Electronic supplementary information (ESI)

Site-Specific Nanopatterning of Functional Metallic and Molecular Arbitrary Features in Nanofluidic Channels

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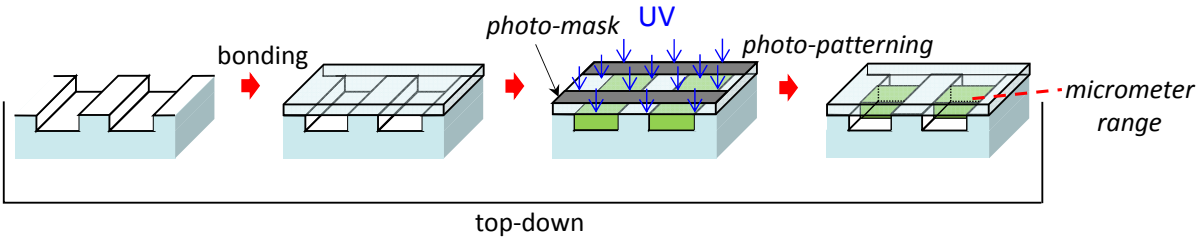

Fig. S1 Schematic drawing of photo-patterning widely used in microfluidics.

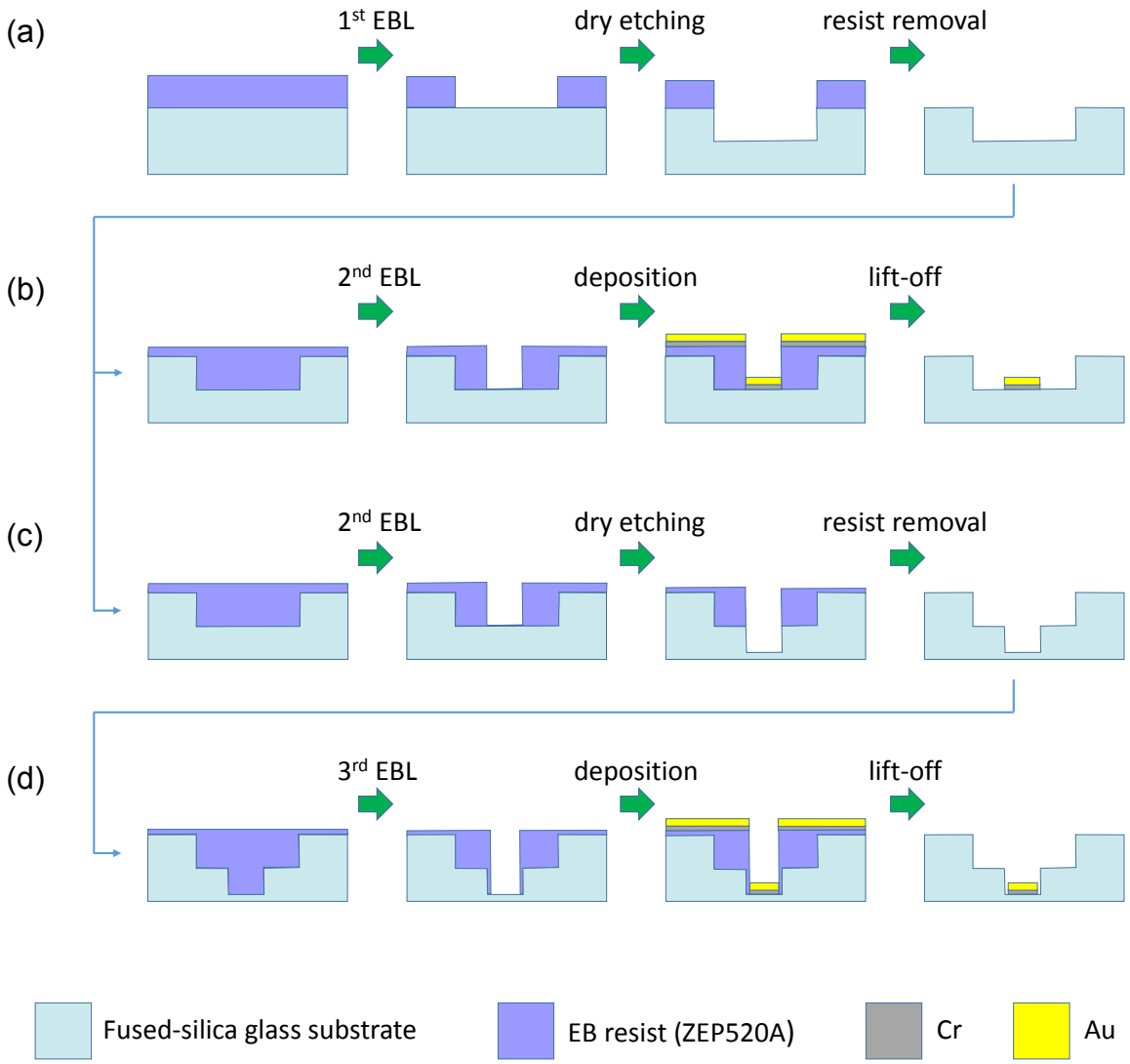

Fig. S2 Nano-in-nano fabrication guided by the high-precision placement control technique. (a) Open nanofluidic channels; (b) gold-nanopatterned open nanofluidic channels; (c) nanowells in open nanofluidic channels; (d) gold-bottomed nanowells in open nanofluidic channels.

