performed with a Dektak 150 surface profilometer (Veeco Instruments, Inc.) using 5 mg of force and a 12.5 µm diameter probe tip to verify the proper feature heights. The dimensions of the photomask for fluidic layer and valve control layer were scaled to 100.42% and 101.67%, respectively, of the final device to compensate for PDMS shrinkage during curing.

The valving regions were patterned on silicon wafers using Shipley Microposit SPR 220-7.0 (Rohm & Haas Electronic Materials, LLC). After the silicon wafers (100 mm, test grade, University Wafer) were primed with HMDS by spin-coating at 4000 rpm for 40 s and incubated for one minute at room temperature, the positive photoresist was then coated on by spinning at 500 rpm for 10 s, then 625 rpm for 45 s and 5000 rpm for 0.3 s at maximum acceleration to help remove edge-beads, and soft-baked at 115 °C for 7 min. The photoresist on the wafer was exposed using a transparency photomask (FineLine Imaging) on a Karl Suss MA6 contact aligner for 130 seconds at 11 mW/cm^2 in hard contact mode. The photoresist was developed in MF-24A (Microposit) for 5-10 min. and then rinsed thoroughly in dH_2O, and dried with nitrogen gas. To produce the rounded features for the valving regions, the photoresist was hard-baked and reflowed by heating at 200 °C for 120 minutes.

The mold for the remaining fluidic features was patterned using SU 8-2025 (MicroChem Corp.) with channel height of 25 µm. were patterned as follows. SU 8-2025 was spin-coated at 3100 rpm onto the wafers then soft-baked at 65 °C for 1 min. and 95 °C for 5 min. The photoresist was patterned with a transparency photomask for 13 seconds at 11 mW/cm^2. The resist was post-baked at 65 °C for 1 min. and 95 °C for 5 min., and developed in SU-8 developer (MicroChem Corp.) for 4 min. After the wafer was rinsed in isopropyl alcohol and dried with nitrogen gas, the photoresist was hard-baked at 150 °C for 10 min. to enhance the mold strength and durability.

For the valve control channel molds, SU 8-2050 (MicroChem Corp.) was spin-coated onto the wafers at 4000 rpm, then soft-baked at 65 °C for 3 min. and 95 °C for 6 min. The photoresist was then exposed using a transparency photomask for 15 seconds at 11 mW/cm^2. The photoresist was baked at 65 °C for 1 min. and 95 °C for 6 min., and then developed in SU-8 developer for 5 min. The wafer was cleaned, dried and soft-baked a described above. Prior to first use, all molds were passivated with tridecafluoro-1, 1, 2, 2-tetrahydrooctyl-1-trichlorosilane (Pfaltz and Bauer) by vapor deposition. The valve control layer and fluidic channel layer were prepared using Sylgard 184 (Dow Corning) with part A to part B ratio of 5:1 and 20:1, respectively. The PDMS prepolymer mix-

ture was degassed and poured onto the mold in a polycarbonate carrier to produce the valve control layer. The degassed PDMS mixture was spin-coated with 1150 rpm for 1 min. onto the fluidic channel mold to a height of about 50 μm to produce the fluidic channel layer. After the PDMS layers were cured at 65 °C for 30 min., the PDMS valve control layer was peeled off the mold and holes were punched for fluidic connections using a 0.75 mm diameter punch (Ted Pella, Inc.). The layer was aligned and laid onto the fluidic channel layer on the mold using a custom system. The two layers were bonded together by heating at 65 °C for 4 hours. The bonded PDMS layers were peeled off mold together and holes were punched for the fluidic channel inlets and outlets. A custom alignment setup comprising of micromanipulators and a stereomicroscope was used to align the valve control layer onto the fluidic channel layer via pre-defined fiducial marks.

3. Chemical bonding of PDMS to glass and selective functionalization of surfaces

The overall process is illustrated in Fig. 2 in the main text. First, the bottom of the PDMS channel layer is covalently bonded onto a glass coverslip by a chemical reaction between the reactive groups functionalized on their surfaces.[4] To do so, the surface of the PDMS layer is functionalized with epoxy groups while the surface of the cover glass is activated with primary amine groups. Borosilicate glass coverslips (50 x 50 mm x 0.17 mm) functionalized with amine groups essentially as described.[5] Briefly, the glass slides were cleaned and then soaked in 2% solution of 3-aminopropyltriethoxysilane in 93% methanol plus 5% glacial acetic acid for 10 minutes, then rinsed with methanol and dried with argon gas. The surface of the PDMS was activated by oxygen plasma in a UV-ozone cleaner (Jelight) for 3 min. and then functionalized by soaking in a 2% solution of 3-glycidoxypropyl triethoxysilane in 93% methanol and 5% glacial acetic acid for 15 min. The PDMS was then rinsed with methanol and blown dry with argon. Finally, the functionalized PDMS was laid on top of the coverslip. Any trapped air bubbles between layers were removed by placing the assembly under vacuum for 15 min. and rapidly equilibrating to atmospheric pressure. The PDMS-glass assembly was then heated at 65 °C for 4 hours to facilitate the crosslinking of the epoxy groups on the PDMS to the amine groups on the coverslip.

