Electronic Supporting Information

One step DNA amplification of mammalian cell in picoliter microwell arrays

Wenwen Liu,[a] Zhao Li, [a] Yuanjie Liu, [a] Qingquan Wei, [a] Yong Liu, [a] Lufeng Ren, [a] Chenyu Wang, [a] and Yude Yu [a] *

[a] State Key Laboratory on Integrated Optoelectronics, Institute of Semiconductors, Chinese Academy of Sciences, P.O. Box 912, Beijing, 100083, China

EXPERIMENTAL SECTION

Chip fabrication and surface modification. The chip is fabricated by traditional etching technique. The detail steps are the same as we have reported previously.[13] Briefly, the silicon wafer is ultrasonic cleaned by acetone, ethanol, deionized water for 15 min each. Then, it is immersed in a Piranha solution (H_2SO_4:H_2O_2=3:1) at 180 ^oC for 30 min, rinsed thoroughly by deionized water, and dried with nitrogen. Next, photoresist is spin-coated on the silicon wafer for subsequent lithography with a photolithography mask. Under the protection of the photoresist, a picoliter microwell array is formed using deep reactive-ion etching and the remaining photoresist is removed with acetone. After cleaning, a 200-nm-thick silicon dioxide (SiO2) layer is added to the wafer in a thermal oxidation furnace at 1050°C.

Methoxy-PEG-silane is covalently modified to the chip surface (2-[methoxy(polyethyleneoxy)propyl]-trimethoxysilane; J&K Scientific, Beijing, China). The modification solution is 1 g of methoxy-PEG-silane dissolved in 100 mL of anhydrous toluene with 1% triethylamine as a catalyst. Then, the bare chip is cleaned by oxygen plasma for 30 s and immersed in the modification solution at room temperature for overnight at least. Do not pick up the chip until using and each time of modification can keep for 3 days. Prior to use, the well-modified chip is rinsed by ethanol and DI water, and dried with nitrogen. The dried chip is better to be used immediately to keep the surface fresh.

Cell culture and pretreating. CaSki, SiHa and C-33A cells were purchased from National Infrastructure of Cell Line Resource, China. These cells were cultured in MEM (SiHa and C-33A) or RPMI 1640 (CaSki) medium supplemented with FBS (10%, v/v), penicillin and streptomycin (1%, v/v), and glutamine (1%, v/v). Cells are grown in culture dish put in a humidified incubator at 37^oC with 5% CO_2. Once cells become confluent, trypsin was used to detach and harvest them from the culture dish. For the analysis of cell/well ratio, live cells were stained by Calcein AM (Invitrogen, c3099) before loading to the chip. For most of the PCR assay, cells were unstained and washed by PBS, fixed by PFA (4%, v/v) for 20 minutes, centrifuged (1000 rpm, 3 min), and resuspended by DNase/RNase-free water. In some special condition (shown in Figure 3), cell were stained by PI after fixiation and washed again. Blood cell counting chamber is used to modulate the density of cell suspension before the loading into chip.

Taqman PCR reagents. Sequence information for the PCR of human Alu elements were as follows: Forward primer 5'-GACCATCCCGGCTAAAACG-3'; Reverse primer 5'-CGGGTTCACGCCATTCTC-3', Taqman probe: 5'-FAM/CCCCGTCTCTACTAAA/BHQ-3'. Sequence information for PCR of the HPV16 E6 PCR as follows: Forward

primer 5'- CTGCAATGTTTCAGGACCCA-3'; Reverse primer 5'- TCATGTATAGTTGTTTGCAGCTCTGT-3', Taqman probe for HPV16 E6 PCR was: 5'- FAM/AGGAGCGACCCGGAAAGTTACCACAGTT BHQ/-3'.

All these primers and Taqman probes were synthetized by Sangon Biotech (Shanghai). THUNDERBIRD Probe qPCR Mix is purchased from TOYOBO. Bovine serum albumin (BSA, Sigma) and DNase/RNase-free water (Life Technology) were used during the mixture preparation. Thermal cycler conditions were 95°C for 5 min followed by 50 cycles of: 95 °C, 15 s and 60 °C, 45 s.

Sample loading and Assemble of the heating chamber. There are two times of sample loading in this work. The first time is the loading of cells to chip. Cells suspended in DNase/RNase-free water were centrifuged (1200 rpm, 5 min) into the wells on chip by using a swing bucket rotor. Then the chip was rinsed by DNase/RNase-free water and dried by nitrogen again. The next is the second sample loading of the PCR reagent. A detailed description was given in our previous work.[13] Briefly, we added the premixed PCR reagent on one side of the chip and wiped them by a piece of silica gel to the other side slowly. After the reagent filled the wells, mineral oil was quickly added on the surface of the chip to prevent evaporation and leakage. Finally the chip was embedded in the middle of a cover glass and a copper sheet to form a chamber, which is fixed by screws. The assembled heating chamber was put on the top of the heating modulate of thermal cycler (Applied Biosystems 2720) and ready for PCR assays.