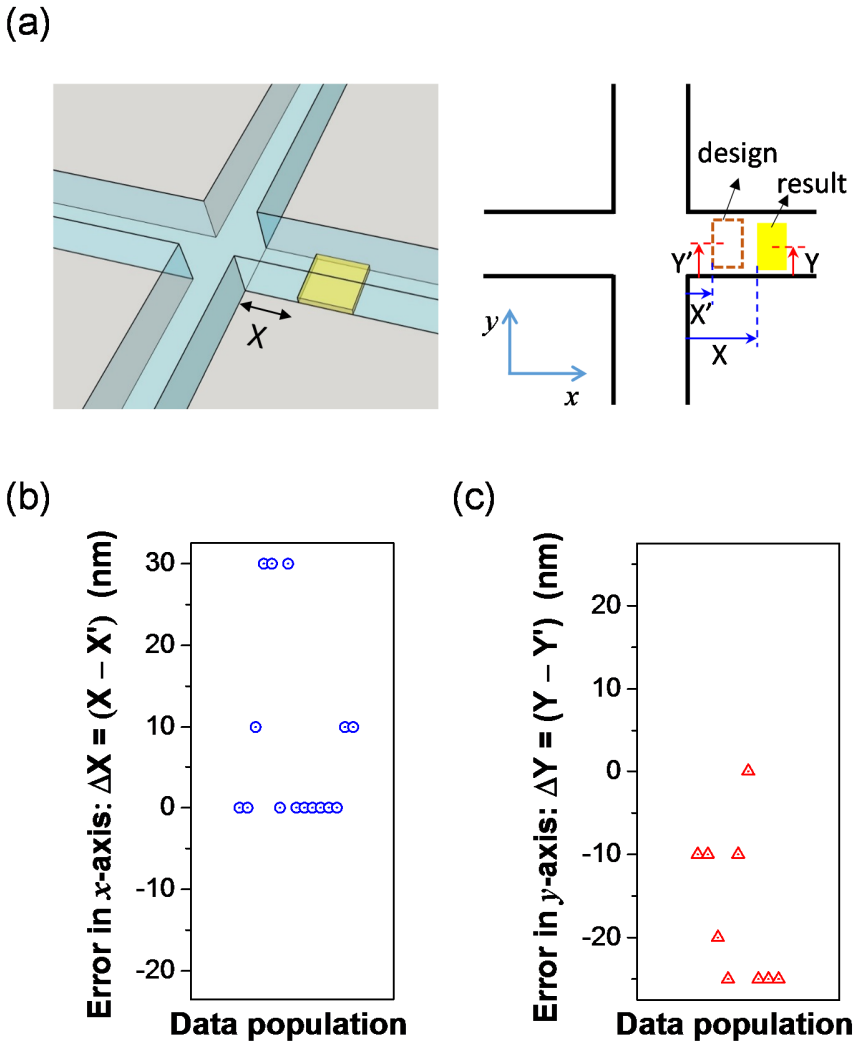

Fig. S3 Evaluation of precision of the placement control technique.

(a) The precision in terms of placement errors in both *x*-axis ($\Delta X = X - X'$) and *y*-axis ($\Delta Y = Y - Y'$) directions was characterized by comparison of the actual (result) placement measured by SEM (X and Y, respectively) and the design placement (X′ and Y′, respectively) of a rectangular gold nanopattern (300 nm × 470 nm) fabricated in an open cross-shaped nanofluidic channel-network (520 nm wide, 240 nm deep) with the placement control technique.

(b) The placement error in *x*-axis direction: (8 ± 16) nm in average, 30 nm in max and 0 nm in min (Data are the mean ± SD, $n \geq 9$).

