Supplementary Methods for "A Nanoliter Scale Nucleic Acid Processor with Parallel Architecture"

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Supplemental Information

Fabrication of the mRNA microfluidic chip. The chip was fabricated by Multilayer Soft Lithography in a "push-up" geometry[1–3]. The microfluidic channels are deep enough for the mammalian cells to pass through, but sufficiently shallow to keep volumes small and, therefore, the mRNA concentration high. Pneumatically actuated microfluidic on-off valves were created to close 100 μm wide and 50 μm deep channels with low actuation pressures (5 psi)[3]. In order to create and actuate those valves, the chip consists of two layers of channels: one layer with the microfluidic channels and a second layer for the pneumatic actuation of the valves. This second layer of channels, called the actuation layer, is separated from the microfluidic channels by a thin membrane. Figure 1 (in the main article) provides the layout of the chip. The active valves are located at the intersection of the fluidic channel and the actuation channel. By increasing the pressure in the actuation channel, the membrane can be deflected toward the roof of the fluidic channel. The fluidic channels have a round cross section thus allowing complete closure. Molds for both layers of the chip were fabricated by standard photolithography using high resolution printed slides as masks. In order to achieve high aspect ratio fluidic channels, a thick positive photoresist (AZ 100 XT PLP) was used. Once developed, the photoresist was heated above its glass transition temperature, thus allowing reflowing of the photoresist in order to get channels with a rounded cross section. Molds for the actuation channels were made using SU-8 50, an epoxy-based negative photoresist. Both molds were then exposed to a vapor of trimethylchlorosilane for 1 minute in order to facilitate mold release. Then the two-component silicone elastomer (GE RTV 615) was poured on the fluidic channel mold placed in a plastic petri dish, and allowed to partially cure. GE RTV 615 was spun onto the actuation channel mold, and partially cured. The actuation channel chip layer was positioned on the fluidic layer and the two layers were bonded. The fluidic channel layer has an excess in curing agent (GE RTV 615 B) whereas the actuation channel layer has an excess in the other component (GE RTV 615 A), thus allowing a strong bond between the two cured layers. Access holes to the actuation channels and to the fluidic channels were punched through the cured PDMS and the final chip was sealed to a precleaned glass slide.

Operation of the microfluidic chip. All the valve actuation lines were filled with fluid (a concentrated aqueous solution of Orange G) in order to avoid bubble formation in the fluidic channels. The membrane separating the fluidic channel and the actuation line is very thin (less than 5 microns) and permeable to air. Each control line is connected to its pressure source. The working pressure at which all pressure sources were set was 2 psi above the actuation pressure of the valves, thus allowing a 1 psi pressure on the fluidic channels. The working pressure was different from one chip to another due to small variations in the membrane thickness, but typically between 8 and 15 psi. All the valves were closed before loading the reagents.

The reagents were delivered through polyethylene tubing, which was connected to the chip using 23 gauge stainless steel pins (New England Small Tubes, Litchfield, NH). The other end of the tubing was connected to a common pressure source for all the fluidic channel inputs (1 psi). The chip was loaded with 10

µl of cell solution, 20 µl of Dynal Lysis buffer, and 5 µl of beads solution. The original Dynabeads were resuspended in Dynal Lysis buffer and reconcentrated to 5_ concentration.

Loading the chip. The lysis buffer was first loaded on the chip by opening valves 1 and 2. Once the lysis buffer flowed through the Lysing Buffer Out channel (in the main article Fig. 1a), valve 1 was closed. The "lysis buffer chamber" (composed of the channel space delineated by valves 1, 2, 3 and 4; in the main article Fig. 1a) was dead-end-filled by pushing the air into the gas-permeable chip. Valve 2 was closed. The cells were loaded by opening valves 5 and 6. The cell suspension flowed through the "cell chamber" (composed of the channel space delineated by valves 4, 5, 6 and 7; in the main article Fig. 1b); valve 5 was closed. Valve 5 could be opened and closed repeatedly until a suitable number of cells were trapped in the "cell chamber" (in the main article Fig. 1c). This number could also be influenced by changing the cell suspension concentration. Typically, 1 to 100 cells could be trapped and lysed on the chip. Valve 6 was kept open until the remaining air in the "cell chamber" was pushed out; valve 6 was then closed.

The bead suspension was loaded by opening valves 9 and 10. Once the bead suspension flowed through the "beads chamber" (composed of the channel space delineated by valves 7, 8, 9 and 10), valve 9 was closed and the chamber was dead-end-filled by pushing the remaining air into the gas-permeable chip. Valves 8 and 11 were then opened and pressure on valve 8 was slowly increased using the pressure regulator until the 2.8 µm diameter beads began to stack up against the partially closed valve. Once the stack was sufficiently long to reach the Beads In channel, valve 10 was closed (in the main article Fig. 1b).

Lysing the cells. Valve 4 was opened thereby allowing the lysis buffer to diffuse into the "cell chamber". As lysis buffer reached an individual cell, the cell would immediately lyse, and then gradually disintegrate (in the main article Fig. 1c).

Trapping the mRNA. The cell lysate was flushed out of the "cell chamber" by opening valves 3, 4, 7 and 11. The lysate was allowed to flow through the stack of beads at a controlled speed (typically 100 µm/s) that could be adjusted by changing the pressure on the Air In channel. The poly A-containing mRNA hybridized to the oligo-dT on the beads and remained on the beads while the majority of the cell debris washed on out of the chip. The air/fluid interface stops at the stack of beads due to surface tension. At that point, valve 3 was closed, valve 2 was opened and lysis buffer was flushed through the beads to wash them free of as much cellular debris as possible. Valve 2 was then closed.