Second, the surface of the PDMS inside the channels was passivated with polyethylene glycol (PEG) by reacting the epoxy groups with a monofunctional amine-PEG2000. The assembled device was heated at 60 °C on a hotplate and an automated syringe pump was used to deliver a solution containing 2 mM amine-PEG and 10 mM triethylamine (TEA) in anhydrous N,N-dimethylformamide (DMF) through the channels. The reaction was carried out at 60 °C for 1 hour. The solution was frequently refreshed to compensate for evaporation.

Third, the amine groups on the glass surface of the channels was conjugated to a bifunctional carboxyl PEG1000 by injecting a solution containing 1 mM of the carboxymethyl-PEG1000, 10 mM EDC and 5 mM TEA in anhydrous DMF into the channels and incubating for two hours at room temperature. The channels were treated then with 10 mM sulfo-NHS acetate in 100 mM sodium borate, pH 8.5 with 0.02% Triton X-100 for 30 minutes at room temperature to acetylate the residual amine groups.

Finally, the glass surface was selectively functionalized with poly$(dT)_{50}$ oligos. The valves above and below the RNA capture and processing chambers (valve lines 1 and 2 in Fig. 1B) were closed to isolate the channel containing the chambers and a solution containing 1 μM amine-labeled poly$(dT)_{50}$, 10 mM EDC in 100 mM (N-morpholino) ethanesulfonic acid (MES) buffer at pH 5.0 was inject into the channel and incubated for 1 hour at room temperature. The channel was then washed and stored in saline sodium citrate buffer (SSC) until use.

4. Microscope system with TIRF setup for single-molecule imaging and kinetics measurements

The system is a based on fully automated inverted fluorescence microscope equipped with an objective-based TIRF system (Zeiss Axio Observer with Laser TIRF 3). The entire TIRF microscope setup without the microfluidic device and syringe pump is shown in Fig. S1. An adaptor (Zeiss TIRF 3 Slider) is used to introduce the laser excitation into the objective (100x oil, NA 1.46, Alpha Plan Apochromat, Zeiss). The lasers (405 nm direct diode, 488 nm direct diode, 532 nm DPSS and 642 nm direct diode lasers) are combined and coupled into the TIRF slider by a polarization preserving single-mode broadband optical fiber (kineFLEX, Point Source). The emitted

fluorescence is reflected by a dichroic mirror (DM), passed through a filter and captured on a back-illuminated EM-CCD camera (512 x 512 pixels, 16 µm x 16 µm pixel size, iXon3 897, Andor Technology).

All dichroic mirrors and interference bandpass filters were from Semrock Inc. The emission from Cy3 was imaged using FF01-560/25 bandpass filter with a transmission center at 560 nm and 25 nm bandwidth, Di01-R532 as dichroic mirror. The emission from Alexa 647 was imaged using FF01-676/29 bandpass filter with a transmission center at 676 nm and 29 nm bandwidth, Di01-R635 as dichroic mirror. The emission from fluorescein was imaged using FF01-536/40 bandpass filter with a transmission center at 536 nm and 40 nm bandwidth, Di01-R488 as dichroic mirror. For kinetics measurement, time-lapse images were captured for the same field of view using 100 ms exposure time and an EM gain of 30. To image the entire mRNA capture chamber, scanning was performed by stepping with an automated stage (MAC 6000, Ludl Electronic Products). A 10 percent overlap between fields of view was employed to ensure that all spots were detected.