(c) The placement error in *y*-axis direction: (−16 ± 9) nm in average, 0 nm in max and −25 nm in min (Data are the mean ± SD, $n \geq 9$)

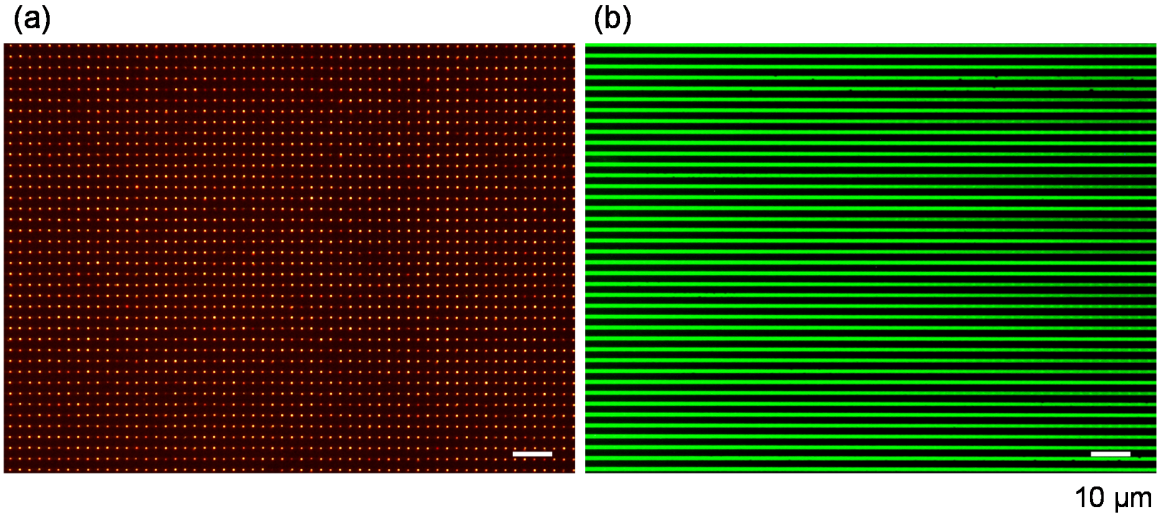

Fig. S4 Evaluation of the gold-pattern-friendly bonding of a gold-patterned nanofluidic chip (Fig. 3b) under a continuous nanofluidic condition at a high pressure (430 kPa) for six days. (a) A bright-field image and (b) a corresponding fluorescence image were taken at the nanofluidic channel area, during the introduction of an ethanol solution of HOOC-$(CH_2)_{10}$-S-S-$(CH_2)_{10}$-CONH-fluoresceine on the sixth day. Fluorescence was not observed in the non-channel area, revealing that no detectable leakage occurred during the continuous operation at the high pressure.

