

Supporting Online Material (SOM) Section S1. Large-Size of DCI Produced Samples

Using the DCI approach, it is possible to generate large microwell arrays with interconnected nanochannels without any defects. Fig. S1 shows a local surface area of a sample with size of 2 cm x 2 cm. More than 10 other areas were examined and all showed defect free 7 μ m microwells and 1 μ m long nanochannels. The samples made by EGDMA were sufficiently robust and remained intact after soaking in Piranha solution for 5 hours. An example is shown in Fig. S2 by SEM.

Fig. S1. Fluorescence micrograph of nanochannels and microwells filled with Rhodamine over a large area.

Fig. S2. SEM image of 1 µm microwells of EGDMA connected by nanochannels after soaking in Piranha solution for 5 hours to form hydrophilic surface.

Section S2. Procedure to Form Hydrophilic EGDMA Sample

The hydrophobic EGDMA polymer samples were treated with Piranha solution of 7:3 v/v of conc. H₂SO₄ and 30% H₂O₂ for 5 hours to turn them hydrophilic. Fig. S3 shows the water contact angles before and after Piranha solution treatment.

Before treatment

After treatment

Fig. S3. Water droplets on EGDMA samples with 7 µm microwells (a) before and (b) after being treated with Piranha solution.

Section S3. DNA Nanostrands on Various Micropillar Designs

 In addition to circular pillars, other shapes including squares, triangles, and diamonds can be used to enhance or direct DNA stretching (See Fig. S4). Long raised ridges that become access grooves upon imprinting can also be integrated with DNA nanostrands and micropillars as shown in Fig. S5 (a-b). DNA would always be stretched towards the closest feature.

Fig. S4. SEM images of stretched DNA nanostrands between (a) 1 µm tapered circular pillars with 1 μ m spacing, (b) 7 μ m square pillars with 1.2 μ m spacing, (c) 10 μ m circular pillars with 10 μ m spacing, (d) 5 μ m diamond pillars with 5 μ m spacing, (e) 5 μ m equilateral triangle pillars with 2 μ m spacing, and (f) 20 μ m diamond pillars with 10 μ m side by side spacing and 20 µm diagonal spacing.

Fig. S5. SEM images of stretched DNA nanostrands between (a) pillars and long micro-ridge and (b) sharp points of saw-tooth shaped micro-ridge.

Section S4. Fabrication of Microgrooves and Inlet/Outlet on the Nanochannel/Microwell Arrays of EGDMA

To make functional devices out of these arrays, inlet/outlet ports with access channels can be added after fabrication of the arrays using micromilling (MM), reactive ion etching (RIE) or femtosecond laser ablation (FLB) to remove part of the EGDMA as shown in Figs. S6 and S7. Micromilling was accomplished by milling channels directly into the EGDMA of the nanochannel/microwell arrays after fabrication. A high-precision CNC micromilling machine (Aerotech) was used with end mills (Performance Micro Tool) of either 10 or 25 µm. The channels were milled down to the depth of the glass surface of the cover slip removing all EGDMA in the channel area. This allowed various reagent solutions to be introduced into the array after sealing. RIE and femtosecond laser ablation have also been successfully used to remove an area of EGDMA in order to create access channels (Figs. S7a and b). The frequency doubled pulses from a mode-locked erbium-doped fiber laser intensified in a Ti: Al_2O_3 regenerative amplifier laser (CPA2161, Clark-MXR) were used for creating

microgroove by a femtosecond laser. The maximum output power of the laser was $P_{av}=2.5W$, pulse duration was $T_p=150$ fs, pulse repetition frequency was $f_p=3$ kHz and collimated beam diameter was 5 mm. Laser beam power was adjusted by a series of optics, including thin-film polarizing beams splitters and ½ waveplates. The attenuated laser beam was delivered by a beam mirror train through a mechanical shutter and then focused on the material. A 50x infinity corrected microscope objective lens with numerical aperture NA= 0.42 (M plan Apo NIR 50x, Mitutoyo) was used for focusing the femtosecond laser beam. Attenuated laser power was measured by a power meter (PM100, Thorlab) placed under the laser focusing lens. The beam quality factors were $M^2=1.2$ in the vertical Y direction and $M^2=1.3$ in the horizontal X direction. The computer controlled motion system (MX80L, Parker) had 0.5 μ m resolution in the X, Y, and Z axes. For microgroove fabrication, laser power of 3 mW and scan speed of 20mm/min was used on EGDMA substrate. 10 multipasses with 1 µm pitch were used to create 10 µm wide channels.

 Although we can incorporate access grooves into the de-wetting design, these techniques of removing the channels from the EGMDA after the array is fabricated allows extra flexibility to add channel designs that would otherwise disrupt the de-wetting and DNA stretching process, such as large channels or complex channel designs.

Fig. S6. (a-b) SEM images of microchannel made by micro milling. (c-d) Bright field images of a microgroove integrated with microwell/nanochannel arrays at higher magnification.

Fig. S7. SEM images of microgrooves made by (a) femtosecond laser and (b) RIE etching.