Collecting the beads. A short tubing was connected to the Output port of the chip and was placed in a 0.1 ml PCR tube. Valves 2 and 8 were opened and the beads were flushed with lysis buffer into the PCR tube. A magnet was used to draw out any beads remaining in the outflow channel. The beads were centrifuged, and then resuspended in 100 µl of fresh lysis buffer containing 1 µl of RNase Inhibitor. The tube was vortexed and the mRNA was either analyzed immediately, or the tube was stored frozen at –80°C.

Bacterial DNA chip Microfabrication. Chips were fabricated by Multilayer Soft Lithography in a "push-down" geometry[1–3]. Mask designs were created with the CAD program FluidArchitect (Fluidigm, South San Francisco, CA) and transferred to high-resolution transparency masks (3,389 dpi). The dimensions of the fluidic channels are 100 μm in width and 10 μm in depth, while the valve actuation channels are typically 200 μm in width and 15 μm in depth. As shown in figure 3f, within 20 _ 20 mm space, we designed 13 fluidic access vias, 54 valves and 14 actuation vias that allow microfluidic flow and control. Mask molds for the fluidic channels are made by spin-coating positive photoresist (Shipley SJR 5740) on a silicon wafer with 2,000 rpm for 1 min, followed by mask exposure and development. These mold channels are rounded at 135 °C for 15 min to create a geometry that allows full valve closure. Another mold for the actuation layer is made by spinning photoresist on a separate wafer at 1,600 rpm for 1 min with a resulting height of 13 μm, followed by mask exposure and development. The devices were fabricated by bonding together two layers of two-part cure silicone (GE Silicone RTV 615) cast from the photoresist molds. The bottom layer of the device, containing the flow channels, is spin coated with 20:1 part A:B RTV615 at 2,400 rpm for 1 min and the resulting silicone layer is an 11 μm thick film. The top layer of the device, containing the actuation channels, is cast as a thick layer (5 mm thick) with 5:1 part A:B RTV615 using a separate mold. The two layers are initially cured for 30 min at 80°C. Actuation channel interconnect holes are then punched through the thick layer with a 20 gauge luer stub, after which it is sealed, channel side down, on the thin layer, after aligning the respective channel networks with an optical microscope. Bonding between the assembled layers is accomplished by curing the assembled devices at 80 °C for more than 90 min, followed by punching the fluidic channel interconnects. The resulting devices are cut to size and mounted on RCA cleaned cover slips (No. 1, 24 mm _ 50 mm), followed by incubation at 80 °C overnight to promote adhesion.

System setup and pneumatic control. The chip is mounted on an inverted microscope (Nikon Eclipse TE2000-S). Fluorescence excitation was provided by a mercury lamp (100W). A FITC filter set (Ex465 - 495, DM 505, BA 515-555) was used and the image was recorded by using a PC controlled color digital camera (Sony DFW-V500).

Each actuation line on the chip was connected with a stainless steel pin (New England Small Tube, Litchfield, NH) and polyethylene tubing to an external solenoid valve controlled by a digital data I/O card (CCA, PPC1-DIO32HS; National Instruments, Austin, TX). Regulated external pressure was provided to the normally closed port, allowing the actuation channel to be pressurized or vented to atmosphere by switching the miniature valve. The fluidic vias for the introduction and collection of sample and buffer were connected to an external pressure source through polypropylene tips (Multiplex tips, Sorenson BioScience, Inc., West Salt Lake City, Utah). The typical pressure for driving liquid inside the chip is 0.5 to 2.0 psi (1 psi = 6.89 kPa).

Sequence of Operations for the Chip. After the chip was mounted on an optical microscope stage and the control channels were connected to external pneumatic control systems, microbeads (Dynabeads® DNA DIRECT™universal, Dynal ASA, Oslo, Norway) with DNA binding capacity were loaded through the

'bead in' port in the upper right part of figure 3f. For this operation, valve 10 'loop exit control' and valve 12 'bead trap control' were closed. The beads were positioned just before valve 12 by controlling the valves 6 and 12. The pressure control for valve 12 was lower than the pressure for the other valve controls, resulting in a partially closed valve that allows buffer to pass through while the beads accumulate into a column. 1 µl of each of the buffer solutions (lysis buffer (Xtra Amp®, lysis buffer series 1, Xtrana, Inc., Ventura, CA), wash buffer (Xtra Amp®, wash buffer series 1) and elution buffer (Tris-EDTA buffer, pH 7.8)) were loaded through the 'lysis in', 'wash in' and 'elute in' ports, respectively.

The sample solution containing bacteria was then loaded through the 'cell in' port. Control valve 4 'vertical control' and valve 1 'lysis control' were opened in order to switch the chip from "loading" mode to "processing" mode, and the solutions were moved into the rotary mixer by pushing with lysis buffer supplied though the 'lysis in' port. At this point the three solutions were isolated in the rotary mixer, and complete mixing was rapidly achieved by actuating the peristaltic pump within the ring. The time for complete mixing of two liquids with the mixer was less than 10 s with 5 Hz operating speed; 5 min of mixing was used to achieve complete lysis of the target cells.

After lysis, valve 2 'wash control', valve 4 'vertical control', valve 10 'loop exit control' and valve 13 'wash control' were opened. Lysed bacterial cell solution containing genomic DNA and other cell debris was removed from the rotary mixer by applying wash buffer. The genomic DNA adsorbs onto the surface of the bead column, while the cell debris was flushed to "waste". Genomic DNA captured onto the surface of the beads was washed and then eluted and recovered through collection ports 1, 2, and 3. For this, valve 13 was closed and valve 14 'collection control' was opened followed by opening valve 3 'elution control'. Collected samples were amplified by conventional PCR and confirmed by gel electrophoresis.

References

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