Fluorescent imaging and Data analysis. To detect the cell loading-rate or the PCR fluorescent signal, a wide-filed and high resolution imaging microscopy was used to resolve all picoliter wells. The detailed setup of microscopy is the same as we previous reported. [13] The filters set for excitation and emission are 480/30 nm and 535/40 nm, respectively. For the cell/well ratio analysis, the cells were stained by Calcein AM.

Image-pro Plus was used to systematically detect and quantify the fluorescent positive microwells and to analyze their density and averaged fluorescent density. Background subtractions and contrast enhancement were performed for quantification of the PCR results. The image acquired at the beginning (cycle-1) was regarded as the background noise of the experiment; other images acquired subsequently subtract it in situ for contrast enhancement.

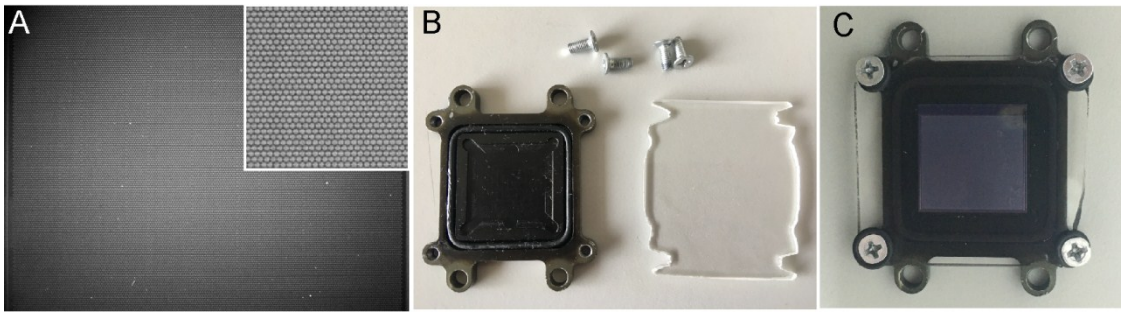

Figure S1 Real photos of chip and heating chamber (cooper-glass chamber). (A) The chip containing 30,600 microwells. (B) Elements for heating chamber assemble. (C) A well-assembled cooper-glass chamber.