Experimental

Fabrication of Reference Marks. First, an EB resist (ZEP-520A; Zeon) was spin-coated on a cleaned fused-silica glass substrate (30 mm × 40 mm ×0.7 mm; Sendai Quartz). Then, a standard EBL process (ELS-7500; Elionix) was performed to transfer CAD designed patterns of reference marks to the resist layer on the substrate. After that, 5 nm-thick chromium (Cr, 99.9%; Nilaco) and 100 nm-thick gold (Au, 99.99%; Tanaka Kikinzoku Kougyo) films were sequentially deposited on the substrate by using a vacuum evaporation equipment (A9858; Seinan Industries) at 10^{-5} Pa. After a lift-off process in a mixture (3/1, v/v) solution of dimethyl sulfoxide (Wako) and xylene (Wako), the remained Au/Cr patterns on the substrate formed the reference marks.

Nano-in-Nano Fabrication. First, single or arrayed open nanofluidic channels were fabricated on the substrate having reference marks by EBL and plasma dry etching (NE-550; Ulvac), according to processes reported previously[1] (Fig. S2a). In the EBL process, the aforementioned placement control technique was used to decide the relative placement of the nanofluidic channels on the substrate. Then, another EBL was performed to form EB-resist nanopatterns with desired features on exact locations of nanofluidic channels. The process was also guided by the placement control technique. Next, Cr (5 nm) and Au (45 nm) were sequentially deposited on the substrate by using the vacuum evaporation equipment at 10^{-5} Pa. After a lift-off process as described above, the gold-nanopatterned open nanofluidic channels (nano-in-nano structures) were obtained (Fig. S2b). For the nano-in-nano-in-nano structures demonstrated in Fig. 2j, additional EBL and plasma dry etching for the fabrication of nanowells (Fig. 2i) in nanofluidic channels were performed, before fabrication of gold nanopatterns (Fig. S2c, d). The feature sizes (width, length, and depth) of the open nanofluidic channels and the nano-in-nano patterns were characterized by using a SEM

(ELS-7500; Elionix), a FE-SEM (SU8010; Hitachi), an AFM (SPA-400; SII), and a stylus surface profiler (Dektak 150; Ulvac).

Chip Fabrication, Liquid Introduction and Molecular Nanoarray Formation. Microfluidic channels (Fig. 3a) were dry-etched on another fused-silica glass substrate after a photolithography process and then the inlet/outlet holes pierced using a diamond-coated drill. The nanofluidic chip (Fig. 3b) was obtained by bonding the microfluidic-channel substrate and the gold-nanopatterned nanofluidic-channel substrate using the gold-pattern-friendly bonding method. The operation of liquid introduction based on the setup shown in Fig. 3c has been described in details previously.[1] The ethanol (99.9%; Merck) solution of HOOC-$(CH_2)_{10}$-S-S-$(CH_2)_{10}$-CONH-fluoresceine (1.0 mM; ProChimia) was flowed through the nanofluidic channels at 100 kPa for 2 h, and then stopped for 12 h to form SAM in the nanofluidic channels at a static condition by closing all inlets and outlets. After the liquid in the nanofluidic channels were flowed out by introducing air at 90 kPa, the molecular nanoarray was observed by using both the fluorescence mode and the bright-field mode of an system microscope (BX53; Olympus), with ultra-long working distance objective lenses of high magnification (LUCPLFLN; Olympus) and a microscope digital camera (DP80; Olympus). The profiles of the fluorescence intensity were analyzed by an image processing program (ImageJ 1.48v; NIH).

References

1. Y. Xu, C. X. Wang, Y. Y. Dong, L. X. Li, K. Jang, K. Mawatari, T. Suga and T. Kitamori, *Anal. Bioanal. Chem.*, 2012, **402**, 1011-1018.