II. Figures

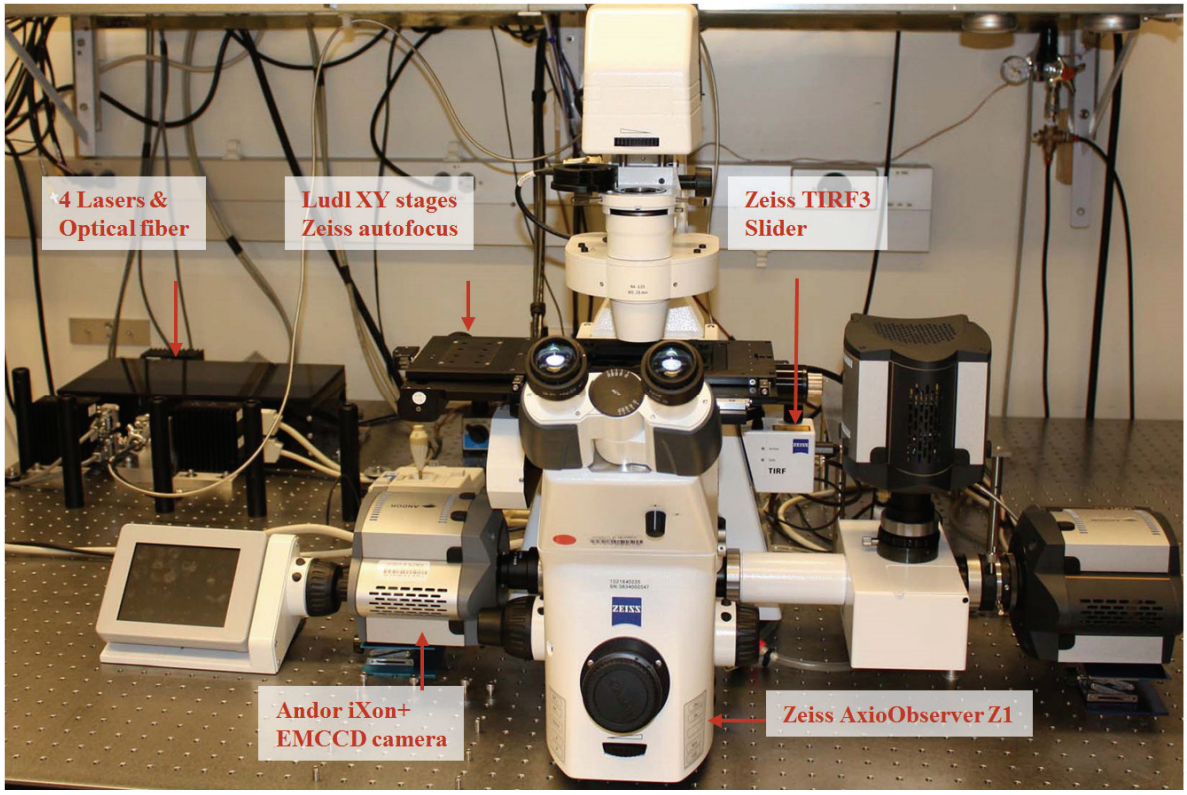

Fig.S1 Microscope system with a TIRF setup for single-molecule imaging and kinetics measurements. The system is based on a fully motorized inverted fluorescence microscope (Zeiss AxioObserver Z1). An adaptor (Zeiss RITF3 Slider) is used to introduce the laser excitation into the objective (100x oil, NA 1.46, Alpha Plan Apochromat, Zeiss). The lasers (405 nm direct diode, 488 nm direct diode, 532 nm DPSS and 642 nm direct diode lasers) are combined and coupled into the TIRF slider by a polarization preserving single-mode broadband optical fiber (kineFLEX, Point Source).

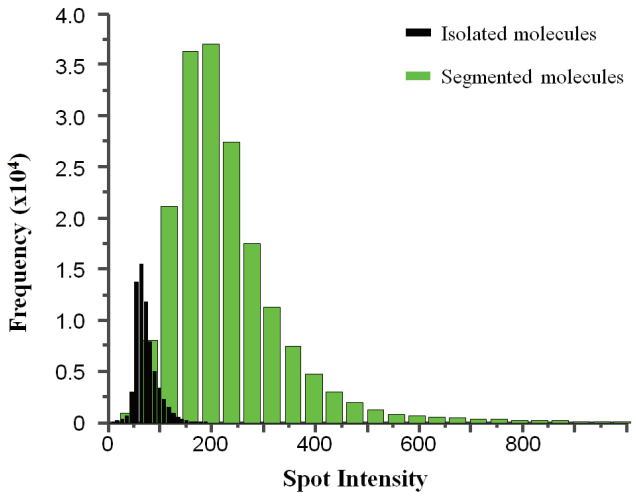

Fig.S2 Histogram of intensity values for isolated and segmented molecules compiled from all images of mRNA capture experiments with 9 HeLa cells. The mean intensity of spots selected by the image processing algorithm as individual molecules is displayed, whose mean was used to approximate the average intensity of a single molecule. The mean intensities of the spots which were segmented using the watershed function are generally higher, indicating that each spot may contain multiple mRNA molecules.

III. Movies

1. Movie M1. Single-cell capture video clip 1.

This video shows that hydrodynamic trapping of single cells takes place automatically with high efficiency. The HeLa cells in PBS were injected into the cell capture channel of a device with multiple single-cell traps in serial. The trap path is designed as a small channel with 8 μm x 8 μm cross section with a round 22 μm x 22 μm cup-shaped mouth.

2. Movie M2. Single-cell capture video clip 2

The video shows that the cells are quite elastic and some cells will be squeezed through the trap for some combinations of cell size/shape/elasticity and dimensions of the traps under certain hydrodynamic pressure or flow rate. The layout of this particular single-cell capture device is slightly different from the ones we used for our single-cell devices.

IV. References

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