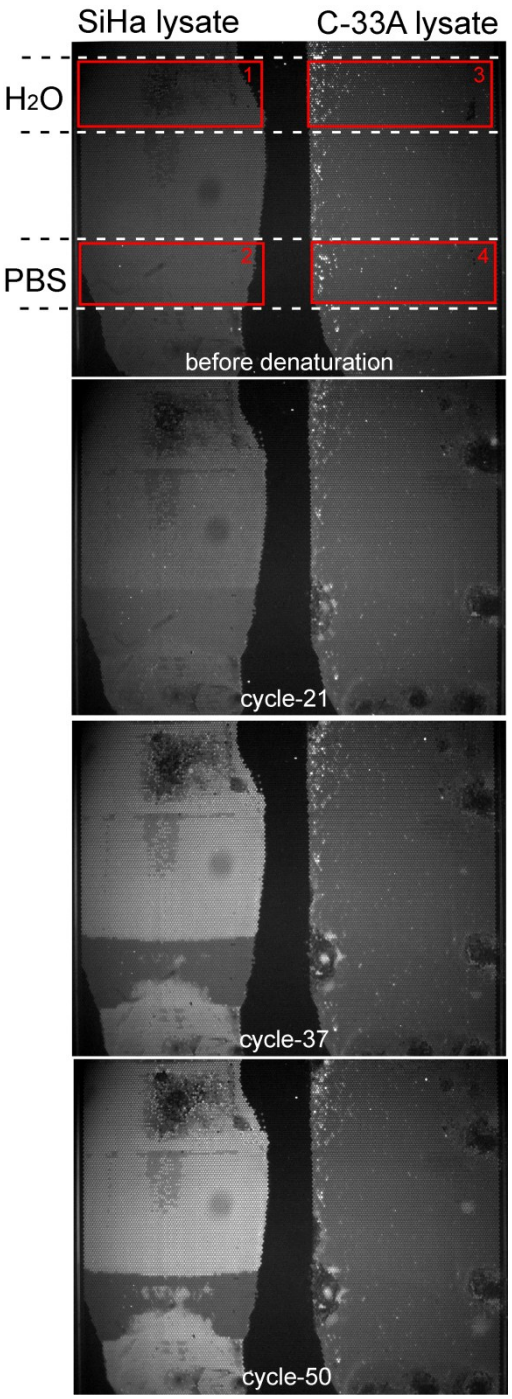

Figure S2 PBS inhibits the PCR reaction on chip. We first loading H_2O and PBS on the PEG modified chip by silica gel in the dotted line marked area. These two areas are separated, thus we can compare them on the same chip. After the evaporation, the dried chip was loaded two samples of PCR reagent in the perpendicular direction. One sample containing SiHa cell lysate (left area) which is lysised by proteinase K, and another one was C-33A cell lysate for comparison (right area). So, there are four cross regions shown by red frame. This PCR result demonstrates that PBS inhibited the PCR reaction completely (region 2). These figures also show the bad effects of air bubbles during the PCR reaction. All the darkest area in figure（especially in the middle） was not filled by PCR reagent and containing air, which generated air bubble during the PCR assays.

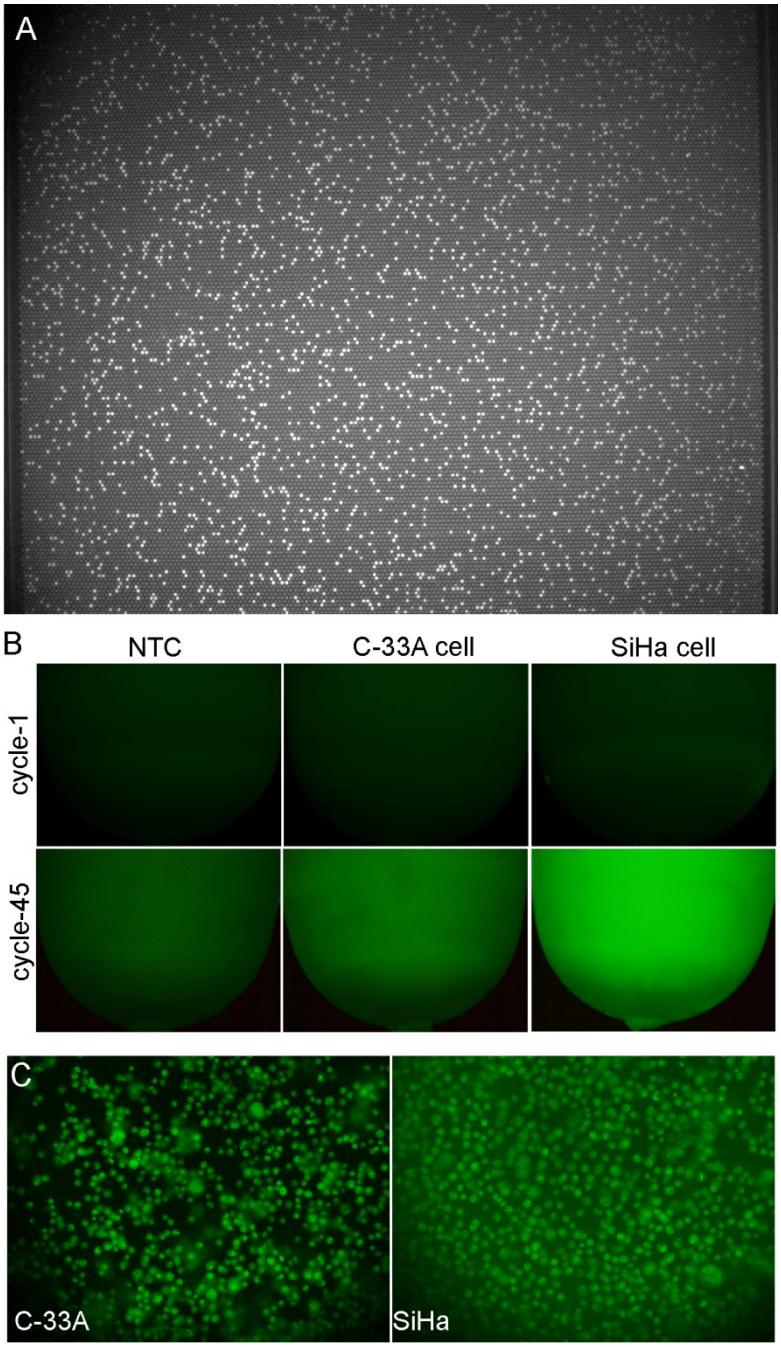

Figure S3 Verification of PCR reagent and PEG modified chip. (A) Image of digital PCR result using PEG modified chip demonstrates the well modification of surface. The templates in this PCR reagent are pure DNA molecules. (B) Detection of HPV 16 E6 in PCR vials (20 μl) using thermal cycler. The fluorescent images show the bottom of PCR vial through microscope and SiHa cells show strong fluorescence indicates the reagents work well. Both C-33A cells and SiHa cells in this experiment are pretreated as the manuscript described. No cells were added in NTC vial. (C) Higher magnification of PCR results of C-33A and SiHa in vial. The cells are still in the bottom of the vial after PCR. We put them on the cover glass and observed by fluorescent microscope. The fluorescence of cell themselves is corresponding with the nonspecific fluorescence on chip (the bright spots in Figure.3A, S4). The fluorescence out cell in solution is PCR signals in SiHa sample, which is darkness in C-33A.

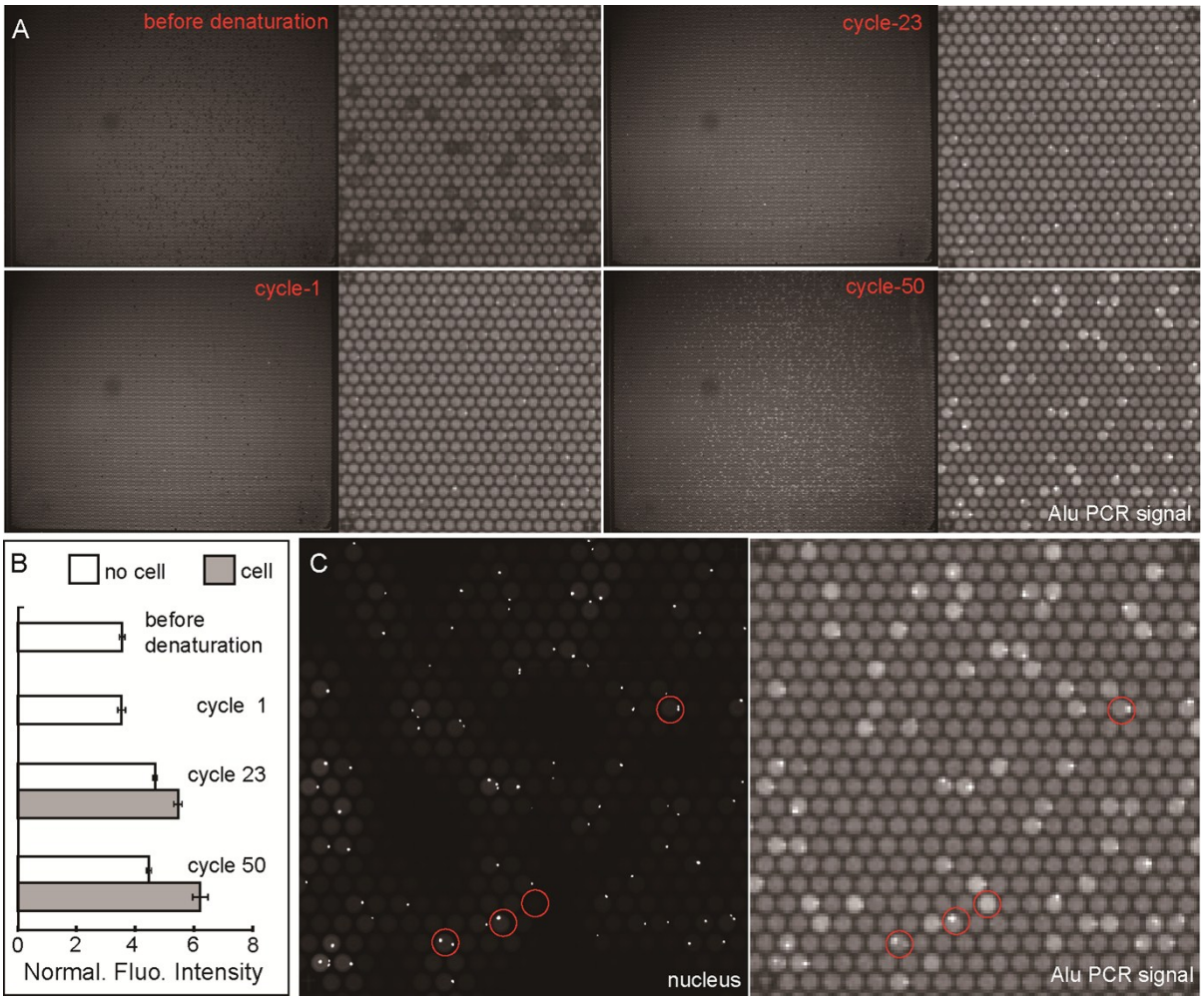

Figure S4. One step PCR of single cell on detection of Alu element. (A) Series images of the same chip during PCR. Bright spots in cycle-1 and cycle-23 are nonspecific fluorescence of cells, and the bright wells in cycle 50 shown the PCR signals. (B) The graph illustrates the difference of fluorescence increase between microwells empty and that containing cells. (C) The comparison between cell nucleus and PCR signals of Alu elements. Red circles marked cell+/fluo- and cell-/fluo+ as we have defined in the manuscript.